

- LIEBAU, F., (1965). *Acta Cryst.* **18**, 352–354.
 LIMINGA, R. (1965). *Acta Chem. Scand.* **19**, 1629–1642.
 LIMINGA, R. (1966). *Acta Chem. Scand.* **20**, 2483–2496.
 McDONALD, W. S. & CRUICKSHANK, D. W. J. (1967). *Z. Kristallogr.* **124**, 180–191.
 MACKIE, P. E., ELLIOTT, J. C. & YOUNG, R. A. (1972). *Acta Cryst.* **B28**, 1840–1848.
 MAMEDOV, KH. S., GAMIDOV, R. & BELOV, N. V. (1961). *Sov. Phys. Crystallogr.* **5**, 91–94.
 MASON, R. (1964). *Acta Cryst.* **17**, 547–555.
 PALACHE, C., BERMAN, H. & FRONDEL, C. (1963). *Dana's System of Mineralogy*, 7th ed. Vol. II, p. 735.
 PIMENTEL, G. C. & MCCLELLAN, A. L. (1960). *The Hydrogen Bond*, p. 280. San Francisco, London: Freeman.
 REA, J. R. & KOSTINER, E. (1972a). *Acta Cryst.* **B28**, 2504–2509.
 REA, J. R. & KOSTINER, E. (1972b). *Acta Cryst.* **B28**, 2525–2529.
 REA, J. R. & KOSTINER, E. (1972c). *Acta Cryst.* **B28**, 3461–3464.
 SCHULTEN, M. A. DE (1904). *Bull. Soc. Fr. Minér.* **27**, 100–103.
 SPENCER, L. J., (1908). *Miner. Mag.* **15**, 1–38.
 STÄHLIN, W. & OSWALD, H. R. (1970). *Acta Cryst.* **B26**, 860–863.
 SUDARSANAN, K. & YOUNG, R. A. (1972). *Acta Cryst.* **B28**, 3668–3670.
 SUDARSANAN, K. & YOUNG, R. A. (1974). *Acta Cryst.* **B30**, 1381–1386.
 SUDARSANAN, K., YOUNG, R. A. & DONNAY, J. D. H. (1973a). *Acta Cryst.* **B29**, 808–814.
 SUDARSANAN, K., YOUNG, R. A. & DONNAY, J. D. H. (1973b). *Acta Cryst.* **B29**, 2649.
 SUTOR, D. J. (1967). *Acta Cryst.* **23**, 418–422.
 TILLMANN, E. & BAUR, W. H. (1971). *Acta Cryst.* **B27**, 2124–2132.
 WHITAKER, A. (1973). *Z. Kristallogr.* **137**, 194–219.
 WHITAKER, A. & JEFFERY, J. W. (1970). *Acta Cryst.* **B26**, 1429–1440.
 WOLFE, C. W. (1940). *Amer. Min.* **25**, 787–809.

Acta Cryst. (1975). **B31**, 2035

The Crystal Structure and Molecular Conformation of Cyclo-L-prolyl-L-prolyl-L-hydroxyproline, a Cyclic Tripeptide

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The cyclization of three peptides to form a nine-membered ring introduces a certain amount of strain in the geometry of the peptide unit. This first crystal structure analysis of a cyclic tripeptide ($C_{15}H_{21}O_4N_3$) has shown that all three peptide units are in the *cis* conformation. The planarity of the peptide units is not unduly disturbed, but the three bonds involving the nitrogen atom are not coplanar. The three pyrrolidine rings are puckered differently, the atoms out of plane in the three cases being N, C $^{\alpha}$ and C $^{\beta}$ respectively. The hydroxyl oxygen of the hypro group in this molecule is disordered, occupying two positions, both of which are able to participate in hydrogen bonding. The crystal belongs to the orthorhombic space group $P2_12_12_1$ with unit-cell parameters, $a=16.284$, $b=9.449$, $c=9.260$ Å and $Z=4$. The structure was solved by direct methods with the *MULTAN* program and refined by full-matrix least-squares calculations to a reliability index of 6.2%.

Introduction

In proteins and polypeptides, the building block is almost invariably the *trans* peptide unit. However there are situations where the *cis* peptide is incorporated in molecules for specific stereochemical reasons. A class of compounds containing *cis* peptides comprises the cyclic peptides having a small number of peptide units. In the diketopiperazines, the two *cis* peptides are cyclized to form a six-membered ring (Corey, 1938; Karle, 1972). It has been predicted that three peptides can be cyclized only if all three are in the *cis* conformation (Venkatachalam, 1968). As the number of peptide units increases, the *trans* peptide is increasingly preferred (Dale & Titlestad, 1970; Kopple, Onishi & Go,

1969; Kopple, 1971). Cyclo-L-Pro-L-Pro-L-Pro is the first cyclic tripeptide to be synthesized (Rothe, Steffan & Rothe, 1965). The derivatives of this compound were later synthesized and studied by n.m.r. spectroscopy by Blout and coworkers (Debar, Torchia & Blout, 1971). The structural parameters of cyclo-L-Pro-L-Pro-L-Pro were deduced from empirical calculations using stereochemical criteria (Venkatachalam, 1968). In these calculations it was shown that the three peptides have to be in the *cis* conformation for ring closure to occur. The two assumptions made in these calculations were: (1) the proline rings were planar and (2) the three bonds connected to the nitrogen atom were coplanar. To avoid short intramolecular contacts between the C $^{\alpha}$ protons, the torsion angle ω (a measure

of non-planarity of the peptide group) was given the value of 25° for all three peptides. On the other hand, n.m.r. studies on the solutions of cyclo-L-Pro-L-Pro-L-Pro and its derivatives showed that the peptide units were, indeed, in the *cis* form, but the pyrrolidine rings were non-planar, with the nitrogen atom out of the plane of the other four atoms (Debar *et al.*, 1971). In order to obtain more precise information about the conformational features of cyclic tripeptides, we carried out the X-ray analyses of cyclo-L-Pro-L-Pro-L-Pro and cyclo-L-Pro-L-Pro-L-Hyp (hereinafter called CTP(1) and CTP(2) crystals, respectively). The results of these studies showed that the three peptide units in both molecules were in the *cis* conformation, but the pyrrolidine rings were non-planar (Kartha, Ambady & Shankar, 1974). This paper describes the results of the X-ray analysis of CTP(2).

Experimental

The crystals used in this study were obtained by recrystallization from ethanol of the material given to us by Professor E. R. Blout, Harvard Medical School. The unit-cell parameters were obtained from the 2θ measurements of 12 high-angle reflections.

Crystal data $a = 16.284$ (2), $b = 9.449$ (2), $c = 9.260$ (2) Å, $Z = 4$, $V = 1425$ Å³, $D_x = 1.437$, $D_m = 1.420$ g cm⁻³, space group $P2_12_12_1$, $Cu K\alpha = 1.5418$ Å, molecular formula $C_{15}H_{21}O_4N_3$.

The density was measured by flotation in bromoform-benzene at $22 \pm 3^\circ C$. The intensity data were collected on a G.E. XRD-3 diffractometer by the θ - 2θ scan up to a maximum of 2θ of 150° . Out of 1725 intensities 1538 were greater than 2.5σ and these were considered non-zero. An approximate correction for

anisotropy of absorption as a function of φ was applied to the data.

Structure determination and refinement

The structure was solved by the multisolution tangent refinement technique with the *MULTAN* program (Germain, Main & Woolfson, 1971). There were 402 reflections with $E > 1.2$ and the reflections 085, 093, 360 and 470 were used to define the origin of the cell and to select the enantiomorph. Phases of the three reflections 591, 10,10,0 and 15,2,0 were permuted to give the starting phase sets which were developed by tangent refinement to give 16 phase sets, out of which the one with the second-highest figure of merit gave the correct electron density map which revealed the complete molecule. The structure was refined by the block-diagonal least-squares method. All hydrogen atoms, except that of the hydroxyl group OH(1), were located from an electron-density difference map. During the refinement, it became apparent that the hydroxyl group was disordered. The electron-density map calculated without this oxygen revealed two peaks [OH(1) and OH(2)] with occupancies in the ratio 3:7. Of these OH(2) with the larger occupancy was connected to the C γ atom of proline 2, while OH(1) was connected to C γ of proline 1. Moreover, in both positions the hydroxyl group was hydrogen bonded with the carbonyl oxygens of two different molecules. The final cycles of refinement were therefore carried out with the hydroxyl group with occupancies in the ratio 3:7 from peak heights. No attempt was made to refine the occupancies themselves. All the non-hydrogen atoms were given anisotropic thermal parameters but the hydrogen atoms were given isotropic ones.

Table 1. Atomic parameters of cyclo-L-Pro-L-Hyp with *e.s.d.*'s within parentheses ($\times 10^4$)

The thermal parameters are in the form $T = \exp[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl)]$.

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(1)A	5732 (3)	5217 (5)	-1605 (6)	22 (2)	56 (5)	81 (6)	0 (2)	2 (3)	1 (5)
C(1)B	6458 (2)	4567 (6)	-2433 (6)	33 (2)	85 (6)	107 (7)	9 (3)	18 (4)	-9 (6)
C(1)G	7031 (3)	4012 (7)	-1256 (8)	29 (2)	115 (8)	154 (9)	20 (4)	6 (4)	0 (8)
C(1)D	6909 (3)	5017 (7)	17 (7)	22 (6)	109 (7)	130 (8)	14 (3)	-17 (3)	5 (7)
C(1)P	5104 (3)	4082 (5)	-1149 (5)	27 (2)	61 (5)	73 (6)	-3 (3)	-4 (3)	4 (3)
C(2)A	3956 (3)	5888 (5)	-1045 (6)	22 (2)	62 (5)	110 (7)	4 (3)	-1 (3)	3 (5)
C(2)B	3088 (3)	5457 (6)	-1609 (8)	21 (2)	95 (6)	169 (9)	3 (3)	-16 (4)	4 (7)
C(2)G	2993 (4)	3902 (7)	-1245 (8)	35 (2)	115 (8)	213 (12)	-18 (4)	-29 (5)	29 (9)
C(2)D	3714 (3)	3487 (6)	-313 (8)	27 (2)	85 (7)	180 (9)	-9 (3)	3 (4)	18 (7)
C(2)P	3899 (3)	6732 (6)	365 (6)	29 (2)	82 (6)	112 (7)	11 (3)	9 (3)	8 (6)
C(3)A	5264 (3)	7923 (5)	-122 (6)	32 (2)	47 (5)	89 (6)	1 (3)	-5 (3)	-10 (5)
C(3)B	5439 (4)	9489 (6)	247 (7)	59 (3)	52 (5)	149 (9)	0 (4)	-24 (5)	-15 (6)
C(3)G	5066 (6)	9702 (8)	1735 (8)	93 (5)	118 (9)	173 (12)	4 (6)	-14 (7)	-75 (9)
C(3)D	4423 (15)	8565 (7)	1964 (7)	70 (4)	122 (8)	118 (9)	16 (5)	7 (5)	-51 (7)
C(3)P	5967 (3)	6980 (5)	431 (6)	26 (2)	64 (5)	95 (6)	-3 (3)	-8 (3)	-1 (5)
N(2)	4356 (2)	4508 (4)	-737 (5)	23 (1)	50 (4)	116 (6)	0 (2)	0 (3)	11 (4)
N(3)	4510 (3)	7615 (5)	699 (5)	36 (2)	76 (5)	81 (5)	7 (5)	3 (3)	-20 (5)
N(1)	6127 (2)	5779 (4)	-298 (4)	22 (1)	72 (5)	87 (5)	2 (2)	-8 (2)	-1 (4)
O(1)	5299 (2)	2831 (4)	-1117 (5)	35 (2)	47 (4)	159 (6)	9 (2)	12 (3)	1 (4)
O(2)	3313 (3)	6595 (6)	1160 (6)	45 (2)	190 (8)	180 (8)	-6 (4)	46 (4)	-26 (7)
O(3)	6370 (2)	7303 (4)	1482 (5)	45 (2)	112 (5)	127 (6)	4 (3)	-32 (3)	-41 (5)
OH(1)	7826 (12)	4162 (20)	-1470 (20)	99 (12)	187 (31)	213 (30)	65 (16)	47 (16)	-39 (26)
OH(2)	3180 (39)	3108 (69)	-2609 (74)	50 (3)	150 (10)	187 (11)	17 (5)	-36 (5)	65 (9)

The final reliability index is 6.2%. At this stage the shifts in coordinates were less than one tenth the standard deviation. The structure was further refined through one cycle of full-matrix least-squares calculations without any change in the reliability index. The atomic scattering factors of non-hydrogen atoms were taken from *International Tables for X-ray Crystallography* (1962) and for the hydrogen atoms, from Stewart, Davidson & Simpson (1965).*

The positional and anisotropic thermal parameters of the non-hydrogen atoms are given in Table 1 and those of the hydrogen atoms in Table 2. The weighting scheme used in the final cycles of refinement was of the type $1/\sigma$, where σ represents standard deviation in $|F_o|$ based mainly on counting statistics and instrumental stability. The e.s.d.'s of all non-hydrogen atoms except C(3)G, OH(1) and OH(2) were 0.003–0.005 Å. For these three atoms they were 0.007, 0.018 and 0.006 Å re-

* A table of observed and calculated structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 30972 (10 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester, CH1 1NZ, England.

spectively. The corresponding accuracy in the bond distances and angles were 0.004 Å and 0.4° respectively.

Table 2. Atomic parameters of hydrogen atoms ($\times 10^3$)

	Bonded to	x	y	z	β
H(1)	C(1)A	549 (3)	586 (6)	-226 (6)	2.9
H(2)	C(2)A	419 (3)	640 (5)	-186 (5)	1.7
H(3)	C(3)A	517 (3)	786 (5)	-109 (6)	2.1
H(4)	C(1)B	634 (4)	388 (7)	-315 (7)	4.3
H(5)	C(1)B	665 (4)	515 (8)	-314 (8)	6.3
H(6)	C(1)G	690 (4)	299 (6)	-84 (7)	5.7
H(7)	C(1)D	689 (3)	462 (6)	98 (6)	2.9
H(8)	C(1)D	744 (4)	555 (8)	44 (8)	6.4
H(9)	C(2)B	302 (5)	568 (9)	-280 (9)	5.9
H(10)	C(2)B	265 (4)	607 (7)	-112 (8)	5.9
H(11)	C(2)G	247 (3)	358 (5)	-72 (5)	2.9
H(12)	C(2)D	354 (4)	362 (7)	93 (8)	7.0
H(13)	C(2)D	392 (4)	263 (7)	-36 (7)	4.4
H(14)	C(3)B	508 (4)	1005 (7)	-26 (7)	6.6
H(15)	C(3)B	602 (5)	977 (9)	29 (9)	10.4
H(16)	C(3)G	567 (5)	952 (9)	264 (9)	8.6
H(17)	C(3)G	490 (6)	1061 (9)	219 (9)	10.4
H(18)	C(3)D	378 (4)	899 (7)	211 (7)	7.5
H(19)	C(3)D	449 (4)	801 (7)	282 (7)	5.2
H(20)	OH(2)	257 (12)	345 (22)	-326 (23)	3.5

Table 3. The conformational angles in CTP(2) obtained from X-ray study compared with results from other studies

		Ring 1	Ring 2	Ring 3	Ave.	N.m.r.
N—C(A)—C(B)—C(G)	(χ_1)	34°	13°	28°	25°	30°
C(A)—C(B)—C(G)—C(D)	(χ_2)	-31	8	-22	-15	-10
C(B)—C(G)—C(D)—N	(χ_3)	17	-26	7	-1	-5
C(G)—C(D)—N—C(A)	(χ_4)	5	36	12	18	25
		1	2	3	Stereochemical criteria*	
C(3)P—N(1)—C(1)A—C(1)P	φ	-100°	-110°	-95°	-78°	
N(1)—C(1)A—C(1)P—N(2)	ψ	84	92	97	109	
C(1)A—C(1)P—N(1)—C(2)A	ω	20	0	1	25	
C(2)D—N(2)—C(2)A—C(2)P	θ''	94	88	92		
$\theta'' - \varphi$ (=180° for planar nitrogen environment)		194	198	187		

* Venkatachalam (1968).

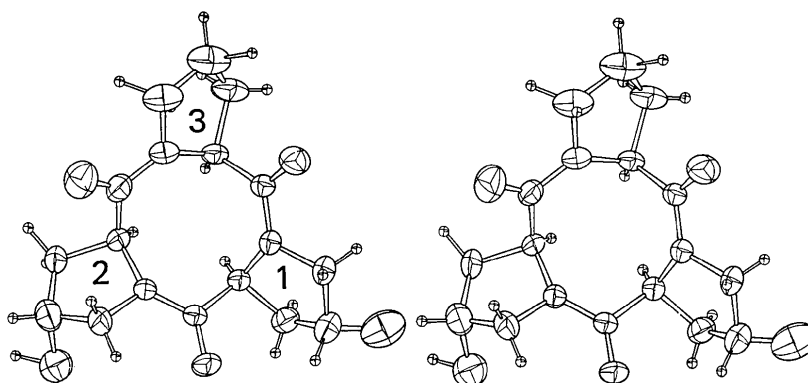


Fig. 1. Stereo view of the molecule of CTP(2) looking down an axis approximately perpendicular to the mean plane of the nine-membered ring (Johnson, 1965). The ellipsoids are of 50% probability.

Molecular structure

The molecule viewed down an axis approximately perpendicular to the mean plane of the nine-membered ring is shown in Fig. 1. The three peptide groups are in the *cis* conformation confirming the prediction based on stereochemical criteria (Venkatachalam, 1968). The distortion in the peptide group may be represented by two parameters, namely the torsion angle ω (IUPAC-IUB Commission on Biochemical Nomenclature, 1970), which is zero for planar peptide units and the quantity $\theta''-\phi$, which is an indication of the coplanarity of the bonds connected to the nitrogen atom. When these bonds are coplanar $\theta''-\phi$ becomes 180° (Balasubramanian, Tegoni, Venkatesan & Ramachandran, 1971). In the molecule of CTP(2), ω is less than 1° for two of the peptides and 19° for the third peptide (Table 3). The C^α protons [H(1), H(2), H(3)] are free of intramolecular short contacts, the shortest contact between them being 2.23 Å. It appears, therefore, that non-planarity of the peptide group is not strictly required to avoid C^α proton short interactions. The average values of the conformational torsion angles ϕ and ψ are -101° and 90.5° respectively as compared with -78° and 109° reported earlier (Venkatachalam, 1968).

Bond lengths and angles

The bond distances and angles in the molecule of CTP(2) are given in Fig. 2. In the proline residues, the introduction of the methylene group at the nitrogen atom instead of the usual hydrogen atom causes appreciable changes in the peptide geometry. When one compares the stereochemical parameters of the average *cis* proline unit (Kantha, Ambady & Shankar, 1974) with the corresponding *trans* proline unit (Ashida & Kakudo, 1974) one finds that the angle $C(A)-C(P)-N$ (119° , 118°) in proline peptides is widened by about 5° while the $O-C(P)-N$ angle (121° , 120°) is significantly reduced. The corresponding values for these angles given by Corey & Pauling (1953) are 121° and 114° respectively. Angles around the nitrogen atom are also appreciably altered; the increase in the angle $C(P)-N-C(A)$ and the corresponding decrease in the angle $C(D)-N-C(A)$ are obviously due to the steric repulsion of the C^α protons in the molecule. In the case of *trans* proline residue, this repulsion is between the C^α proton and C^δ proton of the pyrrolidine ring with a resultant increase in the angle $C'-N-C^\delta$. The increase in the $C'-N$ bond length (1.345 Å) could be due to the strain imposed on the cyclization of the tripeptide or a structural feature of *cis* proline residues. The $C'-N$ bond here is significantly longer than Pauling & Corey's value of 1.32 Å for the standard *trans* peptide unit, but agrees well with the average value of this bond distance obtained from the eight *cis* proline residues in the molecules of sodium and lithium antamanide complexes (1.34 (1) Å; Karle, 1974a).

The pyrrolidine rings

The conformation of the pyrrolidine rings in the oligopeptides has been discussed in detail by Ashida & Kakudo (1974). In general, the proline rings are non-planar and exhibit puckering at the C^γ atom, as seen from many single-crystal studies of both linear and cyclic peptides containing proline residues. For example, in the crystal structures of the sodium and lithium complexes of the cyclic decapeptide antamanide (Karle, 1974b), seven out of eight *cis* prolines show either an *A* or a *B* type of C^γ puckering (Ramachandran, Lakshminarayanan, Balasubramanian & Tegoni, 1970), while in the eighth proline, the C^β atom is puckered. A different type of C^β puckering is found in the molecule of cyclo-L-Pro-L-Leu (Karle, 1972). In the linear

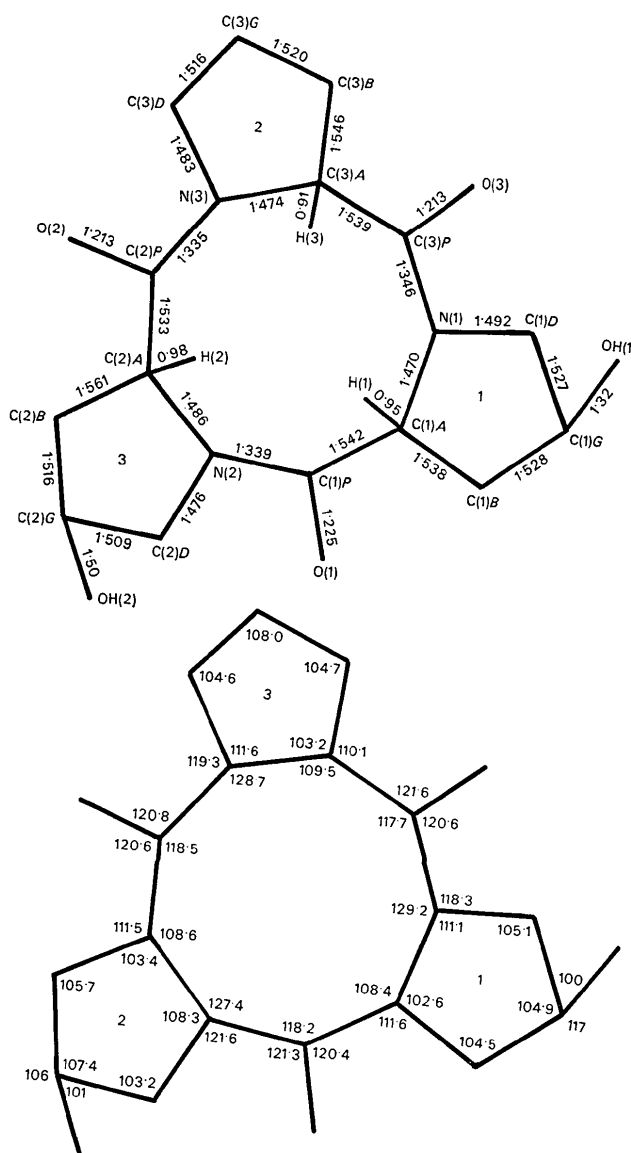


Fig. 2. Bond distances and angles in the molecule of CTP (2).

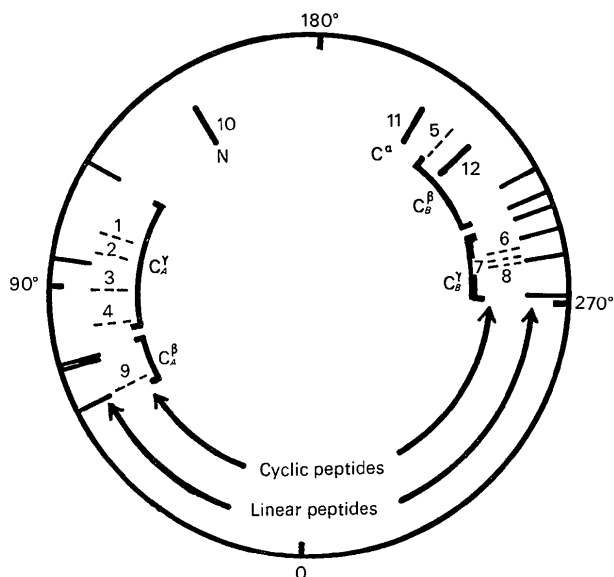


Fig. 3. Pseudorotation map showing the proline conformations. The prolines Nos. 1–8 occur in the two antamanide structures, No. 9 in cyclo-L-Pro-L-Leu and Nos. 10–12 in the molecule of CTP(2). The proline conformations found in the linear peptides are represented in the outer ring.

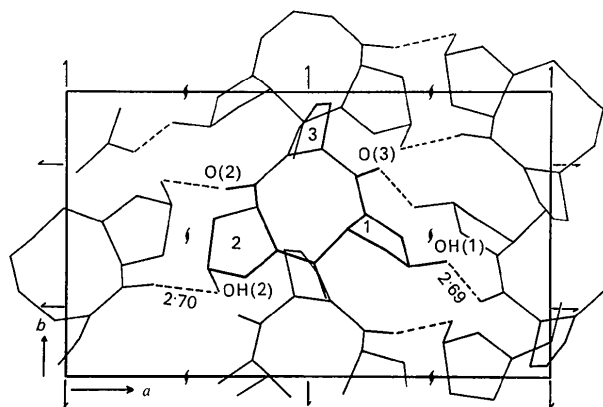


Fig. 4. The structure viewed down the c axis showing the hydrogen bonds.

tripeptide, t -amyloxy-carbonyl-L-Pro-L-Pro-L-Pro, two of the three prolines take up C^β puckering (Kartha, Ashida & Kakudo, 1974). In the highly strained molecule of CTP(2) reported in this paper, in addition to C^β puckering, we find C^α and N puckering also. These proline conformations are elegantly represented in a pseudorotation map (Fig. 3), similar to those used to describe the sugar-ring conformations (Altona & Sundaralingam, 1972). From the map, it is seen that not only C^γ but also C^β puckering is observed to occur in the A and B conformations. Thus, even though C^γ puckering is the most commonly observed for proline,

other types of ring conformations can also be imposed on the pyrrolidine ring in constrained situations like ring closure.

Molecular packing and hydrogen bonding

The molecules are oriented with the nine-membered ring nearly parallel to the ab plane (Fig. 4) and inter-linked by $\text{OH}\cdots\text{O}$ type hydrogen bonds.

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References

- ALTONA, C. & SUNDARALINGAM, M. (1972). *J. Amer. Chem. Soc.* **94**, 8205–8212.
- ASHIDA, T. & KAKUDO, M. (1974). *Bull. Chem. Soc. Japan*, **47**, 1129–1133.
- BALASUBRAMANIAN, R., TEGONI, G., VENKATESAN, K. & RAMACHANDRAN, G. N. (1970). *Biochim. Biophys. Acta*, **221**, 165–182.
- COREY, R. B. (1938). *J. Amer. Chem. Soc.* **60**, 1598–1604.
- COREY, R. B. & PAULING, L. (1953). *Proc. Roy. Soc. B* **141**, 10–20.
- DALE, J. & TITLESTAD, K. (1970). *Chem. Commun.* pp. 1403–1404.
- DEBAR, C. M., TORCHIA, D. A. & BLOUT, E. R. (1971). *J. Amer. Chem. Soc.* **93**, 4893–4897.
- GERMAIN, G., MAIN, P. & WOOLFSON, M. M. (1971). *Acta Cryst.* **A27**, 368–376.
- International Tables for X-ray Crystallography* (1962). Vol. III, pp. 202–203. Birmingham: Kynoch Press.
- IUPAC-IUB COMMISSION ON BIOCHEMICAL NOMENCLATURE (1970). *J. Mol. Biol.* **52**, 1–17.
- JOHNSON, C. K. (1965). *ORTEP*. Oak Ridge National Laboratory Report ORNL-3794.
- KARLE, I. L. (1972). *J. Amer. Chem. Soc.* **94**, 81–84.
- KARLE, I. L. (1974a). *J. Amer. Chem. Soc.* **96**, 4000–4006.
- KARLE, I. L. (1974b). *Biochemistry*, **13**, 2155–2162.
- KARTHA, G., AMBADY, G. & SHANKAR, P. V. (1974). *Nature, Lond.* **247**, 204–205.
- KARTHA, G., ASHIDA, T. & KAKUDO, M. (1974). *Acta Cryst.* **B30**, 1861–1866.
- KOPPLE, K. D. (1971). *Biopolymers*, **10**, 1139–1152.
- KOPPLE, K. D., ONISHI, M. & GO, A. (1969). *J. Amer. Chem. Soc.* **91**, 4264.
- RAMACHANDRAN, G. N., LAKSHMINARAYANAN, A. V., BALASUBRAMANIAN, R. & TEGONI, G. (1970). *Biochim. Biophys. Acta*, **221**, 165–181.
- ROTHER, M., STEFFAN, K. D. & ROTHE, I. (1965). *Angew. Chem. Int. Ed.* **4**, 356.
- STEWART, R. F., DAVIDSON, E. R. & SIMPSON, W. T. (1965). *J. Chem. Phys.* **41**, 3175–3187.
- VENKATACHALAM, C. M. (1968). *Biochim. Biophys. Acta*, **168**, 397–401.