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cis-Proline in the Linear Oligopeptide: Structure of Benzyloxycarbonyl-glycyl-prolyl-leucine

BY T. YAMANE AND T. ASHIDA

Department of Applied Chemistry, Faculty of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464, *Japan*

K. SHIMONISHI AND M. KAKUDO

Institute for Protein Research, Osaka University, Yamada-kami, Suita, Osaka 565, *Japan*

AND Y. SASADA

Laboratory of Chemistry for Natural Products, Tokyo Institute of Technology, Ookayama, Meguro-ku, Tokyo 152, *Japan*

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The crystal structure of a racemic compound of benzyloxycarbonyl-Gly-L-Pro-L-Leu and its enantiomorph has been determined. The crystallographic data are: $P2_1/a$, $a=22{\cdot}126$ (9), $b=9{\cdot}615$ (7), $c=10{\cdot}185$ (5) Å, $\beta=96{\cdot}23$ (4)°, $Z=4$. The final R value is 0·059. The peptide bond between the glycyl and prolyl residues has a *cis* conformation, and the bond angles around the N(Pro) atom are significantly affected by the internal rotation of the peptide bond. The angle $\angle C'-N-C^{\alpha}$ is larger and the angle $\angle C'-N-C^{\delta}$ is smaller, respectively, than those in the *trans* form. The torsion angles of the prolyl residue agree essentially with those of poly-L-proline I.

Introduction

A series of oligopeptides, such as benzyloxycarbonyl(*Z*)-Gly-L-Pro-L-Leu (*Z*-GPL), *Z*-Gly-L-Pro-L-Leu-Gly (*Z*-GPLG) and *Z*-Gly-L-Pro-L-Leu-Gly-L-Pro (*Z*-GPLGP) was synthesized to examine their structural specificity as a substrate of collagenase (Nagai & Noda, 1959; Nagai, Sakakibara, Noda & Akabori, 1960); among them only *Z*-GPLGP has substrate activity for collagenase. The crystal structures of *Z*-GPLG and *Z*-GPLGP have been determined, and the main chains of these peptides are both folded at the prolyl and leucyl residues, showing a 3_{10} bend (Ueki, Ashida, Kakudo, Sasada & Katsube, 1969; Ueki, Bando, Ashida & Kakudo, 1971). On the other hand, in the ORD study (Hamaguchi, 1970) the conformation of *Z*-GPL was found to be significantly different from those of the tetra- and pentapeptide. Thus the structural and physical properties of *Z*-GPL are particularly interesting as compared with *Z*-GPLG and *Z*-GPLGP, and also in comparison with the structure of L-Leu-L-Pro-Gly (Leung & Marsh, 1958) which has the inversed amino acid sequence to *Z*-GPL.

The peptide *Z*-GPL crystallizes in the space group $P2_12_12_1$ (Sasada, Tanaka, Ogawa & Kakudo, 1961), but the crystal was rather unsuitable for the analysis. Therefore the authors synthesized the exactly enantiomorphic peptide, that is, *Z*-Gly-D-Pro-D-Leu. In expectation of obtaining a crystal species with a centre of symmetry, the crystallization of the equimolar mixture of these two enantiomorphs was tried. Two modifications of crystals were obtained, and fortunately one has a convenient symmetry of $P2_1/a$. The other has a $4a$ identity period. The present paper describes the structure of the former crystal, in which the peptide bond between glycyl and prolyl residues was found to have a *cis* conformation.

Experimental

The crystal comprising a 1:1 molar ratio of *Z*-Gly-L-Pro-L-Leu and *Z*-Gly-D-Pro-D-Leu was obtained from an ethyl acetate solution of the equimolar mixture of these peptides, by adding a small amount of water. The crystallographic data are: molecular formula $C_{21}H_{29}N_3O_6$; molecular weight 419·47; space group $P2_1/a$, $a=22{\cdot}126$ (9), $b=9{\cdot}615$ (7), $c=10{\cdot}185$ (5) Å, $\beta=96{\cdot}23$ (4)°; $D_m=1{\cdot}28$, $D_x=1{\cdot}289$ g cm^{-3} (for $Z=4$); $\mu=7{\cdot}97$ cm^{-1} (for Cu $K\alpha$).

The collection of the intensity data from the crystal with dimensions $0{\cdot}2\times0{\cdot}5\times0{\cdot}2$ mm was carried out on a computer-controlled four-circle diffractometer (Rigaku AFC-III, at the Institute for Protein Research), using Ni-filtered Cu $K\alpha$ radiation with a scintillation counter connected to a pulse height analyser. Integrated intensities were measured by the ω–2θ scanning method, with a scanning speed of 4°min^{-1}(2θ). The scanning range was $(1{\cdot}50+0{\cdot}30\tan\theta)°(2\theta)$. A total of 3769 independent reflexions with $2\theta\leq120°$ were obtained, of which 3460 were non-zero reflexions. The absorption correction was not made.

Structure determination

The good starting set of the phases was obtained by *MULTAN* (Germain, Main & Woolfson, 1970). All

the non-hydrogen atoms were found from the E map in which only one significant false peak appeared near the pyrrolidine ring. The refinement was carried out by the block-diagonal least-squares method using *HBLS* (Ashida, 1973). In the refinement, the function minimized was $\sum\omega(|F_o|-|F_c|)^2$, where $\omega=\frac{1}{2}$ for $|F_o|=0$, $\omega=1$ for $0<|F_o|<50$, and $\omega=(50/|F_o|)^2$ for $|F_o|\geq 50$. The final refinement including hydrogen atoms gave an R of 0·059 for all the reflexions (0·054 for non-zero reflexions). The atomic scattering factors of the C, N, and O atoms were taken from *International Tables for X-ray Crystallography* (1962), and that of the H atom from *International Tables for X-ray Crystallography* (1974). Calculations were carried out on the FACOM 230-60 computer of the Computer Centre of Nagoya University.

The final parameters of all the atoms are listed in Tables 1 and 2.* The e.s.d.'s of non-hydrogen and hydrogen atoms are 0·0015–0·0033 Å and 0·020–0·029 Å, respectively.

Discussion

Molecular structure

Bond lengths and angles of non-hydrogen atoms are shown in Fig. 1(*a*) and (*b*). Two N–H bond lengths are 0·88 and 0·86 Å, respectively. The O–H is 0·93 Å. The bond lengths of C–H vary from 0·96 to 1·19 Å

* A list of structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 31621 (18 pp., 1 microfiche). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

Table 2. *Positional* ($\times 10^3$) *and thermal* ($\times 10$) *parameters of hydrogen atoms*

Standard deviations are given in parentheses.

	Bonded to	x	y	z	B
H(1)	C(1)	468 (1)	96 (3)	1062 (2)	66 (7)
H(2)	C(2)	567 (1)	185 (3)	1029 (3)	91 (8)
H(3)	C(3)	639 (1)	248 (3)	1218 (3)	91 (8)
H(4)	C(4)	608 (1)	209 (3)	1433 (3)	87 (8)
H(5)	C(5)	507 (1)	129 (3)	1461 (3)	78 (7)
H(6)	C(7)	415 (1)	−51 (3)	1213 (2)	69 (7)
H(7)	C(7)	416 (1)	4 (3)	1379 (3)	87 (8)
H(8)	N(1)	291 (1)	272 (2)	1163 (2)	42 (5)
H(9)	C(9)	267 (1)	157 (2)	921 (2)	55 (6)
H(10)	C(9)	231 (1)	290 (2)	979 (2)	50 (5)
H(11)	C(11)	167 (1)	238 (2)	784 (2)	42 (5)
H(12)	C(12)	93 (1)	108 (2)	610 (2)	54 (6)
H(13)	C(12)	61 (1)	186 (3)	736 (3)	101 (9)
H(14)	C(13)	100 (2)	−108 (4)	715 (4)	133 (12)
H(15)	C(13)	29 (1)	−30 (3)	768 (3)	71 (7)
H(16)	C(14)	116 (1)	−112 (3)	943 (2)	57 (6)
H(17)	C(14)	74 (1)	26 (3)	973 (2)	67 (7)
H(18)	N(3)	228 (1)	232 (2)	628 (2)	47 (5)
H(19)	C(16)	309 (1)	16 (2)	626 (2)	37 (4)
H(20)	C(17)	368 (1)	175 (2)	508 (2)	47 (5)
H(21)	C(17)	314 (1)	281 (2)	492 (2)	49 (5)
H(22)	C(18)	325 (1)	327 (2)	722 (2)	54 (6)
H(23)	C(19)	429 (1)	137 (3)	733 (3)	78 (7)
H(24)	C(19)	417 (1)	228 (3)	854 (3)	85 (8)
H(25)	C(19)	368 (1)	101 (3)	807 (2)	64 (6)
H(26)	C(20)	439 (1)	355 (3)	609 (3)	70 (7)
H(27)	C(20)	421 (1)	453 (3)	732 (3)	80 (8)
H(28)	C(20)	382 (1)	469 (3)	586 (3)	99 (9)
H(29)	O(5)	220 (1)	69 (3)	282 (3)	96 (9)

Table 1. *Positional* ($\times 10^4$) *and thermal* ($\times 10^5$) *parameters, with their standard deviations in parentheses*

The temperature factor is of the form $\exp(-\beta_{11}h^2-\beta_{22}k^2-\beta_{33}l^2-\beta_{12}hk-\beta_{13}hl-\beta_{23}kl)$.

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(1)	4991 (1)	1235 (3)	11377 (2)	279 (6)	1410 (35)	1016 (27)	53 (26)	172 (22)	110 (51)
C(2)	5555 (2)	1778 (3)	11233 (3)	390 (9)	1648 (44)	1709 (41)	−40 (34)	725 (33)	506 (70)
C(3)	5944 (1)	2118 (3)	12315 (4)	286 (8)	1485 (43)	2705 (58)	−361 (31)	463 (35)	−178 (82)
C(4)	5774 (1)	1943 (3)	13546 (3)	276 (7)	1521 (41)	1958 (44)	−113 (29)	−233 (29)	−976 (71)
C(5)	5205 (1)	1398 (3)	13706 (3)	273 (6)	1406 (35)	1077 (28)	157 (26)	43 (22)	−241 (52)
C(6)	4814 (1)	1019 (2)	12620 (2)	177 (4)	882 (25)	1077 (25)	139 (18)	79 (18)	90 (42)
C(7)	4213 (1)	363 (3)	12784 (3)	183 (5)	1227 (33)	1642 (35)	160 (22)	78 (22)	687 (57)
O(1)	3728 (1)	1334 (2)	12389 (2)	172 (3)	1048 (19)	1233 (19)	96 (13)	−72 (13)	−197 (32)
C(8)	3336 (1)	992(2)	11321 (2)	183 (4)	888 (25)	889 (22)	−100 (18)	219 (17)	−156 (39)
O(2)	3368 (1)	−51 (2)	10667 (2)	302 (4)	967 (19)	1274 (20)	65 (16)	299 (16)	−718 (33)
N(1)	2920 (1)	1994 (2)	11102 (2)	179 (4)	886 (21)	849 (18)	25 (15)	−31 (14)	−472 (32)
C(9)	2470 (1)	1938 (2)	9971 (2)	209 (5)	1021 (27)	818 (21)	−177 (20)	−30 (17)	18 (40)
C(10)	1952 (1)	953 (2)	10159 (2)	181 (4)	816 (23)	626 (19)	54 (17)	115 (15)	−180 (35)
O(3)	1893 (1)	365 (2)	11216 (1)	245 (3)	1208 (20)	558 (13)	−143 (14)	95 (11)	138 (27)
N(2)	1544 (1)	755 (2)	9104 (2)	168 (3)	962 (21)	599 (16)	−110 (15)	83 (12)	86 (30)
C(11)	1583 (1)	1349 (2)	7783 (2)	185 (4)	907 (24)	632 (19)	6 (18)	98 (15)	164 (36)
C(12)	949 (1)	1046 (3)	7078 (2)	216 (5)	1729 (40)	944 (26)	−48 (25)	−81 (20)	308 (53)
C(13)	740 (1)	−209 (4)	7720 (3)	270 (7)	1979 (48)	1570 (38)	−557 (31)	−479 (27)	956 (71)
C(14)	1010 (1)	−142 (3)	9157 (2)	171 (4)	1177 (30)	1000 (25)	−169 (20)	149 (18)	34 (45)
C(15)	2086 (1)	634 (2)	7115 (2)	204 (4)	765 (23)	534 (18)	−43 (17)	59 (15)	20 (33)
O(4)	2199 (1)	−608 (2)	7268 (2)	338 (4)	711 (17)	1238 (19)	59 (15)	544 (16)	371 (30)
N(3)	2387 (1)	1454 (2)	6355 (2)	205 (4)	680 (18)	671 (16)	50 (14)	174 (13)	214 (29)
C(16)	2897 (1)	943 (2)	5706 (2)	185 (4)	755 (22)	632 (19)	22 (17)	117 (15)	33 (34)
C(17)	3353 (1)	2116 (2)	5546 (2)	190 (5)	988 (26)	752 (21)	−85 (19)	127 (16)	−155 (38)
C(18)	3622 (1)	2805 (2)	6828 (2)	207 (5)	963 (27)	953 (24)	42 (20)	−12 (18)	−374 (41)
C(19)	3949 (1)	1789 (3)	7789 (3)	388 (9)	1540 (41)	1239 (32)	254 (32)	−500 (28)	−411 (60)
C(20)	4040 (1)	3982 (3)	6495 (3)	262 (7)	1533 (40)	1716 (39)	−344 (28)	115 (27)	−678 (66)
C(21)	2704 (1)	341 (2)	4343 (2)	183 (4)	790 (24)	796 (21)	−129 (17)	192 (16)	−32 (36)
O(5)	2260 (1)	1038 (2)	3682 (2)	240 (3)	1316 (21)	724 (15)	196 (15)	−26 (12)	−351 (30)
O(6)	2957 (1)	−649 (2)	3911 (2)	257 (4)	790 (17)	1086 (17)	−14 (14)	262 (14)	−463 (29)

Table 3. *Best planes*

(*a*) Equations of the best planes

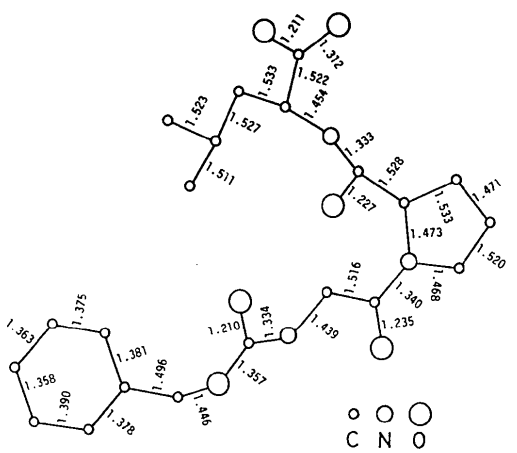
$X=ax+cz\cos\beta,\ Y=by,\ Z=cz\sin\beta$

I	$-0{\cdot}3861X+0{\cdot}9221Y-0{\cdot}0255Z+2{\cdot}9837=0$	Phenyl ring
II	$0{\cdot}6681X+0{\cdot}4672Y-0{\cdot}5791Z+2{\cdot}0866=0$	Peptide *Z*-Gly
III	$0{\cdot}5652X-0{\cdot}7814Y-0{\cdot}2647Z+1{\cdot}6357=0$	Peptide Gly-Pro
IV	$-0{\cdot}5634X-0{\cdot}2141Y-0{\cdot}7980Z+8{\cdot}0398=0$	Peptide Pro-Leu
V	$-0{\cdot}7025X-0{\cdot}6020Y+0{\cdot}3796Z+2{\cdot}4080=0$	Carboxyl Leu
VI	$0{\cdot}5639X-0{\cdot}7907Y-0{\cdot}2386Z+1{\cdot}4148=0$	Pro ring

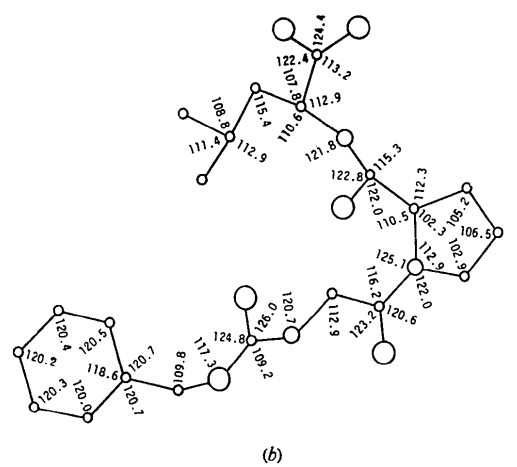
(*b*) Dihedral angles (°) between the planes

	I	II	III	IV	V
II	79·2				
III	158·7	80·5			
IV	87·7	90·8	86·6		
V	107·1	166·0	91·6	77·2	
VI	160·2	81·6	1·6	87·6	90·6

(average 1·02 Å). The e.s.d.'s of the bond lengths are: 0·002–0·005 Å for X–X, 0·03–0·05 Å for X–H (X, non-hydrogen atoms); and for the angles 0·2–0·3° for X–X–X, 0·7–0·8° for X–X–H, and 1·1–1·9° for H–X–H.



(*a*)



(*b*)

Fig. 1. (*a*) Bond lengths. (*b*) Bond angles.

Table 3 (*cont.*)

(*c*) Displacements ($\times 10^3$ Å) of atoms from the planes

Atoms with asterisks are not included in the best plane calculations.

I		II		III	
C(1)	8	O(1)	17	C(9)	−25
C(2)	4	C(8)	−12	C(10)	3
C(3)	−9	O(2)	0	O(3)	22
C(4)	3	N(1)	−31	N(2)	−10
C(5)	8	C(9)	26	C(11)	31
C(6)	−13	C(7)*	38	C(14)	−21
C(7)*	−78	H(8)*	−74	N(1)*	121
H(1)*	16			C(12)*	−303
H(2)*	−37			C(13)*	168
H(3)*	−63				
H(4)*	−106				
H(5)*	35				
IV		**V**		**VI**	
C(11)	−15	C(16)	−4	N(2)	0
C(15)	4	C(21)	15	C(11)	0
O(4)	4	O(5)	−5	C(14)	0
N(3)	26	O(6)	−6	C(9)*	−6
C(16)	−20	H(29*)	−119	C(10)*	38
H(18)*	30			O(3)*	90
				C(12)*	−348
				C(13)*	152
				C(15*)	1373

The torsion angles of the peptide chain are shown in Fig. 2, together with the serial numbering of atoms in the molecule. The definition of the torsion angles given by the IUPAC-IUB Commission on Biochemical Nomenclature (1970) is adopted. The stereoscopic view of the molecule drawn by *ORTEP* (Johnson, 1965) is shown in Fig. 3. The best planes and some related data are given in Table 3.

The pyrrolidine ring of the prolyl residue has a half chair structure. The C^{β}[=C(12)] and C^{γ}[=C(13)] atoms of the ring are on the opposite side of the best pyrrolidine plane, and the C^{γ} and C′[=C(15)] are on the same side of the plane (Table 3). Then the conformation of the ring is C^{β}-*exo*–C^{γ}-*endo* or C_2–C^{γ}-*endo* type ($\theta=-13{\cdot}4, \chi_1=28{\cdot}0, \chi_2=-32{\cdot}9, \chi_3=24{\cdot}0, \chi_4=-5{\cdot}9°$; Ashida & Kakudo, 1974). The torsion angles of the

pyrrolidine ring coincide well with those of the conformation *B* of the ring (Balasubramanian *et al.*, 1971).

The peptide bond between the glycyl and prolyl residues has a *cis* conformation, as shown in Figs. 2 and 3. Therefore the folding occurs at the prolyl residue. On the other hand, L-Leu-L-Pro-Gly has an extended configuration, though the twisting of the main chain occurs at the N–C^{α} bond of the prolyl residue (Leung & Marsh, 1958). Thus the difference in the sequence of the amino acids affects the packing of the side chain, leading to the different configuration of the main chain. Then the difference of this structure from those of *Z*-GPLG and *Z*-GPLGP is essential. The carboxyl group of the leucyl residue is outside of the folded chain, and an intramolecular hydrogen bond, as found in *Z*-GPLG and *Z*-GPLGP, is not seen. The chain from the *Z* group to the prolyl residue and that from the prolyl residue to the leucyl residue are nearly *trans* and zigzag, respectively.

This is the first case of the *cis* conformation of the peptide bond in the linear oligopeptide, and is interesting as the recent study of poly(L-Pro-Gly) and poly(Gly-Gly-L-Pro-Gly) using ^{13}C NMR spectroscopy suggests that 15–20% of the Gly-Pro peptide bonds are *cis* (Torchia & Lyerla, 1974). The (ω, φ, ψ) of the prolyl residue of *Z*-GPL, $(-3{\cdot}8, -71{\cdot}7, 144{\cdot}9°)$ agree essentially with those of poly-L-proline I, $(0, -83, 158°)$ (Ramachandran & Sasisekharan, 1968).

In Fig. 4 the bond angles concerning the *cis* prolyl residue are compared with the mean values of the *trans* forms given by Ashida & Kakudo (1974). The bond angles around the N(Pro) [=N(2)] atom are significantly affected by the internal rotation of the peptide bond between the glycyl and prolyl residues. In the *cis* form, the angle $\angle C'–N–C^{\alpha}$ ($\angle\alpha$) is larger than that in the *trans* form by about 4°, while the angle $\angle C'–N–C^{\delta}$ ($\angle\delta$) is smaller than that in the *trans* form. Thus the steric repulsion between the C^{α}(Gly) and C^{α}(Pro) in the *cis* form is bigger than that between O(Gly) and C^{α}(Pro) in the *trans* form. This seems to be plausible, considering the effect of the side chain of the preceding residue. However, the $\angle C^{\alpha}$(Gly)–C′–N(Pro), 116·2°, becomes smaller than that in the *trans* form, 118°. The C^{α}(Gly)–C^{α}(Pro) distance is 2·862 Å, and C^{α}-

Table 4. *Some bond angles in the cis-prolyl residues*

	∠C′–N–C (°)	∠C′–N–C (°)	Reference
Z[(a)]-Gly-L-Pro-L-Leu	125·1	122·0	1
Actinomycin D	125·5 124·8	119·4 121·5	2
7-Bromoactinomycin C_1	124·1 125·7	121·7 120·7	2
Cyclic-tri-L-Pro (mean)	128	120	3
t-AOC[(b)]-L-Pro-L-Pro-L-Pro	123·5	120·8	4
t-BOC[(c)]-L-Pro-L-Pro-L-Pro-L-Pro-Bz[(d)]	123·6	120·8	5
t-BOC-L-Pro	124·9	121·2	6
trans form (mean)	121·3	126·3	7

(*a*) Benzyloxycarbonyl; (*b*) t-amyloxycarbonyl; (*c*) t-butyloxycarbonyl; (*d*) benzyl ester.
References: (1) This study; (2) Jain & Sobell, 1972; (3) Kartha, Ambady & Shankar, 1974; (4) Kartha, Ashida & Kakudo, 1974; (5) Matsuzaki, 1974; (6) Benedetti, Ciajolo & Maisto, 1974; (7) Ashida & Kakudo, 1974.

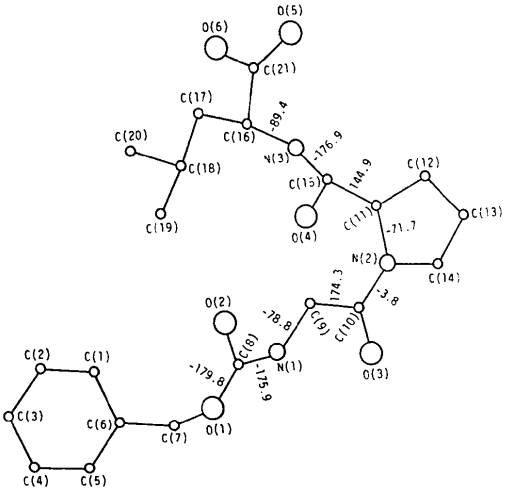


Fig. 2. Torsion angles of the peptide chain. Definitions for these angles are those given by IUPAC-IUB Commission on Biochemical Nomenclature (1970).

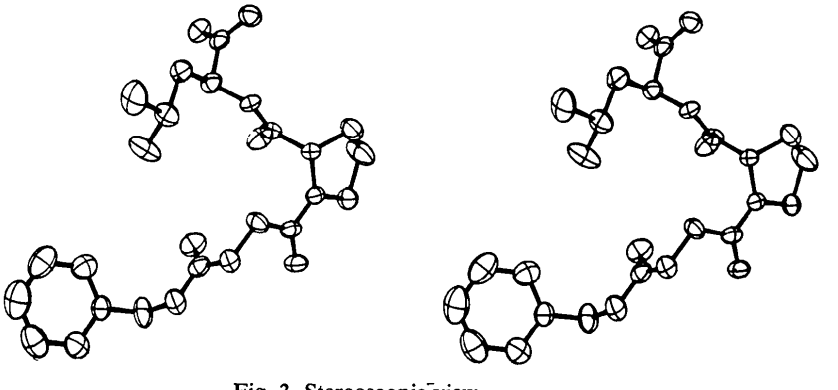


Fig. 3. Stereoscopic view.

(Gly)[=C(9)]–C′(Pro)[=C(15)] is 3·197 Å. The angles α and δ are compared with some related compounds in table 4. The cyclic dipeptides are not included in the Table because of their strong intramolecular constraints. Table 4 shows the similar tendency in all of the compounds. A more exaggerated tendency is shown in cyclic tri-L-proline (Kartha, Ambady & Shankar, 1974; Kartha & Ambady, 1975) since the ring is so small.

Cases in which the *cis* conformation of the peptide linkage occasionally occurs at linkages involving the *N*-methyl amino acid, or prolyl residue in several cyclic oligopeptides, have been reported (Konnert & Karle, 1969; Groth, 1970; Jain & Sobell, 1972; Kartha, Ambady & Shankar, 1974; Iitaka, Nakamura, Takeda & Takita 1974; Kartha & Ambady, 1975). For the *N*-methyl amino acid or prolyl residue, the repulsion energy of the *cis* form may be almost equal to that of the *trans* form. Some examples of the *cis* peptide bonds are given in Table 4. There is one interesting group of an L-proline oligomer in which the *N*-terminal prolyl residue is carboxylated. As shown in Table 4, it has been found that the C=O of the *N*-oxycarbonyl group is *cis* to the N–C$^{\delta}$(Pro) bond (Benedetti, Ciajolo & Maisto, 1974; Kartha, Ashida & Kakudo, 1974; Matsuzaki, 1974).

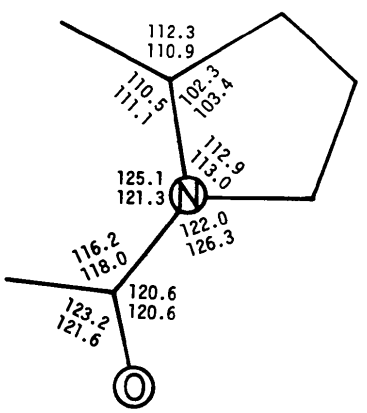


Fig. 4. Bond angles around the nitrogen atom of the prolyl residues. Upper: this peptide; lower: mean values of *trans* form.

Crystal structure

The packing scheme of the molecule is shown in Fig. 5 (viewed along the *b* axis). The hydrogen bonds are given in Table 5. The most prominent feature of the packing is the alternating arrangement of the layer composed of the hydrophobic groups (the phenyl ring, the pyrrolidine ring and the aliphatic side chain of the leucine) and the layer composed of the hydrophilic groups in the direction of the *a* axis. There are no close intermolecular contacts within the former layer. On the other hand, three hydrogen bonds are

Table 5. *Hydrogen bonds*

Donor *D*–H	Acceptor *A*	Distances (Å) *D*···*A*	H···*A*	Angle (°) ∠*D*–H···*A*
N(1)	O(4)^{i}	2·869	1·99	175
N(3)	O(6)^{ii}	2·893	2·04	179
O(5)	O(3)^{iii}	2·636	1·74	163

Symmetry code

Superscript	
(i)	$0{\cdot}5-x, 0{\cdot}5+y, \quad 2{\cdot}0-z$
(ii)	$0{\cdot}5-x, 0{\cdot}5+y, \quad 1{\cdot}0-z$
(iii)	$x, \quad y, -1{\cdot}0+z$

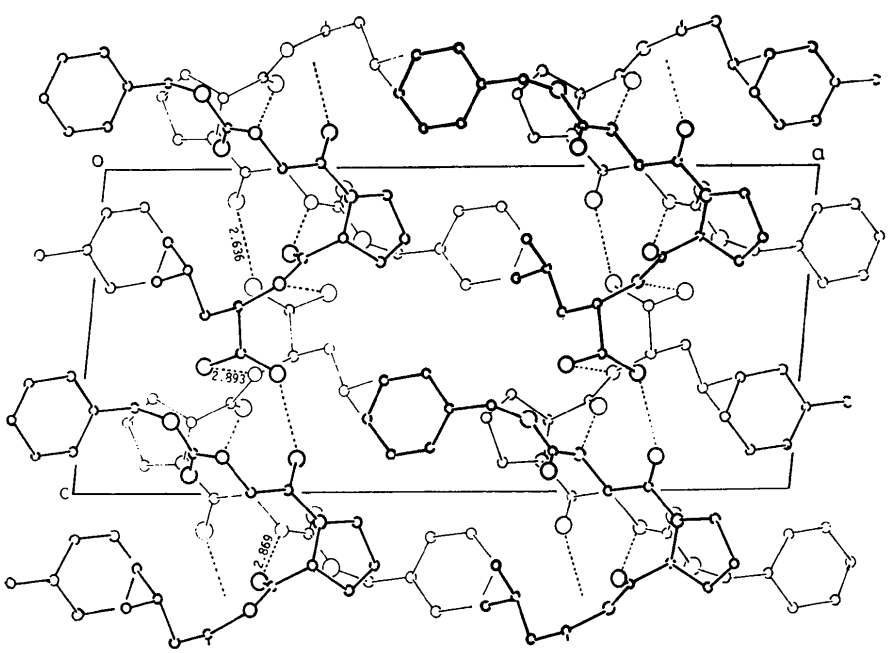


Fig. 5. Crystal structure viewed along the *b* axis. Hydrogen bonds are drawn as dotted lines.

found in the latter layer. No significantly short contacts are observed in this structure: that is, the shortest intermolecular distances are: 3·086 Å between O and O; 3·274 Å between O and N; 3·260 Å between O and C; and 3·572 Å between C and C.

The pyrrolidine ring of the prolyl residue is quite free, so it is interesting that the disorder does not occur at the C^{γ} atom of the pyrrolidine ring, as detected in L-Leu-L-Pro-Gly (Leung & Marsh, 1958). The phenyl ring and the side chain of the leucyl residue are also fairly free, though they have one or two weak contacts with the atoms in the hydrophilic layer.

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