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Structure of *p*-Bromocarbobenzoxy-glycyl-prolyl-leucyl-glycine

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The crystal structure of the synthetic oligopeptide, *p*-bromocarbobenzoxy-Gly-L-Pro-L-Leu-Gly(OH), has been determined. The crystal is orthorhombic, space group $P2_12_12_1$, with four molecules per unit cell of dimensions: $a=14{\cdot}25$, $b=6{\cdot}21$ and $c=29{\cdot}67$ Å. The R value is 0·05 and the average of the estimated standard deviations of the bond lengths is 0·025 Å. The peptide chain is folded back at Pro and Leu so as to make the intramolecular hydrogen bond between NH of Gly(2) and O of Gly(1). The conformation of the peptide backbone is very similar to those in some cyclohexapeptides. The Pro residue in this peptide, unlike those in the structure models of collagen, has the C_α–H bond *cis* to the C′=O bond. The particular conformation may be related to the fact that this peptide is inactive against collagenase while carbobenzoxy-Gly-Pro-Leu-Gly-Pro(OH) is active. The strong hydrogen bond between Gly(2) and the oxycarbonyl group links the peptides to form an endless and slightly deformed helical chain.

Introduction

The X-ray studies on a series of oligopeptides, such as carbobenzoxy(*Z*)-Gly, *Z*-Gly-Pro, *Z*-Gly-Pro-Leu, *Z*-Gly-Pro-Leu-Gly and *Z*-Gly-Pro-Leu-Gly-Pro has been carried out as a long range research project in this laboratory, concerning biologically important substances. They were synthesized in order to examine the relationship between the structure of collagen and the substrate specificity of the enzymatic reaction of collagenase (Nagai & Noda, 1959; Nagai, Sakakibara, Noda, Akabari, 1960; Sakakibara & Nagai, 1960; Kakudo, Sasada, Katsube, Sakakibara & Akabori, 1963). It has been recognized that the above mentioned pentapeptide has a high degree of specificity to the reaction while the tetrapeptide has not, and that the sequence of the amino acid residues, such as -Pro-Leu-Gly-Pro-, has an essential importance for the specificity (Nagai & Noda, 1959; Nagai *et al.* 1960). From this biochemical information it seems natural to expect the existence of substantial difference in the molecular structures of the tetra- and penta-peptide. In fact, the difference has already been suggested on the basis of their crystallographic data (Sasada, Tanaka, Ogawa & Kakudo, 1961; Sasada & Kakudo, 1961; Kakudo

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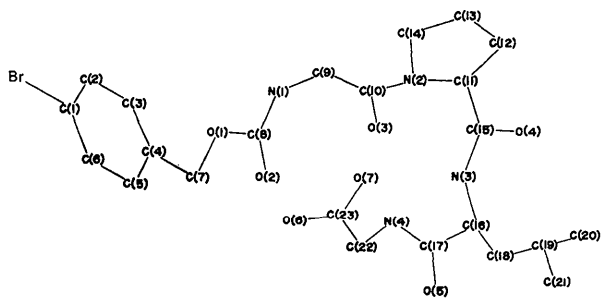
et al. 1963). The X-ray analysis of this series of peptides will yield not only the structural basis for its biochemical behaviour, but also some basic knowledge for the examination of the detailed structure of the characteristic part of collagen and its homologues. Apart from these interests, the comparison of the molecular structures of this series with those of L-Leu-L-Pro-Gly (Leung & Marsh, 1958) and tosyl-L-Pro-L-Hypro (Fridrichsons & Mathieson, 1962) is also of significance from the viewpoint of structural chemistry.

The present account deals with the molecular structure of *p*-bromocarbobenzoxy-glycyl-prolyl-leucyl-glycine.*



* In the present paper the Gly residue at the amino-terminal is called Gly(1) and the other at the carboxyl-terminal, Gly(2).

The substance was synthesized and kindly supplied by Professors Akabori and Sakakibara of this Institute. Some characteristic features of the structure have already been reported (Ueki, Ashida, Kakudo, Sasada, & Katsube, 1967).

Experimental

The crystals, recrystallized from ethyl acetate solution, are colourless transparent needles elongated along the *b* axis. A crystal with dimensions $0{\cdot}07 \times 0{\cdot}21 \times 0{\cdot}08$ mm was mounted on a goniometer head with its needle axis vertical, and was used in the experiment throughout. The cell dimensions were determined on a General Electric XRD-5 diffractometer, using Cu $K\alpha$ radiation ($\lambda = 1{\cdot}5418$ Å). Intensities were measured for each independent reflexion with $\sin\theta/\lambda$ less than 0·417 (spacing larger than 1·2 Å) by the stationary-crystal stationary-counter technique. The fixed-time method was applied with 20 seconds counting time for each reflexion. The intensities with spacing less than 1·2 Å were too weak to be measured. A total of 973 reflexions could be collected exclusive of space group extinctions, of which 57 were recorded as zero intensity. The absorption correction was not made. The extinction effect did not seem appreciable, and the correction was ignored.

Table 1. *The positional parameters (in fractions) and the estimated standard deviations* (Å$\times 10^3$)

	x	$\sigma(x)$	y	$\sigma(y)$	z	$\sigma(z)$
Br	0·1986	2	0·2097	2	0·2051	2
C(1)	0·1449	15	0·3731	17	0·2522	13
C(2)	0·1810	15	0·5653	17	0·2605	14
C(3)	0·1405	14	0·6907	16	0·2969	16
C(4)	0·0732	14	0·6051	16	0·3220	13
C(5)	0·0396	16	0·4088	16	0·3136	13
C(6)	0·0729	16	0·2843	17	0·2779	13
C(7)	0·0312	13	0·7393	16	0·3621	13
C(8)	0·1092	13	0·9688	15	0·4152	12
C(9)	0·2013	14	1·1838	16	0·4658	11
C(10)	0·1946	13	1·0818	14	0·5117	14
C(11)	0·2175	14	1·1132	14	0·5939	13
C(12)	0·2470	17	1·3109	19	0·6224	15
C(13)	0·2828	20	1·4699	17	0·5929	16
C(14)	0·2473	15	1·4275	16	0·5465	16
C(15)	0·1312	14	1·0023	15	0·6099	12
C(16)	−0·0395	14	0·9966	15	0·6081	13
C(17)	−0·0840	14	0·8614	15	0·5701	14
C(18)	−0·1062	14	1·1651	17	0·6271	13
C(19)	−0·0715	15	1·2857	18	0·6672	13
C(20)	−0·0488	23	1·1278	22	0·7064	17
C(21)	−0·1476	19	1·4511	20	0·6822	17
C(22)	−0·0720	14	0·7077	15	0·4970	13
C(23)	−0·0305	14	0·4923	15	0·4899	14
N(1)	0·1934	11	1·0203	14	0·4319	11
N(2)	0·2143	11	1·2054	11	0·5488	10
N(3)	0·0482	10	1·0856	12	0·5950	10
N(4)	−0·0358	11	0·8222	12	0·5343	10
O(1)	0·1155	10	0·7965	12	0·3883	9
O(2)	0·0372	10	1·0602	10	0·4217	9
O(3)	0·1675	9	0·8901	9	0·5168	9
O(4)	0·1343	9	0·8502	11	0·6357	10
O(5)	−0·1684	9	0·8031	14	0·5753	9
O(6)	0·0109	11	0·3920	11	0·5192	10
O(7)	−0·0439	10	0·4112	10	0·4502	9

Crystal data

Crystals of *p*-bromocarbobenzoxy-(*p*-bromobenzyloxycarbonyl)-glycyl-L-prolyl-L-leucyl-glycine, $C_{23}H_{31}N_4O_7Br$ are orthorhombic with

$$a = 14{\cdot}25 \pm 0{\cdot}01,$$
$$b = 6{\cdot}21 \pm 0{\cdot}01,$$
$$c = 29{\cdot}67 \pm 0{\cdot}01 \text{ Å},$$
$$V = 2625 \text{ Å}^3.$$

Assuming four molecules in a unit cell, the density was calculated to be 1·405 g.cm^{-3}, the observed density being 1·40 g.cm^{-3}. From the systematic absences of the reflexions the space group was determined to be $P2_12_12_1$.

Determination of the structure

On the basis of the parameters of the bromine atom, the heavy atom Fourier synthesis and the minimum function maps were made, and only sixteen peaks with reasonable heights were found to be consistent in both maps. Several steps of least-squares calculation followed by Fourier summation had to be carried out before the final structure of the molecule was revealed. In each step, the atoms whose temperature factors became unreasonably large after the least-squares refinement were abandoned in calculating the phases for the following Fourier summation, in which a new set of atoms was picked up for the next step. Thus, after five steps all the atoms were clearly shown up.

A least-squares refinement with anisotropic temperature factors of the form $\exp\{-(\beta_{11}h^2+\beta_{22}k^2+$

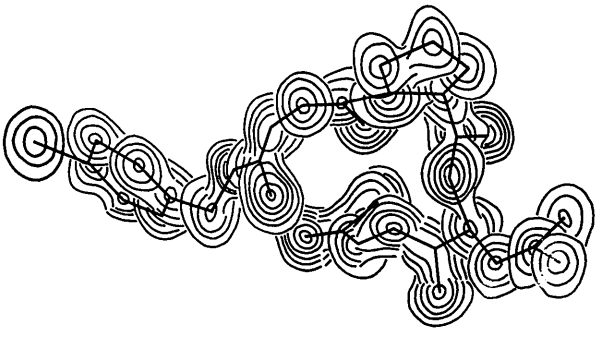


Fig. 1. Composite of the Fourier synthesis. The contours are drawn at intervals of 1 e.Å^{-3} beginning at 1 e.Å^{-3}. Those for the bromine atom are 1, 10 and 20 e.Å^{-3} from the lowest.

Table 2. *Anisotropic thermal parameters* ($\times 10^4$) *and equivalent isotropic temperature factors* (Å^2)

	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}	B
Br	111	567	13	127	10	−47	7·4
C(1)	80	386	6	126	−13	−50	4·8
C(2)	60	348	14	−69	1	−49	5·1
C(3)	61	259	19	−91	−2	15	5·3
C(4)	57	235	12	−11	−17	−21	4·1
C(5)	77	274	10	−27	−10	7	4·7
C(6)	91	265	10	−6	18	−27	5·0
C(7)	46	317	13	27	−29	−85	4·4
C(8)	47	259	8	185	9	−6	3·5
C(9)	62	374	4	−68	−11	30	4·0
C(10)	22	211	19	51	2	10	3·9
C(11)	42	204	12	16	9	36	3·6
C(12)	93	406	17	−205	4	−46	6·6
C(13)	140	263	17	−144	24	−39	7·2
C(14)	62	293	20	−86	−30	18	5·5
C(15)	63	204	7	29	−7	−8	3·5
C(16)	55	268	10	20	−30	−18	4·1
C(17)	55	209	17	−31	−7	9	4·5
C(18)	54	403	11	2	20	−40	4·8
C(19)	73	380	12	46	16	−68	5·3
C(20)	180	689	14	280	−19	−96	10·0
C(21)	118	460	17	130	−3	−65	7·6
C(22)	61	168	11	−4	−17	−51	3·8
C(23)	47	197	15	−68	−10	−18	4·0
N(1)	45	421	13	−30	3	−37	4·9
N(2)	53	203	10	−69	−12	−4	3·7
N(3)	26	221	12	−5	−1	3	3·2
N(4)	45	252	11	62	16	−15	3·8
O(1)	78	462	14	193	−19	−106	6·1
O(2)	70	236	17	105	−10	−56	5·1
O(3)	51	221	13	−9	1	−17	4·1
O(4)	46	410	18	28	−3	103	5·5
O(5)	48	702	17	−194	15	−80	6·9
O(6)	96	283	15	103	−3	14	5·9
O(7)	79	253	14	75	−11	−58	5·1

* The equivalent isotropic temperature factors are those defined by Hamilton (1959).

$\beta_{33}l^2+\beta_{12}hk+\beta_{13}hl+\beta_{23}kl)\}$, for all the atoms, resulted in an R value of 0·068. The difference Fourier synthesis showed up all the hydrogen atoms at this stage; their peak heights are distributed in the range from 0·15 to 0·40 e.Å^{-3}. The final refinement was carried out including the positional parameters of the hydrogen atoms, and the R value decreased to 0·043. The final parameters of all the atoms are listed in Tables 1, 2 and 3. The observed and calculated structure factors are tabulated in Table 4. The final electron-density distribution is shown in Fig. 1.

Table 3. *Positional parameters for hydogen atoms*

	x	y	z	Bonded to
H(1)	0·217	0·680	0·244	C(2)
H(2)	0·185	0·819	0·289	C(3)
H(3)	−0·017	0·331	0·342	C(5)
H(4)	0·036	0·132	0·267	C(6)
H(5)	−0·015	0·665	0·363	C(7)
H(6)	0·009	0·912	0·349	C(7)
H(7)	0·262	0·938	0·422	N(1)
H(8)	0·161	1·297	0·453	C(9)
H(9)	0·248	1·269	0·472	C(9)
H(10)	0·304	1·410	0·530	C(14)
H(11)	0·194	1·528	0·539	C(14)
H(12)	0·280	1·613	0·605	C(13)
H(13)	0·373	1·440	0·587	C(13)
H(14)	0·167	1·363	0·632	C(12)
H(15)	0·282	1·260	0·650	C(12)
H(16)	0·251	0·999	0·595	C(11)
H(17)	0·040	1·264	0·573	N(3)
H(18)	−0·035	0·897	0·631	C(16)
H(19)	−0·111	1·279	0·606	C(18)
H(20)	−0·162	1·111	0·634	C(18)
H(21)	0·006	1·385	0·665	C(19)
H(22)	0·006	1·024	0·694	C(20)
H(23)	−0·022	1·261	0·710	C(20)
H(24)	−0·086	1·176	0·739	C(20)
H(25)	−0·136	1·592	0·657	C(21)
H(26)	−0·135	1·537	0·713	C(21)
H(27)	−0·203	1·386	0·686	C(21)
H(28)	0·059	0·886	0·531	N(4)
H(29)	−0·159	0·681	0·493	C(22)
H(30)	−0·033	0·796	0·466	C(22)
H(31)	−0·009	0·291	0·445	O(7)

Most of the calculations were done on the HITAC 5020E of the University of Tokyo, by use of the programs written by one of the authors (Ashida, 1967). The block-diagonal approximation was used in the least-squares calculation, in which the weighting scheme was: $w=1·0$ for reflexions with $|F_o|\geq 0·1$ and $w=0·1$ for others. The atomic scattering factors were taken from *International Tables for X-ray Crystallography* (1962). The anomalous scattering effect of Br($\Delta f'=-0·9$ and $\Delta f''=1·5$) was also taken into account.

The estimated standard deviations of the positional parameters of C, N, and O are from 0·009 to 0·023 Å; they correspond to the e.s.d.'s of the bond distances from 0·015 to 0·03 Å and of the bond angles from 1·2 to 1·6°. As regards the hydrogen atoms, though they were distinctly found in the difference Fourier synthesis and subjected to the refinements, little can be said about them with any precision. The mean length of 27 C–H bonds is 1·05 Å.

It may be necessary to mention the general effect of using an insufficient number of intensity data in the structure determination. When only the reflexions of lower order are used and their number is unusually small, (i) the e.s.d.'s do not become as small as usually expected from the R value for the normal case; (ii) many more cycles are necessary to get a reasonable termination of the refinement; (iii) peaks in the electron density maps are appreciably broadened. All these effects were clear in the present case, where the ratio of reflexions to the parameters, 973/441, is unusually small, and the minimum spacing of reflexions is 1·2 Å.

Discussion

The bond distances and angles are shown in Fig. 2(*a*) and (*b*). Almost all of these seem to be reasonable within the limit of experimental error. Some of the shorter van der Waals contacts in a molecule are shown in Fig. 2(*c*), and the angles of the internal rotation of the peptide chain in Fig. 3.

The phenyl ring in the Z group, which was substituted in order to make crystallization of this peptide easier, seems to play an important role in the packing of the molecule, while the oxycarbonyl group has a substantial effect in stabilizing the conformation of the molecule with a hydrogen bond. Thus, the Z group stabilizes not only the crystal structure but also the conformation of the molecule.

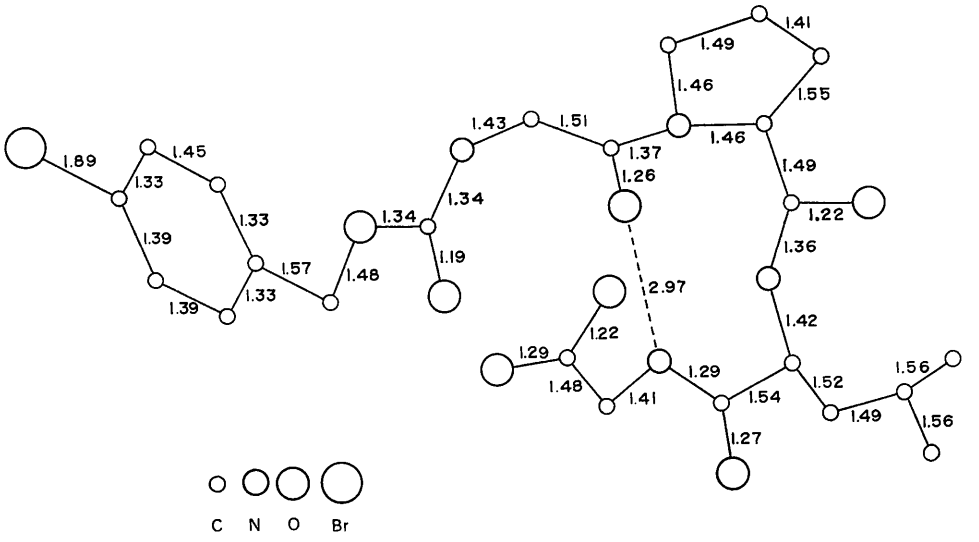
The geometry of the pyrrolidine ring is similar to those so far reported. The four atoms, C_α, C_β, C_δ and N, are coplanar, and C_γ is 0·26 Å out of the plane (see Table 5). In contrast to L-Leu-L-Pro-Gly (Leung & Marsh, 1958), disorder for the C_γ atom does not seem to be present, possibly due to the intermolecular contacts around the pyrrolidine ring. The carbon atom of the amide group, C(15), is situated in the opposite direction to C_γ with respect to the plane.

The leucine residue shows an interesting feature. The bond angle of C_α-C_β-C_γ is 116°, and it is unusually larger than the regular tetrahedral bond angle. The corresponding values are 118° in L-Leu-L-Pro-Gly and 119° in L-leucine hydrobromide respectively (Subramanian, 1967). Thus the widening of this angle seems to be a common characteristic of leucine and leucine residues in peptides. It may be a result of the steric repulsion between the aliphatic side chain and the main polar parts of the peptides or the amino acids.

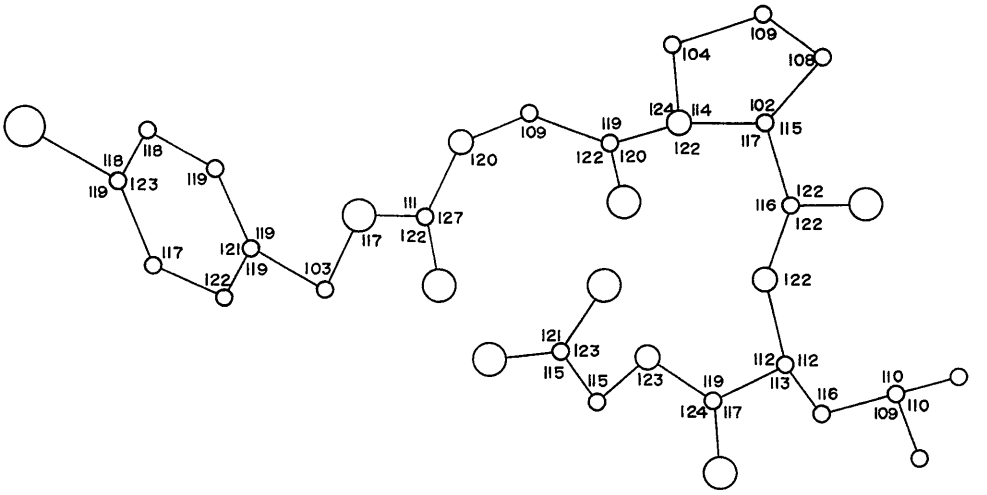
Folding of the peptide chain

There are several planar groups of atoms, and the equations of their best planes and related numerical data are listed in Table 5. Each of the four peptide groups and one terminal carboxyl group are planar within the limit of experimental error. The dihedral angles between the neighbouring peptide or carboxyl

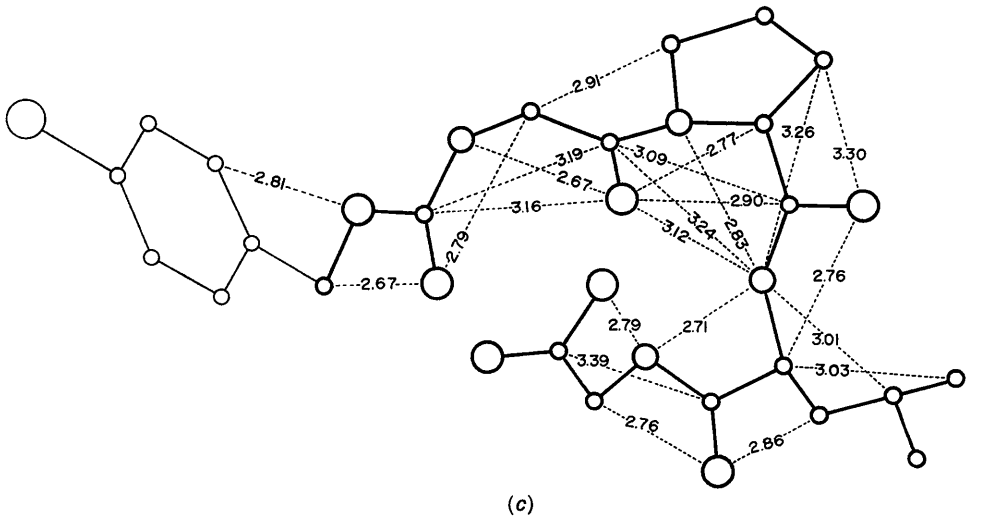
Table 4. *Observed and calculated structure factors*



(*a*)



(*b*)



(*c*)

Fig. 2. (*a*) Bond distances; (*b*) bond angles; (*c*) close contacts of atoms in a molecule.

groups are all close to 90°; this is the case in many peptides analysed so far, except for those including only glycine residues.

The peptide chain is folded back at the Pro and Leu residues, and three hydrogen bonds seem to contribute to this folding. They are one intramolecular hydrogen bond NH[Gly(2)]---O[Gly(1)], and two intermolecular hydrogen bonds NH(Leu)---O(7) of Gly(2) of the next upper molecule in Fig. 6 and OH[Gly(2)]---O(2) of *Z* of the next lower. This folded structure which has not so far been reported in linear peptides is one of the important features of this peptide. The two peptides containing a proline residue, L-Leu-L-Pro-Gly and tosyl-L-Pro-L-Hypro, do not show such twisted conformation. The characteristic of L-Leu-L-Pro-Gly is its highly extended conformation. Such a folded structure, strictly speaking, found in the peptide chain from N(1) of Gly(1) to C(22) of Gly(2), has been found in two kinds of cyclic hexapeptides. Of the conformational isomers in cyclohexaglycyl (Karle & Karle, 1963), the molecules at $y=\frac{1}{2}$ have essentially the same conformation as the present peptide, an intramolecular hydrogen bond of the same type also being found. Another cyclic hexapeptide is that of ferrichrome-A (Zalkin Forrester, & Templeton, 1966) of the sequence -Orn(3)-Orn(2)-Orn(1)-Ser(2)-Ser(1)-Gly-. The internal rotation angles, φ and ψ, are reported to be 103° and 131° for Orn(2) and 76° and 185° for Orn(1).

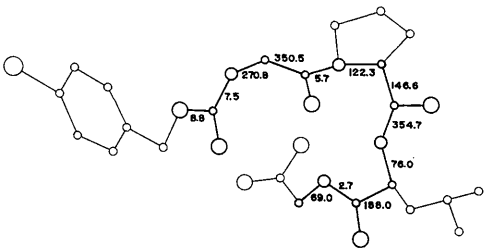


Fig. 3. Internal rotation angles of the peptide chain. The definitions for these angles are those given by Edsall, Flory, Kendrew, Liquori, Nemethy, Ramachandran & Scheraga (1966).

Table 5. *Best planes*

(*a*) Equations*

I	$0{\cdot}1965X+0{\cdot}6187Y-0{\cdot}7606Z=-5{\cdot}3493$	Peptide CBZ–Gly(1)
II	$0{\cdot}9487X-0{\cdot}3005Y-0{\cdot}0981Z=-0{\cdot}8923$	Peptide Gly(1)–Pro
III	$0{\cdot}0314X-0{\cdot}6193Y-0{\cdot}7845Z=-18{\cdot}0031$	Peptide Pro–Leu
IV	$-0{\cdot}3285X+0{\cdot}8479Y-0{\cdot}4160Z=-2{\cdot}0873$	Peptide Leu–Gly(2)
V	$0{\cdot}8577X+0{\cdot}4154Y-0{\cdot}3031Z=-3{\cdot}5206$	Carboxyl Gly(2)
VI	$-0{\cdot}6754X+0{\cdot}3954Y-0{\cdot}6225Z=-5{\cdot}1590$	Phenyl group
VII	$0{\cdot}9467X-0{\cdot}2993Y-0{\cdot}1192Z=-1{\cdot}2724$	Pro ring

(*b*) Dihedral angles between the planes

Between I	and II	85·7°
II	III	73·0
III	IV	102·1
IV	V	78·7
I	VI	54·2
II	VII	1·2

* *X*, *Y* and *Z* in Å units.

(*c*) Displacements (Å) of atoms from the planes.

I		II		III		IV	
O(1)	−0·031	C(9)	0·049	C(11)	−0·005	C(16)	0·013
C(8)	0·007	C(10)	0·015	C(15)	0·011	C(17)	−0·020
O(2)	0·011	O(3)	−0·009	O(4)	−0·004	O(5)	0·003
N(1)	0·064	N(2)	−0·058	N(3)	−0·000	N(4)	−0·011
C(9)	−0·050	C(14)	−0·019	C(16)	−0·003	C(22)	0·016
C(7)*	0·105	C(11)	−0·027	C(12)*	−1·416	N(3)*	0·234
C(10)*	−1·496	N(1)*	0·346	N(2)*	0·688	C(18)*	0·979
		C(12)*	−0·026	C(18)*	−1·122	C(23)*	−1·225
		C(15)*	−0·979	C(17)*	1·384		

V		VI		VII	
C(22)	−0·004	Br	−0·026	N(2)	−0·020
C(23)	0·012	C(1)	0·023	C(14)	0·021
O(6)	−0·005	C(2)	−0·007	C(12)	−0·035
O(7)	−0·004	C(3)	0·020	C(11)	0·036
N(4)*	0·399	C(4)	−0·007	C(13)*	0·257
		C(5)	−0·010	C(10)*	0·076
		C(6)	0·022	C(15)*	−0·979
		C(7)	−0·015	C(9)*	0·139
		O(1)*	−1·169	O(3)*	0·048

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

These values may be compared with those found at Pro and Leu residues in the present molecule. The hydrogen bond was not found between Ser(2) and Orn(3), but this may be the result of the difference in the bond forming conditions caused by accumulation of the slight differences in the internal rotation angles. The other side of this peptide has a closely similar intramolecular hydrogen bond between Orn(3) and Ser(2), but the folding of the chain is substantially different; φ of Ser(1) and ψ of Gly are different by about 180° from those of the Pro and Leu residues in the present peptide, respectively. The finding of this structure in linear as well as in cyclic peptides, regardless of the size and character of the side chains, shows that this structure of peptides is fairly stable and it may be found elsewhere.

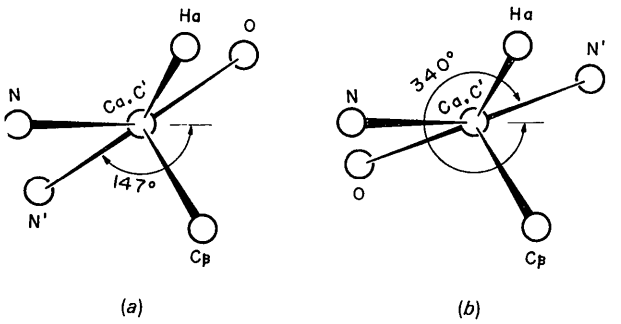


Fig. 4. Internal rotation angles of the C_α–C′ bond of proline residue. (*a*) Present molecule; (*b*) the model used in the collagen model.

Ramachandran, Ramakrishnan & Sasisekharan (1963) have described the sterically allowed region for amino acid residues in peptides, making use of the two internal rotation angles φ and ψ. Since glycine is not optically active, 269° for φ in Gly(1) is essentially equivalent to 91°(=360°−269°) from the stereochemical viewpoint. Thus, the rotation angles in Gly(1) are in the 'fully allowed region' in the conformational map of Ramachandran *et al.* (1963), and they are not far from those of polyglycine II(100° and 330°). The angles in the Leu residue are slightly outside the 'outer limit'. In lysozyme (Blake, Mair, North, Phillips & Sarma, 1967) and carboxypeptidase A (Reeke, Hartsuck, Ludwig, Quiocho, Steitz & Lipscomb, 1967), several amino acid residues have been shown to have closely similar conformations to the present Leu residue. The Orn(1) in ferrichrome-A has also the same rotation angles. Thus, the present conformation, though

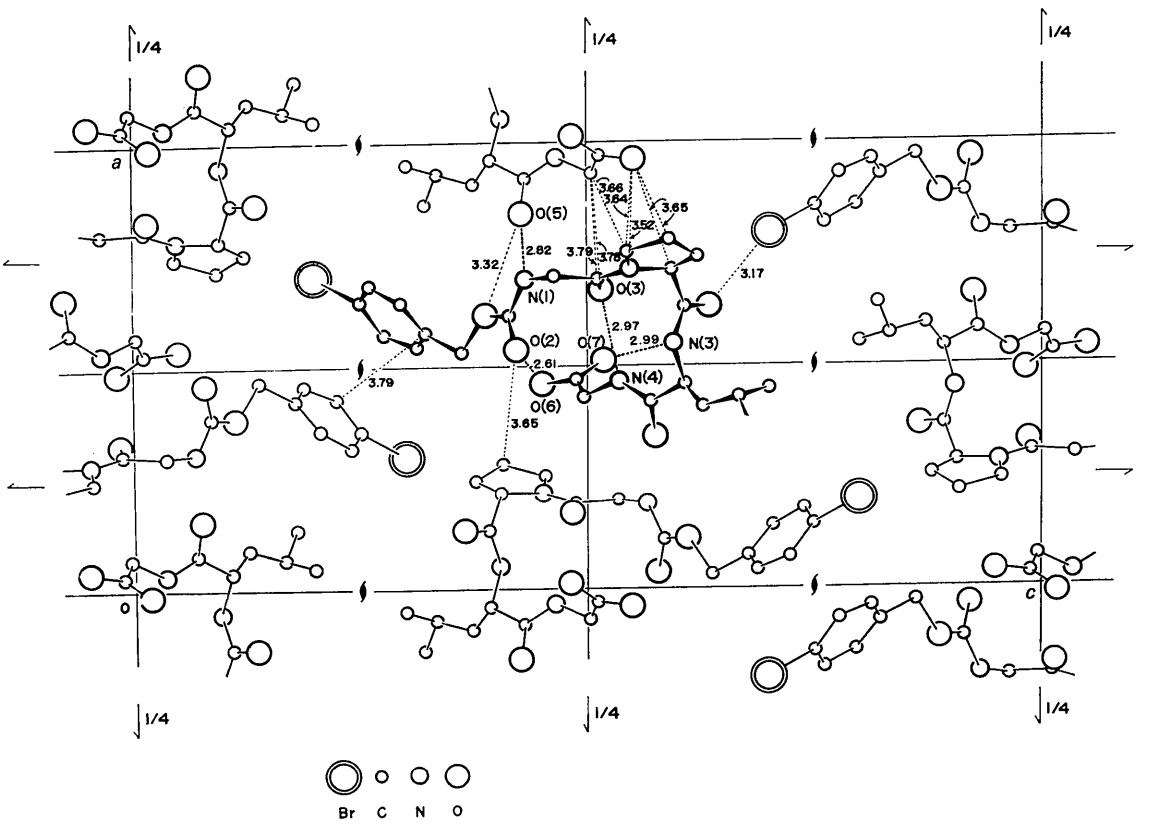


Fig. 5. The crystal structure viewed down the *b* axis. Hydrogen bonds are shown by heavy dotted lines and some intermolecular short contacts by thin broken lines.

it is out of the permissible region, seems to be stable, even if the residue has a fairly large side chain.

The proline residue has some interesting features to be mentioned. The two angles, $\varphi = 111°$ and $\psi = 151°$, are in the outer limit on the conformational map. They are not far from those of the right-handed α-helix ($\varphi = 132°$ and $\psi = 123°$) and $3{\cdot}0_{10}$-helix (Donohue, 1953), but quite different from those of the models of collagen (about 120° and 340°) and polyproline II. This substantial difference in the conformation is a result of the internal rotation around the C_α–C′ bond, as is shown in Fig. 3. Since the rotation around the N–C_α bond is restricted in this amino acid, it rarely affects the conformation of the residue. The present form, in which C_α–H and C′–O are in the *cis* arrangement, appears in both myoglobin and lysozyme structures, while the other form, with C_α–H and C′–O in *trans* configuration has been found in L-Leu-L-Pro-Gly and tosyl-L-Pro-L-Hypro. These two configurations of the C_α–C′ bond may have something to do with the substrate specificity for collagenase, though a conclusion will not be reached until the establishment of the structure of the pentamer, *Z*-Gly-Pro-Leu-Gly-Pro, which investigation is now being carried out. The rotation angles in the Leu residue are also far from those of collagen or its analogues.

Packing of the molecules

The arrangement of the molecules in the crystal is shown in Figs. 5 and 6. There is a strong hydrogen bond between the terminal carboxyl group and the oxycarbonyl group of the lower next molecule along the **b** direction. The hydrogen bond which stabilizes the folded structure of the peptide plays an important part in the packing of the molecules in the crystal. The endless chain of the peptides elongated along the *b* axis is made up by the hydrogen bonding, and the chain as a whole looks like a sort of deformed helix. From such a viewpoint, it is conceivable that the hydrogen bond between NH of Leu and the carboxyl group of Gly(2) contributes to the stability of the chain, and its role seems similar to that in the α-helix. In this endless chain, the carbonyl group of Pro seems to be sterically masked by the pyrrolidine ring and the side chain of Leu, both of which are hydrophobic. Thus, only two polar groups, NH of Gly(1) and CO of Leu, protrude laterally from this chain. These two groups make the hydrogen bond, and connect the chains to a sheet perpendicular to the *c* axis. The surfaces of the sheets so formed are covered by the hydrophobic groups, and the stacking of the sheets along the *c* axis is a result of the van der Waals forces. The interaction

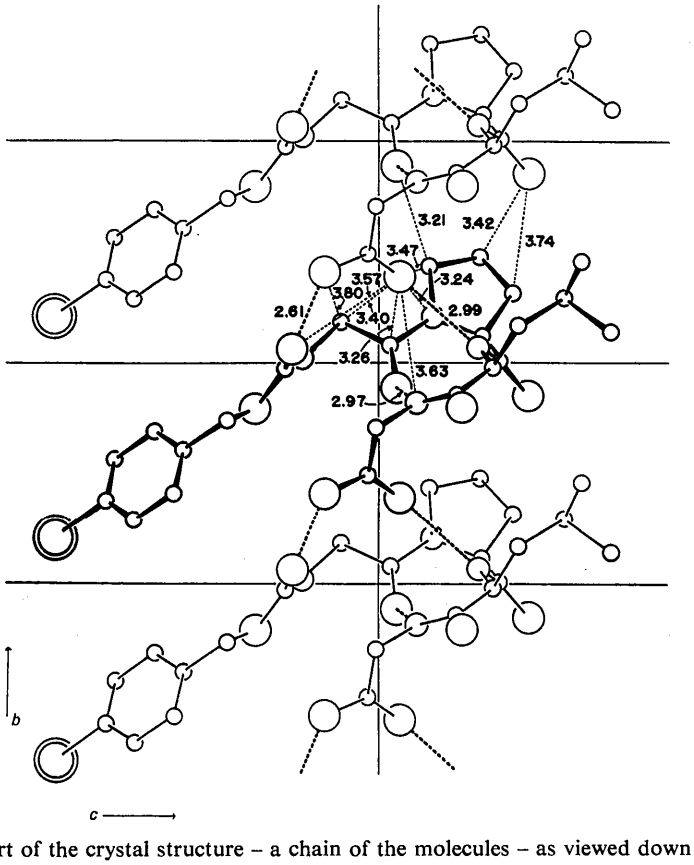


Fig. 6. A part of the crystal structure – a chain of the molecules – as viewed down the *a* axis.

among the helical chains or the sheets seems generally weak. Some of the shorter contacts among the molecules are shown in Figs. 5 and 6.

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The Structure of *trans-p,p'*-Dichloroazobenzene

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Crystals of *trans-p,p'*-dichloroazobenzene are monoclinic, space group $P2_1/c$, $a=9{\cdot}817(5)$, $b=4{\cdot}708(2)$, $c=11{\cdot}710(3)$ Å, $\beta=91{\cdot}12(5)°$, with two molecules in the unit cell. Intensity data (Cu $K\alpha$) were obtained with a Picker automatic diffractometer. The structure was refined to $R=0{\cdot}049$ for 941 observed reflections. The centrosymmetric molecules are nearly planar. A slight deviation from planarity is discussed. Observed bond distances are: N=N, 1·252; C–N, 1·443; C–Cl, 1·737 Å ($\sigma\sim0{\cdot}005$ Å). Observed angles: N=N–C, 112·6°; N–C–C (*cis* relative to N=N), 125·9°; C–C(Cl)–C, 121·7°.

Introduction

Earlier reports of structure determinations of aromatic azo compounds include the crystal structures of *trans*-azobenzene (Brown, 1966*a*), *trans*-azotoluene (Brown, 1966*b*), *trans*-4,4'-azopyridine *N*-oxide (Eichhorn, 1959), and *trans-p,p'*-dibromoazobenzene (Amit & Hope, 1966). The structures of azobenzene and azotoluene are disordered, making it difficult to obtain accurate results, and the reported standard deviations for distances in the other two compounds are rather large (0·015–0·02 Å).

Since dichloroazobenzene was known to be chemically stable, and since the Cl atoms were expected not to be too heavy to preclude a fairly accurate determination of the parameters for the other atoms, we felt that a structure determination of *trans-p,p'*-dichloroazobenzene might provide more reliable data for the geometry of the azo group.

Experimental and structure determination

trans-p,p'-Dichloroazobenzene was prepared by oxidation of *p*-chloroaniline with sodium perborate in acetic acid (Mehta & Vakilwala, 1952). Crystals were obtained without recrystallization in the form of thin,

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