The Crystal and Molecular Structure of Glycyl-DL-phenylalanine*

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Crystals of glycyl-DL-phenylalanine are orthorhombic, space group *Pbca*, with $a = 9.241(2)$, $b = 28.41(1)$, and $c = 8.602(2)$ Å. The structure has been solved by the symbolic addition method and refined to an R index of 0.041 for 2271 reflections. The peptide group is non-planar, the torsion angle about the peptide bond being $-170.2(2)°$ for the L molecules. The structure is stabilized by a network of intermolecular hydrogen bonds N–H$\cdots$O; there is also evidence for the presence of C–H$\cdots$O interactions.

Introduction

The crystal structure of glycyl-DL-phenylalanine was investigated as part of a program of studies on simple linear and cyclic peptides. Such studies should throw further light on the conformations of polypeptide chains and the mode of packing of side groups; they were further motivated by recent observations (Vasantha Pattabhi, Venkatesan & Hall, 1973; Stenkamp & Jensen, 1973; Winkler & Dunitz, 1970) and calculations (Ramachandran, Lakshiminarayanan & Kolaskar, 1973) which indicate that the atoms of a peptide grouping need not be as coplanar as had once been thought.

The preparation and initial survey of the crystals and the solution and initial refinement of the structure were carried out at Bangalore; collection of the intensity data and final least-squares refinements were carried out at Caltech.

Experimental

A sample of glycyl-DL-phenylalanine was obtained from Nutritional Biochemicals Corporation. Crystals in the form of truncated trapezoids, with bases of the form {010} and sides of the form {212}, were formed upon slow evaporation of an aqueous solution at 20 °C. The external morphology of these crystals was clearly hemihedral, of point group C_{2v} (*m2m*); nevertheless, the structure is based on a space group belonging to the holohedral point group D_{2h} (*mmm*).

The crystal used for data collection had approximately square bases of dimensions 0·6 × 0·6 mm and 0·4 × 0·4 mm, and an altitude of 0·5 mm. Preliminary Weissenberg photographs indicated the orthorhombic

space group *Pbca* (0*kl* absent for *k* odd, *h*0*l* for *l* odd, and *hk*0 for *h* odd). The crystal was then oriented with the *b* axis inclined by about 8° to the φ axis of a Datex-automated General Electric diffractometer equipped with a copper X-ray tube, nickel filter, scintillation crystal and pulse-height discriminator. Unit-cell dimensions were obtained from 2θ measurements of the axial reflections; the values of *a* and *c* were in good agreement with values obtained from a Weissenberg photograph of the *h*0*l* zone prepared in a Straumanis-type camera. Crystal data are summarized in Table 1.

Table 1. *Crystal data*

$C_{11}H_{14}N_2O_3$	F.W. 222·25
Orthorhombic	$Z = 8$
Space group: *Pbca*	$F(000) = 944$
$a = 9.241$ (2) Å	$V = 2258$ (2) Å^3
$b = 28.41$ (1)	$D_m = 1.309$ g cm^{-3}
$c = 8.602$ (2)	$D_x = 1.307$ g cm^{-3}
λ(Cu $K\alpha$) = 1·5418 Å	$\mu = 8.11$ cm^{-1}

Intensities were measured from θ–2θ scans at a speed of 1° min^{-1} (in 2θ); the scan width varied from 1·65° at $2\theta = 4°$ to 3·0° at $2\theta = 150°$. Backgrounds were counted for 40 s at each scan extremum. The 042 reflection was checked every 40 reflections; it showed no significant change in intensity. The 41 strongest reflections were re-collected at reduced tube current to preclude errors due to counter overflow or dead-time. Observational variances $\sigma^2(I)$ included counting statistics for scan and background plus a term $(0.02S)^2$, where S is the scan count.

All reflections out to $2\theta = 150°$ were surveyed for the two equivalent octants hkl and $\bar{h}kl$; for each reflection the two intensities were averaged and their weights $\sigma^{-2}(I)$ were summed. The final data set comprised 2326 averaged intensities, of which 54 had negative values. Corrections were then made for Lorentz and polarization factors, but not for absorption ($\mu r \leq 0.3$).

Structure solution and refinement

The structure was solved at Bangalore with the symbolic addition method (Zachariasen, 1952; Karle & Karle, 1963, 1966) and the GAASA system of programs (Lindgren, Lindquist & Nyborg, 1970) modified by R. Ramani to suit an IBM 360/44 computer with a memory of 64 K bytes. All 16 non-hydrogen atoms appeared in the resulting E map, and a structure-factor calculation gave an R value of 0·386. Initial block-diagonal least-squares refinement of positional and

isotropic thermal parameters lowered R to 0·113, and a difference map showed peaks corresponding to all 14 hydrogen atoms. The least-squares program used was that due to Shiono (1968), modified by B. Swaminatha Reddy to suit an IBM 360/44 computer.

Final least-squares refinement was done at Caltech, with the CRYM system on an IBM 370/158 computer. The quantity minimized was $\sum \omega (F_o^2 - F_c^2)^2$, with weights ω equal to $\sigma^{-2}(F_o^2)$. Form factors for C, N, and O were from *International Tables for X-ray Crystallography* (1962) and for H from Stewart, Davidson &

Table 2. *Final parameters and their e.s.d.'s (in parentheses)*

For the heavy atoms, the coordinates have been multiplied by 10^5 and the values of U_{ij} by 10^4; the coordinates of the hydrogen atoms are $\times 10^4$. The temperature factor is of the form $\exp[-2\pi^2(h^2 a^{*2} U_{11} \ldots + 2klb^* c^* U_{23})]$ for the heavy atoms and $\exp(-B \sin^2 \theta/\lambda^2)$ for the H atoms.

	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
C(1)	52263 (16)	33148 (5)	43138 (15)	534 (8)	362 (7)	322 (6)	69 (6)	83 (6)	−7 (5)
C(2)	65418 (18)	34017 (6)	50318 (19)	554 (9)	469 (9)	552 (9)	26 (7)	20 (7)	120 (7)
C(3)	69972 (20)	31330 (7)	62831 (22)	652 (10)	626 (11)	716 (12)	96 (9)	−113 (9)	180 (9)
C(4)	61439 (22)	27750 (7)	68423 (21)	793 (13)	608 (11)	616 (10)	190 (10)	63 (9)	250 (9)
C(5)	48326 (21)	26872 (6)	61496 (22)	748 (12)	525 (10)	666 (11)	32 (9)	217 (9)	207 (8)
C(6)	43729 (19)	29557 (6)	48986 (19)	569 (9)	488 (9)	530 (9)	−3 (7)	71 (7)	25 (7)
C(7)	47555 (17)	35928 (5)	29113 (15)	622 (9)	412 (8)	290 (6)	47 (6)	9 (6)	−37 (6)
C(8)	46150 (12)	41266 (4)	31898 (13)	286 (5)	396 (7)	208 (5)	8 (5)	23 (4)	−21 (5)
C(9)	42311 (12)	43665 (4)	16442 (13)	314 (6)	365 (7)	222 (5)	−41 (5)	0 (4)	−24 (5)
O(1)	51960 (10)	43694 (4)	6296 (10)	375 (5)	751 (7)	258 (4)	50 (5)	94 (4)	84 (5)
O(2)	29792 (9)	45300 (3)	14845 (10)	324 (4)	547 (6)	271 (4)	58 (4)	−17 (4)	11 (4)
N(1)	35588 (10)	42233 (4)	44044 (11)	253 (5)	438 (6)	203 (4)	−28 (4)	18 (4)	−21 (4)
C(10)	39449 (13)	43901 (4)	57941 (13)	268 (5)	419 (7)	201 (5)	32 (5)	1 (4)	11 (5)
O(3)	51625 (9)	45203 (4)	61443 (11)	246 (4)	892 (8)	289 (5)	−24 (5)	−18 (4)	−141 (5)
C(11)	27503 (14)	43925 (5)	69917 (14)	354 (7)	507 (8)	247 (6)	−46 (6)	63 (5)	−43 (5)
N(2)	31049 (11)	47230 (4)	82558 (11)	313 (5)	534 (7)	191 (4)	60 (5)	4 (4)	−37 (4)

|	x	y	z	B		x	y	z	B
H(1)	7130 (19)	3652 (6)	4660 (19)	5·5 (0·4)	H(8)	5527 (14)	4251 (4)	3565 (14)	2·5 (0·3)
H(2)	7947 (21)	3203 (7)	6743 (23)	7·7 (0·6)	H(9)	2602 (16)	4168 (5)	4247 (16)	3·9 (0·3)
H(3)	6450 (20)	2603 (6)	7777 (21)	6·0 (0·4)	H(10)	2648 (18)	4074 (6)	7398 (19)	5·3 (0·4)
H(4)	4213 (20)	2437 (7)	6494 (21)	6·9 (0·5)	H(11)	1854 (19)	4461 (6)	6478 (19)	5·3 (0·4)
H(5)	3471 (18)	2912 (6)	4428 (19)	5·0 (0·4)	H(12)	2573 (22)	4664 (7)	9189 (21)	6·5 (0·5)
H(6)	3838 (17)	3465 (5)	2515 (18)	4·6 (0·3)	H(13)	4050 (23)	4682 (7)	8611 (21)	7·3 (0·5)
H(7)	5438 (17)	3548 (5)	2031 (18)	4·9 (0·4)	H(14)	2925 (23)	5026 (7)	7901 (24)	7·9 (0·6)
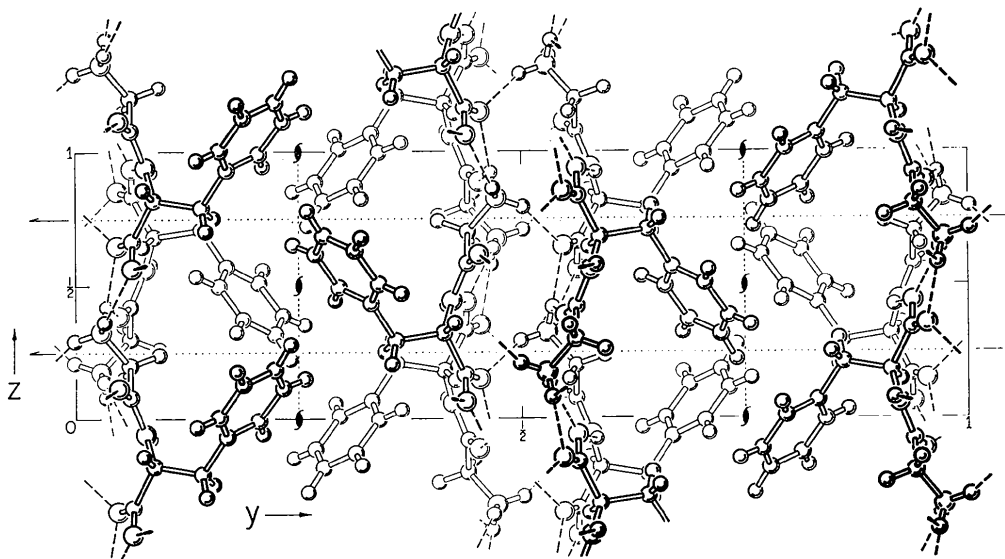
Fig. 1. A drawing of the structure, viewed down a.

Simpson (1965). In the final cycle, coordinates and anisotropic temperature factors for the 16 heavier atoms, a scale factor, and an isotropic extinction parameter (Larson, 1967) were included in one matrix while the coordinates and isotropic temperature parameters of the 14 hydrogen atoms were in a second matrix. One reflection, 5,10,2, was assigned a weight of zero. It showed measurable, but quite different (by about 5 e.s.d.'s), intensities in the two data sets; and since the calculated intensity was very small, the weighted residual $(F_o^2 - F_c^2)/\sigma(F_o^2)$ would have been about 46. Similar but less severe problems with other reflections will be discussed later.

The final R index, $\sum||F_o| - |F_c||/\sum|F_o|$, was 0·041 for 2271 reflections with net intensities greater than zero; the 'weighted R index', $[\sum\omega(F_o^2 - F_c^2)^2/\sum\omega(F_o^2)^2]^{1/2}$, was 0·089, and the goodness of fit, $[\sum\omega(F_o^2 - F_c^2)^2/(m - s)]^{1/2}$, was 3·3 for the 2325 reflections with nonzero weights. There are apparently two reasons for the disappointingly high goodness-of-fit and R values:

(1) The neglect of bonding electrons and other non-spherical scattering effects. In this regard, a final difference map shows features ranging up to 0·2 e Å^{-3} in regions between pairs of bonded atoms, and the goodness-of-fit value for the 2182 reflections with sin θ/λ greater than 0·25 is a somewhat more satisfactory 2·9.

(2) Some sort of systematic error in the measured intensities. Of the 15 reflections (other than 5,10,2) showing residual $|(F_o^2 - F_c^2)/\sigma(F_o^2)|$ greater than 11·0, 13 showed positive residuals and ten of these involved very weak reflections. Moreover, a number of reflections required to be absent by the space-group symmetry showed measured intensities significantly (up to 20 e.s.d.'s) above background (though not above the visual threshold on the Weissenberg photographs). Multiple reflection comes immediately to mind; however, the crystal orientation on the diffractometer was skew, and in a majority of (but not all) instances the two forms that were collected (*hkl* and *hkl*) showed approximately equal intensity. Instrumental errors cannot be ruled out. It is also possible that the crystal contained a very small amount of impurity that did not destroy the lattice but altered the structure, including the space group; this explanation is lent some weight by the hemimorphism of the crystals. The final difference map contained no hint of additional or misplaced atoms, and it is probably fruitless to attempt to explore the myriads of possible structural perturbations on the basis of only a couple of dozen discordant measurements.

The final parameters for the heavy atoms are listed in Table 2.* As is customary, the standard deviations

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* A table of observed and calculated structure factors and weighted residuals $[F^2(\text{obs}) - F^2(\text{cal})]/\sigma[F^2(\text{obs})]$ has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 31130 (12 pp., 1 microfiche). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

reported for these parameters include the goodness-of-fit factor of 3·3, and despite the unexplained intensity discrepancies we believe that they are satisfactory representations of the true uncertainties. This belief is supported by the generally good agreement between bond distances and angles that would be expected to be equivalent, including those involving hydrogen atoms.

Discussion

The crystal structure is shown in Fig. 1. The molecules organize themselves so that the non-polar groups are clustered together in one region (at $y = \frac{1}{4}$ and $\frac{3}{4}$) and the polar groups in another ($y = 0$ and $\frac{1}{2}$). The packing of the non-polar phenyl groups has the typical herringbone pattern. However, it is by no means intimate, the intermolecular H$\cdots$H distances starting at 2·50 Å and the C$\cdots$H distances at 2·91 Å; no C$\cdots$C distance is less than 3·5 Å.

Details of the hydrogen bonding are given in Table 3. All four available protons are involved. One of them, H(12), is involved with two acceptors, its interaction with O(2) being somewhat stronger than that with O(3). The ammonium group has a fifth oxygen neighbor, O(1), at a distance N(2)$\cdots$O(1) of 3·167 Å; however, it is nearly in a head-on position [the C(11)–N(2)$\cdots$O(1) angle is 148·5°] and the interaction must be primarily ionic. The hydrogen bond involving the peptide nitrogen atom N(1) is quite weak, consistent with the observation of Marsh & Donohue (1967) that peptide nitrogen atoms tend to make weaker hydrogen bonds than do terminal ammonium groups.

Table 3. *Details of the hydrogen bonds, D–H$\cdots$A*

						$\angle$D–
	(a)	$-\frac{1}{2}+x,$	$y, \frac{1}{2}-z$			
	(b)	$x,$	$y, 1+z$			
	(c)	$-\frac{1}{2}+x,$	$y, \frac{3}{2}-z$			
	(d)	$\frac{1}{2}-x, 1-y, \frac{1}{2}+z$				
	(e)	$\frac{1}{2}+x,$	$y, \frac{1}{2}-z$			

D	H	A	$D\cdots A$	$H\cdots A$	$\begin{matrix}\angle D-\\H\cdots A\end{matrix}$
N(1)	H(9)	O(1) (a)	3·135 Å	2·30 Å	153°
N(2)	H(12)	O(2) (b)	2·833	2·05	139
	H(12)	O(3) (c)	2·827	2·28	115
	H(13)	O(1) (b)	2·986	2·22	139
	H(14)	O(2) (d)	2·798	1·94	152
C(8)	H(8)	O(2) (e)	3·325	2·40	159
C(11)	H(11)	O(1) (a)	3·265	2·39	152

We include in Table 3 two C–H$\cdots$O interactions which, though rather long, are approximately linear and meet the criterion suggested by Hamilton & Ibers (1968) for the existence of a hydrogen bond. We note that the donor atoms C(8) and C(11) are each bonded to more electronegative atoms, N(1) and N(2), a circumstance suggested by Sutor (1962) as favorable for a C–H$\cdots$O hydrogen bond.

Bond distances and angles involving the C, N, and O atoms are shown in Fig. 2, and a view of the molecule is shown in Fig. 3. The dimensions of the peptide

group are in good agreement with average values in other peptides as reported by Marsh & Donohue (1967); the largest difference is in the C(10)–O(3) bond, which is nearly 0·02 Å shorter than the earlier average. This shortening may well be a consequence of the fact that O(3) is involved in only a weak, shared hydrogen bond (see Table 3) and hence can devote more bonding power to C(10). The dimensions of the carboxylate ion are in similar agreement with the earlier average values (Marsh & Donohue, 1967), the larger C–C–O angle once again being associated with the oxygen atom, O(2), which is cis to the C(8)–N(1) bond. As in other peptides, the C(11)–N(2) bond is longer than the C(8)–N(1) bond.
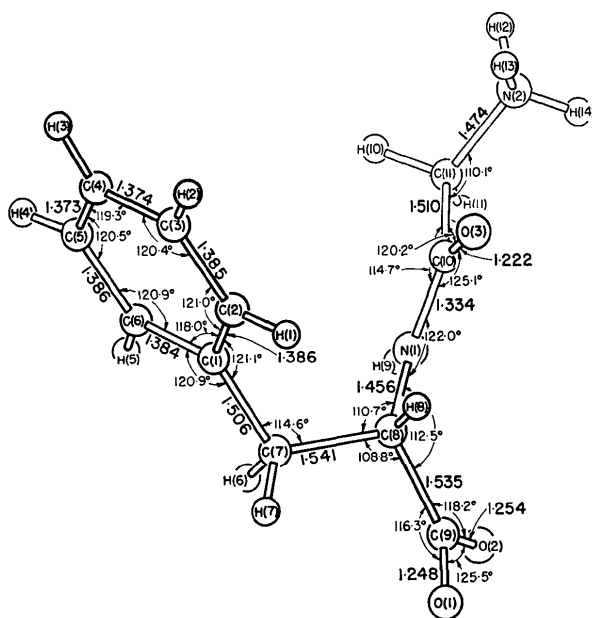
The peptide grouping is appreciably non-planar.

The best four-atom plane is the one passing through C(10), C(11), O(3), and N(1) (see Table 4); the deviation from this plane of −0·202 Å for C(8) corresponds to a twist of about 10° about the peptide bond, the torsion angle C(11)–C(10)–N(1)–C(8) being −170·2(2)° for the L molecule shown in Fig. 2. Even more severe non-planarity was found by Stenkamp & Jensen (1973) in crystals of N-acetyl-L-phenylalanyl-L-tyrosine, where the torsion angle is 162·3(4)°. Clearly, the statement made some years ago by Marsh & Donohue (1967), 'The amide group has invariably been found to be planar within experimental error', is no longer operational.

Bond distances and angles involving the hydrogen atoms are given in Table 5. The H(6)–C(7)–H(7) angle is appreciably smaller than tetrahedral, compensating for the opening of the C(1)–C(7)–C(8) angle that is typical of amino acid side chains. The three H–N(2)–H bond angles are significantly different, the distortions

Fig. 2. The glycyl-L-phenylalanine molecule, showing bond distances and angles involving the heavy atoms. Standard deviations are about 0·003 Å and 0·15°.
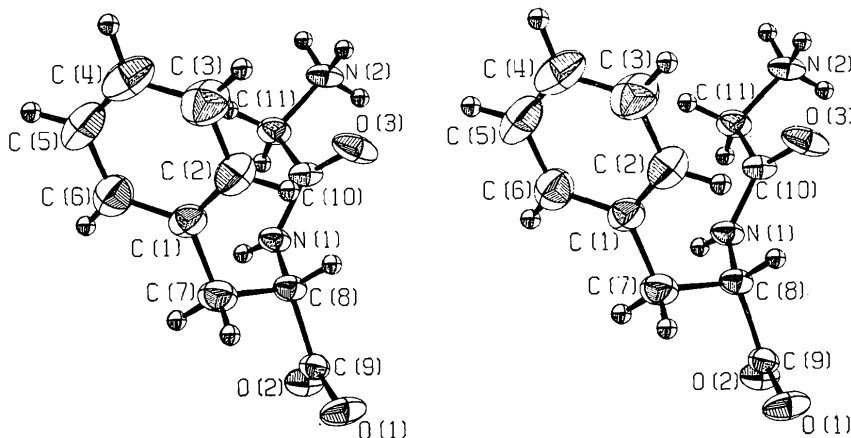
Table 4. *Some planes of interest and atomic deviations* (Å)

Least-square planes were passed through the atoms in the first column, all weighted equally; atoms in the second column were given zero weight. The direction cosines g are relative to the crystallographic axes; D is the origin-to-plane distance.

Benzene ring

$g_a = -0.4421$	C(1)	−0·005	C(7)	−0·062
$g_b = 0.6420$	C(2)	0·003	H(1)	0·02
$g_c = 0.6264$	C(3)	0·001	H(2)	−0·01
$D = 6.241$ Å	C(4)	−0·002	H(3)	0·06
	C(5)	0·000	H(4)	−0·02
	C(6)	0·004	H(5)	0·04

Carboxylate ion

$g_a = 0.3226$	C(9)	0·010	N(1)	0·294
$g_b = 0.8778$	O(1)	−0·004	H(9)	−0·18
$g_c = 0.3541$	O(2)	−0·004		
$D = 12.641$ Å	C(8)	−0·003		

Peptide unit

$g_a = -0.2439$	C(10)	0·012	C(8)	−0·202
$g_b = 0.9279$	N(1)	−0·004	H(9)	0·10
$g_c = -0.2821$	O(3)	−0·004	N(2)	0·481
$D = 9.266$ Å	C(11)	−0·003		

Fig. 3. Stereo diagram of the molecule.

Table 5. *Bond distances and angles involving the hydrogen atoms*

Estimated standard deviations are about 0·02 Å and 1·5°.

C(2)—H(1)	0·95 Å	C(1)—C(2)—H(1)	119°	C(3)—C(2)—H(1)	120°
C(3)—H(2)	0·98	C(2)—C(3)—H(2)	118	C(4)—C(3)—H(2)	121
C(4)—H(3)	0·98	C(3)—C(4)—H(3)	119	C(5)—C(4)—H(3)	121
C(5)—H(4)	0·96	C(4)—C(5)—H(4)	122	C(6)—C(5)—H(4)	118
C(6)—H(5)	0·94	C(5)—C(6)—H(5)	122	C(1)—C(6)—H(5)	117
C(7)—H(6)	0·98	C(1)—C(7)—H(6)	109	C(8)—C(7)—H(6)	110
C(7)—H(7)	0·99	C(1)—C(7)—H(7)	111	C(8)—C(7)—H(7)	107
				H(6)—C(7)—H(7)	104
C(8)—H(8)	0·97	C(7)—C(8)—H(8)	110	C(9)—C(8)—H(8)	109
				N(1)—C(8)—H(8)	106
N(1)—H(9)	0·91	C(8)—N(1)—H(9)	121	C(10)-N(1)—H(9)	117
C(11)-H(10)	0·97	C(10)-C(11)-H(10)	108	N(2)—C(11)-H(10)	110
C(11)-H(11)	0·96	C(10)-C(11)-H(11)	109	N(2)—C(11)-H(11)	114
				H(10)-C(11)-H(11)	106
N(2)—H(12)	0·96	C(11)-N(2)—H(12)	113	H(12)-N(2)—H(13)	101
N(2)—H(13)	0·93	C(11)-N(2)—H(13)	112	H(13)-N(2)—H(14)	113
N(2)—H(14)	0·93	C(11)-N(2)—H(14)	108	H(12)-N(2)—H(14)	110

being in such a direction as to improve the linearity of the N–H···O bonds (see Fig. 4). Related effects, resulting in out-of-plane displacements of hydrogen atoms in ring compounds, have already been noted [see, for example, Holian & Marsh (1970)].

Conformation of the molecule

The notation followed in the description of the conformation of this molecule is that of Edsall et al. (1966). The rotation about the N–C$^\alpha$ and and C$^\alpha$–C' bonds are denoted by torsion angles φ and ψ respectively. In the present case we have one ψ angle for the N-terminal residue, one φ angle and two angles ψ_1 and ψ_2 for the C-terminal residue. For the L enantiomer, the value of φ [C(10)–N(1)–C(8)–C(9)] is − 127·6° and that of ψ [N(2)–C(11)–C(10)–O(3)] is 21·9°; the values of ψ_1 [N(1)–C(8)–C(9)–O(2)] and ψ_2 [N(1)–C(8)–C(9)–O(1)] are − 13·9° and 168·0° respectively. The side-chain conformation is described by the torsion angle χ^1, which corresponds to rotation about the bond C$^\alpha$–C$^\beta$. In the present structure the value of χ^1 [N(1)–C(8)–C(7)–C(1)] is − 59·7°, i.e., C(1) is *trans* to C(9). The value of χ^1 in the structure of glycyl-L-phenylalanylglycine (Marsh & Glusker, 1961) is 185° and in L-phenylalanine hydrochloride (Gurskaya, 1964), 62°.

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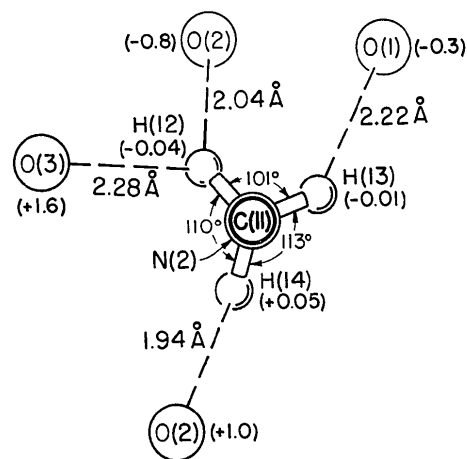KARLE, J. & KARLE, I. L. (1966). *Acta Cryst.* 21, 849–859.

Fig. 4. A view down the C(11)–N(2) bond, showing the hydrogen bonds and their effects on the H–N–H angles. Numbers in parentheses are displacements, in Å, from various planes oriented perpendicular to the C(11)–N(2) bond. For the three hydrogen atoms, the plane passes through their centroid. For the acceptor oxygen atoms, the planes pass through points that would correspond to linear N–H···O bonds.

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