

Crystal and Molecular Structure of Glycyl-L-alanine Hydrochloride*

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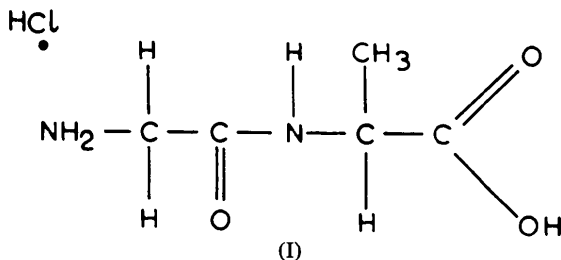
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The crystal structure of glycyl-L-alanine hydrochloride, $C_5N_2O_3H_{11}Cl$, has been determined using three-dimensional photographic data. The crystals are monoclinic, space group $P2_1$, with cell dimensions $a = 8.21$, $b = 5.04$, $c = 10.82$ Å and $\beta = 100.1^\circ$. Refinement was carried out by full-matrix least-squares methods with anisotropic thermal parameters for all non-hydrogen atoms to a final R index of 0.098 for 895 observed reflexions. The crystal structure is stabilized by a three-dimensional network of hydrogen bonds. Significant non-planarity of the peptide linkage with a torsion angle of 10.2° about the peptide bond has been observed. The conformation of the molecule has also been studied.

Introduction

As part of a major scheme of research on the structures of amino acids and simple peptides being carried out in this laboratory, the investigation of the structure of glycyl-L-alanine hydrochloride (I) was undertaken. Glycine and alanine are amino acids that frequently occur in proteins. The monohydrate modification of glycyl-L-alanine hydrochloride has already been studied by Tranter (1953, 1956). In this paper, the molecular features and the conformation of glycyl-L-alanine, as found in the crystal structure of the anhydrous hydrochloride derivative, are presented.



Experimental

The free compound, glycyl-L-alanine, was treated with a slight excess of 2*N* hydrochloric acid, evaporated to dryness in a vacuum, and needle-shaped crystals were separated out. A single crystal of dimensions 0.10×0.15 mm² was selected for X-ray analysis. Rotation, Weissenberg and precession photographs were taken with Cu $K\alpha$ radiation ($\lambda = 1.5418$ Å), and the crystal data as obtained from Weissenberg and precession photograph are:

Crystal system:	Monoclinic
Cell dimensions:	$a = 8.21 \pm 0.02$ Å
	$b = 5.04 \pm 0.01$
	$c = 10.82 \pm 0.02$
	$\beta = 100.1 \pm 0.2^\circ$

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Space group:	$P2_1$ ($0k0$, $k = 2n + 1$ absent)
Number of molecules in unit cell:	$Z = 2$
Molecular formula	$C_5N_2O_3H_{11}Cl$
F.W.	181.6
D_m	1.383 g.cm ⁻³
D_c	1.370 g.cm ⁻³
μ (Cu $K\alpha$)	36 cm ⁻¹

The space group $P2_1/m$ is not compatible with the optical activity of the compound. Density was measured by the flotation method, using a mixture of bromoform and benzene.

Intensity data were collected with Cu $K\alpha$ radiation for layers hkl , $k = 0$ to 4, and hkl , $h = 0$ and 1, by mounting the crystal about the b and a axes respectively, using the equi-inclination Weissenberg technique for higher layers. The intensities were corrected for Lorentz and polarization factors. Corrections for elongation of spots were applied by the method of Phillips (1962). No absorption correction was applied, as it was not considered necessary. The two sets of data, about the b and a axes, were then correlated by the method of Rollett & Sparks (1960). Out of 1057 reflexions, being the total number accessible to the copper sphere, 895 independent reflexions were observed and used in the refinement.

Structure determination and refinement

A chlorine-sharpened Harker section at $V = \frac{1}{2}$ was computed, which revealed the position of the chloride atom clearly. The choice of the origin along Y being arbitrary in the space group $P2_1$, the y coordinate of chlorine was fixed at 0.2500. Therefore, initial coordinates of chlorine were: $x = 0.3250$, $y = 0.2500$, $z = 0.0583$. A structure-factor calculation with the chlorine contribution alone resulted in an R index of 0.48. A chlorine-phased electron-density map was used to locate all light atoms. The R index with chlorine and ten light atoms was 0.33, which indicated that the model was essentially correct.

Refinement of the structure in the initial stages was

Table 1. Final positional (fractional) and anisotropic thermal parameters for non-hydrogen atoms ($\times 10^4$)

The standard deviation is given in parentheses. The b_{ij} 's are defined by

$$T = \exp [-(b_{11}h^2 + b_{22}k^2 + b_{33}l^2 + b_{12}hk + b_{13}hl + b_{23}kl)] .$$

	x	y	z	b_{11}	b_{22}	b_{33}	b_{12}	b_{13}	b_{23}
Cl	3206 (2)	2500 (—)	538 (0)	166 (3)	420 (12)	86 (2)	-1 (9)	24 (3)	0 (8)
C(1)	992 (11)	7112 (23)	-1415 (8)	156 (13)	331 (51)	90 (8)	24 (37)	-24 (15)	-92 (32)
C(2)	338 (10)	4944 (21)	-2339 (7)	127 (11)	291 (40)	65 (6)	-74 (33)	21 (12)	-9 (26)
C(3)	-2135 (11)	2909 (24)	-3597 (9)	159 (14)	346 (51)	88 (8)	-18 (42)	6 (16)	39 (33)
C(4)	-3906 (13)	2444 (34)	-3366 (10)	182 (16)	699 (72)	133 (9)	-241 (66)	-5 (19)	148 (50)
C(5)	-2145 (10)	4193 (22)	-4895 (8)	136 (12)	311 (43)	70 (7)	23 (36)	-11 (14)	-4 (26)
N(1)	2791 (9)	7494 (23)	-1336 (7)	168 (11)	447 (40)	75 (6)	20 (42)	-12 (12)	-17 (35)
N(2)	-1291 (8)	4616 (19)	-2565 (6)	126 (10)	433 (42)	67 (6)	-85 (31)	-8 (11)	-27 (25)
O(1)	1316 (8)	3441 (16)	-2796 (6)	152 (10)	341 (36)	89 (6)	-11 (26)	36 (12)	-29 (21)
O(2)	-2795 (9)	3037 (19)	-5860 (6)	206 (13)	438 (50)	86 (6)	9 (36)	-10 (13)	-24 (27)
O(3)	-1445 (9)	6589 (17)	-4916 (7)	204 (13)	391 (39)	96 (7)	-53 (35)	-1 (15)	-3 (24)

carried out through successive difference electron-density maps. Three such maps were computed, and the R index at this stage was 0.215. Four cycles of full-matrix least-squares refinement were carried out on a CDC 3600 computer using the program of Gantzel, Sparks & Trueblood (1961). The quantity minimized was $\sum w(|F_o| - |F_c|)^2$, where w is the weight of the observation. An analysis of the plot of $\langle |F_o| \rangle$ versus $|\Delta F|^2$, obtained after correlating the data collected about the b and a axes, indicated the weighting function of the form, $w = 1/(2.0 + |F_o| + 0.07|F_o|^2)$, (Cruickshank, Bujosa, Lovell & Truter, 1961) to be appropriate. This refinement of the positional coordinates, individual isotropic vibration parameters and the overall scale factor reduced the R index to 0.120. A difference electron-density map computed at this stage showed peaks at positions where hydrogen atoms were expected to occur, at heights varying from 0.2 to 0.4 $e.\text{\AA}^{-3}$. Two more cycles of refinement were carried out with isotropic temperature factors for the non-hydrogen atoms, while the contribution of hydrogen atoms was included in the structure-factor calculations only; the hydrogen atoms were ascribed the isotropic temperature factors of the heavy atoms to which

they are bonded. This resulted in an R index of 0.11. Two more cycles of refinement, with anisotropic thermal parameters for all non-hydrogen atoms and the overall scale factor, reduced the agreement factor to the final value of 0.098. In the last cycle, the maximum parameter shifts for the non-hydrogen atoms were less than one fifth of their estimated standard deviations. The final positional and thermal parameters, and their standard deviations, for the non-hydrogen atoms are listed in Table 1, and for hydrogen atoms in Table 2. The final electron-density distribution is shown in Fig. 1.

Table 2. Positional (fractional) coordinates of hydrogen atoms ($\times 10^3$) obtained from the difference electron-density map

	Bonded to	x	y	z	B (\AA^2)
H(1)	N(1)	317	800	-217	3.92
H(2)	N(1)	300	900	-83	3.92
H(3)	N(1)	317	617	-100	3.92
H(4)	C(1)	50	900	-183	3.89
H(5)	C(1)	83	667	-67	3.89
H(6)	N(2)	-200	600	-217	3.56
H(7)	C(3)	-167	100	-367	4.48
H(8)	C(4)	-433	100	-417	5.50
H(9)	C(4)	-367	167	-250	5.50
H(10)	C(4)	-450	433	-333	5.50
H(11)	O(3)	-133	700	-567	4.75

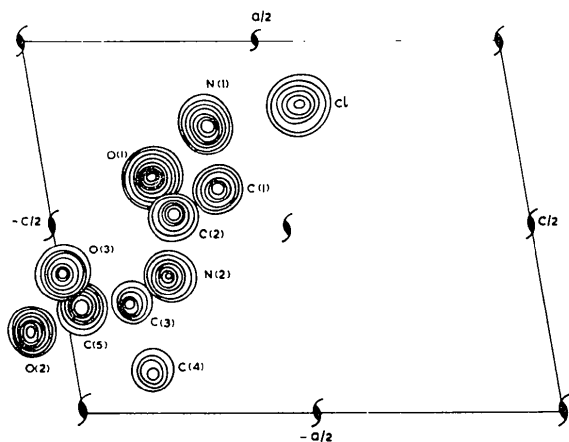


Fig. 1. Composite diagram of final electron-density distribution. Contours are drawn at intervals of 1 $e.\text{\AA}^{-3}$, starting from 1 $e.\text{\AA}^{-3}$, while near chlorine the interval is 5 $e.\text{\AA}^{-3}$.

Atomic scattering factors used for Cl^- , O, N, C, and H are those listed in *International Tables for X-ray Crystallography* (1962). The correction due to the real part of the anomalous dispersion ($\Delta f' = 0.3$ for $\text{Cu } K\alpha$) was included at all stages of refinement. It may be mentioned that the imaginary component of the anomalous dispersion corrections for the chloride ion was not incorporated in the refinements ($\Delta f'' = 0.6$ for $\text{Cu } K\alpha$). It has been found that the neglect of $\Delta f''$ in the refinements of polar structures could give rise to positional errors (Ueki, Zalkin & Templeton, 1966; Cruickshank & McDonald, 1967). Consequently, in the present case, where most of the data were obtained from the photographs recorded about the b axis, the standard deviations of the y coordinates of the light atoms

ton from the carboxyl oxygen O(3) and the hydrogen bond is of length O(3)–H···O(1'), 2.67 Å. The chloride ion accepts three hydrogen bonds. The deviation of the chlorine atom from the plane containing the surrounding atoms is 0.84 Å. Hydrogen bond lengths and angles are listed in Table 4.

Bond distances and angles

The observed bond distances and angles in this dipeptide are shown in Fig. 3. The average e.s.d.'s in bond lengths and angles among C, N and O atoms are 0.013 Å and 1.0°, respectively. In general, no significant deviation from the normal value of bond lengths and angles among different atoms is observed in this structure. The C^α–C' [C(3)–C(5)] length of 1.545 (0.013) Å at the C-terminal alanyl end is not significantly different from the weighted average distance of 1.526 (0.004) Å (Marsh & Donohue, 1967; Sundaralingam & Putkey, 1970). The C(1)–N(1) length of 1.477 (0.013) Å is, within the limit of experimental error, in good agreement with the value of 1.487 Å (Marsh & Donohue, 1967). The C^α–C^β [C(3)–C(4)] distance of 1.536 (0.016) Å agrees well with the values observed in the structures of L-alanine (Simpson & Marsh, 1966) and L-alanyl-L-alanine hydrochloride (Tokuma, Ashida & Kakudo, 1969).

Peptide group

The atoms C(1), C(2), O(1), N(2) and C(3) constitute the peptide group. The dimensions of the peptide group are in good agreement with the values reported by Marsh & Donohue (1967) from an analysis of accurately solved peptide structures. However, it is observed in Fig. 3 that the N(2)–C(3) distance of 1.481 (0.013) Å is slightly larger (about 2σ) than the value of 1.455 Å for the above distance. The least-squares plane passing through the atoms of the peptide group is listed in Table 5(a). Non-planarity of the peptide linkage is

highly significant, since $\chi^2 = \sum A^2/\sigma^2 = 245$, for $\nu = 2$, $P \ll 0.001$ (for $\nu = 2$, $P = 0.001$, $\chi^2 = 13.82$ only; Fisher & Yates, 1965). A similar observation regarding the non-planarity of the peptide linkage has been made in the structure of α-glycylglycine (Biswas, Hughes, Sharma & Wilson, 1968). The best plane among the peptide group of atoms is the one passing through C(1), C(2), O(1) and N(2) [Table 5(b)]. It is interesting to note that the C^α atom at the C-terminal end deviates significantly in both the structures of glycyl-L-alanine hydrochloride and α-glycyl glycine. This appears to be mainly due to the influence of hydrogen bonding involving the carboxyl group. The torsion angle ω (Edsall, Flory, Kendrew, Liquori, Némethy, Ramachandran & Scheraga, 1966) about the C'–N bond [C(1)–C(2)–N(2)–C(3)] in glycyl-L-alanine hydrochloride is 10.2° and in α-glycyl glycine 5.3°.

Table 5. Least-squares planes

Equation of the plane: $aX + bY + cZ = D$, where X, Y, Z are expressed in Å units with respect to the crystallographic axes a, b, c .*

		Deviations (Å)	
(a) Peptide group	C(1)	0.048	$\left\{ \begin{array}{l} a = 0.1336 \\ b = 0.6621 \\ c = -0.7374 \\ D = 3.5819 \end{array} \right.$
	C(2)	0.002	
	O(1)	-0.022	
	N(2)	-0.103	
	C(3)	0.071	
(b) Plane through C(1), C(2), O(1), N(2)	C(1)	-0.007	$\left\{ \begin{array}{l} a = 0.0855 \\ b = 0.6356 \\ c = -0.7673 \\ D = 3.5342 \end{array} \right.$
	C(2)	0.023	
	O(1)	-0.009	
	N(2)	-0.008	
	C(3)	0.246†	
(c) Carboxyl group	C(3)	0.0012	$\left\{ \begin{array}{l} a = -0.8982 \\ b = 0.4361 \\ c = 0.0550 \\ D = 1.3951 \end{array} \right.$
	C(5)	-0.0043	
	O(2)	0.0016	
	O(3)	0.0014	
	N(2)	-0.0110†	

† Not included in the calculation of the plane.

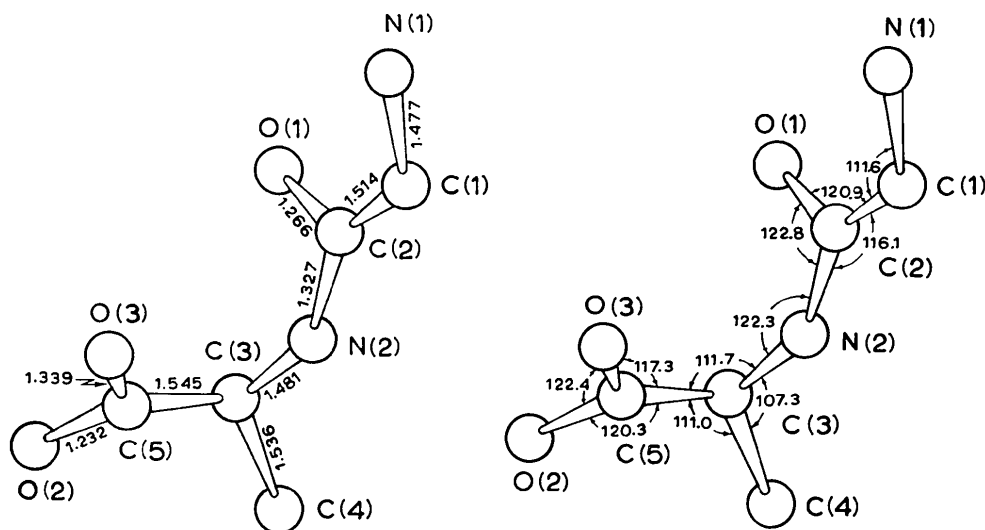


Fig. 3. Bond lengths and angles in the molecule.

Carboxyl group

The carboxyl group in this structure is un-ionized with lengths $C(5)-O(2)=1.232$ (0.012) Å and $C(5)-O(3)=1.339$ (0.012) Å; the proton is attached to O(3). Dimensions are in close agreement with the mean values of 1.23 and 1.32 Å observed in monocarboxylic acids (Kanters, Kroon, Peerdeman & Schoone, 1967). It is interesting to observe that the O(3)H group is *cis* to nitrogen N(2). The least-squares plane passing through the carboxyl group is given in Table 5(c); the deviation of the amino nitrogen N(2) from this plane is quite small (0.01 Å).

Molecular conformation

The notation followed in the description of the conformational parameters of this molecule is that of Edsall *et al.* (1966). The rotations about the $N-C^\alpha$ and $C^\alpha-C'$ bonds are denoted by torsion angles φ and ψ , respectively. In the present case of a dipeptide, we have one angle ψ for the *N*-terminal glycyl residue, one angle φ and two angles ψ_1 and ψ_2 for the *C*-terminal alanyl residue. Fig. 4 gives the values of these torsion angles. The value of ψ [N(1)-C(1)-C(2)-O(1)] in this structure is 11° [Fig. 4(a)]. The torsion angle of [C(2)-N(2)-C(3)-C(5)] is $\varphi=108^\circ$ [Fig. 4(b)]. In many dipeptides this angle is close to 90° (Marsh & Donohue, 1967; Lakshminarayanan, Sasisekharan & Ramachandran, 1967). The conformational angles clearly fall in the allowed regions of the (φ, ψ) diagram of Ramachandran and co-workers for the peptide configuration (Ramachandran, 1962; Ramachandran & Sasisekharan, 1968). Conformational analysis on the dipeptide glycyl-L-alanine has been made through energy minimization methods (Crippen & Scheraga, 1969) and also by a quantum mechanical approach (Hoffman & Imamura, 1969).

The conformational angles obtained in the present study are within the allowed ranges of φ and ψ obtained theoretically by these workers.

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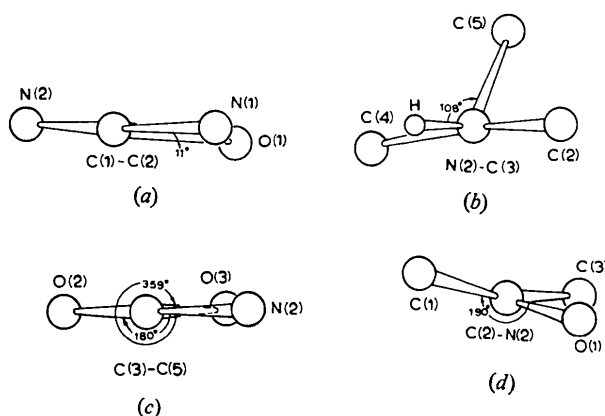


Fig. 4. Conformations about various bonds.