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Crystal Structure of N-Methyl-DL-leucylglycine Hydrobromide*

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Crystals of N-methyl-DL-leucylglycine hydrobromide are orthorhombic, space group Pbca, with $a = 17\cdot21$, $b = 17\cdot80$ and $c = 8\cdot41$ Å, $Z = 8$. The structure has been determined by the heavy atom method using three-dimensional photographic data collected with Cu $K\alpha$ radiation. All the hydrogen atoms in the molecule have been located from a difference-Fourier synthesis. The structure has been refined by full-matrix least-squares methods to a final R index of $0\cdot103$. The molecules in the crystal are stabilized by a network of hydrogen bonds. While the amino group is protonated, the carboxyl group appears to be in the non-ionized form. Surprisingly, the carbonyl oxygen atom in this group does not participate in hydrogen bonding even as an acceptor. The conformational features of the molecule are discussed.

Introduction

As part of a major work on the study of amino acids and simple peptides in this laboratory, an X-ray crystal structure analysis of the dipeptide N-methyl-DL-leucyl-glycine hybrobromide was carried out. The chemical formula of the compound is

$$(CH_3)_2-CH-CH_2-CH-CO-NH-CH_2-COOH.HBr.$$
$$\overset{|}{N}H(CH_3)$$
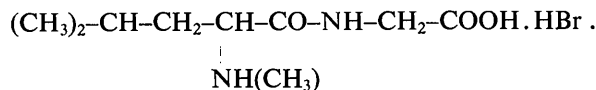
Experimental

Crystals of the compound suitable for X-ray analysis were obtained by evaporating a solution of N-methyl-DL-leucylglycine in 30% hydrobromic acid at room temperature. Preliminary photographs taken with

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Cu $K\alpha$ radiation established the crystallographic data summarized below:

Molecular formula: $C_9H_{18}N_2O_3.HBr$; M.W. = 283.
Orthorhombic; $a = 17\cdot21$, $b = 17\cdot80$, $c = 8\cdot41$ Å.
Systematic absences: $0kl$, k odd,
$h0l$, l odd,
$hk0$, h odd.
Space group: Pbca.
$U = 2576\cdot3$ Å³,
$D_o = 1\cdot47$ g.cm⁻³ (flotation in a mixture of benzene and bromoform),
$D_c = 1\cdot472$ g.cm⁻³,
$Z = 8$.

Absorption coefficient for X-rays, $\mu(\mathrm{Cu}\ K\alpha) = 46$ cm⁻¹.

A thin crystal elongated along c, and sealed in a Lindemann capillary tube, was used to collect photographic intensity data for hkl, $l = 0$–6. These intensities were recorded using Cu $K\alpha$ radiation and multiple-film equi-inclination Weissenberg techniques. Photographs about the a or b axis could not be taken since the crystals crushed on cutting.

Of the 2152 reflexions which were accessible in the various layers, only 1582 reflexions were of measurable

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intensity. The intensities were estimated visually by comparison with a calibrated film strip, and corrected for Lorentz and polarization factors as well as for spot shape (Phillips, 1962). The average thickness of the crystal was less than 0·1 mm. The maximum error in the relative values of F_o because of absorption is about 5%. However, no corrections for absorption were made. The intensities were scaled to their absolute values by Wilson's (1942) method.

Structure analysis

A three-dimensional Lp-sharpened Patterson function was computed on a CDC 3600 computer. Approximate coordinates for the bromine atom were derived from the three Harker sections at $U=\frac{1}{2}$, $V=\frac{1}{2}$ and $W=\frac{1}{2}$. A complete solution to the structure was obtained from a three-dimensional electron density map computed with the phases of the bromine atom. A structure factor calculation for all atoms, assuming an overall isotropic temperature factor of $B=2\cdot6$ Å^2 gave an R index of 0·27. The scattering factors for the atoms were taken from *International Tables for X-ray Crystallography* (1962).

Refinement of the positional and isotropic thermal parameters proceeded by full-matrix least-squares methods, the function minimized being $\Sigma w(|F_o|-|F_c|)^2$. The refinement was carried out on a CDC 3600 computer using a program written by Gantzel, Sparks & Trueblood (1961). For the 1582 observed reflexions, and using $w=1$, three cycles of refinement resulted in an R index of 0·127. At this stage, the 570 unobserved reflexions were also included in the refinement with $|F_o|$ set equal to half the minimum value of $|F_o|$ locally observed. After the introduction of anisotropic thermal parameters as well as the real part of the dispersion correction for the bromine atom, three more cycles of refinement were carried out with unit weights. The reduction in R was only slight ($R=0\cdot121$).

A structure factor calculation at the end of this analysis showed that some of the 570 unobserved reflexions included in the refinement had F_c values very close to zero, while the $|F_o|$ values assigned to them were comparatively large (varying from 2·7 to 8·7). These very weak reflexions, numbering 123, were therefore removed from the data in the subsequent analysis, which was carried out on an IBM 7094 computer at the University of Chicago.

A three-dimensional difference synthesis was now computed using a Fourier program written by Sly, Shoemaker & Van der Hende (1962). The map, shown in Fig.1, clearly indicated the positions of all nineteen hydrogen atoms, the peak heights varying from 0·3 to 1·2 e.Å^{-3}.

Final refinement of the structure was achieved by varying the positional parameters of all the atoms including the hydrogen atoms, anisotropic thermal parameters of the bromine atom and isotropic thermal parameters of the remaining non-hydrogen atoms. The form factors for the hydrogen atoms were taken from

International Tables (1962) and the hydrogen atoms were assigned a value of $B=3\cdot5$ Å^2 which was kept constant during the refinement. Adopting a weighting scheme with $W=1/(A+|F_o|+C|F_o|^2)$ (Cruickshank, Pilling, Bujosa, Lovell & Truter, 1961) where $A=16\cdot0$ and $C=0\cdot025$, a few cycles of full-matrix refinement were carried out using a modified program of Busing, Martin & Levy (1962). Convergence was reached at $R=0\cdot103$. The final shifts in the parameters were all much less than the corresponding standard deviations.

Results and discussion

The final electron density projected down the c axis is shown in Fig.2. The atomic parameters with their standard deviations at the end of the refinement are listed in Table 1. The observed and calculated structure factors are given in Table 2.

Intramolecular features

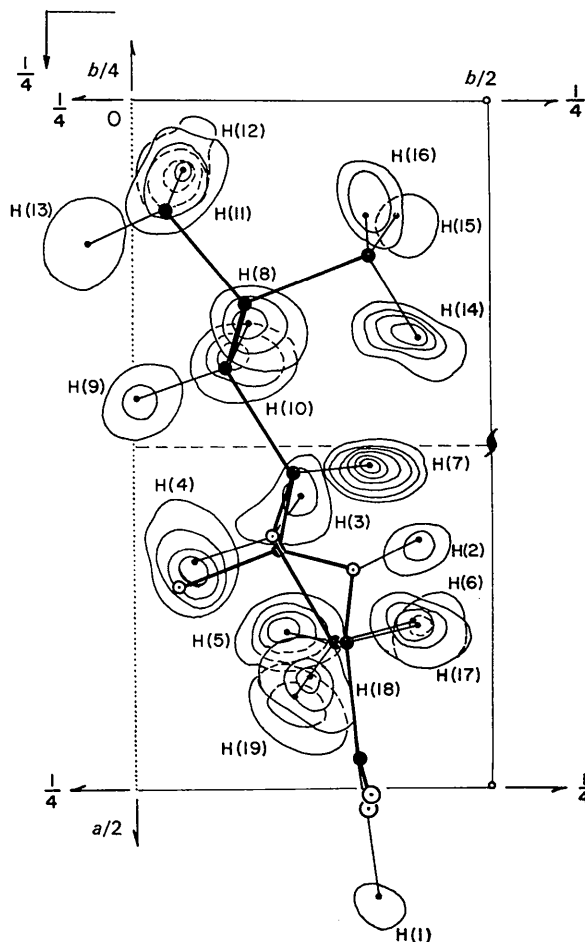The bond lengths and bond angles observed in the molecule are shown in Fig.3. The average standard

Fig. 1. Composite diagram of the three-dimensional difference electron density map. The contours are drawn at intervals of 0·2 e.Å^{-3} from the 0·2 e.Å^{-3} level.

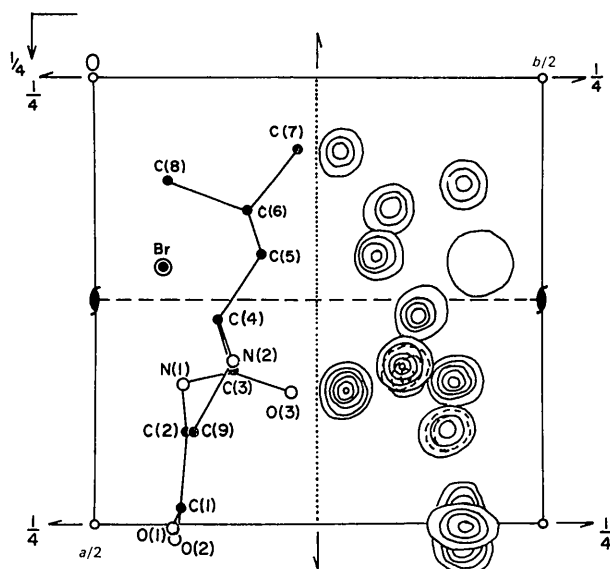Fig. 2. Composite drawing of the final electron density map. The contours are drawn at intervals of 2 e.Å⁻³ from the 2 e.Å⁻³ level. Only the 5 e.Å⁻³ contour is shown for the bromine atom.

deviations for the bond distances and angles are 0·013 Å and 1° respectively for bonds involving non-hydrogen atoms; these values for bonds involving the hydrogen atoms are 0·14 Å and 8° (for angles of the type XXH). The dimensions of the molecule are found to be in good agreement with those observed in other related structures of amino acids and peptides.

The peptide unit is in the *trans* planar configuration. The atoms C(2), N(1), C(3), O(3) and C(4) of the peptide unit are at distances of 0·008, −0·014, 0·009, −0·006 and 0·003 Å from the least-squares plane $0·8093X + 0·1858Y + 0·5572Z = 6·202$ Å. The coefficients of X, Y and Z are the direction cosines of the normal to the plane relative to the crystallographic axes; X, Y and Z are the cartesian coordinates in Å. The hydrogen atom H(2) of the peptide unit is at a distance of 0·20 Å from this mean plane.

The atoms in the carboxyl group and the α-carbon atom C(2) of the C-terminal end are coplanar. The equation of the mean plane passing through the atoms O(1), O(2), C(1) and C(2) is given by

$$-0·1013X + 0·9949Y + 0·0008Z = 6·365 \text{ Å}.$$

The deviations of these four atoms from this plane are

Table 1. *Final positional parameters (fractional), standard deviations (Å) and thermal parameters*

Atom	x	y	z	σ(x)	σ(y)	σ(z)	B
Br	0·2098	0·4245	0·6613	0·001	0·001	0·001	*
O(1)	0·5048	0·4091	0·0476	0·007	0·007	0·008	4·77 Å²
O(2)	0·5230	0·4110	0·2159	0·007	0·006	0·008	4·08
O(3)	0·3513	0·2813	0·0796	0·005	0·006	0·007	3·31
N(1)	0·3449	0·4035	0·0105	0·006	0·007	0·008	3·15
N(2)	0·3222	0·3471	0·3948	0·006	0·006	0·008	2·93
C(1)	0·4789	0·4068	−0·0833	0·008	0·008	0·010	3·17
C(2)	0·3945	0·3983	−0·1283	0·009	0·009	0·011	3·78
C(3)	0·3290	0·3458	0·1034	0·006	0·006	0·008	2·38
C(4)	0·2761	0·3639	0·2468	0·008	0·007	0·009	2·84
C(5)	0·2014	0·3158	0·2435	0·007	0·008	0·010	2·89
C(6)	0·1492	0·3305	0·0994	0·008	0·008	0·010	3·23
C(7)	0·0821	0·2747	0·1015	0·010	0·010	0·120	4·17
C(8)	0·1190	0·4131	0·1034	0·011	0·011	0·015	4·39
C(9)	0·3955	0·3897	0·4059	0·009	0·009	0·012	3·88
H(1)	0·5569	0·4065	−0·1598	0·14	0·12	0·14	3·5
H(2)	0·3396	0·4490	0·0407	0·12	0·12	0·13	3·5
H(3)	0·2755	0·3563	0·4628	0·11	0·12	0·13	3·5
H(4)	0·3295	0·2928	0·4009	0·12	0·12	0·13	3·5
H(5)	0·3872	0·3530	−0·1814	0·12	0·13	0·12	3·5
H(6)	0·3825	0·4540	−0·1923	0·12	0·13	0·13	3·5
H(7)	0·2789	0·4204	0·2387	0·13	0·11	0·15	3·5
H(8)	0·1847	0·3329	0·3521	0·13	0·13	0·13	3·5
H(9)	0·2086	0·2597	0·2497	0·10	0·12	0·14	3·5
H(10)	0·1848	0·3243	−0·0381	0·12	0·12	0·13	3·5
H(11)	0·0402	0·2814	0·2141	0·11	0·11	0·13	3·5
H(12)	0·0378	0·2888	0·0111	0·11	0·11	0·12	3·5
H(13)	0·0970	0·2098	0·0919	0·11	0·12	0·13	3·5
H(14)	0·1513	0·4509	0·1153	0·13	0·12	0·13	3·5
H(15)	0·0809	0·4242	0·0060	0·13	0·11	0·15	3·5
H(16)	0·1027	0·4216	0·1817	0·14	0·12	0·15	3·5
H(17)	0·3833	0·4473	0·3695	0·12	0·13	0·13	3·5
H(18)	0·4245	0·3717	0·5524	0·11	0·11	0·13	3·5
H(19)	0·4392	0·3723	0·3519	0·12	0·12	0·13	3·5

	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
*	0·00189	0·00260	0·01351	0·00004	0·00036	−0·00062

Temperature factor $= \exp\{-(h^2B_{11} + k^2B_{22} + l^2B_{33} + 2hkB_{12} + 2hlB_{13} + 2klB_{23})\}$.

Table 2. *The final observed and calculated structure factors*

−0·001, −0·001, 0·002 and −0·001 Å respectively. The hydrogen atom H(1) of the carboxyl group is at a distance of −0·14 Å from this plane.

It is observed in all the peptide structures that the carboxyl group is nearly either parallel or perpendicular to the adjacent peptide plane (Marsh & Glusker, 1961; Freeman, Robinson & Schoone, 1964). In this structure, these two planes are approximately perpendicular to each other with a dihedral angle of 84°.

The dimensions of the carboxyl group are consistent with the standard values for a pure carboxyl group (Hahn, 1957), confirming that this group exists in the non-ionized form. At the N-terminal end, the amino nitrogen N(2) linked to the α-carbon atom C(4) is also bonded to a methyl group and is protonated. Thus, the charged groups in the structure are $>NH_2^+$ and Br^-. The two $C-NH_2^+$ distances are found to be C(4)–N(2) = 1·506 Å and C(9)–N(2) = 1·476 Å. The average of these two lengths is 1·491 Å, in good agreement with the weighted mean value of the $C-NH_3^+$ distance of 1·487 Å found in accurately solved crystal structures of amino acids (Marsh & Donohue, 1967).

The side chain bond lengths and angles in the N-terminal leucyl residue are close to the expected values for such groups.

Conformational features

The conformation of the molecule in the L-configuration about the various bonds is shown in Fig. 4. Following the standard conventions (Edsall, Flory, Kendrew, Liquori, Nemethy, Ramachandran & Scheraga, 1966; Ramachandran & Sasisekharan, 1968), the angles φ, ψ and χ denoting the dihedral angles in the molecule are marked in the Figure.

For the C-terminal end, there is one value for φ defining the dihedral angle C(3)–N(1)–C(2)–C(1) and two values for ψ, namely ψ' and ψ'', corresponding to the two oxygen atoms of the carboxyl group. In this molecule, the value of φ is 265°. This angle is considered to be 0° when C(3) and C(1) are *trans* about the bond N(1)–C(2). The other two angles ψ' and ψ'' are 352° and 173° respectively. Usually, these values are found to be less than about 360° and 180° in the structures of amino acids.

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

At the N-terminal end the parameter ψ measuring the dihedral angle N(2)–C(4)–C(3)–O(3) has the value 296°. The atoms attached to N(2) and C(4) take up a staggered configuration about the N(2)–C(4) bond with the methyl group carbon atom C(9) *trans* to C(5).

The side chain of the leucyl residue is characterized by three dihedral angles denoted by χ^1, χ^{21} and χ^{22}. Usually, for bonds linking atoms of tetrahedral coordination, each of these angles will be about one of the three values 60°, 180° and 300°. In this molecule, the angles χ^1, χ^{21} and χ^{22} are 181°, 63°, and 185° respectively. The corresponding values are found to be 188°, 58° and 182° in leucine hydrobromide (Subramanian, 1967), 294°, 155° and 272° in leucylglycine hydrobromide (Thyagaraja Rao, 1968) and 279°, 170° and 292° in leucylprolylglycine (Leung & Marsh, 1958). It is interesting to note that the leucyl side chain conformations in these four structures fall basically into

Table 3. *Hydrogen bonding distances and angles*

X–H···Y		X···Y	X–H	H···Y	XH∧XY
O(2)–H···Br(I)		3·26 Å	0·75 Å	2·65 Å	32°
N(1)–H···Br(II)		3·45	0·85	2·61	8
N(2)–H···Br		3·27	1·00	2·35	19
N(2)–H···O(3) (III)		2·81	0·98	2·04	32
O(2)–H(1)···Br(I)	138·8°		N(2)–H(3)···Br		151·2°
C(1)–O(2)···Br(I)	116·2		C(4)–N(2)···Br		99·9
N(1)–H(2)···Br(II)	165·5		C(9)–N(2)···Br		104·3
C(2)–N(1)···Br(II)	121·0		N(2)–H(4)···O(3) (III)		134·8
C(3)–N(1)···Br(II)	114·7		C(4)–N(2)···O(3) (III)		135·5
			C(9)–N(2)···O(3) (III)		103·3

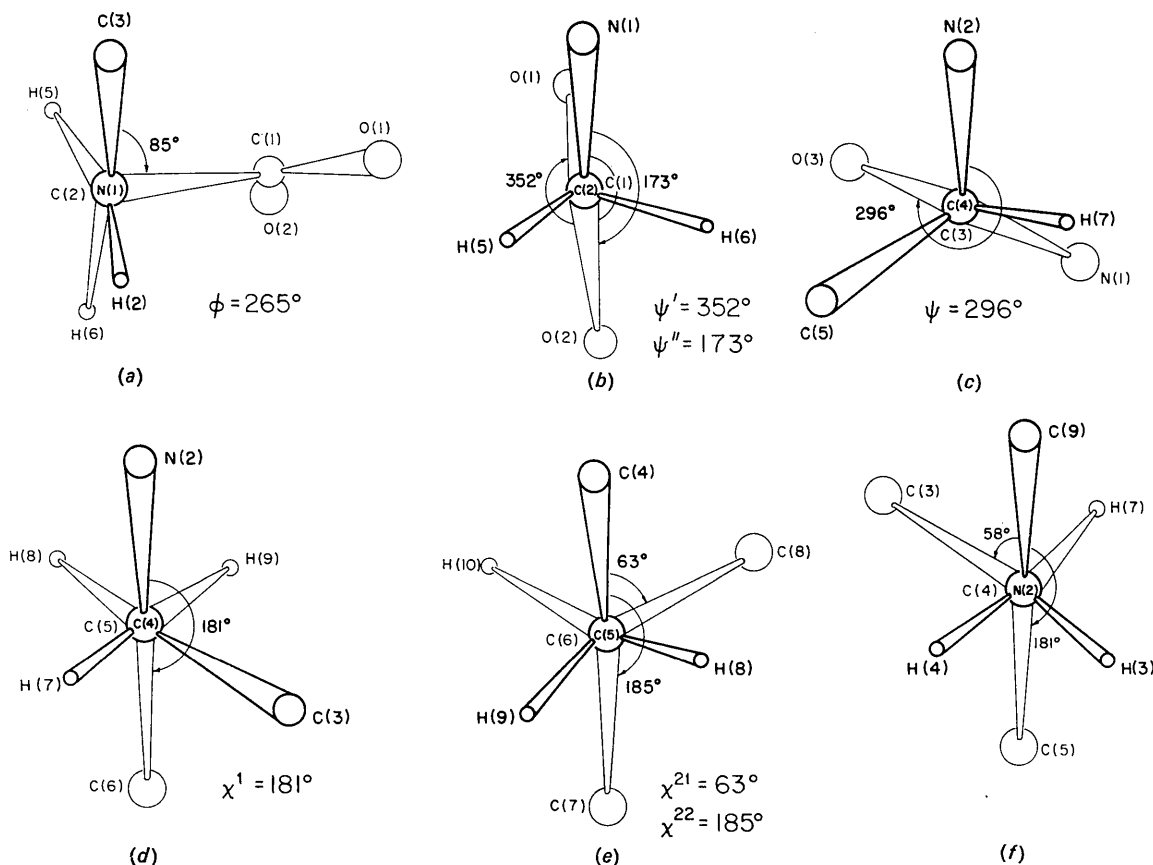| Standard molecule at		x	y	z
Molecule I	at	$\frac{1}{2}+x$	y	$\frac{1}{2}-z$
II	at	$\frac{1}{2}-x$	$1-y$	$-\frac{1}{2}+z$
III	at	x	$\frac{1}{2}-y$	$\frac{1}{2}+z$

Fig. 4. Conformation about the various bonds.

Table 4. *Non-bonding intermolecular contacts between non-hydrogen atoms less than* 4·0Å

Atom i	Atom j	d_{ij}	Atom i	Atom j	d_{ij}
C(9)	O(2) (I)	3·88 Å	O(2)	O(1) (VI)	3·53 Å
Br	N(1) (I)	3·76	C(1)	O(1) (VI)	3·30
Br	C(2) (I)	3·67	C(2)	O(1) (VI)	3·90
C(9)	C(2) (I)	3·92	O(1)	O(1) (VI)	3·34
C(9)	O(3) (II)	3·46	C(1)	C(1) (VI)	3·68
C(3)	N(2) (III)	3·86	C(7)	O(1) (VII)	3·75
C(7)	C(5) (III)	3·98	C(7)	O(2) (VII)	3·60
O(1)	Br(IV)	3·95	C(7)	C(1) (VII)	3·69
C(9)	C(7) (IV)	3·81	C(4)	Br(VIII)	3·84
C(9)	C(8) (IV)	3·87	C(9)	C(8) (IX)	3·89
C(8)	O(1) (V)	3·53			

Standard molecule at	x	y	z
Molecule I	x	y	$1+z$
II	x	$\frac{1}{2}-y$	$\frac{1}{2}+z$
III	x	$\frac{1}{2}-y$	$-\frac{1}{2}+z$
IV	$\frac{1}{2}+x$	y	$\frac{1}{2}-z$
V	$-\frac{1}{2}+x$	y	$\frac{1}{2}-z$
VI	$1-x$	$1-y$	$-z$
VII	$-\frac{1}{2}+x$	$\frac{1}{2}-y$	$-z$
VIII	$\frac{1}{2}-x$	$1-y$	$-\frac{1}{2}+z$
IX	$\frac{1}{2}-x$	$1-y$	$\frac{1}{2}+z$
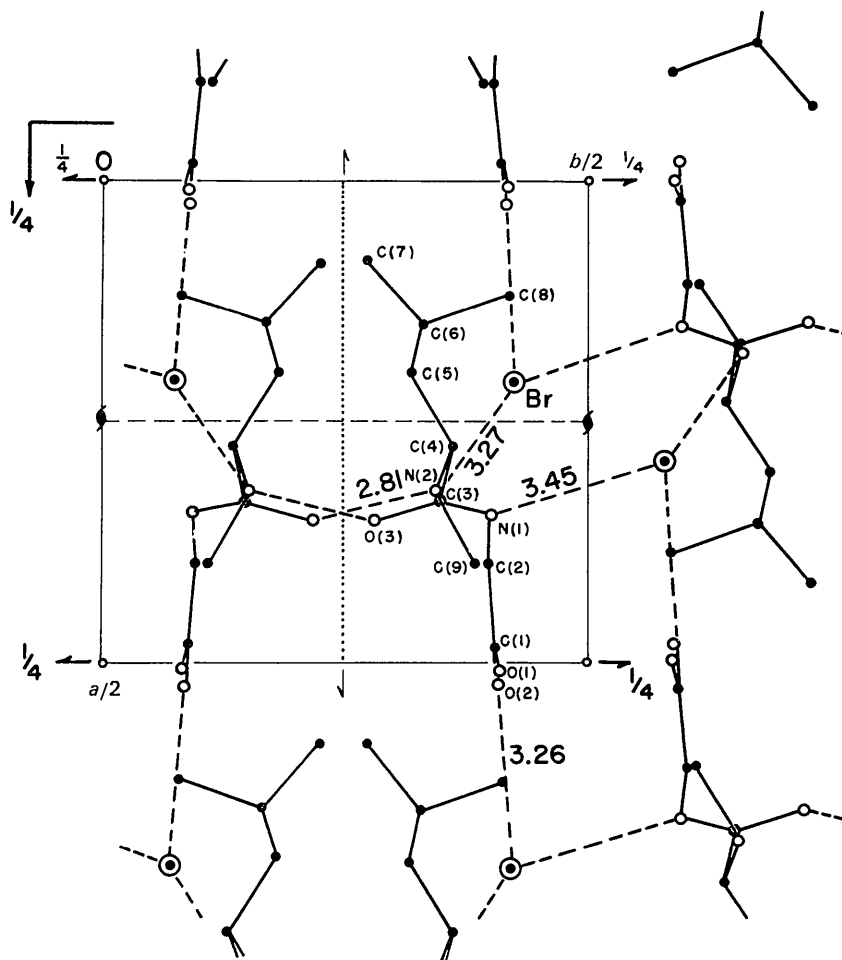
Fig. 5. A view of the structure projected down the c axis.

two distinct groups; the three χ values for the first two structures are around 180°, 60° and 180° and for the other two structures they are nearly 300°, 180° and 300°.

Intermolecular features

The molecules in the crystal are held together by a three-dimensional network of hydrogen bonds. There are four protons in the molecule available for hydrogen bonding. While three of them are hydrogen bonded to neighbouring bromide ions, the other is directed towards an oxygen atom of the peptide unit. The hydrogen bond distances and angles are listed in Table 3. Fig. 5 shows the hydrogen bonding scheme in the structure.

The oxygen atom O(2) of the C-terminal carboxyl group donates its hydrogen atom H(1) to the bromine atom Br(I) to form a hydrogen bond of length 3·26 Å. The non-linearity of this bond is about 32°. The peptide nitrogen atom N(1) with its hydrogen atom H(2) oriented towards Br(II) forms a nearly linear hydrogen bond of length 3·45 Å. The nitrogen atom N(2) of the $>NH_2^+$ group has two close neighbours Br and O(3) (III) at distances of 3·27 and 2·81 Å respectively. The tetrahedral nitrogen atom N(2) has the two hydrogen atoms H(3) and H(4) approximately in the directions of these acceptor atoms.

It is surprising to find that the carbonyl oxygen atom O(1) in this structure is not being used even as an acceptor in the hydrogen bonding scheme. A similar feature has been observed in the crystal structure of L-tryptophan hydrobromide (Ramachandra Ayyar & Chandrasekaran, 1967).

Table 4 gives the non-bonding intermolecular contacts – between the non-hydrogen atoms – less than 4·0 Å. There appear to be no unusually short contacts.

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