

## Structure and Conformation of Amino Acids Containing Sulfur.

### I. Crystal and Molecular Structure of L- $\beta$ , $\beta'$ -Dimethylcysteine (Penicillamine) Hydrochloride Monohydrate\*

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Crystals of L-penicillamine hydrochloride monohydrate,  $\text{HS-C(CH}_3)_2\text{-CH(NH}_3^+\text{)-COOH}\cdot\text{HCl}^- \cdot \text{H}_2\text{O}$ , are monoclinic, space group  $P2_1$  with two molecules in the unit cell. The cell parameters at  $22 \pm 3^\circ\text{C}$  are:  $a = 12.585$  (6),  $b = 6.129$  (2),  $c = 6.910$  (3) Å and  $\beta = 107.32$  (5) $^\circ$  ( $\text{Cu } K\alpha_1 = 1.54051$  Å). Three-dimensional intensity data to the limit  $2\theta = 160^\circ$  were collected using the GE-XRD-5 diffractometer by the stationary-crystal stationary-counter method, with balanced Ross filters. The structure was determined by the heavy-atom method and refined to  $R = 0.07$  by the least-squares method, with the block-diagonal approximation. Significant structural features are: (i) a long bond distance of 1.59 Å for the C(3)–C(4) bond; (ii) the angles  $\text{N-C}^\alpha\text{-C}^\beta = 113.1^\circ$ ,  $\text{N-C}^\alpha\text{-C}' = 105.0^\circ$  at the  $\text{C}^\alpha$  atom are different from tetrahedral values; (iii) the torsion angle  $\chi^1$  of S about the  $\text{C}^\alpha\text{-C}^\beta$  with respect to N is  $289^\circ$ , unlike that in other amino acids containing sulfur, where this angle is close to  $60^\circ$ ; (iv) the direction of the C–S bond is in close correspondence with the maximum value of the  $g$ -tensor as found from e.s.r. spectra; and (v) hydrogen bonds link symmetry-related molecules, mostly along the  $a$  direction and connect nitrogen atoms to chlorines along the  $c$  direction. Water molecules bridge chlorine atoms to nitrogens of penicillamine molecules.

#### Introduction

L-Penicillamine hydrochloride monohydrate is a  $\beta$ , $\beta'$ -dimethyl derivative of cysteine, an amino acid present in many proteins. L-Penicillamine is the most characteristic degradation product of penicillin-type antibiotics (Abraham, Chain, Baker & Robinson, 1943) and is used as a chelating agent in heavy-metal poisoning (Ohlsson, 1962; Goldberg, Smith & Lockhead, 1963) and in the treatment of Wilson's Disease (Hartley & Walshe, 1963).

Electron spin resonance studies on many amino acids containing sulphur have been carried out by several workers (Budzinski & Box, 1971; Kurita & Gordy, 1961; Akasaka, Ohnishi, Suita & Nitta, 1964; Akasaka, 1965) and on L-penicillamine hydrochloride monohydrate in particular by Budzinski & Box (1971). The several e.s.r. absorption spectra observed by Budzinski & Box (1971) on L-penicillamine hydrochloride monohydrate were explained by them as being due to different conformations of the 'sulphur' radical  $\dot{\text{S}}\text{C(CH}_3)_2\text{CH(NH}_3^+\text{Cl}^-)\text{COOH}$ . In view of the above, and also as a part of a program on conformational studies of amino acids containing sulphur, the present investigation was undertaken.

#### Experimental

Single crystals of L-Penicillamine hydrochloride monohydrate, obtained by slow cooling of supersaturated

aqueous solutions, took the form of thin plates. The unit-cell parameters and their e.s.d.'s at room temperature were obtained by least-squares refinement of the  $d$  spacings of 26 large-angle reflections. The cell data at  $22 \pm 3^\circ\text{C}$  are:  $a = 12.585$  (6),  $b = 6.129$  (2),  $c = 6.910$  (3) Å,  $\beta = 107.32$  (5) $^\circ$  ( $\text{Cu } K\alpha_1 = 1.54051$  Å),  $\mu = 49.11$   $\text{cm}^{-1}$ ,  $d_m = 1.34$   $\text{g cm}^{-3}$  and  $d_c = 1.33$   $\text{g cm}^{-3}$ . The crystals are monoclinic, space group  $P2_1$ , with two molecules in the unit cell, consistent with the systematic absences of reflections  $0k0$  with  $k$  odd. In the earlier studies of the cell constants of D-penicillamine hydrochloride monohydrate by Crowfoot, Bunn, Rogers-Low & Turner-Jones (1949), the following values were obtained:  $a = 6.85$ ,  $b = 6.08$ ,  $c = 12.20$  Å,  $\beta = 103.6^\circ$ ,  $d = 1.36$   $\text{g cm}^{-3}$ .

Employing  $\text{Cu } K\alpha$  radiation, complete three-dimensional intensity data to the limit  $2\theta = 160^\circ$  were collected by the stationary-crystal stationary-counter (Furnas & Harker, 1955) method. With a GE-XRD-5 manual diffractometer 1216 non-equivalent reflections were measured, of which 929 had intensities greater than twice the background value in that  $\sin \theta$  range. The crystal used for data collection had the dimensions  $0.11 \times 0.44 \times 0.31$  mm. The crystal was mounted with [010] along the  $\phi$  axis of the goniostat. The difference in absorption as a function of the angle  $\phi$  was measured for 020 and 040 reflections and was used for correcting approximately for the anisotropy of absorption. No other absorption corrections were carried out. The crystal was somewhat unstable and soon showed signs of decay. It turned green in the first week during which the data were collected and, after three weeks of standing, became powdery. However, the variation in the intensities of ten reflections selected as standards ranged from 5 to 10% only during the

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data collection of the last 200 reflections. The intensities were corrected for Lorentz and polarization effects and processed in the usual way. No decay correction was applied since the decay was different for different standards.

### Derivation and refinement of the structure

The positions of the sulphur and chlorine atoms, which were indistinguishable at that early stage of the structure analysis, were obtained from the Harker section ( $u, \frac{1}{2}, w$ ) of the three-dimensional Patterson map. The structure was solved by the heavy-atom method and the atomic and thermal parameters were refined by the least-squares procedure, with the block-diagonal approximation. Refinement with individual isotropic temperature factors, followed by six cycles of refinement with individual anisotropic temperature factors (weighting with  $w = 1/f_c$ ) reduced the  $R$  value from 0.34 to 0.08. The atomic scattering factors and dispersion corrections for sulphur and chlorine atoms were those in *International Tables for X-ray Crystallography* (1962). For the hydrogen atoms, the values given by Stewart, Davidson & Simpson (1965) were used.

Of the fourteen hydrogen atoms in the molecule, ten atoms were located from electron-density difference maps. These atoms were also included in the refinement with isotropic thermal parameters. The refinement was carried out by minimizing  $[\sum w(|F_{\text{obs}}| - 1/k|F_{\text{calc}}|)^2]$ . Reflections for which the intensities were less than twice the value for the background in that range of  $(\sin \theta)$  were given zero weight during refinement and for the  $R$  index calculations. In the final cycles of refinement,  $1/\sigma$  weighting (Evans, 1961) was used. The final  $R$  value is 0.076. The final atomic and thermal parameters, and their standard deviations, were obtained from the inverse of the block-diagonal matrix and are listed in Tables 1 and 2. The observed and calculated structure factors are given in Table 3.

### The geometry of the molecule

The bond distances and angles for L-penicillamine hydrochloride monohydrate are shown in Fig. 1. The

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

	$x$	$y$	$z$	$B$
H(W)	487 (5)	45 (14)	861 (8)	568
H'(W)	579 (5)	-123 (12)	872 (9)	477
H(N)	639 (6)	394 (11)	674 (8)	479
H'(N)	552 (5)	326 (14)	509 (10)	657
H(2)	734 (5)	313 (11)	442 (8)	310
H(4)	916 (5)	100 (10)	950 (8)	300
H'(4)	798 (5)	-8 (10)	804 (7)	219
H(5)	977 (5)	147 (10)	658 (7)	259
H'(5)	921 (6)	226 (14)	464 (10)	503
H''(5)	896 (6)	-21 (12)	562 (8)	509

average standard deviation for bond distances is 0.01 Å and for bond angles 0.6°. The hydrogen atoms located in the electron-density difference maps are also shown in Fig. 1.

The S-C distance of 1.83 Å agrees fairly well with the value of 1.81 Å (Pauling, 1960) and with 1.817 Å (Sutton, 1965). The C(3)-C(4) distance of 1.59 Å appears to be unusually long compared with most  $C(sp^3)$ - $C(sp^3)$  bond distances and any thermal correction is

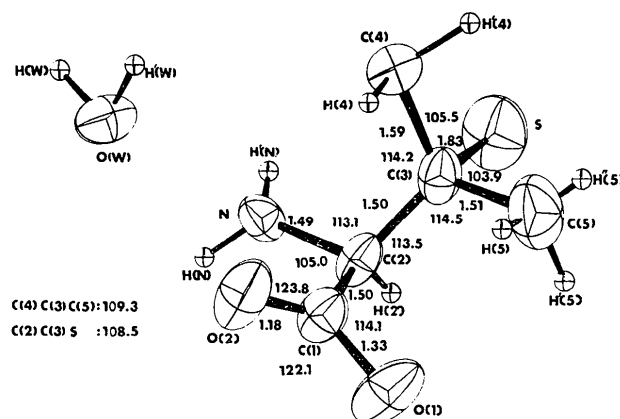


Fig. 1. Bond distances (Å) and bond angles (°). The average e.s.d. for distances is 0.01 Å and for angles 0.6°. One hydrogen on the  $\text{NH}_3^+$  group and the hydrogen on O(1) were not located and are not shown in the figure, although their presence is indicated by short contacts appropriate to hydrogen bonds. The hydrogen atoms on C(4) and S have also not been located and are not shown in the figure.

Table 1. *Coordinates and thermal parameters*

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

Values  $\times 10^4$ ; standard deviations given in parentheses refer to the last digit.

	$x$	$y$	$z$	$b_{11}$	$b_{22}$	$b_{33}$	$b_{12}$	$b_{13}$	$b_{23}$
S	8735 (2)	5000 (5)	7455 (4)	109 (3)	287 (8)	430 (8)	-108 (9)	158 (8)	-224 (14)
Cl	3439 (2)	735 (4)	-73 (3)	128 (2)	217 (6)	222 (4)	8 (8)	189 (5)	93 (11)
O(1)	7292 (5)	-289 (9)	2581 (7)	162 (8)	268 (22)	305 (17)	-163 (22)	296 (19)	-280 (33)
O(2)	6164 (5)	-989 (10)	4391 (8)	119 (7)	398 (25)	369 (18)	-237 (23)	275 (18)	-435 (37)
O(W)	5242 (4)	-250 (10)	7711 (7)	86 (5)	270 (18)	180 (12)	71 (18)	96 (13)	34 (27)
C(1)	6818 (7)	183 (13)	4007 (11)	74 (7)	197 (26)	228 (19)	-55 (24)	123 (19)	-63 (37)
C(2)	7180 (6)	2323 (13)	5049 (10)	64 (7)	175 (23)	213 (19)	-14 (23)	140 (19)	-39 (37)
C(3)	8284 (6)	2216 (14)	6658 (11)	52 (7)	210 (25)	254 (22)	-26 (23)	114 (19)	-123 (41)
C(4)	8255 (6)	985 (12)	8660 (10)	72 (7)	102 (20)	187 (17)	38 (22)	83 (17)	38 (35)
C(5)	9218 (8)	1305 (19)	5947 (13)	68 (9)	524 (52)	424 (32)	-7 (37)	132 (28)	-266 (66)
N	6255 (5)	2991 (11)	5846 (8)	42 (6)	232 (22)	174 (15)	29 (20)	21 (14)	-80 (33)



served values of  $\psi$  for this structure are:  $\psi_7^1 = -23.6^\circ$  ( $156.4^\circ$ ) and  $\psi_7^2 = 156.4^\circ$  ( $336.4^\circ$ ). The values in parentheses are those corresponding to the earlier convention of Edsall *et al.* (1966) and are given mainly to compare our values with those reported by other workers in this field. For the amino group, there are three values for  $\phi$ , namely  $\phi_1^1$ ,  $\phi_1^2$  and  $\phi_1^3$  corresponding to the three hydrogens on the nitrogen atom. In this structure, corresponding to the two hydrogens that have been located on the nitrogen atom, the observed values of  $\phi$  are:  $\phi_1^1 = -61^\circ$  and  $\phi_1^2 = 162^\circ$ . These values are in the allowed ranges of  $\psi$  and  $\phi$  for amino acids (Lakshminarayanan, Sasisekharan & Ramachandran, 1967).

The angle  $\chi^1$  is the torsion angle of  $C^\gamma$  about  $C^\alpha-C^\beta$  with respect to N (Fig. 3) and has a value of  $289^\circ$  in our structure. The  $\gamma$ -atom has been found to be restricted to three possible positions corresponding to a small range of angles around  $\chi^1 = 60, 180$  or  $300^\circ$ . The value of  $\chi^1$  near  $300^\circ$  seems to be preferred, if the  $\gamma$ -atom is not S or O (Lakshminarayanan, Sasisekharan & Ramachandran, 1967). This is the position in which the  $\gamma$ -atom is antiperiplanar to the carboxyl group and -synclinal to N; it lies between two groups of relatively small size, namely N and H. If the  $\gamma$ -atom is sulphur, in eight out of the nine structures (Lakshminarayanan, Sasisekharan & Ramachandran, 1967), the atom  $S^\gamma$  occupies only the position with  $\chi^1 = 60^\circ$ , *i.e.* synclinal to N and -synclinal to the carboxyl group. However, in our case, the S atom is found to occupy position III with  $\chi^1 = 71$  or  $289^\circ$  as in the case of *S*-methylcysteine sulfoxide with  $\chi^1 = 294.9^\circ$  (Lakshminarayanan, Sasisekharan & Ramachandran, 1967).

### Hydrogen bonding and packing

The nitrogen atom has five contacts which are of the right order of distance for hydrogen-bond formation: N-O(2), 2.63; N-O(W), 2.87; N-Cl<sup>i</sup>, 3.29; N-O(2<sup>i</sup>), 3.07; and N-O(W<sup>i</sup>), 2.83 Å (see Tables 5 and 6). The first contact is intramolecular and such contacts are observed in many amino acids (see for example, Koetzle, Hamilton & Parthasarathy, 1972). It is seen from Fig. 4 that the H(N) mediates two short contacts between N and two other acceptor atoms. Such contacts have been frequently observed and recently discussed by Parthasarathy (1969) and Koetzle, Hamilton & Parthasarathy (1972). H(N) hydrogen-bonds to Cl<sup>i</sup> and the third hydrogen on the nitrogen atom is probably oriented between O(W) and O(2). Since the angle C(2)-N-O(W) is  $118.6^\circ$ , it may be inferred that the third hydrogen on N hydrogen-bonds to O(W).

The hydroxyl oxygen atom O(1) of the carboxyl group has a contact of 2.97 Å with the Cl<sup>iii</sup> atom (see Table 5) and the angle C(1)-O(1)-Cl,  $119^\circ$ , is favorable for a hydrogen bond.

The water molecule hydrogen-bonds to Cl<sup>ii</sup> and Cl<sup>vi</sup> and accepts hydrogen atoms from two nitrogens N and N<sup>vi</sup>. The environment of the water molecule is

shown in Fig. 5. The nearest contacts around water form a distorted tetrahedron.

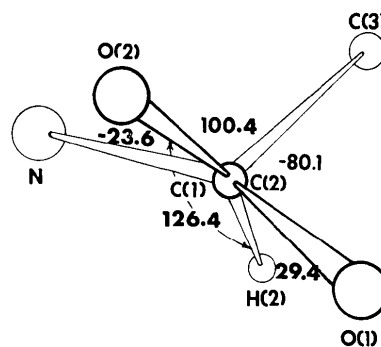


Fig. 2. Projection of the molecule down C(1)-C(2) and the corresponding torsion angles ( $^\circ$ ).

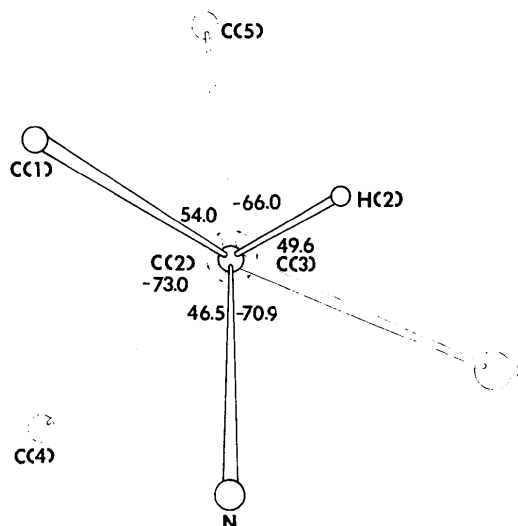


Fig. 3. Projection of the molecule down C(2)-C(3) and the corresponding torsion angles ( $^\circ$ ).

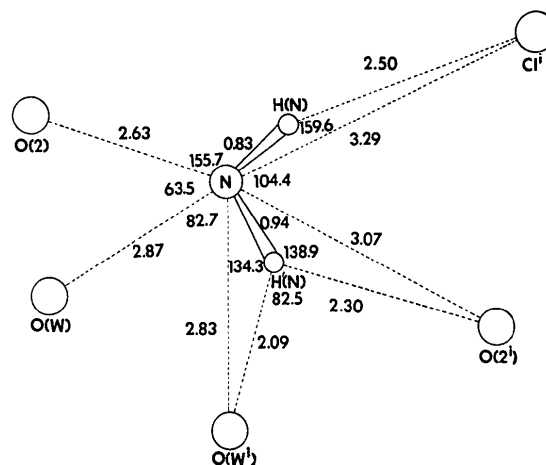


Fig. 4. Schematic diagram of the environment of the nitrogen atom. For superscripts, see Table 5.

There are four short contacts around the  $\text{Cl}^-$  ion:  $\text{Cl} \cdots \text{O}(W^{iv})$ , 3.15 Å;  $\text{Cl} \cdots \text{O}(1^v)$ , 2.97 Å;  $\text{Cl} \cdots \text{O}(W^i)$ ; 3.14 Å and  $\text{Cl} \cdots \text{N}^{vi}$ , 3.29 Å. The environment of the chlorine atom is shown in Fig. 6.

The hydrogen-bond distances and angles are given in Table 6. The packing of the molecules is shown in

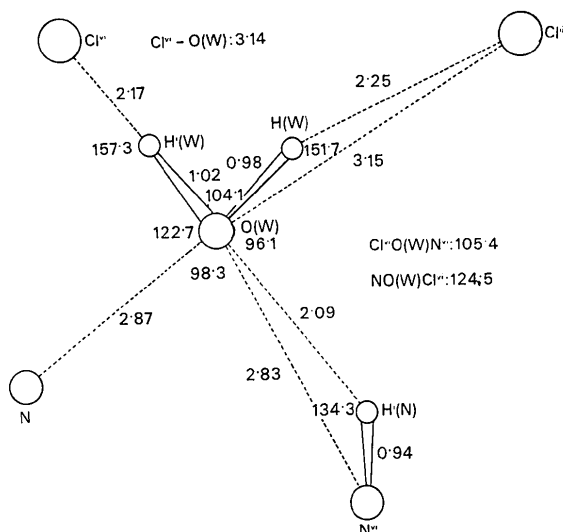


Fig. 5. Environment of the water molecule. For superscripts, see Table 5.

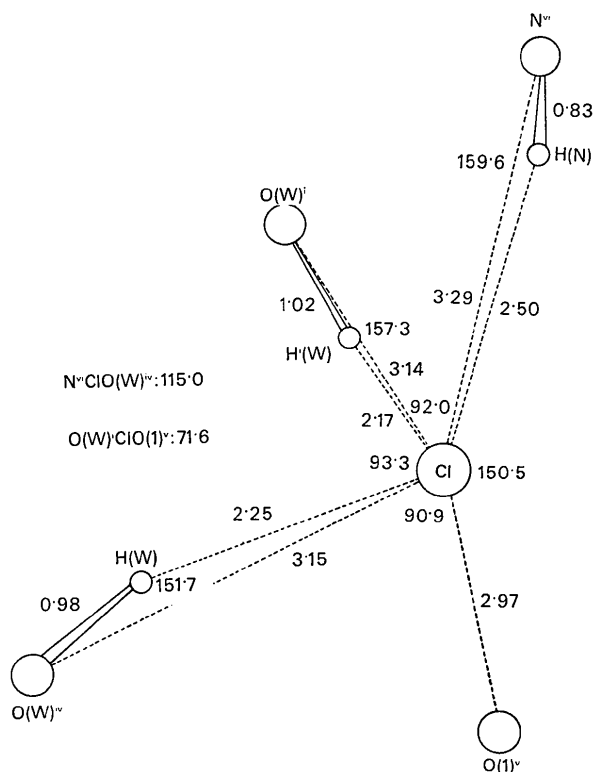


Fig. 6. Environment of the chlorine atom. For superscripts, see Table 5.

Table 5. Symmetry code

Super-script	Translation	Equiv- alent position
None	0 0 0	1
i	1 0 1	2
ii	0 0 1	1
iii	1 $\bar{1}$ 0	2
iv	0 0 $\bar{1}$	1
v	1 0 0	2
vi	1 $\bar{1}$ 1	2
1	$x$ $y$ $z$	
2	$\bar{x}$ $\frac{1}{2}+y$ $\bar{z}$	

Table 6. Hydrogen-bond distances (Å) and angles ( $^\circ$ )

E.s.d.'s are given in parentheses.

$D-H \cdots A^*$	$D \cdots A$	$H \cdots A$	$D-H \cdots A$
$\text{N}-\text{H}(\text{N}) \cdots \text{Cl}^i$	3.293 (6)	2.50 (6)	160 (6)
$\text{N}-\text{H}'(\text{N}) \cdots \text{O}(2^i)$	3.065 (9)	2.30 (6)	139 (5)
$\text{N}-\text{H}'(\text{N}) \cdots \text{O}(W^i)$	2.828 (7)	2.09 (7)	134 (6)
$\text{O}(W)-\text{H}(W) \cdots \text{Cl}^{iii}$	3.151 (6)	2.25 (7)	152 (6)
$\text{O}(W)-\text{H}'(W) \cdots \text{Cl}^{vi}$	3.142 (6)	2.17 (7)	157 (5)

\*  $D$  is the donor and  $A$  is the acceptor atom. For the superscripts, see Table 5.

Fig. 7. It is seen that the hydrophobic groups are separated from the polar groups. The symmetry-related molecules are linked by a series of hydrogen bonds, mostly along the  $a$  direction. The nitrogen-to-chlorine contacts are along the  $c$  direction. The water molecules, in addition, bridge the chlorine atoms to the penicillamine molecules.

One hydrogen atom on the  $\text{NH}_3^+$  group and the hydrogen on  $\text{O}(1)$  were not located, although their presence is indicated by short contacts appropriate to hydrogen bonds. The hydrogen atom on the sulfhydryl group could not be clearly located from electron-density difference maps. The contact  $\text{S} \cdots \text{Cl}^i$  of 3.73 Å and the angle  $\text{C}(3)-\text{S} \cdots \text{Cl}^i$  of  $93^\circ$  indicate that this contact may be interpreted as a possible hydrogen bond.

#### Relation of the structure to the e.s.r.

The e.s.r. absorption spectra  $\beta_1$ ,  $\beta_1'$  and  $\beta_1''$  observed by Budzinski & Box (1971) on L-penicillamine hydrochloride monohydrate have been explained as being due to different conformations of the 'sulphur' radical. Electron spin resonance studies on amino acids containing sulphur (Kurita & Gordy, 1961; Akasaka *et al.*, 1964) suggest that the maximum value of the spectroscopic coupling tensor  $g$  for the S radical should correspond to the direction of the C-S bond. The direction cosines of the principal axes associated with the absorptions  $\beta_1$  and  $\beta_1'$ , 0.25 0.95 0.20 and 0.33 0.91 0.23 corresponding to the maximum value of  $g$  for these absorptions, 2.297 and 2.217 respectively, are nearly the same as the direction cosines 0.22 0.93 0.27 of the C(3)-S bond in L-penicillamine hydrochloride mono-

hydrate (see Budzinski & Box, 1971). The tensor of the  $\beta_1'$  absorption spectra corresponding to room temperature is completely reoriented and was interpreted by Budzinski & Box (1971) as being due to conformational changes of the S radical. Further study in correlating the structure with the e.s.r. data is in progress.

We are indebted to Dr Harker for his interest in this work and for many discussions, and are grateful to Dr Box for bringing this problem to our attention. The following programs were used in the course of this study: *BDL-6*, a modified version of the Gantzel, Sparks & Trueblood block-diagonal least-squares program (ACA No. 317), *ORTEP* by Dr C. R. Johnson, a general Fourier program and a program for torsion-angle calculations by Dr S. T. Rao. We thank Mr Manuel Soriano for help with the drawings.

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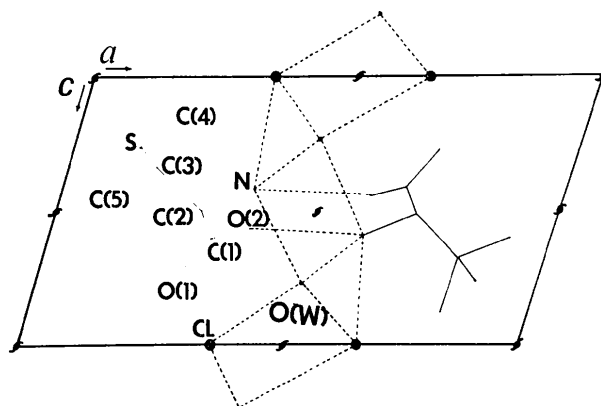


Fig. 7. Packing of the molecules and hydrogen-bonding scheme.