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The Crystal Structure of Glutathione

By W. B. Wright

The Laboratories, J. Lyons and Company Limited, London W. 14, *England*

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The tripeptide glutathione (γ-L-glutamyl-L-cysteinyl-glycine, $C_{10}H_{17}N_3O_6S$) crystallizes in the orthorhombic system with space group $P2_12_12_1$ and unit-cell dimensions $a = 28{\cdot}05 \pm 0{\cdot}02$, $b = 8{\cdot}802 \pm 0{\cdot}002$, $c = 5{\cdot}630 \pm 0{\cdot}002$ Å. The positions of the sulphur atoms in the unit cell were determined from the Harker sections of the three-dimensional Patterson function.

The structure was finally determined from a c-axis Fourier projection pattern based on terms with signs derived by the method of Cochran & Douglas, used in conjunction with a three-dimensional electron-density distribution based on terms with phase angles given by the sulphur atoms only. The structure was refined by two- and three-dimensional Fourier syntheses.

The molecule contains no unusual bond lengths or angles and adopts a curled configuration with the planes of the glycine carboxyl group and the amino–carboxyl group both parallel to the c-axis, and the planar peptide linkages inclined at 94·4° to one another. The sulphur atoms form zigzag chains about alternate screw diad axes parallel to c, the S–S distance being 4·41 Å. The structure is held together by a three-dimensional network of hydrogen bonds, but there are no internal hydrogen bonds in the molecule.

Introduction

The investigation of the crystal structure of the naturally occurring tripeptide glutathione γ-L-glutamyl-L-cysteinyl-glycine,

$$\mathrm{HOOCCHNH_2CH_2CH_2CONH\overset{\overset{\displaystyle CH_2SH}{|}}{C}HCONHCH_2COOH}\ ,$$

was undertaken with a view to providing additional information about the molecular configuration of the amino acids present in proteins. But it was also considered to be of especial interest in that the two peptide linkages present in the molecule might bear the same spatial relationship to one another as do neighbouring peptide linkages in protein chains, for which certain structures such as the α-helix have been postulated (Pauling & Corey, 1951).

Various structures have been postulated for the glutathione molecule in solution (Calvin, 1954), such as those involving internal hydrogen-bond formation to the sulphur atom, which would account for the increased reactivity of the mercaptan group in the presence of urea and guanidinium salts, a phenomenon also observed with proteins. The possibility of this type of hydrogen-bond formation and of the presence of other internal hydrogen bonds in the molecule was considered during the trial structure work.

Most of the well known methods of crystal-structure analysis were tried during the preliminary attempts to determine the structure. The trials were made on the c-axis projection, which was considered to contain the least overlap and has the additional advantage of a centre of symmetry. It was found that most of the methods tried gave essentially the same c-axis projection Fourier pattern, on which it was possible to place the molecule in a variety of different ways; but only one of the patterns, derived by the method of Cochran & Douglas, suggested a configuration of the molecule in which the maximum number of hydrogen bonds could be formed without strain. This pattern, used in conjunction with a three-dimensional electron-density distribution calculated using phase angles based on the coordinates of the sulphur atoms only, served to locate the positions of all the atoms in the unit cell, and the atomic parameters were then refined by two- and three-dimensional Fourier methods.

It is proposed in this account to outline the methods which actually led from the experimental data to the determination of the crystal structure, and in a subsequent account (Wright, 1958) to give an outline of all the other methods which were tried, together with some comparison and discussion of the results obtained in each case.

Experimental

The crystals of glutathione used for the X-ray investigation were grown, in a reducing atmosphere, from solutions in water saturated with hydrogen sulphide, in order to keep the substance in the reduced form. They grew as thin flat plates, normal to the a axis and slightly elongated parallel to the c axis, the predominant form being {100}. The crystals showed straight extinction, and refractive indices parallel to the b and c axes of $1{\cdot}589 \pm 0{\cdot}002$ and $1{\cdot}591 \pm 0{\cdot}002$ respectively. Oscillation and Weissenberg photographs established the crystals as belonging to the orthorhombic system, with space group $P2_12_12_1$. The unit-cell dimensions, determined from high-order equatorial reflexions on oscillation photographs, were found to be

$$a = 28{\cdot}05 \pm 0{\cdot}02,\ b = 8{\cdot}802 \pm 0{\cdot}002,\ c = 5{\cdot}630 \pm 0{\cdot}002\ \text{Å},$$

substantially in agreement with the values found by Bernal (1932). There are four molecules in the unit cell, which gives a calculated value for the density of 1·467 g.cm.$^{-3}$. The density of the crystals, determined by flotation in mixtures of benzene and carbon tetrachloride, is 1·47 g.cm.$^{-3}$.

Three-dimensional intensity data were obtained from equi-inclination Weissenberg photographs, using the multiple-film technique and taken with the crystals oscillating about the b axis (k = 0–6), and about the c axis (l = 0–4). Of the 1830 reflexions which could have been recorded using Cu $K\alpha$ radiation (λ = 1·5418 Å) only 1186 were actually observed. Although, therefore, only 65% of the (hkl) reflexions, 77% of the ($hk0$), and 66% of the ($h0l$), were actually observed, these values represent 90%, 97% and 94% respectively of the total structure amplitude in each case, estimated on the assumption that $|F_o|$ for the unobserved reflexions is equal to half the minimum observable value in each zone. The intensities of the reflexions were estimated visually by comparison with a set of spots of standard intensity, and a correction for variation in spot shape was made by the method given by Broomhead (1948) to allow for the non-uniform cross section of the crystals. As the crystals were very thin, no correction was made for absorption. An empirical correction to allow for the variation in background on the films was applied, and Lorentz and polarization corrections were made by the method of Goldschmidt & Pitt (1948). For the 393 reflexions which were measured about both axes the probable error was calculated and was found to be of the order of 13·3% for $|F_o(hk0)|^2$ and 9·7% for $|F_o(hkl)|^2$, where l = 1, 2 or 3.

The values of $|F_o(hkl)|^2$ were placed on an absolute scale, using Wilson's (1949) method, and the value of B in the temperature factor $\exp\,[-B\,(\sin^2\theta)/\lambda^2]$ was found to be 3·396 Å^2. Atomic scattering-factor curves for carbon, nitrogen and oxygen (McWeeny, 1951), and for sulphur (James & Brindley, 1931), were modified by this temperature factor and used for the two-dimensional work. For the three-dimensional work the values of atomic scattering factors for the different kinds of atoms in the structure, uncorrected for temperature effects, versus values of $2\sin\theta$ at intervals of 0·0185 in the latter, were held in the computer during all structure-factor calculations. For each reflexion the appropriate values of the atomic scattering factors were selected by the machine and multiplied by a calculated temperature factor for which the value of B had been derived, by a least-squares method, in a previous round of the structure factor calculations.

Determination of the positions of the sulphur atoms in the unit cell

The three-dimensional intensity data were used to calculate the three Harker sections and the three zero sections of the three-dimensional Patterson function. From the Harker sections it was possible to recognize the S–S vector peaks at ($2x-\frac{1}{2}$, $2y$, peak height 350) in $P(x,y,\frac{1}{2})$, at ($2x, 2z-\frac{1}{2}$, peak height 250) in $P(x,\frac{1}{2},z)$, and at ($2y-\frac{1}{2}$, $2z$, peak height 503) in $P(\frac{1}{2},y,z)$, where x, y and z are the fractional coordinates of a sulphur atom in the unit cell and the peak heights are in arbitrary units. The sulphur coordinates were found to be (0·291, 0·641, 0·335) so that the sulphur atoms in the structure form zigzag chains along alternate screw diad axes parallel to c, the distance between neighbouring sulphur atoms in the chains being 4·40 Å.

The Cochran & Douglas method (1955) applied to the c-axis projection

By this method the sets of terms for which

$$\chi = \Sigma Y(h,h')P(h,h')$$

is large and positive are determined, as it can be shown that the extent to which χ is positive is a measure of the plausibility of a particular set of signs. $Y(h,h')$ is defined as $S(h)S(h')S(h+h')$, and $P(h,h')$ is defined as $|U(h)U(h')U(h+h')|$, where $S(h)$ is the sign of the unitary structure factor $U(h)$. It has also been noted by Cochran & Douglas (1953) that if the signs of about 20 of the largest structure factors are known correctly, it is often possible from the resulting Fourier map to recognize the correct configuration of the molecule.

The $|U|$ values were calculated for 28 of the strongest ($hk0$) reflexions for glutathione, the signs of two of them were chosen in order to fix the origin of the projection, and the signs of the remaining 26 were represented by letters. Of the largest product terms 47 were calculated and set in order of magnitude, and the χ values corresponding to the various sign combinations were calculated on the electronic computer EDSAC, and set in order of magnitude. Of the nine sets of sign combinations which gave the largest χ values, only four differed from one another by more than one or two signs. These four sets of signs were used to derive the signs of the next largest structure factors, using the sign relationship

$$S(h)S(h') \approx S(h+h'),$$

and from the results four c-axis Fourier projections were calculated. All four patterns have at least one heavy peak which could correspond to a sulphur atom, but only one pattern (Fig. 1) has a heavy peak in the position already chosen for a sulphur atom from the Harker sections. It was possible to place the molecule on the system of peaks as shown in Fig. 1, in a curled configuration in which the maximum number of hydrogen bonds could be formed without straining the molecule.

Calculation of $F(hk0)$ for this molecular arrangement gave a reliability (R) index of 47·5% for 101 terms. By rearrangement of the amino-carboxyl group of the

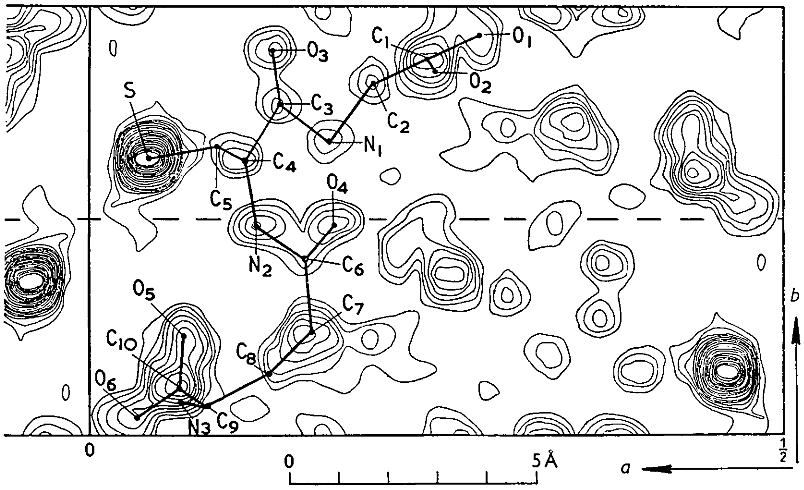

Fig. 1. The c-axis Fourier projection based on 90 terms whose signs were derived by the method of Cochran & Douglas. Contours are at arbitrary intervals.

glutamic acid end of the molecule, so that an external instead of an internal hydrogen bond is formed from N_2 to O_5, R decreased to 38% over 127 terms. Although two atoms, C_4 and C_5, had been placed on one Fourier peak (Fig. 1), the peak height corresponded to only one and a half carbon atoms, and it was therefore concluded that C_5 must lie on the sulphur peak in this projection.

The use of the heavy-atom technique

The z coordinates of the atoms were determined from a three-dimensional electron-density distribution, calculated using the sulphur atom in the asymmetric unit as a heavy atom. The phase angles and structure amplitudes of the 1186 (hkl) reflexions for which values of $|F_o(hkl)|$ were actually observed were calculated from the sulphur coordinates, and the 598 reflexions to which the sulphur was making more than 50% of its maximum contribution were used to calculate a three-dimensional electron-density distribution. The electron density was evaluated over one quarter of the unit cell, at intervals of $a/64$, $b/32$ and $c/16$, on the electronic computer LEO.

This distribution showed the sulphur peak, maximum density 20 e.Å^{-3}, at (0·294, 0·642, 0·337), and immediately beneath it in the c direction at (0·292, 0·644, 0·031) a peak, maximum density 3 e.Å^{-3}, corresponding to carbon atom C_5. None of the other regions of positive electron density was greater than 2 e.Å^{-3}. The x and y coordinates of the atoms determined from the c-axis projection, used in conjunction with this three-dimensional distribution, and with generally accepted values of bond lengths and bond angles, enabled the positions of all the atoms in the asymmetric unit to be located in the unit cell. Neither of the two alternative arrangements of the amino-carboxyl group, allowing for the formation of either an external or an internal hydrogen bond from N_2 to O_5, could be eliminated, as there were small peaks in the three-dimensional distribution which would allow for either possibility.

The b-axis Fourier projection containing 65 terms, whose signs were determined from the sulphur coordinates, was calculated (Fig. 2). The positions of the atoms, which have x coordinates obtained from the c-axis projection and z coordinates obtained from the three-dimensional distribution, are shown, with the two alternative positions for atoms N_3, C_{10}, O_5 and O_6,

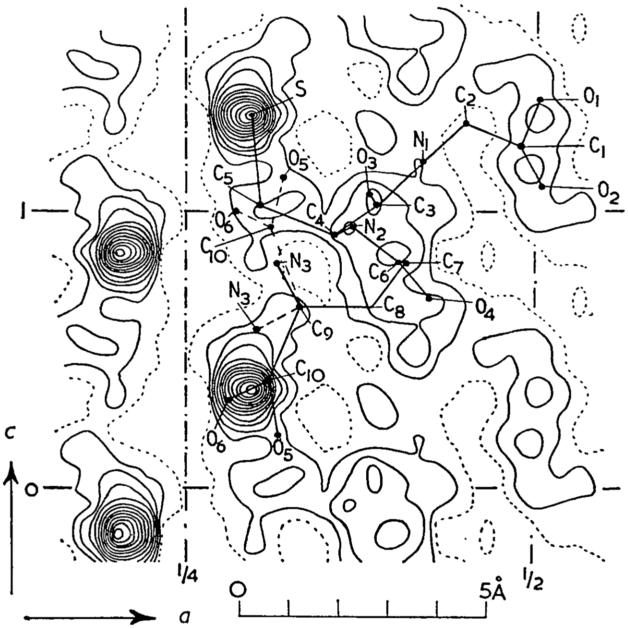

Fig. 2. The b-axis Fourier projection based on 65 terms whose signs were determined from the sulphur atoms only. Contours are at arbitrary intervals.

the arrangement for the formation of an internal hydrogen bond to O_5 being indicated by the broken-line bonding between the atoms, and the arrangement for this end of the molecule allowing for external hydrogen bonding between N_2 and O_5 being shown by the full-line bonding between the atoms. The z para-

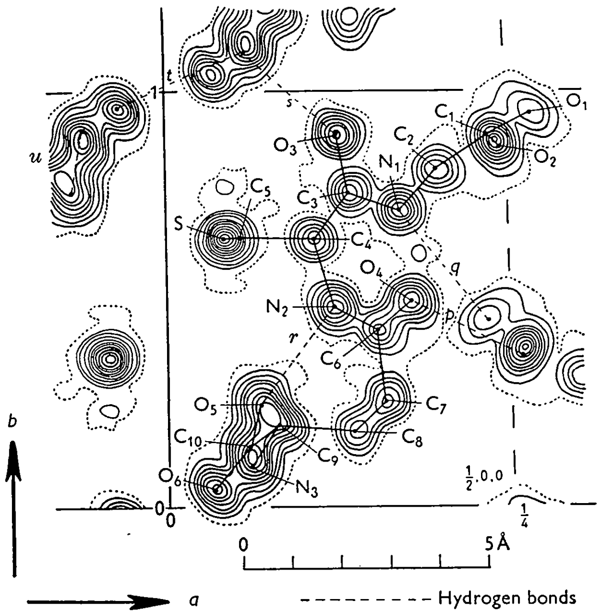

Fig. 3. The c-axis Fourier projection for which $R = 25{\cdot}5\%$ for 246 terms. Contours at intervals of 1 e.Å^{-3}, beginning at 1 e.Å^{-3} (broken line), except in the case of the sulphur atom, which has contours at 2 e.Å^{-3} intervals.

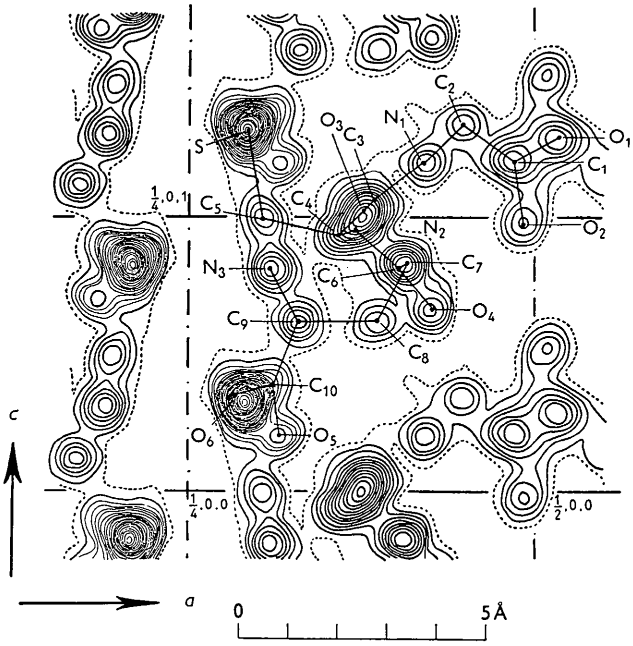

Fig. 4. The b-axis Fourier projection for which $R = 27{\cdot}5\%$ for 132 terms. Contours are at intervals of 1 e.Å^{-3}, beginning at 3 e.Å^{-3} (broken line).

Table 1. *Atomic parameters*

The x parameters

	(i)	(ii)	(iii)	(iv)	(v)	(vi)
C_1	0·487	0·4868	0·4867	0·4868	—	0·4897
C_2	0·450	0·4486	0·4476	0·4478	0·4479	0·4474
C_3	0·384	0·3849	0·3855	0·3862	0·3849	0·3852
C_4	0·358	0·3570	0·3563	0·3563	0·3570	0·3566
C_5	0·304	0·3036	0·3033	0·3031	0·3036	0·3036
C_6	0·402	0·4032	0·4040	0·4039	0·4032	0·4039
C_7	0·410	0·4090	0·4083	0·4084	0·4085	0·4087
C_8	0·385	0·3844	0·3840	0·3841	0·3848	0·3848
C_9	0·331	0·3296	0·3286	0·3293	0·3310	0·3309
C_{10}	0·310	0·3090	0·3083	0·3084	0·3084	0·3088
N_1	0·421	0·4196	0·4184	0·4189	0·4195	0·4196
N_2	0·370	0·3711	0·3719	0·3715	0·3715	0·3724
N_3	0·310	0·3102	0·3103	0·3104	0·3108	0·3109
O_1	0·516	0·5157	0·5155	0·5159	—	0·5186
O_2	0·496	0·4942	0·4929	0·4934	—	0·4920
O_3	0·377	0·3773	0·3775	0·3775	0·3766	0·3761
O_4	0·427	0·4269	0·4268	0·4264	0·4266	0·4270
O_5	0·317	0·3164	0·3160	0·3161	0·3161	0·3161
O_6	0·284	0·2829	0·2821	0·2829	0·2829	0·2829
S	0·293	0·2915	0·2905	0·2908	0·2915	0·2910

The y parameters

	(i)	(ii)	(iii)	(iv)	(v)	(vi)
C_1	0·890	0·8937	0·8963	0·8943	—	0·8833
C_2	0·810	0·8156	0·8195	0·8126	0·8107	0·8126
C_3	0·759	0·7554	0·7529	0·7567	0·7567	0·7577
C_4	0·643	0·6410	0·6396	0·6397	0·6407	0·6399
C_5	0·648	0·6511	0·6533	0·6531	0·6511	0·6526
C_6	0·425	0·4218	0·4196	0·4207	0·4218	0·4207
C_7	0·259	0·2562	0·2542	0·2545	0·2534	0·2520
C_8	0·171	0·1747	0·1773	0·1762	0·1775	0·1775
C_9	0·198	0·1910	0·1861	0·1882	0·1921	0·1909
C_{10}	0·151	0·1528	0·1541	0·1534	0·1534	0·1541
N_1	0·710	0·7105	0·7109	0·7115	0·7119	0·7117
N_2	0·480	0·4822	0·4837	0·4837	0·4822	0·4837
N_3	0·097	0·0936	0·0912	0·0924	0·0922	0·0912
O_1	0·960	0·9586	0·9576	0·9578	—	0·9506
O_2	0·860	0·8626	0·8644	0·8653	—	0·8699
O_3	0·895	0·8945	0·8941	0·8941	0·8951	0·8938
O_4	0·491	0·4928	0·4941	0·4932	0·4937	0·4937
O_5	0·247	0·2484	0·2494	0·2487	0·2487	0·2487
O_6	0·037	0·0380	0·0387	0·0404	0·0404	0·0404
S	0·644	0·6419	0·6404	0·6411	0·6417	0·6415

The z parameters

	(i)	(ii)	(iii)	(iv)	(v)	(vi)
C_1	0·214	0·2211	0·2261	0·2276	—	0·2066
C_2	0·351	0·3406	0·3333	0·3380	0·3521	0·3531
C_3	0·054	0·0589	0·0623	0·0599	0·0589	0·0574
C_4	0·938	0·9284	0·9217	0·9248	0·9240	0·9240
C_5	0·985	0·9989	0·0016	0·0055	0·9989	0·9977
C_6	0·817	0·8157	0·8148	0·8166	0·8157	0·8166
C_7	0·826	0·8219	0·8190	0·8208	0·8219	0·8231
C_8	0·629	0·6239	0·6203	0·6215	0·6160	0·6100
C_9	0·621	0·6185	0·6167	0·6169	0·6167	0·6178
C_{10}	0·379	0·3819	0·3839	0·3832	0·3832	0·3832
N_1	0·188	0·1929	0·1963	0·1951	0·1965	0·2000
N_2	0·978	0·9635	0·9533	0·9571	0·9590	0·9574
N_3	0·805	0·8042	0·8036	0·8045	0·8070	0·8086

Table 1 (*cont.*)

O_1	0·339	0·3390	0·3390	0·3378	—	0·3190
O_2	0	0·9906	0·9840	0·9843	—	0·9777
O_3	0·031	0·0266	0·0235	0·0245	0·0266	0·0245
O_4	0·679	0·6731	0·6690	0·6684	0·6731	0·6712
O_5	0·201	0·2075	0·2121	0·2111	0·2111	0·2140
O_6	0·348	0·3507	0·3526	0·3536	0·3536	0·3560
S	0·321	0·3117	0·3052	0·3072	0·3070	0·3072

meters of the atoms were adjusted slightly as indicated by this Fourier map, and the $(h0l)$ structure factors were calculated for the two alternative arrangements. For the internal hydrogen-bond arrangement R was 52% over 65 terms, and for the external hydrogen bond arrangement R was 35%. It was therefore concluded that the latter arrangement was the correct one, confirming the findings on the c-axis projection.

The atomic parameters were partially refined by three c-axis and three b-axis Fourier projections. The third c-axis projection, for which R is 25·5% over 246 terms, is shown in Fig. 3, and the third b-axis projection, for which R is 27·5% over 132 terms, is shown in Fig. 4. The atomic parameters obtained from these two Fourier maps are given in Table 1, column (i).

The refinement of the structure

Throughout the partial refinement of the structure in projections, oxygen atom O_1, which is resolved in the c-axis projection, gave an electron density peak (Fig. 3) which was much more diffuse than the peaks corresponding to any of the other oxygen atoms. This diffuseness was not considered to be entirely due to thermal motion and for this reason the normal method of Fourier refinement was used in three dimensions, so that the shapes of the peaks in the electron-density function could be ascertained at each stage of the refinement. As the crystals of glutathione were of an unsuitable shape and size for accurate intensity measurements the method of least squares, which minimizes $\Sigma(|F_o|-|F_c|)^2$, was not used for the later stages of the refinement. The positional parameters were therefore refined by two complete rounds of structure-factor calculations followed by full three-dimensional electron-density calculations, several pairs of b- and c-axis difference syntheses in projection, and a final three-dimensional difference synthesis containing 17 atoms, i.e. omitting three atoms which might have been wrongly placed.

1. *First round of the refinement*

The atomic parameters given in Table 1, column (i) were used for the first round of the refinement together with a temperature factor $B = 3{\cdot}396$ Å^2 and a scaling factor $K = 1{\cdot}30$, both determined by Wilson's statistical method. 1557 $|F_c|$ values and phase angles were calculated and for this number of terms, which includes 371 for which the value of $|F_o|$ was taken as one-half the minimum observed value, R was 29·4%. The value calculated for K in this round was 1·435, the high values for both R and K being attributed to the inclusion of the 371 terms with uncertain $|F_o|$ values.

A three-dimensional electron-density function containing 1186 terms was calculated and the peak positions were calculated from the equation representing a Gaussian ellipsoid (Shoemaker, Donohue, Schomaker & Corey, 1950), solving for the 10 unknowns in this equation from the 10ϱ values suitably disposed about the maximum ϱ value for each peak. The atomic parameters calculated in this way are shown in Table 1, column (ii). Column (iii) shows the values obtained if allowance is made for the phase angles of 70% of the structure factors, by taking 1·7 × atomic shifts.

In connection with this electron density distribution the following points were noted:

1. Oxygen atom O_1, which in the c-axis projection produced a peak elliptical in shape, diffuse, and lower in height than the peaks corresponding to the other oxygen atoms, gave a diffuse ovoid-shaped electron-density distribution in three dimensions, the maximum ϱ value being only 6 e.Å^{-3}, whereas all other oxygen atoms, with the exception of O_2, gave peaks with maximum ϱ values of 9–11 e.Å^{-3}.
2. The change in parameters of carbon atom C_1 had resulted in an unusual set of values for the bond lengths at the glycine end of the molecule, the peculiarities being enhanced by taking 1·7 × atomic shifts. Therefore, although this allowance for the phase angles would probably have speeded up the refinement process, on the assumption that some of the atomic positions might have been overshot in the first round, the actual shifts were used in calculating the parameters for the second round of the refinement.

2. *Second round of the refinement*

The structure amplitudes and phase angles for the second round of the refinement were calculated from the atomic parameters shown in Table 1, column (ii). R was 25·7% over 1557 terms and 22·2% for the 1186 terms for which $|F_o|$ values were actually obtained. K and B were 1·450 and 2·983 Å^2 respectively.

Table 2. *Peak electron-density values obtained in the second round of the three-dimensional refinement*

C_1	6·1 e.Å^{-3}	N_1	10·3 e.Å^{-3}
C_2	6·8	N_2	9·1
C_3	7·6	N_3	10·4
C_4	8·0		
C_5	7·0	O_1	7·3
C_6	7·9	O_2	8·0
C_7	8·6	O_3	10·0
C_8	7·0	O_4	9·8
C_9	9·0	O_5	10·9
C_{10}	8·2	O_6	10·3
		S	21·2

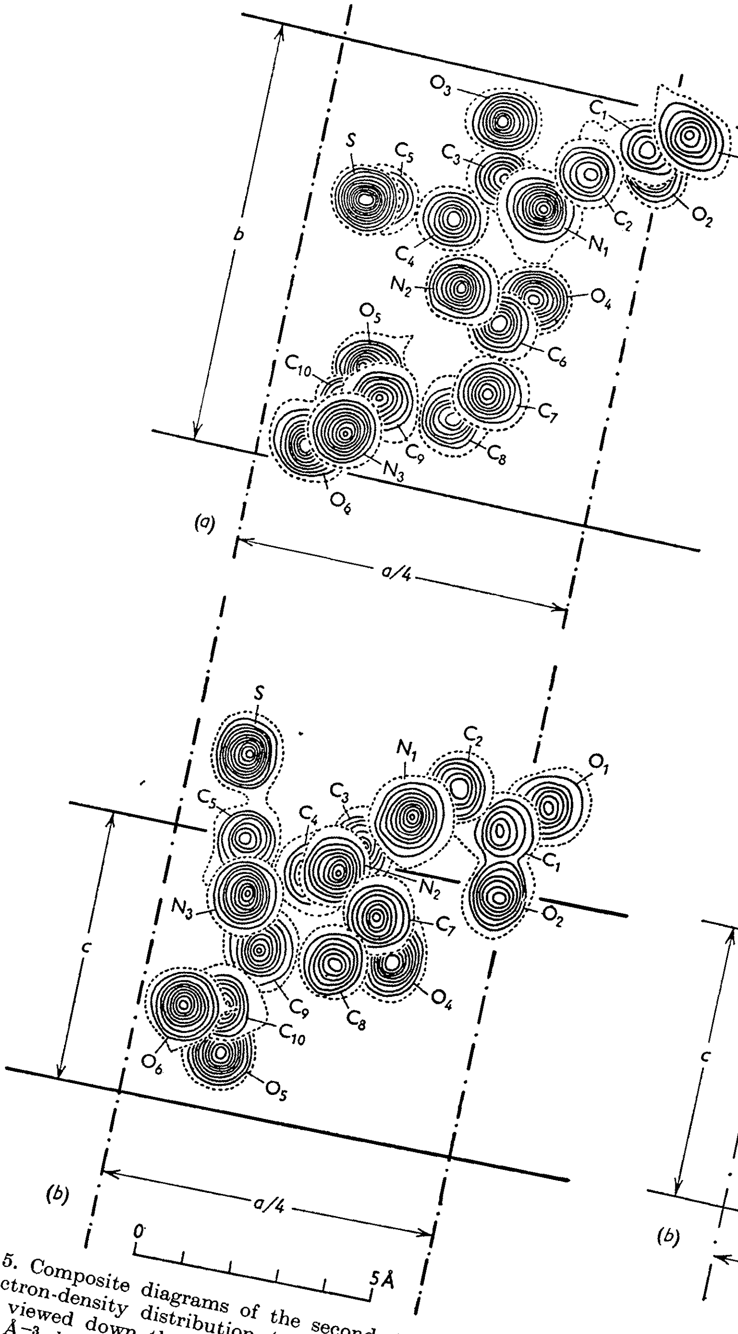

Fig. 5. Composite diagrams of the second three-dimensional electron-density distribution (*a*) viewed down the *c*-axis, (*b*) viewed down the *b*-axis. Contours are at intervals of 1 e.Å^{-3}, beginning at 1 e.Å^{-3} (broken line), except in the case of the sulphur atom, which has contours at intervals of 2 e.Å^{-3}.

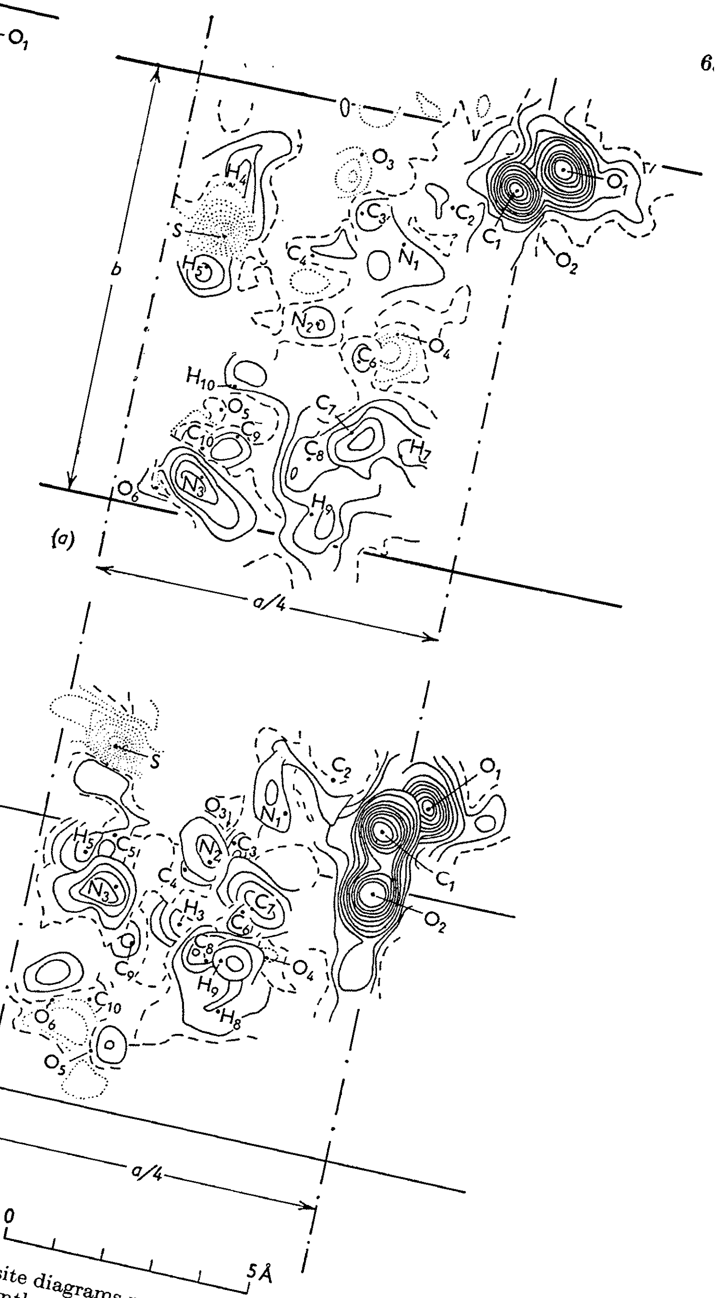

Fig. 6. Composite diagrams representing the three-dimensional difference synthesis (*a*) viewed down the *c*-axis (*b*) viewed down the *b*-axis. Contours are at intervals of 0·25 e.Å^{-3}, with the zero contour line broken and the negative contour lines dotted.

Atomic parameters calculated from the second three-dimensional electron-density distribution are given in Table 1, column (iv). Figs. 5(*a*) and 5(*b*) are composite diagrams of this second distribution, representing views down the *c* and *b* axes respectively. Peak heights for the atoms are given in Table 2. In general, the apparent atomic shifts calculated from this second round were in the same direction as those calculated from the first round, so that taking 1·7 × the shifts would have been justified. The peak for atom O_1 was again diffuse (Fig. 5), and lower in height than the peaks for other oxygen atoms in the structure, while the values for the bond lengths at the glycine end of the molecule were not in agreement with generally accepted values for such bonds.

Three pairs of *c*-axis and *b*-axis difference syntheses in projection were calculated in an unsuccessful attempt to determine the true positions of atoms C_1, O_1 and O_2, and to improve the shape of the peak for O_1. The parameters obtained from the final pair, for the 17 other atoms in the asymmetric unit, are shown in Table 1, column (v).

3. *A three-dimensional difference synthesis*

During the investigation of the structure of vitamin B_{12} (Dr Hodgkin, private communication), it has been found that there is a tendency, in electron-density functions involving phase angles, for a peak one-third of the normal height to be produced for atoms not included in the calculation, or included in incorrect positions, provided that the positions of most of the other atoms in the structure are known with reasonable accuracy. Therefore, a three-dimensional difference synthesis, omitting atoms C_1, O_1 and O_2, and based on the 17 atoms with parameters as in Table 1, column (v), was calculated. The distribution obtained is represented by the composite diagrams showing a view down the *c* axis (Fig. 6(*a*)) and down the *b* axis (Fig. 6(*b*)). The results may be summarized as follows:

1. There are peaks with maximum ϱ values 3 e.Å^{-3} in the difference distribution corresponding to the atoms C_1, O_1 and O_2 omitted from the calculation, as predicted from the work on vitamin B_{12}. The atomic parameters calculated from these three peaks are given in Table 1, column (vi).
2. Other peaks in the difference distribution did not have ϱ values exceeding 1 e.Å^{-3} except for the negative peak at the position of the sulphur atom, indicating that the sulphur atom requires a larger temperature factor than the overall one which was used in the calculation. O_6 appears to have an anisotropic thermal motion, and several other atoms require small adjustments to their temperature factors.
3. Corrections to the atomic parameters for C_2, C_7, C_9 and N_3 were indicated and were calculated from the difference distribution by the usual methods.
4. There are small peaks, maximum ϱ values 0·7–1·0 e.Å^{-3}, (Figs. 6(*a*) and 6(*b*)), in positions corresponding to eight of the covalently-bonded hydrogen atoms, and parameters calculated for these atoms are given in Table 3.

Table 3. *Atomic parameters of the eight covalently bonded hydrogen atoms included in the final structure-factor calculations*

	x	*y*	*z*
H_3	0·3601	0·6589	0·7332
H_4	0·2888	0·7595	0·9325
H_5	0·2840	0·5612	0·9112
H_6	0·3939	0·2088	0·9892
H_7	0·4465	0·2230	0·8231
H_8	0·4002	0·2230	0·4472
H_9	0·3939	0·0568	0·6189
H_{10}	0·3217	0·3091	0·6567

The final atomic parameters calculated from the three-dimensional difference synthesis are given in Table 1, column (vi). The final $|F_c|$ values, which include a contribution from the eight covalently bonded hydrogen atoms, are shown together with the $|F_o|$ values in Table 4. *R* for 1557 terms was 25·3%, and for 1186 terms 21·0%.

Accuracy

An approximate value of the standard deviation $\sigma(x)$ etc. in the atomic parameters was calculated by the method of Cruickshank (1949). $\sigma(x)$ was found to be about 0·020 Å, which gives a value for the standard deviation in bond length of about 0·028 Å. Therefore

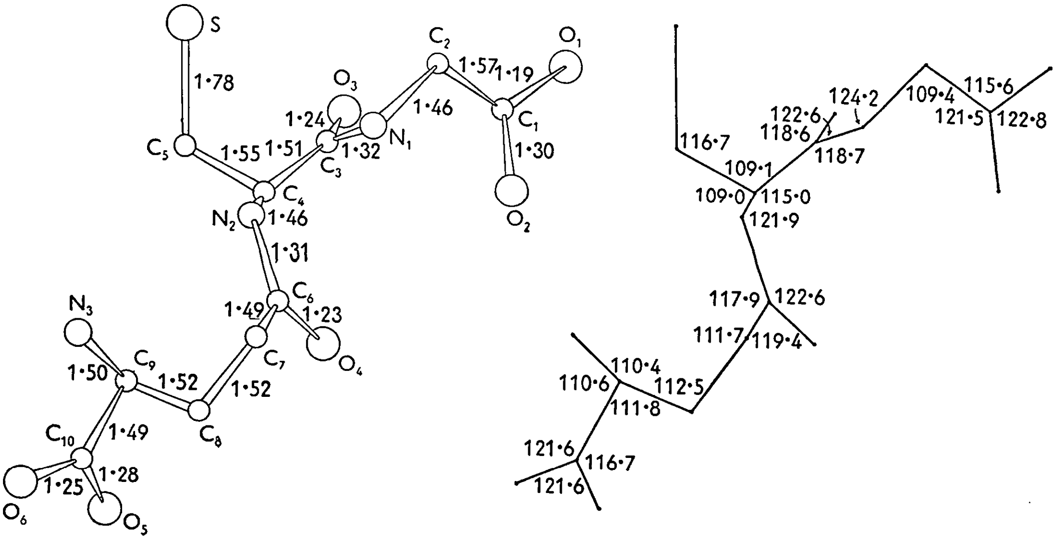

Fig. 7. The glutathione molecule showing values of bond lengths and bond angles

Table 4. *Final values of observed and calculated structure amplitudes*

For each entry the upper is the observed value

$l = 0$

h \ k	0	1	2	3	4	5	6	7	8	9	10	11
0			39.4 39.6		18.2 20.8		9.1 11.2		23.7 24.8		3.4 12.0	
1		10.7 13.6	28.2 34.6	9.3 6.2	45.0 54.8	5.4 5.2	9.6 3.4	28.8 32.6	7.2 3.2	<4.2 1.4	<3.5 5.0	6.6 9.4
2	24.6 19.2	2.3 0.6	18.9 24.2	9.5 7.4	26.2 24.0	43.9 50.6	10.6 9.8	16.3 14.8	<4.4 5.2	7.6 6.0	13.5 13.2	<2.1 2.2
3		47.2 48.6	122.6 169.8	73.4 95.8	28.8 22.2	17.7 25.6	16.5 16.0	11.3 14.4	15.5 21.4	3.4 4.4	5.2 2.6	2.4 2.8
4	50.6 48.0	44.7 36.6	35.8 45.8	30.3 38.4	33.4 43.6	28.2 30.4	10.9 9.8	<3.5 0.4	4.4 1.0	14.8 15.8	7.8 10.2	4.8 6.0
5		18.0 22.4	76.8 69.0	15.8 5.6	27.0 15.6	<3.5 0.2	14.9 14.0	6.4 6.6	12.6 12.0	7.1 3.2	<3.3 0.6	3.2 1.8
6	39.0 35.2	16.8 14.4	10.1 5.4	15.5 10.8	43.5 59.0	35.7 55.8	9.3 0.6	19.1 13.2	<4.4 4.6	<4.0 3.8	<3.3 6.0	<1.8 0.4
7		26.1 16.6	92.0 110.8	9.6 5.8	14.5 18.6	13.9 5.2	13.5 17.0	7.1 6.0	9.1 11.8	<4.0 5.4	11.0 14.2	5.5 6.2
8	42.0 23.2	16.5 12.2	46.4 49.2	18.1 19.0	15.2 22.2	19.1 16.2	10.6 11.6	<4.3 2.4	6.2 3.8	<4.0 5.0	<3.2 2.8	
9		10.7 12.6	10.7 19.6	36.2 43.4	10.9 20.2	3.7 1.0	7.4 7.6	<4.3 11.4	18.3 18.6	7.2 8.8	<3.0 3.2	
10	140.8 154.8	69.6 71.2	21.8 16.8	7.1 1.6	20.4 31.0	17.0 26.6	10.6 11.2	28.0 35.2	14.3 16.2	7.3 8.4	10.1 14.2	
11		22.3 16.0	4.3 9.2	15.5 8.2	19.3 22.0	<4.4 1.0	14.8 13.6	9.5 8.2	10.2 9.4	12.2 12.4	<2.9 5.2	
12	46.8 39.2	56.4 53.8	20.8 17.0	3.8 3.6	35.1 31.4	<4.5 11.0	14.8 18.0	<4.4 0.8	7.7 5.0	6.1 5.8	7.2 9.6	
13		25.9 13.0	21.6 29.2	34.1 42.8	7.1 4.6	6.1 1.6	18.4 19.8	28.3 34.8	12.5 9.2	<3.5 5.8	8.0 3.2	
14	44.0 41.2	29.4 20.8	48.1 40.8	6.6 10.0	18.9 24.6	4.0 5.8	32.3 28.0	<4.4 2.8	15.6 15.0	<3.5 1.4	3.5 4.2	
15		46.3 42.8	24.6 16.2	51.5 41.0	<3.8 5.2	5.3 5.0	16.7 10.8	<4.4 0.8	5.2 6.0	<3.4 1.4	3.7 2.6	
16	36.8 36.0	53.5 50.0	<3.5 6.6	8.5 4.8	<3.9 4.6	28.0 39.8	11.5 1.8	<4.3 4.8	9.1 9.2	<3.2 5.6	5.7 7.2	
17		13.6 15.4	38.9 45.8	23.1 32.6	13.6 12.2	43.0 43.2	20.4 21.2	<4.2 4.0	11.0 8.6	<3.0 4.0		
18	9.5 6.4	17.9 16.2	6.1 4.8	8.5 2.2	5.3 1.4	17.4 11.8	7.2 3.0	15.6 14.2	7.7 6.4	10.1 9.8		
19		26.5 19.2	9.2 11.6	31.1 38.2	10.6 10.0	12.7 9.2	8.0 3.4	5.8 3.0	11.4 14.8	<2.7 1.0		
20	5.0 11.2	34.1 33.6	18.2 20.6	10.5 4.2	26.6 33.4	<4.4 10.4	7.4 2.6	24.4 23.4	<3.4 1.4	8.8 11.2		
21		<3.3 3.4	10.9 8.4	4.9 2.2	14.8 13.6	12.3 11.6	10.1 3.2	5.4 3.8	<3.2 2.2	5.8 6.6		
22	4.2 8.0	7.6 6.4	<4.2 8.0	<4.3 0.8	10.1 7.8	<4.3 3.4	14.3 14.8	11.9 11.0	<3.0 0.2			
23		18.3 18.4	12.1 6.0	13.5 13.4	<4.4 4.2	<4.3 2.2	12.6 11.4	9.1 14.4	3.0 0.6			
24	19.6 16.0	5.0 3.2	8.3 4.4	14.8 9.0	<4.4 7.6	25.1 22.4	13.1 10.2	<3.3 2.8	4.9 5.4			
25		5.0 3.6	<4.4 4.2	6.7 4.8	9.8 8.0	6.7 3.8	5.9 5.2	4.7 2.8	<2.0 0			
26	<4.4 8.8	14.0 20.2	10.4 7.0	14.6 10.6	<4.2 5.8	<3.9 5.2	<3.5 0.8	3.9 4.8				
27		17.4 17.0	10.6 8.8	19.9 19.8	13.0 10.0	17.4 13.2	6.9 8.0	7.1 9.4				
28	<4.3 2.0	<4.3 0	<4.2 0	7.4 4.4	<3.8 0.2	11.1 7.8	6.9 4.4	3.9 3.2				
29		8.7 7.2	8.2 11.8	8.2 4.8	5.6 3.4	<3.2 2.8	<2.6 3.6					
30	8.7 16.8	17.9 18.8	10.4 6.6	11.5 8.6	3.4 4.0	<2.9 5.4	<2.1 4.4					
31		6.6 6.8	10.4 9.4	<3.5 0.8	<3.2 3.2	<2.5 2.0						
32	<4.2 0.4	8.0 5.8	11.9 5.8	<3.1 1.0	<2.8 3.0	<2.1 4.6						
33		4.2 8.4	<3.5 2.6	8.7 8.4	2.4 2.6							
34	<3.5 0	<3.4 1.8	12.0 11.6	5.4 7.8								
35		4.2 2.6	<2.7 4.2									

$l = 1$

h \ k	0	1	2	3	4	5	6	7	8	9	10
0		104.6 90.2	<3.2 3.0	14.5 19.2	<5.2 10.0	30.0 28.4	<7.4 7.4	<8.0 1.8	12.9 13.6	<6.7 2.0	6.8 8.8
1	21.3 23.6	62.3 56.2	41.5 45.1	24.9 22.6	18.2 11.8	34.4 41.3	7.4 7.5	<8.0 8.8	<7.1 5.1	10.0 11.4	<5.4 5.4
2	55.3 67.8	16.8 22.6	44.5 57.8	40.4 40.3	11.0 2.5	10.3 16.8	28.8 25.4	15.9 18.6	21.6 25.6	<6.7 4.6	<5.4 6.0
3	23.0 19.6	96.0 75.6	34.4 34.5	38.8 41.5	22.9 17.2	44.5 42.2	5.2 7.4	13.9 17.3	14.6 18.8	10.0 6.7	9.7 12.0
4	16.7 17.8	70.0 56.3	20.1 14.2	15.9 21.4	17.4 25.9	21.7 12.7	11.7 14.0	17.9 16.4	11.4 10.7	<6.5 6.2	<5.2 4.3
5	102.6 138.0	43.2 37.2	18.3 22.7	29.0 31.9	12.6 12.2	14.2 15.7	19.7 14.8	7.0 10.2	16.4 16.5	5.2 6.5	10.2 16.6
6	68.0 76.0	42.6 45.4	51.0 53.5	25.3 25.9	16.5 20.7	41.8 39.8	6.5 12.4	4.1 10.1	16.4 13.2	5.2 3.1	<5.1 2.6
7	41.2 39.2	35.1 34.0	49.7 52.3	38.8 35.8	32.4 26.0	28.8 34.3	22.4 23.0	14.6 9.3	<7.1 4.0	5.2 7.3	<4.9 8.9
8	18.1 25.4	50.2 48.5	48.8 50.1	61.7 64.4	26.7 17.7	9.3 12.3	18.4 14.0	26.1 28.2	12.0 11.5	<6.2 4.0	<4.8 8.4
9	37.2 44.2	45.8 36.0	36.4 40.8	59.9 61.9	18.1 21.6	16.7 17.0	16.9 11.7	4.1 5.6	12.0 9.1	<6.2 5.9	<4.6 4.6
10	43.4 52.8	25.2 27.3	42.5 41.3	10.1 6.0	19.9 23.9	<6.2 8.5	8.8 6.7	23.9 17.3	14.9 13.5	<6.1 6.1	<4.6 4.8
11	27.5 18.2	29.4 24.8	50.3 55.3	28.5 22.0	16.4 11.9	22.3 34.2	6.8 3.1	17.4 16.9	9.7 7.5	<5.9 7.4	<4.3 6.2
12	28.7 39.4	35.0 37.2	16.4 23.6	13.2 18.9	10.3 8.4	12.5 17.0	22.6 19.8	12.9 7.2	18.3 15.5	<5.8 3.5	<4.1 5.5
13	8.8 12.6	13.0 17.1	14.8 13.2	<5.7 5.3	18.7 16.4	27.2 20.2	<7.0 7.0	14.1 14.6	13.2 11.5	10.8 11.3	<3.8 7.3
14	16.4 6.2	14.9 4.8	36.0 33.4	15.4 16.3	11.2 11.5	25.6 21.1	<7.1 4.8	<7.1 7.7	<6.5 8.8	<5.4 3.4	<3.5 5.3
15	31.4 25.4	24.8 24.8	22.8 26.9	22.4 24.3	15.9 21.4	19.3 17.1	<7.1 0.7	17.1 16.3	<6.4 3.7	8.4 10.9	
16	23.6 24.6	30.8 25.3	33.2 31.2	19.0 19.3	10.0 12.9	18.7 17.8	10.0 8.5	12.6 10.3	8.7 6.6	<4.9 4.5	
17	18.4 16.6	20.2 21.3	9.0 10.1	13.8 9.6	14.5 18.2	14.8 14.9	18.3 19.3	<6.8 2.6	<5.9 2.4	<4.6 5.4	
18	25.2 33.4	16.9 9.6	12.3 8.5	10.7 14.1	24.6 25.2	17.9 15.0	10.7 6.0	16.8 22.7	<5.8 7.6	<4.3 5.3	
19	22.0 17.8	<5.1 3.0	30.4 35.1	10.9 11.0	12.7 18.9	19.7 16.1	16.1 14.6	7.4 9.3	<5.5 7.3	<4.1 0.9	
20	17.4 15.0	10.0 10.7	21.1 8.4	22.2 19.1	<7.1 3.1	7.1 4.7	11.9 9.7	14.5 11.8	<5.2 3.8	<3.5 9.4	
21	20.2 22.2	9.9 12.5	21.3 9.3	15.1 13.1	14.8 15.5	11.4 12.0	15.5 11.4	4.9 8.7	<4.9 3.7		
22	13.3 14.2	9.7 6.4	9.0 9.0	30.3 32.7	10.9 11.7	12.7 9.1	9.3 12.8	<5.7 3.8	<4.5 3.7		
23	11.3 7.4	14.8 20.0	11.2 9.9	17.7 17.1	10.9 7.8	14.2 12.6	<6.3 3.7	<5.4 6.8	<4.1 1.4		
24	<7.1 4.6	7.7 5.9	17.7 21.0	<7.1 7.0	<7.0 3.7	8.6 8.5	4.9 4.4	6.5 6.6	<3.5 5.1		
25	<7.1 4.4	22.3 24.8	14.5 18.6	<7.1 2.7	<5.8 9.9	12.9 13.5	<5.7 6.4	<4.6 3.0			
26	6.5 12.4	16.5 16.9	14.6 14.2	<7.0 1.7	<6.7 2.8	<6.1 6.7	7.5 9.6	<4.1 3.4			
27	<7.1 6.0	<4.1 3.7	9.0 11.3	<6.7 8.3	10.5 12.7	10.6 11.3	5.7 10.6	<3.5 2.9			
28	5.7 8.2	<4.1 5.6	10.3 12.4	<6.7 6.8	<6.1 4.9	<5.4 8.1	<4.3 3.5				
29	<6.7 3.0	<5.4 4.6	<6.7 3.8	<6.2 2.4	7.4 10.9	7.7 9.7	<3.8 2.4				
30	6.4 7.2	<5.2 3.2	<6.1 2.6	10.1 12.2	<5.2 4.0	<4.5 3.6					
31	13.0 19.8	<4.8 3.2	<5.8 1.7	<5.4 1.7	<4.8 4.1	<3.8 1.6					
32	<6.1 5.0	7.1 9.8	<5.4 2.5	<5.7 6.5	<4.1 7.8						
33	<5.5 5.2	<4.1 8.1	<4.8 5.1	8.1 10.1	<3.3 2.6						
34	<4.9 4.0	<3.6 2.9	<4.1 4.6	<4.1 4.4							

$l = 2$

h \ k	0	1	2	3	4	5	6	7	8	9	10
0	40.7 46.4	18.4 18.2	11.4 7.4	40.4 46.2	11.4 20.4	14.0 17.2	12.5 11.4	8.3 5.9	<4.2 1.2	<3.6 1.8	<2.8 3.4
1	42.4 49.0	5.4 9.8	34.6 33.4	26.6 26.4	29.9 32.1	21.1 15.6	6.3 11.4	13.8 17.9	7.7 7.3	6.4 2.7	10.4 11.4
2	15.9 10.4	30.4 38.0	16.8 22.6	19.1 21.8	24.3 29.0	21.6 22.3	21.6 19.6	21.6 21.3	8.8 13.2	6.2 1.4	8.0 8.9
3	38.7 60.4	30.0 30.5	32.6 33.7	24.6 27.4	21.1 23.4	22.5 25.8	14.7 14.0	16.2 17.0	5.8 4.3	7.7 4.7	4.5 4.5
4	40.3 52.2	44.2 52.5	55.3 60.8	17.9 21.6	11.9 9.9	17.7 15.2	11.0 9.1	6.6 8.1	11.2 8.6	7.7 8.6	<2.8 1.2
5	19.6 14.6	40.4 48.2	34.8 41.7	31.1 20.6	9.5 3.8	18.0 12.5	12.8 17.0	16.8 14.3	10.4 9.0	6.1 7.2	5.1 0.7
6	22.1 22.2	16.7 16.3	43.5 44.7	12.8 9.1	16.7 17.4	33.7 33.5	15.5 14.8	11.9 13.0	13.5 14.8	6.7 7.0	<2.7 1.8
7	17.4 8.0	26.2 16.8	15.5 18.5	23.1 21.4	21.6 24.1	18.3 22.4	15.5 10.8	10.3 11.2	<4.0 2.9	5.9 5.8	2.5 2.5
8	69.4 91.8	38.0 41.4	15.2 16.8	11.0 8.4	15.9 9.5	24.4 21.4	11.6 6.2	4.2 3.6	15.5 18.4	6.0 7.5	2.5 3.6
9	28.0 29.2	23.8 15.8	31.7 29.1	8.0 10.3	28.6 27.0	27.2 28.8	14.9 16.0	7.9 8.8	9.4 7.9	3.4 5.0	7.0 9.6
10	28.4 31.8	10.9 2.4	20.8 24.9	16.5 16.0	8.5 5.8	<3.9 3.9	11.7 10.6	3.0 2.7	8.8 4.6	7.7 6.1	<2.1 4.5
11	41.1 30.4	27.2 25.6	17.7 16.5	26.3 25.6	10.1 10.9	22.8 22.8	13.5 13.6	7.9 8.7	6.1 8.1	<3.3 3.5	<2.1 0.7
12	27.2 29.0	38.0 37.9	2.2 5.1	<3.6 8.9	26.9 27.6	13.4 13.4	11.7 12.4	15.5 16.4	7.1 2.9	6.5 4.8	4.9 6.4
13	38.0 52.4	13.7 7.2	4.8 5.5	20.2 22.7	15.5 17.2	12.2 12.7	19.8 16.1	10.9 10.8	5.2 4.5	<3.0 4.1	
14	12.9 11.4	16.9 12.3	19.3 13.3	18.8 24.3	9.2 7.7	17.7 18.7	16.4 16.8	15.5 16.0	8.9 5.1	3.6 5.0	
15	18.2 17.0	17.6 22.4	23.7 27.9	10.1 6.7	10.9 7.3	7.1 6.7	7.9 4.9	9.8 6.7	4.9 7.3	7.6 6.5	
16	28.7 31.6	13.8 12.2	29.6 35.0	13.8 21.0	14.0 15.5	7.9 6.0	8.9 6.5	9.2 9.1	10.1 10.8	3.6 2.4	
17	11.2 23.6	12.0 4.2	11.2 13.1	16.4 10.1	16.8 17.8	14.6 12.4	8.8 3.7	9.5 7.7	<3.3 3.9	8.5 9.2	
18	13.2 15.8	16.5 23.6	12.8 9.7	13.8 13.1	15.3 16.0	13.2 9.2	17.7 12.4	10.3 6.6	6.8 8.2	<2.1 6.2	
19	34.6 37.6	16.5 22.0	17.3 12.2	8.2 8.1	11.4 6.9	15.6 20.7	12.0 9.5	6.3 3.8	8.9 10.5		
20	<4.0 1.6	17.4 19.4	11.9 5.7	9.8 10.7	14.0 13.1	16.1 10.5	4.8 4.1	7.3 6.4	<2.7 2.3		
21	10.4 6.0	13.1 14.6	20.4 19.7	10.3 7.8	12.2 10.2	7.6 7.6	8.5 8.0	4.2 6.1	2.5 3.1		
22	11.0 1.4	14.1 15.4	16.2 14.2	13.2 19.8	15.0 13.0	5.7 4.9	14.1 13.3	5.8 3.9	<2.1 2.4		
23	18.3 15.6	11.4 11.9	13.2 15.4	15.9 14.4	6.5 4.9	8.8 11.8	4.3 1.5	2.1 5.7	7.0 7.3		
24	<4.2 3.0	11.2 13.9	12.2 10.3	10.6 8.3	8.9 8.5	9.2 4.6	8.0 9.3	<2.7 4.5			
25	13.5 16.2	7.9 9.4	6.6 9.2	15.5 17.2	10.3 7.1	12.0 10.6	3.1 3.2	4.5 4.6			
26	14.0 15.0	9.7 9.5	9.5 5.7	4.8 8.4	9.2 9.8	<3.4 4.1	6.4 6.4	<1.9 5.9			
27	<4.0 9.8	8.0 7.2	<4.0 1.4	3.7 4.4	9.4 5.1	3.1 3.1	4.0 5.4				
28	7.9 9.2	4.8 8.7	5.9 2.1	11.7 12.8	8.2 6.7	<3.0 3.7	7.6 5.7				
29	8.3 4.4	8.8 10.2	6.3 6.1	6.0 4.9	8.8 8.0	7.3 8.4					
30	6.6 8.8	8.6 11.7	3.4 4.0	5.1 5.7	7.4 1.2	5.7 3.6					
31	4.6 8.0	8.0 11.1	4.5 5.0	8.2 7.9	6.0 6.8						
32	<3.6 2.6	3.0 4.3	<2.8 5.1	7.7 8.0	<1.9 3.3						
33	<2.7 3.2	<2.7 3.3	<2.5 8.8	3.6 0.3							
34	<2.2 0.2	4.9 8.9									

$l = 3$

h \ k	0	1	2	3	4	5	6	7	8	9
0		45.2 59.4	15.7 17.2	15.5 24.4	29.4 26.0	<5.4 12.8	<5.8 1.2	24.4 21.2	<5.3 2.0	5.0 9.0
1	28.0 27.0	9.1 14.2	27.2 29.1	41.8 33.1	14.3 13.2	19.4 17.4	12.7 11.6	14.2 16.3	15.8 18.2	5.0 7.0
2	34.8 41.0	23.3 25.6	20.6 22.3	5.4 7.3	14.3 14.6	15.7 22.1	29.4 32.2	14.2 14.0	5.9 6.5	5.0 6.3
3	15.6 9.0	24.2 28.1	31.5 36.3	14.8 19.0	17.5 17.7	6.3 7.2	13.0 11.0	9.2 11.8	7.3 9.6	5.0 5.0
4	28.4 34.8	29.2 24.2	28.7 30.0	7.9 7.4	20.3 21.6	21.4 20.4	9.2 7.6	17.2 19.4	7.3 3.3	<5.0 4.9
5	10.2 24.4	26.4 19.4	24.1 19.4	41.4 48.9	25.2 25.5	24.2 21.4	14.7 12.1	7.3 1.1	10.6 11.4	<4.1 3.3
6	15.0 17.2	16.3 12.5	9.6 17.3	19.3 18.3	13.8 17.6	<5.4 4.7	17.8 22.7	<6.4 4.0	6.4 3.2	6.9 9.1
7	20.1 17.4	14.5 28.3	20.1 19.0	22.3 24.6	9.1 3.0	16.7 16.8	8.1 5.7	17.0 19.7	6.4 7.0	5.1 3.4
8	18.0 27.4	19.8 11.5	24.6 25.0	12.4 12.9	10.2 9.4	9.1 6.6	13.2 14.1	<5.4 2.0	6.9 6.1	<4.0 2.4
9	5.3 5.6	22.1 23.7	22.9 24.2	22.3 25.7	14.8 20.4	7.9 6.6	15.5 21.2	8.9 9.8	11.9 9.7	<3.8 1.3
10	38.3 38.6	30.2 33.0	12.7 18.3	15.5 17.2	7.1 9.5	7.3 5.6	14.7 10.6	15.6 11.5	6.6 8.0	4.1 9.4
11	<2.8 3.5	<2.8 2.0	26.2 31.1	14.7 14.0	18.8 10.2	13.8 13.4	10.4 7.5	6.1 3.1	9.9 13.5	<3.5 1.4
12	24.4 32.8	21.6 24.7	20.0 26.1	8.7 7.8	12.5 15.5	19.0 23.5	19.0 19.0	6.1 3.5	<5.1 2.2	<3.3 0.6
13	10.1 13.0	18.6 11.5	7.4 9.1	7.6 8.8	15.5 15.9	9.4 5.0	10.7 15.1	5.9 3.7	6.1 9.3	<3.0 5.2
14	36.2 45.0	15.7 17.3	15.4 6.5	5.4 10.4	<5.6 6.6	23.2 30.2	15.3 9.2	5.9 6.1	5.4 3.5	<2.8 4.9
15	<3.1 5.0	34.8 40.3	18.5 25.7	6.4 4.7	17.0 15.5	<5.8 3.9	<4.5 1.5	5.8 7.5	5.3 7.4	
16	8.9 8.4	12.5 12.2	19.3 13.8	14.5 12.3	11.9 13.6	9.7 6.8	13.0 12.9	6.1 4.2	9.1 12.4	
17	<3.1 5.6	10.6 11.3	12.5 13.5	21.3 27.8	16.1 11.0	7.3 10.7	6.1 0.6	9.9 12.7	6.4 5.6	
18	<4.6 7.0	9.1 3.4	9.7 8.4	15.8 15.5	13.0 12.1	<6.3 6.5	5.9 4.2	5.8 6.9	<3.3 1.5	
19	<4.6 1.2	17.0 16.8	13.8 8.2	18.1 17.0	18.7 20.0	12.0 7.5	10.2 11.5	5.4 5.1	3.3 1.8	
20	33.3 33.6	19.3 16.1	13.5 11.0	11.9 7.3	11.5 5.1	<6.1 5.3	9.1 2.3	5.4 5.9		
21	14.3 8.0	7.4 5.4	14.3 14.6	7.3 7.3	11.7 5.4	<5.9 5.6	5.9 8.0	7.6 10.6		
22	15.6 20.6	11.9 5.9	17.2 18.2	13.5 9.1	<5.3 4.2	9.4 8.5	<4.9 4.6	<3.3 3.6		
23	16.6 7.0	9.7 5.4	10.7 9.5	10.4 6.5	9.4 10.5	<5.4 2.5	5.6 6.5	3.3 2.7		
24	11.9 7.2	<6.3 3.0	10.9 9.3	<6.4 5.1	5.8 5.6	12.2 15.6	<4.1 3.5			
25	<5.4 0.4	11.2 10.2	9.1 7.4	5.8 7.4	6.1 4.0	5.3 1.5	3.8 4.3			
26	9.9 9.0	8.4 9.7	11.1 7.0	8.7 3.7	9.2 9.3	5.4 4.4	<3.1 5.9			
27	<4.9 3.2	<4.0 3.3	10.4 12.7	5.9 6.1	5.3 1.3	4.0 0.9				
28	5.4 4.2	<3.8 3.7	8.7 6.0	<4.3 3.1	<3.8 3.8	<2.8 6.9				
29	8.1 8.0	6.1 9.3	5.4 2.5	9.4 8.7	4.1 6.6					
30	8.7 10.0	<3.3 3.3	<3.8 0.8	<3.5 2.9						
31	9.7 9.4	<3.6 3.7	<3.3 4.0	<2.8 4.9						
32	<3.1 1.6	<3.0 4.8								

Table 4 (*cont.*)

$l = 4$ h k	0	1	2	3	4	5	6	7	8	9
0	12.0 0.8	15.3 19.6	7.7 4.0	16.8 26.8	7.3 12.2	19.7 20.4	16.5 10.8	8.5 12.4	11.2 13.8	<3.0 2.6
1	16.4 22.6	19.5 15.0	16.7 15.4	16.7 10.4	18.3 24.3	17.6 13.8	14.5 8.1	8.5 8.1	<4.3 1.6	5.0 7.9
2	13.7 6.8	20.2 20.5	11.5 7.0	12.8 10.6	14.0 8.1	18.0 13.7	9.2 2.1	12.2 8.0	7.5 9.1	<2.8 3.4
3	12.8 21.8	16.7 22.2	22.0 19.7	16.0 7.3	11.5 7.0	8.2 14.5	21.5 21.5	8.8 11.3	6.0 4.7	5.7 9.8
4	4.2 2.2	12.7 18.5	25.0 27.2	14.8 23.1	14.8 12.5	16.0 11.5	9.2 6.7	16.3 18.8	6.0 2.2	5.3 7.4
5	6.8 0.6	19.5 20.2	12.2 7.7	14.5 7.4	<5.8 4.6	12.5 7.4	<5.5 6.0	<5.0 4.1	<4.2 2.4	
6	10.2 14.6	5.3 1.0	25.5 36.9	14.5 10.5	13.7 5.7	12.8 10.7	16.3 14.0	10.0 4.7	6.7 6.7	
7	11.2 16.2	5.3 11.6	18.0 21.1	13.8 13.8	14.5 13.0	27.2 32.5	17.2 14.9	<5.0 1.9	6.5 3.6	
8	14.3 15.8	11.2 8.6	11.5 12.6	<5.7 3.3	9.5 11.8	<5.7 2.4	14.0 13.4	8.0 7.1	7.8 10.8	
9	20.5 24.8	7.2 12.2	16.5 18.8	15.2 17.1	18.0 21.2	9.3 9.0	<5.3 0.4	<4.7 4.6	5.3 3.1	
10	<4.5 13.6	14.7 17.2	13.8 17.0	17.2 22.8	7.5 8.1	10.8 6.4	8.7 4.1	12.0 14.1	<3.7 7.4	
11	24.5 27.4	13.8 16.2	<5.7 7.9	13.3 10.5	16.5 13.4	9.8 6.3	<5.3 5.5	7.5 8.9	<3.5 7.8	
12	5.7 0.6	10.4 12.2	12.3 12.6	9.5 12.1	<5.8 0.8	14.3 14.5	<5.2 2.8	7.7 8.0	<3.3 2.4	
13	10.9 21.2	22.2 24.2	11.0 5.3	12.5 7.2	13.2 15.2	<5.5 0.9	<5.0 5.4	<4.2 6.1	<3.0 2.5	
14	<5.8 3.6	<4.7 2.6	13.7 8.4	10.0 19.3	11.8 11.3	13.8 13.3	8.5 5.3	<4.0 5.7	<2.8 2.0	
15	5.8 9.2	7.5 8.3	<5.8 4.2	10.0 10.9	<5.5 6.2	<5.3 5.0	<4.7 6.1	<4.0 4.8		
16	12.0 14.8	15.3 15.3	11.5 16.9	<5.7 4.8	9.5 5.7	<5.2 3.3	7.5 8.3	<3.7 2.7		
17	24.5 33.0	12.0 9.6	12.3 11.5	13.8 13.7	10.8 7.5	14.2 16.7	<4.3 5.8	<3.5 4.8		
18	7.3 9.2	8.8 9.8	<5.7 4.5	7.8 5.2	10.7 11.9	7.8 7.6	<4.2 8.2	7.3 2.3		
19	11.8 6.6	10.9 10.1	9.7 9.1	<5.3 4.6	<5.2 2.6	10.4 14.3	<4.0 3.7	<2.8 5.3		
20	<5.5 1.0	13.7 19.5	13.3 19.2	<5.3 4.0	<5.0 4.5	<4.5 2.2	8.5 6.7			
21	12.7 8.6	10.5 14.1	<5.3 8.1	12.2 13.8	<4.7 4.1	<4.2 2.5	<3.3 7.5			
22	<5.3 2.0	7.5 3.1	<5.2 2.4	8.5 10.3	<4.5 4.6	<4.0 5.7	<2.8 0.5			
23	8.0 11.6	5.8 3.0	8.0 8.5	7.7 6.3	9.5 13.4	6.0 8.3				
24	5.0 4.4	6.2 8.4	<4.7 4.0	8.8 9.3	6.7 5.5	6.8 7.2				
25	9.0 10.0	<3.8 0.7	<4.3 4.6	6.7 7.1	5.0 3.3					
26	<4.3 3.2	8.3 9.7	5.8 5.2	6.3 8.1	<3.0 2.2					
27	4.7 7.0	3.2 3.5	<3.7 3.4	7.0 10.0						
28	4.2 2.8	3.0 3.9	4.7 5.7	4.5 4.6						
29	4.3 4.6	5.0 3.8	<2.5 2.7							

$l = 5$ h k	0	1	2	3
0		30.8 22.6	12.4 5.2	<13.6 1.7
1	<8.4 0.4	9.8 11.7	10.3 7.8	<13.6 13.5
2	27.9 22.0	18.9 22.9	10.3 14.7	<13.6 8.0
3	<8.4 5.2	15.3 13.3	<12.4 9.4	13.6 10.3
4	15.3 5.2	4.8 7.2	<12.4 6.8	
5	20.3 22.2	18.2 14.2	<12.4 7.1	
6	<8.4 4.8	16.2 13.1	<10.3 4.3	
7	<8.4 1.2	4.8 10.4	12.4 7.1	
8	<8.4 8.6	6.0 15.2	12.2 3.7	
9	<8.4 5.4	<4.8 8.6	12.2 15.6	
10	12.4 8.2	<4.8 14.6		
11	16.8 15.2	<4.8 5.9		
12	29.0 30.0	4.8 12.1		
13	<6.5 4.0	<4.8 1.6		
14	<6.5 6.4	7.9 8.2		
15	16.5 9.0	6.4 13.3		
16	5.5 5.0	<4.5 6.2		
17	<6.2 6.6	<4.3 3.0		
18	13.2 21.8	<4.1 4.3		
19	<5.7 6.4	<4.1 7.2		
20	10.0 9.8	<4.0 3.3		
21	<5.2 2.4	<3.6 4.5		
22	<4.8 10.2	<3.4 2.8		
23	<4.5 3.0	<3.1 6.1		
24	<4.1 2.8	<2.9 1.5		

$l = 6$ h k	0	1
0	21.5 24.0	<5.4 5.5
1	<9.5 5.0	<5.4 7.5
2	<9.5 2.6	<5.4 8.2
3	<9.5 6.2	<5.4 4.7
4	9.5 9.8	<5.4 6.0
5	10.5 5.2	14.3 20.2
6	9.3 5.4	
7	<9.1 2.2	
8	11.0 8.4	
9	<8.9 2.6	
10	15.1 14.0	

$l = 7$ h k	0	1
0		3.3 4.0
1	<6.2 1.0	<3.3 7.9
2	14.1 7.8	3.3 10.2
3	<4.3 3.2	<3.3 3.0
4	11.5 3.0	<3.3 7.7
5	<4.8 5.0	6.7 9.1
6	5.5 1.0	

differences less than 0·08 Å between the values calculated for the bond lengths in this structure and generally accepted values for such bond lengths cannot be considered as significant.

Discussion of the structure

The values of bond lengths and bond angles determined in the final round of the refinement are shown in Fig. 7. The two peptide linkages in the molecule are planar, with the –NH and –CO groups in the *trans* configuration. The best representative planes, determined by the method of least squares on the assumption that the z parameters are the least accurate, are

$$2{\cdot}5959x+0{\cdot}0436y-0{\cdot}5705z = 1$$

for atoms C_2, N_1, C_3, O_3 and C_4, and

$$1{\cdot}6389x+0{\cdot}1242y+0{\cdot}3552z = 1$$

for atoms C_4, C_6, N_2, O_4 and C_7.

The displacements of the atoms from these planes are shown in Table 5. The planes make angles of 42·4°, 2·0° and 47·6°, and 42·1°, 9·3° and 46·4° with the a, b and c axes respectively, and the angle between the planes is 94·4°.

The structure is represented by the clinographic projection (Fig. 8), which shows two complete molecules and certain portions of others which illustrate the hydrogen bonding arrangement. Molecule M is related to molecules M', M'' and M''' by the screw diad axes at $(\frac{1}{4}, 0, z)$, $(\frac{1}{2}, y, \frac{1}{4})$ and $(\frac{1}{2}, y, \frac{3}{4})$ respectively,

Table 5. *Displacements of the atoms from the planes of the peptide links*

	Displacement (Å)		Displacement (Å)
C_2	+0·034	C_4	−0·090
N_1	−0·045	N_2	+0·121
C_3	−0·002	C_6	+0·049
O_3	−0·009	O_4	−0·005
C_4	+0·022	C_7	−0·075

Table 6. *Interatomic distances and angles*

	Distance (Å)		Angle (°)
$S–O_3$	3·63		
$S–O_{6\ z+1}$	3·53		
$S–O_{5\ z+1}^{\ y+1}$	3·57		
$S–S'$	4·41		
$S–O_{\substack{5\ z+1\\ y+1}}$	3·90		
$N_1–O_4$	3·55	$C_3–N_1–O_1''$	131·5
$N_1–O_{4\ z+1}$	3·28	$C_2–N_1–O_1''$	100·7
$N_1–O_1''$	2·88	$C_1''–O_1''–N_1$	142·1
$O_1''–C_6$	3·00	$C_1–O_2–O_4'''$	108·6
$O_2–O_4'''$	2·66	$C_6'''–O_4'''–O_2$	116·6
$O_1–O_4'''$	3·18	$N_2–O_{5\ z+1}–N_3$	88·6
$O_1–O_{4\ z+1}'''$	3·27	$C_{10\ z+1}–O_{5\ z+1}–N_3$	153·7
$N_2–O_{5\ z+1}$	2·98	$C_6–N_2–O_{5\ z+1}$	110·9
$N_3–O_{6\ z+1}$	3·21	$C_4–N_2–O_{5\ z+1}$	123·8
N_3O_6'	2·89	$C_9–N_3–O_6'$	130·0
		$C_{10}'–O_6'–N_3$	149·9
$N_3–O_{5\ z+1}$	2·68	$C_9–N_3–O_{5\ z+1}$	106·7
		$C_{10\ z+1}–O_{5\ z+1}–N_3$	106·8
$N_3–O_{3\ y-1}$	2·81	$C_9–N_3–O_{3\ y-1}$	115·3
		$N_3–O_{3\ y-1}–C_{3\ y-1}$	142·7

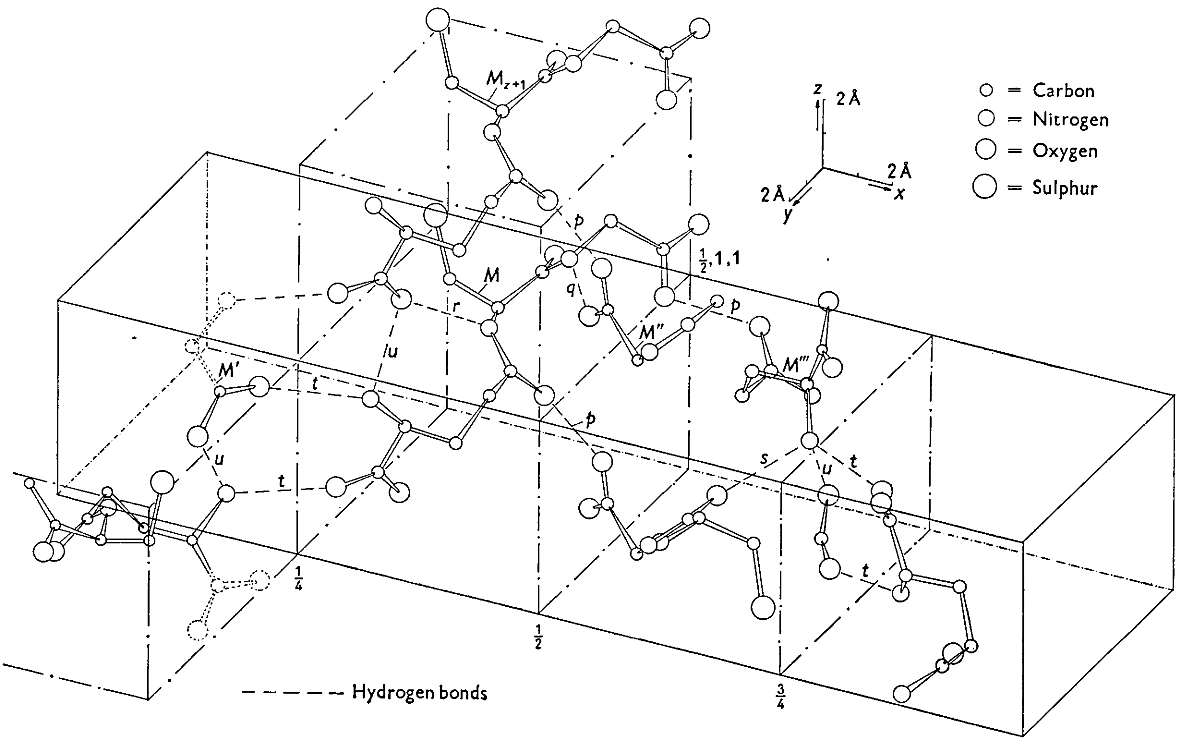

Fig. 8. Clinographic projection of the structure, showing the hydrogen-bonding arrangement.

and to molecules M_{z+1}, M_{y-1} by unit translations in the z and $-y$ directions respectively. The molecule has adopted an S-shaped configuration, running diagonally downwards in the c direction from the glycine carboxyl group at $x = \frac{1}{2}$, $y = 1$, $z = 1+\frac{1}{3}$, to the glutamic acid carboxyl group at $x=\frac{1}{4}$, $y=0$, $z=\frac{1}{3}$ approximately. Certain interatomic distances and angles present in the structure are given in Table 6.

There are no internal hydrogen bonds in the molecule, the shortest distance N_1–O_4 being 3·5 Å, and the nearest oxygen atom, O_3, to the sulphur atom being at a distance of 3·6 Å from it.

The molecules are held together in the structure by a three-dimensional network of hydrogen bonds. Oxygen atom O_2 of the glycine carboxyl group, which lies in a plane almost parallel to the c axis, forms a hydrogen bond with carbonyl oxygen atom O_4 of molecule M'''. This bond is 2·66 Å long, and is represented by bonds of the type p in Figs. 3 and 8. Peptide nitrogen atom N_1 forms a hydrogen bond, q, 2·88 Å long, to glycine–carboxyl oxygen atom O_1 of molecule M''. Peptide nitrogen atom N_2 forms a hydrogen bond of type r, 2·98 Å long, to oxygen atom O_5 of molecule M_{z+1}. As the plane of the amino–carboxyl group lies almost parallel to the c axis, nitrogen atom N_3 is in a position to form three hydrogen bonds: type s, 2·81 Å long, with oxygen atom O_3 of molecule M_{y-1}; type t, 2·89 Å long, with oxygen atom O_6 of molecule M'; and type u, 2·68 Å long, with oxygen atom O_5 of molecule M_{z+1}.

All other non-bonded atoms in the structure are at the usual van der Waals distances from one another, with the exception of C_6 and O_1 of molecule M'', which are 3·00 Å apart. The sulphur atom has four oxygen atoms as nearest neighbours (Table 6), and the distance between neighbouring sulphur atoms is 4·41 Å.

This work, which was submitted as a thesis for the Ph.D. degree at London University, was begun as a part-time study at Birkbeck College, but in the two final years was continued as a full-time study in the Scientific Department of J. Lyons and Company Limited, to whose directors, and particularly the late Dr L. H. Lampitt, the author expresses her gratitude for the opportunity to complete the work, and for the use of the electronic computer, LEO.

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A Comparison of the Methods Used in the Attempt to Determine the Crystal Structure of Glutathione

By W. B. Wright

The Laboratories, J. Lyons and Company Limited, London W. 14, *England*

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Various methods, such as the heavy-atom technique, the Buerger superposition method, the permutation-synthesis method, and the sign-determining methods of Cochran & Douglas and of Hauptman & Karle, were used in the attempted solution of the *c*-axis projection of glutathione. A comparison of the results obtained by the different methods is given.

The heavy-atom technique was also used on the three-dimensional data, using the sulphur atom in the asymmetric unit as the heavy atom. Possible positions for seven atoms nearest to the sulphur atom in the asymmetric unit were selected from the three-dimensional electron-density distribution, and these were used, together with the sulphur atom, in the calculation of a second distribution. The results obtained in this attempt to build up the whole asymmetric unit by addition of a few atoms at a time, for a molecule such as that of glutathione, are discussed.

Introduction

In the preliminary attempts to determine the crystal structure of glutathione, which crystallizes in the orthorhombic system with space group $P2_12_12_1$, most of the methods of crystal-structure analysis were tried on the *c*-axis projection as it was considered likely to have the least overlap, and has the advantage of a centre of symmetry. The methods tried included the heavy-atom technique (using the sulphur atom as the heavy atom), the permutation-synthesis method (Woolfson, 1954), the Buerger synthesis method, the Cochran & Douglas method (1955) and the method of Hauptman & Karle (1953). Most of the methods gave essentially the same *c*-axis projection pattern, and the difficulty lay in the fact that the molecule could be fitted on to the general system of peaks in a number of different ways. This account gives some comparison of the results obtained by the application of these methods to the determination of the configuration of a molecule such as that of glutathione.

What was considered to be essentially the correct molecular configuration was first indicated by the arrangement of peaks on a *c*-axis Fourier projection pattern based on signs derived by the Cochran & Douglas method, but the partial refinement of the structure from this pattern proved unexpectedly difficult. When, therefore, it was decided to use the heavy-atom technique on the three-dimensional data, attempts were made to determine the molecular configuration from it without reference to the results derived from the work on the *c*-axis projection. From the three-dimensional distribution based on the sulphur atoms only, and using standard bond lengths and bond angles, seven atomic positions were chosen in regions of positive electron density near to the sulphur atom in the asymmetric unit, and consistent with the structure of the glutathione molecule. A second distribution based on the sulphur atom, and these seven atoms all ranked as carbon atoms, was calculated. It was thought to be of interest to show the type of results to be expected from a procedure such as this, in which it was hoped to build up the whole asymmetric unit by the addition of a few atoms at a time.