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

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(*Received* 31 *January* 1958)

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.

The heavy-atom technique

The parameters of the sulphur atom in the asymmetric unit of the glutathione structure were determined from the Harker sections of the three-dimensional Patterson function. They were found to be 0·291, 0·641, 0·335, expressed as fractions of the cell edges a, b and c respectively. The sulphur parameters were used to determine the signs of the $(hk0)$ structure factors, and a c-axis Fourier projection containing 180 terms was calculated.

The Fourier map, with contours at arbitrary intervals, is shown in Fig. 1(a) and the contributions of the sulphur atom in the asymmetric unit, expressed as fractions of its maximum possible contribution, to the structure factors are shown in Table 1, column (1). The Fourier pattern (Fig. 1(a)) has a heavy peak A in a similar position to the sulphur peak S close to a screw diad axis parallel to c, a heavy peak B on a screw diad axis parallel to b, and a well defined system of peaks C, D, E and F.

In an attempt to accentuate the arrangement of peaks representing the rest of the molecule, the sulphur peak was decreased to one-third of its height, the Fourier map was sampled to obtain the signs of the structure factors, and a second Fourier map was calculated using these signs. This process was repeated twice more, but as the Fourier pattern appeared to be deteriorating in that a chain of peaks which might represent the backbone of the molecule appeared to be breaking up, the process was discontinued. The molecule could be arranged on the series of patterns resulting from that shown in Fig. 1(a) in a slightly diagonal chain configuration, a typical arrangement being shown in Fig. 1(b), for which the values of $F_c(hk0)$ are given in Table 1, column (2), and the reliability index R is 52% over 209 terms. Various alternative arrangements of the terminal carboxyl and amino–carboxyl groups were tried, but in the course of a set of seven Fouriers, R could not be decreased below 50%. Two main conclusions were drawn:

1. As the peak representing atom N_1 showed a tendency to disappear completely, it was concluded that the chain of the molecule does not cross the edge of the unit cell at $y = 1$.
2. As a marked break in the chain of peaks between C_4 and C_6 persisted in appearing, it was concluded that the chain of the molecule does not cross the screw diad axis parallel to b at $x = \frac{1}{4}$ in the projection.

The permutation-synthesis method

The pattern chosen from the first 256 possibilities given by this method for glutathione, and selected

Table 1. *Structure factors and signs*

$\|F_o\|$	Observed structure amplitudes.
S	Geometrical structure factors calculated from the sulphur atoms.
F_6	Calculated structure factors based on an incorrect arrangement of the molecule.
P.S.	Signs obtained by the permutation synthesis method.
*W*81.	90 signs determined by the Cochran & Douglas and by the sign-relationship methods.
H.K.	Signs determined using the method of Hauptman & Karle.
F.P.	Structure factors calculated for the final c-axis projection in the partial refinement of the structure in two dimensions.
C	Signs chosen in order to fix the origin of the projection.

hkl	$\|F_o\|$	(1) S	(2) F_6	(3) P.S.	(4) *W*81.	(5) H.K.	F.P.
Signs correct		132	134	13	80	123	
Signs incorrect		48	75	3	10	29	
200	23·6	+0·870	− 2·4		+	+	+15·0
400	48·0	+0·514	−38·4	−	+	+	−46·4
600	37·2	+0·025	+63·8	−		+	−46·2
800	40·0	−0·471	+27·9	−	−	−	−27·8
10,0,0	133·6	−0·844	−112·9	−	−	−	−145·0
12,0,0	44·4	−0·999	−17·9		−	−	−38·7
14,0,0	42·0	−0·894	+16·7		−	−	−28·3
16,0,0	35·2	−0·557	−51·1			−	+37·5
18,0,0	8·8	−0·075	−28·7				+17·6
20,0,0	4·8	+0·426	+20·9				− 5·6
22,0,0	4·0		+10·7				+ 7·7
24,0,0	18·4		−26·0		+	+	+13·2
26,0,0	< 4·2						+ 7·9
28,0,0	< 4·1						− 3·8
30,0,0	8·4		− 6·4			?	+ 9·9
32,0,0	< 4·0						− 4·0
34,0,0	< 3·4						+ 0·8
110	10·2	−0·612	+38·4				−11·3
210	2·2	+0·382	− 9·2				+ 8·0
310	44·8	−0·453	+ 5·3	+		?	+55·8
410	42·4	+0·665	+24·2	−	+	+	−42·8

Table 1 (*cont.*)

hkl	$\lvert F_o \rvert$	(1) S	(2) F_6	(3) P.S.	(4) *W*81.	(5) H.K.	F.P.
510	17·2	−0·177	+ 1·0		−	−	−19·4
610	16·0	+0·775	−10·2		+		+17·0
710	24·8	+0·146	−28·3			−	−20·4
810	15·8	+0·684	−34·2		+		+ 8·8
910	10·2	+0·430	+24·4				+13·3
10,1,0	66·0	+0·415	+ 3·4		+	+	+67·2
11,1,0	20·2	+0·603	− 1·5		+	+	−12·4
12,1,0	53·6	+0·039	+18·3		+	+	+46·3
13,1,0	24·6	+0·620	+ 8·8		+	+	+15·0
14,1,0	28·0	−0·347	−32·3		−	−	−30·2
15,1,0	44·0	+0·475	+ 1·5		+	+	+38·6
16,1,0	50·8	−0·644	−42·6		−	+	−50·6
17,1,0	13·0	+0·207	+ 3·4		+		−15·7
18,1,0	17·0	−0·773	+12·8		−	−	−14·3
19,1,0	25·2	−0·115	−36·7		−	−	−25·1
20,1,0	32·4	−0·701	−21·7		−	−	−26·9
21,1,0	< 3·1						− 5·0
22,1,0	7·2		−14·1				− 2·6
23,1,0	17·4		+ 0·7		−	−	−16·4
24,1,0	4·8		+16·9				+ 7·3
25,1,0	4·8		− 0·8				− 5·0
26,1,0	13·4		+ 6·4		+	−	+23·4
27,1,0	16·6		− 8·8		−	−	−15·2
28,1,0	< 4·1						− 0·7
29,1,0	8·2		+ 5·6			?	+10·1
30,1,0	17·0					+	+15·6
31,1,0	6·2					+	+ 9·9
32,1,0	7·6					+	+ 3·3
33,1,0	4·0						+ 8·6
34,1,0	< 3·2						− 3·7
35,1,0	4·0						+ 1·0
020	37·2	−0·200	0	+		−	−37·6
120	26·8	−0·250	+35·0		−	+	+36·8
220	18·0	−0·174	−11·6				−20·7
320	116·4	−0·684	−82·9	−*C*	−*C*	−	−167·6
420	34·0	−0·103	−28·5			+	+42·6
520	73·0	−0·941	−53·3	−	−	−	−76·5
620	9·6	−0·005	−11·8				+ 6·3
720	87·4	−0·954	−65·6	−	−	−	−105·1
820	44·2	+0·094	+20·0	−	+	−	+40·9
920	10·2	−0·718	+ 2·5				+24·4
10,2,0	20·8	+0·169	−36·3		+	+	+19·2
11,2,0	4·0	−0·297	−21·7				+ 3·6
12,2,0	19·8	+0·200	+26·4			+	−15·6
13,2,0	20·4	+0·202	+ 3·7		+	+	+33·7
14,2,0	45·6	+0·179	+24·2		−		−37·5
15,2,0	23·4	+0·648	+21·0		+	+	+24·1
16,2,0	< 3·4						− 8·5
17,2,0	37·0	+0·926	+20·8		+	+	+39·3
18,2,0	5·8	+0·015	− 7·7				+ 9·4
19,2,0	8·8	+0·963	+17·3				+ 7·5
20,2,0	17·2	−0·082	+ 0·8		−	−	−17·7
21,2,0	10·4		−10·3				+ 3·1
22,2,0	< 4·0						+ 5·6
23,2,0	10·6		+17·4				− 9·3
24,2,0	8·0		+ 0·6				− 7·7
25,2,0	< 4·2						− 7·9
26,2,0	9·8		+ 8·5		−	−	+ 8·1
27,2,0	10·0		+ 3·0			+	+ 5·7
28,2,0	< 4·0						+ 0·2
29,2,0	7·8		−10·6				−11·5
30,2,0	9·8		− 1·7			+	− 6·2
31,2,0	9·8					−	− 7·2
32,2,0	11·2					+	+ 5·7
33,2,0	< 3·4						− 0·7
34,2,0	11·4					+	+10·9
35,2,0	< 4·5						+ 5·9

Table 1 (*cont.*)

hkl	$\lvert F_o \rvert$	(1) S	(2) F_6	(3) P.S.	(4) *W*81.	(5) H.K.	F.P.
130	8·8	+0·856	+23·0				+ 3·5
230	9·0	+0·229	−14·9				+ 0·1
330	69·8	+0·634	+39·8	+*C*	+	+	+91·6
430	28·8	+0·399	− 1·8			+	+37·9
530	15·0	+0·247	−14·1				+ 6·2
630	14·8	+0·465	+20·2		+		−10·5
730	9·2	−0·204	−23·3				+ 7·3
830	17·2	+0·410	−21·7		+	+	−17·7
930	34·4	−0·602	+20·5	−	−	−	−42·0
10,3,0	6·8	+0·249	+20·8				− 0·1
11,3,0	14·8	−0·843	−12·8			−	−12·1
12,3,0	3·6	+0·023	+ 2·0				− 6·7
13,3,0	32·4	−0·866	−11·6		−	−	−45·4
14,3,0	6·2	−0·208	+ 7·6				− 4·8
15,3,0	49·0	−0·664	−29·0		−*C*	−	−42·4
16,3,0	8·0	−0·386	− 1·0				− 6·0
17,3,0	22·0	−0·289	− 0·7		−	−	−21·5
18,3,0	8·0	−0·464	+ 4·5				+ 1·1
19,3,0	29·6	+0·160	− 1·4		+	+	+36·0
20,3,0	10·0	−0·421	− 2·8				− 5·0
21,3,0	4·6		+ 2·8				+ 7·5
22,3,0	< 4·1						+ 0·3
23,3,0	12·8		+12·4		+	+	+15·4
24,3,0	14·0		+ 1·3		−	−	− 6·1
25,3,0	6·4		+ 5·5				+ 4·3
26,3,0	14·0		+16·5		+	+	+10·6
27,3,0	19·0		+ 0·6		+	+	+13·1
28,3,0	7·0		− 2·2			+	+ 6·5
29,3,0	7·8		− 1·3			+	+ 1·4
30,3,0	11·0					+	− 6·8
31,3,0	< 3·4						− 1·4
32,3,0	< 3·0						+ 0·8
33,3,0	8·2					−	− 8·8
34,3,0	5·2					+	+ 5·2
040	17·2	−0·920	− 0·9		−	−	−22·4
140	42·8	+0·100	+33·0	+	+	+	+53·2
240	25·0	−0·800	− 9·5		−	−	−27·4
340	27·4	+0·273	+23·7		+	+	+35·0
440	31·8	−0·473	−26·1		−	−	−43·2
540	25·6	+0·375	− 4·7			+	−14·9
640	41·4	−0·023	+24·7	−	+	+	+58·2
740	13·8	+0·380	− 4·1		+	+	−13·9
840	14·4	+0·433	+13·5		+	+	−10·1
940	10·4	+0·287	+17·7				+19·6
10,4,0	19·4	+0·776	+25·3		+	+	+33·4
11,4,0	18·2	+0·118	−10·5		−	+	−20·4
12,4,0	33·4	+0·919	+20·0		+	+	+30·8
13,4,0	6·8	−0·081	−13·4				− 7·9
14,4,0	18·0	+0·822	+12·6		+	+	+18·9
15,4,0	< 3·6						− 4·5
16,4,0	< 3·7						− 8·7
17,4,0	13·0	−0·369	−15·3		−		−13·4
18,4,0	5·0	+0·069	−20·3				− 4·8
19,4,0	10·0	−0·384	−16·5				+10·7
20,4,0	25·2	−0·392	− 1·7		−	−	−32·3
21,4,0	14·0		+11·8		−	−	−14·4
22,4,0	9·6		−12·3				− 9·5
23,4,0	< 4·2						+ 6·7
24,4,0	< 4·2						− 5·4
25,4,0	9·4		− 3·0		+	+	+ 5·2
26,4,0	< 4·0						− 3·0
27,4,0	12·4		+10·9			+	+10·3
28,4,0	< 3·6						+ 3·7
29,4,0	5·2		+ 6·7				+ 4·9
30,4,0	3·2		− 8·6				+ 5·6
31,4,0	< 3·0						+ 3·3
32,4,0	< 2·6						+ 4·0
33,4,0	2·2						− 3·3

Table 1 (*cont.*)

Index.	$\lvert F_o \rvert$	(1) S	(2) F_6	(3) P.S.	(4) W81.	(5) H.K.	F.P.
150	5.2	+.270	+ 16.0				- 3.3
250	41.8	-.473	+ 0.2	-	-	-	- 48.5
350	16.8	+.200	- 29.1			+	+ 19.9
450	26.8	-.824	- 15.1		-	-	- 29.9
550	<3.4						- 1.9
650	34.0	-.960	- 19.5		-	-	- 49.8
750	13.0	-.064	+ 16.4			-	- 5.5
850	18.2	-.847	- 29.0		-	-	- 16.1
950	3.4	-.190	- 14.2				- 0.2
10,50	16.2	-.515	- 10.6		-	-	- 23.1
11,50	<4.2						- 1.1
12,50	<4.3						+ 11.1
13,50	5.8	-.273	+ 11.3				+ 0.8
14,50	<3.8						- 1.7
15,50	5.0		+ 9.4				+ 9.5
16,50	26.6	+.798	- 1.4		+	+	+ 38.5
17,50	40.8	-.091	- 21.1		-	-C	- 37.7
18,50	16.6	+.957	+ 16.0		+	+	+ 13.6
19,50	12.2	+.050	- 4.9		+	+	+ 6.3
20,50	< 4.2						+ 6.2
21,50	11.8		+ 8.0			+	+ 10.5
22,50	<4.1						- 4.4
23,50	<4.1						- 1.8
24,50	23.8		+ 4.5		+	+	+ 16.6
25,50	6.4						+ 3.3
26,50	<3.7						- 5.6
27,50	16.6		+ 3.6			-	+ 8.4
28,50	10.6		- 2.8			-	- 11.1
29,50	<3.0						- 1.4
30,50	<2.8						- 5.1
31,50	<2.4						+ 0.3
32,50	<2.0						- 2.1
060	8.8	+.567	+ 17.5		+		+ 13.6
160	9.2	+.210	+ 5.5				+ 2.2
260	10.0	+.493	+ 7.3				+ 11.5
360	15.8	+.575	+ 13.4		+	+	+ 15.1
460	10.4	+.291	+ 0.2				+ 12.9
560	14.2	+.791	+ 5.1		+	+	+ 13.0
660	8.8	+.014	- 14.9				- 5.0
760	12.8	+.802	+ 4.5		+		+ 15.7
860	10.0	-.267	- 12.4				- 12.2
960	7.0	+.604	- 0.3				- 6.3
10,60	10.0	-.479	- 10.5				+ 7.3
11,60	14.0	+.250	- 9.7			+	+ 10.2
12,60	14.0	-.566	- 4.1			-	- 16.1
13,60	17.6	-.170	- 10.7		-	-	- 19.9
14,60	30.8	-.507	- 9.0		-	-	- 27.1
15,60	15.8	-.545	- 11.4		-	-	- 12.6
16,60	11.0	-.316	- 9.5				+ 2.1
17,60	19.4	-.779	+ 5.1		-	-	- 19.8
18,60	6.8	-.043	+ 6.0				- 1.1
19,60	7.6	-.810	- 1.0				- 1.3
20,60	7.0	+.242	+ 6.4				+ 7.2
21,60	9.6		- 13.8			-	+ 7.5
22,60	13.6		+ 9.6			+	- 11.7
23,60	12.0		+ 5.1			-	+ 10.3
24,60	12.4		+ 6.7			+	+ 9.6
25,60	5.6		+ 8.8				- 2.8
26,60	<3.4						+ 0.3
27,60	6.6		+ 0.6			+	+ 9.0
28,60	6.6		+ 4.1			+	+ 4.9
29,60	<4.5						+ 3.9
30,60	<2.0						- 6.5
170	27.4	-.964	- 2.6		-	-	- 29.5
270	15.4	-.040	+ 19.5			+	- 13.9
370	10.8	-.714	- 0.8				- 14.6
470	< 3.4						+ 2.4
570	6.2	-.278	- 15.2				+ 7.2
670	18.2	-.082	- 3.1		+	-	+ 14.2
770	6.8	+.229	+ 4.4				+ 9.5
870	<4.1						+ 2.6
970	<4.1						+ 10.0
10,70	26.6	-.044	- 0.7		-	-	- 29.4
11,70	9.0	+.950	+ 6.8				+ 9.0
12,70	<4.2						+ 0.2
13,70	26.8	+.976	+ 17.8		+	+	+ 30.6
14,70	<4.2						- 2.2
15,70	<4.2						- 2.0
16,70	<4.1						+ 3.8
17,70	<4.0						- 0.1
18,70	14.8	+.082	- 1.8			-	- 12.8
19,70	5.6	-.180	+ 8.8				- 4.7
20,70	23.2	+.074	+ 1.5			+C	+ 17.5
21,70	5.2		- 2.8				- 5.0
22,70	11.2		+ 7.0			+	+ 10.1
23,70	8.6		- 10.4			-	- 12.1
24,70	<3.1						- 3.0
25,70	4.4		- 0.4				- 2.7
26,70	3.8		+ 1.2				- 3.1
27,70	6.8		+ 5.4			-	- 5.8
28,70	3.8		+ 4.7				+ 1.7

Index.	$\lvert F_o \rvert$	(1) S	(2) F_6	(3) P.S.	(4) W81.	(5) H.K.	F.P.
080	22.8	+.694	+ 6.8		+	+	+ 18.1
180	6.8	-.184	- 6.6				- 1.5
280	<4.2						+ 6.4
380	14.8	-.503	- 18.0			-	- 19.2
480	4.1	+.357	+ 4.7				- 1.5
580	12.0	-.691	+ 7.5			-	- 11.4
680	<4.2						- 4.1
780	8.6	-.701	- 20.2				- 12.3
880	5.8	-.327	- 5.6				- 0.2
980	17.4	-.528	- 18.3		-	-	- 14.2
10,80	13.6	-.586	- 7.0			-	- 13.1
11,80	9.8	-.218	+ 3.9			-	- 8.5
12,80	7.4	-.693	+ 6.6				- 3.8
13,80	11.8	+.148	+ 4.8			-	+ 9.9
14,80	14.8	-.620	+ 6.2			-	- 11.1
15,80	5.0	+.476	+ 1.5				+ 5.3
16,80	8.6	-.387	- 6.3			-	+ 9.6
17,80	10.4	+.680	+ 6.4			+	+ 9.3
18,80	7.4	-.052	- 0.9			+	+ 7.5
19,80	11.0	+.708	+ 12.4			+	+ 12.6
20,80	< 3.2						+ 1.2
21,80	< 3.0						+ 1.7
22,80	< 2.9						+ 0.3
23,80	2.8		+ 0.7				- 0.5
24,80	4.6		- 0.3				+ 3.6
25,80	< 1.9						- 0.1
190	<4.0						+ 1.4
290	7.2	+.490	- 1.6				+ 4.6
390	3.2	+.085	- 2.8				+ 3.5
490	14.0	+.852	- 3.0		+	+	+ 13.9
590	6.8	+.033	+ 1.1			+	- 3.6
690	<3.8						+ 2.8
790	<3.8						- 4.5
890	<3.8						+ 5.2
990	6.8	-.081	- 3.0			-	+ 7.1
10,90	7.0	+.532	- 3.9			+	+ 5.7
11,90	11.6	-.113	+ 1.6			-	- 11.7
12,90	5.8	+.050	- 9.8				- 6.3
13,90	<3.4						+ 2.8
14,90	<3.4						- 3.5
15,90	<3.2						+ 0.1
16,90	<3.0						- 4.5
17,90	<2.9						+ 3.1
18,90	9.6	-.990	- 8.6			-	- 8.2
19,90	<2.5						+ 0.3
20,90	8.4	-.899	- 4.0			-	- 8.8
21,90	5.6		+ 2.8			+	+ 5.1
0,10,0	3.2	-.844	- 8.1				- 11.4
1,10,0	<3.4						+ 3.1
2,10,0	12.8	-.734	- 8.4			-	- 10.6
3,10,0	5.0	-.374	- 2.7				- 1.2
4,10,0	7.4	-.434	- 16.7			-	- 8.6
5,10,0	<3.1						- 2.0
6,10,0	<3.1						+ 6.3
7,10,0	10.4	-.572	- 7.4			-	- 10.1
8,10,0	<3.0						+ 2.2
9,10,0	<2.9						+ 1.2
10,10,0	9.6	+.712	+ 8.0			+	+ 13.0
11,10,0	<2.8						- 1.6
12,10,0	6.8	+.843	+ 8.0			+	+ 7.2
13,10,0	7.6	+.110	+ 5.0			+	- 1.4
14,10,0	3.4	+.755	+ 6.1				+ 3.9
15,10,0	3.4	+.354	- 6.0				- 1.8
16,10,0	5.4	+.470	+ 4.1			+	+ 5.2
1,11,0	6.2	+.918	- 6.4			+	+ 8.1
2,11,0	<2.0						+ 0.5
3,11,0	2.2	+.679	+ 4.8				+ 0.2
4,11,0	4.6	-.270	- 6.2				- 5.6
5,11,0	3.0	+.265	- 3.0				- 1.8
6,11,0	<1.7						- 0.2
7,11,0	3.4	-.218	-2.2				- 4.3

because it has a heavy peak in the position chosen for the sulphur atom, and a general even distribution of electron density, is shown in Fig. 1(*c*) and the signs of the structure factors are given in Table 1, column (3). The pattern shows the same general arrangement of peaks as in Fig. 1(*a*) and two possible configurations of the molecule have been indicated on it. The extended chain configuration has been abandoned in favour of a curled structure, and the molecule does not cross the screw diad axis parallel to *b*. However, with these configurations there was no choice but to cross the edge of the cell at $y = 1$, although from the previous section it was concluded that this was incorrect. Attempts to refine these two similar structures were unsuccessful.

It was concluded that the permutation-synthesis method had not proved successful in this case for the following reasons:

1. The Huggins masks used in the method are designed for a square projection. As $a = 28{\cdot}05$ Å and $b = 8{\cdot}80$ Å in the *c*-axis projection of glutathione it is a particularly unfavourable case, although a cylindrical lens was used for enlarging the patterns in the *a* direction only when choosing the most likely one.
2. As only orders up to 10 were used in the method, and as the maximum *h* value for glutathione is 35 and as it is the higher orders which give the recognizable detail to the pattern, the choice of the correct pattern was rendered very difficult in this case.

Various molecular configurations indicated both by the permutation-synthesis pattern (Fig. 1(*c*)) and by the pattern in Fig. 1(*a*) were tested by forming the optical transform of the four molecules in the unit cell for each of the molecular configurations in turn, and comparing them with the weighted reciprocal lattice. There was some measure of agreement for all of the configurations tried, due to the fact that the arrangement of peaks shown in Fig. 1(*a*) is essentially correct. In no case was the agreement satisfactory.

The Buerger superposition method (1951)

The sharpened *c*-axis Patterson projection pattern was duplicated and the origin of one pattern placed in turn on each of the S–S vector peaks of the second pattern, and regions of overlap of positive peaks were drawn in. The four patterns so obtained were used to produce the composite pattern of Fig. 1(*d*). The familiar arrangement of peaks S, *A*–*F* is again very evident.

Fig. 1(*e*) shows the pattern obtained by the method of Beevers & Robertson (1950). Although the S-peak position, which lies midway between two lattice points in the *x* direction, was taken to be at a lattice point for the purposes of calculation and therefore $\frac{1}{4}$ Å from its true position, the actual atomic positions as finally determined (shown by dots in Fig. 1(*e*)) do tend to lie on peaks on this pattern.

However, the patterns given by application of the superposition method did not indicate any configuration of the molecule or a portion of the molecule which had not already been considered in connection with the pattern of Fig. 1(*a*).

The Cochran & Douglas method

The nine sets of signs for the 28 largest ($hk0$) structure factors which gave maximum χ values in the method

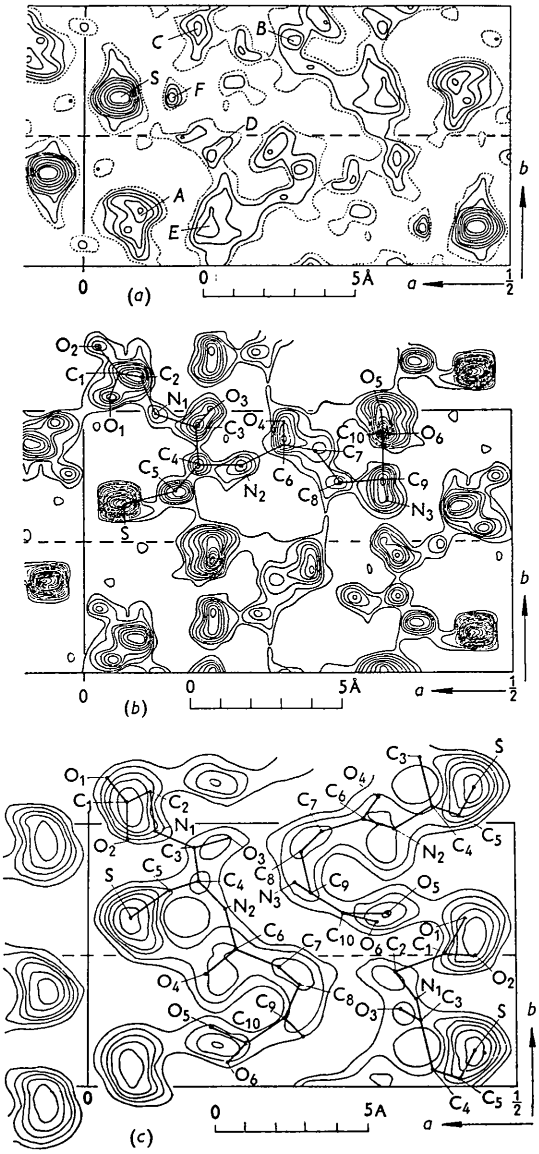
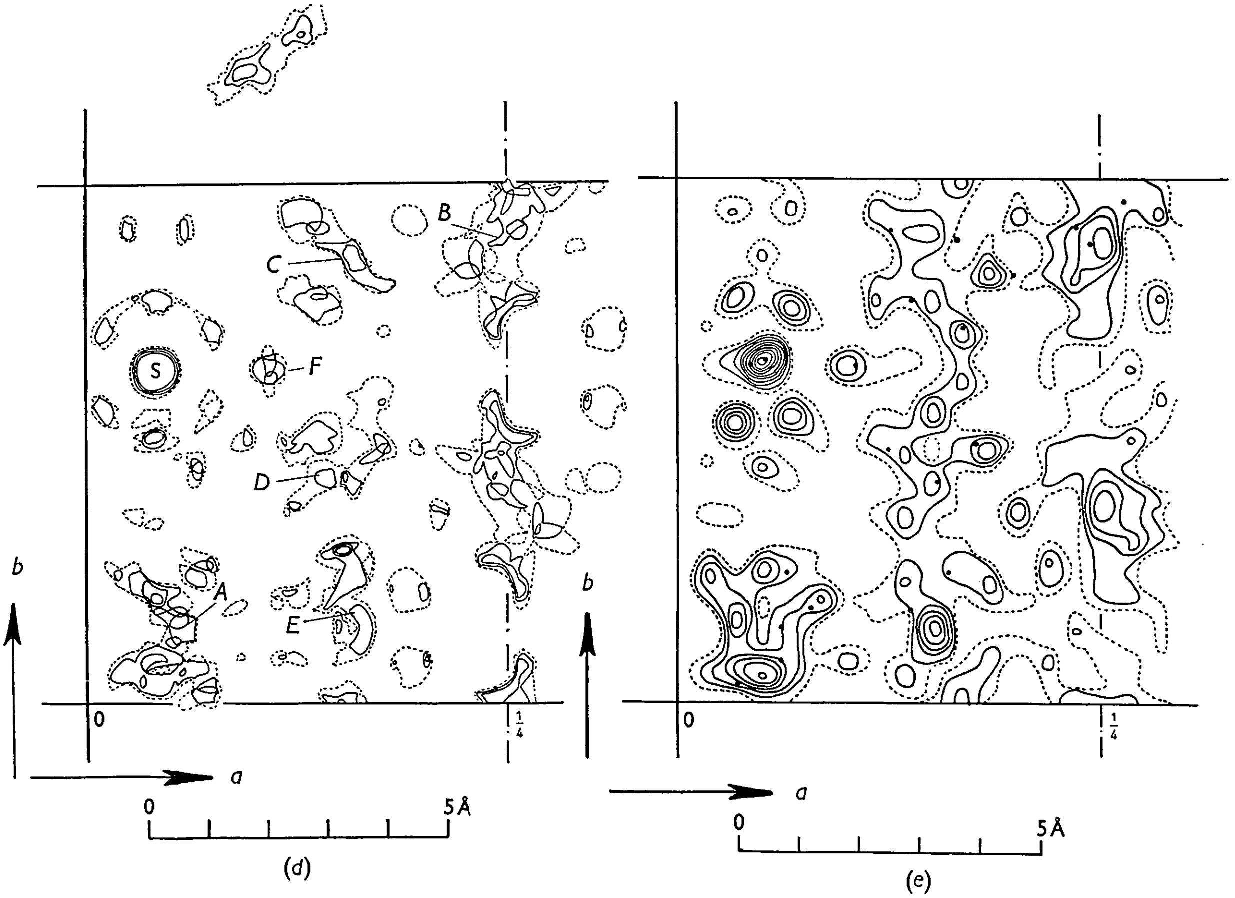


Fig. 1. (*a*) The *c*-axis projection Fourier pattern based on 180 terms with signs determined by the sulphur atoms alone. (*b*) Fourier pattern based on an incorrect configuration of the molecule. $R = 52\%$ over 209 terms. (*c*) Fourier pattern based on 16 terms with signs determined by the permutation-synthesis method.



(*d*)

(*e*)

Fig. 1. (*d*) Pattern given by the Buerger superposition method. (*e*) Pattern given by the method of Beevers & Robertson. All contours are at arbitrary intervals.

of Cochran & Douglas (1955) are shown in Table 2, and the corresponding Fourier patterns based on the four sign sets *W*1, *W*2, *W*5 and *W*8, which differ from one another by more than one or two signs, are shown in Fig. 2(*a*), (*b*), (*c*) and (*d*) respectively. All of these patterns have a heavy peak which could correspond to the sulphur atom in the asymmetric unit, but only in the pattern of Fig. 2(*d*) is the heavy peak in the position already chosen for the sulphur atom from the Harker sections. The sign set *W*8 was used in conjunction with the sign relationship to determine the signs of the 62 next largest structure factors, and these 90 signs (Table 1, column (4)) were used in the calculation of a Fourier pattern. This pattern indicated a configuration of the molecule which had not previously been considered, and in which all the hydrogen bonds could be formed satisfactorily. The atomic parameters were partially refined and the Fourier pattern corresponding to $R = 38\%$ over 127 terms is shown in Fig. 2(*e*).

This projection, although thought to be essentially correct, proved difficult to refine for the following reasons:

1. Peak representing oxygen atom O_1 was very elongated and lower in height than peaks representing other oxygen atoms in the structure. This peculiarity was not considered to be entirely due to thermal motion of the atom but rather to the choice of incorrect atomic parameters, changes in which, however, failed to improve the shape of the peak.
2. The distance between C_6 and atom O_1 of the molecule M'' appeared to be too small, being slightly less than 3·0 Å.
3. Although atoms C_4 and C_5 had been placed on the one peak in this projection it was concluded from the height of this peak that C_5 should be transferred to the sulphur peak, but this move was found to increase the value of R to over 40%.

Although it was considered that this arrangement of the molecule was essentially correct the difficulty in refinement might be caused by certain parts of the projection requiring somewhat different interpretation. In an attempt to solve this difficulty by obtaining a little extra resolution the method of Hauptman & Karle was tried.

Table 2. *The signs for the 28 largest (hk0) structure factors*

hkl	$\|U\|$	*W*1	*W*2	*W*3	*W*4	*W*5	*W*6	*W*7	*W*8	*W*9	Correct
10,0,0	0·364	−	−	−	−	−	−	−	−	−	−
10,1,0	0·186	+	−	−	−	−	−	+	+	−	+
12,1,0	0·176	+	+	+	+	+	+	+	+	+	+
15,1,0	0·185	+	+	+	+	+	+	+	+	+	+
20,1,0	0·203	−	+	+	+	+	+	−	−	+	−
27,1,0	0·189	+	−	+	+	+	+	−	−	+	−
520	0·174	−	+	+	+	+	+	−	−	+	−
720	0·232	+	+	−	−	−	−	−	−	−	−
14,2,0	0·196	+	+	−	−	−	−	−	−	−	−
17,2,0	0·197	−	−	+	+	+	+	+	+	+	+
330	0·195	−	+	−	−	−	−	+	+	−	+
27,3,0	0·237	−	+	−	−	−	−	+	+	−	+
12,4,0	0·172	−	+	−	−	−	−	+	+	−	+
20,4,0	0·210	+	+	−	−	−	−	−	−	−	−
250	0·192	+	+	−	−	−	−	−	−	−	−
650	0·172	+	−	−	+	+	+	−	−	−	−
16,5,0	0·206	−	+	+	+	−	−	−	+	+	+
24,5,0	0·302	−	+	−	−	−	−	+	+	−	+
14,6,0	0·260	+	+	−	−	−	−	−	−	−	−
17,6,0	0·196	+	+	−	−	−	−	−	−	−	−
170	0·209	+	+	−	−	−	−	−	−	−	−
10,7,0	0·243	−	+	+	+	+	+	−	−	+	−
13,7,0	0·281	+	−	−	−	−	+	+	+	+	+
080	0·232	+	+	+	+	+	+	+	+	+	+
980	0·203	+	−	+	+	+	+	−	−	+	−
490	0·183	−	+	+	+	+	+	+	+	+	+
320	0·254	← Chosen negative →									−
15,3,0	0·252	← Chosen negative →									−
No. incorrect		17	13	11	12	13	12	1	0	10	

The method of Hauptman & Karle

The (*hk*0) structure factors were divided into the four groups: *A* with *h* and *k* both even, *B* with *h* and *k* both odd, *C* with *h* odd and *k* even and *D* with *h* even and *k* odd. The symbols are those used in the authors' monograph (1953).

From Σ_4, Σ for (10,0,0), the strongest reflexion in group *A* for glutathione, was found to have a small negative value. As the sulphur position determined from the Harker sections lies practically on (10,0,0) negative, and as such a strong reflexion would require the contribution from the sulphur atom to the structure factor to reinforce that from the rest of the molecule, this sign was therefore accepted as being correct, and no other signs were determined by Σ_4. Six other signs were determined by Σ_1 and Σ_3 together, after which Σ_2, the sign relationship term, made by far the largest contribution to Σ and dominated the sign determinations for the remaining terms in group *A*.

In group *B* the sign of the largest structure factor (17,5,0) was chosen. One sign was determined by Σ_3' and Σ_5 together, three by the Σ_2, Σ_3' and Σ_5 terms together, four by the $\Sigma_2+\Sigma_5$ terms, and the remaining 21 by the Σ_2 term alone, although it should be noted that all the signs, with the exception of those of (17,5,0) and (13,7,0) could equally well have been determined from the Σ_2 term alone. Σ_6 and Σ_7 were not used at all.

In group *D* the sign of (20,7,0) was chosen, thereby fixing the origin of the projection. Of the 30 signs determined in this group two were given by the Σ_3' and Σ_5 terms together, one by the $\Sigma_2+\Sigma_3'+\Sigma_5$ terms, five by the $\Sigma_2+\Sigma_5$ terms, and the remainder by the Σ_2 term alone. However, all but two of the signs could equally well have been determined by the Σ_2 term alone.

In the fourth group *C*, of the 29 signs which were determined, all of them from the Σ_2 term alone, 25 were later found to have been given correctly. Finally, additional signs were determined in all four groups using the Σ_2 sign relationship term alone.

Of the 152 signs which were determined by the method of Hauptman & Karle (1953) for the (*hk*0) structure factors, 123 were later proved to be correct and 3 were indeterminate. 60 signs were actually determined by the Σ_2 sign-relationship term, but 142 of the total number could equally well have been determined by the Σ_2 term alone. The signs determined by this method are given in Table 1, column (5), and the corresponding Fourier projection is shown in Fig. 2(*f*). The Fourier pattern shows the familiar arrangement of peaks, but had no new detail which was of use in the resolution of the structure.

Three-dimensional trial methods

Although the molecular configuration shown in Fig. 2(*e*) appeared to be essentially correct, the fact that it proved difficult to refine suggested that the Fourier peaks might be capable of a different interpretation for certain portions of the molecule. Therefore, when the three-dimensional distribution based on the sulphur atom only was calculated in an attempt to deter-

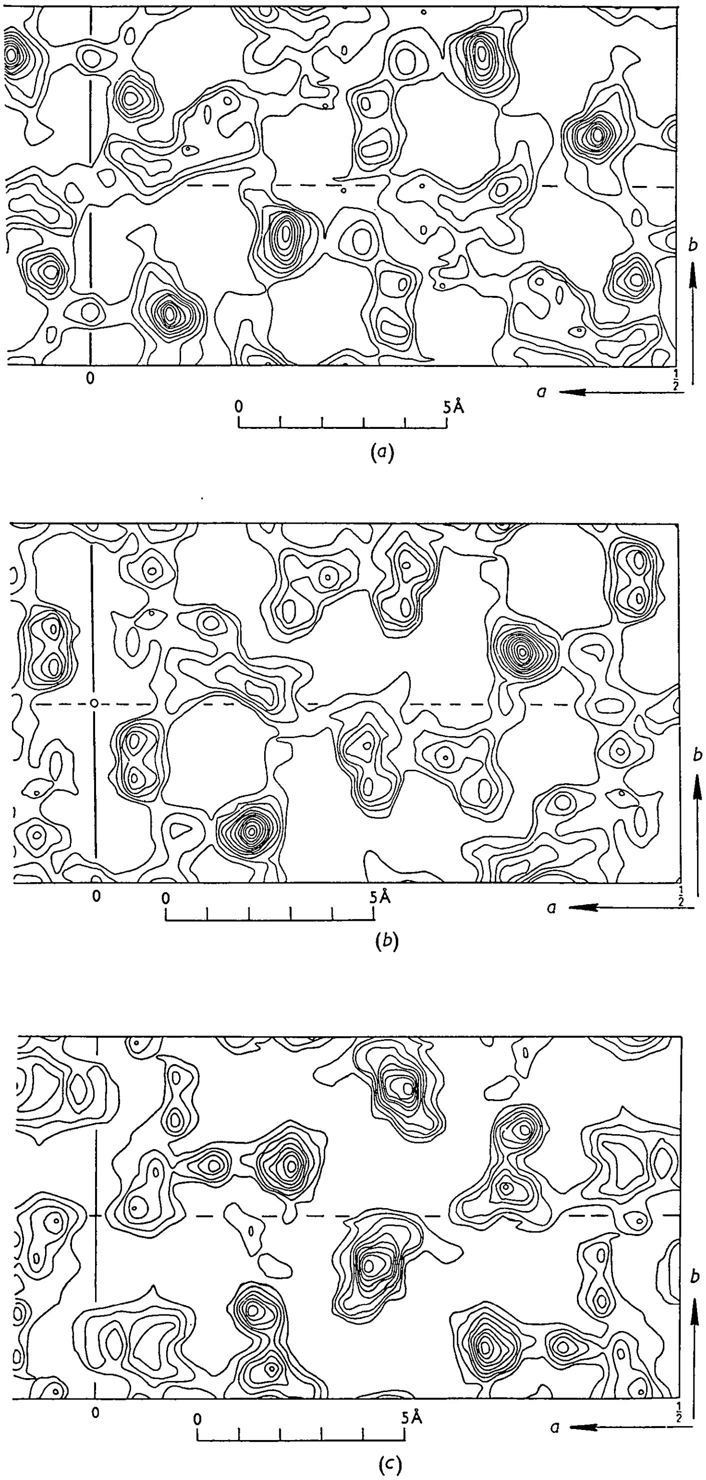


Fig. 2. *c*-axis Fourier projection patterns based on (*a*) sign set *W*1; (*b*) sign set *W*2; (*c*) sign set *W*5.

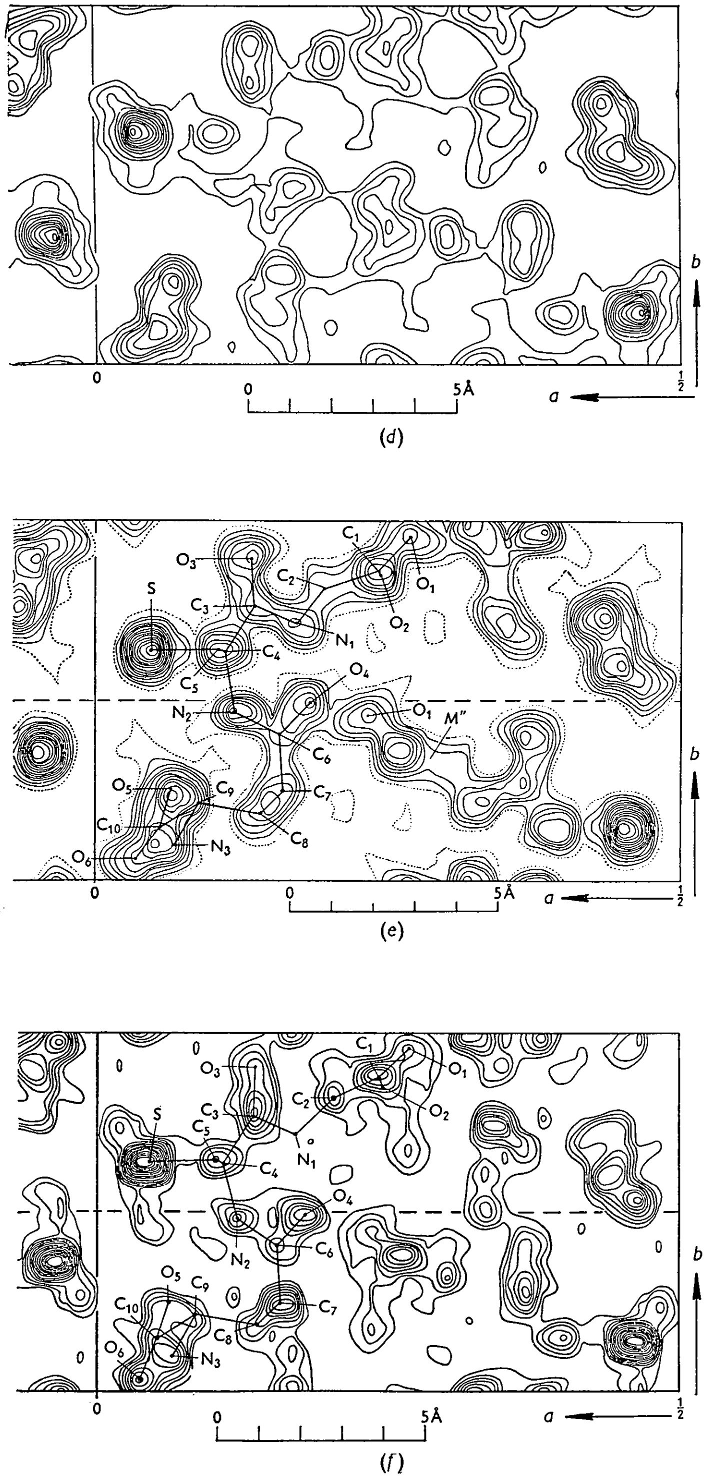


Fig. 2. (*d*) sign set *W*8; (*e*) signs determined by the molecular configuration shown, $R = 38\%$ over 127 terms; (*f*) 152 terms with signs determined by the Hauptman & Karle method. All contours are at arbitrary intervals.

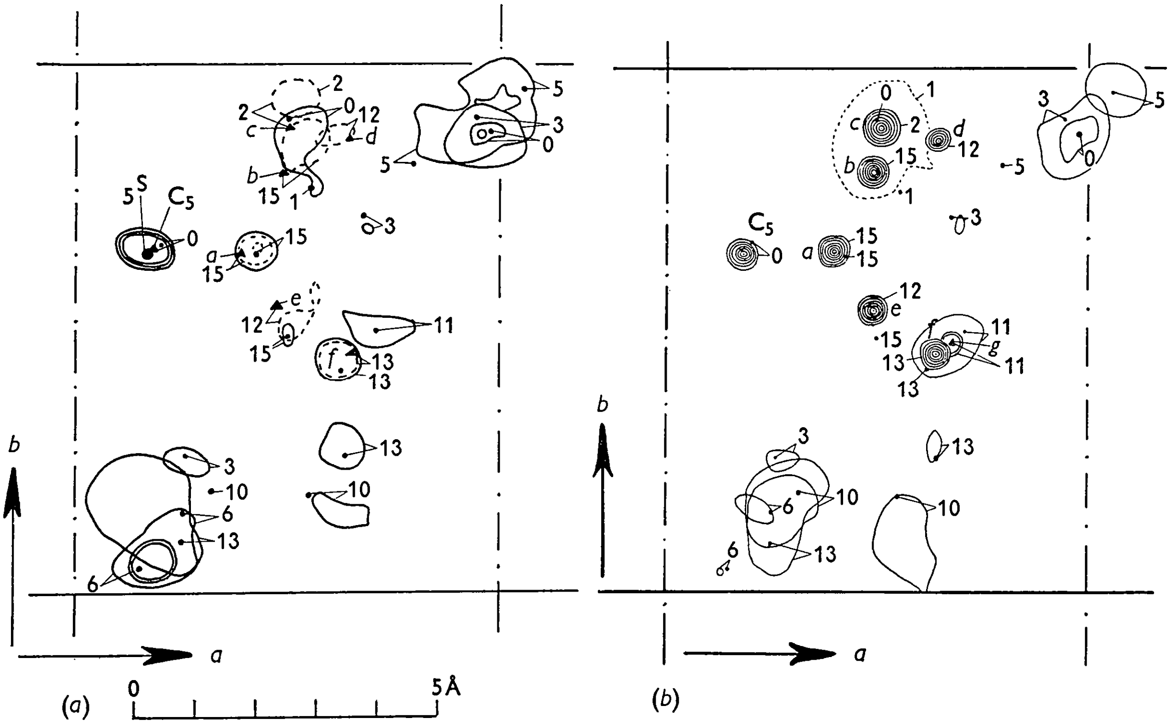


Fig. 3. (*a*) Composite diagram showing seven atomic positions chosen from the three-dimensional electron-density distribution based on 598 terms with phase angles determined by the sulphur atoms. (*b*) Composite diagram representing results obtained from the second distribution based on 900 terms.

mine the positions of the atoms nearest to the sulphur atom in the asymmetric unit, reference was not made to the configuration shown in Fig. 2(*e*).

Using standard bond lengths and bond angles, positions were chosen, lying as far as possible in peak regions of electron density, for seven atoms (C_5, *a*, *b*, *c*, *d*, *e* and *f*) nearest to the sulphur atom. All seven atoms were treated as carbon atoms at this stage. The atomic positions chosen are shown by the triangles in Fig. 3(*a*), the corresponding peak regions in the electron density distribution being outlined by the broken lines; what where later found to be the correct peak regions and atomic positions are indicated by the full lines and by the dots respectively. The numbers attached to the peak regions of the composite diagram (Fig. 3(*a*)) refer to levels in z (z = 0–16) in the three-dimensional distribution.

The positional parameters of the sulphur atom and of the seven carbon atoms were used to calculate the phase angles for a second three-dimensional electron-density distribution containing 900 terms. The results obtained are represented in the composite diagram Fig. 3(*b*) and may be summarized as follows:

1. Wherever an atomic position was postulated a peak appeared in the second three-dimensional distribution, the distance between the postulated and peak positions never being greater than about 0·08 Å, regardless of the fact that several of the postulated positions were of the order of 0·5 Å from the true atomic positions.
2. Atoms *c* and *d* cannot both be present in the glutathione molecule. They were both chosen as possibilities from the first three-dimensional electron-density distribution, which, being very diffuse in the *c* direction, did not indicate any way of choosing between them. It was hoped that in the second distribution the peak representing the wrongly placed atom would be much smaller than the other one.

   The peak representing atom *c* has a maximum ϱ value of 6·7 e.Å^{-3} as shown in Fig. 3(*b*), in which the number of contour lines represents the peak electron-density value, whereas the peak for atom *d* has a maximum ϱ value = 5·3 e.Å^{-3}.
3. Positions postulated for atoms C_5 and *a* were approximately correct, and these atoms gave rise to peaks with ϱ(max.) = 6 and 7 e.Å^{-3} respectively.
4. Although atoms *b* and *c* were of the order of 0·5–0·8 Å from the true atomic positions, this second distribution showed peaks with ϱ(max.) = 7 and 6 e.Å^{-3} respectively at the postulated positions, without any indication of elongation of the peaks in the direction of, or presence of a smaller peak at, the true atomic positions.
5. Atom *f*, placed 0·3 Å from the correct atomic position in the *xy* plane, but at the correct level

in z, produced a peak with $\varrho(\text{max.}) = 6\ \text{e.Å}^{-3}$ at a distance 0·05 Å from the postulated position.

6. There was strong indication for the existence of an atom labelled g in Fig. 3(b) adjacent to atom f, the peak representing g having $\varrho(\text{max.}) = 2{\cdot}5\ \text{e.Å}^{-3}$.
7. No other peaks in this second distribution had $\varrho(\text{max.}) > 1\text{–}2\ \text{e.Å}^{-3}$, but, although they were not recognized at the time, there is a small peak present corresponding to every atom in the structure except one.

From these results a third distribution, calculated using the new positional parameters of the sulphur atom, C_5, and atoms a, b, c, e and f, and omitting atom d and putting in atom g, would probably have produced further information about the molecular configuration. However, as the peaks appeared to move almost imperceptibly from one distribution to the next, and as the difference between correct and incorrect alternatives such as c and d was so small, and as only one new atomic position was clearly indicated, this procedure was not followed, it being considered too time-consuming.

Discussion

Of the methods employed for the determination of the signs of the ($hk0$) structure factors for glutathione the most successful was that of Cochran & Douglas, in which $W8$ had all 28 signs determined correctly. 62 extra signs were determined from these 28 by the use of the sign relationship, and the resulting 90 signs had 89% correct. The Hauptman & Karle method also proved successful, 81% of the 152 signs determined being correct. For the permutation-synthesis method it is claimed that one of the first 256 possibilities will have the signs correct for at least 14 out of the 16 structure factors used in the method. The pattern chosen as the most promising for glutathione corresponds to 13 signs correct out of the 16. The choice was made difficult in this case because of the dimensions of the c-axis projection. In the heavy-atom technique, of the 180 signs determined by the sulphur atom, 73% were correct. For the totally incorrect arrangement of the molecule with $R = 52\%$ over 209 terms 64% of the ($hk0$) structure factors had the correct sign.

All the methods tried gave essentially the same c-axis projection pattern. The Fourier pattern based on the 90 signs derived by the method of Cochran & Douglas was so well resolved that it indicated the correct molecular configuration, and although the one derived by the Hauptman & Karle method had included 62 extra terms it gave no improved useful resolution. The patterns given by the Buerger superposition and Beevers & Robertson methods were very similar to the Fourier pattern based on structure-factor signs determined by the sulphur atoms alone, and, although the system of peaks in these patterns could be interpreted in several different ways, it was not possible at the time to recognize the correct molecular configuration from them.

The heavy-atom technique appears to have worked well as a sign-determining method for this structure using the sulphur atom as the heavy atom, particularly if only those terms are included to which the sulphur atom is making more than 50% of its maximum contribution. This may be due to the fact that for the c-axis projection, in which 89% of the signs of such terms were given correctly by this method, S and C_5 were acting together as a heavy atom, and for the b-axis projection, for which 87% were given correctly, S, O_6 and C_{10} were acting together. In the case of the three-dimensional structure factors 76% of the phase angles determined by the sulphur atoms alone were within ±45° of the correct phase angle.

Starting from the partial three-dimensional distribution based on the sulphur atom in the asymmetric unit, it seems unlikely that the structure could have been determined completely by the gradual building up of the asymmetric unit from one distribution to the next. Such a method would be of greater value in the case of ring structures, the sets of closely related peaks representing the rings in the structure being easier to recognize in the partial three-dimensional electron-density distributions.

The author is glad to have this opportunity of thanking Dr Woolfson for the trial of the permutation-synthesis method on glutathione, Mr G. W. White for assistance with some of the calculations, Miss A. V. Bell for the preparation of the drawings and the Directors of J. Lyons and Company Limited for permission to publish.

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