

peptide bonds. However, the hydrogen bonding scheme in the glycylglycine phosphate structure appears to be largely dominated by the phosphate-carboxyl and phosphate-phosphate interactions. It is possible that, in the absence of a terminal carboxyl group, phosphate-carbonyl hydrogen bonding would be an important mode of phosphate-peptide binding. We are currently investigating this possibility.

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References

- AOKI, K., NAGANO, K. & IITAKA, Y. (1971). *Acta Cryst.* **B27**, 11.
- BISWAS, A. B., HUGHES, E. W., SHARMA, B. D. & WILSON, J. N. (1968). *Acta Cryst.* **B24**, 40.
- BUSING, W. R. & KOSTANSEK, E. C. (1971). *Abs. Amer. Cryst. Assoc. Winter Meeting*, Columbia, South Carolina, p. 35.
- BUSING, W. R., MARTIN, K. O. & LEVY, H. A. (1962). *ORFLS*. Report ORNL-TM-305, Oak Ridge National Laboratory, Oak Ridge, Tennessee.
- CALLERI, M. & SPEAKMAN, J. C. (1964). *Acta Cryst.* **17**, 1097.
- COPPENS, P. & HAMILTON, W. C. (1970). *Acta Cryst.* **A26**, 71.
- EDSALL, J. T., FLORY, P. J., KENDREW, J. C., LIQUORI, A. M., NEMETHY, G. & RAMACHANDRAN, G. N. (1966). *J. Mol. Biol.* **15**, 399.
- HOWELLS, E. R., PHILLIPS, D. C. & RODGERS, D. (1950). *Acta Cryst.* **3**, 210.
- International Tables for X-ray Crystallography* (1962). Vol. III, pp. 202, 214. Birmingham: Kynoch Press.
- JOHNSON, C. K. (1965). *ORTEP*. Report ORNL-3794, revised, Oak Ridge National Laboratory, Oak Ridge, Tennessee.
- MARSH, R. E. & DONOHUE, J. (1967). *Advanc. Protein Chem.* **22**, 235.
- PARTHASARATHY, R. (1969). *Acta Cryst.* **B25**, 509.
- PHILIPPOT, E. & LINDQVIST, O. (1971). *Acta Chem. Scand.* **25**, 512.
- RAMAKRISHNAN, C. & RAMACHANDRAN, G. N. (1965). *Biophys. J.* **5**, 909.
- STEWART, R. F., DAVIDSON, E. R. & SIMPSON, W. T. (1965). *J. Chem. Phys.* **42**, 3175.
- SUNDARALINGAM, M. & PUTKEY, E. F. (1970). *Acta Cryst.* **B26**, 790.
- WEHE, D. J., BUSING, W. R. & LEVY, H. A. (1962). *ORABS*. Report ORNL-TM-229, Oak Ridge National Laboratory, Oak Ridge, Tennessee.
- WILSON, A. J. C. (1942). *Nature, Lond.* **150**, 151.
- ZACHARIASEN, W. H. (1963). *Acta Cryst.* **16**, 1139.

Acta Cryst. (1972). **B28**, 2912

Crystal Structure Determination of Valinomycin by Direct Methods

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The application to the phase determination for valinomycin of the recently devised method of strong enantiomorph discrimination *via* the calculated values of the cosine invariants is described. The initial 552 term *E* map revealed 74 of the 78 nonhydrogen atoms in the structure.

1. Introduction

Crystal structure determination by direct methods is strongly dependent on the tangent formula (Karle & Hauptman, 1956), which in turn requires firstly the evaluation of a more or less broad base of phases. In recent attempts to strengthen the method by solving the latter problem (Hauptman, Fisher, Hancock & Norton, 1969) a least-squares procedure based on an improved method for calculating the values of the cosine invariants was employed. A total of four non-centrosymmetric structures have been solved by this technique. Recent improvements, both in the evaluation of the cosine invariants and in the implementation of the calculated cosines, have led to the solution of some dozen additional noncentrosymmetric crystal structures. However, it has become increasingly apparent that still better procedures for implementing calculated cosine invariants will be needed in order to

tackle successfully non-centrosymmetric structures of great complexity. To this end a method for strong enantiomorph discrimination based on a suitably chosen class of several structure invariants, each approximately equal to $\pm\pi/2$, rather than only a single such invariant, has been recently devised (Hauptman & Duax, 1972).

The method is strongly dependent on the calculated values of certain cosine invariants, in particular invariants of special, well defined types, and the further analysis and interpretation of these invariants. It requires the identification of two (orthogonal) Classes, I and II, of phases φ_{hkl} , with fixed *k*, such that: (1) any two phases in Class I differ from each other by 0 or π , approximately; (2) any two phases in Class II differ from each other by 0 or π , approximately; (3) (orthogonality property) any phase in Class I differs from any phase in Class II by $\pi/2$, approximately. In addition, the associated values of the normalized structure factor

magnitudes, E , are required to be large. Employing the phases of these two classes, it is possible to construct a class, C , of cosine invariants each of which is approximately equal to zero. The corresponding structure invariants, each approximately equal to $\pm\pi/2$, are then used for decisive enantiomorph discrimination.

Three successful applications of this procedure have now been made. The present paper is devoted to a description of the application to the phase determination for valinomycin, $C_{54}N_6O_{18}H_{90}$, space group $P2_1$.

2. Calculated values of the required cosine invariants, $\cos(\varphi_1 + \varphi_2 + \varphi_3)$

The values of some 3000 cosine invariants

$$\cos(\varphi_1 + \varphi_2 + \varphi_3) \tag{2.1}$$

where

$$\varphi_j = \varphi_{h_j}, \quad j = 1, 2, 3, \tag{2.2}$$

$$h_1 + h_2 + h_3 = 0, \tag{2.3}$$

and φ_h is the phase of the normalized structure factor E_h , were calculated by the recently established method of Hauptman *et al.* (1969; but see Hauptman, 1970, for further details). 73 of these cosines, which constitute the hard core of those needed in the phase determination process, are listed in Table 1. In addition, some 80 cosines played a secondary role in that they provided supporting evidence for the identification of the orthogonal classes and aided in the interpretation of the results of the tangent procedures. The parameter A is defined by

$$A = \frac{2\sigma_3}{\sigma_2^{3/2}} |E_1 E_2 E_3| \approx 0.1586 |E_1 E_2 E_3|, \tag{2.4}$$

where

$$E_j = E_{h_j}, \quad j = 1, 2, 3, \tag{2.5}$$

$$\sigma_n = \sum_{j=1}^N Z_j^n, \tag{2.6}$$

Z_j is the atomic number of the j th atom, and N is the number of atoms in the unit cell. (If there is no heavy atom present, then

$$\frac{\sigma_3}{\sigma_2^{3/2}} \approx \frac{1}{N^{1/2}} \tag{2.7}$$

where N is the number of nonhydrogen atoms in the unit cell). Only the cosines with A values greater than 1.5 were calculated. With rare but important exceptions, only those cosines were used whose calculated values were close to or greater than one, the presumption being that the true values of these cosines were then very likely equal to unity, at least approximately, and the identification of these cosines was an important feature of the method. However, the four cosine invariants with serial numbers 636, 1669, 1760, 2315 (Table 1) were calculated to be small or negative and these played a crucial role in the process of phase

determination. For the purpose of comparison, the last column of Table 1 contains the true values of the cosine invariants as obtained from the refined structure. Using identities among the cosine invariants (Karle & Hauptman, 1957; Hauptman, 1970), improved values for a number of these cosines (*e.g.* 3305) were obtained which, however, are not shown in Table 1. Although the better values were for the most part not essential for the present structure determination, these improvements would clearly facilitate the solution of more complex structures.

Table 1. Calculated values of cosine invariants, $\cos(\varphi_1 + \varphi_2 + \varphi_3)$

Serial number	h_1	h_2	h_3	A	Calculated cosine	True cosine
423	11 4	1	10 4 10	1 0 9	1.544	+2.02
557	0 8 0	16 4 9	16 4 9	16 4 9	1.562	+2.14
598	1 4 2	16 4 9	17 0 11	1.568	+1.34	+0.963
636	4 4 3	9 4 10	13 0 7	1.572	+0.30	+0.602
977	1 4 2	11 4 1	10 0 1	1.618	+1.03	+0.992
1115	0 8 0	10 4 10	10 4 10	1.640	+1.61	+0.675
1415	19 4 7	1 4 2 2	20 0 9	1.694	+1.87	+0.962
1669	0 8 0	5 4 3 3	5 4 3	1.743	-0.23	-0.888
1760	0 8 0	9 4 10	9 4 10	1.763	+0.16	-0.689
1876	0 8 0	17 4 7	17 4 7	1.796	+1.06	+0.993
2016	21 3 7	17 3 3	4 0 7	1.832	+1.44	+0.999
2050	11 3 4	18 3 3	7 0 5	1.842	+1.16	+0.969
2212	12 1 4	5 0 2 2	7 1 2	1.892	+1.75	+0.999
2223	7 3 2	5 0 2 2	7 1 2	1.898	+1.30	+0.963
2230	10 1 4	8 8 11	7 0 7	1.901	+1.22	+0.944
2267	0 8 0	11 4 1 1	11 4 1	1.914	+1.44	+0.983
2279	8 1 11	4 0 5	12 1 4	1.917	+1.85	+0.997
2315	0 8 0	21 4 5	21 4 5	1.931	-0.85	-0.860
2329	14 3 2	21 0 4	7 3 2	1.938	+1.47	+0.915
2534	6 1 1	7 0 5	1 1 2	2.018	+1.34	+0.909
2564	6 1 1	4 0 5	5 0 1	2.031	+1.27	+0.955
2599	11 4 10	14 4 8	3 0 2	2.059	+1.69	+0.986
2675	6 2 11	13 1 13	7 1 2	2.101	+1.52	+0.960
2682	1 4 14	4 4 12	3 0 2	2.106	+1.44	+0.999
2739	18 3 8	25 0 7	7 7 2	2.148	+1.01	+0.999
2753	4 3 0	3 0 2	7 7 2	2.155	+1.09	+0.954
2771	14 4 9	4 0 5	18 3 9	2.164	+1.12	+0.997
2796	7 3 2	6 6 11	13 1 13	2.183	+0.94	+0.857
2838	10 7 10	4 4 12	14 3 3	2.230	+1.73	+0.972
2893	12 7 3	2 4 10	21 0 2	2.288	+2.64	+0.979
2909	10 1 11	13 1 12	3 0 2	2.297	+1.80	+0.992
2918	5 1 2	10 4 12	5 0 2	2.309	+1.19	+0.946
2925	9 0 15	4 4 12	5 0 2	2.323	+0.83	+0.898
2941	11 4 11	16 4 9	5 0 2	2.335	+1.28	+0.999
2960	14 3 2	18 3 3	4 0 7	2.366	+1.64	+0.990
2963	19 4 7	5 0 2	14 4 9	2.368	+1.46	+0.995
2981	0 2 0	4 1 0	4 1 0	2.388	+0.90	+0.837
2995	13 1 7	21 0 4	8 1 11	2.407	+1.37	+0.982
2999	5 4 13	16 4 9	21 0 4	2.413	+1.33	+0.990
3001	9 7 1	5 4 3	14 3 2	2.414	+1.27	+0.996
3020	6 1 7	4 1 0	2 0 7	2.445	+1.13	+0.717
3028	1 0 6	4 0 7	5 0 1	2.463	+0.84	+1.000
3091	5 4 3	3 4 10	4 0 7	2.589	+1.22	+0.986
3121	23 3 7	5 0 2	18 3 9	2.661	+1.67	+0.949
3124	16 4 9	19 4 7	3 0 2	2.672	+1.43	+0.999
3130	1 0 9	4 0 7	5 0 2	2.691	+1.91	+1.000
3131	10 0 3	3 0 2	7 0 5	2.694	+0.74	+1.000
3138	19 0 7	2 0 3	21 0 4	2.702	+0.59	-1.000
3139	10 7 10	9 7 1	1 0 9	2.703	+1.76	+0.990
3142	17 3 0	3 0 2	14 3 2	2.709	+1.23	+0.982
3153	11 1 8	6 1 7	5 0 1	2.728	+1.12	+0.908
3159	12 7 3	5 4 3	17 3 0	2.743	+0.97	+0.999
3162	1 1 2	4 1 0	5 0 2	2.747	+1.22	+0.942
3170	21 3 7	3 0 2	18 3 9	2.773	+1.18	+0.999
3171	13 3 11	18 3 9	5 0 2	2.775	+1.33	+0.969
3182	4 4 12	9 4 10	5 0 2	2.815	+1.73	+0.812
3184	3 1 9	8 1 11	5 0 2	2.822	+1.53	+0.999
3204	14 4 8	5 0 2	9 4 10	2.922	+1.67	+0.728
3214	0 2 0	8 1 11	8 1 11	2.974	+0.87	+0.998
3217	9 7 1	9 4 10	18 3 9	2.999	+2.09	+0.994
3218	17 4 7	3 0 2	14 4 9	3.013	+0.86	+0.995
3258	16 0 5	5 0 1	21 0 4	3.375	+0.44	+1.000
3259	7 1 2	4 1 0	3 0 2	3.375	+1.20	+0.974
3263	14 7 3	10 7 10	4 0 7	3.426	+1.23	+0.996
3267	10 0 1	5 0 2	5 0 1	3.478	+0.75	+1.000
3269	8 7 10	4 0 7	12 7 3	3.493	+1.24	+0.939
3282	9 0 5	5 0 2	4 0 7	3.861	+1.23	+1.000
3283	5 0 2	2 0 0	3 0 0	3.888	+1.67	+1.000
3289	14 7 3	9 7 1	5 0 2	4.034	+1.80	+0.975
3291	2 0 7	7 0 5	5 0 2	4.190	+1.18	+1.000
3294	13 1 13	5 0 2	8 1 11	4.250	+1.71	+0.953
3299	4 0 7	7 0 5	3 0 2	4.812	+1.16	+1.000
3305	2 0 3	5 0 1	3 0 2	6.470	+0.55	+1.000

The importance of using calculated values of the cosine invariants should perhaps be stressed. Other methods are strongly dependent on the fact that these cosines, for large A , are likely to be close to unity, but the inability to identify correctly those cosines whose values differ significantly from unity often leads to errors, particularly with complex structures. Restricting attention to those cosines whose values are definitely calculated to be +1 or definitely calculated to be negative, as is done here, greatly reduces the likelihood of error, as inspection of Table 1 shows. In addition, the systematic study of cosine invariants of special type, as required by the method of strong enantiomorph discrimination, is an essential feature of the procedure and should also be emphasized.

3. Determination of seventeen two-dimensional phases φ_{h0l}

The values of 17 two-dimensional phases φ_{h0l} are listed in Table 2 in the order in which they were determined. The method of phase determination is given in column 3 of this table. Thus φ_{302} and $\varphi_{50\bar{1}}$ were arbitrarily set equal to zero as is permitted by the recipe for origin specification in space group $P2_1$ (Hauptman & Karle, 1956). Again $\varphi_{200} = 180^\circ$ was determined by means of the \sum_1 formula (Hauptman & Karle, 1953). The phase $\varphi_{21,0,4} = 180^\circ$ was found from φ_{302} by means of a recently secured formula for cosine invariants, $\cos(\varphi_1 + \varphi_2)$ (Hauptman, 1971, 1972). The phases $\varphi_{70\bar{5}}$ and $\varphi_{25,0,7}$ could not be directly determined but they were needed in the evaluation of additional phases. Hence each was permitted to take on its two possible values and only the correct values (180° for each) are listed in Table 2. For the remaining phases, the serial number of the required cosine invariant, as given in Table 1, is listed in the third column of Table 2. Only the phase $\varphi_{19,0,7}$ was incorrectly calculated, a consequence of the negative cosine invariant 3138 which was calculated to be +0.59 and mistakenly assumed to be +1 (Table 1).

Table 2. Values of 17 two-dimensional phases

$h k l$	$ E $	Phase determined by means of	Calculated phase	True phase
3 0 2	3.732	origin	0°	0°
5 0 $\bar{1}$	3.448	origin	0	0
2 0 $\bar{3}$	3.170	3305	0	0
2 0 0	1.838	\sum_1	180	180
5 0 2	3.574	3283	180	180
10,0,1	1.779	3267	180	180
21,0,4	3.453	$\varphi_{302}, \cos(\varphi_1 + \varphi_2)$	180	180
16,0,5	1.787	3258	180	180
19,0,7	1.556	3138	180	0
7 0 $\bar{5}$	2.866	ambiguous	180	180
4 0 $\bar{7}$	2.836	3299	180	180
2 0 $\bar{7}$	2.579	3291	0	0
9 0 $\bar{5}$	2.401	3282	0	0
10,0, $\bar{3}$	1.588	3131	180	180
1 0 9	1.674	3130	0	0
1 0 6	2.522	3028	180	180
25,0,7	2.291	ambiguous	180	180

4. Determination of 32 three-dimensional phases which are enantiomorph independent

In Table 3 are listed the values of 32 three-dimensional (*i.e.* $k \neq 0$) phases, φ_{hkl} , in the order in which they were determined. The phase φ_{410} was arbitrarily set equal to 180° in accordance with the procedure for origin-fixing in space group $P2_1$. For the remaining phases, the serial numbers of the required cosine invariants, as given in Table 1, are listed in the third column of Table 3. Since the values of all the cosine invariants used in this calculation are, in accordance with Table 1, presumed to have the value +1, every structure invariant $\varphi_1 + \varphi_2 + \varphi_3$ has the same value, 0, for both enantiomorphs permitted by the observed structure factor magnitudes $|E|$. Hence the calculated values (0 or 180°) of all phases listed in Table 3 are enantiomorph independent. The true values of these thirty-two phases are listed in column 5 of the table and the last column gives the error ($\varphi_{\text{calc}} - \varphi_{\text{true}}$) of the calculated phase.

Table 3. Values of 32, enantiomorph independent, three-dimensional phases

$h k l$	$ E $	Phase determined by means of	Calculated phase	True phase	Error
4 1 0	2.554	origin	180°	165°	+15°
7 1 2	2.233	3259	180	-177	-3
1 1 2	1.897	3162	180	-175	-5
6 1 $\bar{7}$	2.340	3020, 2534	180	-149	-31
11, 1, $\bar{8}$	2.131	3153	180	-171	-9
12, 1, 4	1.495	2212	0	3	-3
8, 1, 11	2.850	2279	180	-174	-6
13, 1, 13	2.630	3294	0	-12	+12
3 1 9	1.747	3184	0	-2	+2
13, 1, $\bar{9}$	1.542	2995	180	-159	-21
10, 1, 11	1.476	2909	0	-14	+14
9 1 $\bar{1}$	1.454	2564	180	-177	-3
10, 1, 4	1.630	2230	180	166	+14
5 1 2	2.499	2918	0	4	-4
6, 2, 11	2.255	2675	0	-2	+2
7 3 2	2.320	2796	0	41	-41
4 3 0	1.569	2753	0	5	-5
14, 3, 2	1.526	2329	0	45	-45
18, 3, $\bar{9}$	2.548	2739	0	28	-28
13, 3, $\bar{11}$	1.921	3171	180	-171	-9
21, 3, $\bar{7}$	1.838	3170	0	16	-16
23, 3, $\bar{7}$	1.842	3121	180	-134	-46
14, 3, $\bar{2}$	2.064	2960	180	-170	-10
17, 3, 0	2.217	3142, 2016	180	-161	-19
11, 3, $\bar{4}$	1.590	2050	180	-179	-1
0 2 0	2.309	2981, 3214, 2223	180	-176	-4
14, 4, $\bar{9}$	2.096	2771	0	11	-11
19, 4, $\bar{7}$	1.993	2963	180	-168	-12
16, 4, $\bar{9}$	2.265	3124	180	-168	-12
17, 4, $\bar{7}$	2.428	3218	0	7	-7
11, 4, $\bar{11}$	1.818	2941	0	27	-27
5, 4, 13	1.945	2999	0	20	-20

5. Enantiomorph selection

In accordance with the recently devised procedure (Hauptman & Duax, 1972) for strong enantiomorph discrimination employing calculated cosine invariants and a class of structure invariants with values $\pm \pi/2$,

approximately, two orthogonal classes, I and II, of phases φ_{h4l} were tentatively identified. First, $|E_{080}|$ was observed to be moderately large (1.921). Second, using the modified triple-product procedure (Hauptman *et al.*, 1969), the values of all cosine invariants

$$\cos(\varphi_{h\bar{4}l} + \varphi_{\bar{h}4\bar{l}} + \varphi_{080}), \quad (5.1)$$

with (see equation 2.4)

$$A = 0.1586 |E_{h4l}^2 E_{080}| > 1.5 \quad (5.2)$$

were calculated. In accordance with the method of strong enantiomorph discrimination, those cosines which were calculated to be greater than unity led to four phases φ_{h4l} which were placed unambiguously in Class I. Those cosines calculated to be most negative led to two phases φ_{h4l} which were placed unambiguously in Class II. The results are summarized in Table 4 which also lists, for comparison, the true values of the cosine invariants as obtained from the refined structure.

Table 4. *Tentative definition of the orthogonal Classes I and II of phases, φ_{h4l} , via calculated cosine invariants*

Class	$h\ 4\ l$	$ E $	A	$\text{Cos}(\varphi_{h\bar{4}l} + \varphi_{\bar{h}4\bar{l}} + \varphi_{080})$	
				Calculated	True
I	11,4, $\bar{1}$	2.506	1.914	+1.44	+0.983
	17,4, $\bar{7}$	2.428	1.796	+1.06	+0.993
	10,4, $\bar{10}$	2.320	1.640	+1.61	+0.675
	16,4, $\bar{9}$	2.265	1.562	+2.14	+0.963
II	21,4, 5	2.518	1.931	-0.85	-0.860
	5 4 $\bar{3}$	2.392	1.743	-0.23	-0.888

Next, the membership of Classes I and II was confirmed and the classes themselves extended by calculating appropriate cosine invariants,

$$\cos(\varphi_{h4l} + \varphi_{h'\bar{4}l'} + \varphi_{-h-h'0l-l-l'}), \quad (5.3)$$

in accordance with the procedure. Thus

$$\cos_{\text{calc}}(\varphi_{\bar{1}\bar{1}\bar{4}l1} + \varphi_{10l4l\bar{10}} + \varphi_{109}) = +2.02, A = 1.544, \quad (5.4)$$

confirms the presence of $\varphi_{11l4l\bar{1}}$ and $\varphi_{10l4l\bar{10}}$ in the same class (I). Again, since $\varphi_{16l4l\bar{9}}$ and $\varphi_{11l4l\bar{1}}$ are in Class I, the values of the calculated cosines,

$$\cos_{\text{calc}}(\varphi_{14\bar{2}} + \varphi_{16l\bar{4}l9} + \varphi_{\bar{1}\bar{7}l0l11}) = +1.34, \\ A = 1.568, \quad (5.5)$$

$$\cos_{\text{calc}}(\varphi_{14\bar{2}} + \varphi_{\bar{1}\bar{1}l4l1} + \varphi_{10l0l1}) = +1.03, \\ A = 1.618, \quad (5.6)$$

require that $\varphi_{14\bar{2}}$ be placed in Class I, too. Next, since $\varphi_{16l4l\bar{9}}$ and $\varphi_{14\bar{2}}$ are in Class I,

$$\cos_{\text{calc}}(\varphi_{19l4l\bar{7}} + \varphi_{\bar{1}\bar{8}l\bar{4}l9} + \varphi_{\bar{3}0\bar{2}}) = +1.43, \\ A = 2.672, \quad (5.7)$$

$$\cos_{\text{calc}}(\varphi_{19l4l\bar{7}} + \varphi_{14\bar{2}} + \varphi_{\bar{2}0l0l9}) = +1.87, \\ A = 1.694, \quad (5.8)$$

imply that $\varphi_{19l4l\bar{7}}$ likewise belongs to Class I. Continuing in this way the membership of Class I was extended and firmly established. The final membership of Class II was firmly established in a similar way. The ten phases, φ_{h4l} , finally placed in Class I and the six phases, φ_{h4l} , placed in Class II are shown in Table 5.

Table 5. *Final definition of the orthogonal classes I and II of phases, φ_{h4l} , using Table 4 and calculated cosine invariants,*

Class	$h\ 4\ l$	$ E $	$\text{Cos}(\varphi_{h4l} + \varphi_{h'\bar{4}l'} + \varphi_{-h-h'0l-l-l'})$	φ_{true}
I	11,4, $\bar{1}$	2.506		5°
	4 4 3	2.497		-152
	17,4, $\bar{7}$	2.428		7
	10,4, $\bar{10}$	2.320		-15
	1 4 $\bar{2}$	2.289		-178
	16,4, $\bar{9}$	2.265		-168
	14,4, $\bar{9}$	2.096		11
	19,4, $\bar{7}$	1.993		-168
	5,4,13	1.945		20
	11,4, $\bar{11}$	1.818		27
II	24,4, 4	3.627		89
	21,4, 5	2.518		90
	9,4, $\bar{10}$	2.406		71
	5 4 $\bar{3}$	2.392		-95
	14,4, $\bar{8}$	2.143		-141
	4,4, $\bar{12}$	2.064		-62

Only one cosine invariant directly connecting the Classes I and II had a sufficiently large A value to warrant calculating the cosine. This was

$$\cos_{\text{calc}}(\varphi_{443} + \varphi_{9l\bar{4}l\bar{10}} + \varphi_{\bar{1}\bar{3}l0l7}) = +0.30, \\ A = 1.572, \quad (5.9)$$

sufficiently close to 0 to serve as additional confirmation of orthogonality.

Having firmly established the orthogonal Classes I and II of phases φ_{h4l} , it could be inferred, in view of the theoretical basis, that

$$\varphi_{h14l1} + \varphi_{h2\bar{4}l2} + \varphi_{-h1-h2l0l-l1-l2} \approx \pm 90 \quad (5.10)$$

and

$$\varphi_{h14l1} + \varphi_{\bar{h}2\bar{4}\bar{l}2} + \varphi_{-h1+h2l0l-l1+l2} \approx \pm 90^\circ, \quad (5.11)$$

where φ_{h14l1} is an arbitrary phase in Class I and φ_{h24l2} is an arbitrary phase in Class II. In short, the invariants (5.10) and (5.11) constitute a class of structure invariants each member of which is approximately equal to $\pm 90^\circ$ and is therefore suitable for decisive enantiomorph selection. The corresponding cosines constitute a class C of 65 cosine invariants whose values are approximately 0 and for which the average value of A is 0.50.

Inspection of the last six rows of Table 3 shows that the ten phases φ_{h4l} of Class I must have values equal to 0 or 180° , approximately. Hence the values of the six phases φ_{h4l} of Class II must be equal to ± 90 degrees, approximately. As it turned out, values could be unambiguously assigned to only six phases of Class I (Table 3) and to four phases of Class II (§6).

However, the true values of all ten phases in Class I and all six phases in Class II are shown in the last column of Table 5 and, with minor exceptions, these show good agreement with the expected values, 0 or 180° for Class I, $\pm 90^\circ$ for Class II.

The stage was now set for the determination of the remaining phases, mostly enantiomorph dependent, to be used as input to the tangent procedures.

6. Determination of 12 phases which are mostly enantiomorph dependent

The values of the remaining twelve phases (all but one of which are enantiomorph dependent), needed for use in the tangent procedures, are listed in Table 6. In view of §5 and the description of Table 3, Table 6 is self-explanatory.

Table 6. Values of 12 phases, mostly enantiomorph independent

$hk\ l$	$ E $	Phase determined by means of	Calculated phase	True phase	Error
9,4, $\bar{10}$	2.406	Enantiomorph	90°	+71°	+19°
14,4, $\bar{8}$	2.143	3204 (Enan.)	-90	-140	+50
4,4, $\bar{12}$	2.064	3182 (Enan.)	-90	-62	-28
5,4, $\bar{3}$	2.392	3091 (Enan.)	-90	-95	+5
9,0, $\bar{15}$	2.925	2925	0	0	0
1,4, $\bar{14}$	1.724	2682	-90	-57	-33
11,4, $\bar{10}$	1.623	2599	-90	-149	+59
9,7,1	3.084	3001, 3217	+90	+101	-11
12,7,3	3.262	2893, 3159	+90	+109	-19
10,7,10	3.301	3139, 2838	+90	+113	-23
14,7,3	2.370	3263, 3289	-90	-56	-34
8,7,10	2.380	3269	-90	-62	-28

7. Determination of remaining phases by the tangent procedures

The 61 phases thus far determined were used as input to the tangent formula and modified tangent procedure (Weeks & Hauptman, 1971) to determine the values of 452 phases. Modified and simple tangent figures of merit on the four runs required, due to the $h0l$ reflections of ambiguous phase, showed no preferred solution. Inspection of phasing of some Σ_1 type reflections and the 020 and certain $h1l$ phase values, strongly indicated by the phasing process, suggested the most promising solution. The 51 strongest peaks in that E map (using modified tangent phases) formed an oval. Structure factor calculations based upon those peaks

gave an R value (defined as $\sum||F_o| - |F_c|| / \sum|F_o|$) of 36% for 5982 reflections. Two subsequent difference maps led to the unambiguous identification of the 78 non-hydrogen atoms of the structure. Three cycles of isotropic refinement on all nonhydrogen atoms reduced the R value to 15%.

When comparing the true phases, as determined from the structure, with the phases that led to the solution, it was found that the average magnitude of the error in the 43 three-dimensional phases of the basis set was 18°. This good agreement suggested that an initial E map with more than 452 terms might give the full structure. To test this hypothesis, the 61 basis phases were again used as input to the modified tangent procedure in order to calculate 552 phases. In the initial E map using these 552 phases, 57 of the 60 strongest peaks were found to be at an average distance of 0.15 Å from the refined atomic positions. 17 of the remaining 21 atoms were also present in this initial 552 term E map at an average distance of 0.27 Å from refined positions. The four atoms not in the map were terminal carbons on isopropyl groups exhibiting greatest thermal motion.

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References

- HAUPTMAN, H. (1970). Ottawa Meeting of the ACA. Aug. 16-22, Abstract *J2*.
 HAUPTMAN, H. (1970). *Crystallographic Computing*, pp. 45-51. Edited by F. AHMED, S. R. HALL & C. P. HUBER. Copenhagen: Munksgaard.
 HAUPTMAN, H. (1971). Ames Meeting of the ACA. Aug. 15-20, Abstract *E1*.
 HAUPTMAN, H. (1972). *Acta Cryst.* **B28**, 2337.
 HAUPTMAN, H. & DUAX, W. L. (1972). *Acta Cryst.* **B28**, 2622.
 HAUPTMAN, H., FISHER, J., HANCOCK, H. & NORTON, D. (1969). *Acta Cryst.* **B25**, 811.
 HAUPTMAN, H. & KARLE, J. (1953). *Solution of the Phase Problem. I. The Centrosymmetric Crystal*. ACA Monograph No. 3. Pittsburgh: Polycrystal Book Service.
 HAUPTMAN, H. & KARLE, J. (1956). *Acta Cryst.* **9**, 45.
 KARLE, J. & HAUPTMAN, H. (1956). *Acta Cryst.* **9**, 635.
 KARLE, J. & HAUPTMAN, H. (1957). *Acta Cryst.* **10**, 515.
 WEEKS, C. & HAUPTMAN, H. (1971). Columbia Meeting of the ACA. Jan. 31-Feb. 4, Abstract *H4*.