

Table (cont.)

XII.	<i>G</i>	<i>H'</i>	<i>s</i>	<i>H</i>	Icosahedral			<i>d</i>	Chirality	Symbol
					<i>t</i>	<i>F</i>				
	532	532	1	...	...	$C_1$	1	<i>G</i>	532	
		23	5	1	60	<i>Y</i>	2	<i>G</i>	532 (23 1)	
		52	6	1	60	<i>Y</i>	12	<i>G</i>	532 (52 1)	
		32	10	1	60	<i>Y</i>	9!/6	<i>G</i>	532 (32 1)	
		5	12	1	60	<i>Y</i>	11!/5	<i>G</i>	532 (5 1)	
		222	15	1	60	<i>Y</i>	14!/4	<i>G</i>	532 (222 1)	
		3	20	1	60	<i>Y</i>	19!/3	<i>G</i>	532 (3 1)	
		2	30	1	60	<i>Y</i>	29!/2	<i>G</i>	532 (2 1)	
		1	60	...	...	<i>Y</i>	59!	<i>G</i>	532 (1)	
	$\bar{5}3m$	$\bar{5}3m$	1	...	...	$C_1$	1	<i>N</i>	$\bar{5}3m$	
		532	2	...	...	$C_2$	1	<i>C</i>	$\bar{5}3m(532)$	
		$m\bar{3}$	5	$\bar{1}$	60	<i>Y</i>	2	<i>N</i>	$\bar{5}3m(m\bar{3} \bar{1})$	
		$\bar{5}m$	6	$\bar{1}$	60	<i>Y</i>	12	<i>N</i>	$\bar{5}3m(\bar{5}m \bar{1})$	
		23	10	1	120	$Y \times C_2$	9!/12	<i>C</i>	$\bar{5}3m(23 1)$	
		$\bar{3}m$	10	$\bar{1}$	60	<i>Y</i>	9!/6	<i>N</i>	$\bar{5}3m(\bar{3}m \bar{1})$	
		$\bar{5}$	12	$\bar{1}$	60	<i>Y</i>	11!/5	<i>N</i>	$\bar{5}3m(\bar{5} \bar{1})$	
		52	12	1	120	$Y \times C_2$	11!/10	<i>C</i>	$\bar{5}3m(52 1)$	
		$5m$	12	1	120	$Y \times C_2$	11!/10	<i>C</i>	$\bar{5}3m(5m 1)$	
		$mmm$	15	$\bar{1}$	60	<i>Y</i>	14!/4	<i>N</i>	$\bar{5}3m(mmm \bar{1})$	
		$\bar{3}$	20	$\bar{1}$	60	<i>Y</i>	19!/3	<i>N</i>	$\bar{5}3m(\bar{3} \bar{1})$	
		32	20	1	120	$Y \times C_2$	19!/6	<i>C</i>	$\bar{5}3m(32 1)$	
		$3m$	20	1	120	$Y \times C_2$	19!/6	<i>C</i>	$\bar{5}3m(3m 1)$	
		5	24	1	120	$Y \times C_2$	23!/5	<i>C</i>	$\bar{5}3m(5 1)$	
		222	30	1	120	$Y \times C_2$	29!/4	<i>C</i>	$\bar{5}3m(222 1)$	
		$mm\bar{2}$	30	1	120	$Y \times C_2$	29!/4	<i>C</i>	$\bar{5}3m(mm\bar{2} 1)$	
		$2/m$	30	$\bar{1}$	60	<i>Y</i>	29!/2	<i>N</i>	$\bar{5}3m(2/m \bar{1})$	
		3	40	1	120	$Y \times C_2$	39!/3	<i>C</i>	$\bar{5}3m(3 1)$	
		2	60	1	120	$Y \times C_2$	59!/2	<i>C</i>	$\bar{5}3m(2 1)$	
		$m$	60	1	120	$Y \times C_2$	59!/2	<i>C</i>	$\bar{5}3m(m 1)$	
		$\bar{1}$	60	...	...	<i>Y</i>	59!	<i>N</i>	$\bar{5}3m(\bar{1})$	
		1	120	...	...	$Y \times C_2$	119!	<i>C</i>	$\bar{5}3m(1)$	

point groups with  $H' \neq H$  provide some asymmetric subunits with neighbors of the same color, while others have only differently colored neighbors. Thus, such a group does not treat an object's subunits consistently, if  $H' \neq H$ . The Wittke-Garrido colored point groups are listed in the book, *Symmetry in Science and Art*, by Shubnikov & Koptsik (1974); they will not be considered further here.

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## An Example of the Use of Quartet and Triplet Structure Invariants when Enantiomorph Discrimination is Difficult

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Problems relating to enantiomorph discrimination in the structure determination of the alborixin antibiotic ( $C_{48}O_{14}H_{84}, K^+$ ) are studied. To select the starting set, quartet structure invariants are used to define two orthogonal classes of reflexions, and a variation of the tangent formula refinement using the phases of the triplet invariants is described.

### Introduction

Since the use and automation of multiresolution methods, of which *MULTAN* (Germain, Main & Woolfson,

1970) is the best example, problems rarely occur in crystal structure determination, even if the number of heavy atoms in the asymmetric unit is rather large (50 to 80 for instance). The few failures of *MULTAN*

(certainly less than 1%) are generally due to the nature of the space group and even more to a peculiar distribution of atoms in the unit cell.

It is well known that it is more difficult to deal with some space groups, like  $P2_1$ ,  $P1$ , than others. Troubles are partly due to the fact that the enantiomorph cannot be fixed with the same confidence as in  $P2_12_12_1$  for example. In that case, it is easy, by working only with the reflexions of the principal planes, to find one reflexion which forms with the origin-defining reflexions a structure seminvariant, the phase of which is equal to  $\pm\pi/2$ . In  $P2_1$ , on the other hand, the enantiomorph must be defined through the use of an unknown phase which is supposed sufficiently different from 0 or  $\pi$ . This process does not seem too troublesome for small molecules but problems occur when the number of atoms is large. Duax & Hauptman (1972) have shown that it is then necessary to find, by the use of structure invariants, two sets of orthogonal reflexions (*i.e.* reflexions with phases differing by about  $90^\circ$ ) to find an enantiomorph unambiguously.

Another cause of failure of the multisolution technique is the fact that tangent-formula refinement is based only on the zero mean value of the invariant sine,  $\sin(\varphi_{\bar{H}} + \varphi_K + \varphi_{H-K})$ , and ignores completely the corresponding value of the invariant cosine. Thus it tends to strengthen the dominant features of a structure. For small molecules, it increases the relative weight of heavy atoms or of multibound atoms (Busetta, 1973). An equivalent result is observed in proteins; if multiple-isomorphous-refinement phases are refined by the tangent formula, a strengthening of groups with large electronic density occurs (haeme, cysteine groups) and conversely a weakening of others (alkyl groups) (Weinzierl, Eisenberg & Dickerson, 1969).

In the same way, if a molecule is roughly centrosymmetric, tangent-formula refinement will exaggerate this feature. This drawback appears for molecules with a relatively large number of atoms which present a more or less spherical shape. In the molecule we studied (an antibiotic ionophore for which we only knew the rough chemical composition  $C_{48}O_{14}H_{84}, K^+$ ), the general centrosymmetric character was increased by a heavy atom ( $K^+$ ) at the centre of the molecule (Alleaume, Busetta, Farges, Gachon, Kergomard & Staron, 1975).

### The present problem

This molecule crystallized in the monoclinic space group  $P2_1$ . In the different methods used for structure determination, we worked with the 406 reflexions with  $E > 1.60$ ; that is a little more than six reflexions per atom. The position of the  $K^+$  ion was determined from the Patterson function.

To determine the structure by the multisolution process, it was necessary to refine by the tangent formula 128 different solutions which may be divided in two groups.

(a) About half of the solutions converge to the same

result, a solution where all the phases have values  $\alpha$  or  $\alpha + \pi$  for a fixed value of  $k$ . It is a strongly self-consistent solution, as proved by the abnormally high ABSFOM factor (Germain *et al.*, 1970), 1.55, instead of the theoretical expected value 1.0. The  $\psi_0$  test is also a maximum. On the corresponding  $E$  map the position of the largest peak actually represents the  $K^+$  ion (the  $y$  coordinate of this atom naturally takes different values in accordance with the different starting sets). Furthermore, about thirty peaks may be seen as well as their symmetry mates, with respect to a mirror plane perpendicular to the screw axis and passing through the  $K^+$  ion. As we knew nothing about the molecule, it was impossible to find a solution from this  $E$  map.

In the following, we shall call such a solution a 'mirror solution'. If the  $K$  atom is arbitrarily situated at the level  $y=0$ , the phases of the different reflexions have a value near  $\pm\pi/2$  if  $k$  is odd and near 0 or  $\pi$  if  $k$  is even.

As expected, the mirror solution is also obtained by refining with the tangent formula the phases (about 30) which may be supposed determined by the  $K$  atom.

(b) All the other solutions, for which the ABSFOM factor has normal values, provide  $E$  maps where the position found for the  $K$  atom was incompatible with the Patterson function.

### The use of quartets to define correctly the enantiomorph

Thus it seemed that a starting set converging to a mirror solution was very close to the correct one, since it was the only way to obtain the  $K^+$  ion in the correct position. The final drawback of phase refinement depended on the inability to define correctly the enantiomorph from the reflexions used in the starting set.

If we take into account that about two thirds of the 406 final phases differ from the mirror solution by less than  $30^\circ$ , it is easy to understand how difficult it is to choose correctly a reflexion with a phase which is sufficiently far from the mirror value to allow unambiguous enantiomorph definition.

In Table 1, which reports origins and symbols required by the convergence process (Germain *et al.*, 1970), we see that the final phases of these reflexions are not very different from the values corresponding to the mirror solution. Now, for correct enantiomorph discrimination it is necessary to choose a reflexion, the phase of which differs by about  $90^\circ$  from the mirror solution.

For this purpose, we define two orthogonal classes of reflexions as described by Duax & Hauptman (1972). But, instead of triplet invariants, we examine the phases of quartets built on reflexions of the type  $02k0$  as suggested by Hauptman (personal communication).

Of all quartets constructed from  $02k0$  reflexions we consider only those having two reflexions with a common index and their symmetrically related ones, for example

$$(h_1k_1), (h_1\bar{k}_1), (h_2\bar{k}_2), (h_2k_2)$$

Table 1. *Origin, the two orthogonal classes and their final real phases*

Reflexions defined by $\sum_1$ relations	Phase used	Final phase
12 0 0	0°	0°
$\bar{4}$ 0 6	180°	180°
$\bar{2}$ 0 10	0°	0°
0 0 12	0°	0°
Origin*		
1 1 11	270°	288°
$\bar{6}$ 1 16	270°	286°
7 3 12	90°	100°
Symbols		
6 8 6	0°	2°
8 0 5	0°	0°
$\bar{1}$ 10 8	0°	7°
3 10 4	180°	185°
Class I		
(fixes the enantiomorph)		
$\bar{1}$ 2 2	270°	278°
9 2 3	270°	314°
5 2 4	not used	103°
12 2 0	not used	334°
1 2 10	270°	344°
Class II		
$\bar{3}$ 2 10	180°	186°
$\bar{7}$ 2 8	0°	358°
$\bar{1}\bar{1}$ 2 5	180°	235°
3 2 9	0°	327°

\* The phases of the origin-fixing reflexions were fixed to  $\pm 90^\circ$  ( $k$  is odd) to obtain the  $K^+$  ion at the zero level.

(the sum of the indices is naturally equal to zero).

If the phases of  $h_1k_1l_1$  and  $h_2k_2l_2$  reflexions are respectively  $\alpha_1$  and  $\alpha_2$  the phase of the corresponding quartet is  $\varphi = 2(\alpha_1 - \alpha_2)$ .

If the reflexions  $h_1k_1l_1$  and  $h_2k_2l_2$  belong to the same orthogonality class (*i.e.* their phases differ by  $0^\circ$  or  $180^\circ$ ), the invariant phase of the quartet is  $\varphi = 0^\circ$ .

Conversely, if they belong to different orthogonal classes, the invariant phase of the quartet is  $\varphi = 180^\circ$ .

Hauptman (1974*a,b*) pointed out that the invariant phase of the quartet could be estimated from the moduli of the normalized structure factors  $E_{h_1+h_2, 0, l_1+l_2}$  and  $E_{h_1-h_2, 0, l_1-l_2}$ :

if both are large,  $\varphi$  is probably zero;

if both are weak,  $\varphi$  is probably equal to  $180^\circ$ .

Table 2 illustrates how we separated nine ( $h2l$ ) reflexions into two orthogonal classes, by using the quartets built from the 040 reflexion, assuming that the  $\bar{1}22$  reflexion belongs to the first class. For seven of those reflexions, symbolic addition, applied to the starting set provided by the convergence process, had assigned symbols corresponding to two different sets of correlation, compatible with the existence of two orthogonal classes. We preferred, then, to discard the other two reflexions in the starting set. The relative magnitudes of the structure factor computed from the  $K^+$  ion only allow us to assign a 0 or  $\pi$  phase to reflexions of class II. Then the reflexions of class I, with phases near  $\pm \pi/2$ , can be used to define the enantiomorph correctly. If we examine the final true phases of those nine reflexions we see that the phase assignment of the last four reflexions is not quite satisfactory. However, the new starting set, so obtained, was undoubtedly better than the first one; nevertheless tangent-formula refinement led again to a mirror solution.

Table 2. *Determination of two orthogonal classes of reflexions, using quartets built from the 040 reflexion*

For each quartet (in this case two reflexions and their symmetry-related ones) we used  $E_{h_1+h_2, 0, l_1+l_2}$  and  $E_{h_1-h_2, 0, l_1-l_2}$ . Some of those  $E$  values are unknown and denoted by an interrogation mark.

Class I ( $\pm \pi/2$ )		Class II (0 or $\pi$ )	
(1) $\bar{1}$ 2 2			
(2) 9 2 3	to (1) $\varphi = 0$ $E_1 = 2.75$ $E_2 = 3.10$		
(4) 5 2 4	to (1) $E_1 = 2.54$ $E_2 = 0.84$ $\perp$ to (3) $E_1 = 1.24$ $E_2 = 0.35$	(3) $\bar{3}$ 2 10 $\perp$ to (2) $E_1 = 0.25$ $E_2 = 0.57$ (1) $E_1 = 0.53$ $E_2 = 0.17$	
(6) 12 2 0	to (1) $E_1 = 1.98$ $E_2 = 1.68$	(5) $\bar{7}$ 2 8 $\perp$ to (1) $E_1 = 0.47$ $E_2 = 0.38$ (4) $E_1 = 0.39$ $E_2 = 0.56$ presumption    to (3) $E_1 = 2.34$ $E_2 ?$	
(8) 1 2 10	to class I (1) $E_1 = 2.18$ $E_2 = 0.64$ presumption (4) $E_1 = 2.94$ $E_2 ?$ (6) $E_1 = 1.67$ $E_2 ?$ presumption $\perp$ to class II (3) $E_1 = 0.58$ $E_2 ?$ (5) $E_1 = 0.69$ $E_2 ?$ (7) $E_1 = 0.55$ $E_2 ?$	(7) $\bar{1}\bar{1}$ 2 5 $\perp$ to (1) $E_1 = 0.57$ $E_2 = 0$ presumption $\perp$ to (2) $E_1 = 0.17$ $E_2 ?$ (4) $E_1 = 0.34$ $E_2 ?$ (6) $E_1 = 0.04$ $E_2 ?$ presumption    to (5) $E_1 = 1.49$ $E_2 ?$ (3) $E_1 = 2.75$ $E_2 ?$	
		(9) 3 2 9	presumption    to (3) $E_1 = 3.10$ $E_2 ?$ presumption $\perp$ to (2) $E_1 = 0.38$ $E_2 ?$ (8) $E_1 = 0.04$ $E_2 ?$

### How to introduce the phase of triplet invariants in a tangent refinement

Close examination of the refinement process showed that even if a phase was initially computed far from the corresponding value for the mirror solution, it rapidly converged (generally only one or two cycles of tangent refinement) towards the critical mirror-solution value. *A posteriori*, this result is not surprising if we take into account the fact that two thirds of the final phases are near the mirror solution and the strong internal consistency of such a solution.

To converge to the correct solution it seems necessary to introduce into the refinement process the phases of the triplet structure invariants computed by the modified triple-product formula (Hauptman, Hancock, Fisher & Norton, 1969). The least-squares refinement proposed by these authors may be used only to generate the first phases, but it is too time-consuming to be used in the complete refinement of all phases which are required for crystal structure determination.

The very fast classical refinement by tangent formula is based on the relation:

$$\sum_K W_{H,K} \sin(\varphi_{\bar{H}} + \varphi_K + \varphi_{H-K}) = 0.$$

This statistical relation is correct only if the number of  $K$  terms is sufficiently large; in fact, a single invariant phase  $\varphi_{\bar{H}} + \varphi_K + \varphi_{H-K}$  is generally equal to a non-zero value  $\alpha_{H,K}$  and it would better to base the refinement on the equation:

$$\sum_K W_{H,K} \sin(\varphi_{\bar{H}} + \varphi_K + \varphi_{H-K} - \alpha_{H,K}) = 0.$$

Though it is possible to compute, from the modified triple product formula for instance, the modulus  $|\alpha_{H,K}|$  of the invariant phase, it is impossible to obtain its sign.

As pointed out previously, the phases computed in the first cycle, without any refinement, were probably closer to the true phases than those we get from the mirror solution. Then, if the classical tangent formula provides a phase  $\varphi'_H$  for the  $H$  reflexion we may suppose that the sum  $\varphi'_H + \varphi_K + \varphi_{H-K} = \alpha'_{H,K}$  is not far from the correct invariant phase, the modulus of which is theoretically available ( $|\alpha_{H,K}|$ ); it seems reasonable to assume that the true invariant is  $s(\alpha'_{H,K}) \times |\alpha_{H,K}|$  and then a new refinement process may be based on the equation:

$$\sum_K W_{H,K} \sin(\varphi_{\bar{H}} + \varphi_K + \varphi_{H-K} - s(\alpha'_{H,K}) \times |\alpha_{H,K}|) = 0.$$

That is to say, in a more classical form:

$$\operatorname{tg} \varphi_H = \frac{\sum_K W_{H,K} \sin(\varphi_K + \varphi_{H-K} - s(\alpha'_{H,K}) \times |\alpha_{H,K}|)}{\sum_K W_{H,K} \cos(\varphi_K + \varphi_{H-K} - s(\alpha'_{H,K}) \times |\alpha_{H,K}|)}.$$

In fact we applied the *a posteriori* computed sign  $s(\alpha'_{H,K})$  to the *a priori* computed modulus  $|\alpha_{H,K}|$  in the two following cases:

$$\begin{aligned} &\text{if } \Delta\alpha' = |90^\circ - |\alpha_{H,K}|| < 60^\circ, \\ &\text{if } \Delta\alpha' > 60 \text{ but remains lower than } \Delta\alpha = |90^\circ - |\alpha_{H,K}||. \end{aligned}$$

Then, as in the classical tangent formula, we use a weight  $W_{H,K} = A_{H,K}$  for such an invariant relation.

However, when the sign of the invariant phase could not be determined we used the information given by the structure invariant but with the classical form  $\sin(\varphi_{\bar{H}} + \varphi_K + \varphi_{H-K}) = 0$ , with the modified weight  $W_{H,K} = A_{H,K}$  which we introduced (Busetta & Comberton, 1974) to take into account the discrepancy of the considered invariant phase from its supposed zero value.

Finally, forecasting the existence of a majority of phases near the mirror solution, we tried to favour the enantiomorph discrimination by multiplying the previously defined weight by an arbitrary factor  $f$  equal to one if  $\Delta\alpha > 60^\circ$  and to  $(3 - 2\Delta\alpha/90^\circ)$  otherwise.

After each cycle of refinement the sign assigned to each invariant phase was checked. Although there was usually no change in the sign assigned to an invariant phase near  $90^\circ$ , the signs of phases near  $0^\circ$  or  $180^\circ$  were more doubtful, especially in the first cycles of refinement. After 12 cycles of refinement no further change in the signs of the invariant phases was observed, so we stopped.

On the corresponding  $E$  map it was possible to identify 33 atoms of the desired structure (Fig. 1). If, in some cases, there remained two symmetrical peaks on each side of the pseudo mirror passing through the  $K^+$  ion, the different weights of the peaks were always sufficient to provide a correct and unambiguous position for the atom.

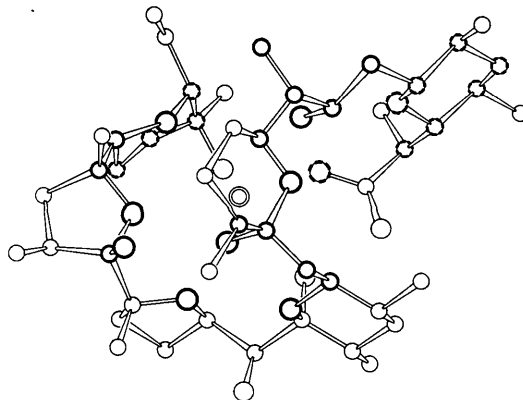


Fig. 1. The alborixin structure (Alleaume *et al.*, 1975). The atoms located by the direct method are represented by bold circles, full above and dashed below the  $K^+$  ion (the double circle). The enantiomorph used during the structure determination is the inverse of the real absolute configuration.

Three successive computations of structure factors and Fourier maps allowed us to find all the missing atoms.

The mean error between the phases computed by the direct method and their true final values for the 406

Table 3. *Comparison of refined and real phases of the four first planes*

We report here only the reflexions of which the real phase differs from the mirror solution by more than 45°.

	Real $\varphi$	Computed $\varphi$	Equivalent mirror solution
3 1 12	26°	71°	90°
$\bar{1}\bar{2}$ 1 11	48	56	90
8 1 4	54	67	90
4 1 4	330	308	270
2 1 0	54	34	90
$\bar{1}$ 2 2	278	309	360
4 2 3	76	102	180
5 2 4	103	107	180
$\bar{1}$ 2 5	97	45	0
$\bar{1}\bar{1}$ 2 5	235	193	180
3 2 10	66	102	180
2 2 11	63	20	0
$\bar{7}$ 2 11	49	-10	0
$\bar{1}$ 2 15	121	134	180
$\bar{6}$ 3 10	331	324	270
$\bar{7}$ 3 7	41	42	90
9 3 6	323	282	270
$\bar{1}\bar{0}$ 3 6	200	232	270
$\bar{1}\bar{1}$ 3 6	29	86	90
$\bar{1}\bar{1}$ 4 5	134	135	180
3 4 10	91	105	180
4 4 10	305	334	360
$\bar{2}$ 4 12	127	151	180
$\bar{4}$ 4 12	105	153	180
2 4 13	94	137	180
1 4 13	307	342	360

reflexions is  $\langle |\Delta\varphi| \rangle$  17.9°. But as we had already noticed, 275 phases differ from the mirror solution by less than 30°; therefore we prefer to illustrate the effectiveness of the refinement process described above by comparing only the phases which actually differ from the mirror solution by more than 30° with those computed by the direct method. In Table 3, we report the 26 corresponding phases with  $k$  ranging from 1 to 4.

Finally we introduced in the classical refinement process, the 406 true final phases obtained for the completely refined structure ( $R=0.07$ ) and once more we obtained the mirror solution.

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