Fig. 2. Difference density in plane of cyclopropane ring. Contour interval 0.01 e.Å^{-3}, zero contour broken. Estimated standard deviation averages 0.017 e.Å^{-3}.

etical difference density that self-consistent-field calculations (Scherr, 1955) place along the bond axis in the N_2 molecule (Bratož, Daudel, Roux & Allavena, 1960).

Intermolecular $N \cdots H$ contacts of 2.73 and 2.81 Å and $N \cdots C(2)$ contacts of 3.10 Å hold the structure firmly together and lead to small departures, up to 0.015 Å, from $3m$ molecular symmetry. An interesting, though expected, feature is the non-linearity of the $C-C\equiv N$ angle; the $C\equiv N$ bond is deflected $1.7°$ towards the molecular symmetry axis. While an intermolecular source cannot be ruled out, the deflection is thought to reflect a bending of the $C(1)-C(2)$ bond,

with consequent rotation of the C(2) valence orbitals (Hirshfeld, 1964), as a result of repulsion between C(2) and H.

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The Extension of the Isomorphous Replacement Method to Include Anomalous Scattering Measurements

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The combination of isomorphous replacement and anomalous scattering data in phase determination of non-centrosymmetric reflexions is discussed. Expressions are derived which take into account the relative reliabilities of the experimental observations and enable any combination of replacement atoms to be used in the phase determination.

North (1965) has proposed an improved method for the combination of isomorphous replacement and anomalous scattering data in phase determination of non-centrosymmetric reflexions which makes use of the greater intrinsic accuracy of the anomalous scattering measurements. North's formulation leads to alternative expressions for the phase probability distribution, and North leaves it open as to which of these expressions should be used in practice. The purpose of this communication is to show that the problem may be exam-

ined in a general way, and to extend the method so that anomalous scattering measurements from isomorphous crystals differentiated by any combination of 'heavy atoms' may be included in the phase determination.

Blow & Crick's (1959) treatment of errors in the isomorphous replacement method can be illustrated by a phase diagram in which the three vectors F, F_H and f_c do not, in general, form a closed triangle. (F and F_H are the observed structure amplitudes of the isomorphous 'parent' and 'derivative' compounds, and f_c is the calculated structure factor of the atoms which differentiate the two compounds). By considering the lack of closure of this triangle and estimating the error in its determination, the probability of F having a given phase may be obtained. Similarly, to illustrate the treatment of errors in phase determination using both isomorphous replacement and anomalous scattering data the phase diagram shown in Fig.1 can be constructed. $F_{H}+$ and $F_{H}-$ are the observed structure amplitudes of a Friedel pair of derivative reflexions; F is the corresponding structure amplitude of the parent compound which is assumed to have negligible anomalous scattering; f and δ are the calculated components of the combined 'heavy atom' scattering and are discussed in more detail later, and F_c is defined as the resultant of F and f. ε_+ represents the lack of closure of the $F_H{}^+$ vector and ε_- the lack of closure of $F_{H}-$. The phase of the heavy atom structure factor has been denoted by ψ in place of North's α. This is in keeping with the nomenclature used in a related paper (Matthews, 1966) dealing with the location of anomalously scattering heavy atoms in protein structures. Blow & Rossmann (1961) have shown that the overall phase probability distribution using both isomorphous replacement and anomalous scattering data can be written as

$$P(\varphi) \propto \exp \left\{ -(\varepsilon_+^2 + \varepsilon_-^2)/2E^2 \right\} \tag{1}$$

where E is the total r.m.s. error in determining ε_+ and ε_-. As North points out, this procedure does not take into account the inherently greater accuracy of the anomalous differences. This accuracy can be taken into account by rewriting the lack of closure terms in a different form, viz.

$$\varepsilon_+^2 + \varepsilon_-^2 = \tfrac{1}{2}[(\varepsilon_+ + \varepsilon_-)^2 + (\varepsilon_+ - \varepsilon_-)^2]$$
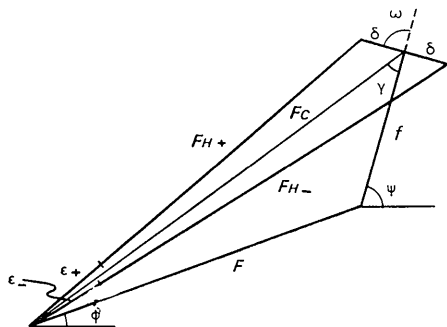$$= \tfrac{1}{2}[x_1(\varphi)^2 + x_1'(\varphi)^2] . \tag{2}$$

Fig.1. Vector diagram showing lack of closure with combined isomorphous replacement and anomalous scattering data.

Having thus defined $x_1(\varphi)$ and $x_1'(\varphi)$ it will now be shown that these quantities can be given simple physical interpretations. Let us define $F_H' = \tfrac{1}{2}(F_H{}^+ + F_H{}^-)$. If there were no anomalous scattering F_h' would equal F_H the derivative structure amplitude. Since δ is normally small compared with F_c, it follows from Fig.1 that

$$x_1(\varphi) = (\varepsilon_+ + \varepsilon_-) = 2(F_c - F_H') . \tag{3}$$

$x_1(\varphi)$ can therefore be considered as the 'total isomorphous replacement lack of closure'. Analogously, $x_1'(\varphi)$ which depends on the anomalous scattering difference $\Delta_H = (F_H{}^+ - F_H{}^-)$ can be considered as the 'anomalous scattering lack of closure'. From the definitions of $x(\varphi)$ and $x'(\varphi)$ given by North, it follows that $x_1'(\varphi) = -x'(\varphi)$ and $x_1(\varphi) = 2x(\varphi)$. As discussed by North, the error in determining $x_1'(\varphi)$ will tend to be less than that for $x_1(\varphi)$. If E_1 and E_1' are defined as estimates of the total r.m.s. error in determining $x_1(\varphi)$ and $x_1'(\varphi)$ respectively, then the overall relative probability $P(\varphi)$ of any particular phase φ being correct is

$$P(\varphi) = \exp \left\{ -\left(\frac{x_1(\varphi)^2}{2E_1^2} + \frac{x_1'(\varphi)^2}{2E_1'^2} \right) \right\} . \tag{4}$$

Clearly $P(\varphi)$ may be written

$$P(\varphi) = P_{is}(\varphi) . P_{an}(\varphi) \tag{5}$$

where $P_{is}(\varphi)$ is the phase probability distribution obtained using isomorphous replacement data, and $P_{an}(\varphi)$ is the probability distribution obtained from anomalous scattering data.

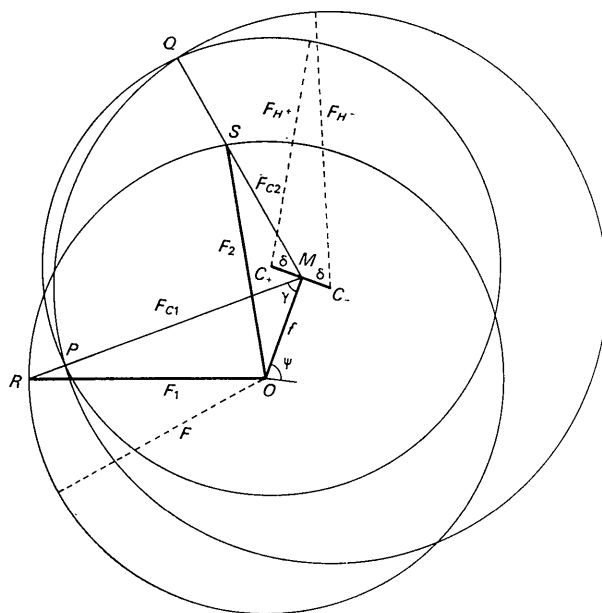$$P_{is}(\varphi) = \exp \left\{ -2(F_c - F_H')^2/E_1^2 \right\} . \tag{6}$$

Fig. 2. Phase diagram illustrating phase determination using anomalous scattering data. F_{c_1} and F_{c_2} give the most probable phases of F_c, and F_1 and F_2 are the corresponding most probable values of F.

Since $E_1=2E$, this is equivalent to North's equations (1) and (2).

In evaluating $P_{an}(\varphi)$ we first consider the case in which f and δ in Fig. 1 are orthogonal. It will be shown later that $P_{an}(\varphi)$ may be evaluated without this assumption; however, this special case illustrates the general method and can be used if all the heavy atoms have the same relative anomalous scattering. If then $\omega=\pi/2$ and we again use the fact that δ is normally small compared with F_c, we have

$$\varepsilon_+-\varepsilon_-= -F_{H-}+F_{H+}-2\delta \sin \gamma$$
$$= -\Delta_H-2\delta \sin \gamma . \quad (7)$$

Now

$$\sin \gamma = (F/F_c) \sin (\psi -\varphi)$$
$$=(F/F_cf)(b \cos \varphi - a \sin \varphi) \quad (8)$$

where $a=f\cos \psi$ and $b=f\sin \psi$ [see also (14) and (14a)]. Substitution in (7) leads to

$$\varepsilon_+-\varepsilon_-= -\Delta_H-(2F\delta/F_cf)(b \cos \varphi - a \sin \varphi) . \quad (9)$$

This result is similar to North's equation (6), but the assumption that the phase triangle should be closed at phase φ has not been needed. From North's derivation it is not clear whether F_c should be replaced by F'_H, and although North suggests that it appears to be valid to do so, a more correct procedure in evaluating the probability distribution for the phase of F is to use

$$P_{an}(\varphi)=\exp \left\{ -\frac{1}{2E_1'^2} [-\Delta_H \right.$$
$$\left. -(2F\delta/F_cf) (b \cos \varphi - a \sin \varphi)]^2 \right\} \quad (10)$$

where E_1' equals North's E' and $F_c=[F^2+f^2+2F(a \cos \varphi+b \sin \varphi)]^{\frac{1}{2}}$. The physical interpretation of this result is illustrated by the construction of Fig. 2. The calculated vectors **f** and **δ** are plotted first, and then circles of radii F_{H+} and F_{H-} are drawn with centres C_+ and C_-. The points of intersection of these circles give the most probable directions of F_c (i.e. along MP and MQ, the alternative solutions symmetrical about **δ**). It may be noted that if the magnitude of $F'_H=\frac{1}{2}(F_{H+}+F_{H-})$ were to change, $\Delta_H=(F_{H+}-F_{H-})$ remaining constant, P and Q would lie on a hyperbola the direction of whose asymptotes is given by $\sin \gamma= -\Delta_H/2\delta$. Using the approximation that δ is small compared with MP and MQ is equivalent to assuming that the hyperbola can be replaced by its asymptotes. The directions of the asymptotes are determined only by the *difference* between F_{H+} and F_{H-}, and are independent of their sum, i.e. the solutions are independent of any experimental error in the magnitude of F'_H. The most probable directions of F_c having been found, the most probable phases of F can now be found by drawing a circle of radius F about the origin. This circle represents the locus of all possible phases of F, and the most probable phases are given by OR and OS where R and S are the intersections of the F_c direction vectors with the F circle.

To show the effect of using F'_H instead of F_c in calculating $P_{an}(\varphi)$ and hence the joint phase probability distribution, two examples are given. In the first (Fig. 3) the isomorphous replacement and anomalous scattering data give a 'good' phase determination in the sense that $P_{is}(\varphi)$ and $P_{an}(\varphi)$ have maxima which approximately coincide. In the second (Fig. 4) the isomorphous replacement and anomalous scattering data give somewhat conflicting phase determinations and a compromise has to be made. In the calculations E' was put equal to a third of E. As might be expected the alternative calculations using F'_H and F_c do not lead to widely diverging results; however, in both cases the form of the $P_{an}(\varphi)$ distribution changes appreciably. The difference is rather striking in the case of Fig. 3(b) where the use of F'_H results in a bimodal distribution for $P_{an}(\varphi)$ whereas using F_c gives a unimodal distribution as would be expected from Fig. 3(a). For comparison, the most probable phases of F obtained with the use of $P_{an}(\varphi)$ only are shown on the phase diagrams. Clearly the use of F_c gives a physically more acceptable result; i.e. the direction of the most probable F is such that F_c is directed toward the points of closure of the F_{H+} and F_{H-} phase circles. In the 'good' phase determination neither the overall most probable phase nor the 'best' phase is changed very much, but in the poorer determination the 'best' phase is changed by 11 degrees and the most probable phase by 20 degrees. It may be noted that the treatment here, following that of Blow & Crick, assumes that any errors in F may be considered as lying in F'_H. Using F'_H in place of F_c in evaluating $P_{an}(\varphi)$ is *not* equivalent to assuming that all the errors reside in F rather than in F'_H. If this assumption were made, then in Fig. 2 the most probable phases of F would be given by OP and OQ rather than OR and OS. From Fig. 2 [and from Figs. 3(a) and 4(a)] it is clear that the use of either assumption would generally lead to much the same values for the most probable phases.

Phase determination
with the use of heavy atoms of different types

Suppose that two isomorphous crystal structures are differentiated by N 'heavy atoms' which may or may not exhibit significant anomalous scattering. Let the position vector of the nth atom be r_n and its scattering factor

$$f_n=f'_n+if''_n .$$

For a reflexion with indices (h, k, l) the calculated structure factor of the N atoms is

$$f_c(\mathbf{h})= \sum_{n=1}^{N} f'_n(\mathbf{h}) \exp (2\pi i \mathbf{h} . r_n)$$
$$+i \sum_{n=1}^{N} f''_n(\mathbf{h}) \exp (2\pi i\mathbf{h} . r_n) \quad (11)$$
$$=\mathbf{f}(\mathbf{h})+ \boldsymbol{\delta}(\mathbf{h}) . \quad (12)$$

For simplicity we write

$$\mathbf{f}(\mathbf{h}) + \boldsymbol{\delta}(\mathbf{h})=\mathbf{f}+\boldsymbol{\delta}$$

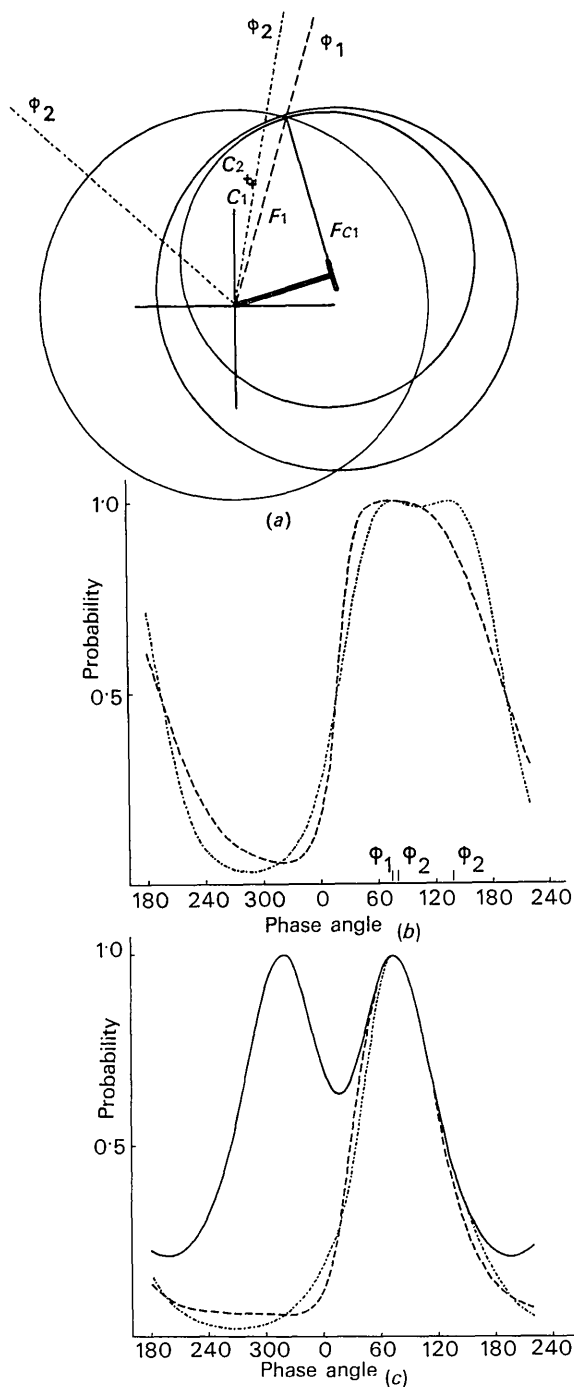and this defines f and δ in Fig. 1.

Fig. 3. (a) Phase diagram for a 'good' phase determination. φ_1 and φ_2 are the most probable phases of F obtained with the use of $P_{an}(\varphi)$ only. C_1 and C_2 are the centroids of the joint probability distributions $P(\varphi)$. The subscripts 1 and 2 refer to calculations using respectively F_c and F_H' in evaluating $P_{an}(\varphi)$. (b) Alternative anomalous scattering phase probability distributions $P_{an}(\varphi)$. The broken line is obtained with F_c, the chain line with F_H'. (c) Combination of isomorphous replacement probability distribution $P_{is}(\varphi)$ and anomalous scattering probability distribution $P_{an}(\varphi)$ to give joint phase probability $P(\varphi)$. $P_{is}(\varphi)$ is drawn solid and the alternative joint distribution $P(\varphi)$ is drawn similarly to the $P_{an}(\varphi)$ curve in (b) from which it was derived.
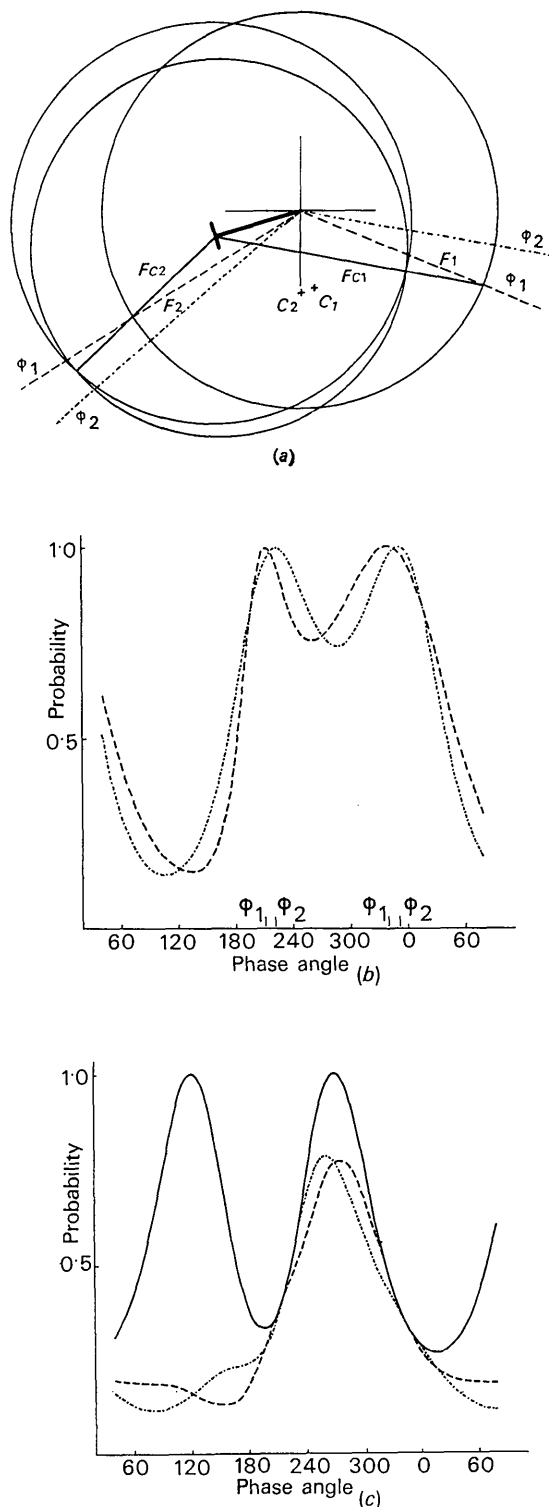
Fig. 4. (a) Phase diagram for a 'poor' phase determination. Otherwise as for Fig. 3(a). (b) Alternative anomalous scattering phase probability distribution $P_{an}(\varphi)$ for the 'poor' phase determination. (c) Combination of $P_{is}(\varphi)$ with alternative $P_{an}(\varphi)$ distributions shown in (b) to give alternative combined phase probability distributions $P(\varphi)$.

If, for this reflexion, the N atoms all have the same ratio $k = f'_n/f''_n$, i.e. they are all 'of the same type' (Rossmann, 1961) then

$$f_c(\mathbf{h}) = \mathbf{f} + \boldsymbol{\delta} = \mathbf{f} + ik^{-1}\mathbf{f} \qquad (13)$$

and $\mathbf{f}$ and $\boldsymbol{\delta}$ are orthogonal. In this case the magnitude of $\boldsymbol{\delta}$ can be found directly from that of $\mathbf{f}$ and the phase determination carried out as outlined in the previous section. In a more general case the isomorphous derivative may differ from the parent in such a way that the ratio f'_n/f''_n will not be a constant. For example, the derivative may contain heavy atoms of different atomic species, or it may contain heavy atoms covalently bonded to the parent structure through atoms of known position but lower atomic number. Also, in the case of complex ions, which are often used as 'heavy atoms', the magnitude of the ratio f'/f'' may change as a function of the angle of scattering (Matthews, 1966). A method of treating such cases will now be described.

It was shown above [equation (12)] that the calculated heavy atom structure factor $\mathbf{f}_c$ could be written as the sum of the vectors $\mathbf{f}$ and $\boldsymbol{\delta}$. Let ω be the angle between these components (see Fig. 1). It is customary to express $\mathbf{f}$ in the form

$$\mathbf{f} = a + ib \qquad (14)$$

where

$$a = \sum_{n=1}^{N} f'_n(\mathbf{h}) \cos(2\pi i\, \mathbf{h} \cdot \mathbf{r}_n)$$

and

$$b = \sum_{n=1}^{N} f'_n(\mathbf{h}) \sin(2\pi i\, \mathbf{h} \cdot \mathbf{r}_n) . \qquad (14a)$$

Similarly, $\boldsymbol{\delta}$ may be written

$$\boldsymbol{\delta} = a' + ib' \qquad (15)$$

where, from the definition of $\boldsymbol{\delta}$,

$$a' = - \sum_{n=1}^{N} f''_n(\mathbf{h}) \sin(2\pi i \mathbf{h} \cdot \mathbf{r}_n)$$

and

$$b' = \sum_{n=1}^{N} f''_n(\mathbf{h}) \cos(2\pi i \mathbf{h} \cdot \mathbf{r}_n) . \qquad (15a)$$

In deriving the expression for $P_{is}(\varphi)$, the phase probability distribution using isomorphous replacement, no assumption was made about the value of ω; therefore equation (6) can still be used, and we only need reconsider the derivation of $P_{an}(\varphi)$.

Previously the anomalous scattering lack of closure was given by equation (7). This result can be rewritten in the more general form

$$\varepsilon_+ - \varepsilon_- = - \varDelta_H + 2\delta \cos(\gamma + \omega) . \qquad (16)$$

Thus, in the most general case, the expression for $P_{an}(\varphi)$ the phase probability distribution using anomalous dispersion data is

$$P_{an}(\varphi) = \exp\left\{ - \frac{1}{2E_1'^2}[-\varDelta_H + 2\delta \cos(\gamma + \omega)]^2 \right\}. \qquad (17)$$

This may be evaluated by using the following relationships which are readily verified.

$$\sin \gamma = [F(b \cos \varphi - a \sin \varphi)]/(F_c f)$$
$$\cos \gamma = [f^2 + F(a \cos \varphi + b \sin \varphi)]/(F_c f)$$
$$\sin \omega = (ab' - a'b)/(\delta f)$$
$$\cos \omega = (aa' + bb')/(\delta f) . \qquad (18)$$

It may be noted that in practice if a' and b' are determined at the same time as a and b, little extra computing will be needed, and in fact the evaluation of the phase probability distribution in the most general case involves a comparatively small increase in computation above that involving in using the isomorphous replacement method alone.

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