ANOMALOUS DISPERSION METHOD OF DETERMINING STRUCTURE AND ABSOLUTE CONFIGURATION OF CRYSTALS

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ABSTRACT

It is possible to determine the phases of structure factors of a non-centro-symmetric crystal from an experimental measurement of the intensity-differences between pairs of inverse reflections, produced by anomalous dispersion. But the determination is ambiguous. An interesting possibility arises when the group of anomalous scatterers possesses a centre of inversion. It is found that in this case, a Fourier synthesis calculated by using both the possible values of the phase angle will contain, in addition to the peaks of the real structure, an equal number of negative peaks at positions related to the former by inversion about the inversion-centre of the group of anomalous scatterers. This idea has been used to determine the absolute configuration of L-ephedrine hydrochloride.

1. INTRODUCTION

During recent years the anomalous dispersion effect has been increasingly used by many workers to determine the absolute configuration of a number of crystals. In all the cases, the structures had to be solved beforehand by conventional methods of X-ray crystal structure analysis (which give only the structure and not the configuration) and the anomalous dispersion effect was used only to determine the configuration of the structure. However, the effect can be put to far greater use than mere determination of the configuration. In a recent paper (Ramachandran and Raman, 1956), it has been shown that it is sometimes possible to determine both the structure and the absolute configuration of a non-centrosymmetric crystal in a straightforward way by making full use of the effect of anomalous dispersion. It is found that the phases \( a(hkl) \) of the structure factors \( F'(hkl) \) can be determined, but for an ambiguity, from an experimental measurement of the intensity differences between pairs of inverse reflections \( hkl \) and \( \overline{hkl} \), produced by the imaginary component \( \Delta f'' \) of the atomic scattering factors. The nature of the ambiguity as well as methods for its resolution have been...
discussed and the validity of the method was also tested in the case of the alkaloid, L-ephedrine hydrochloride. The phase angles determined by the anomalous dispersion method were found to agree very well with those calculated from known atomic co-ordinates. In this paper, further results which tend to confirm the various conclusions previously arrived at are reported. The absolute configuration of L-ephedrine hydrochloride, which has been determined by an interesting application of the new method, is also presented.

2. PHASE DETERMINATION FROM ANOMALOUS DISPERSION

The basic theoretical relations have already been derived in the earlier paper and only a short account is given here so as to make this paper self-contained. Let the crystal contain one atom or a set of \( p \) crystallographically equivalent atoms \( A \) for which the imaginary component \( \Delta f'' \) is significant, while for the rest, \( R \), of the structure \( (N - p \text{ atoms}) \) this component is negligible. Let \( F_A' \) and \( F_A'' \) denote the contributions from the real and imaginary parts of the scattering factors of atoms \( A \) and let \( F_R \) denote the contribution from the atoms \( R \). These vectors are shown in Figs. 1 (a) and 1 (b) for both the reflection \( hkl \) and its inverse \( \bar{h}k\bar{l} \). (In the latter case, the vectors are represented by a bar over the symbols.) From these figures it is clear that

\[
|F|^2 = |F'|^2 + |F_A''|^2 + 2 |F'| |F_A''| \cos \theta
\]  

(1)

Fig. 1. Diagram showing the different components of the structure factors \( F \) and \( \bar{F} \).
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and

\[ |\mathbf{F}|^2 = |\mathbf{F}'|^2 + |\mathbf{F}_A''|^2 - 2 |\mathbf{F}'| \cdot |\mathbf{F}_A''| \cos \theta \]  \hspace{1cm} (2)

whence

\[ 
\Delta |\mathbf{F}|^2 = |\mathbf{F}'|^2 - |\mathbf{F}'| \\
= 4 |\mathbf{F}'| \cdot |\mathbf{F}_A''| \cos \theta 
\]  \hspace{1cm} (3)

where \( \theta \) is the angle between the vectors \( \mathbf{F}' \) and \( \mathbf{F}_A'' \). It is obvious that \( \mathbf{F}' \) is the structure factor when the anomalous dispersion effect is zero. If \( a \) be the phase of \( \mathbf{F}' \) and \( a_A \) the phase of \( \mathbf{F}_A'' \) then,

\[ a = \frac{1}{2} \pi + a_A - \theta. \]  \hspace{1cm} (4)

Equations (3) and (4) may be used to determine \( a \) from experimentally measured \( \Delta |\mathbf{F}|^2 \) provided the positions of the anomalous scatterers \( A \) are known. It is not difficult to know the latter, since the anomalous scatterers are usually heavy atoms.

But there is an ambiguity in the determination. This ambiguity arises because \( \theta \) derived from \( \cos \theta \) [equation (3)] may be positive or negative and correspondingly there are two values possible for \( a \), given by

\[ a_{1,2} = \frac{1}{2} \pi + a_A \pm \theta. \]  \hspace{1cm} (5)

3. THE "ANOMALOUS" SYNTHESIS

It is interesting to investigate the nature of the Fourier synthesis in which both the possible values, \( a_1 \) and \( a_2 \), obtained by the anomalous dispersion method, are used. This synthesis may be called the "anomalous" synthesis: \( \rho_{an}(xyz) \). It is given by

\[ \rho_{an}(xyz) = \frac{1}{2V} \sum_{h}^{+\infty} \sum_{k}^{+\infty} \sum_{l}^{+\infty} |\mathbf{F}'(hkl)| \{\exp ia_1(hkl) \\
+ \exp ia_2(hkl)\} \exp -2\pi i (hx + ky + lz) \]  \hspace{1cm} (6)

and the two values of \( a \) are related by

\[ a_2 = 2a_A + \pi - a_1. \]  \hspace{1cm} (7)

In general, the synthesis (6) may be shown to contain peaks at the correct atomic positions, in addition to a background which may contain spurious peaks. An interesting possibility arises in the special case when the group of anomalous scatterers possesses a centre of inversion. In this case,
the value of $a_A$ is 0 or $\pi$ and the two possible values of the phase angles are $a$ and $(\pi - a)$. If both these values are used, the resulting synthesis is simply the Fourier sine synthesis $\rho_s$, and is given by

$$\rho_{an}(xyz) = \rho_s(xyz) = \frac{2}{V} \sum_0^\infty \sum_{-\infty}^{+\infty} \left| F'(hkl) \right| \sin a(hkl) \times \sin 2\pi(hx + ky + lz)$$

This synthesis may be shown to contain, in addition to the positive peaks of the true structure, an equal number of negative peaks at positions related to the former by inversion at the inversion-centre of the group of anomalous scatterers. The proofs for both the cases will be given later.

Thus it is possible to determine the structure and absolute configuration from the anomalous synthesis. However it is to be noted that the anomalous synthesis will give no information about those parts of the structure which by accident happen to be centrosymmetric about the inversion-centre of the anomalous group. In such cases it is necessary to use a certain amount of care and judgment and confirm the deductions with other methods like the heavy atom method suggested in Section 5.

This method of determining the structure in its absolute configuration seems even more promising than the method of Pepinsky and Okaya (1956) using the Patterson sine synthesis ($P_s$). These authors have investigated the nature of the Patterson function obtained from the intensity data alone when some of the atoms are anomalous scatterers. It is found that both the structure and absolute configuration can be derived from the imaginary part $P_s(xyz)$ of the complex Patterson function. However the interpretation of the Patterson sine synthesis is much more difficult than that of the Fourier sine synthesis. This is obvious from the fact that, whereas the latter consists of only the structure and its negative inverse, the $P_s$-function will consist of a series of $p$ images of the structure as seen from each one of the $p$ atoms in the set $A$ and will contain, in addition, a duplicate of the entire series in the form of negative peaks by inversion at the origin of the diagram.

4. **Comparison of the "Anomalous" and the "Isomorphous" Synthesis**

It is interesting to compare the superposed synthesis obtained with the anomalous dispersion method with a similar synthesis which may be obtained in the parallel case of phase determination by the isomorphous replacement
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technique. In this case also the phase determination is ambiguous and the two solutions $a_2$ and $a_4$ obtained for $a$ are related by

$$a_4 = 2a_A - a_2,$$  \hspace{1cm} (9)

where $a_A$ is the phase of the contribution from the group of replaceable atoms. The Fourier synthesis obtained by using both the solutions may be called the "isomorphous synthesis," $\rho_{\text{is}}(xyz)$, and is given by

$$\rho_{\text{is}}(xyz) = \frac{1}{2V} \left\{ \sum_{h=-\infty}^{\infty} \sum_{k=-\infty}^{\infty} \left| F'_{\text{is}}(hkl) \right| \exp \{ia_2(hkl)\} + \exp \{ia_4(hkl)\} \exp -2\pi i (hx + ky + lz) \right\} \hspace{1cm} (10)$$

As in the case of the anomalous synthesis, this synthesis also will give peaks at the correct atomic positions in addition to a background. In the case when the group of replaceable atoms possesses a centre of inversion, the two solutions are $a$ and $-a$ and the isomorphous synthesis calculated using both the possible values becomes merely the Fourier cosine synthesis ($\rho_{\text{c}}$ say).

$$\rho_{\text{is}}(xyz) = \rho_{\text{c}}(xyz) = \frac{F(000)}{V} + \frac{2}{V} \sum_{h=-\infty}^{\infty} \sum_{k=-\infty}^{\infty} \left| F_{\text{is}}'(hkl) \right| \cos a(hkl) \cos 2\pi (hx + ky + lz). \hspace{1cm} (11)$$

It can be shown that this $\rho_{\text{c}}$ function will contain two sets of peaks, both positive and the two sets will be related by inversion about the inversion-centre of the group of replaceable atoms. Thus $\rho_{\text{is}}$ will give the structure and its positive inverse whereas $\rho_{\text{an}}$ will give the structure and the negative inverse.

Obviously a happy example is one where the replaceable atoms are also anomalous scatterers. Then the isomorphous method can be used to determine values of $a$ and $-a$ and the anomalous dispersion method determines values of $a$ and $(\pi - a)$. Using both methods in conjunction it is possible to fix uniquely the value of $a$ and calculate the electron-density,

$$\rho(xyz) = \frac{F(000)}{V} + \frac{2}{V} \sum_{h=-\infty}^{\infty} \sum_{k=-\infty}^{\infty} \left| F'_{\text{is}}(hkl) \right| \cos a(hkl) \hspace{1cm} \times \cos 2\pi (hx + ky + lz)$$

$$+ \frac{2}{V} \sum_{h=-\infty}^{\infty} \sum_{k=-\infty}^{\infty} \left| F_{\text{is}}'(hkl) \right| \sin a(hkl) \hspace{1cm} \times \sin 2\pi (hx + ky + lz). \hspace{1cm} (12)$$
Thus the two methods are complementary. The isomorphous replacement method determines the first synthesis given by equation (12) (i.e., the structure and its positive inverse) and the anomalous dispersion method determines the second synthesis (i.e., the structure and its negative inverse) and the two together add up to give the electron-density $\rho (xyz)$ (i.e., the structure).

It is also interesting to note that both methods are based on more or less the same principle, that phase determination is possible by introducing a change in the scattering powers of a few atoms and studying its effect on the intensities of the X-ray reflections. In the isomorphous replacement method, this change is achieved by isomorphously replacing some of the atoms. In the anomalous dispersion method there is no replacement as such, but use is made of the fact that when anomalous dispersion takes place, the intensities of the inverse reflections $hkl$ and $\overline{hkl}$ are different. The phases are determined from the intensity differences between pairs of inverse reflections. Thus in this case the isomorphous substances appear to be merely the structure and its inverse and the analogy is clearer if it is remembered that the reflection $hkl$ of the structure is the reflection $\overline{hkl}$ of the inverse.

Between the two methods, preference should be given to the anomalous dispersion method because it requires only one crystal, whereas the other method demands a series of isomorphous substances, with a minimum of at least two.

5. Experimental Verification

The method has been experimentally verified in an investigation of the structure of L-ephedrine hydrochloride. The structure of this compound has been determined by conventional methods (Phillips, 1954). Our aim was to test the new method both as regards the determination of phase and the determination of absolute configuration. The crystal belongs to the monoclinic space group and the unit cell contains two chlorine atoms. These show a measurable, though small, anomalous effect ($\Delta f^\prime \epsilon_{l} = 0.69$) even for CuKα radiation. The crystals are elongated along the $b$-axis but the $h0l$ reflections are not useful for our purpose. Therefore it was necessary to make measurements in the $c$-zone. The differences in the intensities of the pairs of inverse reflections $hk0$ and $\overline{hk0}$ were measured with a Geiger counter spectrometer. The measurements were put on an absolute basis by direct comparison with the reported values of $F_c$. The values of $\alpha$ and
(\pi - \alpha) were obtained by using an approximate form of expression (3), namely,
\[
\sin \alpha = \Delta F / 2 F_A''
\]
which is valid if \(F_A'' \ll F_A'\). The phase angles thus derived were found to be in good agreement with the correct values calculated from the atomic co-ordinates. The measurements were also put on an absolute basis by the method of Wilson (1942) which is the usual method adopted when the structure is not known. The phase angles derived in this way were not much different from the above values. A table showing the phase angles derived by the former method and the correct values has been given in the earlier paper (Ramachandran and Raman, 1956).

Using these values and the observed values of \(|F_o|\) the \(\rho_{an}\)-synthesis was calculated (Fig. 2). The phases of some of the reflections could not be determined because the contribution from the chlorine atoms to these reflections is zero. The corresponding coefficients had to be dropped from the synthesis. Further, the phases of a few reflections disagreed by more than twenty degrees from the correct phases calculated from known atomic co-ordinates. In spite of this, these were included in the synthesis, as this is what one would do if the phases were not known to start with. To see what would be the effect of both these defects, the synthesis was calculated using the correct values of \(\alpha\) and \((\pi - \alpha)\) taken from Phillips’ paper and this is shown in Fig. 3. It is found that the two diagrams agree in their broad
features, though the latter is in better agreement with the correct structure. In interpreting the diagrams, the important fact should be borne in mind that the positive and the negative density distributions are superposed and will interfere. This effect will be more serious in a two-dimensional projection than in a three-dimensional synthesis. To see the nature of this interference, the two molecules are drawn in both the diagrams, using the atomic co-ordinates given by Phillips and their negative inverses have also been drawn. If now the phases are calculated, taking only the positions of the positive peaks then the ambiguity in the solution of \( \alpha \) may be resolved.

![Diagram](image)

**Fig. 3.** \( \rho_{an} \)-function projected on (001) using correct values of the phase angles. The contours have been drawn at arbitrary intervals of 20. The full lines represent positive contours and broken lines represent negative contours.

It may be resolved in another way also (Ramachandran and Raman, 1956). Of the two possible values of \( \alpha \), the one which is nearer to the phase of the chlorine atoms may be chosen for a preliminary Fourier synthesis. This procedure is justified for those reflections for which the contribution from the heavy chlorine atoms is fairly large. This synthesis is shown in Fig. 4, which is seen to be a good approximation to the structure. This use of the heavy atom method has been suggested by Peerdemann and Bijvoet also (1956).

6. **Indexing the Reflections for Finding Absolute Configuration**

The \( \rho_{an} \)-diagram can be used to determine the absolute configuration of the compound. The positive peaks of the diagram correspond to the structure in its absolute configuration provided correct signs have been assigned to the indices \( hko \). This is easily done by choosing a particular co-ordinate system on the crystal and indexing the reflections on the basis of this system.
We have conventionally taken this frame of reference to be a right-handed system of co-ordinates. For a monoclinic crystal, the senses of the co-ordinate axes can be chosen in two equivalent ways to get a right-handed system, as shown in Figs. 5 (a) and 5 (b). The sense of either the c- or a-axis can be arbitrarily chosen and then the senses of the other two axes are automatically fixed by the following two stipulations:—

(a) The angle between the positive directions of c- and a-axes should be obtuse; this fixes the sense of a, once the sense of c has been arbitrarily chosen;

(b) The sense of b is fixed by the fact that the three axes should form a right-handed system.

Fig. 4. Electron-density projected on (001). Contours have been drawn at intervals of approximately 1 e. A\(^{-2}\) except near the chlorine atom where the interval is 2 e. A\(^{-2}\). The one electron contour is not shown.

Fig. 5. The two equivalent right-handed co-ordinate systems possible for monoclinic crystals.
In the particular example, the crystal was set with the c-axis vertical. The directions of a- and b-axes were determined from the known positions at which the corresponding planes (100) and (010) reflect in the spectrometer. The positive direction of c was arbitrarily chosen to be vertically upwards. The sense of a can then be fixed from stipulation (a) provided it is known whether the chosen direction of a makes an obtuse or an acute angle with the chosen c direction. This point was decided by studying the direction of shift of the higher layer spots of an oscillation-photograph taken about the c-axis. The sense of the remaining axis, namely b-axis, can be fixed from stipulation (b). This procedure of assigning correct signs to the indices is essential, for a reversal in the sign will lead to the inverse configuration. Some aspects of the method of assigning the indices correctly in an orthorhombic crystal have been discussed by Peerdemann and Bijvoet (1956).

7. Absolute Configuration of L-Ephedrine Hydrochloride

The absolute configuration of L-ephedrine hydrochloride has thus been determined (Fig. 6). The alkaloid ephedrine \(\text{C}_6\text{H}_11\text{CH} (\text{OH}) \text{CH} (\text{CH}_3)\text{NH CH}_3\) has four isomers, namely, (−)-ephedrine, (+)-ephedrine, (−)-psi-ephedrine and (+)-psi-ephedrine. Their conformations have been studied by stereochemical methods (Henry, 1949; Close, 1950) and relative configurations have been assigned to the isomers from their relation, established by chemical means, to D-glyceraldehyde and hence to D-tartaric acid whose absolute configuration is now known (Bijvoet and others, 1951). The present analysis gives an independent determination of the absolute configuration of L-ephedrine hydrochloride. The configuration found by us is the one assigned to (+)-ephedrine. The earlier X-ray analysis of Phillips did not make use of the effect of anomalous dispersion and so it is consistent with this configuration as well as its mirror-image. Of the two possibilities Phillips chose the configuration which was in agreement with the one deduced by a number.
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of chemical workers as the correct configuration. The present analysis confirms this choice.

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REFERENCES