# Recovering Phase Information from Intensity Data

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THE CONCEPT OF A CRYSTAL IS THAT OF A SOLID BODY IN which the atomic or molecular units are so arranged as to form an array having three-dimensional periodicity. Because of the periodicity, it is possible to describe the arrangements of the atomic composition by means of Fourier series. The type of Fourier series that is used in crystal structure analysis represents the electron density distribution in a crystal. This is indeed equivalent to representing the structure of a crystal since the atomic locations are represented by the regions of highest electron density in the electron distributions.

The experimental technique used for examining the structure of crystals is called diffraction. In a diffraction experiment, rays are made to impinge on a crystalline substance of interest, and, given the proper geometric conditions, the rays are scattered as if they were bouncing off large numbers of different planes imagined to be cutting through the crystal. The collected intensities of scattering (often 5,000 to 10,000 in number) are called a scattering pattern or diffraction pattern and comprise the experimental data from which the structure of the crystal of interest is to be elucidated. The most commonly used rays are Roentgen rays or x-rays, as they are usually called. Other rays, composed of neutrons or electrons, also have their purposes.

The problem that the analyst faces is to be able to take the diffraction pattern and from it determine the atomic architecture of the crystal which cannot be observed directly. There is a special problem in taking the intensity information from a diffraction pattern and calculating from it the electron density distribution of a crystal by use of the Fourier series. The coefficients in the Fourier series are, in general, complex numbers. Only the magnitudes of the complex numbers appear to be available from the measured intensities of scattering. The required phase angles of the complex numbers seem to be lost in an ordinary x-ray diffraction experiment. It was therefore generally thought that it was not possible to go directly from a diffraction pattern to a determination of a crystal structure. The impasse was overcome in a series of steps that involved recognition that the required phase information was contained in the experimental intensity information, the derivation of a foundation mathematics that displayed relationships between phases and magnitudes and even among phases alone and, finally, the development of practical procedures for structure determination, strategies that brought together in a more or less optimal fashion the mathematical relationships with suitably adjusted and refined experimental data.

The results of structure determinations have been playing a valuable role in a number of areas of scientific endeavor. Crystallization, for example, is a very common phenomenon and many types of substances form crystals ranging from metals and minerals to huge macromolecules such as viruses. Knowledge of structure allows one to relate structure to function, that is, understand physical, chemical, or biological properties and activities; provides the chemist with useful information for syntheses, modifications, and reaction mechanisms; and can also be used to identify very small quantities of scarce material. It often provides the theoretical chemist with a starting point for his calculations. Structural research provides a conceptual basis for many associated scientific disciplines and it is the opportunity to interact with a variety of such disciplines that has made structural research particularly appealing to me.

## Electron Density Distribution

The electron density distribution, $\rho(\mathbf{r})$, is expressed in terms of the three-dimensional Fourier series

$$\rho(\mathbf{r}) = V_c^{-1} \sum_{\mathbf{h}=-\infty}^{\infty} F_{\mathbf{h}} \exp(-2\pi i \mathbf{h} \cdot \mathbf{r}) \qquad (1)$$

where $V_c$ is the volume of the unit cell of the crystal, the basic structural unit from which, through three-dimensional periodicity, the crystal is formed. The coefficients

$$F_{\mathbf{h}} = |F_{\mathbf{h}}| \exp(i\phi_{\mathbf{h}}) \qquad (2)$$

are the crystal structure factor associated with the plane labeled with the vectors $\mathbf{h}$. The $\mathbf{h}$ have integer components, $h$, $k$, and $\ell$, the Miller indices, whose values are inversely proportional to the intercepts on the $x$, $y$, and $z$ axes, respectively, of planes cutting through the crystal. The angle $\phi_{\mathbf{h}}$ is the phase associated with $F_{\mathbf{h}}$, and $\mathbf{r}$ labels the position of any point in the unit cell. $F_{\mathbf{h}}\mathbf{E}$ is the amplitude of the scattered wave associated with the plane labeled by $\mathbf{h}$, where $\mathbf{E}$ is the electric field vector of the incident beam. The measured intensities of x-ray scattering are proportional to $|F_{\mathbf{h}}|^2$. If the values of the $\phi_{\mathbf{h}}$ were also obtained directly from experiment, structures could be immediately calculated from (1). The seeming absence of this information gave rise to the so-called "phase problem."

The Fourier inversion of (1), followed by the replacement of the integral by the sum of contributions from the $N$ discrete atoms in the unit cell gives, for the Fourier coefficient,

$$|F_{\mathbf{h}}| \exp(i\phi_{\mathbf{h}}) = \sum_{j=1}^{N} f_{j\mathbf{h}} \exp(2\pi i \mathbf{h} \cdot \mathbf{r}_j) \qquad (3)$$

where $f_{j\mathbf{h}}$ represents the amplitude of scattering of the $j$th atom in the unit cell and $\mathbf{r}_j$ is its position vector.

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## Overdeterminacy

A system of simultaneous equations is formed by the definition of the crystal structure factors given by (3) since the values of the scattered intensities are measured for a large number of $\mathbf{h}$. The unknown quantities in (3) are the phases $\phi_{\mathbf{h}}$ and the atomic positions $\mathbf{r}_j$. The known quantities are the $|F_{\mathbf{h}}|$ obtained from the measured intensities and the $f_{j\mathbf{h}}$, which differ little from the theoretically calculated atomic scattering factors for free atoms. Since each equation in (3) involves complex quantities, there are really two equations, one for the real and one for the imaginary part. In order to determine the overdeterminacy, a comparison is made of the number of unknown quantities with the number of independent data available. With the use of CuK$\alpha$ radiation, the overdeterminacy can be as great as a factor of about 50 for crystals that have a center of symmetry and about 25 for those that do not. In practice, somewhat fewer than the maximum available data are measured, but the overdeterminacy is still quite high.

## Some Attempts, Some Successes

There were some early attempts to obtain structural information or phase information from the structure factor equations. The names Ott (*1*), Banerjee (*2*), and Avrami (*3*) are associated with early attempts. The computational demands of these approaches limited their application to very simple structures and the limitations cannot be suitably overcome even now with modern computers.

A significant advance in the attempt to obtain structural information from the measured intensities was made in 1934 by A. L. Patterson (*4, 5*). He developed a Fourier series which has as its coefficients the magnitude of the square of the structure factors rather than the structure factors themselves. The phases may be eliminated from (3) by multiplying by the corresponding complex conjugates to obtain

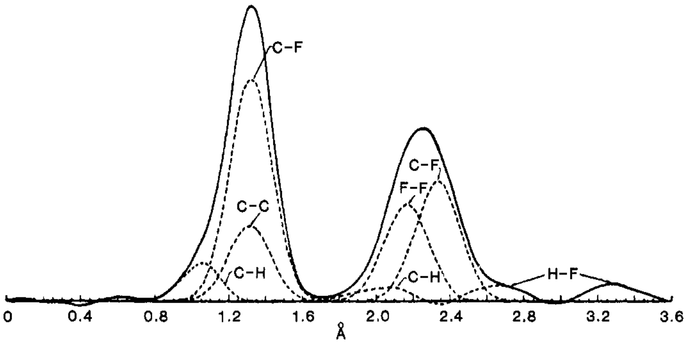
$$|F_{\mathbf{h}}|^2 = \sum_{j=1}^{N}\sum_{k=1}^{N} f_{j\mathbf{h}} f_{k\mathbf{h}} \exp[2\pi i\mathbf{h}\cdot(\mathbf{r}_j - \mathbf{r}_k)] \qquad (4)$$

The Fourier transform of (4) is known as the Patterson function (*4, 5*),

$$P(\mathbf{r}) = \sum_{\mathbf{h}=-\infty}^{\infty} |F_{\mathbf{h}}|^2 \exp(-2\pi i\mathbf{h}\cdot\mathbf{r}) \qquad (5)$$

The maxima of a Patterson function represent the interatomic vectors in a structure. Evidently the values of the coefficients are directly obtainable from the measured intensities of scattering. This function has been very useful in locating the heavier atoms in a structure, if they are not too numerous, since the interatomic vectors associated with them would predominate in a map computed from (5) and the atomic positions for them could then be readily deduced. The coordinates for the heavy atoms may be used with (3) to compute an initial set of approximate phases. Depending upon the scattering power of the heavy atoms, such a computation may be suitable for structures containing up to a few hundred atoms. There are numerous procedures for developing a complete structure from the initial phase information obtained from the heavy atoms. The use of the Patterson function with structures containing heavy atoms has found widespread application and remains one of the major methods of crystal structure determination.

The difficulty with using the Patterson function with experimental data in a general way in the absence of heavy atoms arises from the lack of resolution that occurs for the $N(N-1)$ interatomic vectors as well as inaccuracies. The Patterson function becomes somewhat accessible when it is used in combination with known atomic groupings (*6–8*).



| Distance | C-H | C-C | C-F | C-H | F-F | C-F | H-F | H-F |
|---|---|---|---|---|---|---|---|---|
| $nZ_iZ_j$ | 4 | 12 | 36 | 4 | 27 | 36 | 6 | 6 |
| Actual area | 4.2 | 12.1 | 36.0 | 4.1 | 27.2 | 36.0 | 5.4 | 7 |

Fig. 1. The essentially non-negative radial distribution function (solid curve) for $CH_2CF_2$, computed from the experimental molecular intensity data extracted by use of a properly formed background intensity curve. The solid curve is a probability density that gives the probability of finding interatomic distances in some distance interval along the horizontal axis. The dashed lines represent the decomposition of the main peaks into their component individual interatomic distances. The individual peaks have a definite width related to the internal vibrations in the molecule.

Relationships between phases and magnitudes that anticipated the later developments were the inequalities of Harker and Kasper (*9*). They derived a number of inequalities by application of the Schwarz and Cauchy inequalities to the structure factor equations (3) in the presence of crystallographic symmetry. The Harker-Kasper inequalities have provided valuable insights. For example, the simple inequality formulas can provide useful phase information as shown by Kasper, Lucht, and Harker (*10*) in their solution of the structure of decaborane. In addition, work with the inequalities indicated that they may have probabilistic characteristics. Gillis (*11*), for example, noted that the implication of an inequality was probably correct even when the magnitudes of the structure factors were too small to permit a definitive conclusion to be drawn. Gillis speculated that the smallness of the structure factor magnitudes may have been due to thermal effects and employed an appropriate function to increase the values of the structure factor magnitudes so that the inequalities could be applied. The probabilistic interpretation, however, remained a possible alternative, namely, that, although an inequality does not quite determine the value of a phase definitively, it still does so with a high probability that the value is correct. This could be important because it would imply that the inequalities have probabilistic implications that could extend their range of usefulness.

## Non-Negativity and General Inequalities

The initial motivation to investigate the mathematics of crystal structure determination arose from experiences in the development of an analytical procedure for obtaining accurate radial distribution functions for determining the structures of gaseous molecules by electron diffraction. A problem arose, namely, to find an accurate background intensity so that the molecular interference intensity could be accurately extracted from the total intensity of scattering. The Fourier transform of the molecular intensity can be interpreted as representing the probability of finding interatomic distances in a molecule. Therefore, this transform must be non-negative and the non-negativity imposed a very useful constraint on the shape of the background intensity (*12, 13*). Figure 1 shows a radial density function for $CH_2CF_2$ (*14*) derived from application of the non-negativity constraint and the component distances in the molecule.

The attendant accuracy of the result permitted not only equilibrium interatomic distances to be determined but also estimates of the root-mean-square amplitudes of vibration associated with the interatomic distances.

At about the time this work in gas electron diffraction was proceeding, Herbert Hauptman joined our group at the Naval Research Laboratory and, in view of the success of the non-negativity criterion, we decided to explore the possibility that this criterion might be useful in other areas of structural research. This led us to investigate the determination of electron density distributions around free atoms (*15*) which found a very fine application in the determination of the electron distribution about argon by Bartell and Brockway (*16*).

We were also quite interested in seeing what the consequences of the application of non-negativity would be for crystal structure analysis since the electron density distribution defined in (1) is constrained not to be negative. This brought in the work of Toeplitz (*17*) early in this century on non-negative Fourier series and subsequent development by others. We discussed the theory in three-dimensions and wrote it in a form that would have particular relevance to crystallographic data.

The fundamental result was that the necessary and sufficient condition for the electron density distribution in a crystal to be non-negative is that an infinite system of determinants involving the crystal structure factors be non-negative. A typical determinant is (*18*)

$$\begin{vmatrix} F_{000} & F_{-\mathbf{k}_1} & F_{-\mathbf{k}_2} & \cdots & F_{-\mathbf{h}} \\ F_{\mathbf{k}_1} & F_{000} & F_{\mathbf{k}_1-\mathbf{k}_2} & \cdots & F_{\mathbf{k}_1-\mathbf{h}} \\ F_{\mathbf{k}_2} & F_{\mathbf{k}_2-\mathbf{k}_1} & F_{000} & \cdots & F_{\mathbf{k}_2-\mathbf{h}} \\ F_{\mathbf{h}} & F_{\mathbf{h}-\mathbf{k}_1} & F_{\mathbf{h}-\mathbf{k}_2} & \cdots & F_{000} \end{vmatrix} \geq 0 \qquad (6)$$

The subscripts in the first column start with 0,0,0 but are otherwise arbitrary. The subscripts in the first row are the same but of opposite sign. The subscript of the element in the *i*th row and the *j*th column is the sum of the subscripts of the elements of the *i*th row and first column and the first row and *j*th column. The third order inequality

$$\begin{vmatrix} F_{000} & F_{-\mathbf{k}} & F_{-\mathbf{h}} \\ F_{\mathbf{k}} & F_{000} & F_{\mathbf{k}-\mathbf{h}} \\ F_{\mathbf{h}} & F_{\mathbf{h}-\mathbf{k}} & F_{000} \end{vmatrix} \geq 0 \qquad (7)$$

contains a relationship among the structure factors that has played a most important role in direct crystal structure analysis. This may be seen by rewriting (7) in the form (*18*)

$$\left| F_{\mathbf{h}} - \frac{F_{\mathbf{k}} F_{\mathbf{h}-\mathbf{k}}}{F_{000}} \right| \leq \frac{\begin{vmatrix} F_{000} & F_{-\mathbf{k}} \\ F_{\mathbf{k}} & F_{000} \end{vmatrix}^{1/2} \begin{vmatrix} F_{000} & F_{-\mathbf{h}+\mathbf{k}} \\ F_{\mathbf{h}-\mathbf{k}} & F_{000} \end{vmatrix}^{1/2}}{F_{000}} \qquad (8)$$

For structure factors of unusually large magnitude, the right side of (8) becomes quite small and then

$$F_{\mathbf{h}} \sim F_{\mathbf{k}} F_{\mathbf{h}-\mathbf{k}} / F_{000} \qquad (9)$$

One evident conclusion from (9) is

$$\phi_{\mathbf{h}} \sim \phi_{\mathbf{k}} + \phi_{\mathbf{h}-\mathbf{k}} \qquad (10)$$

This states that for large structure factor magnitudes, the value of $\phi_{\mathbf{h}}$ may be defined in terms of the values of two other phases. This may also be seen from a construction based on (8), Fig. 2, in which $\delta = F_{\mathbf{k}} F_{\mathbf{h}-\mathbf{k}} / F_{000}$ and $r$ is equal to the right side of (8). The form for (8) is then

$$|F_{\mathbf{h}} - \delta| \leq r \qquad (11)$$

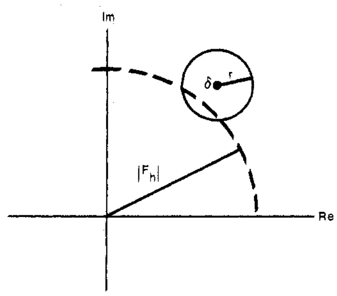


Fig. 2. The determinantal inequalities can be written in the general form $|F_{\mathbf{h}} - \delta| \leq r$. This means that $F_{\mathbf{h}}$ is bounded by a circle of radius $r$ in the complex plane centered at $\delta$. If $|F_{\mathbf{h}}|$ is known, the $F_{\mathbf{h}}$ is confined to a line within the circle.

It can be readily shown (*18*) that all determinants (6) can be written in the form of (11). As the order of the determinants increases, there is a tendency for $r$ to decrease in size, making the determination of $\phi_{\mathbf{h}}$ rather definitive.

Formula (10) has found wide application beyond the range of usefulness of (8). This is because of the probabilistic characteristics of the inequalities (*19*) which imply that the most likely value of $\phi_{\mathbf{h}}$ is that of $\phi_{\mathbf{k}} + \phi_{\mathbf{h}-\mathbf{k}}$ and the probability decreases the farther the value of $\phi_{\mathbf{h}}$ deviates from that of $\phi_{\mathbf{k}} + \phi_{\mathbf{h}-\mathbf{k}}$. Therefore, even when the radius, $r$, of the bounding circle is large, the most likely value of $\phi_{\mathbf{h}}$ is $\phi_{\mathbf{k}} + \phi_{\mathbf{h}-\mathbf{k}}$.

The structure factors in (6) can be replaced by quasi-normalized structure factors, $\mathscr{E}$, that represent point atoms (to an approximation when atoms of unequal atomic number are present) rather than atoms with electron distributions,

$$\mathscr{E}_{\mathbf{h}} = F_{\mathbf{h}} \Big/ \left( \sum_{j=1}^{N} f_{j\mathbf{h}}^2 \right)^{1/2} \qquad (12)$$

Structure factors representing point atoms are the type of quantity normally used in phase-determining procedures. Instead of (9), we have

$$\mathscr{E}_{\mathbf{h}} \sim \mathscr{E}_{\mathbf{k}} \, \mathscr{E}_{\mathbf{h}-\mathbf{k}} / \mathscr{E}_{000} \qquad (13)$$

For centrosymmetric crystals, we have

$$s\mathscr{E}_{\mathbf{h}} \sim s\mathscr{E}_{\mathbf{k}} \mathscr{E}_{\mathbf{h}-\mathbf{k}} \qquad (14)$$

where s means "sign of," a plus sign implying that the phase is equal to zero and a minus sign that it is equal to $\pi$. A one-term tangent formula also follows from (13),

$$\tan\phi_{\mathbf{h}} \simeq \frac{|\mathscr{E}_{\mathbf{k}} \mathscr{E}_{\mathbf{h}-\mathbf{k}}| \sin(\phi_{\mathbf{k}} + \phi_{\mathbf{h}-\mathbf{k}})}{|\mathscr{E}_{\mathbf{k}} \mathscr{E}_{\mathbf{h}-\mathbf{k}}| \cos(\phi_{\mathbf{k}} + \phi_{\mathbf{h}-\mathbf{k}})} \qquad (15)$$

The tangent formula composed of the sum of terms over **k** both in the numerator and the denominator is another formula that has played a major role in the practical applications of the theory for structure determination.

The variety of phase determining formulas contained within the determinantal inequalities (6) have their counterpart in probability theory, that is, similar formulas can be derived with the use of probability theory. The pursuit of probabilistic formulas was motivated by the expectation that the usefulness of the formulas from the inequalities could be extended because of the great overdeterminancy of the structure problem. One of their virtues is that measures of reliability can be attached to them and the judicious use of such measures was an important feature in bridging the gap between mathematical theory and practical application.

In order to characterize the probabilistic aspects of this subject, we initially decided to develop a facility in the use of the random walk (*20*), but subsequently changed to the joint probability distribution (*21, 22*) which culminated in a monograph (*23*) that contained for the first time, a set of probabilistic formulas and measures for attacking the phase problem, in this case limited to crystals that have a center of symmetry. It was in the monograph (*23*) also that the theory of invariants and semi-invariants was introduced for the purpose of solving the problem concerning how many and what types of phases to specify to fix the origin in a crystal and what values are permitted. The practical aspects of solving crystals that lack a center of symmetry were developed later on and it was not until 1964 that the structure of the first crystal lacking a center of symmetry was solved (*24*) by the "direct method" for obtaining phase information by direct use of the measured intensities of scattering. It is interesting that fairly recent developments in the mathematics of the random walk have made this technique quite accurate, stimulating revived interest in its application to the probabilistic aspects of phase determining formulas (*25*).

## Formulas for Phase Determination

The main formulas for phase determination are now listed. They will suffice to characterize the nature of the phase determining procedures. There are additional formulas that play a variety of helpful roles and may be found in the referenced literature of this article.

*Centrosymmetric crystals.* The $\Sigma_2$ formula is (*23*),

$$sE_{\mathbf{h}} \approx s \sum_{\mathbf{k}_r} E_{\mathbf{k}} E_{\mathbf{h}-\mathbf{k}} \qquad (16)$$

where s means the "sign of" and $\mathbf{k}_r$ represents restricted values of $\mathbf{k}$ for which the corresponding $|E_{\mathbf{k}}|$ and $|E_{\mathbf{h}-\mathbf{k}}|$ values are large. A plus sign refers to a phase of zero and a minus sign to a phase of $\pi$, the only two values possible for a centrosymmetric crystal when an origin in the crystal is properly chosen. The quantities, $E$, are normalized structure factors which arise as appropriate quantities to use with probability theory and are the same as the quasi-normalized structure factors, $\mathscr{E}$, except for a reweighting (*26*) of certain subsets of the $E$. The treatment of the intensity data to obtain normalized structure factors (*27*) arises from the work of Wilson (*28, 29*), the earliest application of probability methods to crystal structure analysis. Formula (16) is the probability equivalent of the set of inequalities (8) as $\mathbf{k}$ varies over the set $\mathbf{k}_r$. The appropriate probability function, $P_+(\mathbf{h})$, which represents the probability that the sign of $E_{\mathbf{h}}$ be positive, was given in the monograph (*23*). It is conveniently applied in the form derived by use of the central limit theorem by Woolfson (*30*) and Cochran and Woolfson (*31*),

$$P_+(\mathbf{h}) \approx \frac{1}{2} + \frac{1}{2}\tanh \sigma_3\sigma_2^{-3/2}|E_{\mathbf{h}}| \sum_{\mathbf{k}} E_{\mathbf{k}} E_{\mathbf{h}-\mathbf{k}} \qquad (17)$$

where

$$\sigma_n = \sum_{j=1}^{N} Z_j^n \qquad (18)$$

and $Z_j$ is the atomic number of the $j$th atom in the unit cell containing $N$ atoms.

*Noncentrosymmetric crystals.* The sum of angles and tangent formulas are, respectively,

$$\phi_{\mathbf{h}} \approx \langle \phi_{\mathbf{k}} + \phi_{\mathbf{h}-\mathbf{k}} \rangle_{\mathbf{k}_r} \qquad (19)$$

$$\tan\phi_{\mathbf{h}} \approx \frac{\sum_{\mathbf{k}} |E_{\mathbf{k}}E_{\mathbf{h}-\mathbf{k}}| \sin(\phi_{\mathbf{k}} + \phi_{\mathbf{h}-\mathbf{k}})}{\sum_{\mathbf{k}} |E_{\mathbf{k}}E_{\mathbf{h}-\mathbf{k}}| \cos(\phi_{\mathbf{k}} + \phi_{\mathbf{h}-\mathbf{k}})} \qquad (20)$$

Formulas (19) and (20) are comparable to (10) and (15), respectively, and result from combining a number of individual terms as $\mathbf{k}$ varies over some chosen set. An appropriate measure of the reliability of (19) and (20) is a variance, $V$ (*32*), given by

$$V = \frac{\pi^2}{3} + [I_0(\alpha)]^{-1} \sum_{n=1}^{\infty} [I_{2n}(\alpha)/n^2] - 4[I_0(\alpha)]^{-1} \sum_{n=0}^{\infty} [I_{2n+1}(\alpha)/(2n+1)^2] \qquad (21)$$

where $I_n$ is a Bessel function of imaginary argument (*33*),

$$\alpha = \left\{ \left[ \sum_{\mathbf{k}_r} \kappa(\mathbf{h},\mathbf{k}) \cos(\phi_{\mathbf{k}} + \phi_{\mathbf{h}-\mathbf{k}}) \right]^2 + \left[ \sum_{\mathbf{k}_r} \kappa(\mathbf{h},\mathbf{k}) \sin(\phi_{\mathbf{k}} + \phi_{\mathbf{h}-\mathbf{k}}) \right]^2 \right\}^{1/2} \qquad (22)$$

and

$$\kappa(\mathbf{h},\mathbf{k}) = 2\sigma_3\sigma_2^{-3/2}|E_{\mathbf{h}}E_{\mathbf{k}}E_{\mathbf{h}-\mathbf{k}}| \qquad (23)$$

Expression (21) gives the variance of $\phi_{\mathbf{h}}$ as determined from a given set of $\phi_{\mathbf{k}} + \phi_{\mathbf{h}-\mathbf{k}}$. This variance formula has its origin in a probability distribution (in somewhat different notation) of Cochran (*34*) for $\phi_{\mathbf{h}}$, given a particular $\phi_{\mathbf{k}} + \phi_{\mathbf{h}-\mathbf{k}}$ and the accompanying $|E|$ values. The tangent formula (20) can be derived in many ways. It has arisen, for example, in theoretical investigations of noncentrosymmetric space groups by use of the joint probability distribution (*35*) and can be shown to occur (*32*) in a generalization of the Cochran formula (*34*) for a particular $\phi_{\mathbf{k}} + \phi_{\mathbf{h}-\mathbf{k}}$ to take into consideration a set consisting of several or more $(\phi_{\mathbf{k}} + \phi_{\mathbf{h}-\mathbf{k}})$ rather than just one (*32, 34*). The average in (19) is to be taken in the context of maximum clustering, that is, a minimum deviation of the contributions of individual addition pairs, $\phi_{\mathbf{k}} + \phi_{\mathbf{h}-\mathbf{k}}$ from the average value. All $\phi$ are kept in a range $-\pi < \phi \leq \pi$ and maximum clustering requires the addition of 0, $2\pi$, or $-2\pi$ to each addition pair. A practical method for effecting appropriate clustering has been described (*32*).

## Practical Phase Determination

In this part, the various aspects of practical phase determination will be outlined in terms of the first procedure that had broad practical applications to both centrosymmetric and noncentrosymmetric crystals, the symbolic addition procedure (*24, 32, 36, 37*). It arose mainly from the efforts of my wife, Dr. Isabella Karle, to bridge the gap between the mathematics of phase determination and the world of experimental data and practical application.

Once the intensity data are collected, they are transformed into normalized structure factors magnitudes defined by

$$|E_{\mathbf{h}}| = |F_{\mathbf{h}}| \Big/ \left( \epsilon \sum_{j=1}^{N} f_{j\mathbf{h}}^2 \right)^{1/2} \qquad (24)$$

where $\epsilon$ reweights certain subsets of the data (*26*). A procedure for doing this is described in the *International Tables for X-ray Crystallography* (*38*).

It is apparent on examining (16), (19), and (20) that it is necessary to know the values of some phases before additional ones can be evaluated. There are several sources of such information, from certain phase specifications associated with establishing an origin in the crystal (*38*), the assignment of symbols to some phases for later evaluation, and the use on occasion of auxiliary formulas, such as $\Sigma_1$ and $\Sigma_3$, that define individual phases in terms of structure factor magnitudes alone (*38*). The number and types of phases to be used for specifying the origin in a crystal has been determined by use of the theory of invariants and semi-invariants that was developed for this purpose. Depending upon the type of space group involved, the number can vary from none at all to as many as three. Suitable tables (*38*) are available for carrying out this task.

The phase determining procedure is a stepwise one with few contributors to (15) or (19) at the start. Use at the start of phases associated with the largest possible values of the normalized structure factor magnitudes, $|E|$, will assure that the probability measures, (17) and (21) will be as large as possible. The large overdeterminacy of the problem helps to ensure that initial probabilities will be large enough to proceed in a stepwise fashion to build up a sufficiently reliable set of phase values to effect a solution to the structure problem. Because the nature of phase determination is inherently probabilistic and contingent in a stepwise and interdependent fashion, the problem of establishing optimal procedures based on experimental data was not at all straightforward. There are a very large number of paths through a phase determination. Among many of them are pitfalls in which there arise, for example, temptations to take a path in which the interconnections between phase evaluations flow easily at the expense somewhat of the probability measures. Such paths are more likely to lead to missteps and cumulative errors that could damage or defeat a phase determination than ones that are based only on the highest values of the probability measures.

There is also an ambiguousness inherent in procedures for phase determination which is controlled by the use of symbols. The symbols can assume more than one value. For centrosymmetric crystals, they can have only two phase values, 0 or $\pi$. For noncentrosymmetric crystals, experience has shown that, whereas phase values for the general reflections can have any value in the range from $-\pi$ to $\pi$, it is usually sufficient to use only four possible values for the symbols spaced $\pi/2$ apart. One of the virtues of using symbols is that, as the phase determination develops, relationships develop among the symbols reducing the number to be assigned values.

An additional specification, whose character also derives from the theory of invariants and semi-invariants, is required for most noncentrosymmetric space groups. In making the specification, a choice is made of enantiomorph or axis direction or both. A good way in which this specification is achieved in the symbolic addition procedure is to find that a symbolic representation of a phase value most likely has a magnitude that differs significantly from 0 and $\pi$. The specification is accomplished by assigning a plus or minus sign arbitrarily to the magnitude of the phase.

In the course of applying the procedures of the monograph (*23*), two important features were found that ultimately played an important role in the symbolic addition procedure. The first feature was that if probability measures were carefully employed at each step of a phase determination, it was possible to carry out the procedure with a small set of starting phases. It was also apparent that the use of symbols could greatly increase the efficiency of the procedure by carrying along in their alternative values a residual ambiguity that could not be easily overcome. A sufficient number of reliable relationships among the symbols usually developed in the course of a phase determination to reduce the alternative possible sets of phases to consider to just a few. A further reduction could be obtained, if desired, by applying auxiliary phase determining formulas at the end of a phase determination to help evaluate the remaining symbols.

There were other early attempts to develop procedures for centrosymmetric crystals. They did not have the degree of reliability for initiating a phase determination, the method for application of probability theory, or the facility to handle ambiguousness that could be found in the procedures given in the monograph (*23*) or the symbolic additional procedure.

The procedural features of the symbolic addition procedure for centrosymmetric crystals were extended, in the main by the efforts of Isabella Karle, to noncentrosymmetric crystals (*24*) with the use of (19), (20), and (21). Several problems arose in developing the technique for phase determination for noncentrosymmetric crystals concerning, for example, the assignment and handling of symbols, the use of the probability measure (21), the number of possible values to assign to the symbols that represent phase values that range continuously from $-\pi$ to $\pi$, the combined use of (19) and (20) for phase determination, the proper use of the tangent formula for the processes of phase refinement and phase extension, the development of techniques for specifying an enantiomorph or axis directions or both, and special considerations such as the avoidance of certain troublesome triplet phase invariants involving one- and two-dimensional centric reflections. These various aspects of the symbolic addition procedure are to be found in (*32*) and (*38*) and in papers concerning applications.

A considerable virtue of the symbolic addition procedure is that, because of its efficiency, a main part of the procedure for phase determination can be carried through by hand. For many years, the procedure for centrosymmetric crystals was carried out in our laboratory completely by hand. In the case of noncentrosymmetric crystals, the first stage, which involved the use of formula (19), was performed by hand until about 100 phases were evaluated and useful relationships developed among the symbols. Only after selected numerical values were assigned to the few remaining symbols was the tangent formula (20) applied with the aid of a computer. The benefits of this aspect of the efficiency of symbolic addition have been the opportunity for those with modest computing facilities to carry out structure determinations, the possibility of close interaction with the phase determination as it progresses, and the educational value for those newly learning about phase determination to be able to witness and carry through the procedure by hand.

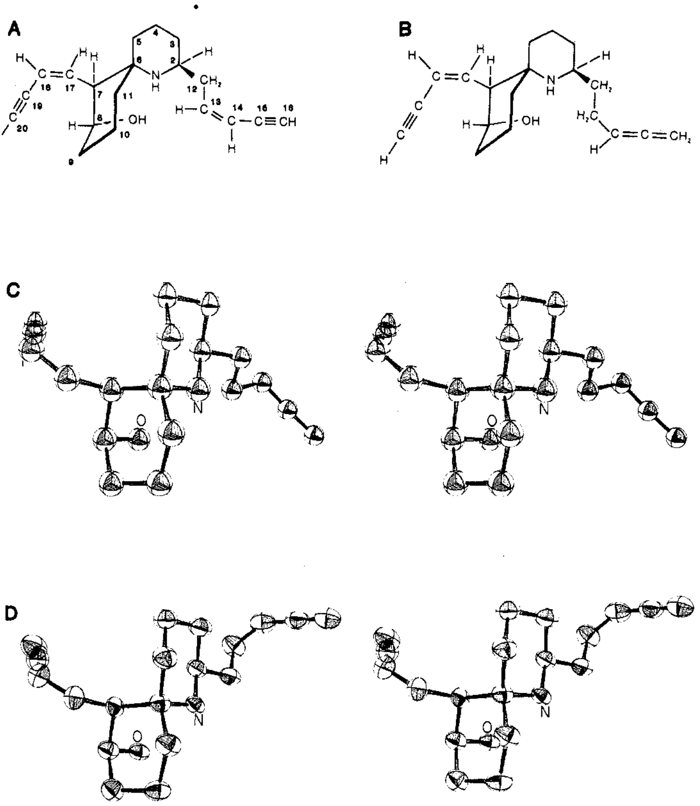


Fig. 3. Histrionicotoxin (A) and the corresponding stereodiagram (D). Dihydroisohistrionicotoxin (B) and the stereodiagram (C).

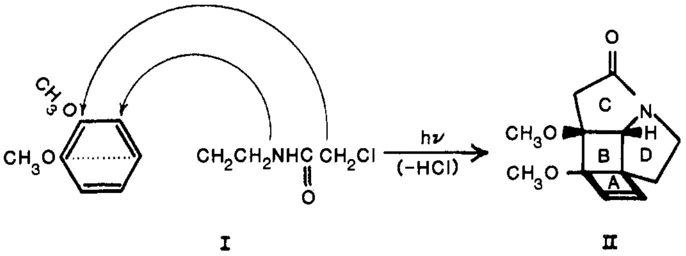


Fig. 4. The photorearrangement of *N*-chloroacetyldimethoxyphenethylamine (I) to a fused ring system consisting of two four- and two five-membered rings (II).

As the application of direct phase determination began to increase during the 1960's and structure determination became more and more a part of research programs, there began to be developed at the end of the 1960's "program packages," software for determining structures from x-ray diffraction data. Insight into the contents and philosophy of the programs can be obtained from several publications of the Commission on Crystallographic Computing of the International Union of Crystallography (*39–43*). On occasion, an answer will not be forthcoming from the use of a program package. In that case, crystallographers may pursue the problem with special techniques and the application of insights and acumen that have been too specialized to be found in current programs.

## Applications

A few examples will be given that illustrate types of applications that have been made accessible by the development of direct structure determination. They are representative of studies that now produce thousands of structural investigations each year.

The earliest applications after the publication of the monograph (*23*) were collaborations with colleagues at the U.S. Geological Survey on colemanite (*44*) and mayerhofferite (*45*). This was followed by the initial investigations based on the experimental work of Isabella Karle, for example, on *p*, *p*′-dimethoxybenzophenone (*46*) and *N*-benzyl-1,4-dihydronicotinamide (*47*) that, in time, led to the symbolic addition procedure.

*Identification and stereoconfiguration.* The problems concerning the determination of molecular formula and stereoconfiguration can become especially acute when the amount of sample available is very small, when the chemical linkages are new and unusual, or when the number of asymmetric centers is large. Under such circumstances, the use of crystal structure analysis can be not only quite helpful but also essential. An example of this is provided by histrionicotoxin and dihydroisohistrionicotoxin, two toxic alkaloids secreted by the skin of a small frog, *Dendrobates aurotaenia*, occurring in Colombia and Ecuador. These alkaloids are quite unique, having a spiropiperidine system with acetylenic and allenic moieties (Fig. 3). The molecular structures, stereoconfigurations, and absolute configurations were established by crystal structure analyses of a hydrochloride and hydrobromide of the histrionicotoxin and a hydrochloride of the dihydroiso compound (*48, 49*). Space groups $C2$ and $P2_12_12_1$ were involved in the analyses. The histrionicotoxins appear to offer the first examples of the occurrence of acetylene and allene moieties in the animal kingdom. Other congeners that occur in smaller quantities were shown, subsequently, by means of mass and nuclear magnetic resonance spectra, to differ only in the saturation of the two side chains [(*50*) and references therein]. The spiro ring system, with the internal NH. . .O hydrogen bond, remained unchanged.

## Rearrangements

In the case of rearrangement reactions, crystal structure determination can again play a particularly useful role because many rearrangement reactions give products that are the result of vast and unanticipated changes in the starting materials.

A photorearrangement reaction in which a major rearrangement takes place is illustrated by the reaction shown in Fig. 4. A crystal structure investigation of a single optically active crystal, selected from a racemic conglomerate, established the structural formula and configuration of the photoproduct (*51, 52*). The substance crystallized in space group $P2_12_12_1$. The structure analysis showed that the photoproduct consisted of four ring systems, two five-membered and two four-membered rings.

Ultraviolet irradiation of *N*-chloracetyltyramine, where there is a hydroxyl group on the phenyl ring in contrast to the two methoxy groups in the previous example, yields entirely different photorearrangement products. HCl was eliminated and two unusual photodimers, shown in Fig. 5, were produced. Their molecular formulas and stereoconfigurations have been identified by use of crystal structure analysis (*53*). It is interesting to note that dimer (II) is the more stable since it is produced from dimer (I) by use of additional ultraviolet radiation. Dimer (I) crystallizes in space group $P2_1/c$, and dimer (II) crystallizes in space group *Pbca*. Dimer (I) is seen to have a central cage bounded by four six-membered rings and two four-membered rings. Each four-membered ring is puckered, with the torsion angles around the ring bonds having values of about 20°. The six-membered rings assume distorted boat conformations. Dimer (II) is seen to have a more complex, partially open, cage bounded by one three-, two five-, two six-, and one seven-membered ring. The six-membered rings are again in a distorted boat conformation. Once the structural characteristics of the photoproducts are known, it is possible to consider possible reaction mechanisms that describe the intermediate changes that occur in the rearrangements of the initial materials resulting in the final products. Postulated mechanisms for the formation of dimers (I) and (II) have been presented (*54*).

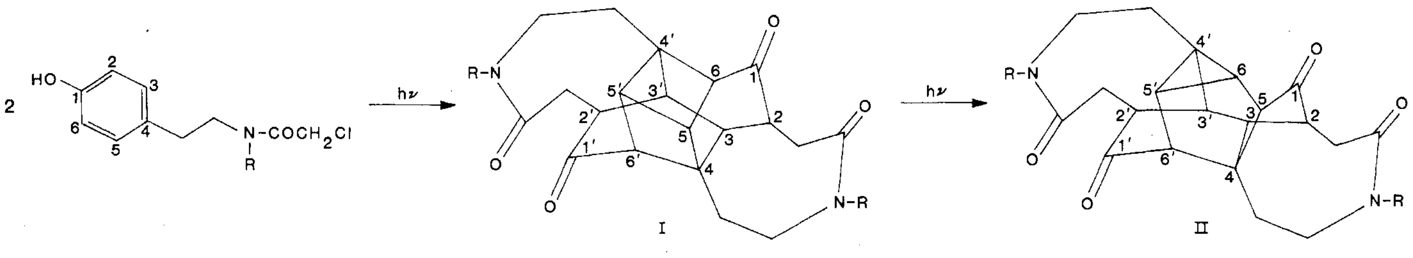


Fig. 5. The configurations of two dimers formed by photorearrangement from *N*-chloroacetyltyramine. Dimer (II) is seen to be formed from dimer (I) by continued irradiation with ultraviolet light.

## Conformation

The conformations of molecules can be importantly related to their chemical and physiological behavior. It may be argued, and rightly so, that biologically active materials may assume conformations in the crystalline state that they would not assume in solution. There are, however, numerous instances of conformational studies in which the results of crystal structure analyses are either highly suggestive or rather definitive. One way in which the crystalline state can imitate the circumstances found in solution is to include in the crystallization relatively large amounts of solvent.

The cyclic decapeptide antamanide acts as an antidote to the toxin phalloidin found in the deadly poisonous mushroom *Amanita phalloides*. Antamanide can also be isolated from the same mushroom but occurs in much smaller quantities. The synthetic analog of antamanide in which all four phenylalanyl residues are hydrogenated to cyclohexylalanyl residues (Cha), cyclic(ValProProAlaChaChaProProChaCha), has no antitoxic potency despite its ability to form ion complexes in the same manner as antamanide. A conformational analysis of the hydrogenated antamanide was carried out by means of a crystal structure analysis of Li$^+$ · perhydroantamanide which crystallizes in space group $P2_12_12_1$ (*55*) (Fig. 6). The backbone encapsulates a Li$^+$ ion in quite the same fashion as in Li$^+$ · antamanide. In the Li$^+$ · antamanide, however, the four phenyl groups are folded against the backbone, thus providing a hydrophobic surface for the complex, whereas in Li$^+$ · perhydroantamanide the four cyclohexyl moieties are extended away from the folded backbone, with the consequent exposure of large portions of the polar backbone to the surrounding environment. As a result, elements of the backbone that would be otherwise shielded from the environment were found to make hydrogen bonds and ligands that would not occur in the Li$^+$ · antamanide complex. It would appear that the large change of hydrophobicity around the backbone in the perhydroantamanide complex is related to the loss of biological activity.

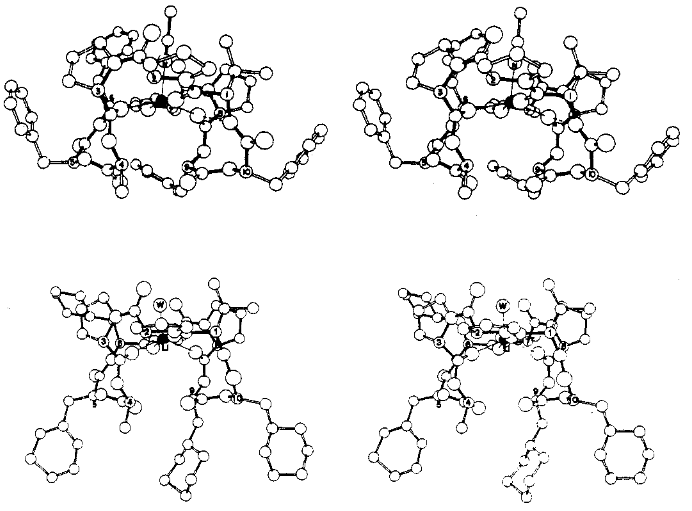


Fig. 6. Phenyl rings folded up around the backbone of a biologically active antamanide complex with Li$^+$ or Na$^+$ providing a lipophilic surface (top). In the inactive analogue, Li$^+$ · perhydroantamanide, the cyclohexyl groups are folded down, thus exposing the peptide backbone to the environment (bottom).

These few applications represent only a miniscule portion of the broad range of topics and individual studies represented by the thousands of structural investigations performed each year. They do illustrate, however, how structure determination can play a useful and often indispensable role in the progress of many research disciplines.

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