X-RAY STUDIES OF THE CRYSTAL STRUCTURES OF SOME BIOLOGICALLY SIGNIFICANT MOLECULES

A Thesis presented for the Degree of

DOCTOR OF PHILOSOPHY OF THE UNIVERSITY OF LONDON

by

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August 1970

Summary

The principles and techniques of crystal-structure analysis are discussed, together with a brief description of some associated computer programs.

An account of the determination of the absolute configurations of optically active molecules by X-ray methods is presented. The absolute configuration of α-D-glucose monohydrate, a compound of previously known structure, has been determined utilising the anomalous scattering from the oxygen and carbon atoms of the molecule.

The phase problem for three biologically-significant compounds has been solved by the heavy-atom method.

The crystal and molecular structure, and absolute configuration, of 2(S),S(R)-methionine sulphoximine, a potent inhibitor of the enzyme glutamine synthetase,have been determined The structure has been refined to an R factor of 0.093 for 483 visually-estimated reflection intensities. The geometry and conformation of the molecule, and the hydrogen bonding in the crystal lattice, are discussed in some detail.

The crystal and molecular structure of the anti-viral compound 16-hydroxyphenoxymethyl-3,4-dihydroisoquinoline hydrochloride monohydrate has been determined from 1577 reflections measured on an automatic diffractometer, and refined to a final R of 0.0777. The overall conformation of the compound, predicted on chemical grounds, has been confirmed.

The conformation of the 3,4-dihydropyridine group is unusual; it has been described as a 'skew-boat'. The probable existence of a bifurcated hydroxonium ion has been postulated to account for the hydrogen bonding and packing in the crystal.

The crystal and molecular structure, and absolute configuration, of streptomycin oxime selenate have been determined The structure has been refined to an R factor of 0.0862 for 3236 visually-estimated reflection intensities. The structure and absolute configuration deduced chemically for the antibiotic, have been confirmed. The arrangement of molecules in the crystal lattice is determined by a complex arrangement of hydrogen bonds and ionic interactions.

A review of direct methods of solving the phase problem is presented. The crystal structure of the steroid viridin, a non heavy-atom compound crystallising in space group $P2_12_12_1$, has been determined by application of a direct method for non-centrosymmetric structures. The final R factor is 0.0916 for 1580 visually-estimated reflection intensities. The molecular geometry of the compound, and the method of structure solution, are discussed.

Acknowledgements.

I am deeply grateful to Professor D. Rogers for his wise and stimulating guidance throughout the past three years. I should also like to thank Dr. Mick Hursthouse for allowing me to carry on from his early work on streptomycin and viridin, and for much useful discussion on these subjects, and also Dr. Frank Allen who collaborated in the preparation of the absolute configuration lists.

It is a pleasure to thank other members of the Chemical Crystallography Laboratory, Imperial College, for their excellent camaraderie, and constant discussions on many topics. I am grateful to Glyn Millhouse for his careful photography of the many diagrams.

I thank the staffs of both the Imperial College and London University Computer Centres for their co-operation in the use of the computer facilities.

The University of London is thanked for the award of a Research Studentship, during the tenure of which this work was carried out.

I am very much indebted to my fiancée Andrea, who undertoo the onerous task of typing this manuscript, and for her encouragement at all times.

"In Nature's infinite book of secrecy
A little I can read."

William Shakespeare.

To my

parents.

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PART ONE - THE THEORY OF CRYSTAL STRUCTURE ANALYSIS.

CHAPTER 1

Crystallographic Principles.

1.1. Historical Introduction.

Man has been interested in, and studied crystals, almost from his first recorded history. Whereas animals and plants have curved edges and shapes, natural crystals have well-defined flat faces and straight edges, and often exhibit remarkable symmetry. Indeed, the word "crystal" stems directly from the Greek, "krystallos" - rock crystal.

Until the seventeenth century studies were restricted to the description of the external forms of crystalline minerals and precious gem stones. However, in 1665, Robert Hooke attempted to explain the regular features of particularly alum crystals by the close packing of spheres. About the same time Huygens went even further and described in detail a model for calcite, which he envisaged as built up of ellipsoids, not spheres, lightly stuck together. He speculated that, "the regularity comes from the arrangement of the small invisible equal particles of which they are composed." In this way he explained the cleavage and angles

of the calcite crystal, which is of lower symmetry than the alums. Thus his approach was a somewhat more general one. We shall see that his statement regarding internal regularity has proved to be a remarkably accurate one.

The next step in the development of the subject came with the work of the Abbe Hauy in 1784. He regarded crystals as repeating units of what he called, "molecules elementaires," thus preceding Dalton's atomic theory by several years. His "Law of rational indices" implies the regular stacking of these units in sets of parallel straight lines - the crystal lattice.

In 1839 W.H. Miller systematised these ideas on the "lattice" in a more quantitative manner. The basic structure unit he called the unit cell. He defined a set of reference axes a, b and c for the whole lattice, based on the three cell axes. For these one can choose the intersections of any three non-parallel external faces - though some choices are more convenient than others. With respect to any such axes one can find a sequence of parallel equi-spaced planes with intercepts ma/h, mb/k and mc/ℓ, and the plane with m=1 i.e. the plane adjacent to that through the origin, has intercepts a/h, b/k and c/ℓ where h, k and ℓ must be integers. Miller used this trio to define the planes and they are therefore known as the Miller indices of the planes.

1.2. Lattices and Symmetry.

The cell axes are labelled a, b, c and the angles between them, α, β, γ, are often not 90°. The sequence of axes is chosen however, to constitute a right-handed reference system.

The symmetry of crystals was recognised even by the early gem-cutters, but later was recognised as a characteristic feature of the physical properties e.g. the anisotropy of refractive index - even for irregularly shaped single crystals. The concept of symmetry means that if some operation is applied to a figure, it will bring every point of it into some position which was previously occupied by some other point of the figure. By virtue of its repeating structure a crystal possesses only the following symmetry elements:-

a) 1, 2, 3, 4 and 6-fold rotatory axes.

b) $\bar{1}, \bar{2}, \bar{3}, \bar{4}$ and $\bar{6}$-fold inversion axes.

c) mirror planes (which are the same as $\bar{2}$-fold axes).

The number of different sets produced by combining these operations is obviously limited. There are in fact only 32 possible combinations known as the 32 crystallographic point groups.

The seven crystal systems of classical crystallography are based on the exhibition by the crystal of seven characteristic symmetries.. Bravais showed that without

departing from these symmetries, it was possible to define 14 distinct lattice types; seven of these are primitive.

Crystals at the molecular level represent an infinitely extended repeating pattern in three dimensions. Thus, the symmetry operations can now involve translation as well as rotation and/or inversion. A screw axis (n_m) is obtained by combining a rotation of $2\Pi/n$ with a fractional translation of m/n parallel to the screw axis. Similarly a glide plane derives from a reflection through a mirror plane, combined with translation parallel to the plane in a specified direction; the direction of translation is used to designate the glide plane.

Combination in a three-dimensional repetition of this extended list of symmetry elements results in just 230 possible space groups. These have been tabulated in a standard form [1].

1.3. Diffraction of X-rays by a Crystal Lattice.

X-rays were discovered in 1895 by Röntgen. Assuming that they were a form of electro-magnetic radiation, Laue and his school calculated the form the diffraction pattern would take from a three-dimensional grating, such as a crystal. They reasoned that diffraction would occur in

such a case because X-rays were thought to have a wavelength of the order of an Angstrom, the same order of magnitude as inter-atomic distances in crystals. Friedrich and Knipping in 1912 succeeded in obtaining diffraction photographs from crystals of copper sulphate and several other minerals. This experiment thus both established unequivically X-rays as electro-magnetic radiation, and provided for the first time direct evidence for the idea of the crystal as a three-dimensional repeating lattice. The consequences of this have been vast; in one direction the development of X-ray spectroscopy has led to fundamental advances in the knowledge of atomic structure, and in the other to the science of crystal-structure analysis. The latter, the "new crystallography" of the 20th century, has enabled the intimate details of atomic arrangements of molecules in crystalline matter to be elucidated.

Laue, using classical diffraction theory, derived the equations which bear his name:-

$$\vec{a}.\vec{S} = h; \quad \vec{b}.\vec{S} = k; \quad \vec{c}.\vec{S} = \ell \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 1.1$$

$\vec{a}$, $\vec{b}$ and $\vec{c}$ are the primitive lattice translations. $\vec{S}$ is the scattering vector. It is the vector difference between unit vectors parallel to the incident and diffracted rays. (Figure 1.1).

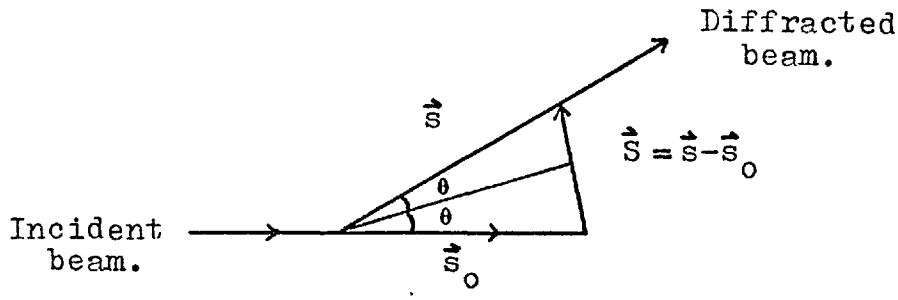

Figure 1.1.

When these three conditions are simultaneously satisfied, a diffracted beam is produced. The integers h, k and ℓ specify the 'order' of diffraction.

W.L. Bragg (1913) gave a simpler interpretation of diffraction. He considered it as arising from multiple reflections of X-rays from the lattice planes. For constructive interference of the rays 'reflected' from successive lattice planes of spacing d, the rays must be inclined to the planes at an angle θ, given by the Bragg equation:-

$$n\lambda = 2d\sin\theta \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 1.2$$

where both d and θ relate to reflections from planes of the sequence hkℓ.

For each family of lattice planes (hkℓ) draw the normals radiating from a common origin and each of length $1/d_{hk\ell}$. The set of terminal points forms a regular array or three-dimensional lattice, known as the reciprocal lattice,

and each point of termination, P, (Figure 1.2) is termed a reciprocal lattice point. This lattice can be defined by three vectors $\vec{a}^*$, $\vec{b}^*$, and $\vec{c}^*$, and every point is therefore expressed as

$$\overrightarrow{OP} = h\vec{a}^* + k\vec{b}^* + \ell\vec{c}^* \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 1.3$$

In other words, lattice planes in direct space are represented by reciprocal lattice points in reciprocal space.

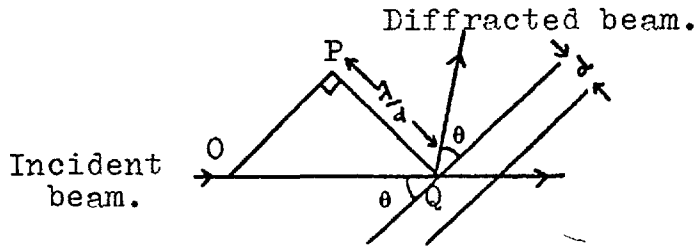

Figure 1.2.

The points OPQ lie on the circumference of a circle because the angle $\hat{OPQ}$ is a right angle. A geometrical construction, devised by Ewald, sets the radius of the circle (or more generally a sphere), as unity. Then the Bragg reflecting condition is fulfilled when the reciprocal lattice point P lies on the surface of the sphere - which is thence known as the reflecting (or Ewald) sphere. This is invaluable in simplifying the treatment of the geometry of diffraction for the many different recording devices that are now available.

1.4. The Structure Factor.

The structure factor $F_{hk\ell}$ is the resultant of N waves scattered in the direction of the reflection from the planes

hkl by the N atoms in the unit cell. The amplitude of each contributing wave is proportional to f_j, the scattering factor of the jth atom.

$$F_{hk\ell} = \sum_{j=1}^{N} f_j^o \exp 2\Pi i \vec{r}_j \cdot \vec{S} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 1.4$$

Where $\vec{r}_j$ represents the position of the jth atom in the unit cell.

$$\vec{r}_j = x_j\vec{a} + y_j\vec{b} + z_j\vec{c} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 1.5$$

x_j, y_j and z_j are its fractional co-ordinates.

Substituting the Laue equations (1.1) and equation (1.5) into 1.4, we obtain

$$F_{hk\ell} = \sum_{j=1}^{N} f_j^o \exp 2\Pi i(hx_j + ky_j + \ell z_j) \quad \ldots\ldots\ldots 1.6$$

This is a complex quantity, and may be represented as:-

$$F_{hk\ell} = A + iB \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 1.7$$

where

$$\left.\begin{aligned} A &= \sum_{j=1}^{N} f_j^o \cos 2\Pi (hx_j + ky_j + \ell z_j) \\ \text{and} \\ B &= \sum_{j=1}^{N} f_j^o \sin 2\Pi (hx_j + ky_j + \ell z_j) \end{aligned}\right\} \quad \ldots\ldots\ldots\ldots\ldots\ldots 1.8$$

We can also write the structure factor in the form

$$F_{hk\ell} = |F_{hk\ell}| \exp(i\phi) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 1.9$$

The corresponding intensity is $|F^2| = F.F^* = A^2 + B^2$, and forms a measure of $|F^2|$. We can, therefore, only derive its amplitude, $|F|$, and not its phase. This loss of essential information constitutes the most important problem in crystal-structure analysis, and calculation of this unobservable phase, becomes one of its prime objectives.

1.5. Thermal Effects.

The above treatment assumes the atoms to be stationary. When isotropic thermal motion is taken into account, the atomic scattering factors must be modified by the term

$$f_j = f_j^o \exp(-B\sin^2\theta/\lambda^2) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 1.10$$

where B (in Å^2), the isotropic temperature factor, is a measure of the root-mean-square amplitude of the thermal oscillations, and is known as the Debye-Waller factor [2].

In fact it is rare that the thermal vibrations of atoms have the same values in all directions, and it is usually necessary to represent this motion by an ellipsoid, with six temperature factors for each atom defining the principal axes and the direction cosines of the ellipsoid. The correction now becomes

$$f_j = f_j^o \exp\left[-(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+\beta_{12}hk+\beta_{13}hl+\beta_{23}kl)\right] \ldots \quad 1.11$$

If an atom lies on one or more of the symmetry elements, it is said to be at a special position. The number of anisotropic temperature parameters may then be reduced [3].

1.6. Electron Density.

If $\rho(xyz)$ is the electron density at the point (x,y,z) the amount of scattering matter in the volume element Vdxdydz is ρVdxdydz;this will make a contribution to the resultant amplitude for the whole unit cell, and for the general plane hkl the phase change from the origin to the point (x,y,z) is $2\Pi(hx + ky + \ell z)$. The resultant vector is the sum of all

these elements in the unit cell. Thus for this form of the structure-factor equation we can write:-

$$F_{hkl} = \int_0^1 \int_0^1 \int_0^1 V\rho(xyz)\exp\left[2\Pi i(hx+ky+\ell z)\right]dxdydz \quad \ldots\ 1.12$$

where V is the volume of the unit cell.

Since a crystal is periodic in three dimensions, the electron density can be represented by a three-dimensional Fourier series. It can be shown that the coefficients of this series are the values of the structure factor, divided by the unit-cell volume:-

$$\rho(xyz) = \frac{1}{V}\sum_{h}\sum_{kl=-\infty}^{\infty}\sum^{\infty} F_{hkl}\exp\left[-2\Pi i(hx+ky+\ell z)\right] \quad \ldots\ 1.13$$

Thus, once a set of structure factors is known, a direct representation of the unit-cell contents of a crystal can be obtained. High points of electron density denote atom sites, of which there are N in the unit cell, but as explained in Section 1.5, this calculation cannot be done until the phase is known for each term in the series.

1.7. The Intensity of the Diffracted X-rays.

The structure amplitudes, ($|F|$'s), can be obtained directly from the measured intensities of the (hkℓ) reflections However there are several corrections which have to be applied to the raw intensity data before it can yield structure amplitudes.

The Lorenz factor is a geometrical correction which represents the rate at which a set of reflecting planes passes

through the reflecting position. The exact form of the correction depends on the geometry of the data-collection method.

The polarisation correction arises from the fact that the incident X-ray beam is usually unpolarised, whereas the diffracted beam is partly polarised. The correction is dependent only on the Bragg angle .

The combined correction, known as the (LP) correction, is given for the equi-inclination Weissenberg technique, by the expression

$$(LP) = \frac{1 + \cos^2 2\theta}{2\xi \cos\theta} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 1.14$$

where ξ is the radial cylindrical co-ordinate of the reciprocal lattice point.

Absorption of X-rays by crystals sometimes affects the measured intensities significantly. The intensity of a beam is reduced by an amount $(e^{-\mu t})$, where μ is the linear absorption coefficient and t the total path length of a ray through the sample. For the majority of organic compounds, is small (<30) and except where crystals are markedly anisotropic in shape and/or considerable accuracy in the final results is required, the absorption effect can safely be ignored. Provided the crystal dimensions are accurately known, and a description made of the faces in terms of their (hkl) indices, an absorption correction A can be calculated;-

$$A = \frac{1}{V}\int \exp[-\mu(\ell_1 + \ell_2)]\, dV \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 1.15$$

where V is the crystal volume, and ℓ_1 and ℓ_2 are respectively the path lengths within the crystal along the primary and diffracted beams. The computer program used to evaluate the correction is based on the method of Coppens et al [4]; a three-dimensional grid of sampling points within the crystal is used.

Crystals are not perfectly continuous and consist of a 'mosaic' of tiny blocks with orientations varying over a few minutes of arc. Primary extinction occurs with multiple reflection from a set of planes within a single mosaic block. Since there is a phase change of $\Pi/2$ on reflection, the doubly reflected rays differ in phase by Π from the incident beam. The net result is that the primary beam is weakened by destructive interference, and is continuously attenuated as it penetrates deeper into the crystal. Secondary extinction is caused by the screening of the lower blocks by the upper ones, which can reflect away an appreciable amount of the incident radiation. Both effects reduce the observed intensities and are most pronounced for strong, low-order reflections. No corrections for such reflections have been applied in this work; they have simply been removed from the main body of data.

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CHAPTER 2

The Phase Problem and Crystal Structure Analysis.

2.1. Introduction.

We have seen in section 1.4 that the structure factor is a complex quantity, but that the phase is not experimentally observable. In general, the value of the phase angle , can be in the range $-\Pi \leq \phi \leq \Pi$. However, a considerable simplification occurs if the structure is a centro-symmetric one. Then, for every atom at (x,y,z), there is a corresponding one at $(\bar{x},\bar{y},\bar{z})$. The structure-factor equation therefore, reduces to

$$F_{hkl} = 2\sum_{j=1}^{N/2} f_j \cos 2\Pi (hx_j + ky_j + \ell z_j) \quad \ldots\ldots\ldots\ldots \quad 2.1$$
$$= \pm |F|$$

Hence, for a centro-symmetric structure, the phase angle can only be 0 or Π.

2.2. The Vector Representation of Crystal Structures.

In 1935, A.L. Patterson proposed a method of solving the phase problem [1] which rapidly found wide-spread use, and today is still the most popular phase-determining procedure. The Patterson method, suitably modified, has had its greatest triumph in the unravelling of the structures of a number of proteins; several different but isomorphous heavy-atom

derivatives have to be compared when tackling proteins.

Patterson defined a function P(uvw) such that

$$P(uvw) = V\int_0^1\int\int \rho(xyz)\,\rho(x+u, y+v, z+w)\,dx\,dy\,dz \quad \ldots\ldots\ 2.2$$

This is the product of the electron densities at points (x,y,z) and $(x+u, y+v, z+w)$, integrated over the volume of the unit cell. If we substitute in this expression the values for the electron density we eventually obtain a new equation:-

$$P(uvw) = \frac{1}{V}\sum_{h,k,l=-\infty}^{\infty}\sum\sum |F_{hkl}|^2 \cos 2\Pi(hu + kv + lw) \quad \ldots\ldots\ 2.3$$

The quantities $|F_{hkl}|^2$ are directly derivable from the X-ray intensities and so this series can be summed directly.

Now, from 2.2, the function P(uvw) can only have a large value when both $\rho(xyz)$ and $\rho(x+u, y+v, z+w)$ are large. This will occur if there are atoms both at (x,y,z) and at $(x+u, y+v, z+w)$, separated by the vector distance (u,v,w). Thus a peak in the Patterson map corresponds to an inter-atomic vector (u,v,w), and there is a peak for each vector; if there are N atoms in the unit cell, there will be N^2 peaks in the Patterson. Of these, N coincide at the origin, and the remaining $(N^2 - N)$ are distributed through the cell, in two centro-symmetrically related sets (since the function 2.3 is

itself centro-symmetric).

The total height of the origin peak should represent $\sum_{n=1}^{N} z_n^2$ electrons, and a peak corresponding to a vector between x_m and x_n will contain $z_m z_n$ electrons. Each peak in the Patterson represents the 'convolution' of two atom peaks in the crystal lattice and so has a width equal to the sum of the widths of the two atoms.(Figure 2.1).

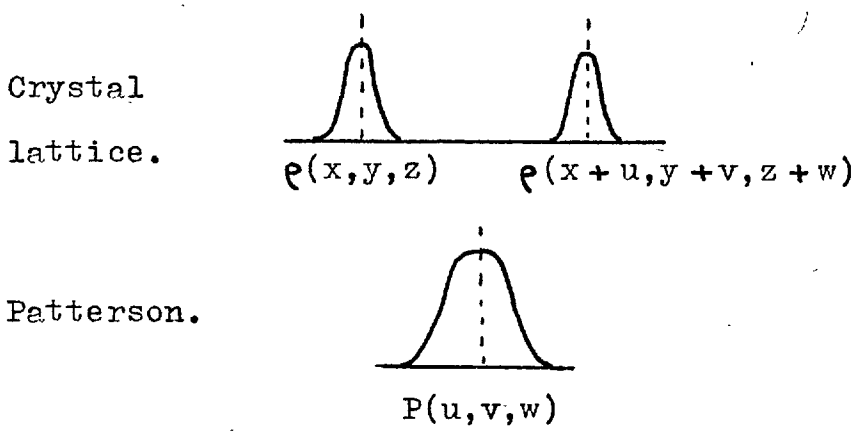


Figure 2.1

Generally speaking, then, Patterson peaks have twice the width of the corresponding atoms, and in three dimensions the peak occupies about eight times the volume of an atom.

Much useful information is contained in certain sections of the three-dimensional Patterson - the Harker sections [2]. The symmetry elements of the crystal space group produce particular concentrations of vectors in the Harker sections; characteristic of these elements, and much useful information

can be obtained by identifying the vectors. For example, in space group Pm there is a mirror plane perpendicular to the b-axis, and thus for every atom at (x,y,z) there is one at $(x,\bar{y},z)$. The vectors between these symmetry-related atoms all have co-ordinates (0,2y,0) i.e. are concentrated on the Harker line which is the v-axis of the Patterson map.

2.3. The Heavy-atom Method.

In general, equi-atom structures produce rather featureless Pattersons that can only be analysed in certain simple cases. Since the cell of the Patterson synthesis is the same size as that of the crystal, the peaks are much more densely packed; this together with their increased size means that except for simple structures, there is very considerable Patterson peak overlap, and the individual peaks cannot be identified.

Obviously if the unit cell contains a few atoms heavy relative to the remaining contents, then vectors between heavy atoms will be clearly visible in a Patterson, as relatively dense peaks, and hence the heavy-atom position(s) relatively easily calculated; these are most easily obtained from the relevant Harker sections. The heavy-atom method, developed largely by Robertson and his school [3], uses these heavy-atom co-ordinates. The total structure factor can be written as the sum of the heavy and light-atom contributions:-

$$\vec{F}_{hk\ell} = \vec{F}^{H}_{hk\ell} + \vec{F}^{L}_{hk\ell} \quad \ldots\ldots\ldots\ldots\ldots\ldots 2.4$$

Provided $F^{H}_{hk\ell}$ is sufficiently large, it will dominate and define the phases for a considerable number of reflections, both in the centro-symmetric and non-centro-symmetric cases. Thus, the computation of a Fourier summation with $F^{H}_{hk\ell}$ as coefficients enables some at least of the light atoms to be located. Successive Fouriers then reveal the complete structure, each utilising the phases calculated from the atoms recognised in the previous map.

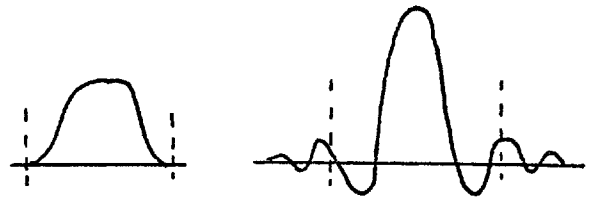
The heavy-atom method works best when the ratio $\sum z_H^2 / \sum z_L^2$ is approximately unity [4]. This corresponds to the sign of 80% of the structure factors being determined by the heavy atom in the centro-symmetric case; for non-centro-symmetric structures, about a third of the phases will have errors of less than 20° and four-fifths will have errors of less than 60°. For decreasing values of $\sum z_H^2 / \sum z_L^2$ the phases are determined with decreasing precision. With care structures with ratios rather less than unity can be solved. Streptomycin oxime selenate (see chapter seven) has a ratio of 0.66, whilst vitamin B12 [5] has the very low ratio of 0.17.

Peaks near the origin of the Patterson are often obscured by the very large origin peak which may have associated

series-termination ripples, but this trouble can be eliminated by calculating a summation with coefficients $|F'_{hk\ell}|^2$ where

$$|F'_{hk\ell}|^2 = |F_{hk\ell}|^2 - \sum_{j=1}^{N} f_j^2 \quad \text{..... 2.5}$$

The interpretation of the Patterson is often more difficult for molecules with only moderately heavy atoms, such as chlorine. It is not uncommon for there to be a build-up of light-light-atom vectors; these, together with the light-heavy vectors tend to make the identification of the heavy-heavy vectors rather ambiguous. Now the scattering factors of heavy atoms do not fall off so quickly as those of light atoms, with increasing Bragg angle. The method of 'sharpening' gives greater weight to these higher order terms, as well as removing the effects of thermal attenuation, by using a modifying function to the $|F|^2$ data, such as $\hat{f}\exp(-2B\sin^2\theta/\lambda^2)$, where $\hat{f}$ is the average form factor per atom. The overall temperature factor B (and the absolute scale for the intensity data) can be estimated by the method of Wilson [6]. The sharpening will reduce the width of all Patterson peaks similarly and assist resolution, but the heavy-heavy peak heights will be preferentially increased. Some care must be taken with the occurence of series-termination ripples since the data is truncated at an arbitary limit. (Figure 2.2).



Peak before sharpening.

Peak after excessive sharpening.

Figure 2.2

It is often useful to calculate a difference Fourier synthesis with coefficients $|F_o| - |F_c|$, when only a partial structure has been obtained. It often shows those parts of the structure ommitted from the structure-factor calculation rather more clearly than a normal Fourier, and has the advantage of eliminating any ripples arising from premature termination of the Fourier series.

2.4. The Refinement of Crystal Structures.

A measure of the correctness of a structure is given by the reliability index, R, where

$$R = \frac{\sum \left| |F_o| - |F_d| \right|}{\sum |F_o|} \qquad \text{2.6}$$

The summation is over the entire data list. Obviously the lower the value of R, the more reliable and accurate is the proposed structure model. Wilson [7] has calculated the values of R which correspond to a completely random i.e. wrong model. Values of roughly 0.59 and 0.83 respectively correspond to random centro-symmetric and non-centro-symmetric structures, whilst values of R = 0.25 indicate a generally

correct structure.

Once a rough trial structure has been obtained, it is necessary to improve the fit between observed and calculated structure factors. A very powerful method of refinement is based on the method of least squares, due to Legendre, in which the most acceptable values of a set of variables are such as to make the sum of the squares of the observations a minimum.

Consider a set of observations $f_1,.....f_s$ with their linearly related parameters $x_1,.....x_m$; these form s linear equations:-

$$f_s = a_{1s}x_1 + a_{2s}x_2 \quad .....a_{ms}x_m \quad ..... \quad 2.7$$

The a_{ms}'s are known constants. The problem is to find the values of $x_1,.....x_m$ which minimise

$$D = \sum_{i=1}^{s}(f_s - \sum_{j=1}^{m}a_{ms}x_m)^2 \quad ..... \quad 2.8$$

We can rewrite 2.7 in matrix notation as

$$F = AX \quad ..... \quad 2.9$$

where A is of order m x s and F and X are column matrices of order s and m respectively.

The conditions for D to be a minimum are that $\partial D/\partial x_j = 0$ for $j' = 1,.....m$. This gives from equation 2.8,

$$\sum_{i=1}^{s} a_{ij}\,(f_i - \sum_{j=1}^{m} a_{ij}x_j) = 0$$

i.e. $$\sum_{j=1}^{m}\left[\sum_{i=1}^{s} a_{ij}\,a_{ij}x_j\right] = \sum_{i=1}^{s} a_{ij}\,f_i \quad \ldots\ldots\ldots\ldots\ldots \quad 2.10$$

These m equations, the 'normal equations', give the best values of $x_1,\ldots\ldots x_m$. Equation 2.10 is in matrix form

$$BX = C \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 2.11$$

From 2.9, $B = A^TA$ and $C = A^TF$, where A^T is the transpose of A.

So,

$$X = M^{-1}C$$
$$= (A^TA)^{-1}A^TF \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 2.12$$

Crystal-structure refinement involves the minimisation of $\sum_{i=1}^{s} w_i(|F_o| - |F_c|)_i^2$ where w_i is the weight of an observation.[8] The structure factors are non-linear functions of the positional and thermal parameters; they are expanded as a Taylor series and second and higher powers neglected, thus obtaining linear relationships. The set of normal equations now obtained is of the form

$$\sum_{j=1}^{m} \Delta x_j\left[\sum_{i=1}^{s} \frac{\partial |F_c|_i}{\partial x_j}\cdot\frac{\partial |F_o|_i}{\partial x_j}\right] = \sum_{i=1}^{s}(|F_o| - |F_c|)_i \frac{\partial |F_c|_j}{\partial x_j} \quad \ldots \quad 2.13$$

where Δx_j is the error in the variable x_j. This can similarly be written in the matrix form 2.12 since it is a system of m equations in m unknowns, the Δx_j's. The

least-squares treatment is valid only if all parameters are tolerably well known.

Solution of these equations is a cyclic process, involving successive solution for the parameter shifts, until these are minimal. With N atoms, the matrix A^TA is of order 9N x 9N, for nine variables per atom. Even on the largest computers currently available, building and inverting this matrix is only practical in terms of both computing time and storage requirements, with less than about 200 variables. The full-matrix least-squares program used here at Imperial College, has a restriction of 180 variables. An approximation which allows for the simultaneous refinement of a very much larger number of variables, rests on the assumption that there is no correlation between parameters of different atoms. The matrix now used has non-zero elements of 9 x 9 blocks about the diagonal, all others (apart from considerations of scale) are neglected. This is the block-diagonal approximation. It is much faster in operation than the full-matrix method, but converges slower; thus, one cycle of the latter is equivalent to about three of block-diagonals. Also, block-diagonals refinement, tends to underestimate standard deviations of parameters by about 50%.

The choice of a particular form of least-squares refinement for a problem thus depends largely on the size

of the problem; obviously for less than about 20-atom structure the full-matrix approach is to be preferred.

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CHAPTER 3.

Data Collection and Treatment; Computer Programs Used.

3.1. Data Collection.

Intensity data were collected by both standard photographic methods and by means of an automatic diffractometer.

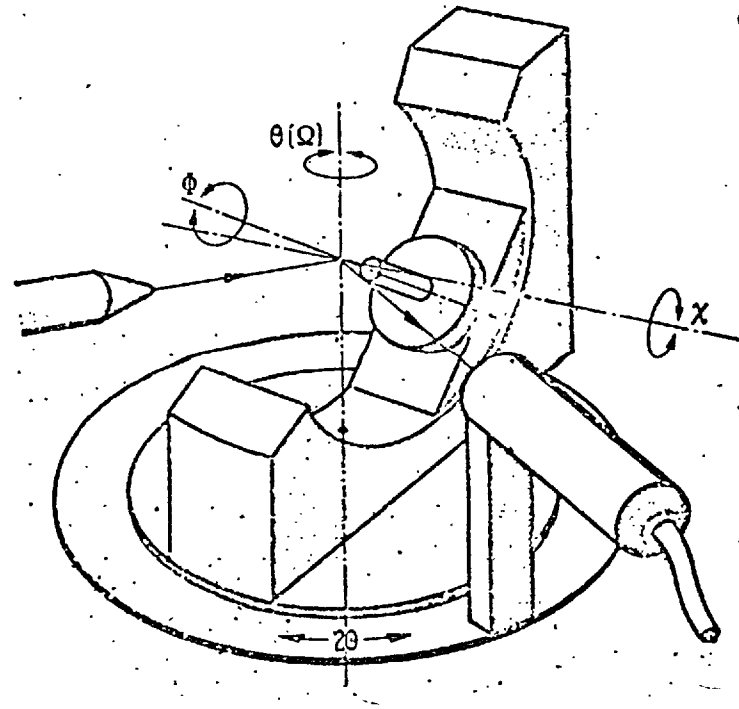
All photographic intensity data were collected using the equi-inclination Weissenberg technique and multi-film packs with filtered Cu-K_α radiation. Two four-film packs were exposed for each layer of the reciprocal lattice, so that the relative exposure times were roughly in the ratio 3:1. The intensities were estimated visually using a calibrated wedge and inter-film scales were calculated and applied by hand. Accurate cell dimensions were obtained from oscillation and Weissenberg photographs by measuring accurate θ-values on a Buerger scale and applying a least-squares fit procedure (see section 3.2) to these indexed θ-values. The camera radius had been previously checked by calibration with powder lines of d-spacings known from the ASTM index.

Intensity data were collected electronically using a Siemens off-line automatic diffractometer. This is a four-circle instrument employing the principle of the Eulerian cradle [1]. Full details of the operating procedure and associated computer programs used in this laboratory have previously been published.[2]

The position of any reflection vector, hkℓ, can be defined uniquely in terms of the three Eulerian angles, θ, ϕ and χ (Figure 3.1). These setting angles are pre-computed by the program SEKO, from the lattic parameters, radiation wavelength and Miller indices. A punched paper "steering tape" with the setting angles is input to the machine, which then automatically drives the circles to each reflecting position in turn. The intensities of the diffracted beams are measured by a Na(Tl)I scintillator, pulse-height analyser and counter, and are output on a paper tape for later processing.

A suitable crystal was chosen, of optimium size about 0.6 mm. in each direction. It was approximately aligned using photographic methods, with either a real or a reciprocal axis parallel to the goniometer axis. It was left, (attached by a quartz fibre), on the goniometer head, in the diffractometer room for 24 hours to ensure temperature stability. After mounting the crystal on the diffractometer, horizontal half-shutters were used for accurate adjustment of its orientation. A constant ratio $N_{\frac{1}{2}}/N_{whole}$ for a set of representative reflections meant that the adjustment was satisfactory. Then, accurate cell dimensions were obtained by measurement of optimum θ-values for a number of hkℓ reflections and refinement of these values by a least-squares program. We are then ready to compute the steering tape.

Figure 3.1. The Eulerian cradle of the diffractometer.



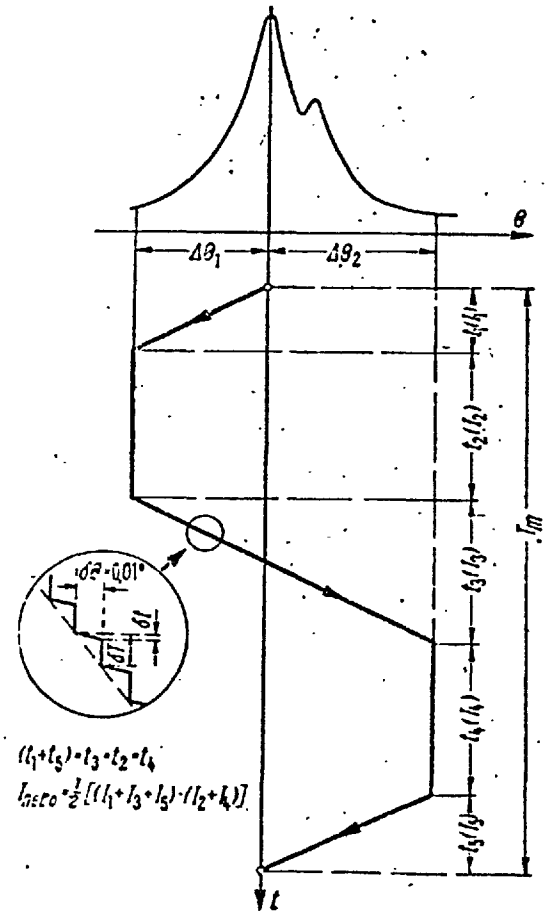


Figure 3.2. Scheme of the 'five-value' measurement technique.

Reflection intensities can be measured in several modes, some of which are used during the adjustment of the crystal, but for routine intensity measurements we always use a 'five-value' technique. (Figure 3.2). This enables the intensity of each peak above the background level to be accurately assessed, to limits of $\Delta\theta_1$ and $\Delta\theta_2$ on either side of the peak maximum. The actual values of $\Delta\theta_1$ and $\Delta\theta_2$ used for each reflection were obtained by interpolation of a scan table derived from the scans of a few reflections representative of the θ range to be covered. All reflections were measured with a coupled θ:2θ (moving-crystal/moving-counter) scan; this is most likely to give a realistic interpolation of the background without careful selection of the detector aperture size.

When collecting data the machine automatically selects an appropriate measuring time up to a maximum pre-set by the user (say, of 0.6 seconds per 0.01° step of a scan). If any reflection is too strong to be measured in the shortest time available without involving counting losses, up to five attenuators are automatically inserted in the incident X-ray beam. It keeps this attenuator in the beam for all measurements on that reflection. The cumulative effect is that approximately constant counts and hence constant statistical accuracy, are obtained for all but the weakest, of a set of reflections.

During the later processing of the five counts, tests

are applied, some of which check the statistical reliability and also examine the ratio of the counts integrated in the left and right-hand half-scans. This ratio is a sensitive indication of whether the reflection peak is where it was expected to be and so draws attention to any movement of the crystal or goniometer head.

Various position checks are carried out
(a) before each reflection by a series of optical digitisers. These are self-correcting to $\pm 0.20^{\circ}$. Thus, no data is measured in the wrong place and there are no needless stoppages
(b) The absolute positions of the circles was checked every 40 reflections by turning them back to their expected zeros and verifying them by digitiser checks, to modulo 0.5°.
(c) More rarely still, the latter check is supplemented by automatically examining a group of strong reflections (the 'control' reflections), with and without half-shutters, every few hundred reflections.

A further check on the stability of the generator and counter is made by periodic measurement of a strong reflection chosen as a 'reference'; this was done, say, once every 20 reflections. This also gives warning of radiation damage in the crystal. During data processing a separate scale factor was computed for each batch of 20 reflections, thereby eliminating variations of generator or detector performance. If radiation damage occurs it is our habit to replace the

crystal when about 10% reduction of intensity of the reference reflection has occured.

3.2. Computer Programs Used.

The majority of crystallographic programs used in this work form part of the X-RAY-63 system [3]. This consists of a number of linked segments which may be called off magnetic tape into core as required. The system is so arranged that as many routines as are required may be employed consecutively in a single run. It has been implemented both on the Imperial College IBM 7094 configuration and on the University of London Atlas.

DATRDN ..... applies LP^{-1} correction and organises the data in a suitable form on magnetic tape for processing by the structure factor and other links.

DATFIX ..... estimates the overall scale and temperature factor using the K-curve method[5], a modified form of the Wilson plot [4]. A set of normalised structure factors (E values) can also be produced

FOURR ..... calculates Patterson and Fourier maps, the latter using as input a set of structure factors calculated in the FC structure factor link. The program is very flexible and a variety of modifications of the basic Fourier map can be produced, such as difference and E-syntheses.

ORFLS ..... this is a version of the Busing-Martin-Levy

full-matrix least-squares refinement program [6]. It allows the simultaneous refinement of up to 180 variables, and such options as isotropic-anisotropic conversion at the user's discretion and a correction for dispersion, are built into the system. For refinement of large structures the block-diagonal least-squares approximation called BLOKLS has been used.

DELSIG ..... gives value of delta-F as a function of $|F_o|$, over a range of $|F_o|$. This enables a suitable weighting scheme to be decided upon.

BONDLA ..... calculates inter-and-intra molecular bond distances and angles, and their estimated standard deviations. Hydrogen atom positions can also be generated, either to sp^3, sp^2 or sp carbon atoms.

LSPQL ..... the best plane through a set of atoms is calculated, together with the displacement of the atoms from the plane, and the displacement of any additional atoms specified. The angle between planes and/or lines can also be obtained.

The programs for processing of the diffractometer data (all written by P.G.H. Troughton), run on the Atlas computer:

SEKO .... calculates least-squares cell parameters and setting angles (see section 3.1) and generates steering tape. The raw intensity data are processed by SODI which applies a

LP^{-1} correction and puts the data on a common (arbitary) scale. An absorption correction using the program ABSO can also be applied.

The following programs are mainly Imperial College products:-

POLO .... (written by M.G.B. Drew). This puts a set of photographic data, collected about two or three axes, on an arbitary common scale. The inter-layer scale-factors are obtained in the course of this, using the common reflections and the least-squares method of Hamilton, Rollett and Sparkes.[7] The program has a storage limitation of about 2500 reflections.

DIDD .... (written by D.J. Hunt). Calculates the best plane through a set of atoms.

MOJO .... (written by F.H. Allen). This computes orthogonalised Angstrom co-ordinates and dihedral angles for a set of atoms, using the "bonding array" method.[8]

The thermal-ellipsoid plot program ORTEP[9] has been used for crystal-structure drawings. Given a set of atoms, cell data and plotting instructions, a perspective drawing can be obtained on an off-line plotter. The program has many flexible features such as facilities for unit-cell outlines, stereoscopic pairs, and anisotropic thermal ellipsoids for individual atoms.

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CHAPTER 4

The Determination of Molecular Chirality by X-ray Methods.

4.1. Introduction

The phenomenon of optical activity, in which certain molecules can rotate the plane of polarised light either to the left or to the right, has been known since the observations of Biot on several natural products, in the early 19th century. These compounds could in principle occur in two forms, one of which rotates to the left, and the other to the right - these are termed optical enantiomers. For many natural products only one enantiomer is known. The theory of the tetrahedral carbon atom propounded by van't Hoff and le Bell in 1875, provided a rationalisation for these findings; if four different groups of atoms, A,B,C and D are attached to a tetrahedral carbon atom, the resulting molecule can exist in just two forms, which are non-superimposable mirror images of each other, both of which are optically active. The ab initio prediction of the sign of rotation for a given isomer is only possible for a few very simple molecules, and correlations of absolute configuration in a group of compounds by means of optical rotatory dispersion studies (for example, using the 'octant rule'), are based on the knowledge of the absolute configurations of a number of reference compounds, and are not

completely reliable.

Knowledge of absolute configurations is of vital importance in understanding the detailed chemistry of life processes, which are almost without exception, stereo-specific. In general, even for the most complex molecules with many chiral centres, only one of the very many possible optical isomers occurs naturally. Thus, almost all natural amino-acids have the L configuration (using the Fischer-Rosanoff convention). The reasons for the origin and role of optical stereo-specificity in life have excited much speculation and theories, none of which are really satisfactory Laboratory syntheses of amino-acids under pre-biotic conditions have produced equal amounts of possible isomers; however, Garay has found that L-tyrosine in solution is more stable to the action of beta-particles than the D-form[1]. He suggests that in pre-biotic times the atmospheric radiation was responsible for the selection of left-handed isomers; these would then be favoured to an ever-increasing extent in subsequent protein formation and replication.

The arbitrary convention of configurational assignment chosen by Fischer, was based on tartaric acid as a standard (Figure 4.1). Chemical correlation can relate configurations at a chiral centre to these; however, apart from being lengthy processes, their basis is of course an arbitrary one. The X-ray methods described in this chapter have enabled

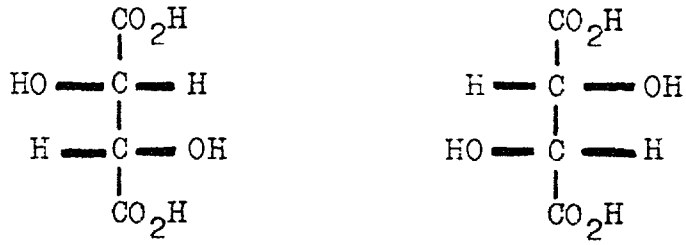

Figure 4.1

D - tartaric acid L - tartaric acid

---

all assignments to be put on a firm absolute foundation for the first time.

4.2. Friedel's Law.

In centro-symmetric crystals, reflections (hkl) and ($\bar{h}\bar{k}\bar{l}$) are identical, by symmetry. For non-centro-symmetric crystals, the sequence of atoms from the (hkl) side is the reverse of that from the ($\bar{h}\bar{k}\bar{l}$)side. However, since the wave scattered by any atom is normally $\Pi/2$ out of phase with the incident wave, the resultant phase change can be ignored for both views. This may be shown more quantitatively:-

Consider the structure factors F_h and $F_{\bar{h}}$ for a structure and its enantiomorph. We can write

$$F_h = |F_h| e^{i\phi_h} \quad \ldots\ldots\ldots 4.1$$

and

$$F_{\bar{h}} = |F_h| e^{-i\phi_h} \quad \ldots\ldots\ldots 4.2$$

since

and thus $\phi_h = -\phi_{\bar{h}}$

Equations 4.1 and 4.2 are the complex conjugates of each other. Thus we can write

$$|F_h|^2 = |F_{\bar{h}}|^2$$

and $$I_h = I_{\bar{h}} \qquad \ldots\ldots\ldots\ldots 4.3$$

This is Friedel's Law. It means that X-ray intensities cannot normally distinguish a structure from its inverse. Optically active molecules can only crystallise in the 65 non-centro-symmetric space groups which involve rotatory symmetry elements. The other non-centro-symmetric space groups involving mirrors, glides or $\bar{4}$ axes can only accomodate racemates i.e. equal amounts of both isomers. Centro-symmetric space groups obviously cannot accomodate optically active molecules. Friedel's Law applies to all these space groups.

4.3. Anomalous Scattering.

Friedel's Law was derived assuming the frequency of the incident radiation to be large in comparison with any natural absorption frequency of the scattering atoms. Von Laue suggested that the Law might not hold if the two frequencies were close [2], but it was not until 1930 that Coster, Knol and Prins showed experimentally that the Law could indeed break down [3]. They used a crystal of zinc sulphide, with gold Lα radiation, which just excites the K electrons of zinc, and found that there was unequal scattering from the $(\bar{1}\bar{1}\bar{1})$ and $(\bar{1}\bar{1}\bar{1})$ planes.

In general, if the incident wavelength is slightly shorter than that of an atomic absorption edge of one or more atoms in the crystal, there is an anomalous phase-shift on scattering, which has the effect of advancing the waves relative to those from the rest of the atoms. This effect of anomalous scattering, or dispersion, causes the scattering factor for the affected atoms to be a complex quantity:-

$$f_j = f_j^o + \Delta f_j + i\,\Delta f_j'' \qquad \ldots\ldots\ldots\ldots 4.4$$

where $\Delta f_j'$ and $\Delta f_j''$ are the real and imaginary correction terms. Theoretical values of these terms were first calculated by Höhl using quantum scattering theory [4], for a variety of anomalous scatterers and wavelengths. $\Delta f_j'$ is sometimes negative, and $\Delta f_j''$, the imaginary part, is always positive, i.e. ahead of the real part by $\Pi/2$. Both factors are virtually independent of the scattering angle as the electrons responsible for these effects are confined to a very small volume in the vicinity of the nucleus. Hence the effects of dispersion are relatively greater at high $\sin\theta$ since the normal scattering factor f_j^o, decreases with increasing angle.

For a structure with both normal and anomalously scattering atoms, the total scattered wave can be written as

$$F_T = F_N + F_A \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 4.5$$

The real and imaginary components of F_A are F_A' and F_A''. Thus, we may write:-

$$F_T = |F_N| \exp(i\phi_N) + |F_A'| \exp(i\phi_A) + |F_A''| \exp(i\phi_A) \quad \ldots \quad 4.6$$

$$= |F_N| (\cos\phi_N + i\sin\phi_N) + |F_A'| (\cos\phi_A + i\sin\phi_A) + |F_A''| (i\cos\phi_A - \sin\phi_A) \quad \ldots \quad 4.7$$

$$= \left\{|F_N| \cos\phi_N + |F_A'| \cos\phi_A - |F_A''| \sin\phi_A\right\} + i\left\{|F_N| \sin\phi_N + |F_A'| \sin\phi_A + |F_A''| \cos\phi_A\right\} \quad \ldots\ldots \quad 4.8$$

Similarily,

$$\overline{F}_T = \left\{|F_N| \cos\phi_N + |F_A'| \cos\phi_A + |F''| \sin\phi_A\right\} + i\left\{|F_A''| \cos\phi_A - |F_A'| \sin\phi_A - |F_N| \sin\phi_N\right\} \quad \ldots\ldots \quad 4.9$$

On multiplying F_T and $\overline{F}_T$ by their complex conjugates, we obtain for the difference

$$|F_T|^2 - |\overline{F}_T|^2 = 4|F_N||F_A''| \sin(\phi_N - \phi_A) \quad \ldots\ldots\ldots\ldots \quad 4.10$$

$$\propto \Delta I$$

Thus, for a non-centro-symmetric structure ($\Delta f'' \neq 0$), Friedel's Law is not obeyed. This is shown in the vector diagrams 4.2-4.4.

Friedel's Law still holds for centro-symmetric structures, but in this case, the phases of reflections $(hk\ell)$ and $(\bar{h}\bar{k}\bar{\ell})$ are equal, but differ slightly from 0 or Π , and the structure factors become complex. (Figure 4.5).

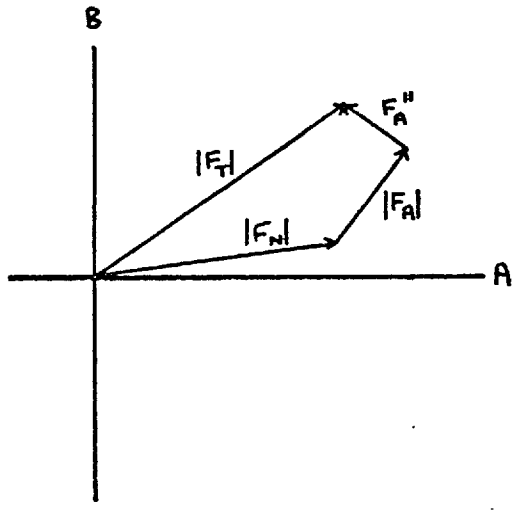

Figure 4.2.

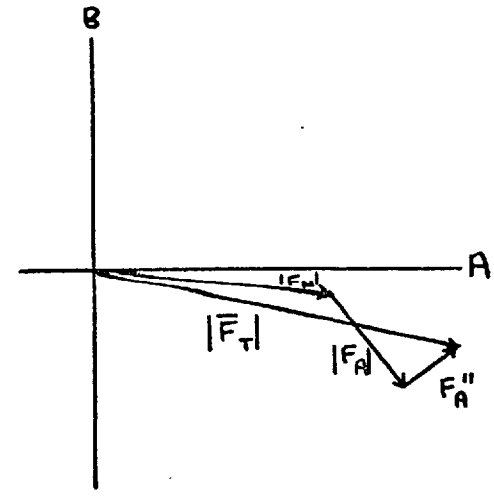

Figure 4.3.

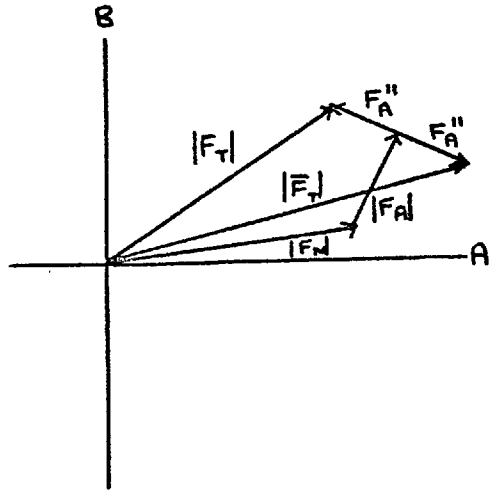

Figure 4.4.

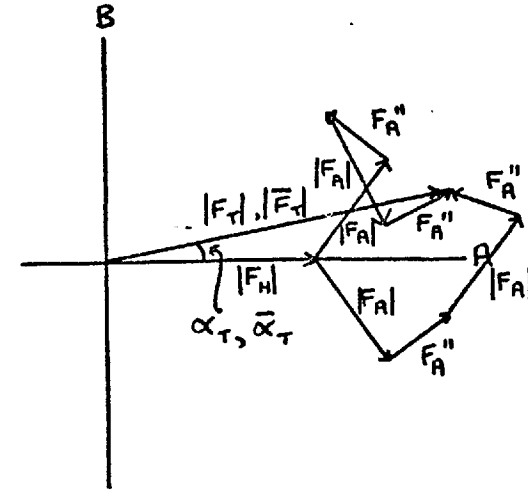

Figure 4.5.

In Figure 4.4, $|\bar{F}_T| > |F_T|$

4.4. Anomalous Dispersion and Absolute Configuration.

Bijvoet in 1949 realised that comparison of (hkℓ) and ($\bar{h}\bar{k}\bar{\ell}$) pairs under suitable conditions of anomalous scattering, could be used to distinguish between optical enantiomers and thus to determine absolute configurations.[5] The first structure to be so analysed was that of sodium rubidium D - (+) - tartrate, an historically apt one.[6] Monochromated zirconium radiation (λ_{a_1}= 0.79010Å; λ_{a_2}= 0.78588Å) was used, which was anomalously scattered by the rubidium atom (λ_{abs}= 0.81549Å). The inequalities in intensities for fifteen pairs were noted, and they agreed with the calculated inequalities, thus establishing the absolute configuration of the tartrate molecule in the crystal as D - (+)[7]; Fischer's arbitrary assignment was shown to be the correct one.

The technique is a simple one; in principle just one 'Bijvoet' pair is measured, and the resulting intensity inequality compared with that calculated from an Argand diagram. (Figure 4.4). If they both tend in the same direction, the absolute configuration chosen is the correct one; if not, it is the reverse. In practice it is wise, since the differences $\left|I_{hk\ell} - I_{\bar{h}\bar{k}\bar{\ell}}\right|$ are relatively small, to examine a number of pairs. If a statistically meaningful number tend the same way, then the determination is a reliable one.

Both Peterson[8] and Templeton[9] have pointed out that even if the incident wavelength is on the longer side of the

K absorption edge it may have sufficient energy to excite the L or even the M electrons. This means that measurable anomalous scattering effects can often be observed with radiation of wavelength quite far from an absorption edge. Thus, it is now common practice to determine the absolute configuration of a bromine derivative of a compound (λ_{abs} for L electrons of bromine = 7.989Å), with Cu-Kα radiation (λ = 1.54178Å); this was first done for strychnine hydrobromide dihydrate. [10] Most elements of atomic number greater than 20 show some measurable effect with all the common radiations used in X-ray work. Several tables of calculated $\Delta f'$ and $\Delta f''$ values are available [11,12], and from these the optimum radiation for a particular situation may be selected.

It is imperative in the application of the technique to have all the measured reflections correctly indexed, since errors in assignment could well lead to a wrong absolute configuration. Normally they are indexed on a right-handed set of axes. Particular care is needed when data is obtained by the equi-inclination Weissenberg method.[13] It is necessary to correlate the sense of direction of motion of the camera film with that of the direction of motion of the crystal Imagine a right-handed co-ordinate system whose c axis is that of the crystal i.e. that of the axis of rotation. (Figure 4.5). The direction of the b axis follows from a consideration of

the sense of rotation of the camera - as shown, it is right-handed. This system, in conjunction with the camera geometry and use of the reflecting sphere concept, enables the correct sequence of recording of axial rows to be determined. In our example these rows will slope upwards from left to right, and their sequence is h0O, 0k0, $\bar{h}$0O and 0$\bar{k}$0 from right to left on the film. Diffractometer data is normally collected according to a right-handed axial system, the counter and angle geometry being so designed. Figure 4.6 shows the axial sequences.

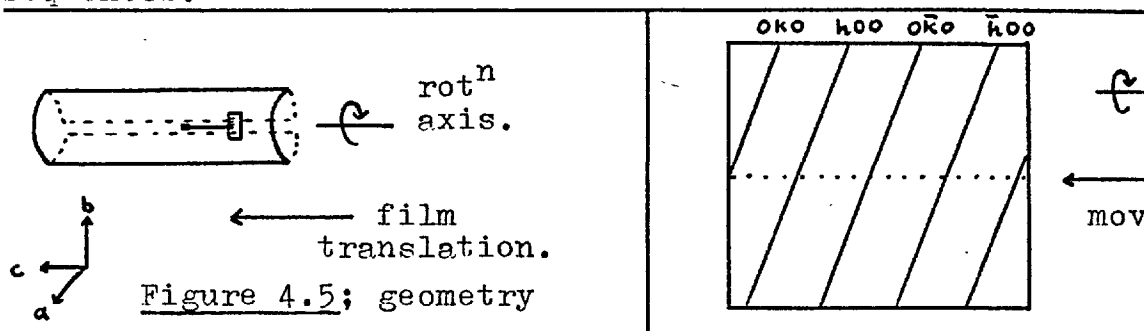

Figure 4.5; geometry of the Weissenberg camera.

Figure 4.6.

Care is also needed in measurement of the Bijvoet pairs, since the intensity differences are often small. They must be recorded under identical conditions ie. on the same film when collected photographically. It is best to rely on microdensitometer estimation of spot intensity, rather than on visual comparison. Small discrepancies are best observed on pairs of high-angle reflections, as stated previously. For these, electronic measurement is the most reliable recording procedure, differences of two per cent being easily observable.

Experience has shown that the calculated differences are best obtained after a structure has been fully refined; in particular, where absorption effects are even moderately severe, a correction should be applied. The latter especially, can produce errors which in adverse circumstances, reverse an assignment.

It should also be mentioned that, due to the particular space-group symmetry of a crystal, reflections other than $(hk\ell)$ and $(\bar{h}\bar{k}\bar{\ell})$ may be compared. For example, with the mono clinic space group $P2_1$, we have the conditions when Friedel's Law is obeyed.

$$F(hk\ell) = F(\bar{h}\bar{k}\bar{\ell}) = F(h\bar{k}\ell) = F(\bar{h}k\bar{\ell}) \neq F(\bar{h}k\ell) \neq F(hk\bar{\ell})$$

Thus, since we can only have Bijvoet pairs from two of the first four groups, which have k and $\bar{k}$, a photograph showing anomalous dispersion effects can only be taken with the crystal mounted about a non-unique axis.

The Bijvoet technique thus only necessitates (sometimes) the taking of an extra photograph, and a few simple calculations. The latter are usually done on a computer, and it is not even necessary to draw a series of Argand diagrams. The past few years have seen a tremendous increase in the use of the method; it is still the only reliable method of absolute configurational assignment available to chemists. The author, in collaboration with Dr. F.H. Allen, has performed an exhaustive literature search of all the

organic structures so established. The initial survey (by Dr. Allen alone), showed that 54 assignments had been made between 1951 and 1966 [14]. Subsequent lists, compiled annually, showed up to the end of 1969, a total of 132 more [15]. These reference lists have been of considerable service to organic chemists generally.

A relatively recent use of anomalous scattering has been in phase determination of complex structures, provided the position of the anomalous scatterer is known, from equation 4.10, the only unknown, ϕ_N, can be determined [16]. The method is of use with a heavy-atom compound in which the heavy-atom is not sufficient to dominate enough phases for the straightforward heavy-atom method to be proceeded with (Section 2.3); the correct absolute configuration is an automatic outcome of anomalous dispersion phasing. An example of the method is the solution of the structure of "factor Vla" ($C_{46}H_{66}O_9N_{11}Co.11H_2O$) [17]. The cobalt atom ($\Delta f' = -2.2$; $\Delta f'' = 3.9$) produced very large Bijvoet differences which were easily observable visually, and it was possible to calculate the phase of nearly 2000 reflections, sufficient to solve the structure. In conjunction with multiple isomorphous derivative procedures, the technique has seen recent application in the solution of a number of enzymes [18]; it has the considerable advantage that a

smaller number of derivatives than hitherto are needed.

4.5. The Method of Internal Comparison.

Mathieson suggested that if a known asymmetric centre was introduced into a molecule, structure solution would then lead to both the relative and absolute stereo-chemistry of the molecule [19]. The method has a number of advantages over the Bijvoet approach:-

(i) no extra photography and calculation is needed.

(ii) a heavy-atom derivative is not needed. The reference centre can be incorporated either in the molecule, or in the crystal lattice, as solvent of crystallisation.

(iii) the structure does not have to be fully refined for unambiguous conclusions to be made.

(iv) in favourable cases, a result can be obtained from a two-dimensional projection.

Rogers and Hine, independently of Mathieson, provided the first example of the use of this method by the determination of the absolute configuration of S-methyl-L-cysteine sulphoxide [20]. The known configuration at the amino acid enables that at the chiral sulphur to be related to it. We have recently determined the absolute configuration of 2(S),S(R) methionine sulphoximine [21] by using the known 2(S)-methionine configuration as a reference centre, and thus obtaining the configuration at the chiral sulphur atom.

4.6. Significance Tests and the Reliability Index.

We have previously defined in Section 2.4 a reliability index, R, which can be used as the measure of correctness of a structure model. Now if a set of structure factors is calculated corresponding to both possible enantiomers (with a dispersion correction included), the correct absolute configuration should give the lower R factor. Hamilton[22] has suggested the use of linear hypothesis tests with which it is possible to assess the statistical significance of an absolute configuration being the correct one.

The weighted R factor, R^w, is here defined as:-

$$R^w = \left[\frac{\sum_{i=1}^{m} w_i (|F_i|_o - |F_i|_c)^2}{\sum_{i=1}^{m} w_i |F_i|_o^2} \right]^{1/2} \quad \ldots\ldots\ldots\ldots\ldots 4.11$$

for m observations and n parameters. The test is based on the ratio of the R factors for the two possible configurations, R_1^w and R_2^w, which is

$$\frac{R_2^w}{R_1^w} = \mathscr{R}_{b,m-n,\alpha} \quad \ldots\ldots\ldots\ldots\ldots\ldots 4.12$$

where b is the dimension of the hypothesis, (m-n) the number of degrees of freedom, and α the significance level of the test. The value of the ratio can be obtained from a set of tables [22], suitably interpolated if necessary. The one-dimensional hypothesis that the second absolute configuration (corresponding to R_2^w), is the correct one,

is then tested, by comparing the ratio obtained from the tables, with that calculated. If

$$\mathcal{R} < \mathcal{R}_{b,m-n,\alpha}$$

then the hypothesis is only correct at the significance level α, which may well be $< 0.5\%$. In such a case, the hypothesis is decisively rejected.

This method has the obvious advantage that no Bijvoet pair measurement is needed. All that is necessary is that the factors R_1^w and R_2^w are obtained from parallel refinement on the data corrected for anomalous dispersion, which means only a relatively small amount of extra computation. However, it is important that the tests are carried out on a fully refined structure, with systematic errors such as absorption effects eliminated as far as possible, otherwise misleading results can be obtained. [23] It is unwise to try the tests on structures with R^w values greater than 0.10. Although formally, properly weighted reliability indices are required, the ratio $\mathcal{R}$ is likely to be relatively insensitive to how the reliability index is computed. Hence, it has often been found adequate to use the conventional R factor, as we have done in checking the absolute configuration of 2(S),S(R)-methionine sulphoximine.

Hamilton's method is now used widely as a speedy

method of absolute configurational determination. However, care is needed in its application, particularly when the significance level attained is not decisive. This indicates, especially when a strong anomalous scatterer is present, that systematic errors have not been properly eliminated. Indeed, without adequate care, it is easier with this method to obtain the wrong assignment, than with the Bijvoet technique.

4.7. The Absolute Configuration of α-D-glucose monohydrate.

Normally in absolute configuration determination by one of the anomalous scattering methods, the anomalous effects are relatively large and hence easily detectable. Until recently, the lightest element so used has been sulphur, with a $\Delta f''$ of 0.6 [21,24]. The advent of diffractometers and scintillation counters has meant that much smaller differences than hitherto can be measured accurately. In a pioneering paper [25], Hope and de la Camp have detected differences for a crystal containing only carbon, oxygen and hydrogen atoms, and they were thus able to determine the absolute configuration of (+)-tartaric acid, $C_4H_6O_6$, (previously known from the classic studies of Bijvoet et al [7]). Both in this study and the subsequent one of the sesquiterpene solstitialin, $C_{15}H_{20}O_5$ [26], a varient of the Hamilton test was employed. Moncrief and Sims, on the other hand, collected a number of Bijvoet

pairs for the determination of the absolute configuration of cellobiose.[27]

A set of data for α-D-glucose monohydrate, $C_6H_{12}O_6 \cdot H_2O$, of known structure[28], was recently collected in this laboratory, as a test on the accuracy of the diffractometer installed here.[29] A total of 912 intensities (the unique set in reciprocal space) was collected using Cu-K_α radiation. After anisotropic refinement of the non-hydrogen atoms, and isotropic refinement of the hydrogens, the unweighted R factor had converged to 0.03012. No absorption or extinction corrections had been applied, but they were thought to be small.

In view of the large percentage of oxygen in this structure we decided that it would be of interest to investigate the effects of allowing for oxygen and carbon anomalous dispersion. We used the values of $\Delta f'$ and $\Delta f''$ adopted by earlier workers:-

Carbon[25,26]:	$\Delta f' = 0.02$.	Oxygen:	$\Delta f' = 0.05$
	$\Delta f'' = 0.01$		$\Delta f'' = 0.04$

These had been calculated by Hönl's method[4], and were felt to be more reliable than the somewhat higher values employed by Moncrief and Sims.[27]

Since no anomalous pairs were included in the measured data, and the space group ($P2_1$), is polar i.e. non-centrosymmetric with an undefined origin, it is invalid to attempt least-squares refinement with a dispersion correction included,

for then refinement tends to minimise the very differences one is looking for, and the parameter shifts are indeterminate. A complete set of pairs is thus needed for polar space groups [30,31]. Accordingly, a straightforward structure-factor calculation was performed on both enantiomorphs, with the dispersion corrections included.

With both $\Delta f''$ values positive for the conventional absolute configuration, the unweighted reliability index summed over all observed reflections, R(+), was 0.03524. On reversing the signs of both $\Delta f''$ values, which is equivalent to taking the reverse absolute configuration, R(-) was 0.03538. Now, using the nomenclature of Hamilton (Section 4.6), we have

$$\mathcal{R} = \frac{R(-)}{R(+)} = 1.0040$$

From the significance tables [22],

$$\mathcal{R}_{1,912,0.01} = 1.0035$$

and $$\mathcal{R}_{1,912,0.025} = 1.0027$$

Thus, the one-dimensional hypothesis that the second absolute configuration corresponding to R(-) is the correct one, can be rejected at about the one per cent confidence level. This was felt to be encouraging, bearing in mind that it was based on the structure factors from all the reflections in the

unique set, and that some (probably small) corrections to the data, had still to be applied. It was accordingly decided to select the most sensitive reflections in order to somewhat enhance the discrimination between the two enantiomers, a procedure analogous to that of Hope et al.

Accordingly, 247 reflections were selected whose calculated dispersion ratios deviated most markedly from unity, this ratio being defined as the ratio of the structure factors before and after inclusion of the anomalous scattering terms. For this restricted set the corresponding R factors were

$$R'_{(+)} = 0.05073 \text{ and } R'_{(-)} = 0.05769$$

Now,

$$\mathscr{R}' = \frac{R'_{(-)}}{R'_{(-)}} = 1.138$$

and $\mathscr{R}'_{1,240,0.005} = 1.017$

Thus, Hamilton's test very decisively rejects the $R_{(-)}$ configuration at much more than the 99.5 per cent confidence level. (This is in fact the limit of the tables in Hamilton's paper). The conventional absolute configuration for α -D(+)-glucose [32] is thus confirmed; this is the first direct demonstration for glucose itself although a number of molecules incorporating known derivatives of D or L-glucose have been studied. [15]

It is evident that anomalous scattering effects from oxygen are sometimes significant and are then worth allowing for, but their influence on the usual overall R factor is slight. They are often large enough to permit the assignment of absolute configuration, preferably by direct measurement on a number of the Bijvoet pairs; in the case of α-D-glucose monohydrate here, the determination was an afterthought, and was successful on a limited set of "sensitive reflections"[33]. In such a case our method is still capable of giving an unambiguous answer.

Some preliminary studies were also made on the mould metabolite viridin, $C_{20}H_{16}O_6$ (Chapter 9), in order to determine its absolute configuration. However, since the structure was determined from photographic data, with obviously less precision than from diffractometer intensities, and since the molecule contained a rather smaller percentage of oxygen than, say, α-D-glucose monohydrate, the differences were so minute as to be statistically insignificant. Thus, in the absence of really good quality data, this attempt was abandoned.

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PART TWO; THE HEAVY-ATOM METHOD IN PRACTICE.

CHAPTER 5

The Crystal and Molecular Structure of 2(S),S(R)-Methionine Sulphoximine.

5.1. Introduction.

In 1946, Mellanby discovered that flour which had been treated with "Agene" (nitrogen trichloride), produced hysteria in dogs [1]. The toxic factor involved was subsequently isolated and chemical degradative work identified it as a sulphoximine derivative of L-methionine.[2] Infra-red spectra confirmed this suggestion [3]. The compound has subsequently been used quite widely, in controlled doses, as a means of inducing epileptiform convulsions in clinical studies[4].

It has been established that methionine sulphoximine is a potent inhibitor of the enzyme glutamine synthetase [5]. This widely distributed enzyme has been isolated both from bacterial sources (in particular, Escherichia Coli [6]), and from mammalian tissue, particularly brain [7]. In both cases, the enzyme performs the same function, namely catalysing the synthesis of glutamine from glutamic acid and ammonia:-

$$\underline{L}\text{-glutamic acid} + NH_3 + ATP \overset{Mg^{2+}}{\rightleftharpoons} \underline{L}\text{-glutamine} + ADP$$

Glutamine, one of the building blocks of proteins, is an essential amino-acid for the growth of living cells. It thus performs a central regulatory role in nitrogen metabolism. It is interesting to note that the enzymes from the two sources differ in a number of respects; thus, the E.Coli product has been shown by electron-microscopic studies to consist of twelve identical sub-units, each of molecular weight about 50,000 [8]. The cerebral enzyme on the other hand, has only eight sub-units, each of molecular weight about 65,000[9]. Both forms show qualitatively similar inhibitor reactions, as well as performing the same function. The significance of the differences between them is as yet obscure.

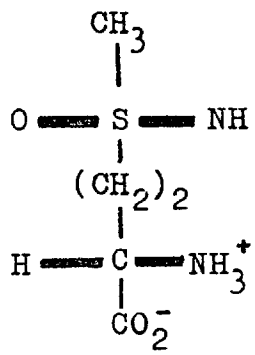
Thus there seems to be a relationship between the enzyme inhibition by methionine sulphoximine, and the latter's effect on the central nervous system. Indeed, it is possible, although work in this field is still in its infancy, that this compound is implicated in a number of neurological disorders in man, since its effects on administration are at least very similar to the natural symptoms of these disorders.

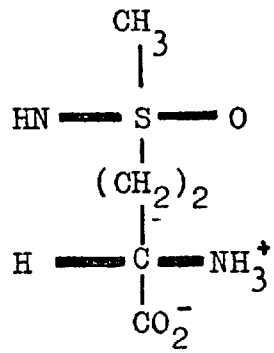
The active sites of many enzymes have been widely investigated by the method of systematic variation of

substrates and inhibitors; in fact, most of the considerable knowledge now available for a number of enzymes, derives directly from an interplay of such studies with X-ray crystallographic investigations. Meister and his co-workers have been prominent in studies of the interaction of (cerebral) glutamine synthetase with a large number of substrates (10). They have been able to obtain some insight into the nature of the active sites, of which there is one per sub-unit. This binding is unusually stereo-specific, for example, only L and not D- -methylglutamic acid, can bind.

Now, 2(S)-methionine sulphoximine has two diastereoisomers, I and II, 2(S),S(R) and 2(S),S(S) respectively:-

I





II

It has been found that only one isomer reacts with the enzyme, whilst the other when pure is totally devoid of activity (11). Thus, knowledge of the absolute configuration of the active isomer should be very useful in investigating the mechanism of inhibition, as well as providing some knowledge of the

geometry and nature of the binding sites involved. Our X-ray investigations have shown that the active molecule is the 2(S),S(S) one [12,13].

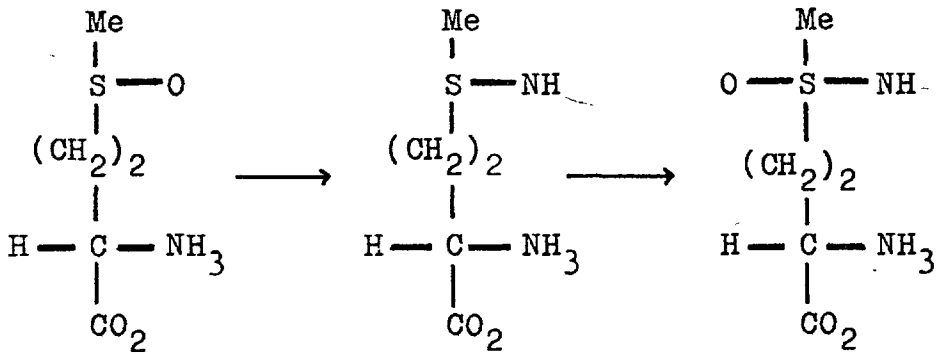
Binding of L-glutamic acid involves an intermediate enzyme-bound acyl phosphate, by initial reaction with ATP. The active site has two carboxyl and one amino-group binding sites. A necessary condition for binding is that the molecule must be in an extended conformation; this was elegantly demonstrated with the finding that L-cis-1-amino-1,3-dicarboxy-cyclohexane,(whose geometry corresponds precisely to extended L-glutamic acid), possesses strong inhibitory properties [14].

Studies on the stereo-chemistry of reactions at an asymmetric sulphur atom, by a number of workers [15], have in particular, focused attention on the stereo-chemical course of the conversion sulphoxide ⟶ sulphoximine, which proceeds via an intermediate sulphimine. Kjaer and Christensen have used the configurationally defined 2(S),S(S)-methionine sulphoxide III,in their studies. On suitable treatment this gives predominately the isomer 2(S), S(R)-sulphoximine I. [11] Thus, either the reactions sulphoxide ⟶ sulphimine ⟶ sulphoximine, both proceed predominately with inversion, or with retention. Since the initial oxidation is thought certainly to proceed with retention, the second possibility is considered more likely [16].

Thus, knowledge we have provided of the absolute config-

uration of the sulphoximine involved, the 2(S), S(R) one, has been vital in elucidating the sequence of these reactions. Our X-ray studies have also provided the first description of the geometry of a sulphoximine.

We thank Professor A. Kjær of the Technical University of Denmark for providing samples, and for much useful discussion.



III I

5.2. Experimental.

Crystals of (I) are colourless needles elongated along a. Oscillation and Weissenberg photographs exhibited Laue symmetry mmm. Cell parameters were obtained by a least-squares technique applied to 25 θ-values measured on Weissenberg photographs.

Crystal Data:- $C_5H_{12}N_2O_3S$, M = 180.0, orthorhombic
a = 5.33(1), b = 7.16(1), c = 21.50(2) Å,
U = 820.5 Å^3, D_m = 1.46(3) g.cm.$^{-3}$ (by flotation),
D_c = 1.46 g.cm.$^{-3}$ for Z = 4 molecules/cell,
μ = 27.5 cm.$^{-1}$ for Cu-K$_\alpha$ radiation (λ_{mean} = 1.54178Å),
F(000) = 384.0. Space group $P2_12_12_1$ (No. 19, D_2^4) from systematic absences hOO, OkO, OOl for h, k, l $\neq$ 2n.

The layers O-3kl were photographed with Ni-filtered Cu-K radiation using the equi-inclination Weissenberg method and multi-film packs. Owing to the rapid 'thermal' attenuation of intensities, the amount of data collected was limited. The intensities of 476 reflections were estimated visually with a calibrated wedge. The layers were brought to a common scale by use of 58 common reflections from the hOl zone, which contributed a further seven unique reflections, making a total of 483 non-zero intensities. The layer scale factors were obtained by hand calculation. A further 92 reflections

were treated as accidental absences.

Isomer (II) was microcrystalline and gave a powder pattern, but repeated attempts failed to produce any satisfactory single crystals.

5.3 Structure Solution and Refinement.

A three-dimensional Patterson synthesis unambiguously gave the co-ordinates of the sulphur atom. A structure-factor calculation based on the phases from the sulphur atom alone, with an assigned isotropic temperature factor of 3.0 $\mathring{A}^2$, gave an R of 0.63. A three-dimensional electron-density map was computed by use of these phases and only those terms for which $|F_c| > 0.5\,|F_o|$. Three peaks around the sulphur atom were identified; otherwise resolution was poor, and many of the 'peaks' were later shown to be spurious. The three atoms were included in the structure-factor calculation and treated as carbon atoms with B-values of 3.0 $\mathring{A}^2$, and three cycles of ORFLS least-squares were done to check their authenticity. R fell to 0.34. A second electron-density map with the same rejection test revealed six more sensible peaks; these were treated as carbon atoms and checked by least-squares, when R fell to 0.23. The third map revealed the one remaining atom. Three peaks were then treated as oxygen and two as nitrogen atoms, and three cycles of least-squares refinement brought R down to 0.146. The isotropic temperature factors of N(1) and O(1) became 1.51

and 5.82 $\mathring{A}^2$ respectively. When the assignment of N(1) and O(1) was reversed, R fell to 0.142, and the temperature factors for O(1) and N(1) became 3.57 and 3.61 $\mathring{A}^2$. Difference maps supported this latter assignment. Subsequent consideration of the crystal structure shows that only this assignment sensibly explains the hydrogen-bonding scheme and gives acceptable S - O and S - N bond lengths.

After a few misindexed reflections were corrected and the 014, 103, 111 and 102 reflections discarded for suspected extinction, four more cycles of least-squares, refining positional parameters, isotropic temperature factors, and inter-layer scale factors, gave R=0.114. Unit weights were used throughout the refinement: an agreement analysis indicated no more appropriate weighting scheme.

A difference map revealed all the hydrogen atoms, in positions comparing well with those predicted. These were included in the final stages of refinement, each being given a B-factor 0.5 $\mathring{A}^2$ greater than that of the atom to which it was bonded. The hydrogen parameters were not refined. A dispersion correction was also applied to the sulphur atom, of $\Delta f' = 0.3$ and $\Delta f'' = 0.6$. After three cycles R was 0.103.

The B-factors were converted into their anisotropic

Table 5.1.

Final positional parameters for the non-hydrogen atoms, as fractions of the unit-cell edges. Standard deviations are in parentheses.

Atom	x	y	z
S	0.03783(90)	0.51727(48)	0.42138(14)
O(1)	-0.12245(281)	0.64991(153)	0.45615(46)
C(5)	0.28541(362)	0.43548(189)	0.46914(53)
N(1)	-0.07920(310)	0.33548(163)	0.39517(50)
C(4)	0.18936(367)	0.65161(186)	0.36048(57)
C(3)	0.31788(379)	0.82521(219)	0.38230(61)
C(2)	0.37911(397)	0.95988(191)	0.33008(61)
C(1)	0.49608(453)	0.87257(173)	0.27498(58)
O(2)	0.72362(302)	0.82543(156)	0.27951(44)
O(3)	0.35753(275)	0.84269(165)	0.22680(45)
N(2)	0.13340(287)	1.06830(147)	0.30941(44)

Table 5.2.

Final anisotropic thermal parameters (β_{ij}'s) for the non-hydrogen atoms, (x 10^5), defined by the equation

$$f_i = f_i^o \exp-(\beta_{11}h^2+\beta_{22}k^2+ \beta_{33}l^2+ 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl)$$

Atom	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
S	3903(250)	1225(64)	116(6)	-19(126)	-24(36)	-10(18)
O(1)	5874(816)	2117(262)	230(25)	-673(452)	19(123)	-291(71)
C(5)	4829(1054)	1724(344)	97(25)	370(504)	22(134)	305(74)
N(1)	4270(864)	1577(255)	189(27)	317(435)	-48(136)	0(73)
C(4)	3939(1037)	1041(283)	154(31)	96(449)	-74(148)	34(76)
C(3)	4202(1144)	1920(353)	166(33)	65(543)	104(159)	-140(96)
C(2)	4449(1092)	984(299)	199(33)	20(467)	12(158)	69(81)
C(1)	3042(1367)	942(253)	125(28)	-95(526)	-389(165)	-56(69)
O(2)	1992(768)	1866(236)	171(24)	-566(386)	-201(106)	-156(64)
O(3)	5348(854)	2668(288)	142(23)	-1013(439)	125(119)	-105(67)
N(2)	2647(777)	1326(270)	123(22)	-235(355)	-63(108)	-23(62)

Table 5.3.

Observed positional parameters for the hydrogen atoms, as fractions of the cell edges.

Atom	x	y	z
H(1)-C(5)	0.375	0.550	0.445
H(2)-C(5)	0.160	0.370	0.500
H(3)-C(5)	0.270	0.470	0.517
H(4)-N(1)	-0.200	0.395	0.370
H(5)-C(4)	0.171	0.679	0.326
H(6)-C(4)	0.318	0.567	0.338
H(7)-C(3)	0.476	0.797	0.406
H(8)-C(3)	0.211	0.890	0.413
H(9)-C(2)	0.475	1.057	0.340
H(10)-N(2)	0.150	1.100	0.350
H(11)-N(2)	0.175	1.137	0.280
H(12)-N(2)	0.025	0.995	0.300

Each hydrogen atom was given a B factor 0.5$\mathring{A}^2$ greater than that of the atom to which it is attached.

The average H-X distance is 0.958$\mathring{A}$.

Table 5.4.

Final observed and calculated structure factors; column headings are h, k, ℓ, followed by ℓ, $10|F_o|$, $10|F_c|$. The accidental absences are marked (*); the value of F_o quoted for such a reflection is the minimum observable. The 200 and 101 reflections were partly obscured by the backstop. The four reflections omitted for suspected extinction were:

h	k	ℓ	$10\|F_o\|$	$10\|F_c\|$
0	1	4	979	1190
1	0	2	359	463
1	0	3	814	985
1	1	1	516	668

0.0.L

2	248	240
4	56	54
6	528	538
8	190	214
10	102	105
12	69	87
14	149	133
16	217	222
18	34*	33
20	323	298
22	120	101
24	74	13

0.1.L

1	297	316
2	357	342
3	129	153
5	20*	22
6	20*	0
7	56	49
8	28*	50
9	63	80
10	63	101
11	245	266
12	181	191
13	69	52
14	170	174
15	80	46
16	248	232
17	20*	2
18	20*	2
19	20*	11
20	155	138
21	123	91
22	170	158
23	80	95
24	106	108

0.2.L

0	359	402
1	438	435
2	310	333
3	361	397
4	135	118
5	293	237
6	40	40
7	466	536
8	428	506
9	56	62
10	49	49
11	248	237
12	377	398
13	56	46
14	157	87
15	85	93
16	28*	30
17	248	268
18	295	249
19	253	298
20	198	166
21	85	57

0.3.L

1	20*	22
2	20*	16
3	20*	8
4	261	259
5	206	199
6	89	79
7	63	51
8	407	420
9	69	74
10	162	144
11	20*	10
12	135	105
13	230	216
14	179	158
15	20*	21
16	270	262
17	20*	26
18	155	139
19	20*	22
20	20*	19
21	20*	34
22	247	204
23	132	64

0.4.L

0	174	193
1	317	338
2	261	249
3	69	62
4	196	192
5	102	96
6	74	98
7	279	305
8	276	274
9	271	281
10	113	102
11	85	87
12	319	350
13	28*	22
14	89	109
15	56	72
16	89	73
17	20*	29
18	204	172
19	85	76
20	138	86
21	20*	12
22	116	117
23	20*	10
24	89	105
25	74	54

0.5.L

1	28*	35
2	167	164
3	20*	9
4	188	160
5	230	205
6	20*	37
7	120	98
8	190	195
9	20*	28
10	215	213
11	251	236
12	89	69
13	200	169
14	89	90
15	20*	19
16	149	133
17	20*	25
18	80	103
19	20*	9
20	98	68
21	85	54

0.6.L

0	361	357
1	49	54
2	20*	38
3	28*	15
4	89	104
5	126	123
6	237	252
7	126	119
8	20*	21
9	183	170
10	20*	13
11	94	74
12	85	54
13	20*	33
14	219	145
15	109	74

0.7.L

1	152	123
2	196	179
4	89	70
5	85	74

1.0.L

4	18*	21
5	143	150
6	362	352
7	44	39
8	426	410
9	478	449
10	362	325
11	141	117
12	254	238
13	63	52
14	96	101
15	73	55
16	184	173
17	258	271
18	112	90
19	180	195
20	81	97
21	25*	26
22	18*	32
23	123	118

1.1.L

0	139	115
2	305	343
3	254	237
4	199	203
5	567	606
6	212	195
7	426	464
8	244	240
9	129	116
10	77	78
11	139	137
12	167	140
13	318	299
14	184	157
15	25*	25
16	77	108
17	77	92
18	112	107
19	180	135
20	81	85
21	112	117
22	18*	38
23	73	91

1.2.L

0	554	665
1	224	225
2	399	372
3	519	555
4	283	282
5	352	331
6	388	386
7	263	273
8	152	148
9	455	441
10	148	120
11	178	193
12	93	81
13	215	216
14	163	133
15	199	215
16	77	52
17	225	214
18	18*	9
19	18*	14
20	112	120
21	134	137
22	103	102
23	96	101
24	63	17

1.3.L

0	196	179
1	18*	15
2	305	294
3	36	43
4	294	284
5	157	163
6	263	242
7	285	263
8	25*	34
9	157	160
10	25*	21
11	310	246
12	25*	34
13	322	318
14	109	118
15	136	117
16	81	80
17	81	84
18	18*	20
19	193	187
20	18*	39
21	126	108

1.4.L

0	106	129
1	150	161
2	89	77
3	63	54
4	143	139
5	260	275
6	163	155
7	193	161
8	171	155
9	269	239
10	129	132
11	186	196
12	134	141
13	112	106
14	81	92
15	227	239
16	31*	41
17	136	116
18	109	89
19	73	93
20	18*	37
21	112	140

1.5.L

0	73	56
1	270	236
2	103	73
3	25*	38
4	180	167
5	211	202
6	131	76
7	171	163
8	220	201
9	77	99
10	25*	51
11	196	178
12	25*	28
13	81	83
14	112	122
15	77	67

1.6.L

0	112	92
1	25*	50
2	81	75
3	226	247
4	139	117
5	112	94
6	180	178
7	115	97
8	139	118
9	112	92
10	81	80
11	77	90
12	134	130
13	77	25
14	18*	21
15	89	81
16	93	86

1.7.L

1	136	156
2	77	69
3	77	71
4	109	110
5	77	76
6	91	98
7	106	110
8	73	78
9	18*	13
10	25*	52
11	25*	38
12	93	98

1.8.L

0	115	123
1	18*	18
2	18*	31
3	112	106

2.0.L

1	137	147
2	178	196
3	261	259
4	243	235
5	234	243
6	390	376
7	381	329
8	294	280
9	169	154
10	122	110
11	17*	26
12	326	339
13	297	305
14	117	112
15	173	179
16	17*	19
17	75	73
18	75	83
19	167	159
20	75	67

2.1.L

0	219	208
1	122	93
2	281	254
3	206	186
4	342	353
5	119	111
7	222	196
8	329	331
9	129	130
10	313	297
11	230	228
12	61	76
14	96	89
15	175	154
16	156	156
17	25*	46
18	75	90
19	106	121
20	17*	24
21	50	73
22	82	96

2.2.L

0	370	342
1	185	166
2	284	257
3	43	59
4	137	142
5	285	264
6	389	412
7	164	167
8	134	137
9	25*	29
10	148	137
11	141	141
12	229	237
13	50	47
14	236	233
15	103	114
16	55	70
17	25*	32
18	75	79
19	50	50
20	100	119

2.3.L

0	158	152
1	145	136
2	207	198
3	289	266
4	214	206
5	148	145
6	141	130
7	167	136
8	137	117
9	156	144
10	256	229
11	96	85
12	173	149
13	70	76
14	127	109
15	25*	39
16	185	175
17	75	53
18	103	86
19	50	60
20	98	109
21	29*	46
22	95	76
23	58	64

2.4.L

0	246	218
1	175	135
2	152	137
3	274	258
4	180	163
5	195	158
6	317	303
7	117	93
8	119	113
9	25*	48
10	103	72
11	75	71
12	120	118
13	55	72
14	152	121
15	25*	49
16	17*	26
17	50	79
18	119	131
19	66	74
20	86	82

2.5.L

0	17*	1
1	218	192
2	225	217
3	100	84
4	176	173
5	125	96
6	164	151
7	25*	43
8	150	146
9	79	58
10	75	69
11	55	37
12	55	70
13	17*	32
14	90	84
15	17*	28

2.6.L

0	75	77
1	152	119
2	160	160
3	75	57
4	25*	45
5	106	90
6	120	114
7	50	49
8	106	116
9	25*	38
10	70	70
11	25*	39
12	96	107
13	114	133
14	17*	23
15	86	98

2.7.L

0	17*	10
1	100	85
2	100	87
3	55	62
4	122	118
5	70	57
6	90	68
7	25*	33
8	17*	34
9	90	85
10	61	79
11	55	83

2.8.L

0	96	116
1	43	48
2	55	72
3	35	39
4	25*	29
5	75	82
6	86	89
7	17*	19
8	17*	28

3.0.L

1	98	108
2	76	81
3	155	144
4	148	149
5	193	164
6	124	140
7	142	168
8	175	165
9	155	121
10	234	232
11	107	96
12	96	96
13	82	82
14	62	82
15	124	118
16	124	130
17	62	25
18	87	103
19	82	85
21	76	77
22	69	65
23	107	128

3.1.L

0	178	177
1	188	213
2	161	190
3	102	87
4	145	131
5	93	72
6	188	204
7	297	308
8	62	70
9	43	94
10	102	93
11	131	113
12	196	182
13	155	140
14	148	149
15	62	77
16	31*	51
17	145	97
18	87	88
19	145	142

3.2.L

0	261	263
1	53	55
2	212	227
3	277	249
4	111	120
5	138	123
6	93	81
7	21*	32
8	69	72
9	127	105
10	188	197
11	196	192
12	142	111
13	62	65
14	69	65
15	124	116
16	62	53
17	82	85

3.3.L

0	275	297
1	221	230
2	69	87
3	43	62
4	21*	35
5	203	194
6	224	252
7	53	36
8	21*	34
9	62	63
10	142	168
12	82	72
12	43*	54
13	31*	47
14	87	113
15	76	109

3.4.L

0	111	113
1	158	159
2	178	166
3	138	128
4	138	123
5	116	107
6	175	160
7	128	111
8	87	89
9	87	83
10	107	83
11	98	93
12	21*	14
13	82	25
14	87	94

3.5.L

0	69	55
1	62	57
2	124	146
3	152	145
4	31*	39
5	124	118
6	87	95
7	87	89

3.6.L

0	21*	10
1	124	107
2	87	92
3	62	46
4	76	65
5	76	63
6	69	75
7	69	61

equivalent β_{ij} values. Six further cycles of least-squares refinement with fixed layer scale factors gave convergence with all parameter shifts less than 0.01 of the corresponding standard deviation. A final check on the layer scale factors showed no significant changes. R was now 0.093 and refinement was judged to be complete. The final parameters together with their estimated standard deviations (σ) in Tables 5.1 and 5.2, and the observed hydrogen positions in Table 5.3, were used to calculate the final structure factors listed in Table 5.4.

5.4. Absolute Configuration.

The absolute configuration of the molecule was obtained by the method of internal comparison (See section 4.5), using the known chirality, (S), at the α-carbon C(2). The configuration at the sulphur atom was deduced to be (R).

A check on this assignment was made by use of Hamilton's method (See section 4.6), in which the significance of the discrepancy between the R-factors for the two absolute configurations is estimated. When the sign of the $\Delta f''$ component of the dispersion ratio for the sulphur atom was reversed, a final R of 0.097 was obtained, compared with 0.093 previously. The one-dimensional hypothesis that the absolute configuration where R = 0.097 is correct, was then tested. The number of reflections exceeds the number of variables by 384; this is the so-called number of degrees of freedom.

$$\mathcal{R} = 0.097/0.093 = 1.042$$

$$\mathcal{R}_{1,384,0.005} = 1.009$$

Thus, the hypothesis has less than 0.5% significance, and can be rejected. This result confirms the absolute configuration of L-methionine as well as that of the sulphoximine. A similar argument rejects the reversed assignment of N(1) and O(1): this also gave R = 0.097.

5.5. Description of the Molecule.

The [100] projection of the molecule (see Figure 5.1) displays the absolute stereo-chemistry, (2(S),S(R)). The bond lengths and angles are listed in tables 5.5 and 5.6, together with their standard deviations. No program was available for the application of libration corrections, but in view of the large standard deviations they are thought to be negligible.

The S-C(5) (methyl) bond length of 1.77(2)Å compares well with that in DL-methionine (1.77A)[17], (+)-S-methyl-L-cysteine sulphoxide (1.81Å)[18b], dimethyl sulphone di-imide (1.76Å)[19] and a copper-methionine complex (1.785Å)[20]. The S-C(4) (methylene) bond length of 1.81(2)Å is also quite normal, the corresponding one in the copper-methionine complex being 1.806Å. This length indicates virtually pure single-

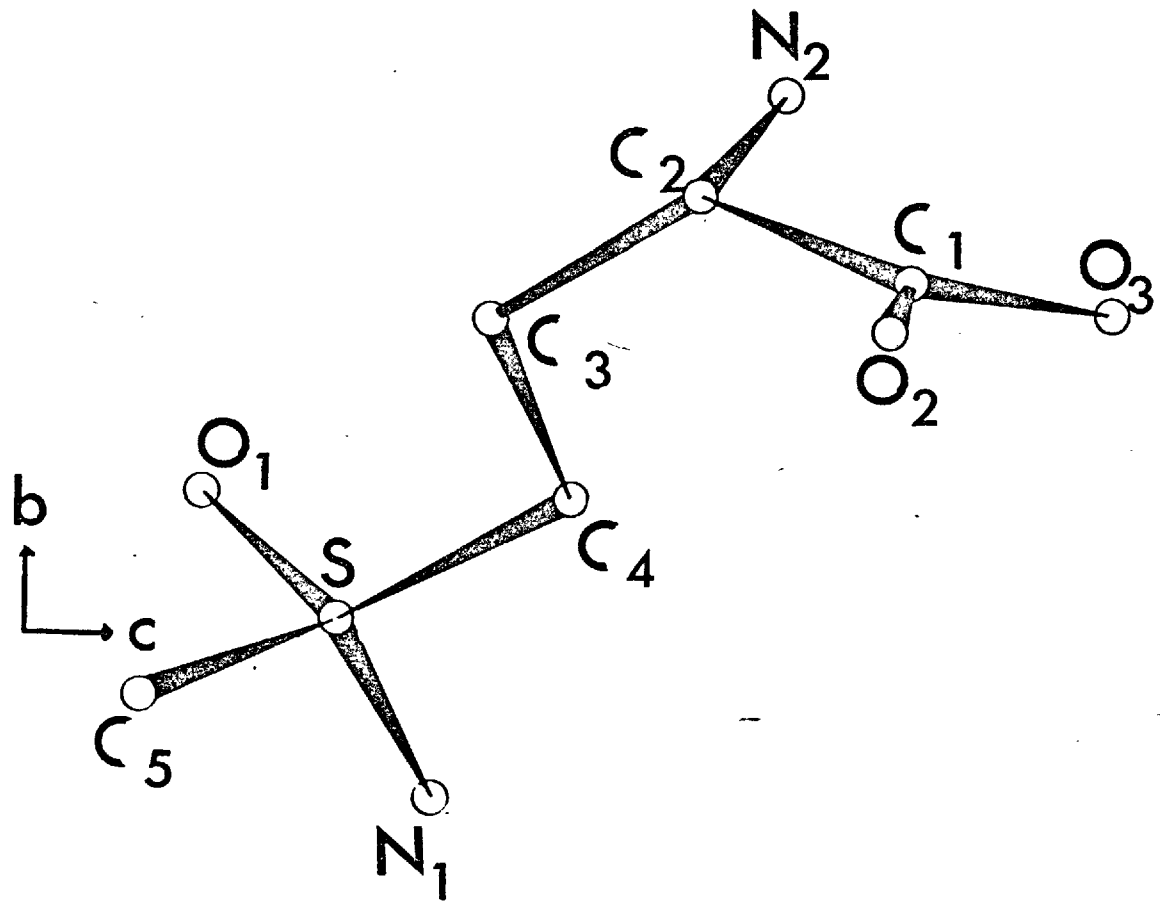


Figure 5.1. The [100] projection of one molecule of 2(S),S(R)-methionine sulphoximine.

Table 5.5.

Intramolecular bonded distances (r), with their estimated standard deviations (σ) in parentheses.

Atoms	r(Å)
S-O(1)	1.480(13)
S-C(5)	1.772(17)
S-N(1)	1.550(13)
S-C(4)	1.814(15)
C(4)-C(3)	1.495(22)
C(3)-C(2)	1.515(20)
C(2)-C(1)	1.477(21)
C(2)-N(2)	1.586(24)
C(1)-O(1)	1.263(28)
C(1)-O(3)	1.290(21)

Table 5.6.

Valence angles (θ) in the asymmetric unit, with their estimated standard deviations in parentheses.

Atoms	θ (°)
O(1)-S-C(5)	110.4(7)
O(1)-S-N(1)	119.4(8)
O(1)-S-C(4)	106.3(7)
C(5)-S-N(1)	103.5(7)
C(5)-S-C(4)	105.2(8)
N(1)-S-C(4)	111.2(6)
S-C(4)-C(3)	114.7(9)
C(4)-C(3)-C(2)	113.3(11)
C(3)-C(2)-C(1)	114.6(12)
C(3)-C(2)-N(2)	109.9(15)
C(1)-C(2)-N(2)	109.3(12)
C(2)-C(1)-O(2)	117.2(14)
C(2)-C(1)-O(3)	118.2(19)
O(2)-C(1)-O(3)	124.6(14)

bond character.

The S-O(1) distance of 1.48(1)Å agrees well with the value of 1.49Å in (+)-S-methyl-L-cysteine sulphoxide [18b], and moderately well with a number of other S-O double-bond lengths, within the limits of experimental error [21]. However, methanesulphonanilide has an average S-O length of 1.438(4)Å [22], and saccharin (o-sulphobenzoi-imide) a corresponding one of 1.420(4)Å [23]. This suggests that 2(S),S(R)-methionine sulphoximine has slightly incomplete $d\Pi - p\Pi$ overlap of the sulphur d orbitals and oxygen $p(sp^3)$ ones, compared to these two compounds. The S-N(1) bond is of some interest. The only other S-NH bond length reported, in dimethyl sulphone di-imide [19], has a length of 1.53(4)Å, compared with a single-bond value of 1.73(1)Å reported for $S_6(NH)_2$ [24], and an estimated double-bond value of c.a. 1.54Å [25]. The compound $NPCl_2(NSOCl)_2$ [26] has a system of N-S double bonds with lengths between 1.54 and 1.58(1)Å; the higher value is thought to indicate incomplete S-N Π-orbital overlap and partial delocalisation. Thus, S-N(1) can be considered as essentially a double bond. The largest deviation from a tetrahedral angle at sulphur is in the O(1)-S-N(1) angle (119°), and is probably a consequence of non-bonded repulsion between O(1) and N(1), especially because of their short bonds. A similar angular deviation occurs in

β-sulphanilamide [21] and in most molecules having non-equivalent tetrahedral substitution at sulphur [27].

The rest of the molecule shows a few departures from the expected geometry. Thus the angles at C(2),C(3) and C(4) are significantly enlarged; many other amino-acids with carbon-carbon chains show similar effects; in L-arginine an angle of 114.4° has been reported [28] for the corresponding C(2)-C(3)-C(4) angle. The C(2)-C(3) bond length is normal but C(4)-C(3) is slightly (0.045Å, 2σ) shorter than the normal C-C single-bond length. Similar deviations from the normal sp^3-sp^3 value of 1.54Å have often been noted in other amino-acids [17,18b]. This may be due to a modification of the normal configuration by the hydrogen bonding in the crystal structure; support for this comes from the angular deviations above. The bond lengths C(2)-C(1) [1.48(3)Å] and C(2)-N(2) [1.59(2)Å] are anomalous, and differ considerably from the values found for almost all other amino-acids; however, in α-methionine C(2)-C(1) has been found to be 1.47Å [17]. This is a significant agreement; in view of this and the normality of the remainder of the carboxy-group geometry, a systematic error in the data can be discounted. The C(2)-N(2) bond is c.a. 0.1Å (4σ) longer than expected. There are two possible explanations for this; either the three hydrogen bonds to this one nitrogen atom weaken the

bond appreciably, or there is a hyperconjugation effect involving C(1),C(2) and N(2), resulting in alternate bond shortening and lengthening. It is probable that the three strong hydrogen bonds around N(2) (Table 5.9) impose rather more formal positive charge on the atom than is usual, so that both the above factors may contribute. A bond length of 1.59Å has been reported for the C-N bond in choline chloride [29], and L-(+)-acetyl-β-methylcholine iodide has a C(2)-C(1) length of 1.43(5)Å and a C(2)-N(2) length of 1.59(4)Å [30]; both of these also have the nitrogen atom with some positive charge.

The dimensions of the carboxy-group show no significant differences from those of other amino-acids; they compare well with those found in the very accurate analysis of L-alanine [31] (Table 5.7). The carboxy-group is planar within the accuracy of the analysis. The amine-nitrogen atom lies -0.5Å out of this plane (Figure 5.2) differing from the planar configuration of almost all other amino-acids except DL-methionine [17] which has the nitrogen atom -0.7Å out of plane, and L-arginine, with a deviation of -0.28Å. It is felt that this is both a consequence of the increased length of the carbon chain forming the 'backbone' of the molecule, compared to the simpler amino-acids, and of the mode of packing and hydrogen bonding in the crystal lattice. Figure 5.3 shows the projection of the amino-acid

Table 5.7.

	This work.	L-alanine.[31]
C(1)-O(2) length (Å)	1.26	1.26
C(1)-O(3) length (Å)	1.29	1.25
C(2)-C(1)-O(2) angle (°)	117.2	116.1
C(2)-C(1)-O(3) angle (°)	118.2	118.3
O(2)-C(1)-O(3) angle (°)	124.6	125.6
Deviation (Å) of C(1) from best plane through C(2), C(1),O(2),O(3).	-0.015	-0.004
Dihedral angles (°)		
N(2)(C(2),C(1))O(2)	161.2	161.5
N(2)(C(2),C(1))O(3)	338.3	340.7

Equations of least-squares planes in direct space in the form $lx + my + nz = d$, for 2(S),S(R)-methionine sulphoximine.

Atoms in the plane.	l	m	n	d
C(2),C(1),O(2),O(3)	0.139	0.643	-0.758	0.421
C(1),C(2),N(2)	0.289	0.592	-0.325	0.570
S,N(1),C(4),C(3),C(2)	0.464	-0.352	0.548	-0.133

The distances of the relevant atoms from these planes are shown in Figures 5.2 - 5.4.

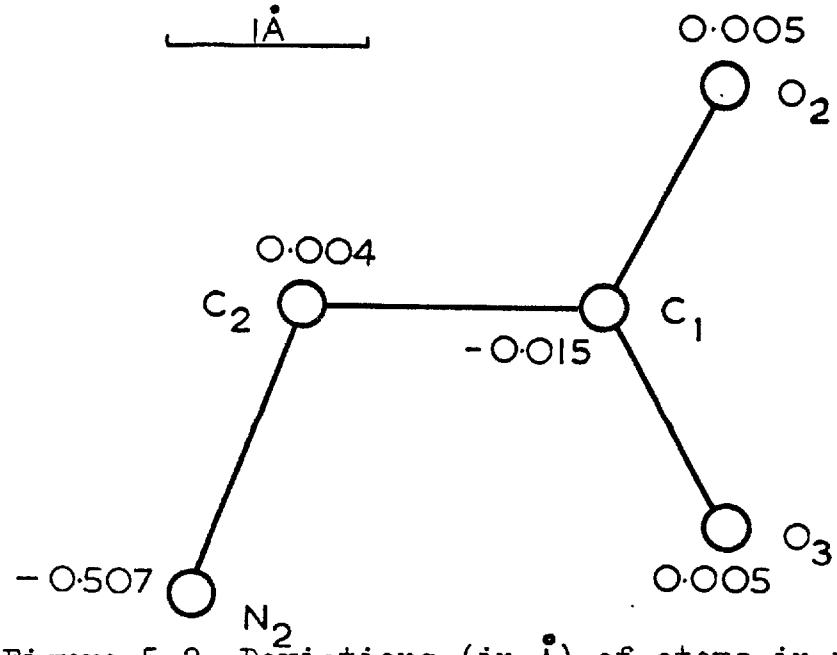
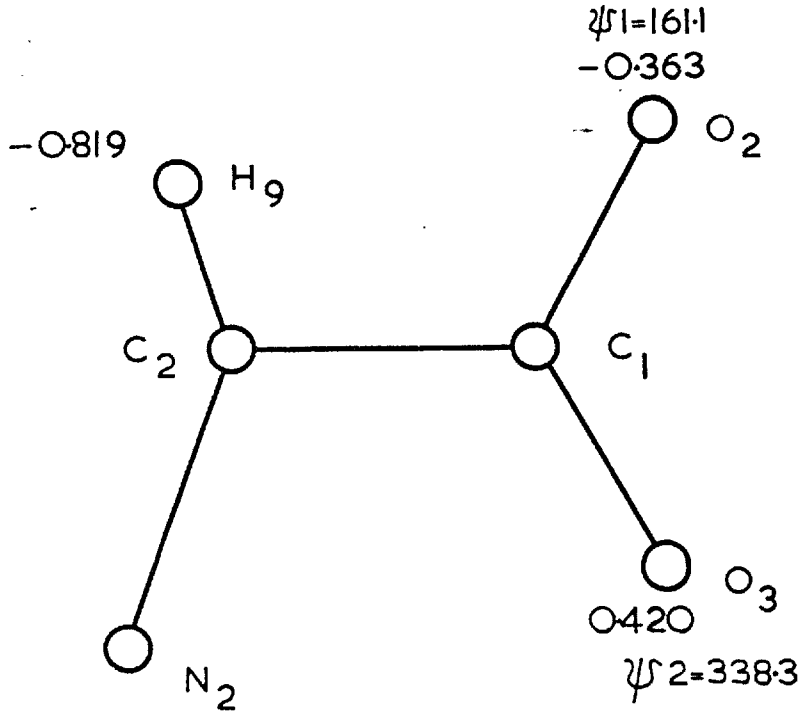


Figure 5.2. Deviations (in Å) of atoms in the amino-acid group from the best plane through atoms C(2),C(1), O(2) and O(3).

Figure 5.3. Deviations (in Å) of atoms in the amino-acid group from the plane defined by atoms N(2),C(2) and C(1).



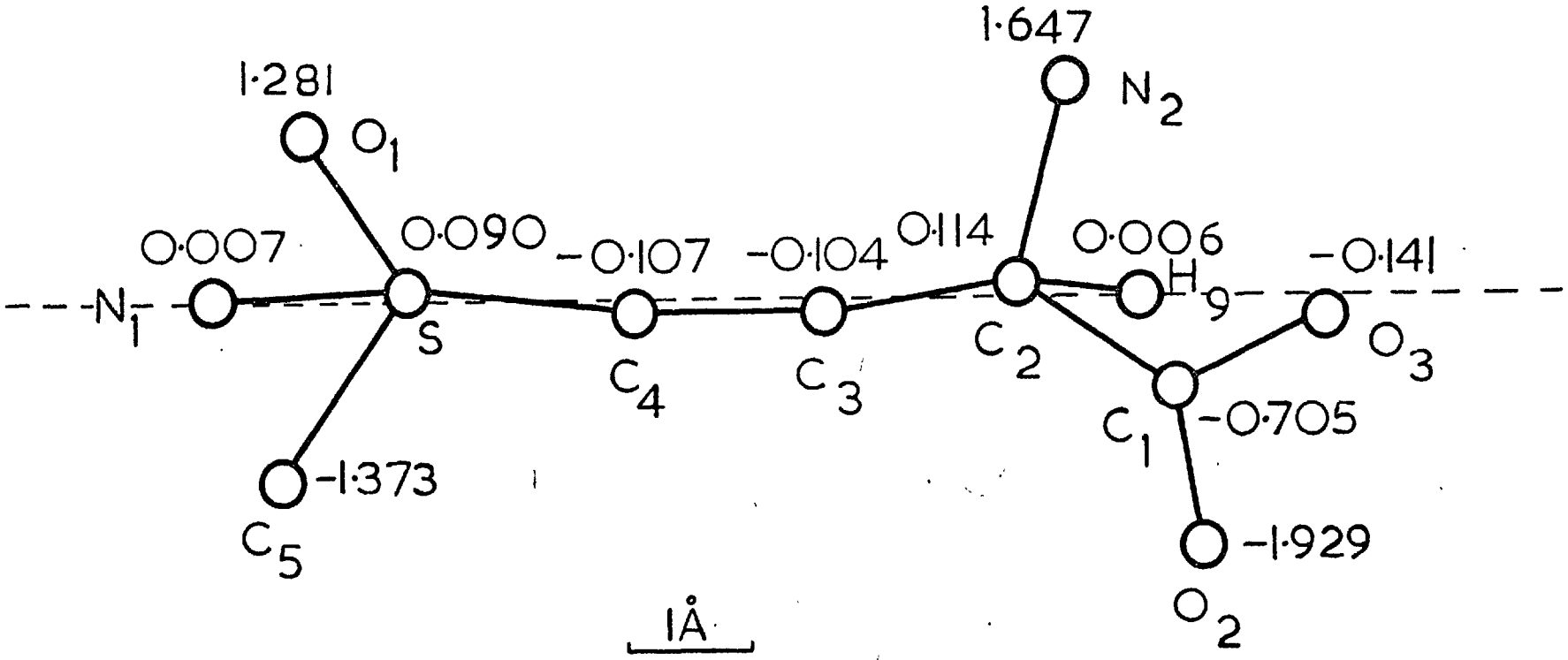


Figure 5.4. Deviations (in Å) of atoms from the best plane (shown in section as a broken line) through atoms N(1),S,C(4),C(3), and C(2).

Table 5.8.

Dihedral angles (ω) in the asymmetric unit. Format is atom 1 (atom 2, atom 3)atom 4. This is looking down the 2 ⟶ 3 bond and the angle quoted is that required to rotate the projection of bond 2 ⟶ 1 to coincide with that of bond 3 ⟶ 4. A positive sign denotes clockwise rotation.

Constituent atoms.	(°)	Notation used in ref.32.
O(1)(S,C(4))C(3)	51.3	
C(5)(S,C(4))C(3)	-65.9	
N(1)(S,C(4))C(3)	182.7	x_3
S(C(4),C(3))C(2)	195.9	x_2
C(4)(C(3),C(2))C(1)	-46.6	
C(4)(C(3),C(2))N(2)	77.0	x_1
C(3)(C(2),C(1))O(2)	-74.9	
C(3)(C(2),C(1))O(3)	102.2	
N(2)(C(2),C(1))O(2)	161.2	ψ_1
N(2)(C(2),C(1))O(3)	338.3	ψ_2

group on the reference plane N(2) C(2) C(1); the deviations from this plane of H(9),O(2), and O(3) are given, the last two also as the dihedral angles $\psi 1$ and $\psi 2$ (notation of Lakshminarayanan et al.[32]). Table 5.8 lists the dihedral angles for the molecule. They compare well with other reported values [32]. The dihedral angles are all close to multiples of 60°, except where the carboxy-group is involved.

It is possible to recognise a nearly planar zigzag chain of atoms in the molecule; it runs from N(1) via S,C(4),C(3), C(2) to H(9). Figure 5.4 shows the departure of these and other atoms from this plane. The presence of N(1) in this plane rather than the methyl-carbon or oxygen atoms is probably due to extension of the molecule by hydrogen bonding.

5.6. Hydrogen Bonding and Molecular Packing.

Figures 5.5 and 5.6 show the [100] and [001] projections of the crystal structure. The main chain C(1)-C(5) is curled, mainly it is believed, by the hydrogen bonding and packing. The molecules are stabilised in the lattice by hydrogen bonds to form infinite sheets parallel to (001) with two layers per cell; Table 5.9 gives the hydrogen-bonded contacts for one molecule. As expected, the amine-nitrogen atom uses its maximum number of contacts. The N(2)-O(2) and N(2)-N(1) hydrogen-bonded distances are normal [33], but the intramolecular N(2)-O(3) bond (2.68Å) is short for an amino-acid. The curling of the molecule in the crystal thus seems to be

Table 5.9.

Hydrogen-bonded contacts (Å).

N(2)-H.....O(3)	intra	2.68
N(2)-H.....O(2)^{I}	inter	2.86
N(1)-H.....O(3)^{II}	inter	3.01
N(1).....H-N(2)^{III}	inter	2.89

Roman numerals refer to equivalent positions relative to the reference molecule at x, y, z.

I $1 + x, y, z.$

II $-x, \frac{1}{2} + y, \frac{1}{2} - z.$

III $x, y - 1, z.$

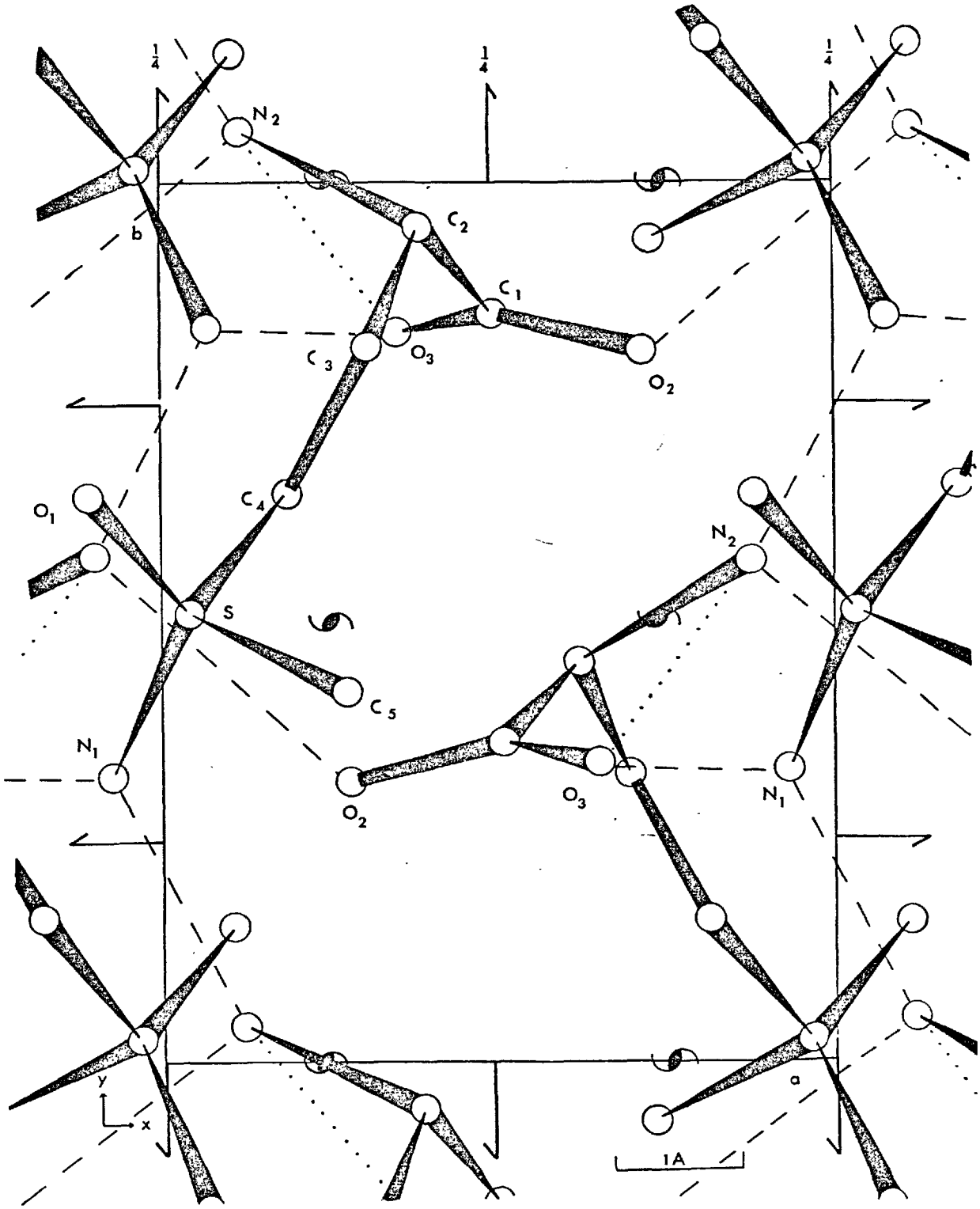


Figure 5.6. The [001] projection of the crystal structure showing a plan view of one of the sheets. ..... and - - - represent intra- and inter-molecular hydrogen bonds respectively.

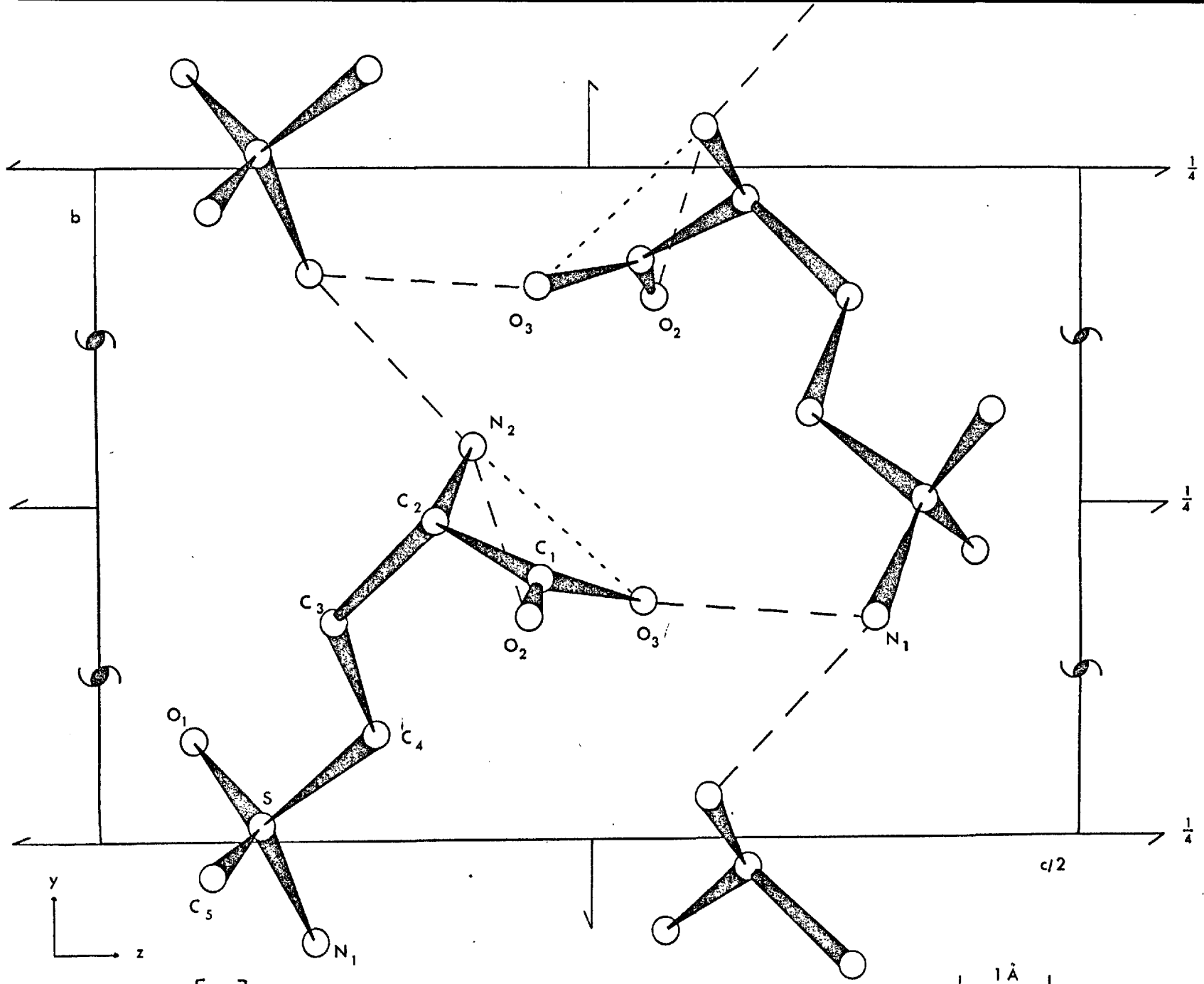


Figure 5.5. The [100] projection of the crystal structure of 2(S),S(R)-methionine sulphoximine, showing one of the two hydrogen-bonded sheets traversing the cell.

controlled by these hydrogen bonds, and especially by the participation of N(1).

In solution, however, the molecules seem to adopt an extended conformation. There are two lines of evidence supporting this view. First, the NMR spectra of the two isomers in solution are indistinguishable [34], which suggests that both molecules adopt similar conformations in which the chiral atoms S and C(2) are as far apart as possible. This could be achieved by a comparatively minor rotation around C(2)-C(3), which seems quite feasible once the constraints imposed in the crystal lattice are removed. Secondly, by comparison studies on numerous substrates and by molecular model considerations, Meister concluded [10,13] that the biologically active molecule must attach itself to the enzyme in a fully extended form with both nitrogen atoms on the same side of the molecular chain and with the methyl group directed away from the enzyme surface.

The irreversible inhibition of glutamine synthetase by 2(S),S(S)-methionine sulphoximine takes place in the presence of ATP and Mg^{2+} (or Mn^{2+}), and Meister considers that the molecule is phosphorylated, probably on the imino nitrogen atom [1]. This phosphate (as yet uncharacterised), acts as a bifunctional reagent [35] binding to both the glutamate and ammonia binding sites, (Figure 5.7). The 2(S),S(R) isomer cannot fulfil these rather precise steric requirements,

and hence is not an inhibitor [36].

One aspect of the inhibition which has so far received little attention concerns the (essential) role of the metal ion. It would perhaps be of some interest to compare the structures of the S(R) and S(S) phosphate-metal ion complexes by X-ray crystallography, assuming that they can be obtained in a suitable form.

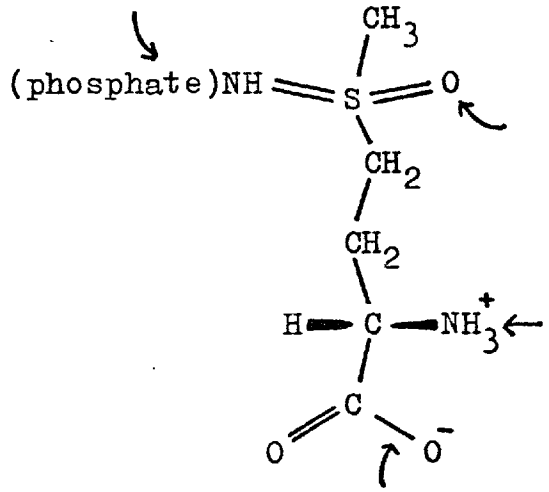


Figure 5.7. Schematic attachment of 2(S),S(S)-methionine sulphoximine phosphate to glutamine synthetase. The arrows indicate possible sites of binding.

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CHAPTER 6

The Crystal and Molecular Structure of 16-Hydroxyphenoxymethyl-3,4-dihydroisoquinoline Hydrochloride Monohydrate, a Topical Anti-viral Agent.

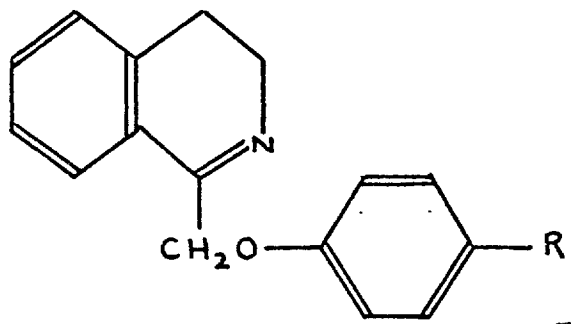
6.1. Introduction.

The influenza family of viruses, although not normally lethal to humans, are responsible for a tremendous amount of illness; there are annually several million victims of "flu" in this country alone. Recent years have seen a great deal of research in order to develop anti-viral vaccines and drugs, but without marked success.

The enzyme neuraminidase has been found to be present as a component of the surface protein in all strains of influenza viruses. The exact role of the enzyme has not as yet been elucidated, but it seems that it assists in the release of infectious virus from cells [1]-[3]. This step plays an important part in determining the replication of a virus population, and if it could be controlled, would affect the rapidity of spread of virus to adjacent tissue and so make it easier for the host defence mechanisms to attack the virus. Thus, it was thought that if a suitable neuraminidase inhibitor

i.e. a compound which combined with the enzyme, could be found, then it was at least possible that virus replication might be slowed down.

A search for suitable compounds by Tute et al., lead to the discovery that certain 1-phenoxymethyl-3,4-dihydro-isoquinolines, in the form of their hydrochloride salts (I), possessed anti-viral activity [4]. This activity is pronounced both in vitro and in vivo [5]; tests on humans have produced encouraging results. Suitable preparations are currently being considered for use in an aerosol spray form against influenza.



(I)

A series of studies [6,7] of structure-activity relationships has shown that R can be hydroxy-,methoxy-, or chloro-, with little difference in activity. The tetrahydroisoquinoline a strong base is quite inactive to the enzyme, and other findings have suggested that the nitrogen atom in the dihydro-isoquinoline I (which does inhibit the enzyme), should not be capable of ionisation. However, I is known to be capable of

forming well-defined salts, which would seem to be of the type $RN^{\oplus}\ X^{\ominus}$, which seems to contradict the seeming lack of basicity of the nitrogen. The tetrahydro compound would also have a different conformation since atom C(1) would be sp^3 hybridised, with a tetrahedral arrangement of substituents. Steric considerations have indicated that for maximum binding, the nitrogen and oxygen atoms should be cisoid, with the aromatic rings approximately coplanar; this conclusion has been supported by dipole moment studies, which indicate a value for the dihedral angle N(2)(C(1),C(11))O(12) of about 20 [8].

A crystal-structure analysis of one of the active compounds was thought to be worthwhile, in view of the questions regarding steric and electronic effects raised above. It would establish the stereo-chemistry of the series, and might also provide information as to the mechanism of neuraminidase inhibition. The 16-hydroxy compound, as its hydrochloride salt was chosen for this study. This particular derivative in fact, is one of the most active so far prepared. Crystals were supplied by Dr. M.S. Tute of the Pfizer Company (U.K.) Limited, whom we thank for his generous hospitality and useful discussions.

6.2. Experimental.

The crystals are yellowish, somewhat fibrous needles elongated along a. They show oblique extinction under the polarising microscope. Oscillation and Weissenberg photographs exhibited Laue symmetry 2/m. Cell parameters were obtained by a least-squares fit to 19 θ-values measured on the diffractometer.

Crystal Data:- $C_{16}H_{18}O_3NCl$, M = 307.5, monoclinic
a = 5.501(1), b = 25.500(3), c = 11.080(2)Å,
β = 100.41(1), U = 1528.8Å^3, D_m = 1.33(3) g.cm.$^{-3}$ (by flotation), D_c = 1.335 g.cm.$^{-3}$ for Z = 4 molecules/cell, μ = 22.9 cm.$^{-1}$ for Cu-K$_\alpha$ radiation (λ_{mean} = 1.54051Å), F(000) = 632.0. Space group $P2_1/c$ (No.14, C_{2h}^5) from systematic absences h0ℓ, 0k0 for ℓ,k $\neq$ 2n.

Intensity data were collected for a crystal of approximate dimensions 0.7 x 0.1 x 0.05 mm, mounted about the a-axis, using a Siemens automatic four-circle diffractometer, with Cu-K$_\alpha$ radiation set at a take-off angle of 45° , a nickel β filter, and a Na(Tl)I scintillation counter. The θ-2θ scan technique was employed throughout, with a 'five-value' measuring procedure. The maximum measuring time per step was selected to be 0.6 second. The 045 reflection was used as a reference every 20 reflections; there was no significant decline in the net count of this reflection over

the seven-day period of data collection, indicating very little decomposition or radiation damage. A check on the crystal setting was made by measurement of a set of 'control' reflections after every set of 250 five-value measurements. 1577 reflections were measured ($\theta \leq 50^{\circ}$) of which 274 were judged insignificant as the net count was 2.58σ (i.e. below the 99% confidence level). The latter were assigned a count equal to this value. The counts were adjusted to a common arbitrary scale using the reference reflection, and Lorentz and polarisation corrections were applied.

6.3. Structure Solution and Refinement.

Since the single chlorine atom is not very heavy relative to the rest of the scattering matter in the asymmetric unit ($\sum f_H^2 / \sum f_L^2 = 0.346$), it was decided to compute a sharpened, origin-removed Patterson map, which would permit it to be located rather more easily than with a normal Patterson. The position of the chlorine atom was deduced from the Harker sections at $(x, \frac{1}{2}, z)$ and $(0, y, \frac{1}{2})$ to be (-0.176, 0.092, 0.185). It was assigned a temperature factor of 3.0Å^2, and its positional and thermal parameters refined for three cycles of ORFLS least squares. R fell from 0.63 to 0.59. Both three-dimensional Fourier and difference Fourier maps were now computed using the structure-factors from the refinement, with reflections for which $|F_c| < 0.5|F_o|$ omitted from the summation. Both were characterised by

a surprisingly large number of peaks, many of which were later shown to be spurious; however, six of the highest peaks when displayed on a ball-and-spoke model, were recognised as a six-membered ring. A structure-factor calculation followed by three cycles of ORFLS, gave an R of 0.532, with these six peaks treated as carbon atoms and given initial temperature factors of $3.0 Å^2$. The difference map now computed with a rejection ratio of 0.3, revealed seven more chemically sensible peaks; these were the highest on the map. The model showed that they could be identified as the aromatic ring part of the isoquinoline system, a fragment of side-chain, and the water oxygen atom. Further structure-factor calculations treating all the identified atoms as either chlorine or carbon reduced R to 0.397. A third difference map revealed the remaining seven atoms; R then fell to 0.200. The oxygen and nitrogen atoms were now assigned their correct scattering factors and values appropriate to $Cl^{\ominus}$ were used for the chloride ion. After four cycles of ORFLS, R was now 0.181. A difference map computed at this stage showed no spurious features, but revealed the presence of considerable anisotropy throughout the structure. The temperature factors were converted to their anisotropic (β_{ij}) equivalents, and these together with the positional parameters and the single scale factor, were refined for ten cycles of

BLOKLS block-diagonal least-squares. R was now 0.106, and no parameter shift was greater than 0.1 of the corresponding standard deviation.

At this stage, an absorption correction was applied to the intensity data. Figure 6.1 shows the crystal dimensions and face indices. In fact, the crystal did not conform exactly to a rectangular block; there was a slight twist of about half a degree along the [100] axis. This would only very slightly affect the absorption correction of course, but was certainly a factor in the intensity data being only of moderate quality. A 24 x 8 x 8 grid of sampling points was found to be appropriate for calculating the absorption corrections. Six subsequent cycles of BLOKLS reduced R to 0.102. Thus,as expected, absorption was not very significant.

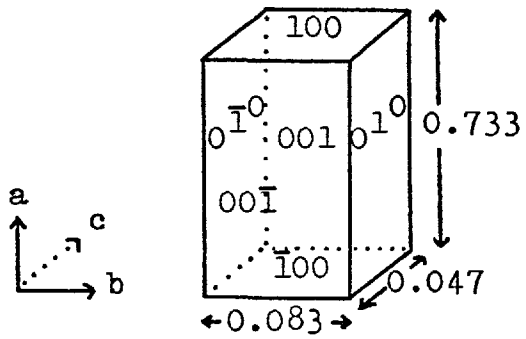


Figure 6.1.

Distances are in mm.

A difference map was now computed. This revealed the hydrogen atoms in their expected positions, at heights varying from 0.4e/Å^3 to 0.8e/Å^3; peaks corresponding to what were

thought to be the two hydrogens of the water molecule and the single hydrogen at the positive nitrogen atom were also seen. The hydrogens were assigned isotropic temperature factors of 0.5$\mathring{A}^2$ greater than the atoms to which they were bonded. Inclusion of the hydrogens as fixed-atom contributions in a structure-factor calculation, with the non-hydrogen parameters refined by five cycles of BLOKLS, reduced R to 0.088. The hydrogen positional and isotropic thermal parameters were now refined for five cycles of full-matrix least-squares; the non-hydrogen parameters were kept fixed. R was now 0.0825 and all but one of the hydrogens refined quite satisfactorily. The "amino hydrogen" had wandered very considerably in position until it was nearly on top of the nitrogen; its temperature factor had risen to about 25$\mathring{A}^2$. This indicated the absence of a hydrogen atom bonded to the nitrogen, the least-squares refinement having "blown up" for the suggested atom. A difference Fourier showed no peaks around the nitrogen; however, rather surprisingly, there was a distinct but rather diffuse peak of height 0.4e/$\mathring{A}^3$ near the water oxygen and in a position such as to suggest it was an $OH_3^{\oplus}$ ion. Three cycles of ORFLS with this new position included as a hydrogen atom reduced R to 0.0812. However, the final positions of the three hydrogens round this water oxygen were geometrically unacceptable, especially as regards their lengths. These four atoms were therefore refined

together, everything else being fixed; the hydrogens were given isotropic temperature factors and the oxygen anisotropic ones. R fell to 0.0792. The hydrogens appeared to have moved an average of 0.1Å from their positions indicated in the difference map, whereas the nine variables of the oxygen atom altered very little.

The geometry around this oxygen was still unacceptable and it was decided to omit the three hydrogens from any further structure-factor calculations. In the final stages of refinement the limited capacity of the full-matrix program made it necessary to refine only a part of the structure at a time; accordingly sixteen of the non-hydrogen atoms were refined by four cycles of ORFLS. The remaining five and fifteen hydrogens were treated as fixed-atom contributions. R was now 0.0781, and all parameter shifts were less than 0.005 of their corresponding standard deviations. The final five atoms, together with eleven of the earlier set, one of which was the chlorine atom, were refined for five more cycles of ORFLS. The parameters of these eleven atoms underwent negligible shifts, and all parameter shifts were less than 0.003 of their corresponding standard deviations. R was now 0.0777 and refinement was judged to be complete. The final parameters, together with their estimated standard deviations in Tables 6.1, 6.2 and 6.3, were used to calculate the final structure factors listed in Table 6.4.

Table 6.1.

Final positional parameters for the non-hydrogen atoms, as fractions of the unit-cell edges. Standard deviations are in parentheses.

Atom	x	y	z
C(1)	0.88813(116)	0.10883(24)	0.72610(61)
N(2)	0.94832(99)	0.08375(20)	0.82773(49)
C(3)	0.82152(148)	0.03620(30)	0.85621(67)
C(4)	0.55568(147)	0.03622(31)	0.80182(76)
C(5)	0.51452(128)	0.05575(25)	0.67011(62)
C(6)	0.30143(135)	0.04070(28)	0.58566(73)
C(7)	0.25818(144)	0.06426(32)	0.47089(73)
C(8)	0.40883(148)	0.10049(31)	0.43627(66)
C(9)	0.62206(135)	0.11507(29)	0.51916(65)
C(10)	0.66870(119)	0.09272(24)	0.63483(59)
C(11)	1.03597(119)	0.15505(25)	0.70020(58)
O(12)	1.24671(83)	0.15618(17)	0.79966(41)
C(13)	1.41233(122)	0.19606(25)	0.80192(58)
C(14)	1.39955(134)	0.23645(27)	0.72004(66)
C(15)	1.57780(140)	0.27536(26)	0.73349(70)
C(16)	1.77276(131)	0.27441(25)	0.83343(65)
C(17)	1.78691(138)	0.23299(28)	0.91565(67)
C(18)	1.60974(129)	0.19422(25)	0.90191(60)
O(19)	1.95279(93)	0.31140(18)	0.85326(47)
O(20)	1.26978(98)	0.09020(24)	1.04486(47)
$Cl^{\ominus}$	0.82341(33)	0.09147(7)	1.18248(17)

Table 6.2.

Final anisotropic thermal parameters ($\beta_{ij's}$) for the non-hydrogen atoms, x 10^5.

Atom	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(1)	3348(279)	94(11)	887(75)	-43(45)	-334(117)	-4(24)
N(2)	4064(249)	106(10)	788(60)	-103(41)	-502(98)	77(20)
C(3)	5543(395)	202(16)	940(81)	-256(65)	-569(143)	73(30)
C(4)	4887(386)	210(17)	1457(104)	-350(65)	-1026(160)	233(34)
C(5)	4109(323)	105(12)	955(79)	-17(53)	-759(134)	42(25)
C(6)	4159(350)	164(14)	1243(95)	-88(57)	-821(150)	-16(31)
C(7)	4324(351)	212(17)	1068(95)	-41(67)	-1109(149)	-52(32)
C(8)	4966(372)	204(16)	878(81)	-145(66)	-776(146)	31(30)
C(9)	4463(336)	198(15)	840(77)	-183(59)	-622(136)	99(28)
C(10)	3561(286)	88(11)	824(75)	-35(49)	-664(119)	3(23)
C(11)	3566(290)	123(12)	843(72)	-106(47)	-512(118)	17(23)
O(12)	4155(209)	134(8)	1002(52)	-243(36)	-1106(85)	123(17)
C(13)	3767(297)	117(13)	818(75)	-173(52)	-504(122)	29(25)
C(14)	4548(340)	146(14)	1071(84)	-200(59)	-957(133)	106(29)
C(15)	4990(356)	118(13)	1183(88)	-134(58)	-527(148)	102(27)
C(16)	4433(336)	107(13)	1017(82)	-168(54)	-236(137)	-48(27)
C(17)	4900(358)	153(14)	1027(83)	-183(61)	-878(136)	-40(30)
C(18)	4513(322)	125(13)	816(74)	-124(55)	-477(128)	60(24)
O(19)	5187(241)	133(9)	1402(63)	-325(41)	-613(97)	-12(19)
O(20)	5108(250)	351(14)	1020(58)	-148(50)	-738(96)	29(23)
$Cl^{\ominus}$	3688(76)	154(3)	1069(20)	93(13)	-530(31)	-14(7)

Estimated standard deviations are in parentheses.

Table 6.3.

Final positional and isotropic temperature factors for the hydrogen atoms. Standard deviations are in parentheses.

Atom	x	y	z	B(Å^2)
H(1)	0.153(13)	0.030(3)	0.636(6)	4.3(18)
H(2)	0.135(13)	0.062(3)	0.427(6)	4.7(19)
H(3)	0.442(16)	0.106(3)	0.350(8)	7.9(26)
H(4)	0.736(11)	0.135(2)	0.479(6)	2.8(15)
H(5)	1.275(14)	0.240(3)	0.655(7)	6.2(22)
H(6)	1.562(12)	0.303(3)	0.683(6)	3.6(17)
H(7)	1.951(18)	0.227(4)	0.946(9)	9.2(29)
H(8)	1.613(11)	0.164(2)	0.957(6)	3.0(15)
H(9)	0.924(12)	0.019(3)	0.804(6)	3.6(17)
H(10)	0.815(13)	0.038(3)	0.937(7)	4.8(19)
H(11)	0.472(18)	0.076(4)	0.859(8)	8.2(26)
H(12)	0.478(11)	0.002(2)	0.807(5)	2.3(14)
H(13)	0.900(11)	0.189(2)	0.720(6)	2.7(15)
H(14)	1.142(12)	0.151(3)	0.632(6)	3.7(17)
H(15)	1.905(12)	0.335(3)	0.806(6)	3.7(17)
H(16)	1.464(18)	0.092(4)	1.038(8)	7.0(26)
H(17)	1.099(12)	0.094(3)	1.131(6)	2.8(15)
H(18)	1.249(20)	0.112(3)	1.058(10)	0.3(28)

H(16)-H(18) were not included in the final structure-factor calculation.

Table 6.4.

Final observed and calculated structure factors; column headings are marked h, k, ℓ, followed by ℓ, $10|F_o|$, $10|F_c|$. The accidental absences are marked (*); these are not significantly greater than the background level.

0.0.L

2	1218	1156
4	143	138
6	479	446
8	411	385
10	114	65

0.1.L

0	115	A2
2	468	456
3	726	682
4	172	162
5	347	370
6	252	212
7	112	100
8	32	14
9	138	120
10	127	105

0.2.L

0	118	116
1	686	611
2	484	469
3	391	382
4	134	109
5	151	138
6	246	244
7	212	187
8	80	75
9	50	42
10	84	69

0.3.L

1	36	30
2	942	940
3	216	192
4	146	126
5	136	107
6	39	33
7	220	198
8	87	73
9	28*	11
10	87	89

0.4.L

0	686	645
1	80	67
2	145	149
3	42	54
4	199	211
5	561	537
6	264	268
7	218	181
8	166	145
9	236	210
10	27*	14

0.5.L

1	128	122
2	41	45
3	511	488
4	212	176
5	295	231
6	45	55
7	128	128
8	97	97
9	267	237
10	25	8

0.6.L

0	1480	1401
1	392	367
2	118	119
3	442	419
4	54	41
5	78	48
6	144	144
7	43	31
8	145	133
9	204	204
10	99	99

0.7.L

1	236	193
2	747	743
3	89	98
4	671	671
5	97	100
6	253	253
7	133	132
8	45	38
9	27*	22
10	152	134

0.8.L

0	537	526
1	1044	990
2	48	57
3	66	68
4	420	376
5	34	6
6	258	225
7	375	348
8	64	66
9	142	137
10	27*	19

0.9.L

1	162	138
2	31	31

3	22*	22
4	192	178
5	78	68
6	95	98
7	42	36
8	92	81
9	74	76
10	28*	16

0.10.L

0	574	515
1	185	174
2	21*	22
3	237	235
4	150	116
5	196	157
6	123	108
7	61	56
8	56	42
9	27*	16

0.11.L

1	321	287
2	131	125
3	292	266
4	144	135
5	372	356
6	101	90
7	86	66
8	41	38
9	144	141

0.12.L

0	363	325
1	213	203
2	259	232
3	82.	82
4	25*	0
5	111	105
6	171	175
7	167	164
8	173	160
9	150	142

0.13.L

1	255	226
2	177	174
3	24*	6
4	133	113
5	57	56
6	120	116
7	42	37
8	29*	15
9	28*	34

0.14.L

0	399	354
1	112	113
2	46	33
3	24*	15
4	121	115
5	27*	40
6	39	42
7	147	133
8	37	41
9	63	67

0.15.L

1	122	111
2	143	136
3	57	73
4	26*	26
5	129	136
6	59	56
7	58	60
8	28*	13

0.16.L

0	618	560
1	95	66
2	342	332
3	28*	26
4	27*	22
5	74	71
6	133	106
7	29*	8
8	174	163

0.17.L

1	311	276
2	222	221
3	258	220
4	87	87
5	306	300
6	29*	16
7	29*	15
8	48	43

0.18.L

0	101	125
1	128	104
2	61	60
3	26*	4
4	236	198
5	92	95
6	86	72
7	36	36

0.19.L

1	148	126

2	75	84
3	28*	32
4	28*	19
5	35	33
6	27*	1
7	62	51

0.20.L

0	307	241
1	85	81
2	88	80
3	152	146
4	59	55
5	46	48
6	28*	29

0.21.L

1	50	62
2	47	32
3	28*	7
4	108	94
5	95	97
6	49	52

0.22.L

0	269	252
1	113	95
2	84	84
3	109	114
4	180	185
5	63	62

0.23.L

1	62	60
2	72	72
3	93	92
4	195	193

0.24.L

0	120	114
1	312	303
2	29*	29
3	121	122

0.25.L

1	104	95

1.0.L

-10	31	45
-8	38	36
-6	25*	41
-4	309	321
-2	141	124
0	261	252
2	517	524
4	923	883
6	577	561
8	28*	21
10	29*	7

1.1.L

-10	48	41
-9	62	73
-8	71	81
-7	92	93
-6	212	225
-5	231	263
-4	241	275
-3	316	335
-2	241	234
-1	251	235
0	460	515
1	525	473
2	44	61
3	594	587
4	356	341
5	253	247
6	25*	19
7	162	158
8	130	183
9	404	399
10	47	42

1.2.L

-10	45	63
-9	57	75
-8	27*	8
-7	39	31
-6	71	77
-5	294	327
-4	203	224
-3	96	91
-2	496	536
-1	173	220
0	40	50
1	113	123
2	23*	25
3	405	381
4	132	150
5	195	193
6	120	108
7	96	93
8	33	49
9	98	108
10	27*	1

1.3.L

-10	69	73
-9	27*	3
-8	103	117

-7	120	152
-6	475	514
-5	147	143
-4	176	192
-3	835	892
-2	792	797
-1	780	832
0	25	26
1	277	252
2	959	960
3	23	46
4	85	91
5	256	253
6	188	184
7	204	204
8	157	152
9	42	49
10	39	41

1.4.L

-10	94	98
-9	115	127
-8	47	42
-7	26*	24
-6	69	66
-5	348	389
-4	496	547
-3	779	845
-2	1563	1694
-1	286	334
0	480	531
1	322	319
2	58	61
3	315	325
4	222	205
5	383	354
6	116	132
7	73	73
8	137	143
9	69	67
10	32	28

1.5.L

-10	28*	30
-9	58	68
-8	40	43
-7	64	66
-6	46	45
-5	371	420
-4	28	32
-3	414	459
-2	871	947
-1	856	899
0	328	352
1	497	470
2	246	246
3	802	760
4	380	367
5	46	41
6	236	239
7	27*	8
8	164	170
9	249	253
10	28*	28

1.6.L

-10	50	47
-9	28*	5
-8	120	134
-7	35	26
-6	130	130
-5	170	177
-4	151	137
-3	185	183
-2	232	270
-1	988	1071
0	156	190
1	22*	28
2	161	130
3	164	169
4	214	221
5	25	14
6	25*	10
7	109	113
8	28*	13
9	231	229

1.7.L

-10	30*	32
-9	28*	23
-8	27*	1
-7	71	74
-6	39	42
-5	317	350
-4	50	21
-3	69	57
-2	199	201
-1	22*	25
0	510	539
1	195	179
2	438	428
3	650	625
4	23*	20
5	25*	26
6	254	262
7	27*	10
8	188	180
9	108	122

1.8.L

-10	28*	22
-9	38	48
-8	31	30
-7	131	114
-6	25*	14

-5	41	35
-4	99	111
-3	284	280
-2	73*	41
-1	148	131
0	47	50
1	158	184
2	54	50
3	264	250
4	24*	45
5	477	462
6	148	147
7	229	242
8	79	79
9	29*	7

1.9.L

-10	56	68
-9	36	38
-8	122	147
-7	76	84
-6	175	183
-5	201	207
-4	32	44
-3	144	173
-2	464	466
-1	259	256
0	333	336
1	235	207
2	490	488
3	183	191
4	323	287
5	137	129
6	27*	16
7	65	82
8	42	53
9	29*	29

1.10.L

-10	27*	7
-9	27*	15
-8	28*	8
-7	29	16
-6	141	145
-5	110	112
-4	173	193
-3	64	69
-2	615	646
-1	70	113
0	135	127
1	189	197
2	31	14
3	24*	8
4	298	263
5	272	251
6	162	149
7	145	152
8	52	45
9	85	86

1.11.L

-9	28*	22
-8	33	43
-7	128	131
-6	137	156
-5	299	289
-4	29	48
-3	23*	29
-2	33	15
-1	40	30
0	454	453
1	73	47
2	300	262
3	173	204
4	277	247
5	68	55
6	27*	12
7	87	102
8	113	114
9	136	153

1.12.L

-9	79	100
-8	103	125
-7	27*	9
-6	156	177
-5	24*	24
-4	195	221
-3	234	246
-2	513	540
-1	98	102
0	466	418
1	325	312
2	82	127
3	272	291
4	243	231
5	156	152
6	139	129
7	29*	0
8	141	149

1.13.L

-9	28*	15
-8	32	39
-7	79	97
-6	213	242
-5	116	114
-4	104	121
-3	44	34
-2	118	115
-1	279	286
0	321	264
1	494	458
2	223	247
3	620	591

4	27*	29
5	26*	9
6	55	49
7	30*	39
8	28*	6

1.14.L

-9	27*	3
-8	34	32
-7	97	123
-6	28*	29
-5	162	181
-4	37	46
-3	236	244
-2	34	24
-1	203	223
0	24*	22
1	259	232
2	195	201
3	51	71
4	102	86
5	138	130
6	26*	20
7	53	24
8	92	91

1.15.L

-8	28*	14
-7	50	71
-6	73	75
-5	28*	19
-4	111	130
-3	26*	11
-2	25*	29
-1	71	57
0	86	74
1	361	333
2	140	163
3	235	233
4	34	40
5	109	116
6	81	67
7	110	116
8	102	112

1.16.L

-8	29*	34
-7	30*	29
-6	99	117
-5	156	177
-4	191	203
-3	50	43
-2	214	215
-1	46	42
0	153	148
1	40	34
2	26*	1
3	66	62
4	238	234
5	26*	21
6	123	134
7	169	166

1.17.L

-8	34	41
-7	29*	31
-6	124	134
-5	77	75
-4	42	52
-3	27*	3
-2	42	24
-1	129	109
0	45	74
1	122	123
2	151	173
3	151	169
4	124	131
5	29*	17
6	41	35
7	146	153

1.18.L

-7	49	56
-6	121	154
-5	28*	30
-4	52	56
-3	100	102
-2	44	43
-1	27*	6
0	131	133
1	34	40
2	27*	21
3	174	194
4	166	169
5	64	63
6	124	136
7	41	31

1.19.L

-7	49	52
-6	220	250
-5	45	49
-4	184	197
-3	27*	5
-2	28*	31
-1	27*	29
0	72	69
1	67	73
2	131	151
3	51	48
4	29*	21
5	78	88
6	27*	7

1,20,L

-6	[illegible]	72
-5	[illegible]	101
-4	[illegible]	[illegible]
-3	[illegible]	265
-2	[illegible]	219
-1	[illegible]	[illegible]
0	[illegible]	[illegible]
1	[illegible]	92
2	[illegible]	[illegible]
3	[illegible]	133
4	[illegible]	70
5	[illegible]	101

1,21,L

-6	[illegible]	62
-5	29*	15
-4	27*	3
-3	[illegible]	[illegible]
-2	[illegible]	245
-1	[illegible]	97
0	[illegible]	265
1	[illegible]	90
2	37	31
3	[illegible]	148
4	69	68
5	[illegible]	27

1,22,L

-5	[illegible]	135
-4	21	20
-3	71	59
-2	74	74
-1	151	157
0	29*	9
1	118	121
2	28*	18
3	79	70
4	134	134

1,23,L

-4	28*	10
-3	159	193
-2	135	134
-1	43	38
0	148	168
1	130	131
2	45	54
3	102	111

1,24,L

-3	55	66
-2	68	66
-1	28*	29
0	29*	37
1	46	55
2	27*	3

2,0,L

-10	48	45
-8	270	189
-6	173	176
-4	62	71
-2	305	256
0	364	320
2	619	596
4	75	75
6	142	159
8	119	108

2,1,L

-10	28*	14
-9	84	82
-8	272	211
-7	90	97
-6	157	179
-5	69	74
-4	119	83
-3	94	90
-2	378	396
-1	310	321
0	465	432
1	125	98
2	267	247
3	346	334
4	101	82
5	276	217
6	27*	24
7	221	248
8	60	56
9	37	48

2,2,L

-10	46	44
-9	35	19
-8	27	26
-7	133	131
-6	75	71
-5	218	222
-4	77	72
-3	150	152
-2	152	158
-1	[illegible]	[illegible]
0	[illegible]	[illegible]
1	[illegible]	453
2	77	50
3	[illegible]	317
4	[illegible]	495
5	[illegible]	317
6	[illegible]	178
7	[illegible]	430
8	[illegible]	106
9	112	131

2,3,L

-10	67	73
-9	44	35
-8	136	134
-7	95	91
-6	133	123
-5	24*	28
-4	57	63
-3	187	179
-2	576	627
-1	313	330
0	334	354
1	151	145
2	267	260
3	583	527
4	68	67
5	270	272
6	141	128
7	174	166
8	166	183
9	27*	7

2,4,L

-10	27*	20
-9	36	38
-8	47	40
-7	88	88
-6	89	84
-5	95	105
-4	22*	14
-3	275	281
-2	105	131
-1	122	147
0	137	171
1	776	766
2	195	183
3	462	427
4	409	379
5	77	20
6	61	50
7	411	438
8	27*	37
9	50	57

2,5,L

-10	31	27
-9	71	73
-8	43	38
-7	46	35
-6	93	101
-5	158	178
-4	109	111
-3	61	16
-2	54	41
-1	361	422
0	302	336
1	202	194
2	135	112
3	27	10
4	231	223
5	151	125
6	210	202
7	341	365
8	165	175
9	27*	18

2,6,L

-10	25*	13
-9	25*	0
-8	58	66
-7	126	105
-6	229	216
-5	23*	5
-4	375	376
-3	208	205
-2	188	190
-1	177	204
0	369	427
1	163	166
2	52	85
3	111	105
4	269	281
5	83	85
6	65	101
7	90	89
8	27*	30
9	55	57

2,7,L

-10	52	58
-9	37	37
-8	237	234
-7	56	50
-6	148	151
-5	243	237
-4	200	193
-3	246	255
-2	358	365
-1	75	63
0	291	305
1	21*	1
2	135	124
3	69	54
4	48	45
5	25*	15
6	[illegible]	75
7	20*	19
8	46	56

2,8,L

-10	28	13
-9	39	30
-8	35	32
-7	120	122
-6	108	105
-5	205	205
-4	410	412
-3	149	171
-2	198	180
-1	137	144
0	211	198
1	499	490
2	177	177
3	273	172
4	62	60
5	68	53
6	54	60
7	273	284
8	38	38

2,9,L

-10	56	58
-9	26*	5
-8	208	211
-7	26*	31
-6	97	101
-5	197	197
-4	359	364
-3	565	561
-2	648	718
-1	68	65
0	263	288
1	212	215
2	246	271
3	92	82
4	172	162
5	46	59
6	321	331
7	167	175
8	145	152

2,10,L

-9	27*	20
-8	83	90
-7	138	143
-6	107	116
-5	95	93
-4	63	65
-3	281	284
-2	52	45
-1	119	121
0	68	89
1	177	109
2	111	95
3	155	137
4	169	166
5	252	283
6	89	84
7	69	68
8	33	41

2,11,L

-9	47	63
-8	26*	13
-7	33	26
-6	107	114
-5	68	57
-4	55	66
-3	126	116
-2	347	360
-1	462	499
0	67	55
1	240	203
2	132	117
3	152	152
4	111	126
5	120	117
6	146	156
7	27*	24
8	106	110

2,12,L

-9	38	36
-8	26*	4
-7	132	106
-6	139	149
-5	26*	37
-4	57	59
-3	23*	11
-2	72	78
-1	23*	0
0	239	215
1	43	28
2	121	114
3	189	159
4	164	169
5	326	345
6	40	41
7	26*	7

2,13,L

-9	32	30
-8	174	174
-7	139	133
-6	28	37
-5	27	26
-4	135	[illegible]
-3	[illegible]	[illegible]
-2	413	411
-1	39	45
0	320	315
1	23*	8
2	128	117
3	45	52
4	98	87
5	139	155
6	159	148
7	262	[illegible]

2,14,L

-8	77	45
-7	31	42
-6	71	70
-5	50	51
-4	179	176
-3	187	174
-2	25*	18
-1	64	43
0	24*	8
1	248	245
2	49	21
3	238	233
4	59	62
5	137	141
6	133	135
7	134	154

2,15,L

-8	149	143
-7	58	59
-6	112	127
-5	27*	9
-4	28	30
-3	26*	15
-2	63	61
-1	170	173
0	25*	22
1	59	52
2	113	100
3	54	50
4	30	14
5	172	195
6	111	114
7	260	279

2,16,L

-8	28	25
-7	87	96
-6	209	220
-5	110	107
-4	250	251
-3	249	248
-2	101	101
-1	25*	7
0	315	299
1	118	106
2	176	149
3	98	94
4	27*	13
5	197	198
6	27*	13

2,17,L

-7	27*	37
-6	48	40
-5	123	136
-4	216	230
-3	88	91
-2	202	196
-1	215	195
0	25	27
1	153	153
2	51	50
3	89	93
4	142	154
5	79	95
6	95	97

2,18,L

-7	25*	3
-6	26*	1
-5	122	130
-4	108	128
-3	182	190
-2	26*	1
-1	26*	27
0	27*	17
1	86	83
2	40	49
3	265	265
4	32	30
5	76	88

2,19,L

-6	26*	17
-5	100	113
-4	155	154
-3	89	98
-2	320	322
-1	32	36
0	183	176
1	28	15
2	169	176
3	116	122
4	42	37
5	112	117

2,20,L

-6	35	21
-5	[illegible]	[illegible]
-4	33	31
-3	25*	[illegible]
-2	[illegible]	17
-1	44	24
0	27*	13
1	70	54
2	75	72
3	252	252
4	57	59

2,21,L

-5	50	52
-4	26*	20
-3	25*	3
-2	50	53
-1	152	159
0	93	72
1	30	22
2	140	149
3	[illegible]	136

2,22,L

-4	93	87
-3	26*	14
-2	25*	14
-1	37	33
0	82	84
1	45	49
2	27*	7

2,23,L

-2	26*	11
-1	80	61
0	56	78
1	121	129

3,0,L

-10	26*	13
-8	101	97
-6	155	159
-4	251	222
-2	366	340
0	115	129
2	266	248
4	326	347
6	136	153

3,1,L

-10	26*	14
-9	81	33
-8	54	35
-7	25*	1
-6	25*	28
-5	110	112
-4	267	173
-3	152	133
-2	32	45
-1	22*	2
0	212	192
1	490	441
2	98	92
3	330	312
4	79	94
5	40	37
6	29	25
7	26*	29

3,2,L

-10	44	27
-9	26*	16
-8	53	52
-7	175	170
-6	57	54
-5	111	69
-3	29	13
-4	84	90
-2	172	169
-1	129	154
0	205	192
1	365	324
2	240	224
3	104	98
4	139	131
5	90	106
6	172	194
7	26*	6

3,3,L

-10	111	83
-9	27*	35
-8	55	53
-7	26*	22
-6	58	62
-5	37	43
-4	416	393
-3	116	116
-2	120	119
-1	103	92
0	346	361
1	42	52
2	153	136
3	41	19
4	176	174
5	146	153
6	224	245
7	26*	24

3,4,L

-9	25*	22
-8	36	46
-7	27*	27
-6	45	43
-5	77	67
-4	231	213
-3	72	29
-2	214	233
-1	135	140
0	53	43
1	115	91
2	53	38
3	38	39
4	151	151
5	165	160
6	71	67
7	61	72

3,5,L

-9	26*	[illegible]
-8	32	27
-7	26*	[illegible]
-6	77	72
-5	64	104
-4	140	185
-3	50	53
-2	141	134
-1	142	164
0	50	55
1	340	330
2	48	30
3	294	273
4	112	123
5	70	75
6	192	173
7	59	62

3,6,L

-9	63	59
-8	144	128
-7	91	97
-6	115	112
-5	90	94
-4	35	35
-3	23*	12
-2	23*	5
-1	73	82
0	294	272
1	284	286
2	426	393
3	25*	14
4	92	95
5	196	275
6	25*	27
7	109	121

3,7,L

-9	52	36
-8	73	53
-7	26*	[illegible]
-6	86	87
-5	127	136
-4	316	390
-3	124	132
-2	49	53
-1	96	155
0	166	190
1	30	24
2	131	116
3	27*	25
4	41	39
5	257	235
6	157	160
7	26*	0

3,8,L

-9	56	57
-8	58	48
-7	64	86
-6	50	56
-5	125	190
-4	25*	2
-3	114	124
-2	167	174
-1	192	297
0	36	27
1	371	393
2	130	123
3	25*	25
4	151	151
5	399	422
6	74	69
7	210	233

3,9,L

-9	90	63
-8	43	32
-7	27*	5
-6	75	80
-5	61	65
-4	137	147
-3	98	85
-2	193	197
-1	97	94
0	190	96
1	45	47
2	425	416
3	27*	30
4	316	311
5	27*	1
6	94	92
7	103	124

3,10,L

-9	44	29
-8	119	114
-7	27*	24
-6	209	194
-5	26*	5
-4	45	51
-3	114	[illegible]
-2	93	121
-1	123	123
0	24*	5
1	146	153
2	170	169
3	72	82

3,10,L

4	206	211
5	165	177
6	287	297

3,11,L

-8	121	101
-7	118	100
-6	91	100
-5	67	74
-4	31	44
-3	161	163
-2	25*	37
-1	24*	12
0	27	32
1	81	91
2	26*	0
3	302	295
4	27*	29
5	225	230
6	65	64

3,12,L

-8	54	63
-7	27*	14
-6	49	53
-5	149	128
-4	170	176
-3	156	140
-2	120	120
-1	25*	16
0	36	28
1	81	93
2	154	152
3	315	313
4	193	191
5	263	278
6	98	99

3,13,L

-8	25*	8
-7	159	150
-6	27*	3
-5	211	200
-4	64	58
-3	26*	2
-2	82	87
-1	61	54
0	25*	15
1	45	52
2	28*	28
3	89	95
4	112	119
5	179	183
6	133	139

3,14,L

-7	152	147
-6	97	102
-5	338	330
-4	61	60
-3	27*	35
-2	60	65
-1	136	146
0	33	36
1	449	454
2	150	145
3	248	247
4	45	52
5	30	15

3,15,L

-7	92	88
-6	83	78
-5	75	84
-4	90	102
-3	122	119
-2	40	53
-1	55	57
0	70	61
1	227	228
2	31	22
3	206	207
4	26*	2
5	28	26

3,16,L

-7	26*	6
-6	26*	12
-5	28	18
-4	123	132
-3	83	60
-2	78	67
-1	60	54
0	136	126
1	160	160
2	82	81
3	28*	11
4	186	190

3,17,L

-6	60	56
-5	77	72
-4	116	107
-3	180	185
-2	25*	1
-1	25*	35
0	25*	10
1	181	181
2	206	207
3	240	234
4	92	100

3,18,L

-5	63	53
-4	78	94
-3	26*	2
-2	98	85
-	29	37
L	62	74
1	206	225
2	141	148
3	26*	15

3,19,L

-5	37	27
-4	26*	2
-3	43	28
-2	33	32
-1	80	71
0	28	25
1	55	85
2	68	82

3,20,L

-4	44	25
-3	83	65
-2	48	35
-1	99	110
0	26*	10
1	144	138

3,21,L

-2	27*	32
-1	100	111
0	26*	25

4,0,L

-6	27*	29
-6	219	181
-4	212	194
-2	236	225
0	28*	32
2	143	151
4	140	156

4,1,L

-8	28	20
-7	65	42
-6	28	24
-5	60	54
-4	92	83
-3	26*	23
-2	42	41
-1	129	132
0	92	87
1	95	88
2	87	93
3	157	154
4	120	142
5	67	78

4,2,L

-8	27*	1
-7	36	33
-6	27*	8
-5	57	53
-4	93	78
-3	179	184
-2	52	65
-1	154	158
0	33	37
1	73	84
2	28*	33
3	27*	36
4	26*	6
5	111	132

4,3,L

-8	101	68
-7	26*	3
-6	107	99
-5	40	29
-4	27*	38
-3	27*	6
-2	97	91
-1	100	104
0	142	138
1	43	47
2	74	84
3	90	86
4	88	89
5	61	65

4,4,L

-8	25*	12
-7	27*	15
-6	57	36
-5	28*	21
-4	59	66
-3	76	77
-2	111	115
-1	88	100
0	52	50
1	27*	20
2	221	220
3	135	144
4	172	178
5	250	284

4,5,L

-8	27*	17
-7	81	46
-6	26*	9
-5	134	117
-4	27*	32
-3	47	45
-2	26*	5
-1	322	342
0	175	163
1	78	80
2	27*	21
3	50	55
4	87	93
5	132	148

4,6,L

-8	36	28
-7	25*	1
-6	85	53
-5	37	25
-4	115	110
-3	26*	4
-2	192	203
-1	50	64
0	54	56
1	35	34
2	116	110
3	27*	6
4	27*	27
5	44	53

4,7,L

-8	77	61
-7	99	74
-6	88	76
-5	109	84
-4	79	73
-3	27*	27
-2	41	39
-1	134	144
0	27*	17
1	293	283
2	178	179
3	207	208
4	121	122
5	28*	41

4,8,L

-8	26*	11
-7	106	81
-6	63	60
-5	27*	24
-4	31	14
-3	41	47
-2	93	82
-1	27*	2
0	122	125
1	274	285
2	138	131
3	140	148
4	181	181
5	93	72

4,9,L

-7	32	14
-6	46	28
-5	55	50
-4	27*	0
-3	26*	18
-2	26*	3
-1	276	290
0	103	109
1	94	104
2	33	21
3	180	185
4	139	134

4,10,L

-7	92	69
-6	59	29
-5	43	53
-4	126	117
-3	69	75
-2	178	196
-1	100	113
0	228	248
1	56	61
2	28*	44
3	89	83
4	120	131

4,11,L

-7	134	101
-6	26*	10
-5	60	64
-4	43	45
-3	29	23
-2	27*	18
-1	211	230
0	27*	21
1	168	172
2	33	34
3	27*	10
4	27*	22

4,12,L

-6	71	46
-5	27*	20
-4	102	84
-3	105	96
-2	125	126
-1	27*	19
0	39	46
1	214	214
2	94	96
3	154	148

4,13,L

-6	49	32
-5	102	93
-4	65	61
-3	38	32
-2	27*	0
-1	169	164
0	26*	14
1	142	136
2	82	91
3	109	110

4,14,L

-5	70	68
-4	26*	4
-3	70	66
-2	36	54
-1	134	150
0	152	161
1	28*	10
2	42	33

4,15,L

-5	38	44
-4	28*	2
-3	44	37
-2	35	36
-1	124	155
0	102	100
1	31	14
2	157	156

4,16,L

-4	25*	3
-3	37	36
-2	26*	10
-1	26*	20
0	26*	10
1	26*	16

4,17,L

-2	52	37
-1	64	61

5,0,L

-6	75	60
-4	115	81
-2	70	54
0	177	159
2	208	222

5,1,L

-6	28*	12
-5	44	27
-4	89	70
-3	136	110
-2	44	37
-1	83	89
0	95	80
1	28*	17
2	75	74

5,2,L

-6	86	54
-5	73	43
-4	43	30
-3	35	16
-2	29*	26
-1	85	68
0	141	123
1	123	112
2	28*	10

5,3,L

-6	32	11
-5	28*	10
-4	60	60
-3	35	15
-2	30	30
-1	129	119
0	199	181
1	108	101
2	33	24

5,4,L

-5	29*	24
-4	46	43
-3	27*	17
-2	110	99
-1	186	167
0	31	23
1	113	107
2	28*	18

5,5,L

-5	27*	18
-4	28*	5
-3	104	92
-2	129	124
-1	106	97
0	78	79
1	184	173
2	41	43

5,6,L

-5	27*	9
-4	68	60
-3	42	35
-2	70	66
-1	60	62
0	56	51
1	42	43

5,7,L

-5	36	33
-4	68	44
-3	87	78
-2	76	67
-1	129	121
0	86	83
1	32	14

5,8,L

-4	37	29
-3	30	28
-2	35	39
-1	106	104
0	27*	16

5,9,L

-3	26	19
-2	26*	0
-1	26*	15
0	50*	60

5,10,L

-2	89	87
-1	27*	5

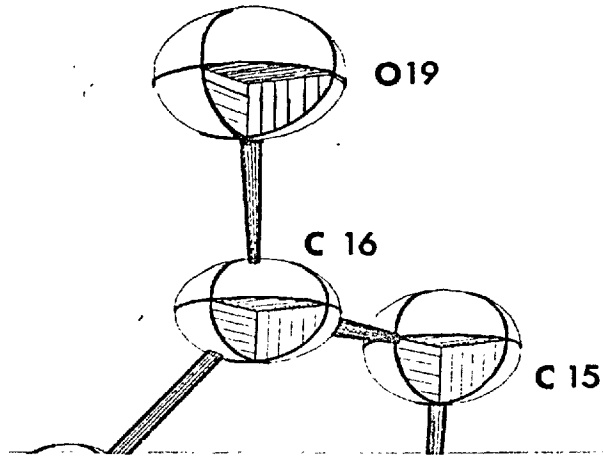
The relatively high value of the final R factor is a direct consequence of the somewhat inferior quality of the data. The crystal chosen for the data collection was the best of a rather poor batch, but was not ideal both as regards crystal texture (it was extremely striated along the needle-axis direction), and as regards the twist along this axis mentioned previously. Also, its small size, together with the relatively weak scattering power of the unit-cell contents, meant that reflection intensities were rather weak, as well as being somewhat diffuse, and thus could not be measured with high precision.

However, the final standard deviations of the atomic parameters (which give an average standard deviation in the bond lengths of 0.010Å) are considered to be sufficiently low for a detailed and meaningful discussion of the molecular geometry to be presented.

6.4. Description of the Molecule.

Figure 6.2 shows the a-axis projection of a molecule of 16-hydroxyphenoxymethyl-3,4-dihydroisoquinoline. Intramolecular bond lengths and angles, together with their standard deviations, are listed in Tables 6.5 - 6.7, and are shown in Figure 6.3. The phenyl atoms have an appreciable anisotropic movement which seems to get larger as one approaches O(19). Unfortunately, no rigid-body program was

Figure 6.2. The a-axis projection of a single molecule of 16-hydroxyphenoxymethyl-3,4-dihydroisoquinoline. The thermal ellipsoids are scaled to include 50% probability.



Warning to the Reader:- subsequent to the preparation of this thesis a computational error was discovered in the version of ORTEP used at Imperial College, which has the effect of making the orientations of the ellipsoids unreliable. The diagrams will be corrected in future publications.

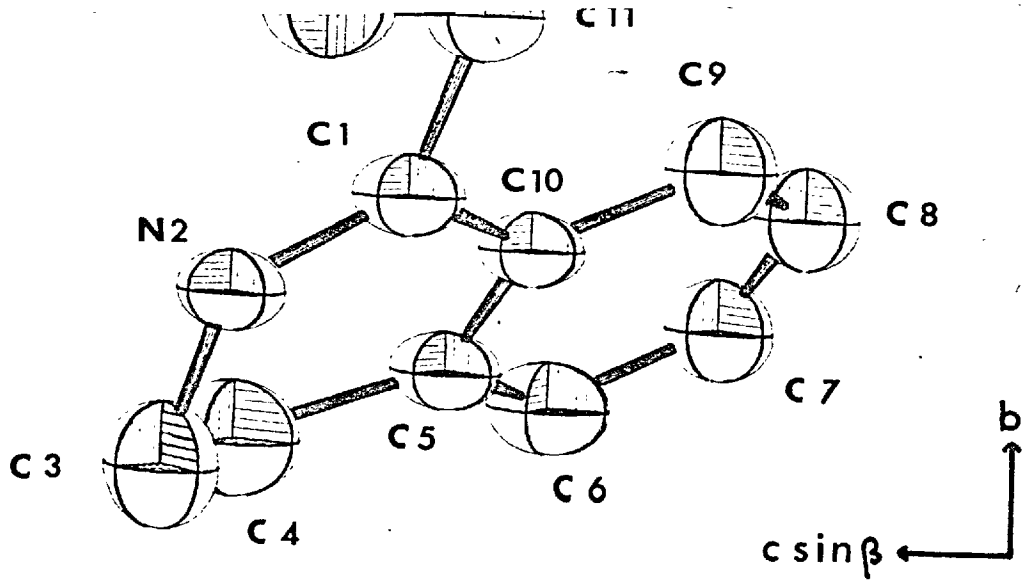


Table 6.5.

Intramolecular bonded distances for the non-hydrogen atoms, in Å. Estimated standard deviations (σ) are in parentheses.

Atoms	r(A)	
C(1)-N(2)	1.285(8)	
C(1)-C(10)	1.486(8)	
C(1)-C(11)	1.489(9)	
N(2)-C(3)	1.461(10)	
C(3)-C(4)	1.478(11)	
C(4)-C(5)	1.520(11)	
C(5)-C(6)	1.414(9)	
C(5)-C(10)	1.371(10)	
C(6)-C(7)	1.388(11)	$\langle r \rangle$= 1.384 Å
C(7)-C(8)	1.343(11)	
C(8)-C(9)	1.403(10)	
C(9)-C(10)	1.384(9)	
C(11)-O(12)	1.448(7)	
O(12)-C(13)	1.363(8)	
C(13)-C(14)	1.366(10)	
C(13)-C(18)	1.405(9)	
C(14)-C(15)	1.384(10)	
C(15)-C(16)	1.396(9)	$\langle r \rangle$= 1.386Å
C(16)-C(17)	1.388(10)	
C(17)-C(18)	1.377(10)	
C(16)-O(19)	1.357(8)	

Table 6.6.

Valence angles in the asymmetric unit, (θ), with their estimated standard deviations in parentheses.

Atoms	θ (°)	Atoms	θ (°)
N(2)-C(1)-C(10)	120.5(6)	O(12)-C(13)-C(14)	126.6(6)
N(2)-C(1)-C(11)	120.0(5)	O(12)-C(13)-C(18)	114.1(6)
C(10)-C(1)-C(11)	119.5(6)	C(14)-C(13)-C(18)	119.3(6)
C(1)-N(2)-C(3)	123.0(5)	C(13)-C(14)-C(15)	121.2(6)
N(2)-C(3)-C(4)	112.8(6)	C(14)-C(15)-C(16)	120.1(7)
C(3)-C(4)-C(5)	110.9(7)	C(15)-C(16)-C(17)	118.6(6)
C(4)-C(5)-C(6)	120.6(7)	C(15)-C(16)-O(19)	123.4(6)
C(4)-C(5)-C(10)	120.4(6)	C(17)-C(16)-O(19)	118.0(6)
C(6)-C(5)-C(10)	118.6(6)	C(16)-C(17)-C(18)	121.3(6)
C(5)-C(6)-C(7)	118.2(7)	C(13)-C(18)-C(17)	119.6(6)
C(6)-C(7)-C(8)	123.2(7)		
C(7)-C(8)-C(9)	118.8(7)		
C(8)-C(9)-C(10)	119.4(7)		
C(1)-C(10)-C(5)	117.7(6)		
C(1)-C(10)-C(9)	120.6(6)		
C(5)-C(10)-C(9)	121.8(6)		
C(1)-C(11)-O(12)	105.0(5)		
C(11)-O(12)-C(13)	118.0(5)		

Table 6.7.

Bonded distances (in Å) involving hydrogen atoms, with their estimated standard deviations (σ), in parentheses.

Atoms	r(Å)	
H(1)-C(6)	1.10(8)	$\langle r_1 \rangle = 0.96$Å
H(2)-C(7)	0.76(7)	
H(3)-C(8)	1.02(9)	
H(4)-C(9)	0.97(7)	
H(5)-C(14)	0.91(7)	$\langle r_2 \rangle = 0.92$Å
H(6)-C(15)	0.89(7)	
H(7)-C(17)	0.92(9)	
H(8)-C(18)	0.97(6)	
H(9)-C(3)	0.99(7)	
H(10)-C(3)	0.90(8)	
H(11)-C(4)	1.22(10)	
H(12)-C(4)	0.98(6)	
H(13)-C(11)	1.18(6)	
H(14)-C(11)	1.04(7)	
H(15)-O(19)	0.80(6)	
H(16)-O(20)	1.08(10)	
H(17)-O(20)	1.46(7)	
H(18)-O(20)	0.60(9)	

$\langle r_{1-15} \rangle = 0.97$Å

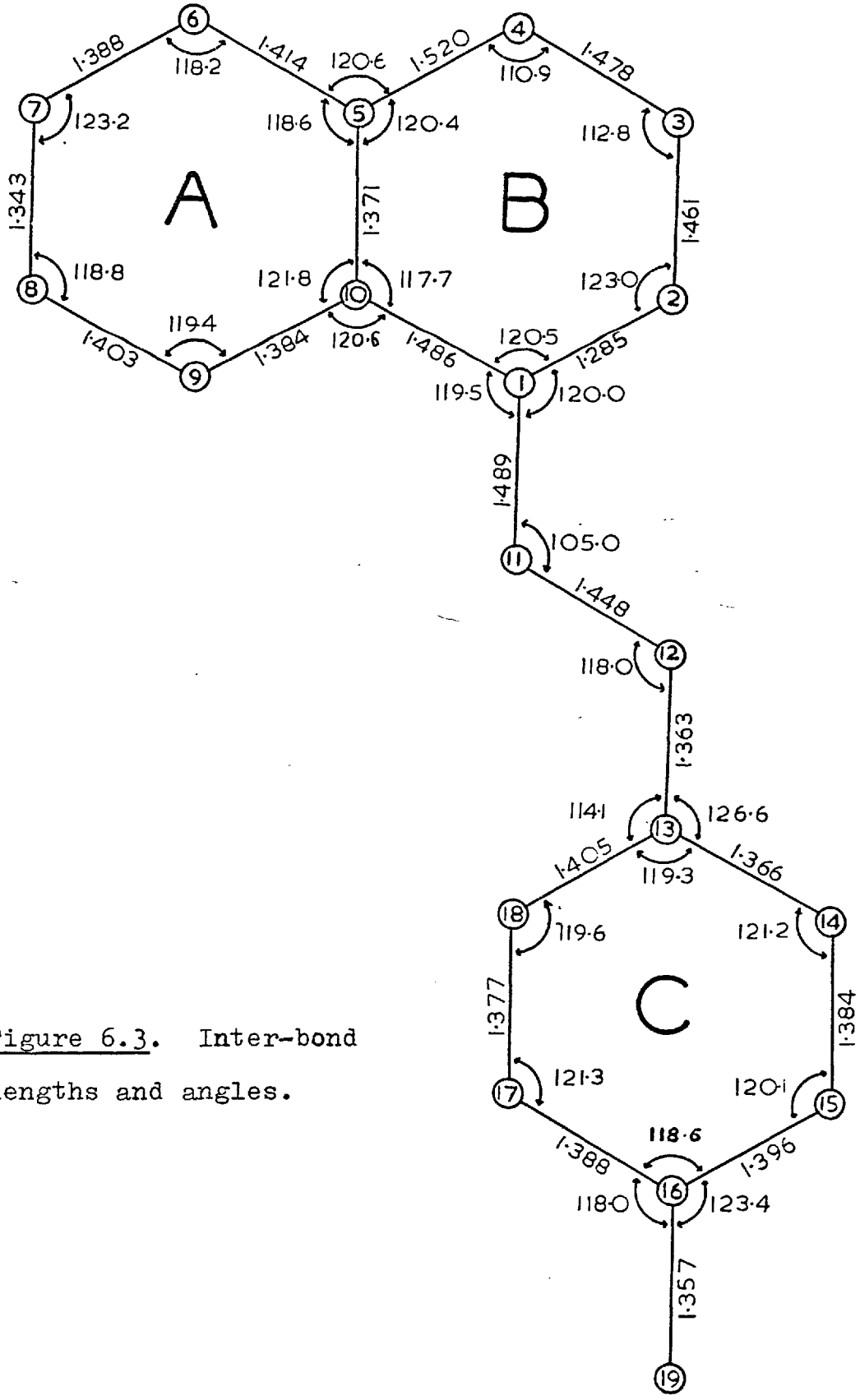


Figure 6.3. Inter-bond lengths and angles.

available to investigate this movement more thoroughly.

The bond distances and angles in both benzene rings conform very closely to their expected values. Thus, the average C-C bond length in rings A and C are 1.384Å and 1.386Å respectively, and the average C-C-C angles are 120.0° in both cases, comparing well with the tabulated ones of 1.394Å and 120.0° [9]. Both rings are of course planar, the average deviations from the best planes through them being 0.004Å and 0.005Å respectively (Table 6.8). The substituents to these rings do not lie exactly in their planes. In particular, C(1) and C(4) are very significantly distorted out of plane; this is due to the strain in the fused heterocyclic ring B. Atoms O(12) and O(19) are displaced by small amounts out of the plane of the benzene ring, but the reasons for this are not clear. The deviation of O(12) is $\sim 3\sigma$ and may be due either to molecular packing forces or to interactions between its lone pair(s) and the proton on C(18). Atom O(19) deviates by about 1σ in the direction of a hydrogen bond to a chlorine atom (See Figure 6.7).

The two bonds O(12)-C(13) and C(16)-O(19) have virtually identical bond lengths, which compare well with values found in similar systems such as phenol [9]. This indicates some degree of Π-orbital overlap in both bonds, and suggests that both oxygens are partly sp^2 hybridised. The angles external

Table 6.8.

Deviations from the least-squares planes, in Å. Those atoms in brackets have not been included in the calculation of the plane.

	A	B1	B2	B3	C	D
C(1)	(-0.042)	0.094	-0.006	-0.040	(0.136)	-0.006
N(2)	(0.111)	0.068	0.002	0.022	(0.319)	0.012
C(3)	(0.434)	-0.238		(0.278)	(0.332)	-0.003
C(4)	(-0.163)	0.236		(-0.288)	(0.885)	
C(5)	-0.001	-0.076		-0.020	(0.341)	
C(6)	0.003	(-0.212)			(0.283)	
C(7)	0.000				(-0.076)	
C(8)	-0.005				(-0.372)	
C(9)	0.008	(-0.232)			(-0.332)	
C(10)	-0.005	-0.084	0.002	0.040	(0.037)	
C(11)	(-0.248)	(0.328)	0.002		(0.049)	
O(12)	(-0.114)				(0.035)	
C(13)	(-0.321)				0.002	
C(14)	(-0.701)				0.002	
C(15)	(-0.905)				-0.008	
C(16)	(-0.750)				0.010	
C(17)	(-0.349)				-0.006	
C(18)	(-0.142)				0.000	
O(19)	(-0.945)				(0.014)	
O(20)						-0.002

Table 6.9.

Equations of the least-squares planes. These are of the form $lx + my + nz = p$, where x, y and z are in Å units.

Plane	l	m	n	p
A	-0.326	0.185	0.499	0.270
B1	-0.301	0.180	0.598	0.354
B2	-0.369	0.154	0.606	0.281
B3	-0.329	0.176	0.567	0.306
C	-0.356	0.135	0.725	0.344
D	-0.408	0.140	0.569	0.202

to the ring at C(13) and C(16) show very considerable deviations from the expected value of 120°. Deviations of this magnitude have been observed in a large number of structure determinations of molecules with benzene rings and side-chain substituents [10]. The reasons for these deviations are obscure, but are possibly induced by molecular packing, steric interactions between the protons on C(11) and C(14), and the tug of a chlorine ion on O(19). Both the values of the bond length C(11)-O(12) and the angle at O(12), agree well with expected values [9], for example in 1-p-(2-dimethylaminoethoxyphenyl)-1,2-cis-diphenylbut-1-ene hydrobromide [11], for a $C(sp^3)$-O bond and a $C(sp^3)$-O-C_{arom} angle, and are also suggestive of considerable sp^2 character for O(12).

The heterocyclic ring B is of some interest; it may be considered as a 3,4-dihydropyridine. Unfortunately, no structures containing this unusual system seem to have been examined by X-ray methods, so it is only possible to make comparisons with similar, but not identical systems. Bond C(5)-C(10) is fully aromatic. The length of C(1)-N(2) (1.285Å) corresponds well with other reported values for carbon-nitrogen double bonds, for example in 10-methylisoalloxazine hydrobromide (1.299Å) [12] and in 2-(1-imidazolin-2-yl) benzophenone hydrobromide (1.27Å) [13]. The latter compound has an analogous C_{arom}-C=N group, and unsurprisingly, the $C(sp^2)$-$C(sp^2)$

single-bond lengths are very comparable:- 1.486Å in our study compared with 1.49Å. The carbon-nitrogen double bond here is very significantly shorter than the corresponding one in pyridine (of 1.35Å), which is a delocalised compound. The dihedral angle between the two double bonds here is -9.4°. All this evidence strongly suggests that there is little conjugation between these two double bonds. The angles at atoms C(1), N(2), C(5), and C(10) are those expected for sp^2 hybridisation at these atoms, and the group of four atoms C(1), N(2), C(10) and C(11) are in a planar arrangement (Plane B2 in Table 6.8).

Ring B is non-planar; Table 6.8 gives the deviations of the atoms from the best plane through this ring (plane B1), as well as their deviations from the ring A plane (plane A). The former are also shown in Figure 6.4. The two double bonds C(1)-N(2) and C(5)-C(10) both have their respective substituents distorted out of plane; thus, C(1) and C(4) are -0.042Å and -0.163Å out of the plane of the benzene ring. Atoms C(1), N(2), C(5) and C(10) i.e. the double-bond system, form an approximately coplanar arrangement. (Plane B3). The other two ring atoms are equi-spaced (0.28Å) above and below this plane. Figure 6.4 is probably the best overall view of the ring conformation, which can be described as a somewhat flattened skew-boat, although in this type of context the terms 'boat' and 'chair' become somewhat meaningless. The 1,3-cyclohexadiene

Figure 6.4. Deviations (in Å) of the atoms in the heterocyclic ring B from the best plane through all six atoms.

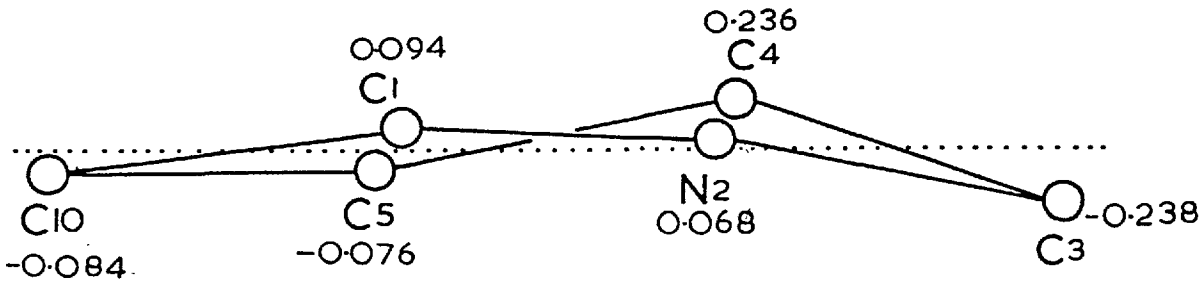
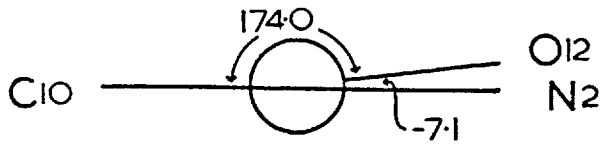
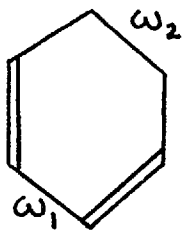


Figure 6.5. Newman projection along bond C(1)-C(11).



system in gliotoxin [15] is not exactly similar, but is probably the nearest example. It has what is described as a skew-chair conformation, with the ethylenic substituents undistorted from coplanarity, in contrast to the situation here. The dihedral angle ω_1 (shown here) has the value of 14.0° in gliotoxin, and 9.4° in the present compound. Angle ω_2 is here 43.6°; the corresponding value for gliotoxin is 44.8°. Thus, the ring conformis rather similar in both cases, in spite of the above-mentioned deviations from coplanarity, which do not seem to be crucial to the overall shape of the ring. A microwave investigation of 1,3-cyclohexadiene itself [16] less directly, finds the ring to be non-planar, with values for the two dihedral angles of 17.5° (ω_1) and 45.0° (ω_2). The non-planarity is considered to arise both from the necessary angle strain present, and from the interaction of the non-bonded protons in the two methylene groups.



The average of the carbon-hydrogen and oxygen-hydrogen bond lengths is 0.97Å (Table 6.7), with no significant departures from this mean. This shortening relative to the values from spectroscopic and neutron diffraction data of about 1.08Å, has been noted in many X-ray structure investigations [17]. The effect has been considered to arise from the shift of the hydrogen K electron towards the heavy atom on bonding, relative to the nucleus [18]. All the hydrogen isotropic temperature

factors have acceptable values.

The inability of the X-ray determination to satisfactorily locate the hydrogen atoms attached to the water oxygen can be traced to a number of possible causes. As will be discussed in more detail in a later section, this oxygen atom participates in, and indeed is crucial to, the hydrogen-bonding network in the crystal lattice. This induces a markedly abnormal electron charge distribution around the oxygen, which would preclude any real location of associated hydrogen atoms.

6.5. The Overall Conformation and Shape of the Molecule.

The value of the dihedral angle N(2)(C(1),C(11))O(12) is 7.1° (Figure 6.5). Thus, the arrangement around the C(1)-C(11) bond is cisoid. This is to be expected, since a trans conformation would mean unacceptable interactions with the proton attached to C(9). However, the $N^{\oplus}$-C-C-O system has been found in a number of compounds to have a dihedral angle in the range 60° to 90° [11,19], the so-called syn-clinal conformation. This minimises interactions of substituents on the nitrogen atom, with the oxygen orbitals. Here, there are no bulky substituents on N(2), in the direction of O(12). A hydrogen atom on the nitrogen would probably be sufficiently big to force the dihedral angle to about 30. This suggests that the nitrogen has no associated proton (and hence no associated positive charge). Additional conformational stabilisation might well derive from interaction of both N(2) and O(12) with the nearby water oxygen O(20), in

Figure 6.6. Deviations (in Å) of the atoms in the molecule from the best plane through ring C.

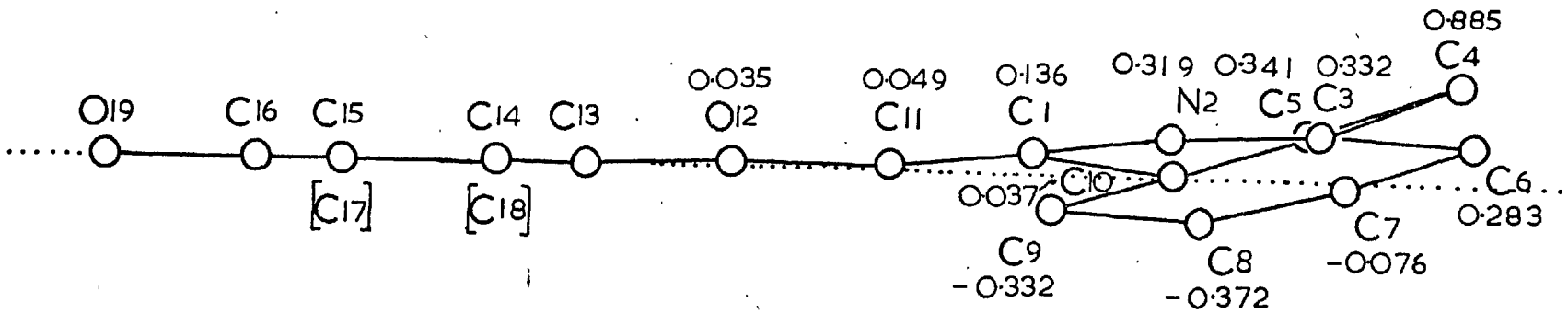


Table 6.10.

Dihedral angles (ω), in degrees, in the asymmetric unit.

Constituent atoms.	ω	Constituent atoms.	ω
C(1)(N(2),C(3))C(4)	33.1	C(6)(C(5),C(10))C(9)	0.6
C(1)(C(10),C(5))C(4)	-5.7	C(6)(C(7),C(8))C(9)	-0.7
C(1)(C(10),C(5))C(6)	-178.6	C(7)(C(6),C(5))C(10)	0.2
C(1)(C(10),C(9))C(8)	177.7	C(7)(C(8),C(9))C(10)	1.4
C(1)(C(11),O(12))C(13)	177.9	C(9)(C(10),C(1))C(11)	-9.7
N(2)(C(1),C(10))C(5)	-9.4	C(10)(C(1),C(11))O(12)	174.0
N(2)(C(1),C(10))C(9)	171.5	C(11)(O(12),C(13))C(14)	-1.1
N(2)(C(1),C(11))O(12)	-7.1	C(11)(O(12),C(13))C(18)	0.4
N(2)(C(3),C(4))C(5)	-43.6	O(12)(C(13),C(14))C(15)	1.2
C(3)(N(2),C(1))C(10)	-5.1	O(12)(C(13),C(18))C(17)	177.9
C(3)(N(2),C(1))C(11)	176.1	C(13)(C(14),C(15))C(16)	1.3
C(3)(C(4),C(5))C(6)	-155.0	C(13)(C(18),C(17))C(16)	-0.9
C(3)(C(4),C(5))C(10)	32.2	C(14)(C(13),C(18))C(17)	0.1
C(4)(C(5),C(6))C(7)	-172.7	C(14)(C(15),C(16))C(17)	-2.0
C(4)(C(5),C(10))C(9)	-173.5	C(14)(C(15),C(16))O(19)	-0.6
C(5)(C(6),C(7))C(8)	-0.1	C(15)(C(14),C(13))C(18)	-0.3
C(5)(C(10),C(1))C(11)	169.5	C(15)(C(16),C(17))C(18)	1.8
C(5)(C(10),C(9))C(8)	-1.4	C(18)(C(17),C(16))O(19)	0.5

The signs of the angles for the centrosymmetrically related molecule are the reverse of the above.

the crystal lattice This is discussed in the next section.

The molecule is approximately flat along its length. Figure 6.6 shows a projection along the plane of the aromatic ring C. The two rings A and C are inclined to each other at an angle of 16.2°.

6.6. Molecular Packing and Hydrogen-bonding in the Crystal Lattice.

Figures 6.7 and 6.8 show stereoscopic views of the unit-cell contents viewed along the a and b axes respectively. The water molecules and chlorine ions form hydrogen-bonded chains parallel to the x-axis, with O-Cl repeating distances of 3.150Å and 3.116Å. The Cl-O-Cl angle is 122.8°. These distances correspond well with other O-Cl hydrogen-bonded separations reported, for example, in hypoxanthine hydrochloride monohydrate [20], with a water-chlorine separation of 3.107Å. The water molecule must then provide both hydrogen atoms for each pair of Cl...O...Cl hydrogen atoms. Each chlorine ion also hydrogen-bonds to the phenolic O(19) in adjacent molecules, at a distance of 3.120Å. Thus, the overall structure consists of sheets of dihydroisoquinoline molecules parallel to the a-axis, hydrogen-bonded to each other through water and chlorine ions.

The water-N(2) distance is 2.721Å, which is well within the accepted range of distances for oxygen-nitrogen hydrogen bonds. The vast majority of such bonds tend to be somewhat

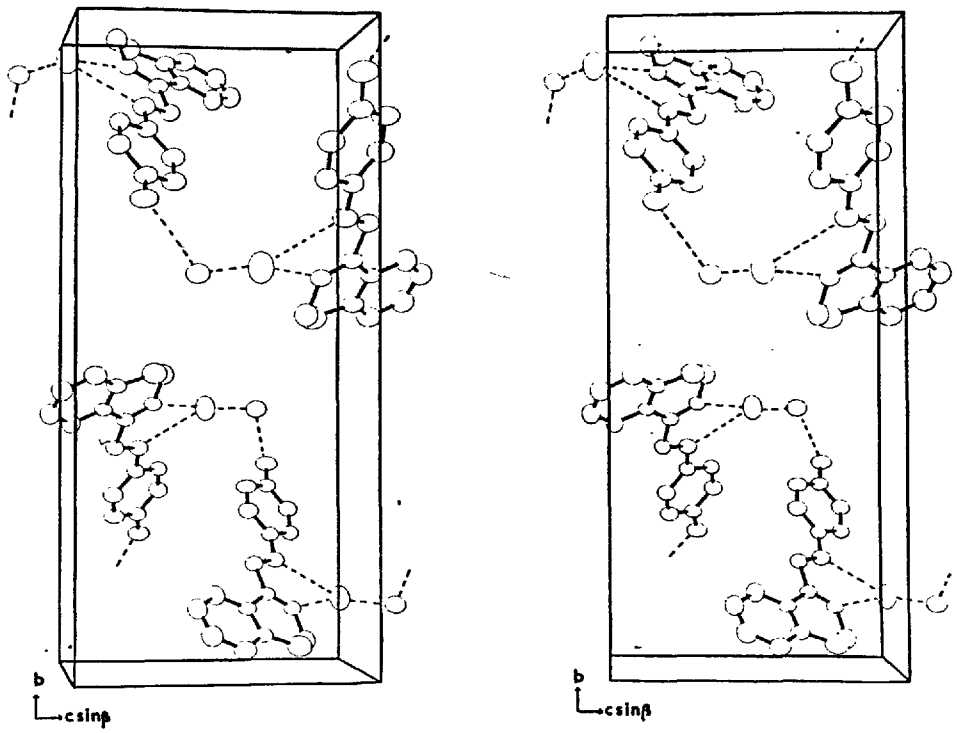


Figure 6.7. A stereodiagram of the unit-cell contents viewed along the a-axis. Dashed lines represent hydrogen bonds.

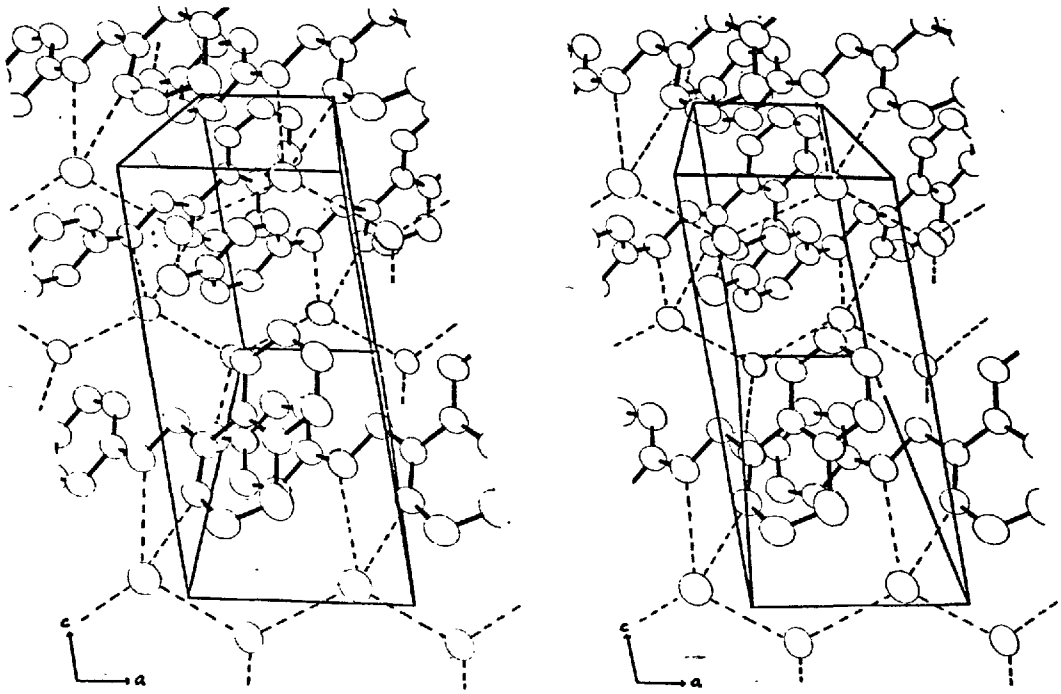


Figure 6.8. A stereodiagram of the unit-cell contents viewed along the b-axis. Dashed lines represent hydrogen bonds.

longer; they range from ca. 2.80Å to 3.10Å [21], and have the nitrogen atom acting as proton donor. The few recorded examples of oxygen acting as donor to nitrogen, include dimethylglyoxime [22] (2.77Å) and deoxyadenosine monohydrate [23] (2.78A). Thus, the short distance involved here is suggestive of a O-H...N type bond. Also, the system C(1),N(2),C(3),O(20) is planar (Plane D1 in Table 6.8). Now, since two hydrogens from the water are already accounted for, linked to the chlorines, we must therefore have a hydroxonium ion, $H_3O^{\oplus}$, here. As Figure 6.9 (a more detailed diagram of the hydrogen bonding), shows, the three hydrogen bonds are roughly equi-spaced in a pyramidal arrangement round the oxygen. This is identical to that found in several other crystal structures with well-authenticated hydroxonium ions, notably hydrogen chloride monohydrate ($H_3O^{\oplus}Cl^{\ominus}$) [24] and caesium chloride.1/3 hydronium bichloride [25]. No studies to date of the hydroxonium ion have been of more than moderate accuracy, and in no case have the hydrogen atoms been located. Indeed, in the case of $H_3O^{\oplus}Cl^{\ominus}$ some rather curious 'spurious' peaks around the oxygen were observed, but were not investigated further because of the limited accuracy of the data. Thus, although the evidence for the hydroxonium ion here in 16-hydroxy-phenoxymethyl-3,4-dihydroisoquinoline hydrochloride monohydrate is largely indirect, it is nevertheless felt to be quite

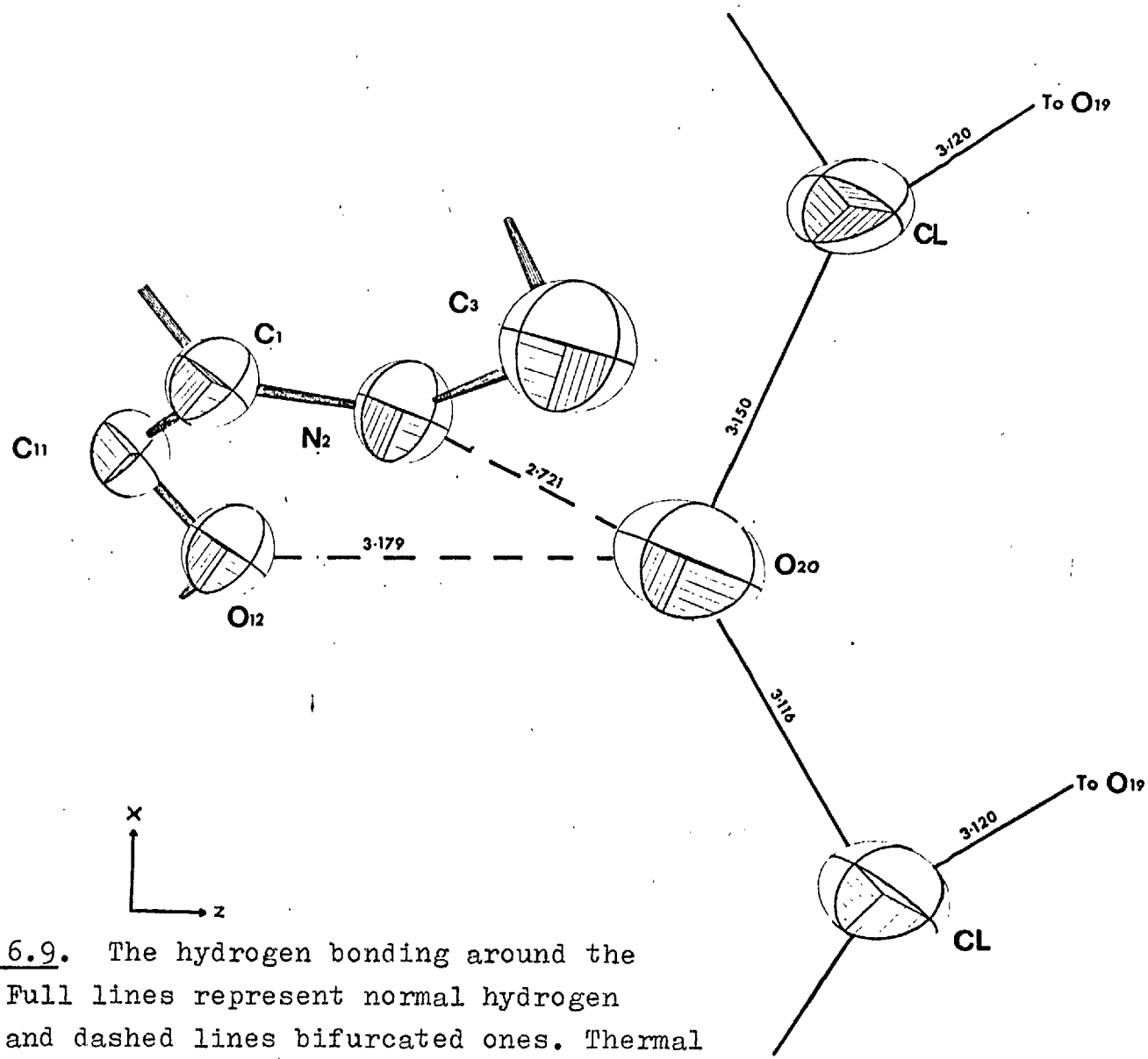


Figure 6.9. The hydrogen bonding around the water. Full lines represent normal hydrogen bonds, and dashed lines bifurcated ones. Thermal ellipsoids are scaled to include 50% probability.

definite. No proton was found attached to the nitrogen. Mention of the fact that the nitrogen has been found chemically to be non-basic [6] lends further support to this hypothesis. In all other hydrochloride monohydrates investigated to date, there is definite interaction between a positively-charged nitrogen atom and chlorine ions [20,26]. It appears here that O(20) and not N(2), is the more basic atom, otherwise the latter would be hydrogen-bonded to a chlorine ion. The nitrogen atom here is never near a chlorine ion, but the water is and bridges between nitrogen and chlorine. Of course, the matter would only be finally settled by a neutron-diffraction study. (Infra-red spectra in the solid state were inconclusive [27]). This appears to be the first reported example of a hydroxonium ion present in the lattice of an organic molecule.

An even more intriguing situation arises when one considers the fact that O(12) is only 3.179Å away from O(20). This is considerably longer than normal strong O-H...O interactions (typically, about 2.75Å [21]). However, if one postulates a bifurcated hydrogen bond here, the hydrogen atom would be shared between N(2) and O(12) and would be lying roughly on the bisector of the O(12)-O(20)-N(2) angle. The angle N(2)-O(20)-Cl is 89.4°and the O(12)-O(20)-Cl angle is 107.1°. Thus, a hydrogen shared between N(2) and O(12) would make the three H-O(20) bonds roughly equi-spaced. The

distance O(12)-hydrogen would be ~2.2Å, which is within the Van der Waals distance [28]. Magnesium sulphate tetrahydrate has a well-authenticated bifurcated hydrogen bond with O...O lengths of 3.282Å and 3.042Å to one of the waters [29]; the hydrogens were located by a neutron diffraction study. Thus, it is probable that there is at least some O(12)...O(20) hydrogen bond interaction here, although probably of a relatively weak nature. The 'semi-bifurcated' hydrogen bond is rather unbalanced since the short N(2)-O(20) length suggests that the proton is more tightly associated with this 'bond'. Any O(12)...O(20) interaction will, of course, lower the potential energy of the system (by only a small amount, probably less than one kilocalorie per mole), and thus, the approximately coplanar arrangement N(2), C(1), C(11), O(12) will be more favoured.

This postulated bifurcated hydrogen bond-hydroxonium ion system is, as far as is known, a unique one. It provides an explanation of the inability in structure solution and refinement, to locate the hydrogen atoms around O(20) in anything like their correct positions. The electron distribution for the oxygen has almost certainly aspherical symmetry, for which aspherical scattering factors are needed [30]. For this particular situation with the oxygen having a positive charge and having three quite strong hydrogen bonds pulling

aspherically, the model needed would not be a simple one, and no such treatment was attempted. (Use of $O^{\oplus}$ scattering factors made negligible difference). The inability in the earlier studies on $H_3O^{\oplus}$ salts to locate the hydrogen atoms was no doubt due to this type of problem. The bifurcated proton would quite likely have its electron density 'smeared out', and even after the above corrections, would probably not be found by X-ray methods. In any case, the electron density of a proton attached to a positive centre almost certainly does not represent the true nuclear position, since the relative departure from spherical symmetry is greatest for a hydrogen atom, and even more so for one in this type of situation. An example of this has been previously reported[31] where a proton 'peak' in a difference map was observed only 0.476Å away from a positively-charged nitrogen atom.

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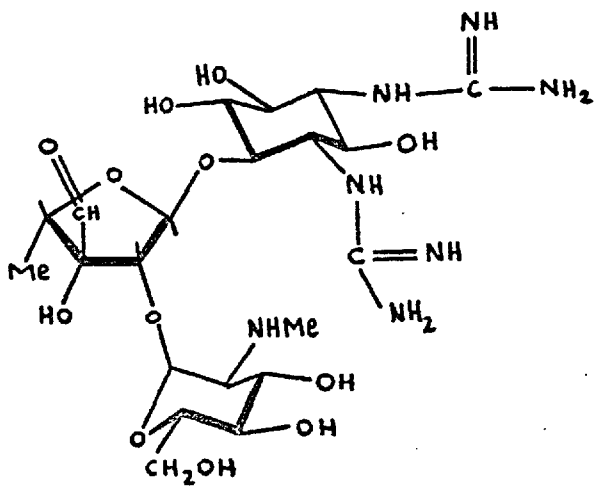
CHAPTER 7

The Crystal and Molecular Structure of Streptomycin Oxime Selenate.

7.1. Introduction.

The antibiotic streptomycin was discovered in 1944 after an extensive search for substances active against gram-negative bacteria, such as those of tuberculosis, against which penicillin was totally ineffective. It was isolated from strains of the micro-organism Streptomyces griseus, which is still the major source of supply for this important antibiotic [1].

Extensive chemical work [2a,b] has shown that streptomycin contains three fragments, N-methyl-L-glucosamine, L-streptose and streptidine, linked together by glycosidic bonds. (I).



(I)

There has been some dispute about the nature of the glycosidic links, in particular that between the streptose and streptidine rings. The original (1947) assignment was α-L- [3], based on optical rotational arguments on polyacetyl derivatives [2a]. This was reversed in 1954 [4] to β-L-, from rotational arguments on polybenzoyl derivatives. This made the two glycosidic links in I cis. However, recent chemical and NMR evidence [5] agrees with the original α-L- assignment. This also confirms as α-L- the earlier stereochemical assignment [2a] of the link between N-methyl-L-glucosamine and streptose. Our X-ray studies of streptomycin oxime selenate have confirmed all the configurational details (both relative and absolute) of structure I. In particular, the original trans assignment of the ether linkages has been vindicated, and the three independent correlation routes (with all their consequential implications) by which the absolute configurations of the three rings were deduced, have been validated. The X-ray work has also confirmed that the L-streptose fragment, which has never been isolated as a degradation product, is 5-deoxy-3-C-formyl-L-lyxose [6].

The NMR spectra of streptomycin and its salts show surprisingly no sign of a free aldehyde group in solution [7], and it is characteristic in our experience that all derivatives of streptomycin (of both the α- and β- forms [8]), are

exceptionally poorly crystalline unless the aldehyde group has been altered, as in dihydrostreptomycin, various schiff's bases or the oxime. Aronson et al. [7] have sought to explain the NMR observations in terms of a link between the aldehyde and the N-Me groups, but a link with a guanyl group might also be possible. Their explanation takes no cognisance of similar NMR observations for the β-streptomycin salts [9]. The two forms of streptomycin are readily interconvertible; the α is the more stable of the two in solution and is the form used medicinally. They have similar, but not identical, X-ray powder patterns, and Young [8] has suggested that the β-form has a link between the aldehyde and the N-Me groups (cf. Aronson et al.). We have been unable to prepare single crystals of any α- or β-streptomycin salt in a quality suitable for structure analysis, so our studies could not elucidate these points. As stated above, only when the aldehyde group was altered, was a suitable derivative obtained. Such compounds did not form α-β isomers, indicating that the aldehyde group is indeed implicated in such tautomerism. An extensive screening program of possible heavy-atom derivatives [10] suggested that the oxime selenate would be suitable for an X-ray study; this was in fact the one chosen.

A preliminary account of this work has been published [11].

7.2. Experimental.

Crystals of streptomycin oxime selenate are colourless needles, when recrystallised from methanol. Oscillation and Weissenberg photographs exhibited Laue symmetry 2/m. The systematic absences were $hk\ell$, $h + k \neq 2n$, which together with the known optical activity of the compound, define the space group uniquely as C2. This was confirmed by the structure analysis. Cell parameters were obtained from a least-squares fit of some 20 θ-values measured on Weissenberg photographs. Crystal data:- $C_{21}H_{40}N_8O_{12}\ 1\frac{1}{2}(H_2SeO_4)\ .4H_2O$, $M = 885.5$ orthorhombic C2 (No. 5, C_2^3), $a = 17.10(1)$, $b = 14.36(1)$, $c = 16.13(1)$Å $\beta = 108.0(2)^\circ$, $U = 3767$Å^3, $D_m = 1.54(2)$gcm.$^{-3}$ (by flotation), $D_c = 1.54$ g.cm.$^{-3}$, $Z = 4$, $\mu = 29.77$ cm^{-1} for Cu-K$_\alpha$ radiation ($\lambda_{mean} = 1.54178$Å). $F(000) = 1840$.

A single crystal of dimensions $0.5 \times 0.2 \times 0.1_5$ mm. was used for intensity data collection. The layers $h0\ell$-$h11\ell$ were photographed with Ni-filtered Cu-K$_\alpha$ radiation using the equi-inclination Weissenberg method and multi-film packs. The intensities of 3236 reflections were estimated visually with a calibrated wedge.* The layers were brought to an approximate common scale using 251 common reflections from the hkl zone, by comparing the ratios $\sum F^2_{main} / \sum F^2_{subsid.}$ for each layer. The effects of absorption were judged to be small, so no correction for it was made.

*All the work, up to this point, was that of Dr. M.B Hursthouse; he was unable to proceed further.

7.3. Structure Solution.

A three-dimensional Patterson synthesis was computed in order to determine the positions of the selenium atoms in the unit cell.

There are four general equipoints in space group C2, viz x,y,z; $\bar{x}$,y,$\bar{z}$; $\frac{1}{2}+x$, $\frac{1}{2}+y$, z; $\frac{1}{2}-x$, $\frac{1}{2}+y$, $\bar{z}$, and the following two pairs of special positions (on diads):-

0,y,0; $\frac{1}{2}$, $\frac{1}{2}+y$, 0 and 0, y, $\frac{1}{2}$; $\frac{1}{2}$, $\frac{1}{2}+y$, $\frac{1}{2}$

Two of the six selenium atoms must occupy special positions, while the remaining four may occupy either a set of general positions or two more independent special positions. In the latter event, at least two crystallographically independent selenium atoms must lie on the same diad, a situation which should be readily detected from the Patterson, but was not found. Evidently there is only one pair of seleniums in special positions, and, as the origin can be taken anywhere on one of the diads, their co-ordinates were set arbitrarily at 000; $\frac{1}{2}\frac{1}{2}$0.

There is only one Harker vector between members of the general set that can give information about their co-ordinates, namely $(x,y,z) - (\bar{x},y,\bar{z}) = 2x, 0, 2z$. A strong peak was found in this Harker section at (0.62, 0, 0.84), whence x = 0.31

and z = 0.42. The y co-ordinates of this set can only be found from intervectors between the set of four and the set of two, and, as one of the latter is at the origin we can get y directly from the strong peak at (0.31, 0.36, 0.42). A check showed that all the strong peaks could be accounted for on this basis.

A structure-factor calculation based on these selenium positions gave an R factor of 0.475 using an assumed B of 2.5$\mathring{A}^2$. A Fourier synthesis based on the selenium phases was then computed. The presence of selenium atoms at levels of y = 0.00, 0.36, 0.50, 0.86 destroyed the pseudo mirror plane normally found for space groups in class 2 when only one set of heavy atoms occurs. This was a tremendous help especially in view of the size of this structure. The map immediately revealed the six crystallographically-independent oxygens of the selenate ions at the positions:-

	x	y	z
O(1)	0.060	0.050	0.000
O(2)	0.510	0.430	0.085
O(3)	0.320	0.360	0.320
O(4)	0.210	0.350	0.410
O(5)	0.350	0.290	0.480
O(6)	0.340	0.450	0.470

Each oxygen atom was assigned an individual isotropic temperature factor of 4.0$\mathring{A}^2$. The selenate ions were then

refined by two cycles of ORFLS full-matrix least-squares refinement. The positional parameters of the special selenium atom were kept fixed, as were the inter-layer scale factors. However, these latter had been rescaled in a preliminary structure-factor calculation, to a set of scale factors K_i, where $K = \sum|F_o| / \sum|F_c|$, for each layer. R was now 0.360. The Fourier map obtained from this new set of phases showed 33 peaks with heights greater than 2.5 e/Å^3. However, no chemical sense could be made of them. Nevertheless, a structure factor calculation was performed including all the 33 peaks as carbon atoms, with isotropic temperature factors of 3.5Å^2. R fell to 0.284, and a considerable number of the included atoms appeared with an enhanced height on the subsequent Fourier map. Examination of a ball-and-spoke model constructed from these peaks clearly showed the outline of a six-membered ring in a chair conformation. Further careful examination of the Fourier enabled the ring substitution pattern to be elucidated; this established the ring as the glucosamine one predicted from chemical evidence. Three cycles of ORFLS gave an R of 0.323, with sensible temperature factors for all these 14 chemically identified atoms. The resulting Fourier map which was computed using these phases and rejecting all reflections for which $|F_c| > 0.5|F_o|$ (this or a similar rejection ratio was used in the calculation of all subsequent Fouriers), revealed the five-membered ring linked to the glucosamine ring. Three

cycles of ORFLS in which 19 carbon atoms were included, but only the five new ones were refined, gave an R of 0.306, with all B's sensible.

Considerable difficulty was experienced in obtaining new information from the next Fourier map. The only peaks that could be assigned with any degree of certainty were those corresponding to the substituents on the five-membered ring, and the oxygen of the bridge to the third ring. These were in the sterically expected positions on the ball-and-spoke model. Three cycles of ORFLS varying only the five new atoms, gave an R of 0.292. No clear indication of peaks corresponding to the third ring could be observed in either the Fourier or the difference Fourier maps. Six peaks (of heights about 2.4 $e/\mathring{A}^3$) seemed to make some sense, although they did not form a ring. These were input for two cycles of ORFLS, the data having been rescaled in the structure-factor link. (It was not considered advisable to refine these by least-squares on only a part structure). R fell to 0.285, and the temperature factor of one of the new input positions increased to 33.1$\mathring{A}^2$, indicating it to be spurious. The Fourier map obtained with these phases (omitting the one erroneous atom from the calculations), was still not free from spurious peaks; however, it was possible without much difficulty to recognise the rest of the streptidine ring with the exception of parts of the substituents. The seven new positions were verified by two cycles of ORFLS, with

the other 29 atoms kept fixed, when R fell to 0.268. The resulting Fourier maps clearly revealed the rest of the structure; a structure-factor calculation, rescaling the data layer by layer, with all 41 atoms included, and assigned their correct atom type, reduced R still further to 0.207.

A difference Fourier revealed the presence of four water molecules at heights of about 4e/Å^3. (This corrects a chemical analysis of the compound which indicated the presence of only three waters of crystallisation per asymmetric unit). Inclusion of these in a structure-factor calculation reduced R to 0.186. Two of the water oxygens had rather high temperature factors; these were watched during refinement in case partial water site occupancy was indicated, but they converged on fairly normal values.

7.4. Refinement.

Due to the large number of atoms involved (53), all subsequent refinement was carried out using the block-diagonal least-squares program BLOKLS. Four cycles of BLOKLS refining all the positional and isotropic thermal parameters, apart from the co-ordinates of the special-position selenium, gave an R of 0.153 The refinement was proceeding slowly, and showed some signs of oscillation. Five more cycles, this time refining the twelve scale factors as well, and with a 'fudge factor' of 0.5 to damp oscillations, produced some improvement. R fell to 0.142. Examination of the structure-factor listing, and the intensity

data and films, revealed three festoons to have been mis-indexed, involving 33 reflections. After these were corrected, five more cycles of BLOKLS reduced R to 0.136.

The temperature factors of the selenium atoms were now converted to their anisotropic equivalents. β_{12} and β_{23} for the selenium atom on the diad were set to zero and were not refined, on account of the two-fold axis. Six cycles of BLOKLS reduced R to 0.121, with slow convergence. At this point, a total of 14 reflections were removed for suspected extinction (See Table 7.3) For all these reflections, F_o was considerably less than F_c; re-measurement of their intensities showed that these large differences were not due to poor intensity estimation. A plot of ΔF against $|F_o|$ for each layer showed that a Hughes-type weighting scheme was appropriate, of the form $\sqrt{w} = 1$ for $F_o < F^*$ and $\sqrt{w} = F^*/F_o$ for $F_o > F^*$, where w is the weight applied and F^* is a constant. The values of the latter used are detailed in Table 7.1:-

Table 7.1.

Layer	F^*	Layer	F^*
h 0 l	70	h 6 l	120
1	140	7	100
2	140	8	70
3	80	9	70
4	140	10	90
5	100	11	60

Four cycles of BLOKLS with the selenates now anisotropic and the scale factors fixed, gave an R of 0.114. A difference Fourier calculated at this stage showed only slight anisotropy in the structure; the hydrogen positions could not be seen at all. The temperature factors of the remaining atoms were now converted to their anisotropic equivalents; after four more cycles of BLOKLS, R was 0.102. In order to bring the layer scale factors to more nearly correct values, the temperature factors for all atoms were reconverted to isotropic B's. Six cycles of refinement varying positional and temperature parameters, and the scale factors gave convergence with R at 0.117; the largest shift was approximately 0.1 of a standard deviation.

Inclusion of the improved scale factors in the next set of anisotropic refinement improved the general agreement; R fell to 0.092 after three cycles.

As stated above, it was not possible to locate any hydrogen atoms in a difference Fourier, which was also free of any spurious features. Accordingly, positions were calculated for all except the methyl and water hydrogen atoms. These were included in the structure-factor calculations with an assigned isotropic temperature factor 0.5$\mathring{A}^2$ greater than the atom to which they were bonded. Six final cycles of BLOKLS varying the parameters of all non-hydrogen atoms, and including a dispersion correction for the selenium atom (with the sign

Table 7.2.

Final observed and calculated structure factors; column headings are h, k, ℓ, followed by ℓ, $10\,|F_o|$, $10\,|F_c|$. The 001, 002 reflections were partly obscured by the backstop. The fourteen reflections omitted for suspected extinction were:-

h	k	ℓ	$10\,\|F_o\|$	$10\,\|F_c\|$
0	0	3	1433	1900
0	0	4	971	1197
2	0	$\bar{8}$	1357	1522
2	0	$\bar{4}$	2397	2813
2	0	$\bar{3}$	1394	1681
2	0	$\bar{2}$	1227	1540
2	0	$\bar{1}$	1822	2524
2	0	1	1262	1526
2	0	2	1436	1726
2	0	3	2898	3102
4	0	$\bar{2}$	1805	2220
4	0	$\bar{1}$	1377	1661
6	0	$\bar{2}$	1571	1805
8	0	1	1620	1842

0,0,L

5	460	458
6	284	464
7	1015	1131
8	582	615
9	240	291
10	678	737
11	65	43
12	775	839
13	304	319
14	163	140
15	175	136
16	97	107
17	293	296
18	187	138
19	255	172

2,0,L

-19	117	115
-18	351	285
-17	215	205
-16	359	339
-15	128	134
-14	572	613
-13	557	578
-12	65	53
-11	852	1026
-10	473	504
-9	500	543
-7	275	363
-5	577	481
0	247	154
4	108	56
5	884	1042
6	721	541
7	325	312
8	1345	1787
9	240	216
10	65	58
11	203	201
12	244	222
13	695	778
14	73	47
15	247	195
16	65	83
17	255	204

4,0,L

-20	215	175
-19	146	129
-18	56	14
-17	318	302
-16	77	6
-15	408	410
-14	73	29
-13	494	529
-12	544	518
-11	65	56
-10	961	1118
-9	378	304
-8	73	131
-7	845	1053
-6	463	357
-5	1514	1708
-4	113	59
-3	122	209
0	443	261
1	1127	1284
2	884	1060
3	491	429
4	433	502
5	46	93
6	514	459
7	336	380
8	418	407
9	637	710
10	320	301
11	73	9
12	443	449
13	261	242
14	172	156
15	347	314
16	508	418

6,0,L

-20	103	75
-19	282	225
-18	65	22
-17	65	59
-16	286	296
-15	73	57
-14	547	572
-13	244	254
-12	65	121
-11	565	539
-10	475	428
-9	630	700
-8	1054	1128
-7	516	618
-6	401	331
-5	206	133
-4	429	462
-3	681	804
-1	674	664
0	224	81
1	413	496
2	731	778
3	246	249
4	665	727
5	666	663
6	240	203
7	435	376
8	624	483
9	215	195
11	215	226
10	304	288
12	710	712
13	122	91
14	378	101

8,0,L

-20	948	188
-19	117	86
-18	257	199
-17	92	82
-16	172	171
-15	143	232
-14	242	290
-13	678	725
-12	267	314
-11	351	395
-10	495	509
-9	358	309
-8	491	577
-7	571	586
-6	1720	1903
-5	275	298
-4	48	48
-3	146	247
-2	1150	1183
-1	1022	1120
0	416	418
2	221	176
3	56	39
4	588	629
5	649	713
6	612	588
7	117	53
8	553	522
9	128	129
10	146	58
11	564	581
12	213	147
13	282	217
14	56	58
15	46	63

10,0,L

-20	143	141
-19	46	86
-18	142	120
-17	560	470
-16	158	105
-15	160	183
-14	564	591
-13	146	135
-12	168	159
-11	445	479
-10	804	831
-9	302	209
-8	166	181
-7	610	662
-6	643	685
-5	1226	1409
-4	343	319
-3	1154	1253
-2	335	303
-1	66	48
0	541	659
1	36	24
2	1045	1110
3	156	160
4	230	194
5	153	222
6	315	335
7	717	641
8	73	60
9	413	375
10	73	41
11	160	133
12	237	204
13	245	219
14	248	220

12,0,L

-19	46	25
-18	261	242
-17	56	46
-16	366	327
-15	73	50
-14	406	410
-13	450	374
-12	73	89
-11	488	508
-10	172	142
-9	841	864
-8	326	219
-7	92	29
-6	649	636
-5	65	72
-4	1076	1140
-3	65	96
-2	275	306
-1	92	60
0	545	540
1	275	289
2	324	308
3	1112	1111
4	73	20
5	143	166
6	438	459
7	139	135
8	156	98
9	368	303
10	468	359
11	56	58
12	108	105

14,0,L

-18	73	65
-17	259	182
-16	65	12
-15	342	331
-14	97	57
-13	224	215
-12	193	212
-11	408	398
-10	759	759
-9	73	19
-8	853	841
-7	46	47
-6	73	53
-5	601	631
-4	474	349
-3	73	14
-2	461	394
-1	907	940
0	175	160
1	253	217
2	400	396
3	73	49
4	250	236
5	273	331
6	134	90
7	65	13
8	187	183
9	242	161
10	46	28

16,0,L

-15	65	66
-14	244	196
-13	65	51
-12	503	436
-11	73	119
-10	73	58
-9	354	321
-8	103	163
-7	242	223
-6	73	29
-5	436	360
-4	228	71
-3	73	76
-2	534	492
-1	146	151
0	175	124
1	316	239
2	97	77
3	269	263
4	65	94
5	407	344
6	56	14
7	293	218
8	46	39

18,0,L

-16	46	144
-15	46	72
-14	73	5
-13	108	101
-12	142	116
-11	243	245
-10	65	27
-9	160	97
-8	134	130
-7	65	62
-6	190	178
-5	214	209
-4	458	430
-3	134	119
-2	163	75
-1	248	306
0	126	68
1	209	154
2	56	79
3	288	229
4	46	39
5	46	78
6	32	129

20,0,L

-12	149	134
-11	46	47
-10	184	163
-9	233	148
-8	73	77
-7	122	164
-6	56	51
-5	134	110
-4	73	85
-3	406	336
-2	46	39
-1	46	33
0	175	179

1,1,L

-19	151	162
-18	129	114
-17	118	124
-16	451	400
-15	203	198
-14	633	624
-13	542	544
-12	338	378
-11	405	440
-10	446	415
-9	1129	1156
-8	487	524
-7	868	862
-6	902	928
-5	1421	1323
-4	2494	2563
-3	1396	1438
-2	1044	963
1	1543	1619
2	1168	1256
3	2748	2641
5	1253	1212
6	784	799
7	385	506
8	695	775
9	387	344
10	812	801
11	375	403
12	842	797
13	559	598
14	137	115
15	370	345
16	278	265
17	193	239
18	200	179

3,1,L

-20	216	260
-19	196	166
-18	210	173
-17	196	190
-16	133	138
-15	304	247
-14	272	290
-13	305	428
-12	390	324
-11	659	646
-10	614	631
-9	744	713
-8	1099	1228
-7	595	548
-6	426	443
-5	468	460
-4	1149	990
-3	1872	1958
-2	340	303
-1	2040	2451
0	1313	1331
1	2049	2217
2	1761	1568
3	1718	1574
4	1435	1453
5	400	458
6	455	511
7	375	338
8	535	529
9	349	343
10	214	215
11	920	960
12	235	247
13	298	356
14	233	215
15	143	134
16	346	348
17	101	113
18	143	123

5,1,L

-20	91	45
-19	143	146
-18	237	202
-17	297	273
-16	95	45
-15	167	147
-14	443	428
-13	162	140
-12	597	644
-11	115	122
-10	523	534
-9	105	96
-8	751	739
-7	1223	1234
-6	779	852
-5	697	823
-4	523	615
-3	1432	1628
-2	493	679
-1	1575	1417
0	2016	2094
1	725	818
2	737	847
3	220	264
4	506	433
5	717	649
6	319	370
7	457	515
8	355	324
9	633	616
10	394	372
11	270	325
12	547	598
13	313	294
14	91	113
15	114	96
16	122	108

7,1,L

-18	181	151
-17	321	271
-16	282	298
-15	237	217
-14	495	492
-13	485	422
-12	272	230
-11	597	826
-10	491	534
-9	995	1346
-8	146	117
-7	420	350
-6	476	465
-5	1019	1030
-4	653	691
-3	312	355
-2	1545	1582
-1	535	477
0	377	349
1	956	935
2	469	512
3	678	635
4	315	331
5	245	173
6	210	240
7	513	541
8	436	439
9	998	523
10	561	477
12	210	178
13	308	287
14	159	203
15	267	249
16	158	105

9,1,L

-20	262	294
-19	118	98
-18	118	148
-17	378	378
-15	458	426
-14	137	182
-13	316	321
-12	335	205
-11	277	255
-10	657	713
-9	429	416
-8	941	888
-7	469	479
-6	805	777
-5	749	806
-4	371	365
-3	593	678
-2	1017	1021
-1	916	862
0	677	672
1	437	443
2	772	872
3	453	495
4	442	384
6	622	558
7	182	204
8	359	368
9	534	575
10	322	328
11	323	295
12	144	128
13	182	118
14	223	187
15	149	168

11,1,L

-19	164	143
-18	106	78
-17	203	208
-16	237	168
-15	289	258
-14	346	358
-13	335	298
-12	768	758
-11	297	268
-10	368	368
-9	768	742
-8	512	448
-7	835	785
-6	412	398
-5	591	488
-4	178	130
-3	567	514
-2	834	892
-1	392	421
0	706	712
1	417	352
2	420	472
3	555	544
4	434	462
5	696	721
6	225	182
7	237	183
8	218	125
9	114	86
10	359	384
11	178	174
12	265	275

13,1,L

-19	162	138
-18	229	234
-17	75	77
-16	166	166
-15	156	161
-14	133	85
-13	687	618
-12	167	178
-11	482	468
-10	287	221
-9	175	153
-8	385	371
-7	432	419
-6	515	467
-5	542	480
-4	619	537
-3	361	343
-2	385	329
-1	1045	982
1	274	292
2	336	326
3	214	284
4	137	122
5	289	291
6	482	501
7	110	135
8	415	349
9	193	169
10	122	120
11	227	169

15,1,L

-18	115	87
-17	275	242
-16	75	71
-15	250	175
-14	159	158
-13	91	170
-12	329	240
-11	391	262
-10	259	267
-9	68	44
-8	641	573
-7	546	361
-6	347	327
-5	750	774
-4	272	243
-3	451	448
-2	147	146
-1	247	222
0	278	263
1	347	351
2	615	476
3	133	100
4	171	161
5	168	274
6	173	127
7	200	219
8	150	147

17,1,L

-16	262	224
-15	68	61
-14	248	219
-13	140	172
-12	268	218
-11	159	358
-10	158	137
-9	343	342
-8	243	240
-7	248	178
-5	257	254
-4	426	446
-3	143	190
-2	153	117
-1	129	129
0	348	248
1	163	174
2	263	275
3	170	166
4	163	262
5	66	62
6	101	190

19,1,L

-15	147	116
-14	133	65
-13	218	165
-12	43	25
-11	265	156
-9	110	130
-8	411	346
-7	140	145
-6	114	72
-5	114	124
-4	140	114
-3	147	40
-2	119	150
-1	140	240
0	259	91
1	96	57
2	46	49
3	63	65
4	75	148

21,1,L

-10	122	97
-8	96	78
-7	101	91
-6	146	147
-5	164	123

0,2,L

2	2131	1976
3	1543	1695
4	1915	1767
5	852	795
6	1175	1295
7	745	713
8	1301	1073
9	992	1002
10	541	655
11	247	295
12	660	687
13	378	346
14	113	137
15	132	174
16	153	190
18	305	252
19	159	151

2,2,L

-19	292	210
-18	226	193
-17	315	272
-16	265	251
-15	315	294
-14	195	175
-13	458	397
-12	657	715
-11	850	847
-10	1082	1075
-9	902	891
-8	375	392
-6	2241	2157
-5	845	1919
-4	1958	2097
-3	628	687
-2	279	236
-1	1118	1176
0	1496	1496
1	814	692

2,2,L

2	1677	2080
3	731	687
4	265	162
5	1035	1026
6	794	803
7	829	852
8	376	388
9	802	786
10	435	395
11	495	490
12	705	723
13	364	306
14	795	798
15	282	284
16	118	112
18	136	125

4,2,L

-20	118	95
-19	123	86
-18	226	186
-17	290	255
-16	387	352
-15	274	276
-14	444	465
-13	518	484
-12	147	126
-11	482	451
-10	922	912
-9	791	752
-8	558	566
-7	635	620
-6	628	681
-5	627	769
-4	1598	1728
-3	2304	2639
-2	1452	1405
-1	1629	1578
0	624	652
1	636	663
2	842	815
3	646	721
4	441	454
5	371	363
6	138	130
7	546	543
8	683	696
9	705	743
10	570	579
11	148	288
12	250	271
13	192	211
14	344	321
15	325	311
16	255	184
17	192	155

6,2,L

-20	322	242
-19	125	143
-18	147	124
-17	272	231
-16	409	350
-15	274	259
-14	250	266
-13	485	451
-12	348	292
-11	388	366
-10	691	665
-9	692	613
-8	618	675
-7	205	254
-6	530	469
-5	542	507
-4	206	161
-3	597	685
-2	1113	1082
-1	1556	1472
0	1771	1895
1	94	25
2	470	456
3	368	358
4	862	880
5	444	461
6	486	485
7	433	404
8	205	186
9	127	106
10	538	519
11	503	506
12	505	466
13	321	267
14	138	73
15	87	108
16	178	171

8,2,L

-20	151	129
-19	268	154
-18	225	186
-17	171	181
-15	160	140
-14	355	376
-13	646	560
-12	467	500
-11	518	508
-10	417	405
-9	247	281
-8	591	552
-7	840	897
-6	538	539
-5	638	701
-4	270	316
-3	787	777
-2	654	644
-1	614	652
0	373	413
1	1122	1137
2	237	228
3	411	416
4	520	561
5	560	564
6	412	367
7	741	455
8	350	325
9	160	210
10	251	216
11	346	294
12	235	274
13	248	294
14	159	136

10,2,L

-19	211	162
-18	335	293
-17	277	218
-16	258	280
-15	270	250
-14	371	288
-13	166	159
-12	355	321
-11	721	678
-10	736	693
-9	333	290
-8	621	572
-7	703	627
-6	474	478
-5	756	764
-4	123	190
-3	745	688
-2	373	354
-1	457	480
0	456	467
1	[illegible]	1183
2	511	519
3	307	261
4	343	347
5	126	128
6	533	562
7	513	489
8	256	321
9	272	286
10	250	248
11	307	294
12	155	111
13	247	101
14	178	150

12,2,L

-19	160	127
-18	158	92
-17	240	243
-16	311	285
-15	240	289
-14	270	260
-13	113	117
-12	427	416
-11	345	364
-10	448	422
-9	556	524
-8	736	670
-7	147	164
-6	529	489
-5	547	624
-4	969	874
-3	448	461
-2	576	581
-1	165	184
0	341	267
1	507	485
2	721	724
3	345	410
4	409	424
5	246	271
6	307	298
7	227	202
8	167	183
9	311	266
10	143	142
11	138	135

14,2,L

-18	167	135
-17	229	156
-16	256	226
-15	242	200
-14	245	268
-13	307	253
-12	222	291
-11	145	215
-10	552	545
-9	674	594
-8	412	364
-7	343	390
-6	707	743
-5	247	259
-4	419	435
-3	348	395
-2	528	528
-1	444	476
0	316	343
1	246	282
2	443	475
3	247	276
4	155	156
5	262	287
6	[illegible]	91
7	138	121
8	145	129
9	145	172
10	134	104

16,2,L

-17	138	112
-16	107	98
-15	192	163
-14	208	185
-13	223	249
-12	208	174
-11	250	223
-10	277	259
-8	247	204
-7	354	344
-6	242	210
-5	192	174
-3	138	163
-2	223	192
-1	272	293
0	205	217
1	359	369
2	151	136
3	192	213
4	138	158
5	160	147
6	143	116
7	220	176
8	133	123

18,2,L

-15	94	49
-14	129	89
-13	143	131
-12	123	137
-11	133	126
-10	192	155
-9	123	112
-8	101	47
-7	272	214
-6	231	226
-5	231	259
-4	163	128
-3	123	152
-2	175	171
-1	71	102
0	234	224
1	202	232
2	171	173
3	113	97

20,2,L

-12	133	104
-11	113	111
-10	167	164
-9	113	124
-8	113	116
-7	129	114
-6	129	157
-5	202	177
-4	163	170
-2	80	61
-3	175	163
-1	71	84
0	147	128
1	151	122

1,3,L

-19	58	79
-18	135	120
-17	255	265
-16	222	227
-15	416	405
-14	366	366
-13	598	638
-12	361	362
-11	342	369
-10	470	461
-9	684	709
-8	949	967
-7	820	863
-6	886	847
-5	1602	1540
-4	450	321
-3	1934	1926
-2	810	710
1	1207	1064
2	1324	1311
3	1988	1036
4	2033	1959
5	441	389
6	966	1015
7	812	858
8	651	703
9	1172	1157
10	413	425
11	564	574
12	371	328
13	147	152
14	393	383
15	268	282
16	326	325
17	82	98
18	214	177

3,3,L

-19	310	268
-18	165	167
-17	131	149
-16	268	211
-15	297	338
-14	395	413
-13	326	338
-12	699	711
-11	295	271
-10	246	218
-9	1035	988
-8	342	329
-7	945	1029
-6	208	199
-5	1806	1868
-4	1392	1357
-3	861	661
-2	1864	1999
-1	687	470
0	2627	2724
1	1640	1443
2	1121	1031
3	765	793
4	787	751
5	902	963
6	844	773
7	692	634
8	353	281
9	67	77
10	458	433
11	445	421
12	664	635
13	182	164
14	101	100
15	95	107
17	219	243

5,3,L

-20	169	129
-19	179	157
-18	297	304
-17	135	138
-16	271	269
-15	165	154
-14	131	72
-13	504	594
-12	494	559
-11	695	719
-10	216	225
-9	232	218
-8	1043	954
-7	545	601
-6	681	697
-5	694	735
-4	772	654
-3	241	216
-2	1518	1546
-1	1374	1349
0	796	819
1	1641	1724
2	662	642
3	793	779
4	577	533
5	518	446
6	464	460
7	412	430
8	660	668
9	440	419
10	397	419
11	333	290
12	147	154
13	458	463
14	67	56
15	151	146
16	75	34

7,3,L

-20	95	71
-19	147	128
-18	58	58
-17	343	308
-16	143	195
-15	445	436
-14	107	121
-12	460	422
-11	407	340
-10	736	737
-9	465	491
-8	643	645
-7	135	67
-6	926	931
-5	1707	1795
-4	782	758
-3	1002	1059
-2	1479	1475
-1	229	146
0	709	691
1	1235	1138
2	857	901
3	339	331
4	985	982
5	229	174
6	224	199
7	695	673
8	348	348
9	465	449
10	182	229
11	314	313
12	227	221
13	176	151
14	239	229
15	176	167
16	151	129

9,3,L

-20	126	126
-19	211	153
-18	165	122
-17	216	190
-16	222	221
-15	232	229
-14	333	363
-13	234	202
-12	399	393
-11	345	371
-10	419	364
-9	1035	1037
-8	553	550
-7	82	50
-6	581	529
-5	453	460
-4	393	347
-3	561	654
-2	1532	1596
-1	164	227
0	433	486
1	518	522
2	536	486
3	1142	1170
4	279	348
5	423	434
6	176	147
7	253	229
8	446	425
9	364	342
10	425	352
11	169	167
12	222	220
14	117	122

11,3,L

-19	169	142
-18	206	206
-17	191	165
-16	165	134
-15	335	343
-14	147	154
-13	448	482
-12	393	374
-11	275	274
-10	731	684
-9	67	112
-8	624	686
-7	312	265
-6	707	776
-5	338	310
-4	297	266
-3	739	754
-2	312	316
-1	865	958
0	279	267
1	306	362
2	364	342
3	172	166
4	671	667
5	369	377
6	333	304
7	75	83
8	301	305
9	101	97
10	321	324
11	366	361
12	143	112

13,3,L

-19	255	273
-18	165	145
-17	211	197
-16	89	120
-15	165	177
-14	266	281
-13	275	26[illegible]
-12	268	31[illegible]
-11	258	226
-10	446	441
-9	429	435
-8	75	9[illegible]
-7	917	894
-6	246	196
-5	481	496
-3	260	166
-2	310	349
-1	454	484
0	606	559
1	208	254
2	277	316
3	224	199
5	277	329
6	176	201
7	427	425
8	158	132
9	206	186
10	179	149

15,3,L

-18	179	159
-17	165	144
-16	268	272
-15	82	121
-13	330	290
-11	308	262
-10	218	154
-9	246	246
-8	324	297
-7	75	61
-6	333	285
-5	435	395
-4	641	836
-2	374	446
-1	473	497
1	371	353
2	253	280
3	352	353
4	67	20
6	122	124
7	58	76
8	185	164

17,3,L

-16	101	91
-15	218	190
-14	75	91
-13	208	203
-12	112	86
-11	95	94
-10	361	348
-9	101	127
-8	347	363
-7	67	130
-5	249	245
-4	143	143
-3	248	233
-2	200	142
-1	172	186
0	67	75
2	271	274
3	122	136
4	208	277

19,3,L

-14	185	148
-13	47	66
-12	143	147
-11	131	110
-9	247	257
-8	122	125
-7	273	241
-6	151	111
-5	151	130
-4	82	87
-2	321	303
-1	158	143
0	107	99
1	67	66
3	122	117

0,4,L

1	1568	1852
2	1158	1092
3	703	489
4	1352	1375
5	162	142
6	1748	1774
7	258	233
8	660	710
9	374	362
10	606	569
11	865	895
12	345	362
13	353	322
15	469	448
16	99	139
17	60	74
18	302	265
19	153	137

2,4,L

-19	248	210
-18	179	173
-17	161	176
-16	292	193
-15	556	541
-14	76	82
-13	214	299
-12	1133	1231
-11	507	601
-10	1094	1047
-9	270	255
-8	880	818
-7	820	840
-6	656	648
-5	805	818
-4	751	793
-3	1524	1377
-2	1151	925
-1	508	529
0	1449	1459
1	874	727
2	1913	1808
3	452	392
4	791	673
5	559	550
6	913	910
7	848	949
8	726	762
9	848	883
10	243	240
11	179	146
12	402	368
13	306	308
14	572	571
15	70	23
16	157	142
17	111	102
18	60	58

4,4,L

-19	70	44
-18	121	122
-17	236	217
-16	527	564
-15	214	200
-14	78	79
-13	337	316
-12	524	463
-11	569	546
-10	343	370
-9	830	970
-8	411	363
-7	949	976
-6	330	272
-5	1444	1462
-4	236	266
-3	823	841
-2	534	659
-1	1097	1017
0	765	483
1	2035	2082
2	542	449
3	819	684
4	326	219
5	1142	1132
6	650	554
7	153	180
8	761	753
9	222	235
10	766	823

16,2,L

4,4,L

11	588	543
12	131	111
13	105	180
14	70	27
15	208	247
16	126	142
17	168	144

6,4,L

-20	263	214
-19	49	19
-18	86	69
-17	179	196
-16	70	38
-15	341	372
-14	172	145
-13	650	688
-12	192	201
-11	180	119
-10	318	355
-9	348	303
-8	1150	1243
-7	572	545
-6	311	423
-5	121	177
-4	763	738
-3	614	658
-2	199	219
-1	621	574
0	842	652
1	798	774
2	937	904
3	891	834
4	1261	1283
5	283	263
6	546	558
7	246	175
8	351	361
9	538	447
10	79	79
11	553	554
12	336	336
13	236	250
14	214	203
15	60	27
16	195	162

8,4,L

-19	274	258
-18	88	94
-17	266	258
-16	126	117
-15	131	110
-14	532	534
-13	332	342
-12	642	664
-11	270	260
-10	239	196
-9	78	84
-8	472	469
-7	697	637
-6	332	410
-5	1170	1204
-4	798	739
-3	606	588
-2	752	777
-1	165	64
0	1220	1252
1	312	362
2	410	404
3	518	520
4	475	365
5	872	878
6	482	480
7	668	654
8	153	127
9	328	359
10	341	307
11	204	147
12	414	416
13	86	108
14	175	120

10,4,L

-18	253	231
-17	93	74
-16	312	317
-15	145	118
-14	216	241
-13	408	401
-12	153	194
-11	776	801
-10	428	384
-9	788	819
-8	327	310
-7	265	230
-6	764	808
-5	310	329
-4	690	768
-3	153	138
-2	126	134
-1	542	556
0	93	93
1	1013	1036
2	333	357
3	454	516
4	362	342
5	211	198
6	214	211
7	375	378
8	506	528
9	187	188
10	177	171
11	228	232
12	160	19
13	205	204

12,4,L

-19	111	81
-18	49	65
-17	277	298
-16	238	200
-15	246	251
-12	353	352
-11	265	243
-10	426	432
-9	258	247
-8	476	530
-7	349	333
-5	613	634
-4	172	161
-3	422	492
-2	342	288
-1	434	462
0	550	573
1	115	184
2	651	682
3	261	301
4	544	688
5	216	218
6	214	198
7	314	310
9	151	182
10	131	153
11	242	223
12	111	90

14,4,L

-16	344	345
-16	344	345
-15	70	61
-14	244	261
-12	365	395
-11	214	175
-10	172	217
-9	575	592
-8	195	157
-7	154	216
-6	266	307
-5	166	233
-4	536	494
-3	517	525
-2	560	580
-1	324	330
0	111	148
2	[illegible]	253
3	[illegible]	319
4	335	87
5	255	272
7	126	141
8	116	149
9	70	71
10	175	171

16,4,L

-17	168	159
-16	136	134
-14	172	176
-13	362	347
-12	70	84
-11	246	234
-10	265	189
-9	76	51
-8	266	284
-7	166	154
-6	253	275
-4	153	151
-3	251	209
-2	168	154
-1	276	302
0	68	46
1	324	332
3	[illegible]	23
4	68	238
5	215	65

18,4,L

-12	248	225
-11	80	69
-10	253	239
-9	131	108
-8	131	84
-7	165	183
-5	272	274
-4	93	123
-3	233	250
-2	73	56
-1	126	142
0	324	303
1	68	38
2	136	146
4	121	137

20,4,L

-11	321	128
-10	111	112
-9	241	250
-8	49	65
-7	46	37
-6	165	203
-5	49	73
-4	251	293
-3	121	140
-2	116	118
-1	149	178

1,5,L

-17	147	187
-16	203	205
-15	352	339
-14	258	278
-13	437	448
-12	180	183
-11	120	176
-10	391	460
-9	761	767
-8	1111	1096
-7	1697	1544
-6	939	849
-5	1260	1161
-4	1231	993
-3	1184	1000
-2	1991	1706
-1	774	806
0	701	731
1	1200	953
2	1937	1531
3	2264	1932
4	870	823
5	1225	1156
6	712	586
7	312	256
8	403	403
9	888	875
10	587	572
11	883	697
12	180	196
13	127	110
14	140	222
15	224	265
16	220	242
17	94	94
18	104	136

3,5,L

-19	127	174
-18	134	139
-17	189	205
-16	169	153
-15	199	160
-14	429	464
-13	315	353
-12	621	611
-11	409	424
-10	761	770
-9	735	714
-8	877	812
-7	916	863
-6	691	695
-5	678	535
-3	350	266
-2	1446	1351
-1	165	301
0	701	672
1	351	256
2	733	642
3	1953	1755
4	993	667
5	958	961
6	471	468
7	700	698
8	469	365
9	275	197
10	590	540
11	160	144
12	593	554

5,5,L

-19	112	136
-18	194	238
-17	194	168
-16	169	240
-15	199	268
-14	258	276
-13	339	339
-12	134	160
-11	723	757
-10	297	312
-9	597	674
-8	663	663
-7	738	755
-6	648	840
-5	704	718
-4	565	606
-3	169	208
-2	424	410
-1	775	751
0	418	288
1	1608	1625
2	1049	973
3	692	920
4	1505	1292
5	379	373
6	603	651
7	823	794
8	540	517
9	540	629
10	240	264
11	457	463
12	150	135
13	297	340
14	158	179
15	140	151
16	112	113

7,5,L

-19	112	162
-18	140	146
-17	261	242
-16	120	145
-15	268	241
-14	127	85
-13	94	144
-12	251	281
-11	437	400
-10	677	659
-9	232	196
-8	914	944
-7	667	878
-6	491	491
-5	1041	970
-4	649	606
-3	1003	1083
-2	339	366
-1	1075	1143
0	1624	1430
1	983	988
2	569	645
3	859	802
4	493	552
5	416	395
6	437	448
7	440	413
8	563	534
9	489	492
10	367	417

11,5,L

0	540	576
1	398	424
2	275	240

7,5,L

11	251	245
12	120	117
13	175	205
14	169	185
15	73	77

9,5,L

-19	104	121
-18	94	82
-17	153	191
-16	372	416
-15	194	173
-14	254	242
-13	203	205
-12	587	598
-11	494	516
-10	364	397
-9	673	730
-8	437	427
-7	345	343
-6	278	244
-5	370	391
-4	936	904
-2	750	744
-1	436	515
0	496	906
1	337	243
2	608	792
3	845	812
4	537	646
5	387	348
6	355	354
7	203	212
8	323	369
9	94	[illegible]
10	275	315
11	84	124
12	147	197

11,5,L

-17	112	140
-16	120	174
-15	240	244
-14	216	257
-13	435	439
-12	303	343
-11	94	96
-10	294	259
-9	569	542
-8	644	652
-7	405	390
-6	437	455
-5	112	159
-4	379	343
-3	823	819
-2	550	503
-1	635	734
3	232	246
4	441	423
5	127	145
6	240	237
7	254	304
8	194	225
10	216	221
11	232	293

13,5,L

-18	73	[illegible]
-17	150	224
-16	194	156
-15	134	203
-14	120	142
-13	291	306
-12	312	343
-11	254	275
-10	409	429
-9	224	197
-8	140	144
-7	392	392
-6	445	477
-5	435	479
-4	306	117
-3	175	173
-2	334	332
-1	284	396
0	254	240
1	339	399
2	275	429
3	203	254
4	495	251
5	251	512
6	244	251
7	164	247

15,5,L

-17	158	186
-16	68	76
-15	94	97
-14	169	172
-13	278	307
-12	128	147
-11	175	193
-10	328	352
-9	236	249
-8	127	120
-7	407	449
-6	447	453
-5	319	334
-3	168	186
-2	268	345
-1	158	186
0	228	252
1	309	356
2	247	286
3	128	127
4	148	162
5	153	134

17,5,L

-11	153	168
-10	251	292
-9	112	107
-8	128	218
-7	128	142
-6	169	152
-5	240	268
-4	208	194
-3	224	220
-2	84	102
-1	213	221
2	288	244
3	169	186
4	153	165
5	112	104

0,6,L

1	968	1031
2	816	956
3	994	1002
4	628	61
5	234	256
6	1226	1174
7	906	986
8	433	436
9	414	368
10	364	326
11	414	418
12	564	608
13	272	304
14	262	235
15	165	176
16	42	[illegible]
17	77	100
18	133	118

2,6,L

-19	91	[illegible]
-18	236	196
-17	179	187
-16	190	186
-15	163	166
-14	73	57
-13	272	276
-12	364	420
-11	778	769
-10	729	742
-9	231	242
-8	524	512
-7	313	272
-6	752	768
-5	679	548
-4	1536	1408
-3	1437	1268
-2	344	358
-1	542	542
0	1055	1124
1	994	1062
2	324	336
3	349	468
4	1162	117
5	653	656
6	1219	1103
7	916	817
8	665	728
9	425	432
10	293	278
11	344	324
12	126	124
13	297	306
14	332	347
15	196	196
16	42	57
17	267	174

4,6,L

-19	146	121
-18	114	114
-17	216	221
-16	239	246
-15	264	254
-14	218	230
-13	73	114
-12	355	342
-11	528	554
-10	564	571
-9	719	755
-8	516	516
-7	538	495
-6	216	248
-5	784	791
-4	959	993
-3	1129	1091
-2	821	764
-1	1243	1292
0	798	682
1	777	881
2	1230	1226
3	658	664
4	893	946
5	543	469
6	954	951
7	334	310
8	496	495
9	639	678
10	745	771
11	179	134
12	73	100
13	206	207
14	241	274
15	152	165
16	165	171
17	97	74

6,6,L

-19	142	154
-18	73	82
-17	59	84
-16	149	197
-15	307	313
-14	355	363
-13	524	532
-12	277	245
-11	218	287
-10	249	240
-9	694	698
-8	318	342
-7	456	516
-6	347	490
-5	557	483
-4	574	694
-3	679	668
-2	1317	1274
-1	735	744
0	527	458
1	526	517
2	514	515
3	265	261
4	666	680
5	1324	1310
6	522	500
7	172	179
8	122	144
9	471	457
10	267	211
11	265	313
12	239	294
13	242	270
14	136	68
15	80	81
16	131	134

8,6,L

-19	148	137
-18	201	196
-17	262	258
-16	195	194
-15	166	121
-14	288	297
-13	349	384
-12	474	592
-11	429	459
-10	546	598
-9	416	367
-8	545	633
-7	342	427
-6	735	751
-5	393	287
-4	762	728
-3	256	325
-2	286	305
-1	606	651
0	744	819
1	570	697
2	753	779
3	476	438
4	461	445
5	815	551
6	326	275
7	373	423
8	341	304
9	193	72
10	140	56
11	177	160
12	288	255
14	126	145

10,6,L

-19	149	115
-18	165	112
-17	398	295
-16	146	197
-15	214	247
-14	323	342
-13	73	144
-12	272	251
-11	622	691
-10	548	622
-9	344	423
-8	365	312
-7	466	477
-6	273	267
-5	769	874
-4	433	444
-3	543	492
-2	142	142

10,6,L

-1	42	21
0	522	543
1	829	812
2	580	590
3	293	276
4	181	144
5	276	238
6	272	266
7	557	471
8	276	267
9	181	141
10	47	77
11	80	84
12	170	192
13	144	119

12,6,L

-18	213	208
-17	219	192
-16	226	199
-15	167	153
-14	135	96
-13	48	74
-12	44	61
-11	170	199
-10	412	424
-9	375	399
-8	378	379
-7	150	131
-6	432	425
-5	333	293
-4	268	257
-3	415	467
-2	471	476
-1	372	356
0	283	284
1	286	267
2	386	403
3	387	383
4	167	152
5	100	182
6	172	180
7	65	80
8	161	151
9	225	214
10	226	193
11	80	76

14,6,L

-17	105	106
-16	170	159
-15	197	181
-14	371	306
-13	84	71
-12	40	69
-11	211	164
-10	304	286
-9	266	247
-8	456	476
-7	262	254
-6	193	173
-5	296	276
-4	177	176
-3	246	216
-2	226	216
-1	540	546
0	161	143
1	124	96
2	490	451
3	65	57
4	106	132
5	184	198
6	211	212
7	142	96
8	148	135
9	116	119

16,6,L

-16	84	83
-15	135	126
-14	165	187
-13	80	77
-12	327	239
-11	162	147
-10	45	43
-9	267	266
-8	119	126
-7	405	353
-6	237	222
-5	122	143
-4	110	142
-3	122	144
-2	240	252
-1	314	277
0	165	137
1	236	202
2	126	102
3	94	116
4	224	125
5	195	143
6	109	104
7	133	133

18,6,L

-13	114	123
-12	193	173
-11	202	194
-10	140	109
-9	144	114
-8	177	155
-7	47	46
-6	257	198
-5	182	150
-4	256	235
-3	42	64

-2	59	79
-1	138	144
0	131	121
1	214	167
2	211	159
3	131	98

20,6,L

-10	146	172
-9	148	127
-8	142	126
-7	163	159
-6	135	89
-5	126	95
-4	144	116
-3	134	158
-2	166	135
-1	61	69

1,7,L

-18	49	94
-17	36	82
-16	218	281
-15	167	166
-14	356	365
-13	320	303
-12	51	79
-11	522	491
-10	265	264
-9	451	456
-8	314	295
-7	1254	1328
-6	553	529
-5	240	248
-4	645	653
-3	640	644
-2	573	651
-1	257	240
0	261	291
1	342	343
2	633	604
3	1151	1242
4	451	428
5	951	1015
6	49	37
7	252	229
8	474	449
9	404	371
10	841	865
11	259	260
12	201	136
13	126	110
14	124	114
15	228	254
16	43	97
17	202	182

3,7,L

-18	157	156
-17	126	120
-16	46	35
-15	363	405
-14	247	218
-13	325	288
-12	256	247
-11	451	477
-10	314	308
-9	252	270
-8	587	558
-7	251	245
-6	734	860
-5	154	187
-4	531	481
-3	1006	933
-2	208	206
-1	854	770
0	541	523
1	844	773
2	1109	1060
3	347	322
4	1274	1196
5	457	496
6	654	647
7	254	220
8	116	134
9	173	166
10	407	385
11	337	311
12	317	295
13	356	358
14	167	170
15	86	104
16	232	168
17	125	137

5,7,L

-19	157	175
-18	56	78
-17	186	194
-16	114	103
-15	232	201
-14	347	366
-13	253	263
-12	776	778
-11	236	221
-10	511	465
-9	285	280
-8	157	162
-7	767	808
-6	43	118
-5	284	291
-4	335	331
-3	1085	1088
-2	836	865
-1	456	498
0	646	627
1	555	587

2	396	363
3	645	645
4	346	338
5	490	454
6	544	538
7	787	777
8	346	405
9	489	466
10	354	367
11	183	175
12	360	333
13	233	239
14	237	221
15	89	115
16	93	95

7,7,L

-18	210	178
-17	103	76
-16	281	316
-15	51	42
-14	51	46
-13	200	155
-12	145	180
-11	585	621
-10	539	568
-9	776	757
-8	147	141
-7	359	361
-6	480	505
-5	364	380
-4	440	456
-3	179	145
-2	768	784
-1	438	423
0	938	945
1	1234	1267
2	116	119
3	768	723
4	103	115
5	412	430
6	554	557
7	51	70
8	375	266
9	262	262
10	355	353
11	51	32
12	93	98
13	230	237
14	46	57
15	144	174

9,7,L

-18	93	87
-17	289	277
-16	69	64
-15	268	214
-14	379	459
-13	387	413
-12	51	32
-11	237	245
-10	390	413
-9	215	212
-8	687	726
-7	529	490
-6	378	363
-5	44	52
-4	118	122
-3	732	752
-2	562	553
-1	381	385
0	224	159
1	316	290
2	617	798
3	379	360
4	762	775
5	228	202
6	548	567
7	159	144
8	139	147
9	352	348
10	132	149
11	142	129

11,7,L

-18	141	138
-17	155	143
-16	144	140
-15	146	107
-14	317	335
-13	115	117
-12	289	272
-11	200	187
-10	233	221
-9	659	699
-8	244	196
-7	646	638
-6	277	288
-5	325	289
-4	573	537
-3	242	267
-2	445	460
-1	529	514
0	533	619
1	51	53
2	219	180
3	348	361
4	312	287
5	353	358
6	187	169
7	134	122
8	144	170
9	44	45
10	197	193
11	116	151
12	141	167

13,7,L

-16	167	156
-15	167	172
-14	93	94
-13	344	379
-12	73	67
-11	360	385
-10	73	101
-9	165	179
-8	370	342
-7	302	296
-6	261	191
-5	199	224
-4	520	526
-3	73	33
-2	340	346
-1	500	519
0	51	47
1	159	149
2	349	335
3	51	72
4	146	148
5	185	172
6	221	235
7	85	45
8	177	162
9	44	29

15,7,L

-15	109	108
-14	36	19
-13	44	48
-12	230	231
-11	204	156
-10	245	226
-9	51	28
-8	230	249
-7	321	302
-6	123	129
-5	375	390
-4	103	79
-3	139	133
-2	195	159
-1	171	165
0	270	295
1	166	171
2	181	178
3	44	42
4	224	193
5	153	144
6	73	114
7	157	144

17,7,L

-14	109	97
-13	36	68
-12	34	47
-11	36	78
-10	134	139
-9	281	259
-8	63	48
-7	121	114
-6	69	58
-5	44	31
-4	193	225
-3	186	150
-2	226	223
-1	44	34
0	171	143
1	216	164
2	36	59
3	291	235

19,7,L

-11	144	146
-10	153	133
-9	36	21
-8	221	223
-7	132	122
-6	134	98
-5	112	115
-4	73	34
-3	159	156
-2	36	58
-1	185	145

0,8,L

1	674	903
2	574	715
3	472	487
4	347	353
5	880	813
6	435	487
7	555	566
8	352	377
9	620	639
10	141	151
11	315	315
12	447	424
13	127	43
14	276	242
15	73	72
16	67	43
17	154	176

2,8,L

-18	147	142
-17	60	48
-16	219	253
-14	79	47
-13	280	270
-11	426	433
-10	255	248
-9	783	723
-8	237	224
-7	808	720
-6	932	896
-5	349	333
-4	671	687
-3	534	536
-2	306	405
-1	495	596
0	116	167
1	1076	1053
2	389	452
3	612	452
4	277	307
5	506	512
6	575	553
7	966	983
9	257	286
10	131	97
11	147	186
13	345	316
14	73	80
15	235	245
16	127	163

4,8,L

-17	195	247
-16	85	61
-15	249	275
-13	244	247
-12	134	107
-11	301	425
-10	601	584
-9	253	202
-8	400	374
-7	475	437
-6	442	434
-5	685	635
-4	471	395
-3	735	687
-2	394	374
-1	671	654
0	443	453
1	167	197
2	1078	1317
3	355	337
4	981	1037
5	435	424
6	464	443
7	521	497
8	354	387
9	437	397
10	269	273
11	293	256
12	163	138
13	153	157
14	129	97
15	59	72
16	211	23

6,8,L

-17	68	7
-16	227	227
-15	52	67
-14	309	317
-12	225	247
-11	237	266
-10	116	155
-9	516	545
-8	193	157
-7	1069	1112
-6	251	281
-5	559	537
-4	672	654
-3	221	234
-2	654	664
-1	275	217
0	546	497
1	473	437
2	369	394
3	766	797
4	351	367
5	571	557
6	416	437
7	555	577
8	225	177
10	452	457
11	59	77
12	149	167
13	153	167

8,8,L

-18	127	136
-16	136	147
-15	145	217
-13	367	377
-12	314	347
-11	564	52
-10	264	194
-9	214	19
-8	618	617
-7	127	74
-6	442	427
-5	221	197
-4	645	647
-3	127	127
-2	324	255
-1	627	637
0	368	326
1	718	806
2	336	287
3	582	584
4	419	407
5	331	283
6	455	472
7	225	201
8	134	117
9	85	67
11	251	243
12	67	87

13	176	173

10,8,L

-18	98	122
-17	244	267
-16	95	128
-15	178	170
-14	112	101
-13	246	221
-12	465	510
-10	340	348
-8	341	364
-7	418	422
-6	304	308
-5	928	949
-4	199	179
-3	227	142
-2	164	153
0	249	300
1	526	524
2	475	484
3	144	134
4	233	225
5	342	294
6	147	94
7	248	254
8	112	97
9	257	273
11	40	49
12	163	175

12,8,L

-16	145	157
-15	104	107
-14	137	146
-13	52	59
-12	79	82
-11	301	296
-10	328	327
-9	242	229
-7	145	82
-6	298	194
-5	412	397
-4	525	520
-3	319	320
-2	476	490
-1	160	164
0	190	201
1	361	364
3	354	367
5	257	236
6	174	144
7	172	154
8	253	251
9	42	57
10	127	126

14,8,L

-16	79	101
-15	167	162
-14	47	26
-13	217	212
-12	104	113
-10	240	248
-9	79	107
-8	441	465
-7	164	154
-6	167	161
-5	253	224
-4	296	159
-3	231	194
-2	120	121
-1	249	249
0	144	114
1	163	149
2	341	356
3	164	134
4	264	313
6	60	40
7	52	45
8	42	30

16,8,L

-14	126	79
-13	116	154
-12	149	168
-10	201	176
-9	275	261
-8	141	112
-7	265	258
-6	124	124
-5	359	353
-4	154	136
-2	217	221
-1	131	111
0	251	221
2	119	122
3	164	114
5	147	159

18,8,L

-11	242	220
-9	45	95
-8	47	43
-5	258	244
-4	262	194
-3	47	47
-2	47	119
-1	45	119
0	40	60
1	104	134

1,9,L

-17	194	147
-16	170	147

1,9,L

-15	119	137
-14	256	263
-13	228	214
-12	233	235
-11	445	409
-10	415	384
-9	611	576
-8	452	499
-7	766	746
-6	287	277
-5	323	282
-4	612	579
-3	559	565
-2	486	589
-1	336	423
0	583	436
1	478	569
2	747	761
3	604	918
4	302	253
5	476	444
6	233	199
7	383	402
8	212	207
9	500	551
10	499	525
11	239	218
12	97	94
13	97	71
14	85	82
15	147	144
16	120	121

3,9,L

-15	220	228
-14	260	272
-13	325	354
-12	270	245
-11	443	432
-10	226	212
-9	85	65
-8	815	851
-7	516	583
-6	634	787
-5	126	151
-4	327	298
-3	412	382
-2	315	299
-1	697	823
0	767	808
1	786	876
2	328	305
3	241	180
4	751	747
5	665	652
6	239	256
7	334	320
10	191	192
11	297	271
12	217	219
13	205	218
15	135	148

5,9,L

-17	205	197
-15	130	108
-14	110	76
-13	163	139
-12	392	412
-11	378	403
-10	327	316
-9	218	175
-8	473	438
-7	366	356
-6	660	626
-5	734	711
-4	343	326
-3	382	394
-2	135	120
-1	159	141
0	567	490
1	419	355
2	431	472
3	212	144
5	470	488
6	224	227
7	590	626
8	267	269
9	405	419
10	187	180
12	253	284
13	157	189
14	130	106

7,9,L

-17	110	113
-16	181	198
-15	203	195
-14	125	105
-13	130	122
-12	299	318
-11	425	383
-10	338	354
-9	518	492
-8	177	197
-7	147	82
-6	544	552
-5	619	445
-4	596	550
-3	565	575
-2	632	641
-1	283	263
0	240	228
1	811	889
2	674	686
3	476	490
4	470	473
5	274	286
6	174	196
7	141	223
8	367	356
9	246	252
10	152	159
11	49	33
12	69	68
13	194	182

9,9,L

-17	200	203
-16	155	113
-15	231	230
-14	184	189
-13	223	222
-11	92	90
-10	415	398
-9	471	502
-8	512	527
-7	143	198
-6	458	524
-5	243	250
-4	346	331
-3	543	572
-2	544	540
-1	316	248
0	435	378
1	410	475
2	346	431
3	417	440
4	453	494
5	72	96
6	141	207
8	47	89
9	257	291
10	244	263
11	152	162

11,9,L

-17	55	64
-16	36	73
-15	125	119
-14	241	248
-13	251	225
-12	258	247
-10	231	223
-9	352	373
-8	233	271
-7	510	490
-6	358	374
-5	446	510
-4	427	374
-3	278	235
-2	349	321
-1	311	278
0	441	451
2	251	288
3	243	255
4	147	193
5	177	191
6	177	171
7	170	144
8	175	106
9	46	86
10	46	123

13,9,L

-16	155	157
-15	110	114
-14	141	198
-13	144	146
-12	155	109
-11	315	298
-10	141	161
-9	156	136
-8	143	142
-7	135	142
-6	249	265
-5	117	196
-4	210	273
-3	92	124
-2	92	135
-1	214	244
0	96	112
1	243	309
2	149	139
3	177	170
4	135	111
5	214	159
6	77	196
7	62	69

15,9,L

-12	217	199
-11	217	205
-10	236	178
-9	66	66
-8	265	241
-7	153	151
-6	152	119
-5	236	189
-4	177	201
-3	134	81
-2	55	48
1	38	134
2	147	168
3	147	105
4	98	140
5	130	125
6	115	56

17,9,L

-12	104	107
-10	110	83
-9	181	164
-8	89	101
-7	177	180
-5	139	138
-4	104	134
-3	125	126
-2	125	116
-1	120	115
0	110	114
1	104	108
2	85	90

0,10,L

1	426	570
2	386	414
3	483	482
4	693	745
5	332	273
6	410	423
7	332	273
8	303	235
9	254	216
11	391	392
12	228	221
13	225	229
14	78	56

2,10,L

-16	61	137
-15	185	200
-14	153	212
-13	66	132
-12	342	346
-11	172	155
-10	223	208
-9	352	268
-8	402	400
-7	410	355
-6	411	412
-5	522	467
-4	554	576
-3	543	557
-2	202	153
-1	61	100
0	663	733
1	639	650
2	487	452
3	433	385
4	513	457
5	313	311
6	111	68
7	464	454
8	594	575
9	273	264
10	61	43
11	239	217
12	268	250
13	141	164
14	223	282

4,10,L

-17	127	156
-16	132	150
-15	199	222
-14	196	157
-13	127	104
-11	422	403
-10	223	208
-9	618	619
-8	117	89
-7	239	199
-6	199	148
-5	740	737
-4	261	261
-3	682	582
-2	641	566
-1	584	580
0	239	250
1	504	473
2	256	273
3	619	582
4	413	453
5	578	540
6	515	512
7	179	123
8	354	329
9	205	179
10	340	355
12	78	127
13	145	164
14	66	134

6,10,L

-16	111	124
-15	247	273
-14	256	263
-13	153	140
-11	446	435
-9	411	388
-8	442	465
-7	205	234
-6	799	760
-5	622	657
-4	105	82
-3	663	706
-2	889	846
-1	408	389
0	397	345
2	794	831
3	271	318
4	117	140
5	938	979
6	537	518
7	239	247
8	157	156
9	157	160
10	141	137
11	199	218
12	161	148

8,10,L

-17	132	128
-16	179	159
-14	228	179
-13	172	190
-12	294	274
-10	356	409
-9	366	361
-8	468	448
-7	772	773
-6	632	696
-5	186	192
-4	396	410
-3	590	590
-2	841	916
-1	319	278
0	301	300
1	474	495
2	247	214
3	217	211
4	241	217
5	478	544
6	183	227
7	127	116
8	86	76
10	169	143
11	117	113
12	117	135

10,10,L

-16	169	157
-15	93	97
-14	132	154
-13	247	236
-12	86	100
-11	359	359
-10	214	254
-9	183	172
-8	275	236
-7	183	199
-6	301	245
-5	477	464
-4	347	394
-2	228	207
-1	220	205
0	256	269
1	439	435
2	234	234
3	93	89
4	93	96
5	254	271
6	247	270
7	202	159
8	132	171
10	117	153

12,10,L

-15	61	86
-14	61	90
-13	49	48
-12	141	143
-11	169	114
-10	231	149
-9	261	244
-8	340	290
-7	241	148
-6	389	345
-5	275	242
-3	319	330
-2	368	345
-1	225	205
0	205	229
2	254	238
3	165	155
4	145	158
5	78	30
6	105	118
7	236	259
8	99	128

14,10,L

-14	153	141
-13	61	32
-12	122	109
-11	132	140
-10	211	159
-9	340	303
-8	293	251
-7	122	69
-6	136	75
-5	176	144
-3	214	193
-4	196	191
-2	172	149
-1	229	250
0	179	144
1	165	111
2	169	154
3	157	195
4	78	108
5	122	172
6	117	144

16,10,L

-12	199	195
-9	117	129
-8	286	307
-7	179	138
-6	149	149
-5	169	190
-4	117	87
-3	127	130
-2	127	132
-1	157	183
0	179	146
1	61	87
2	111	117

1,11,L

-15	190	210
-14	130	163
-13	206	234
-10	253	276
-9	80	82
-8	509	511
-7	326	304
-6	235	257
-5	593	545
-4	339	354
-3	523	589
-2	92	140
-1	403	496
0	469	472
1	216	227
2	273	264
3	410	464
4	789	790
5	292	304
6	172	155
7	88	104
8	172	183
9	378	414
10	113	122
11	333	320
14	146	122
15	92	127

3,11,L

-14	235	259
-13	80	54
-12	190	217
-11	113	125
-10	196	222
-9	352	344
-8	216	195
-7	469	466
-6	345	386

9,11,L

-9	113	94
-7	89	84
-6	345	297
-5	296	267
-4	330	314
-3	364	332
-2	480	424

5,11,L

-13	235	231
-12	160	100
-11	330	304
-10	196	216
-9	336	337
-8	450	466
-7	211	193
-6	558	614
-5	253	194
-4	221	216
-3	216	167
-2	179	172
-1	296	287
0	261	237
1	658	574
2	729	697
3	573	594
4	694	747
5	80	91
6	248	214
7	196	153
8	299	325
9	160	186
10	89	77
11	240	265
12	65	57
13	172	187

7,11,L

-12	190	177
-11	196	159
-10	323	334
-9	80	40
-8	316	362
-7	190	170
-6	512	474
-5	269	294
-4	431	374
-3	774	723
-2	497	500
-1	558	591
0	918	897
1	590	670
2	466	416
3	320	492
4	296	258
5	140	192
6	80	81
7	389	416
8	80	54
9	253	266

9,11,L

-14	153	201
-13	65	29
-12	103	152
-11	190	194
-10	140	111
-9	546	515

3,11,L

-5	558	633
-4	459	513
-3	80	80
-2	661	682
-1	333	333
0	537	613
1	46	77
2	113	124
3	261	273
4	92	139
5	389	394
6	352	323
7	206	229
8	160	143
9	80	75
10	160	150
11	153	133
12	271	233

9,11,L

-1	221	227
0	248	282
1	211	152
2	138	133
3	342	376
4	122	97
5	196	261
6	190	172
7	130	134
8	216	244
9	62	131
10	198	296

11,11,L

-15	146	152
-14	65	44
-13	291	220
-12	135	140
-11	130	55
-10	323	297
-9	113	136
-8	372	315
-7	80	91
-6	535	598
-5	253	218
-4	80	135
-3	323	248
-2	196	178
-1	471	417
0	80	66
2	206	265
4	194	170
6	253	282
7	92	116

13,11,L

-14	153	137
-13	46	74
-12	172	170
-11	92	87
-10	172	186
-9	248	270
-8	146	114
-7	339	315
-6	130	92
-5	241	265
-4	221	186
-3	80	59
-2	248	279
-1	50	60
0	144	193
1	103	120
2	160	160
3	65	78
5	179	214

15,11,L

-10	201	91
-9	92	93
-8	92	41
-7	65	68
-6	211	198
-5	65	70
-4	190	143
-3	92	79
-2	92	109
-1	55	63
0	65	48
1	221	227

Table 7.3.

Final positional parameters for the non-hydrogen atoms, as fractions of the unit-cell edges. Standard deviations are in parentheses.

Atom	x	y	z
Se(1)	0.00000(000)	0.00000(000)	0.00000(000)
Se(2)	0.30867(18)	0.36800(33)	-0.41931(18)
O(1')	0.07971(162)	0.06393(217)	-0.00880(201)
O(2')	0.01404(181)	-0.05968(193)	-0.08665(145)
O(3)	0.32084(129)	0.35986(243)	-0.32481(128)
O(4)	0.21161(136)	0.36332(247)	-0.41200(146)
O(5)	0.36140(191)	0.28764(225)	-0.48614(170)
O(6)	0.34678(180)	0.47362(255)	-0.46290(174)
O(1)	0.11834(166)	0.56030(194)	0.11625(146)
C(2)	0.12202(217)	0.59430(263)	0.03406(229)
C(3)	0.16246(148)	0.52353(206)	-0.03841(169)
C(4)	0.24377(174)	0.48838(261)	-0.03706(193)
C(5)	0.27817(164)	0.41079(247)	-0.10269(184)
C(6)	0.21536(178)	0.33772(216)	-0.09749(177)
C(7)	0.13400(171)	0.38002(219)	-0.09678(161)
O(8)	0.14632(112)	0.41888(147)	-0.18241(115)
C(9)	0.07533(170)	0.45209(193)	-0.19796(177)
C(10)	0.02933(143)	0.37613(203)	-0.23432(157)
C(11)	-0.00588(184)	0.43783(268)	-0.29581(224)
O(12)	0.05897(126)	0.50192(162)	-0.33262(124)

Table 7.3. (Continued).

Atom	x	y	z
C(13)	0.10123(157)	0.52174(180)	-0.27168(160)
O(14)	0.07484(114)	0.61012(141)	-0.22697(112)
C(15)	0.08763(155)	0.69066(191)	-0.28316(158)
C(16)	0.00961(157)	0.74763(212)	-0.24979(169)
C(17)	0.01683(153)	0.84027(193)	-0.30234(159)
C(18)	0.09309(150)	0.89392(173)	-0.29602(152)
C(19)	0.17268(152)	0.83581(174)	-0.32928(167)
C(20)	0.16184(157)	0.74432(209)	-0.27847(164)
N(21)	0.23653(137)	0.68925(172)	-0.31093(159)
C(22)	0.28587(155)	0.66509(233)	-0.26631(235)
N(23)	0.27855(196)	0.69637(241)	-0.18781(188)
N(24)	0.34701(164)	0.60093(270)	-0.30396(259)
O(25)	0.23929(117)	0.88688(164)	-0.31374(151)
N(26)	0.10249(143)	0.97997(156)	-0.35068(128)
C(27)	0.07451(191)	1.06346(219)	-0.31700(196)
N(28)	0.08266(213)	1.13330(198)	-0.31720(191)
N(29)	0.03707(181)	1.08005(190)	-0.23410(157)
O(30)	-0.05220(105)	0.89669(132)	-0.26775(122)
O(31)	-0.06074(117)	0.69986(143)	-0.25217(142)
C(32)	-0.02595(227)	0.38392(312)	-0.36750(235)
O(33)	0.08441(126)	0.30679(145)	-0.28383(133)
C(34)	-0.03671(171)	0.33029(227)	-0.16317(208)
N(35)	-0.02876(168)	0.24861(177)	-0.13698(179)

Table 7.3. (Continued).

Atom	x	y	z
O(36)	-0.09454(142)	0.22348(180)	-0.06614(160)
O(37)	0.10531(113)	0.44572(161)	-0.02955(125)
O(38)	0.29827(133)	0.56546(181)	-0.05180(174)
O(39)	0.34815(106)	0.36934(215)	-0.08904(138)
N(40)	0.25093(149)	0.27389(199)	-0.17204(156)
C(41)	0.20774(269)	0.18764(283)	-0.17428(287)
W(42)	0.14835(287)	0.81608(326)	-0.07284(256)
W(43)	0.31923(174)	0.98862(258)	-0.46313(169)
W(44)	0.42595(198)	0.14176(266)	-0.42315(231)
W(45)	0.75612(235)	0.14795(247)	-0.48542(170)

Table 7.4.

Final anisotropic thermal parameters (β_{ij}'s) for the non-hydrogen atoms, x 10^5. Standard deviations are in parentheses.

Atom	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
Se(1)	408(17)	490(31)	412(17)	000	185(14)	000
Se(2)	390(11)	597(21)	356(10)	74(15)	89(84)	-36(15)
O(1')	579(124)	775(208)	1081(198)	24(134)	512(134)	147(167)
O(2')	920(166)	588(186)	381(101)	-98(137)	98(104)	-21(108)
O(3)	447(92)	1182(231)	414(92)	-55(141)	119(75)	-228(144)
O(4)	502(101)	1045(215)	609(116)	-15(149)	286(91)	-123(159)
O(5)	909(172)	767(221)	555(131)	361(161)	43(119)	130(136)
O(6)	747(155)	1243(301)	588(139)	-292(174)	-1(116)	-383(166)
O(1)	773(144)	575(175)	448(108)	110(131)	132(100)	41(113)
C(2)	560(163)	412(238)	635(185)	112(157)	227(142)	128(166)
C(3)	260(91)	343(207)	404(119)	-6(98)	83(83)	-34(112)
C(4)	384(117)	499(233)	501(141)	88(137)	159(104)	-53(148)
C(5)	298(104)	607(237)	420(128)	7(120)	102(94)	-126(135)
C(6)	414(121)	402(233)	379(118)	2(113)	142(96)	-16(115)
C(7)	489(119)	168(184)	384(108)	-93(119)	160(92)	-83(117)
O(8)	358(77)	356(127)	361(81)	-31(78)	125(64)	-82(80)
C(9)	419(116)	123(168)	450(124)	17(102)	244(100)	-11(107)
C(10)	342(93)	89(159)	444(110)	54(104)	156(82)	67(116)
C(11)	340(119)	629(256)	626(175)	16(137)	222(120)	-3(167)
O(12)	558(97)	291(129)	480(94)	21(101)	254(79)	19(99)

Table 7.4. (Continued).

Atom	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(13)	375(106)	60(169)	363(108)	62(91)	71(85)	-27(94)
O(14)	415(82)	257(123)	336(80)	8(78)	82(65)	-52(78)
C(15)	361(104)	114(160)	353(110)	1(97)	131(86)	-48(100)
C(16)	314(100)	312(190)	387(116)	-6(105)	109(88)	-109(114)
C(17)	307(97)	283(201)	335(104)	84(94)	58(80)	-63(100)
C(18)	342(98)	63(169)	333(100)	16(87)	53(80)	-33(91)
C(19)	313(98)	100(174)	412(114)	3(86)	122(85)	-23(96)
C(20)	333(101)	284(182)	352(110)	3(103)	141(86)	-41(109)
N(21)	345(93)	176(148)	539(123)	80(90)	137(86)	5(105)
C(22)	207(91)	358(216)	860(210)	-77(104)	166(112)	-254(161)
N(23)	698(161)	715(241)	546(146)	-189(159)	372(128)	-121(149)
N(24)	283(107)	822(271)	1215(263)	132(140)	54(132)	-309(222)
O(25)	334(77)	413(162)	816(132)	-71(87)	251(83)	-40(115)
N(26)	513(109)	113(147)	274(85)	-8(92)	74(77)	30(81)
C(27)	510(141)	157(189)	526(150)	-6(123)	174(118)	-87(130)
N(28)	901(197)	219(191)	571(150)	123(146)	195(138)	37(128)
N(29)	677(147)	270(180)	365(111)	-52(121)	-100(101)	126(106)
O(30)	277(68)	229(133)	505(94)	113(67)	2(63)	40(81)
O(31)	369(82)	240(132)	662(119)	-132(79)	202(79)	-184(95)
C(32)	677(183)	653(297)	712(191)	-150(193)	439(161)	54(199
O(33)	486(93)	136(126)	551(107)	-5(85)	93(80)	68(91)
C(34)	302(109)	430(234)	582(158)	-31(111)	88(106)	39(139

Table 7.4. (Continued).

Atom	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
N(35)	541(127)	94(161)	607(143)	-76(105)	2(106)	-32(111)
O(36)	498(106)	455(165)	663(133)	-79(105)	-116(94)	-98(118)
O(37)	304(75)	554(155)	402(89)	-86(84)	51(66)	15(91)
O(38)	419(97)	510(167)	940(166)	-217(103)	333(105)	-185(133)
O(39)	240(66)	852(183)	661(113)	122(113)	100(70)	-147(146)
N(40)	397(105)	474(191)	426(114)	39(111)	67(88)	45(116)
C(41)	790(232)	306(259)	860(259)	18(190)	107(195)	-69(203)
W(42)	1559(321)	1415(378)	1059(252)	335(287)	591(242)	355(255)
W(43)	753(150)	1018(248)	610(138)	-131(175)	152(115)	-119(162)
W(44)	772(171)	1071(280)	1033(218)	-62(184)	355(158)	-98(197)
W(45)	1164(206)	1040(283)	423(132)	335(202)	42(131)	235(142)

Table 7.5.

Calculated positional parameters for the hydrogen atoms, as fractions of the cell edges.

Atom	x	y	z
H(1)-C(2)	0.159	0.660	0.021
H(2)-C(2)	0.061	0.612	0.032
H(3)-C(3)	0.171	0.557	-0.101
H(4)-C(4)	0.236	0.462	0.028
H(5)-C(5)	0.296	0.442	-0.168
H(6)-C(6)	0.207	0.300	-0.038
H(7)-C(7)	0.088	0.327	-0.088
H(8)-C(9)	0.034	0.478	-0.137
H(9)-C(11)	-0.062	0.478	-0.255
H(10)-C(13)	0.167	0.517	-0.305
H(11)-C(15)	0.097	0.670	-0.350
H(12)-C(16)	0.000	0.766	-0.182
H(13)-C(17)	0.024	0.826	-0.369
H(14)-C(18)	0.084	0.913	-0.229
H(15)-C(19)	0.183	0.821	-0.379
H(16)-C(20)	0.155	0.760	-0.211
H(17)-C(34)	-0.093	0.371	-0.132
H(18)-N(21)	0.252	0.667	-0.378
H(19)-N(23)	0.274	0.724	-0.124
H(20)-N(24)	0.395	0.553	-0.334
H(21)-N(26)	0.134	0.974	-0.420

Table 7.5. (Continued).

Atom	x	y	z
H(22)-N(28)	0.091	1.192	-0.415
H(23)-N(29)	0.006	1.093	-0.165
H(24)-N(40)	0.307	0.294	-0.224
H(25)-O(1)	0.115	0.534	0.180
H(26)-O(25)	0.290	0.927	-0.303
H(27)-O(30)	-0.106	0.941	-0.241
H(28)-O(31)	-0.116	0.663	-0.253
H(29)-O(33)	0.126	0.255	-0.321
H(30)-O(36)	-0.146	0.204	-0.010
H(31)-O(38)	0.340	0.625	-0.063
H(32)-O(39)	0.401	0.340	-0.077

Each hydrogen atom was assigned a temperature factor 0.5$\mathring{A}^2$ greater than the atom to which it is attached.

of $\Delta f''$ correct - see next section), converged to a final R value of 0.0862. The largest parameter shift was less than 0.1 of a standard deviation and refinement was judged to be complete. The final structure factors listed in Table 7.2 were calculated from the final atomic parameters in Tables 7.3 - 7.5.

7.5. Absolute Configuration.

The absolute configuration of the molecule was found by the anomalous dispersion method. The intensities of 27 Bijvoet pairs from the hk0 and hkl Weissenberg photographs were estimated visually, and were compared with those calculated from the final refined positional parameters, using values for the dispersion correction terms of $\Delta f' = -1.0$ and $\Delta f'' = 1.3$ for selenium and Cu-K_α radiation. Table 7.6 gives a comparison of the observed and calculated differences. All the pairs except one (that for the 550 reflection), indicate that the true absolute configuration is the reverse of that indicated by the co-ordinates then in use, when indexed on a right-handed system of axes. Therefore, the sign of the y co-ordinates for all atoms was reversed, to give Tables 7.3 and 7.5 corresponding to the correct absolute configuration for streptomycin oxime selenate.

After this switch, a check on this assignment was made by Hamilton's method [12]. R factors for the two absolute configurations were calculated. With the sign of $\Delta f''$ negative,

Table 7.6.

Comparison of the observed and calculated intensity ratios for the Bijvoet pairs examined.

h	k	ℓ	$I_{hk\ell}/I_{h\bar{k}\ell}$	$\lvert F_{hk\ell}\rvert^2/\lvert F_{h\bar{k}\ell}\rvert^2$
8	2	0	> 1	< 1
14	2	0	< 1	> 1
16	2	0	< 1	> 1
18	2	0	> 1	< 1
12	4	0	> 1	< 1
5	5	0	> 1	> 1
15	5	0	> 1	< 1
14	6	0	> 1	< 1
8	8	0	> 1	< 1
10	8	0	> 1	< 1
4	10	0	> 1	< 1
17	10	0	> 1	< 1
5	1	1	> 1	< 1
7	1	1	> 1	< 1
10	1	1	< 1	> 1
11	1	1	< 1	> 1
2	2	1	> 1	< 1
4	2	1	< 1	> 1
12	2	1	< 1	> 1
9	3	1	> 1	< 1
11	3	1	< 1	> 1
8	4	1	< 1	> 1
11	5	1	< 1	> 1
9	7	1	< 1	> 1
4	8	1	> 1	< 1
3	11	1	> 1	< 1
2	12	1	< 1	> 1

R_- was 0.0894. On reversing the sign, R_+ was 0.0862. The one-dimensional hypothesis, that the absolute configuration corresponding to $R_- = 0.0894$ was then tested:-

$$\mathscr{R} = \frac{0.0894}{0.0862} = 1.036$$

and $$\mathscr{R}_{1,2750,0.005} = 1.001$$

(from Table 1. of ref. 12).

Thus, the hypothesis has much less than 0.5% significance, and can be rejected. This result agrees with that obtained using the Bijvoet technique, and agrees with the chemical assignment[5,29].

7.6. Discussion of the Structure.

The intramolecular bonded distances and inter-bond angles are listed in Tables 7.7 and 7.8, together with their associated standard deviations, and are shown in Figures 7.1 - 7.3. The standard deviations are doubtless under-estimates since a block-diagonal approximation was used in the refinement. Figure 7.4 shows the [001] projection of a single molecule of streptomycin oxime.

The [001] projections of the two selenate ions are shown in Figure 7.5. Both are regular tetrahedra within experimental error. The average Se-O bond length is 1.631Å, and the average O-Se-O angle is 109.8°. These values agree well with those reported for the selenate ion in sodium selenate [13] (1.654(20)Å and 109.5(10)°), potassium selenate [13] (1.644(10)Å and

Table 7.7.

Intramolecular bonded distances (r) for the non-hydrogen atoms, with their estimated standard deviations in parenthesis.

Atoms	r(Å)	Atoms	r(Å)
Se(1)-O(1)'	1.613(29)	C(10)-C(11)	1.581(48)
Se(1)-O(2)'	1.593(25)	C(10)-O(33)	1.432(32)
Se(2)-O(3)	1.605(23)	C(10)-C(34)	1.492(36)
Se(2)-O(4)	1.629(24)	C(11)-O(12)	1.422(39)
Se(2)-O(5)	1.630(29)	C(11)-C(32)	1.516(58)
Se(2)-O(6)	1.714(34)	O(12)-C(13)	1.417(38)
O(1)-C(2)	1.396(46)	C(13)-O(14)	1.461(32)
C(2)-C(3)	1.542(44)	O(14)-C(15)	1.444(33)
C(3)-C(4)	1.486(42)	C(15)-C(16)	1.515(37)
C(3)-O(37)	1.462(35)	C(15)-C(20)	1.506(40)
C(4)-C(5)	1.524(46)	C(16)-C(17)	1.562(40)
C(4)-O(38)	1.419(42)	C(16)-O(31)	1.396(36)
C(5)-C(6)	1.485(45)	C(17)-C(18)	1.545(38)
C(5)-O(39)	1.413(39)	C(17)-O(30)	1.598(30)
C(6)-C(7)	1.515(44)	C(18)-C(19)	1.544(34)
C(6)-N(40)	1.485(37)	C(18)-N(26)	1.497(33)
C(7)-O(8)	1.444(33)	C(19)-C(20)	1.529(38)
C(7)-O(37)	1.406(34)	C(19)-O(25)	1.440(36)
O(8)-C(9)	1.397(37)	C(20)-N(21)	1.455(35)
C(9)-C(10)	1.561(42)	N(21)-C(22)	1.314(46)
C(9)-C(13)	1.512(37)	C(22)-N(23)	1.313(49)

Table 7.7. (Continued).

Atoms	r(Å)
C(22)-N(24)	1.385(43)
N(26)-C(27)	1.342(37)
C(27)-N(28)	1.309(43)
C(27)-N(29)	1.314(37)
C(34)-N(35)	1.268(42)
N(35)-O(36)	1.381(32)
N(40)-C(41)	1.448(51)

Table 7.8.

Interbond angles (θ) for the non-hydrogen atoms, in degrees. Estimated standard deviations are in parentheses.

Atoms	(θ)	Atoms	(θ)
O(1')-Se(1)-O(1")	110.6(1.5)	C(5)-C(6)-N(40)	105.7(2.2)
O(1')-Se(1)-O(2')	109.2(1.5)	C(7)-C(6)-N(40)	112.7(2.7)
O(1')-Se(1)-O(2")	106.1(1.6)	C(6)-C(7)-O(8)	106.2(2.0)
O(2')-Se(1)-O(2")	114.9(1.3)	C(6)-C(7)-O(37)	111.2(2.6)
O(3)-Se(2)-O(4)	110.3(1.1)	O(8)-C(7)-O(37)	114.0(2.6)
O(3)-Se(2)-O(5)	110.1(1.6)	C(7)-O(8)-C(9)	114.7(2.0)
O(3)-Se(2)-O(6)	109.4(1.6)	O(8)-C(9)-C(10)	113.3(2.4)
O(4)-Se(2)-O(5)	109.9(1.7)	O(8)-C(9)-C(13)	108.0(2.1)
O(4)-Se(2)-O(6)	109.8(1.7)	C(10)-C(9)-C(13)	103.1(2.4)
O(5)-Se(2)-O(6)	107.3(1.4)	C(9)-C(10)-C(11)	99.7(2.6)
O(1)-C(2)-C(3)	109.0(3.1)	C(9)-C(10)-O(33)	111.8(2.2)
C(2)-C(3)-C(4)	115.2(3.0)	C(9)-C(10)-C(34)	111.6(2.4)
C(2)-C(3)-O(37)	107.8(2.2)	C(11)-C(10)-O(33)	112.2(2.3)
C(4)-C(3)-O(37)	109.1(2.6)	C(11)-C(10)-C(34)	110.7(2.4)
C(3)-C(4)-C(5)	113.4(3.0)	O(33)-C(10)-C(34)	110.5(2.5)
C(3)-C(4)-O(38)	107.7(2.9)	C(10)-C(11)-O(12)	102.5(2.6)
C(5)-C(4)-O(38)	109.9(2.3)	C(10)-C(11)-C(32)	113.7(3.2)
C(4)-C(5)-C(6)	110.1(2.3)	O(12)-C(11)-C(32)	108.2(2.7)
C(4)-C(5)-O(39)	109.8(2.9)	C(11)-O(12)-C(13)	109.3(2.4)
C(6)-C(5)-O(39)	109.1(3.0)	C(9)-C(13)-O(12)	108.9(2.3)
C(5)-C(6)-C(7)	111.1(2.7)	C(9)-C(13)-O(14)	102.0(2.0)

Table 7.8 (Continued).

Atoms	(θ)	Atoms	(θ)
O(12)-C(13)-O(14)	111.4(2.3)	N(21)-C(22)-N(24)	118.3(3.3)
C(13)-O(14)-C(15)	114.4(1.8)	N(23)-C(22)-N(24)	117.8(3.4)
O(14)-C(15)-C(16)	105.1(1.9)	C(18)-N(26)-C(27)	121.7(2.1)
O(14)-C(15)-C(20)	109.6(2.5)	N(26)-C(27)-N(28)	117.6(2.7)
C(16)-C(15)-C(20)	111.5(2.4)	N(26)-C(27)-N(29)	124.4(3.0)
C(15)-C(16)-C(17)	110.3(2.0)	N(28)-C(27)-N(29)	118.0(3.1)
C(15)-C(16)-O(31)	112.8(2.5)	C(10)-C(34)-N(35)	120.0(2.6)
C(17)-C(16)-O(31)	109.0(2.5)	C(34)-N(35)-O(36)	111.0(2.5)
C(16)-C(17)-C(18)	108.4(2.4)	C(3)-O(37)-C(7)	112.1(1.9)
C(16)-C(17)-O(30)	111.0(1.9)	C(6)-N(40)-C(41)	116.9(2.5)
C(18)-C(17)-O(30)	107.3(2.2)		
C(17)-C(18)-C(19)	111.3(2.2)		
C(17)-C(18)-N(26)	108.6(2.4)		
C(19)-C(18)-N(26)	107.9(1.9)		
C(18)-C(19)-C(20)	109.7(1.9)		
C(18)-C(19)-O(25)	108.5(2.2)		
C(20)-C(19)-O(25)	108.0(2.5)		
C(15)-C(20)-C(19)	111.3(2.5)		
C(19)-C(20)-N(21)	108.9(2.0)		
C(15)-C(20)-N(21)	111.8(2.5)		
C(20)-N(21)-C(22)	126.1(2.6)		
N(21)-C(22)-N(23)	123.8(2.9)		

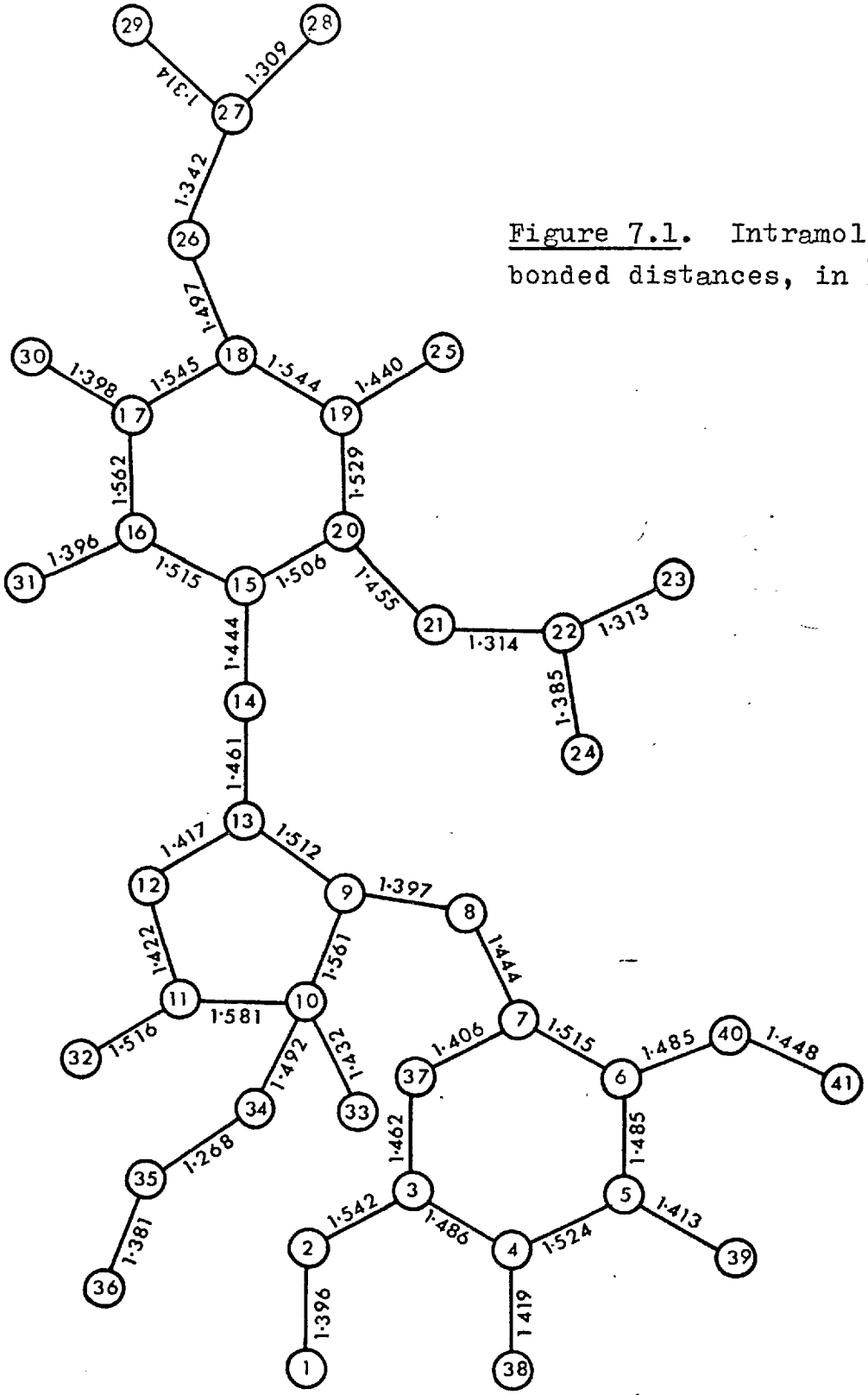


Figure 7.1. Intramolecular bonded distances, in Å.

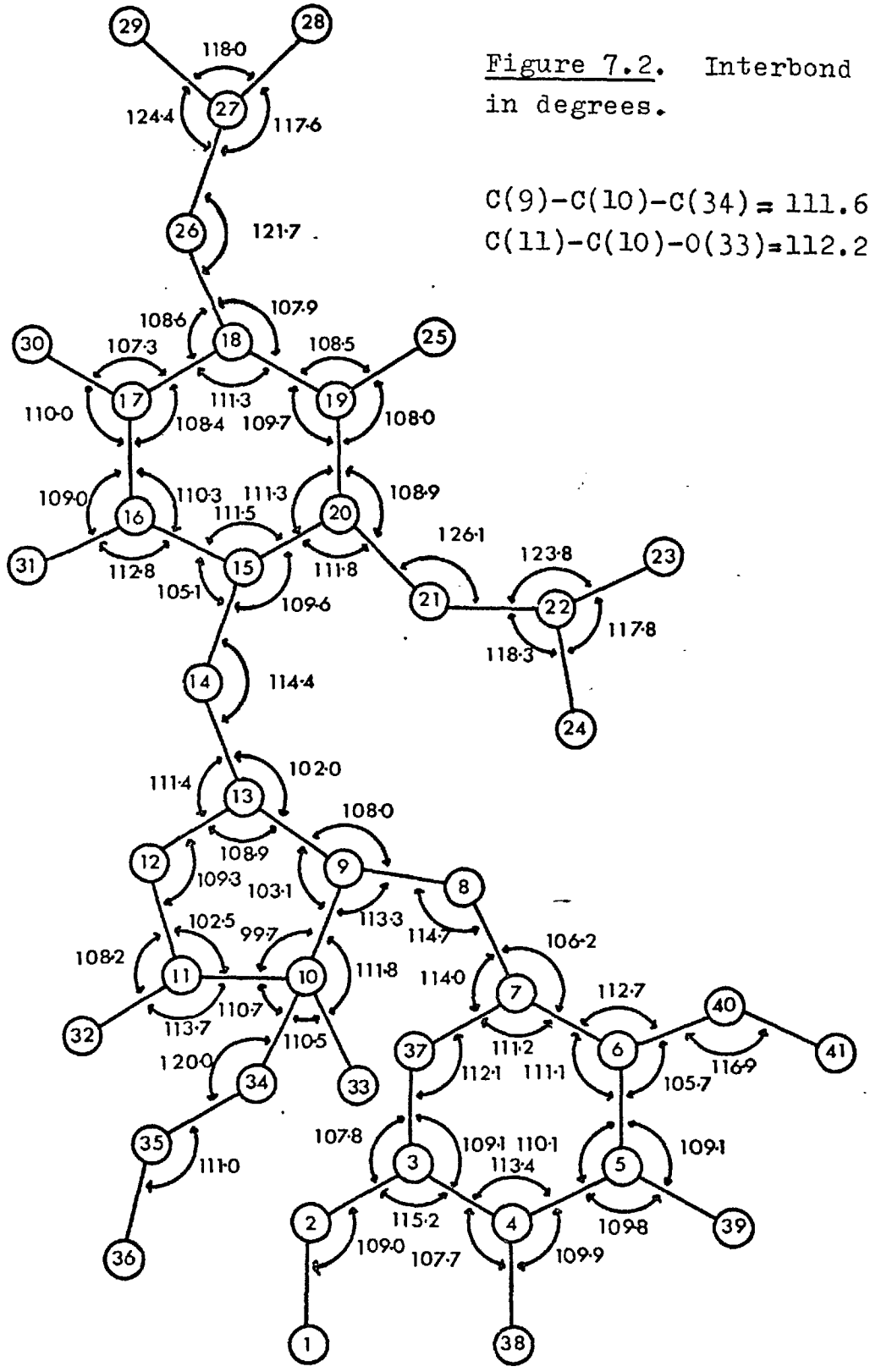
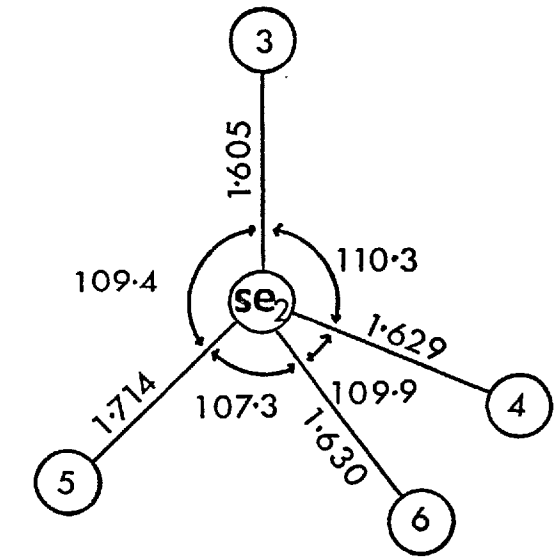
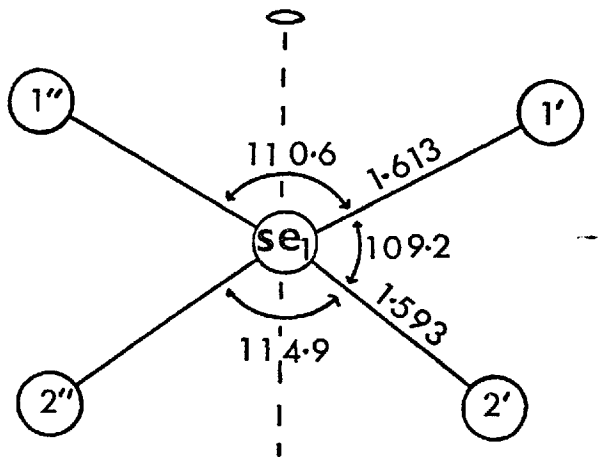


Figure 7.2. Interbond angle
in degrees.

C(9)-C(10)-C(34) = 111.6°
C(11)-C(10)-O(33)=112.2°



O(3)-Se(2)-O(5)=110.1°
O(4)-Se(2)-O(6)=109.8°



O(1′)-Se(1)-O(2″)=106.1°

Figure 7.3. Intramolecular distances and angles for the two selenate ions.

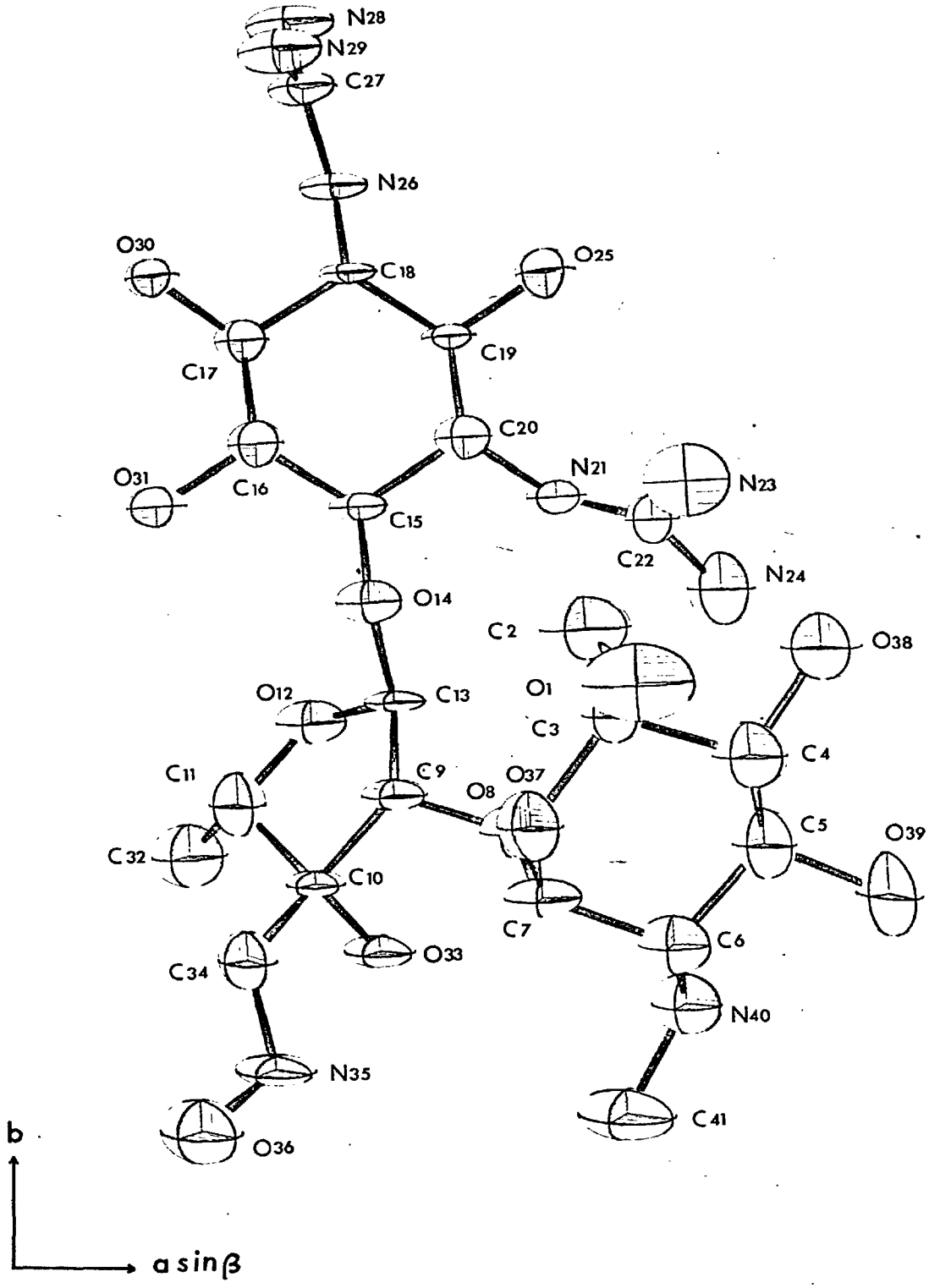


Figure 7.4. c-axis projection of a single molecule of streptomycin oxime. The thermal ellipsoids are scaled to include 50% probability.

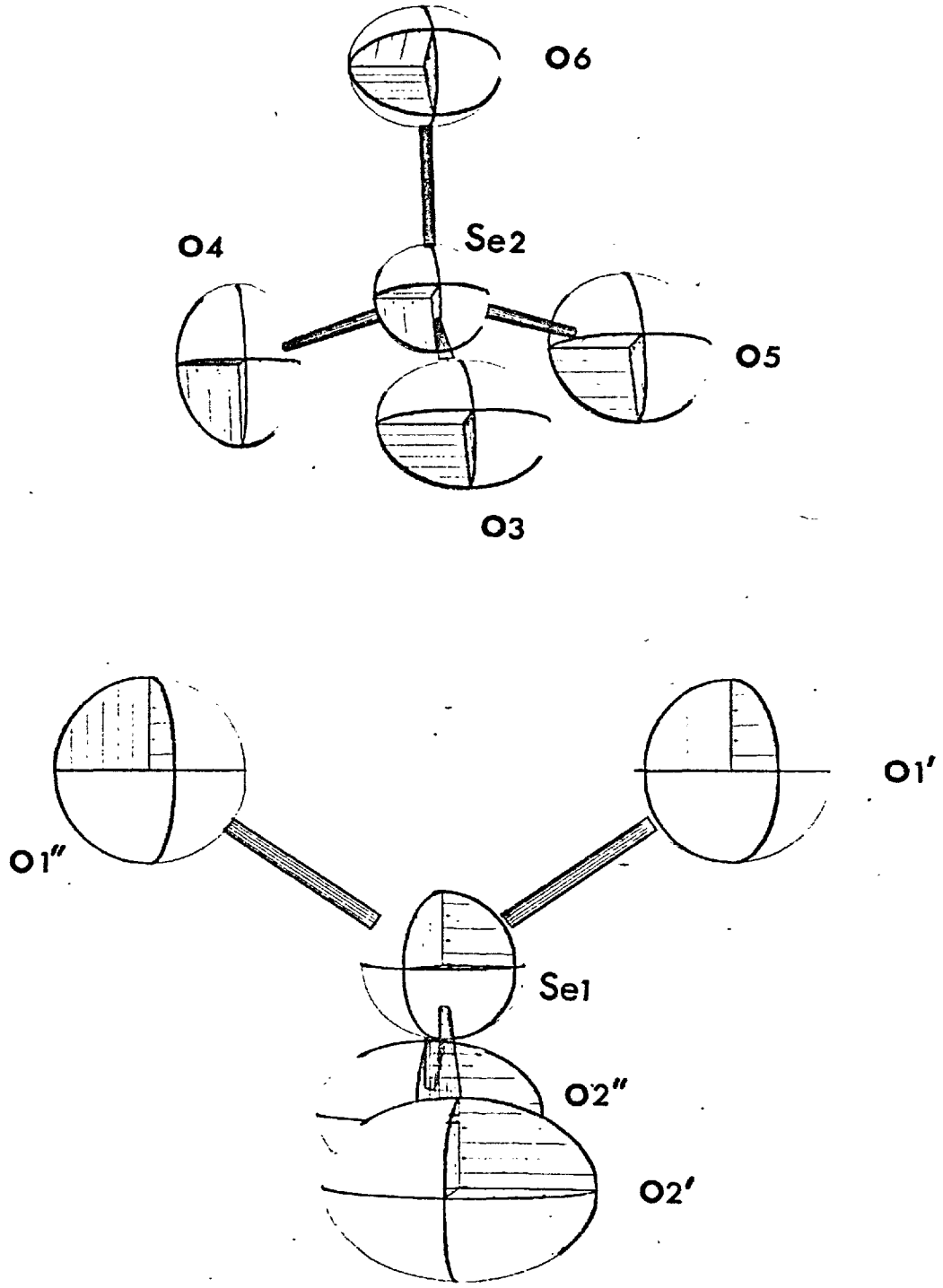


Figure 7.5. c-axis projections of the two selenate ions. The thermal ellipsoids are scaled to include 50% probability.

109.4(6)°), and copper ammonium selenate [13] (1.643(8)Å and 109.4(5)°). The deviation of bond Se(2)-O(5) from this average - it is 1.714(34)Å long - is not considered to be significant.

There are no significant deviations from the average C-C single-bond length of 1.528Å, and average C-C-C angle of 109.3°, both of which compare well with accepted values [15]. Similarily, the average C-O single-bond length of 1.423Å and C-O-C angle of 112.9° compare well with analogous values in other carbohydrates.[16] Within the limits of our necessarily rather imprecise analysis, the exocyclic and endocyclic glycosidic bonds are equivalent.

The two guanyl groups are of some interest. They are equivalent within the limits of experimental error, as are the six carbon-nitrogen bonds involved (with an average bond length of 1.330A). Both groups are planar (planes 1 and 2 in Table 7.9). These results agree well with the average parameters found, for example, in methylglyoxal bisguanylhydrazone dihydrochloride monohydrate [17] (1.330(10)Å), zinc guanidium sulphate (1.314(4)Å) [18], L-arginine dihydrate [19] (1.330(10)Å), and nitroguanidine [20] (1.34(3)Å). All these studies have the three carbon-nitrogen bonds of equivalent length and suggest that they have approximately 50% double-bond character i.e. the groups are partly delocalised. (The standard C-N single- and double-bond distances have been estimated to be 1.47 and 1.27Å respectively [17]).

The dimensions of the oxime group concur well with those

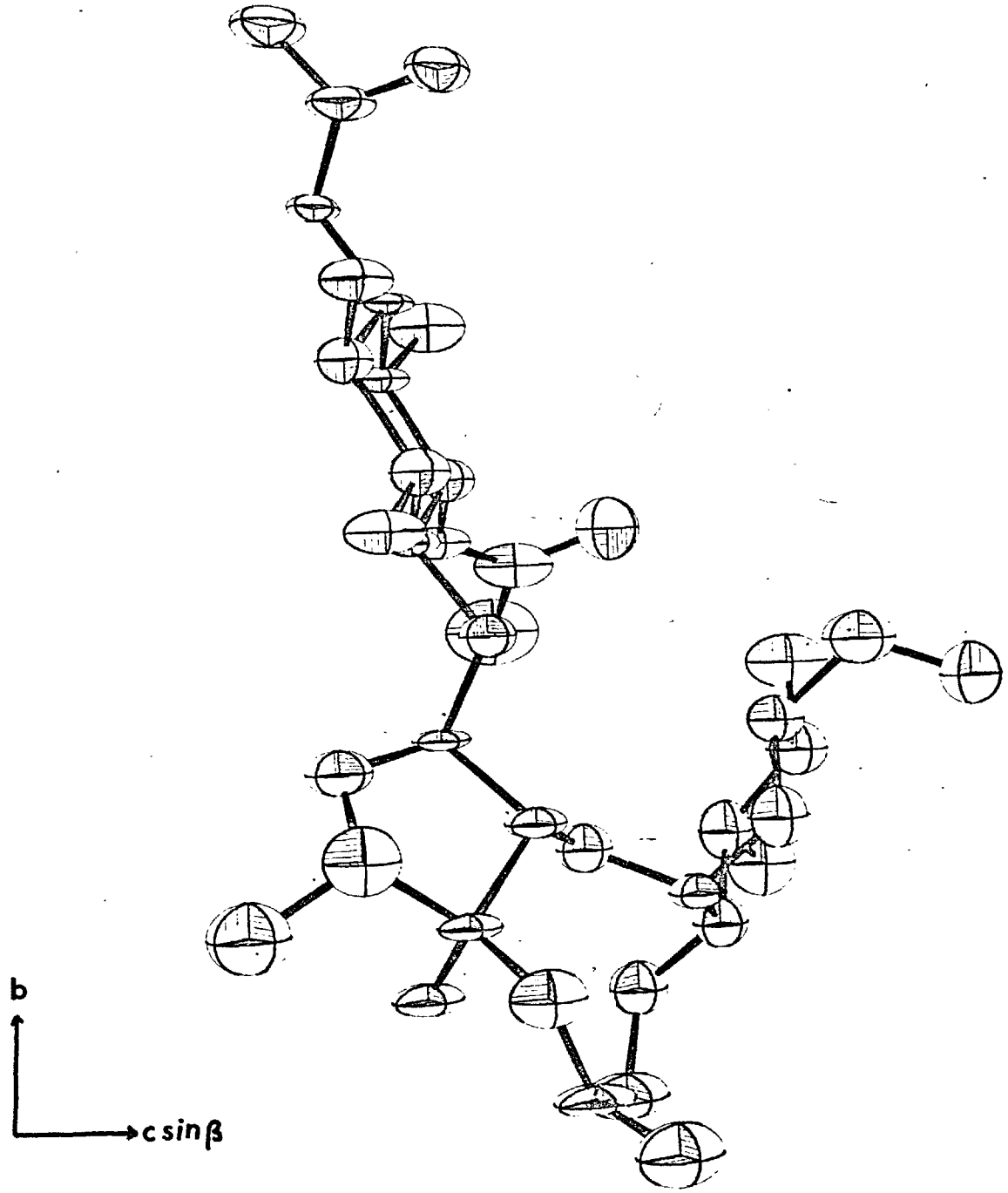


Figure 7.6. a-axis projection of a single molecule of streptomycin oxime. The thermal ellipsoids are scaled to include 50% probability.

reported in a wide variety of oximes. Thus, the carbon-nitrogen distance of 1.268Å corresponds closely to what one would expect for such a double bond [17,21]. The other lengths and angles for the oxime here are in good agreement within the limits of experimental error with, for example, those determined in the accurate analyses of syn-p-chlorobenzaldoxime [22] and glyoxime.[23]

	This work.	Syn-p-chloro-benzaldoxime.	Glyoxime.
C-C length	1.492(36)Å	1.486(8)Å	1.449(5)Å
C-N length	1.268(42)Å	1.260(8)Å	1.284(5)Å
N-O length	1.381(32)Å	1.408(7)Å	1.397(6)Å
C-C-N angle	120.0(26)°	120.8(5)°	117.6(3)°
C-N-O angle	111.0(25)°	110.8(5)°	111.0(3)°

As expected, the oxime group is planar (plane 3 in Table 7.9.).

Both the streptidine and the L-glucosamine rings are in the chair conformation; this is shown more clearly in Figure 7.6, the [100] projection of streptomycin oxime. Thus, in the streptidine ring, C(15) and C(18) are -0.613Å and 0.627Å out of the plane of the other four atoms, with an analogous situation in the L-glucosamine ring (planes 4 and 5 in Table 7.9). There are no significant deviations from the conformation of other known pyranoside and cyclohexane rings. The average dihedral angle in the L-glucosamine ring is 61.2°, (Table 7.10), which agrees, within the limits of experimental error, with the averages found for sucrose [24] (55.0°) and the trisaccharide

Table 7.9.

Deviations of selected atoms from their least-squares planes, in Å, and the equations of these planes in direct space, in the form $\ell x + my + nz = d$.

Plane 1 (guanyl):-

N(21),0.004; C(22),-0.011; N(23),0.004; N(24),0.003.

(W(45)^{III},-0.394; W(42),0.311; O(6),0.873; C(20),0.207).

$$0.968x + 0.118y - 0.399z = 0.116$$

Plane 2 (guanyl):-

N(26),-0.003; C(27),0.009; N(28),-0.003; N(29),-0.003.

(C(18),-0.045; O(33)^{V},0.914; W(45)^{VI},1.408; O(2')^{V},0.142; N(35)^{V},0.005; O(6)^{IV},-0.346).

$$0.133x + 0.244y + 0.538z = 0.186$$

Plane 3 (oxime):-

C(10),0.020; C(34),-0.025; N(35),-0.015; O(36),0.019.

(C(9),1.082; C(11),-0.658; O(33),-0.265; Se(1)^{I},0.627; O(1')^{I}, 0.271; Se(2),0.578; O(4),-0.216; N(28),-1.832; N(29),-1.071).

$$0.872x + 0.392y + 0.997z = -0.627$$

Plane 4 (L-glucosamine):-

C(3),-0.022; C(5),0.022; C(6),-0.022; O(37),0.022.

(C(4),0.492; C(7),-0.529).

$$0.493x - 0.271y + 0.130z = -0.109$$

Plane 5 (streptidine):-

C(16),0.006; C(17),-0.006; C(19),0.006; C(20),-0.006.

Table 7.9. (Continued).

(C(15),-0.613; C(18),0.627).

$$0.245x + 0.683y + 0.126z = 0.198$$

Plane 6 (streptose):-

C(9),-0.077; C(10),0.051; O(12),-0.061; C(13),0.088.

(C(11),-0.647).

$$0.142x - 0.798y - 0.480z = -0.151$$

Plane 7 (streptose):-

C(9),-0.045; C(11),0.045; O(12),-0.076; C(13),0.076.

(C(10),-0.661).

$$-0.992x + 0.114y + 0.565z = 0.333$$

Plane 8 (streptose):-

C(9),0.000; O(12),0.000; C(13),0.000.

(C(10),0.388; C(11),-0.338).

$$0.121x - 0.999y - 0.517z = -0.258$$

Plane 9 (first glycosidic link):-

C(6),-0.034; C(7),0.028; O(8),0.045; C(9),-0.040.

$$0.237x - 0.514y - 0.263z = -0.188$$

Plane 10 (second glycosidic link):-

C(9),-0.015; C(13),0.011; O(14),0.021; C(15),-0.017.

$$0.168x + 0.252y + 0.601z = 0.227$$

Plane 11 (amino):-

C(6),0.000; N(40),0.000; C(41),0.000.

(O(3),-1.110).

$$0.951x - 0.499y + 0.881z = -0.496$$

Atoms in parentheses have not been included in the calculation of the planes.

raffinose pentahydrate [25] (58.0°), and is within the range 55.8° to 61.7° suggested by Kim and Jeffrey [26]. The average dihedral angle for the streptidine ring substituents is 59.3, which also is well within the range of substituted cyclohexanes of between 55.0° and 60.0° [27].

All the streptidine substituents are equatorial, as are those for the L-glucosamine ring, apart from its glycosidic link. The confirmation of the absolute configuration of L-glucosamine indirectly confirms the absolute configurations of D-glucose (see Section 4.7), and all related carbohydrates. The orientations of the two guanyls are influenced by the orbitals on O(25); they are thus both approximately at right-angles to the plane of the streptidine ring. Both glycosidic-link systems are coplanar (planes 9 and 10), indicating considerable rigidity in the structure.

The geometry of the streptose ring is rather unusual compared to most other known furanosyl systems. It is well known that the furanose ring in general is puckered, mainly because of the torsional forces about the single bonds [28,29]. In most structures incorporating this system that have been investigated by X-rays, one or other of the carbon atoms analogous to C(9) or C(10) here, is displaced from the plane of the other four. For example, in ethyl-1-thio-α-D-glucofuranoside [30], the C(9)-equivalent is -0.595Å out of the plane of

the other four. Methyl α-D-lyxofuranoside [31] has the same carbon atom in the ring displaced by 0.652Å from the plane. The streptose ring in streptomycin oxime cannot be described so simply. Planes 6 and 7 in Table 7.9 show that two sets of four atoms are approximately planar, viz C(9), C(10), O(12) and C(13), and C(9), C(11), O(12) and C(13), with the fifth atom in both cases displaced over 0.6Å from the planes. Plane 8 shows that both C(10) and C(11) are displaced by roughly equal amounts, but in opposite directions, from the plane of C(9), O(12), C(13). Thus, the conformation of the streptose ring can be described as a 'half-chair'. The dihedral angles ω_1 to ω_5 (Table 7.10) may be contrasted, for example, with the corresponding values for the furanose ring in sucrose [24], of ω_1 (27.4°), ω_2 (-8.3°), ω_3 (-14.5°), ω_4 (31.0°) and ω_5 (-34.9°). This type of situation seems to be without precedent for furanose rings, although there have been two structure analyses reported recently with the C(10)-equivalent atom out of the plane of the other four. Adenosine 3', 5'-cyclic phosphate has this atom displaced by 0.60Å [32], and 6-azido-5,6-dideoxy-5-iodo-1,2-O-isopropylidene-β-L-idofuranose [33] by 0.7Å. A possible explanation is that the very bulky ring substituents, especially on C(10), would tend to stagger as much as possible and produce complex ring puckering. The half-chair conformation has been noted for the five-membered ring in several steroids, for example, in 2β,3α-dichloro-5α-cholestane [34].

Table 7.10.

Selected dihedral angles (ω) in the asymmetric unit, in degrees.

Constituent atoms.	(ω)	
O(1)(C(2),C(3))C(4)	-69.8	
O(1)(C(2),C(3))O(37)	50.9	
C(2)(C(3),C(4))O(38)	-71.9	
O(8)(C(7),C(6))N(40)	-51.9	
O(8)(C(9),C(13))O(14)	-127.2	
O(8)(C(9),C(10))O(33)	-28.2	
O(8)(C(9),C(10))C(34)	115.1	
C(9)(C(10),C(11))O(12)	49.7	ω_1
C(10)(C(11),O(12))C(13)	-47.6	ω_2
C(11)(O(12),C(13))C(9)	11.7	ω_3
O(12)(C(13),C(9))C(10)	18.1	ω_4
C(13)(C(9),C(10))C(11)	-39.8	ω_5
O(14)(C(15),C(20))N(21)	72.2	
O(14)(C(15),C(16))O(31)	-46.0	
N(21)(C(20),C(19))O(25)	-62.4	
O(25)(C(19),C(18))N(26)	52.6	
N(26)(C(18),C(17))O(30)	-69.8	
O(30)(C(17),C(16))O(31)	52.5	
C(32)(C(11),C(10))C(34)	-102.0	
O(38)(C(4),C(5))O(39)	71.5	
O(39)(C(5),C(6))N(40)	-51.1	

7.7. Hydrogen Bonding and Packing in the Crystal Lattice.

The crystal structure is held together by an elaborate system of hydrogen-bonded and ionic forces in which the water molecules and selenate ions figure prominently. Table 7.11 details the hydrogen-bonded and ionic contacts, shown diagramatically in Figure 7.7. For reasons of clarity, no composite packing diagram is presented.

Each asymmetric unit of streptomycin oxime selenate contains one and a half selenate ions. Therefore, to balance these three negative charges, three protons must be present, presumably attached to various sites in the molecule. Possible sites for protonation are the two guanyl groups, the nitrogen of the oxime, and the amine nitrogen; attachment to water molecules to form hydroxonium ions is a more remote possibility and can almost certainly be ruled out.

It is possible to determine which atoms are hydrogen-bonded to the guanyls by consideration of the various possibilities regarding their deviations from the planes through the guanyls (planes 1 and 2 in Table 7.9). The hydrogens attached to the nitrogens of the guanyls are normally in the guanyl plane (since the nitrogens are sp^2 hybridised) [17]. So, any hydrogen bonds would also be approximately in this plane. For the N(21), C(22), N(23), N(24) group, the only two likely N-H... bonds are to W(45) and W(42); O(6) is over 0.8Å out of plane and is too distant from N(24) anyway for a

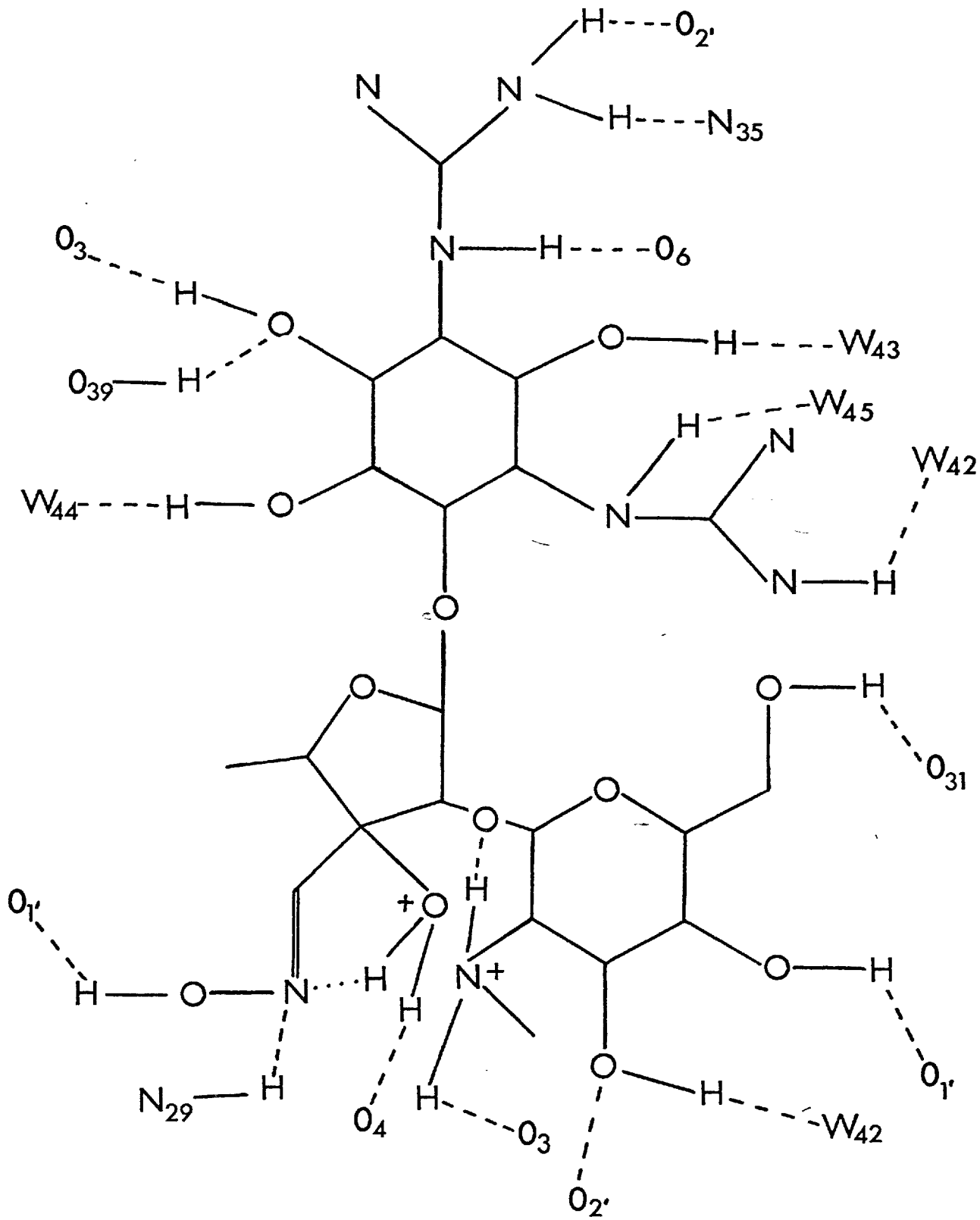


Figure 7.7. Diagramatic representation of the hydrogen-bonding scheme for a single molecule of streptomycin oxime.

Table 7.11.

Hydrogen-bonded and other close contacts, in Å.

O(1)	O(31)^{I}	2.904
O(8)	N(40)^{II}	2.768
N(21)....	W(45)^{III}	2.809
N(23)....	W(42)^{II}	2.968
N(24)....	O(6)^{II}	3.135 (non-bonded).
O(25)....	W(43)^{II}	2.777
N(26)....	O(6)^{IV}	2.859
N(28)....	O(33)^{V}	2.855 (non-bonded).
....	W(45)^{VI}	3.006 (non-bonded).
N(29)....	O(2')^{V}	3.026
....	N(35)^{V}	2.901
O(30)....	O(3)^{III}	2.672
....	O(39)^{III}	2.887
O(31)....	W(44)^{III}	2.967
O(33)....	O(4)^{II}	2.617
....	N(35)^{II}	2.678 (electrostatic).
O(36)....	O(1')^{I}	2.642
....	O(38)^{VII}	2.861 (non-bonded).
O(38)....	O(1')^{VIII}	2.567
O(39)....	O(2')^{IX}	3.004 (ionic).
....	W(42)^{X}	2.739
N(40)....	O(3)^{II}	2.655

Table 7.11. (Continued).

W(42)....	$O(2')^{V}$	2.996
W(43)....	$O(4)^{IV}$	2.877
....	$W(44)^{V}$	3.063
....	$W(45)^{VI}$	2.854
W(44)....	$O(5)^{II}$	2.708
....	$W(44)^{XI}$	2.904 (non-bonded).
W(45)....	$O(5)^{XI}$	2.962
....	$O(6)^{XII}$	3.041

Roman numerals refer to equivalent positions relative to the reference molecule at x, y, z.

I $-x, y, -z.$

II $x, y, z.$

III $x - \frac{1}{2}, y + \frac{1}{2}, z.$

IV $\frac{1}{2} - x, y + \frac{1}{2}, -1 - z.$

V $x, 1 + y, z.$

VI $1 - x, 1 + y, -1 - z.$

VII $x - \frac{1}{2}, y - \frac{1}{2}, z.$

VIII $\frac{1}{2} - x, \frac{1}{2} + y, z.$

IX $\frac{1}{2} + x, \frac{1}{2} + y, z.$

X $\frac{1}{2} - x, y - \frac{1}{2}, -z.$

XI $1 - x, y, -1 - z.$

XII $\frac{1}{2} + x, y - \frac{1}{2}, z.$

hydrogen bond (See Figure 7.8). Thus, rather surprisingly, this guanyl does not utilise its full complement of four hydrogens. Similar considerations indicate that the other guanyl has both O(33) and W(45) too far out of its plane to be considered. It is unlikely then that either guanyl is protonated since this would presumably induce additional hydrogen bonding with now participation of all guanyl nitrogens.

The situation regarding the oxime is rather interesting. Atom O(33) is only -0.265Å out of the plane of the oxime. This, together with the O(33)-N(35) separation of 2.678Å, suggests that there is some interaction between the two atoms, which would explain why the oxime group, which can rotate around bond C(10)-C(34), takes up this particular conformation. A proton on the oxygen could interact electrostatically with the electron density around the nitrogen atom. The oxygen is probably sp^2 hybridised, since the O(33)...O(4) vector (which contains a proton), is only slightly inclined to the oxime plane. The hydrogen bond between N(29) and N(35), which is in the plane of the relevant guanyl, is markedly out of the oxime plane. This is unexpected in view

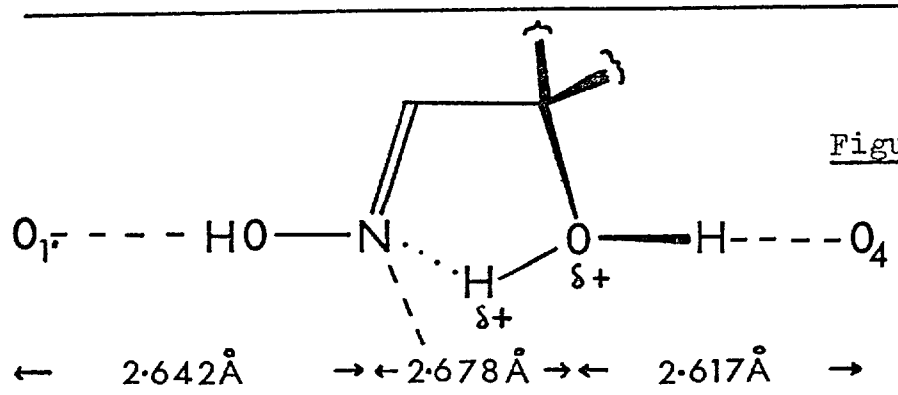


Figure 7.9.

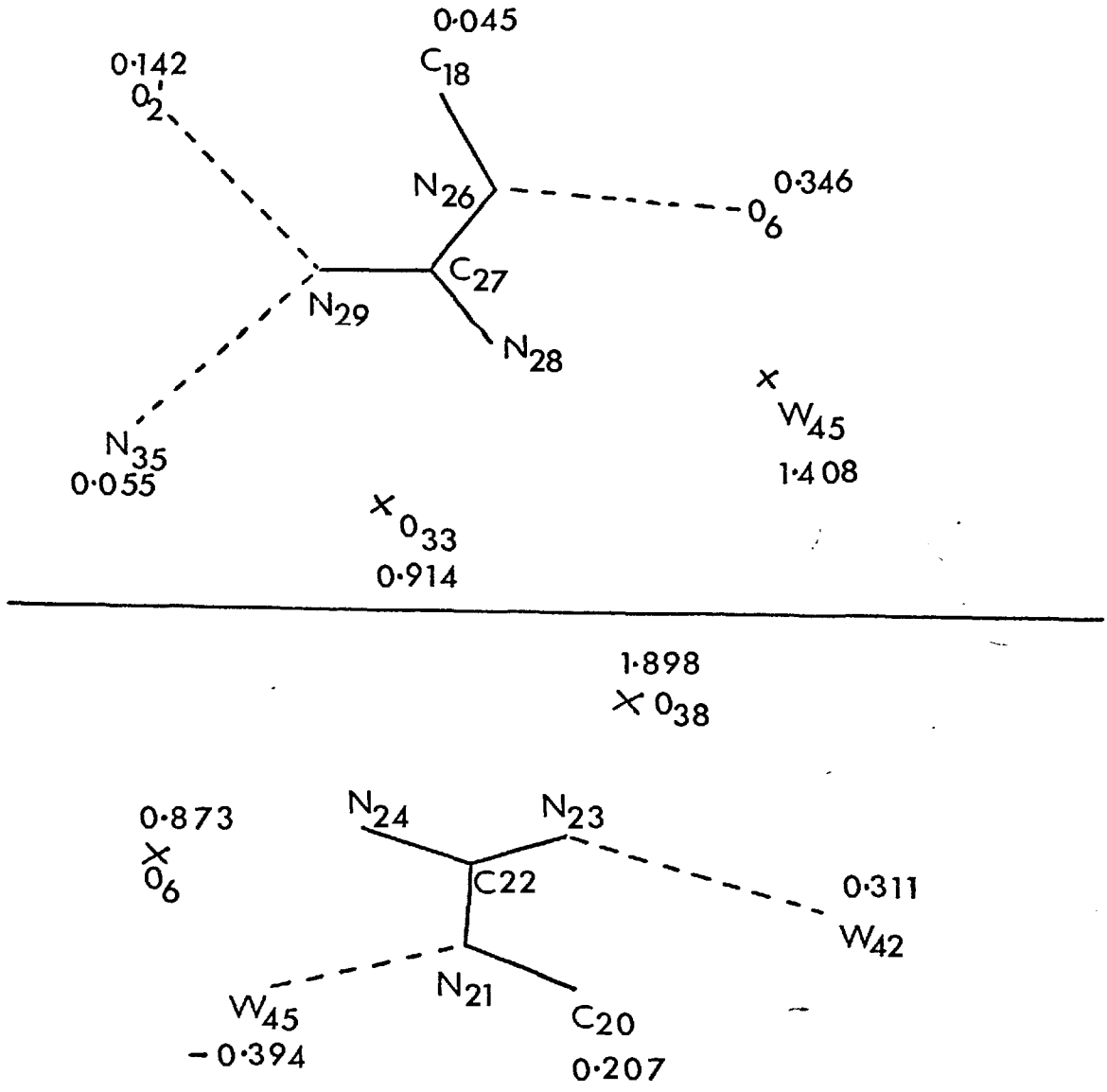


Figure 7.8. Deviations, in Å, of various atoms from the planes of the guanyl groups. Dashed lines indicate probable hydrogen bonds.

of the sp^2 nitrogen hybridisation, but may well be a result of this electrostatic interaction (Figure 7.9). The precise situation here is obviously a matter of some controversy, although it is considered likely that the oxime is indeed protonated in some manner. No structural studies of a charged oxime system have been reported.

The amine nitrogen is sp^3 hybridised since O(3), to which it is strongly hydrogen bonded (the N(40)...O(3) distance is 2.655Å), is -1.110Å out of the plane of C(6),N(40),C(41). Thus, it is possible for N(40) to accept a proton. The O(8)...N(40) distance is short (2.768Å), which suggests that there may be a hydrogen bond present here. It is spatially possible, and could be partly responsible for the curled-up conformation of this part of the molecule.

The probable location of the third positive charge remains somewhat of a mystery. None of the waters (Figure 7.10) can each donate more than two hydrogens in order to explain the hydrogen-bonding pattern. In fact, their co-ordination is satisfactorily explained by our scheme. The W(44)...W(44) separation of 2.904Å is a non-bonded one. The two waters are diad-related and all possible W(44) hydrogens are utilised elsewhere. Figure 7.11 shows the co-ordination around the two selenate ions.

The waters and selenates thus perform an essential role in the hydrogen bonding and are doubtless responsible for

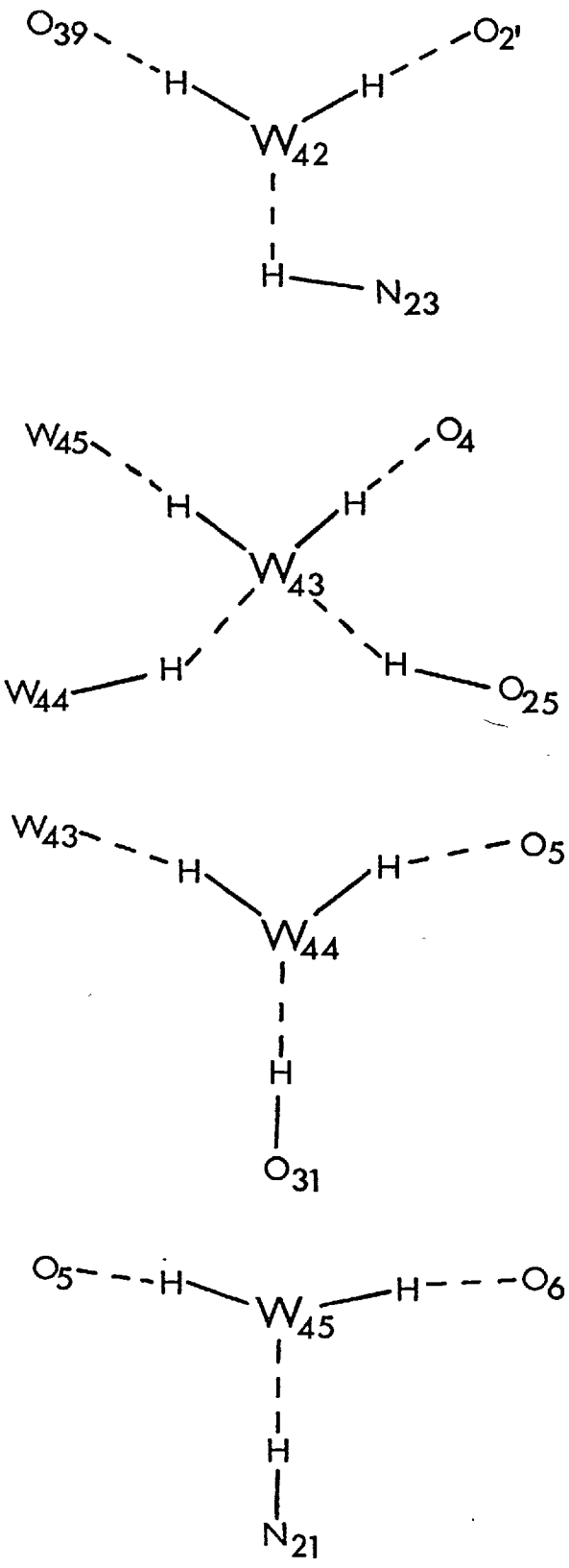


Figure 7.10. The co-ordination around the four water molecules.

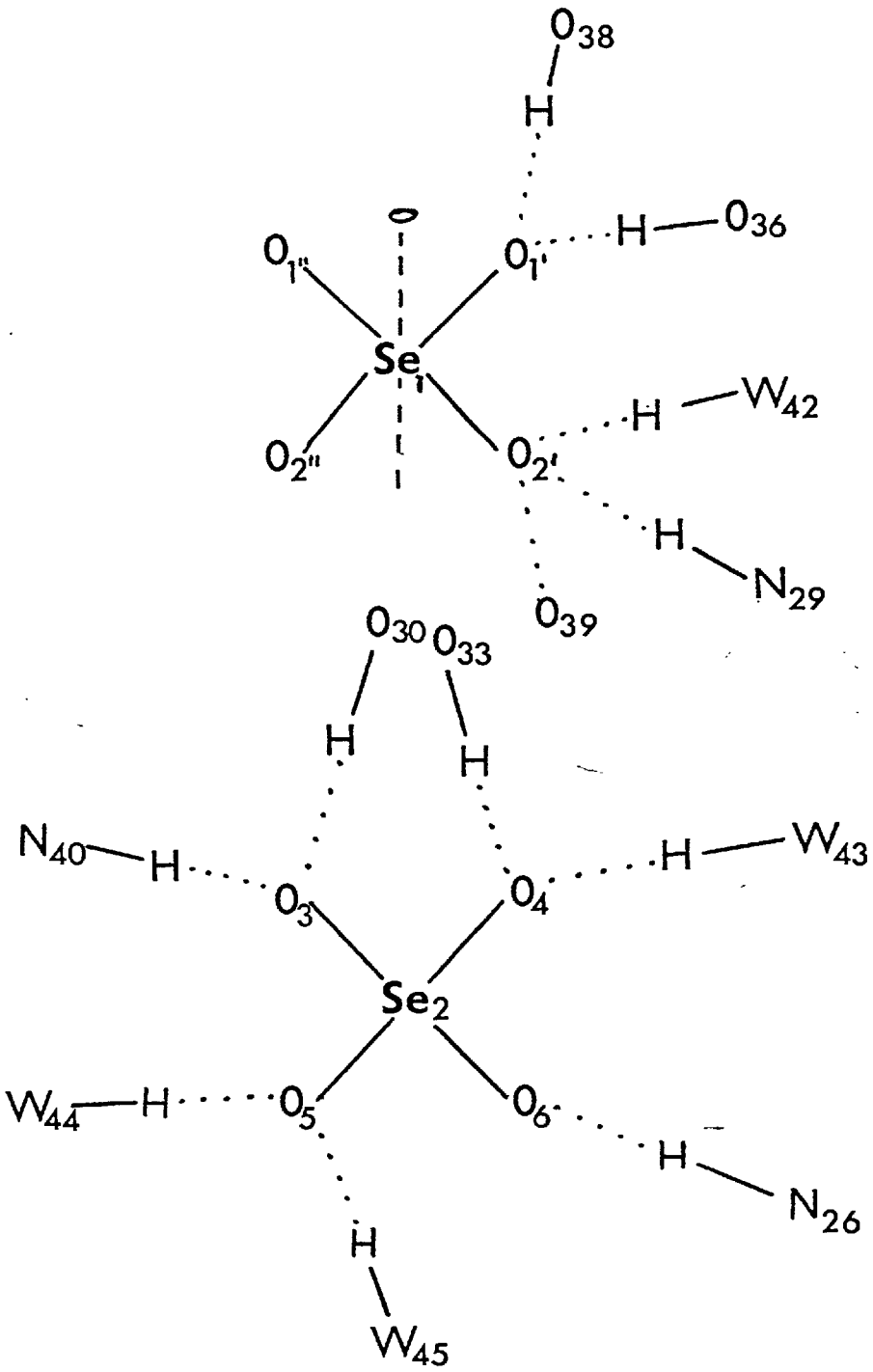


Figure 7.11. The co-ordination around the selenate ions.

making the rather ill-disciplined streptomycin molecule form this crystalline derivative. Thus, all the short (i.e. strong) interactions are between selenate oxygens and other atoms.

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PART THREE. DIRECT METHODS: THEORY AND PRACTICE.

CHAPTER 8

Some Theoretical Considerations of Direct Methods in Crystallography.

8.1. Introduction.

The "heavy-atom" methods of structure solution, employed for all the compounds so far discussed in this thesis, rely on the presence of one (or at most a few) atoms in a compound, which are heavy relative to the others, and which can be readily located from a Patterson synthesis. All-light-atom molecules cannot, in general, be solved by such an approach; however, if at least part of the molecular geometry can be accurately defined, structure solution is sometimes possible by Patterson search techniques [1]. We shall not discuss these further; they are of limited applicability and are relatively little used.

It is frequently difficult to prepare heavy-atom derivatives of many organic compounds and natural products. Indeed, there is a danger that in doing so the very features that are of interest may be modified or destroyed. Thus, the development of phase-determining methods for solving such problems has been a central theme in crystallography for the

past twenty years. In essence, 'direct methods' attempt to determine the phases of the structure factors directly by mathematical means using only the raw diffraction data; no chemical assumptions at all are introduced. The literature on the subject is now very extensive, so only a review of the most important ideas and methods will be presented here.

8.2. Unitary and Normalised Structure Factors.

The unitary structure factor, U_h, is defined as

$$U_h = F_h / \sum_{j=1}^{N} f_j \quad \ldots\ldots\ldots\ldots\ldots\ldots 8.1$$

and thus expresses the structure factor as a fraction of its maximum possible value. f_j is the thermally attenuated scattering factor of the jth atom, so the effect of dividing by $\sum f_j$ is to eliminate the natural decrease of f with θ, and thus gives structure factors due to point atoms at rest.

We can rewrite equation 8.1 in the form

$$U_h = \sum_{j=1}^{N} n_j \exp 2\pi i \vec{h}.\vec{r}_j \quad \ldots\ldots\ldots\ldots\ldots\ldots 8.2$$

where

$$n_j = f_j / \sum_{j=1}^{N} f_j \quad \ldots\ldots\ldots\ldots\ldots\ldots 8.3$$

is the unitary atomic scattering factor, or the fractional scattering power of the jth atom in a structure,

and

$$\vec{h} = \vec{ha}^* + \vec{kb}^* + \vec{lc}^*$$

For a structure with N equal atoms in the unit cell,

$$n_j = f_j / N f_j = 1/N \quad \ldots\ldots\ldots\ldots\ldots, 8.4$$

Another useful quantity is σ, defined as

$$\sigma = \sum_{j=1}^{N} n_j^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 8.5$$

The probability distributions of the structure factors for both centrosymmetric and non-centrosymmetric structures containing a large number of atoms of similar scattering power, randomly distributed in the unit cell, have been calculated by Wilson [2]. They can be expressed in terms of unitary structure factors as

$$P_1(U) = (2\Pi\sigma)^{-\frac{1}{2}}\exp(-U^2/2\sigma)$$
$$P_1(U) = \left(\frac{2}{\sigma}|U|\right)\exp(-|U|^2/\sigma) \quad \ldots\ldots\ldots\ldots \quad 8.6$$

For both the centric and acentric distributions we have

$$\sigma = \langle U_h^2 \rangle \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 8.7$$

This forms the basis of a method of calculating unitary structure factors [3]; the set of data is divided into ranges of $\sin\theta$, and values of $\Phi^2 = \langle U_h^2 \rangle / \langle I_h \rangle$ are found for each $\sin\theta$. Φ, the factor needed to convert a F into the corresponding U, can be obtained for any reflection, from a Φ:$\sin\theta$ graph by interpolation.

The normalised structure factor, introduced by Karle and Hauptman [4], is somewhat more convenient to use in much of the probability theory developed by these workers. It is defined as

$$E_h^2 = U_h^2/\varepsilon\langle U_h^2 \rangle \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 8.8$$
$$= |F_h|^2/\varepsilon\sum_{j=1}^{N} f_j^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 8.9$$

$\mathcal{E}$ is an integer which takes account of the effects of symmetry elements in space groups. For example, in $P2_12_12_1$, $\mathcal{E}=2$ for the h00, 0k0 and 00l reflections.

Both unitary and normalised structure factors may conveniently be obtained by first obtaining the absolute scale factor, K, and overall temperature factor for the intensity data, from a Wilson plot [5]:

$$\ln\left(\frac{\langle I_o\rangle}{\sum_{j=1}^{N} f_j^2}\right) = \ln K - \frac{2B\sin^2\theta}{\lambda^2} \qquad \ldots\ldots\ldots\ldots\ldots\ldots \quad 8.10$$

The set of data is divided into ranges of $\sin\theta$, and $\ln(\langle I_o\rangle/\sum_{j=1}^{N} f_j^2)$ is plotted against $\sin^2\theta/\lambda^2$. The straight line obtained has a slope of -2B, and an intercept of lnK. This is essentially the analogue of the empirical method discussed above for obtaining U's. The accuracy of the results is only fair (about 15%), and several workers have suggested improved procedures [6]. Principal departures from Wilson's expectation are due to non-random positions. Karle and Hauptman's method, the so-called, 'K-curve method' [7], takes the data as found and doesn't introduce the notion of randomness - at least when scaling. They directly plot $\langle I_o\rangle$ against $\sum_{j=1}^{N} f_j^2$ for ranges of values of $\sin\theta/\lambda$, and the slope of the curve gives the absolute scale factor K, which is then used with the observed data to calculate the normalised structure factors, from equation 8.9.

The distribution of the E values is in general, independent of the size and content of the unit cell. However, they have statistical properties which often enable centric and acentric distributions to be readily distinguished. These are a useful supplement to the tests of Rogers and Wilson [8]. Table 8.1 details some of the more useful average properties of the normalised structure factors.

Table 8.1.

	Centric	Acentric
$\langle\|E\|^2\rangle$	1.000	1.000
$\langle\|E\|\rangle$	0.798	0.886
$\langle\|E^2-1\|\rangle$	0.968	0.736

Deviations from these averages, which have been calculated assuming a random distribution of atoms, are sometimes very considerable. They may be considered to arise from two distinct effects. Rational dependence arises when several atomic coordinates are simple fractions of the cell edges; this can occur, for example, with atoms in special or semi-special positions. A systematic procedure for correcting for rational dependence is available, which does not necessitate making any structural assumptions [9]. The second effect, termed hypersymmetry, arises when a molecule itself introduces non-crystallographic symmetry in the unit cell, and is quite common with certain types of

planar molecule [10], and especially with structures containing marked periodicity, such as polymers and proteins.

8.3. Inequalities.

The first major step towards a solution of the phase problem was made in 1948 by Harker and Kasper [11]. They found that relationships existed between certain structure factors which could be expressed in the form of inequalities.

We use the well-known Cauchy inequality

$$\left|\sum_{j=1}^{N} a_j b_j\right|^2 \leq \left(\sum_{j=1}^{N} |a_j|^2\right)\left(\sum_{j=1}^{N} |b_j|^2\right) \quad \ldots\ldots \quad 8.11$$

From equation 8.2, we have an expression for the unitary structure factor. Now take

$$a_j = \sqrt{n_j}$$

and

$$b_j = \sqrt{n_j}\exp(2\pi i \vec{h}.\vec{r}_j)$$

The left-hand side of the inequality is then $|U_h|^2$. Thus, we have

$$|U_h|^2 \leq \left(\sum_{j=1}^{N} n_j\right)\left(\sum_{j=1}^{N} n_j \left|\exp 2\pi i \vec{h}.\vec{r}_j\right|^2\right)$$

$$\leq \left(\sum_{j=1}^{N} n_j\right)^2$$

Hence,

$$|U_h|^2 \leq 1 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 8.12$$

This result is a trivial one. However, when symmetry elements are introduced into the structure-factor expressions, some more interesting results are obtained. When the structure is centrosymmetric, we can write for the unitary structure factor:-

$$U_h = \sum_{j=1}^{N} n_j \cos 2\pi \vec{h}.\vec{r}_j \quad \ldots\ldots\ldots\ldots\ldots\ldots 8.13$$

From the inequality 8.11 we can now write

$$|U_h|^2 \leq \left(\sum_{j=1}^{N} n_j\right)\left(\sum_{j=1}^{N} \cos^2 2\pi \vec{h}.\vec{r}_j\right)$$

$$\leq \tfrac{1}{2} \sum_{j=1}^{N} n_j (1 + \cos 2\pi . 2\vec{h}.\vec{r}_j)$$

Hence, $|U_h|^2 \leq \tfrac{1}{2}(1 + U_{2h})$ $\ldots\ldots\ldots\ldots\ldots\ldots\ldots$ 8.14

This is a relation between the signs of structure factors, and under certain conditions the sign of U_{2h} may be determined to be positive. For example, if $U_h = 0.6$ and $U_{2h} = 0.5$, we then have $0.36 \leq \frac{1}{2}(1 + 0.5)$, which can only be true with the positive sign for U_{2h}. If $|U_h|^2$ is less than 0.5, no conclusions regarding sign can be made. U_{2h} must also be large for a definite indication.

The above inequality is incapable of predicting negative signs. (A Fourier map calculated with all positive signs has just a large positive peak at the origin, a physically unacceptable situation). However, for symmetry elements such as glide planes and screw axes, the more complex inequalities which can be derived, can lead to the introduction of negative signs. In particular, with unitary structure factors of sufficient magnitude, it can be shown that

$$s(h)s(h')s(h \pm h') = 1 \quad \ldots\ldots\ldots\ldots 8.15$$

where $s(h)$ is the sign of U_h.

A number of structures have been solved using inequalities, a notable one being that of decaborane [12]. However, there is

a very real limit to the size of problem that can be tackled; if about 10% of the U's are equal to or greater than 0.4, then the structure will probably succumb. This restriction has been been discussed by Hughes [13] and Woolfson [3], who consider that about 16 atoms in a unit cell is a rough upper limit, a conclusion which has been borne out by experience.

Karle and Hauptman have shown that the inequalities are directly derivable from the condition that the electron density in a crystal is everywhere positive. [14]

In general, the sign of a structure factor can change with change of origin. This can be illustrated in the centrosymmetric case by considering a unit cell (Figure 8.1), with each centre of symmetry considered as an origin, making eight in all. A simple consideration shows that, for example,

$$(F_{hkl})_{\frac{1}{2}00} = (-1)^h (F_{hkl})_{000}$$

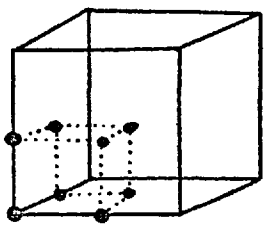

Figure 8.1.

Each of the circles (•) represents a centre of symmetry

i.e. there is a sign change for h odd. The structure factors may be divided into eight groups with different behaviour, distinguished by the various possible combinations of the parities of h, k and l. The (even, even, even) ones do not change sign; they are thus fixed by the structure and are

known as the structure invariants. It is possible to select three structure factors quite arbitrarily as positive, which together will define a unique origin, provided no combination of their parities gives a structure invariant; they are then said to be linearly independent. This process of origin definition is common to all direct methods, and provides a small starting set of signs.

8.4. The Sayre Equation.

The next impetus to the development of direct methods was initiated by Sayre in 1952, and has since been extended by many others. Sayre showed that for a structure containing equal resolved atoms, the structure factors are inter-related by precise equations. We shall now derive Sayre's equation in some detail.

Figure 8.2(a) shows a one-dimensional representation of the electron density $\rho(r)$, for a structure of equal resolved atoms. The squared electron density $\rho(r)^2$, Figure 8.2(b), is very similar, differing only in that the peaks have a sharper profile.

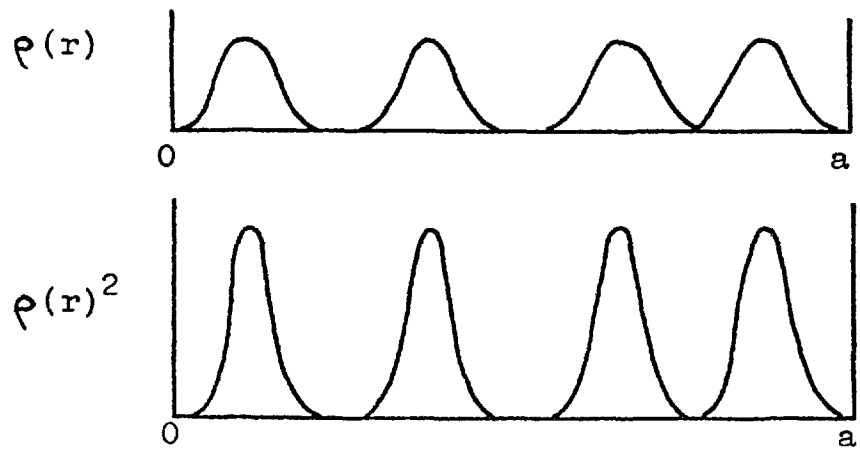

Figure 8.2.
(a)
(b)

The electron density is given by

$$\rho(r) = \frac{1}{V} \sum_{h} F_h \exp(-2\Pi i \vec{h}.\vec{r}_j) \quad \ldots\ldots\ldots\ldots\ldots\ldots 8.16$$

and the squared electron density can also be represented by a Fourier series as

$$\rho(r) = \frac{1}{V} \sum_{h} G_h \exp(-2\Pi i \vec{h}.\vec{r}_j) \quad \ldots\ldots\ldots\ldots\ldots\ldots 8.17$$

where G_h is the hth Fourier coefficient of the squared structure. We can then write

$$F_h = \Theta_h G_h \quad \ldots\ldots\ldots\ldots\ldots\ldots 8.18$$

where Θ_h is a factor to take account of the difference in shape.

We can rewrite equation 8.17 as

$$\rho(r)^2 = \left[\frac{1}{V} \sum_{h} F_h \exp(-2\Pi i \vec{h}.\vec{r}_j)\right]^2$$

$$= \frac{1}{V} \sum_{h_1} \left[\frac{1}{V} \sum_{h_2} F_{h_1} F_{h_2}\right] \exp(-2\Pi i(\vec{h}_1 + \vec{h}_2).\vec{r}_j) \ldots 8.19$$

Now let $\vec{h}_1 + \vec{h}_2 = \vec{h}$ and $\vec{h}_1 = \vec{h}'$. We can then rewrite equation 8.19 as

$$\rho(r)^2 = \frac{1}{V} \sum_{h} \left[\frac{1}{V} \sum_{h'} F_h F_{h-h'}\right] \exp(-2\Pi i \vec{h}.\vec{r}_j) \quad \ldots\ldots\ldots 8.20$$

Now comparing equations 8.17, 8.18 and 8.20, we see that

$$F_h = \frac{\Theta_h}{V} \sum_{h'} F_{h'} F_{h-h'} \quad \ldots\ldots\ldots\ldots\ldots\ldots 8.21$$

This precise relationship is a general one, holding for both centrosymmetric and non-centrosymmetric structures. Thus, any structure factor is related to all the other pairs whose indices add up to give hkl. It can be used also for one- or two-dimensional data, with A the area, or L the length of the unit-cell projection, replacing V.

Sayre solved the structure of the amino-acid hydroxyproline (in two-dimensional projection), by determination of the sign of 53 terms in a week, a tremendous achievement in pre-computer days. At first sight, the equation 8.21 seems rather useless, since to determine one structure factor it is necessary to know the magnitudes and phases of all others. However, if one product is so large that it dominates the sum on the right of equation 8.21, the sign of the sum is controlled by that product. Thus, in the centrosymmetric case, for three reflections with large structure factors, we can write

$$s(h) = s(h')s(h-h') \quad \ldots\ldots\ldots\ldots\ldots \quad 8.22$$

Even when the structure factors are not sufficiently large for this to hold precisely there is a strong probability that the relationship is true. So now

$$s(h) \approx s(h')s(h-h') \quad \ldots\ldots\ldots\ldots\ldots \quad 8.23$$

Both Cochran [16] and Zachariasen [17] have investigated Sayre's equation further; Zachariasen derived the equation

$$s(h) \approx \overline{s(s(h')s(h-h'))} \quad \ldots\ldots\ldots\ldots \quad 8.24$$

as a better approximation than 8.23 to the Sayre equation. He employed this to successfully solve the structure of metaboric acid.

The question of the quantitative estimate of the probability of these sign-determining relationships being correct, is obviously a very important one. Cochran and Woolfson [18] showed that the probability of the sign relationship 8.22 holding, is given by

$$P_+(h) = \tfrac{1}{2} + \tfrac{1}{2}\tanh\left\{\frac{\sigma_3}{\sigma_2^3}\,|U_h U_{h'}, U_{h-h'}|\right\} \quad \ldots\ldots\ldots\ldots \quad 8.25$$

where $\sigma_3 = \sum_{j=1}^{N} n_j^3$ and $\sigma_2 = \sum_{j=1}^{N} n_j^2$

For a structure containing N equal atoms in the unit cell, expression 8.25 becomes

$$P_+(h) = \tfrac{1}{2} - \tfrac{1}{2}\tanh(N|U_h U_{h'}, U_{h-h'}|) \quad \ldots\ldots\ldots \quad 8.26$$

Very often, for a particular value of h, there may be several pairs of known signs of the type s(h') and s(h-h'). We can then write for the sign of h:-

$$s(h) \approx s\left(\sum_{h'} U_{h'}, U_{h-h'}\right) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 8.27$$

and the associated probability is now

$$P_+(h) = \tfrac{1}{2} + \tfrac{1}{2}\tanh\left\{\frac{\sigma_3}{\sigma_2^3}\,|U_h| \sum_{h'} U_{h'}, U_{h-h'}\right\} \quad \ldots\ldots \quad 8.28$$

Klug, in a rigorous and comprehensive analysis of the problem [19], has shown that these probability equations, especially 8.28, are not strictly correct; however, since the

unitary structure factors are themselves determined with some inaccuracy, the probabilities are adequate for most purposes.

In general, the relationship 8.27 is a better approximation to the full Sayre equation than is equation 8.23, and signs determined with it should have a higher probability of being correct.

8.5. The Hauptman-Karle Approach.

These workers have derived sign-determining relationships using the magnitude of the structure factors, based on their concept of the joint probability of structure factors [4,20], and assuming a random distribution of atoms within the unit cell This is in some respects an extension of Wilson's work on the probability distributions of intensities [2]. Their mathematical analyses are lengthy and complex - we shall merely quote their most important results.

For the space group $P\bar{1}$, the sign of E_h is given by

$$\sum_1 + \sum_2 + \sum_3 + \sum_4$$

where

$$\sum 1 = \frac{\sigma_3}{4\sigma_2^{3/2}} \sum_{h'} (E^2_{\frac{1}{2}h} - 1) \quad \ldots\ldots\ldots\ldots \quad 8.29$$

$$\sum 2 = \frac{\sigma_3}{2\sigma_2^{3/2}} \sum_{h'} E_{h'} E_{h-h'} \quad \ldots\ldots\ldots\ldots\ldots \quad 8.30$$

$$\sum 3 = \frac{\sigma_4}{4\sigma_2^2} \sum_{h'} E_{h'} (E^2_{\frac{1}{2}(h-h')} - 1) \quad \ldots\ldots\ldots \quad 8.31$$

and $$\sum 4 = \frac{\sigma_5}{8\sigma_2^{5/2}} \sum_{h'} (E^2_{\frac{1}{2}(h-h')} - 1)(E^2_{h'} - 1) \; . \quad 8.32$$

$\sum_1$ is similar to the Sayre-Cochran-Zachariasen equation with $h = h'$, when $s(2h) = 1$. It can only give the sign of E_{2h} with a high degree of probability when both E_{2h} and E_h are large. It also of course bears a resemblance to the Harker-Kasper inequality 8.14. Serious criticism has been made of the usefulness of both $\sum_1$ and $\sum_2$, (which alone depend on the magnitude of the E's), by several workers [21,22]. They point out that $\sum_1$ is the coefficient of a sharpened, origin-removed Patterson, and $\sum_4$ is the coefficient of the squared Patterson function. Thus, both formulae represent no more than can be obtained by Patterson methods; $\sum_4$ in particular, is considered to be somewhat unreliable.

The $\sum_2$ formula (8.30) is analogous to the Sayre relationship; it can be rewritten more conveniently as

$$s(E_h) \approx s \sum_{h'} E_{h'} E_{h-h'} \quad \ldots\ldots\ldots\ldots 8.33$$

The probability of the sign of E_h being positive, now becomes (c.f. 8.28)

$$P_+(h) = \tfrac{1}{2} + \tfrac{1}{2}\tanh \frac{\sigma_3}{\sigma_2^{3/2}} |E_h| \sum_{h'} E_{h'} E_{h-h'} \quad \ldots\ldots\ldots\ldots 8.34$$

For equal-atom structures, $\sigma_3/\sigma_2^{3/2}$ reduces to $1/N^{\frac{1}{2}}$.

The $\sum_1$ relationship assumes more useful forms for space groups of higher symmetry than $\bar{P}1$. For example, in the space group $P2_1/c$, we have [4]

$$s(E_{2h,0,2l}) \approx s \sum_{\kappa} (-1)^{k+1} (E^2_{hkl} - 1) \quad \ldots\ldots\ldots 8.35$$

with the appropriate probability of the sign being positive, being

$$P(E_{2h,0,2l}) = \tfrac{1}{2} + \tfrac{1}{2}\tanh\left\{\frac{\sigma_3}{2\sigma_2^{3/2}} |E_{2h}| \sum_{\kappa} (-1)^{k+1} (E^2_{hkl} - 1)\right\} \quad \ldots\ldots 8.36$$

The general validity and usefulness of the Hauptman-Karle approach has been fully vindicated by the successful use of their formulae in solving a large number of complex structures. The objections that have been raised really imply that all the information contained in the equations 8.29 - 8.32 could have been obtained from an examination of the Harker sections in a Patterson map, resolved for point atoms. This is certainly not true except for the simplest of structures.

In retrospect, probably the greatest value of the work of Hauptman and Karle lies in the stimulus they have provided to the crystallographic community as a whole. In spite of some initially rather intemperate criticism they have shown that the phase problem for centrosymmetric structures can be overcome with relative ease in many cases. In the next section, we shall consider a number of practical approaches used in applying these methods to real structures.

8.6. Some Procedures for Solving Centrosymmetric Structures.

Early methods devised for structure solution all relied

on the Sayre-Cochran-Zachariasen equation, sometimes in conjunction with inequalities. Cochran and Penfold solved the structure of L-glutamine [23] by first finding the signs of thirteen of the largest U's from inequalities and the signs of most of the rest by use of equations 8.22 and 8.27 in terms of two initially unknown sign symbols. The values of these two were in fact correctly indicated by a number of the relationships.

A multi-solution method was devised by Cochran and Douglas, which was designed for digital computer operation [24], and with it they solved the structure of salicylic acid. Each of the largest U's was given a symbol, and all the sign relations between them were found. Thus, a very large number of possible sets of signs were obtained for the twenty unitary structure factors employed. The correct structure was obtained by computing all the possible Fourier syntheses and selecting the most plausible one. Some elegant tests have also been devised for finding the correct solution in a less time-consuming and more systematic manner [25].

Grant, Howells and Rogers [26] have devised the 'coincidence method', based on the Sayre equation, which has the advantage of allowing large amounts of data to be handled compactly, and for two-dimensional data does not require a computer. From Friedel's Law we can write

$$s(h)s(h') = s(h)s(\bar{h}')$$

Thus,

$s(h)s(h')s(h + h') \approx 1$ with probability P_1

and $s(h)s(h')s(h - h') \approx 1$ with probability P_2

imply that $s(h + h') \approx s(h - h')$ 8.37

with probability $P = 2P_1P_2 - P_1 - P_2 + 1$.

Thus, 8.37 is only useful for large U values since both P_1 and P_2 must both be close to unity for P to have useful values. The sign-determining procedure employs equation 8.37 in a systematic way to find 'coincidences' between signs and sets of signs, which can indicate their value, all the U's having been given sign symbols. The structures of both harmine ($C_{13}H_{12}N_2O$) and hydroxydihydroeremophilone ($C_{15}H_{24}O_2$) have been solved in projection by sign coincidences.

The widespread advent of large, fast computers in the past decade has meant that direct methods, which are often well-suited to machine computation, have come into extensive use, although it must be emphasised that it is advisable to process the initial sign-determining steps by hand. Karle and Hauptman have systematised the whole process in what they term the, 'symbolic-addition method' [27]. This involves iterative use of their $\sum_2$ relationship (equation 8.33), with just a few symbolic phases. Usually only three are needed. Firstly, as much information as possible is

obtained from the $\sum_1$-type relationships, although these rarely give the signs for more than two or three reflections. Then, application of $\sum_2$ to all E values greater than a value of, say, 1.5, enables a considerable number of symbolic phases to be generated. Of course, the starting set of origin reflections and symbols have to be chosen so that they propagate well and with high probability. There are at most 2^n possible solutions (where n is the number of unknown symbols), which means that there are usually 2^3 different Fourier maps to be calculated, a not very difficult task. However, this number can often be very much reduced:- the solution with all possible signs can at once be rejected as physically implausible, and the iteration using $\sum_2$ usually indicates preferred signs for some of the symbols. A Fourier map with the E's as coefficients (a so-called E-map) can then be calculated. This has the advantage of not allowing the large E values (which correspond mostly to the weaker reflections with high $\sin\theta$ values), to be swamped by the lower-order ones. The former contain much of the structural information. E-maps show atoms as very sharp peaks since they correspond to Fourier syntheses of point atoms. The symbolic-addition procedure has proved to be a very powerful and simple one, and many complex structures have now been solved with it, some containing as many as sixty atoms.

Its chief disadvantage seems to be that in the initial stages, a set of phases is being built up with often just a single pair of contributors to $\sum_2$, which can occasionally give an erroneous sign. These wrong signs would then be propagated in subsequent iterations and made more numerous. However, with proper use of probability criteria this type of error can be largely avoided.

The process of choosing the origin-defining set of phases has previously been outlined in Section 8.3. The allowed linear combinations for the centrosymmetric space groups have been tabulated by Hauptman and Karle [4,29]. In the case of $P\bar{1}$ and the primitive monoclinic and orthorhombic groups, the procedure is quite straightforward; three linearly-independent reflections with high E's are chosen. These must also enter into a large number of $\sum_2$ combinations with the other reflections. Sometimes a reflection is used which is subsequently found to be unsatisfactory with regard to this last condition. It must then be rejected and a new choice made. For the alkaloid jamine [30], the combination

	$\lvert E \rvert$
$1\ \bar{1}\ \bar{7}$	3.76
2 1 4	6.89
3 5 2	4.74

was used, all three with an assigned positive sign.

8.7. Phase Determination for Non-centrosymmetric Structures.

The problem of the general case, that for a non-centrosymmetric structure, is a much more difficult one, since in general phases can lie anywhere between 0 and 2Π radians. There has been remarkable progress in this field in the past few years, mainly due to the techniques pioneered by Hauptman and the Karles.

The Sayre equation (8.21) is a quite general one, and so for non-centrosymmetric structure factors, can be written as

$$|F_h| \exp(i\phi_h) = \frac{\theta_h}{V} \sum_{h'} |F_{h'}||F_{h-h'}| \{\exp i(\phi_{h'} + \phi_{h-h'})\} \quad \ldots.8.38$$

This equation can equally well be rewritten using E's or U's. When only a few of the largest terms on the right-hand side are known, the most probable value of the phase angle $_{h'}$ is that given by these terms:-

$$\langle \phi_h \rangle = \langle \phi_{h'} + \phi_{h-h'} \rangle_{h'} \quad \ldots\ldots\ldots\ldots\ldots\ldots 8.39$$

This equation has been derived by Cochran [31] and the Karles.[27] The probability of the correctness of ϕ_h increases considerably with increasing E values. However, we now have to consider a probability distribution in terms of the variance of the phase angle, instead of the relatively simple sign probability formulae for centrosymmetric structures. (Variance is defined as the square of the standard deviation). It can be shown that the variance V is given by the rapidly converging series

$$V = \frac{\Pi^2}{3} + \left[I(\alpha)\right]^{-1} \sum_{n=1}^{\infty} \frac{I_{2n}(\alpha)}{n^2} + 4\left[I(\alpha)\right]^{-1} \sum_{n=1}^{\infty} \frac{I_{2n+1}(\alpha)}{(2n+1)^2} \ldots$$

..... 8.40

where I_o is a Bessel function and α is defined as

$$\alpha = \frac{2\sigma_3}{\sigma^{3/2}} |E_h| (A^2 + B^2)^{\frac{1}{2}} \quad \ldots\ldots\ldots\ldots\ldots\ldots 8.41$$

with

$$A = \sum_{h'} |E_{h'} E_{h-h'}| \sin(\phi_{h'} + \phi_{h-h'})$$

and

$$B = \sum_{h'} |E_{h'} E_{h-h'}| \cos(\phi_{h'} + \phi_{h-h'})$$

Phase indications from 8.39 are only accepted if their variance is less than about 0.5 square radians. Experience has shown that this value, which corresponds to an alpha limit of about 2.5, is a reasonable cut-off point [27].

The $\sum_2$-type formula 8.39, must be used with some care since ambiguities can sometimes arise in the averaging. [32] For example, if two estimates of a phase are respectively $\Pi/10$ and $-2\Pi/10$ (which is $18\Pi/10$), the average $\langle\phi\rangle$ is $-\Pi/20$, (or $19\Pi/20$), which are apart. This ambiguity in phase, which would produce entirely misleading results, can be overcome by only accepting phase indications which are all completely concurrent; the average of all the contributors is then the same as that of a single contributor. If some contributors vary in value, it is wise to consider the phase ϕ_h as undetermined. It is perfectly possible to include symbolic phases as contributors to 8.39, analogous to the centrosymmetri

$\sum_2$ formula.

It can be shown that (27)

$$\tan\phi_h \approx A/B \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 8.42$$

This tangent formula is capable of refining phases found approximately, say, from equation 8.39. and for extending the list of known phases in a set of E-values once the phases of a number are approximately known numerically. A rough guide is that if a phase is initially within about $\Pi/4$ radians of its true value, then the tangent formula is capable of refining it. If symbolic phases are used i.e. from prior application of 8.39, they are assigned numerical values. The formula is valid for the whole range of E's, not just the large ones. Use of it completely avoids any possible phase ambiguities, as discussed above. The phases calculated from the tangent formula have a variance given by 8.40. Thus, in using it, phases which fall outside a reasonable limit of variance, are not employed in the next cycle of iterations.

An R index can also be computed for a set of phases:-

$$R_k = \frac{\sum_{h'} |E_h|_o - |E_h|_c}{\sum_{h'} |E_h|_o} \quad \ldots\ldots\ldots \quad 8.43$$

This has been found to give a good indication of the consistency, and hence, the correctness of a particular set. Both $|E_h| \sin\phi_h$ and $|E_h| \cos\phi_h$ can be readily calculated. The

sums of their squares are rescaled by comparison with E_o, and hence a calculated $|E_h|^2_c$ obtained; the R_k index then follows.

The important question as to how a unique origin is defined, now arises; it is fundamental to the whole procedure. In addition, non-centrosymmetric structures can exist in two enantiomorphic forms, which give the same intensities; this introduces an ambiguity of $\pm\Pi/2$ radians in the origin set, and so it is necessary to 'fix' the enantiomorph. The procedure for origin and enantiomorph definition is straightforward for space group $P2_12_12_1$. (This is fortunate since many natural products crystallise in this group). The reason for this is that in this space group an origin, once chosen, is uniquely defined; whereas, in $P2_1$, for example, there is no such restriction. Hauptman and Karle have derived a series of rules which are useful in specifying the origin and enantiomorph-defining reflections for both primitive [33,34] and centred [35] space groups.

Let us consider an example in space group $P2_12_12_1$, that of the mould metabolite viridin (see Chapter 9). In this space group any one- or two-dimensional reflection has a phase of 0 or Π, or $\pm\Pi/2$, depending on its parity. This is because all three projections are centrosymmetric. Thus, it is a relatively straightforward matter to choose three zonal reflections which are linearly independent, analogous to the

centrosymmetric case. The sign of each phase (0 or Π, or $\Pi/2$), can be arbitrarily fixed. So, for viridin, the set chosen was:-

h	k	ℓ	E	ϕ	Parity
6	13	0	3.23	0	gu0
1	14	0	2.97	$\Pi/2$	ug0
0	17	1	2.24	$\Pi/2$	0uu

where g and u denote even and odd parities respectively.

The phases of reflections of type uu0, uuu, 0gu and g0u are linearly dependent on these three, and are formed by combining them in pairs. For example, the gu0 and the 0uu give ggu with phases of $\Pi/2$. However, an 0gu reflection (which is in ggu) must have a phase of 0 or Π, from structure-factor algebra. This ambiguity can only be resolved by the choice of a suitable fourth reflection which can combine with any of the others to give linearly dependent phases with no ambiguities. The ugu reflections are obtained by summing all three phases and will be 0 or Π if derived from these three. But the u0u must be $\Pi/2$ and so can be chosen independently. The u0u combined with the ug0 now gives 0gu phases of 0 or Π, and the g0u will also correctly be $\Pi/2$. The reflection chosen in the case of viridin was the 15 0 1 one (E = 2.02), which was given a phase of $\Pi/2$.

For space group $P2_1$, it is not possible to define the enantiomorph in this manner; restriction of a symbolic phase to between 0 and Π radians is however quite satisfactory in fixing it. The origin set has to be of the type $h_1 0 \ell_1$, $h_2 0 \ell_2$

and $h_3 1 \ell_3$, where $h_1 0 \ell_1$ and $h_2 0 \ell_2$ are two different terms from the parities u0g, g0u and u0u. The triclinic space group P1 has an even more stringent condition for a linearly independent set. This is that

$$\begin{vmatrix} h_1 k_1 \ell_1 \\ h_2 k_2 \ell_2 \\ h_3 k_3 \ell_3 \end{vmatrix} = 1$$

where the phases may be specified arbitrarily.

The basic set of starting phases, together with any symbolic ones used, should be associated with large E values and should be capable of entering into a large number of $\sum_2$-type relationships, so that they propagate well. This latter point is of crucial importance especially for the origin set, and considerable judgement is required to obtain the correct set. Germain et al. have recently devised a computer procedure for systematically obtaining the best starting set of phases [36], which promises to be of considerable use for $P2_12_12_1$. However, the stage has not yet been reached when non-centrosymmetric structures can be solved in a routine automatic manner, as can often now be done for centrosymmetric ones. It is unlikely that such a situation will be arrived at in the foreseeable future except in the most favourable cases.

The next section gives an outline of the technique used

by the author in implementing these ideas to solve a non-centrosymmetric structure. There is as yet no generally agreed procedure and individual workers favour their own particular approach.

8.8. A Procedure for General Phase determination.

The computer program used is a local version of the PHASEM program written by M.G.B. Drew [37], modified by the author for use on the University of London Atlas Computer. Additional features include provision for calculation of the reliability index R_k, and output of tangent-refined phases in a form suitabl for direct calculation of E-maps using the X-RAY-63 system.

The set of normalised structure factors greater than 1.5 is used. The first part of the program calculates the phases of these input reflections throughout the sphere of reflection, since all the reflections in the sphere must be used in phase-determining formulae. A simple set of algorithms is used for this purpose:-

$$\phi_{hk\bar{l}} = \Pi/2\left[1 + (-1)^{rh + sk + t\ell + 1}\right] - \phi_{hk\ell}$$

$$\phi_{h\bar{k}\ell} = \Pi/2\left[1 + (-1)^{uh + vk + w\ell + 1}\right] - \phi_{hk\ell}$$

$\phi_{\bar{h}k\bar{l}}$ is then fixed by these two. A few examples of 'generating integers' illustrate the method.

	r	s	t	u	v	w
For P1	0	0	0	0	1	0
$P2_1$	0	0	0	0	0	0
$P2_12_12_1$	1	0	1	0	1	1

When h is reversed, $\phi_{\bar{h}\bar{k}\bar{l}}$ is set equal to $-\phi_{hkl}$. Consequently phases of $-\phi_{hkl}$ and $\Pi + \phi_{hkl}$ can occur in the $\bar{h}$ region. The shortcomings of the version of the program used in this work arise when $-\phi_{hkl}$ and $\Pi + \phi_{hkl}$ are needed also in the h region. This occurs with space groups involving mirrors or glides which thus cannot be accomodated by the program as it at present stands.

Listings of all the combinations of h' and h-h' for a given h are now compiled using the $\sum_2$-type relationship. These enable a choice of origin- and enantiomorph-defining reflections to be made, together usually with three symbolic symbolic phases. Employment of the equation 8.39 in a symbolic addition process enables the phases of a number of reflections in the list to be uniquely determined in terms of symbols. Sometimes a phase will be absolutely determined. We define an alpha value as

$$\alpha_D = \alpha/2\sigma_3\sigma_2^{-3/2} \quad \ldots\ldots\ldots\ldots 8.44$$

For equal-atom structures this reduces to $\alpha \sqrt{N}/2$. If the lower limit of 2.5 is taken for α, then α_D is $1.25N^{\frac{1}{2}}$, as an approximate guide. Thus, during the symbolic addition, if a triple product $E_h E_{h'} E_{h-h'}$ has a value than this, the phase indication is rejected.

The tangent formula 8.42 is now used to find the best set of phases corresponding to numerical values for the symbols. For example, if a symbol can range between 0 and 2Π radians,

eight different sets in steps of say $\Pi/4$ radians are tried. So for three symbols, there are many possible solutions corresponding to the combinations of the symbols tried. During tangent refinement a phase has to satisfy two criteria. First, its alpha value must be greater than the minimum preset - usually the same as used previously in symbolic addition. Also, its consistency index t_h, defined as

$$t_h = \frac{(A^2 + B^2)^{\frac{1}{2}}}{\sum_{h'} |E_{h'} E_{h-h'}|} \quad \ldots\ldots\ldots\ldots\ldots \quad 8.45$$

must be greater than its preset value. This is a measure of how well the sum of the contributing phases $(\phi_{h'} + \phi_{h-h'})$ agree with each other. Its value falls within the range $0 < t < 1.0$. Preset values of 0.25 to 0.40 have been found to be satisfactory. The refinement can be varied so that only a small number of new phases are introduced in a batch of refinement cycles.

The set of phases with the lowest R_k index and the highest $\langle \alpha \rangle$ and $\langle t \rangle$ values is usually the most consistent and correct one. An additional measure of the consistency of a set of phases is provided by the R_D index, introduced by Drew. This is defined as

$$R_D = \frac{\sum_h |E_h| (1 - t_h)}{\sum_h |E_h|} \quad \ldots\ldots\ldots\ldots\ldots \quad 8.46$$

R_D does not seem as reliable an indicator as the others. (See next Chapter).

An E-map can then be calculated from these 'best' phases which may then reveal the structure. Karle has shown that even if an incomplete structure is revealed in the map, subsequent tangent refinement using the phases calculated from the part structure, can improve them sufficiently for the complete structure to be eventually obtained [38]. This method, although not needed for viridin, has recently been very useful for the solution of some large structures [39].

Sometimes it is not possible to recognise even a part structure in an E-map, for no apparent reason. The phases in such a case are often quite consistent. Some improvement is obtained by calculating the map with phases weighted as to their probability of being correct.

8.9. Some Future Developments.

The introduction of some knowledge concerning structure into the direct-method formulae offers the prospect of tackling many larger molecules with greater ease than is at present possible. Such knowledge could concern inter-atomic distances or the rigid geometry of a known fragment. A promising start in this difficult field has recently been made by Kroon and Krabbendam, who have been able to incorporate information concerning molecular orientation (derived from the Patterson function), into a triple-product sign relationship [40]. A fair improvement of sign prediction was obtained.

A number of years ago, Perutz and Scatturin attempted to

derive a set of signs for a centrosymmetric projection of methaemoglobin, but without real success [41]. This is hardly surprising considering the weakness of the reflections from such a large and complex structure. They considered that direct methods would not in general be of use in the solution of protein structures. More recently, interest has revived in the possibilities of using direct methods in some form for this type of problem. Coulter has performed some successful model compound calculations using the tangent formula to extend and refine a small set of known phases [42]. He further suggested that phases from a single isomorphous protein derivative can be extended and improved in this manner. This idea has been tested by two groups of workers, with somewhat conflicting results. It seems that, for some proteins at least, tangent refinement using high-resolution data (about 3A resolution), does produce a slight but significant improvement in the phases [43]. This was judged by an improvement in the quality of the electron-density maps produced. Reeke and Lipscombe [44] also find that for carboxypeptidase the lower resolution phases (6Å) are definitely superior to those obtained by the standard multiple isomorphous replacement technique. These studies indicate that this type of approach may assume some importance in the future, provided optimal procedures for utilising the tangent formula with protein data are developed.

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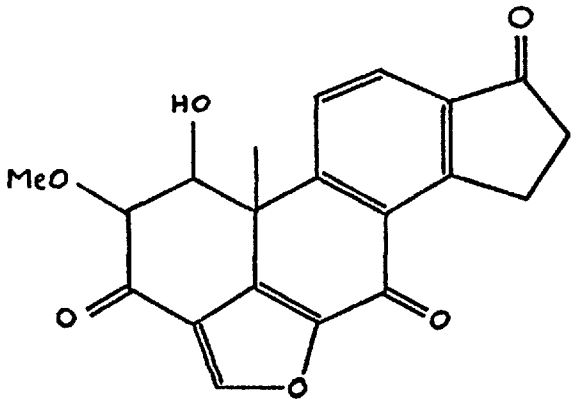
CHAPTER 9

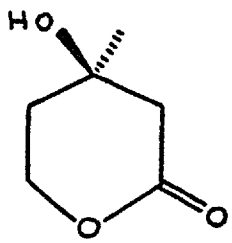
The Crystal and Molecular Structure of Viridin

9.1. Introduction.

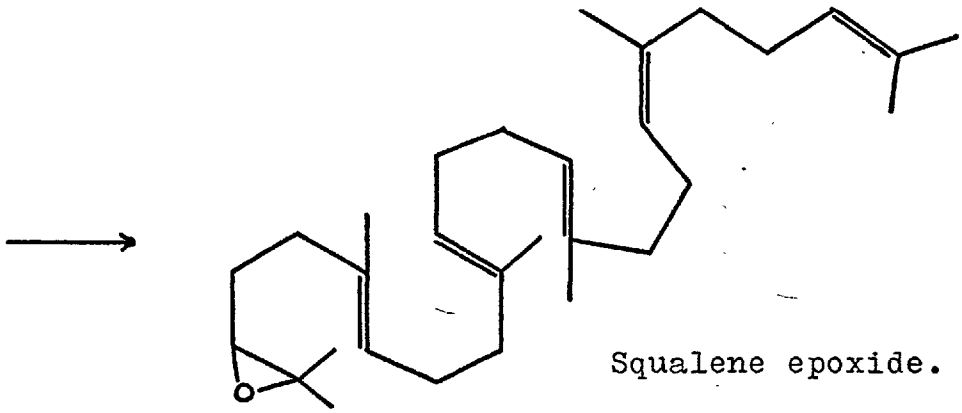
The highly active antifungal metabolite viridin was first described in 1945, as isolated from Gliocladium virens [1]. On the basis of extensive chemical degradations and spectroscopic data, the structure (I) was proposed for viridin [2]. This structure strongly suggests a steroidal biogenetic pathway from (R) - mevalonic acid lactone by way of farnesyl pyrophosphate, squalene epoxide, and lanosterol. (See scheme). Labelling studies [3] strongly supported this hypothesis, and rejected the possibility of viridin being derived from a diterpenoid. The angular methyl group C(18) is given a β absolute configuration, in accordance with that for all natural steroids; viridin has thus been assigned the absolute configuration (II). The relative configurations of the three asymmetric centres were inferred from NMR



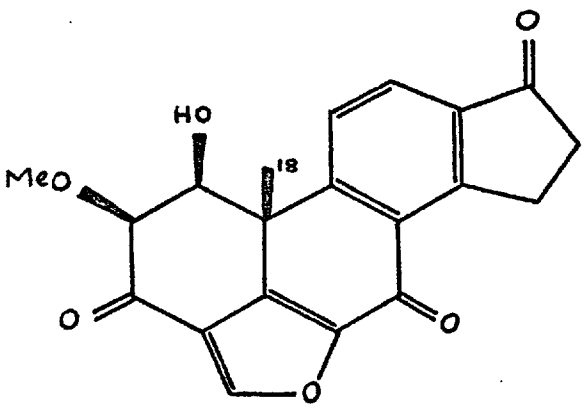
(I)



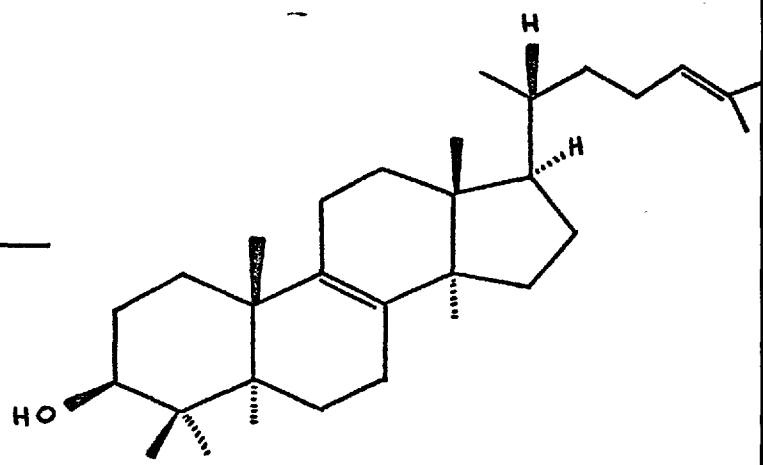
(R)-mevalonic acid lactone.



Squalene epoxide.



(II)



Lanesterol.

studies [2]. Both the aromatic ring C and the oxygenation pattern of ring A are unique among natural steroids.

The mould Trichoderma viride is known to produce as its major metabolite the heterocyclic compound gliotoxin [4], $C_{13}H_{13}O_4N_2S_2$. In support of the chemical studies on gliotoxin in progress in this Department, it was decided to determine its crystal and molecular structure. (This was before the crystal structure was published by Fridrichsons and Mathieson). On crystallising the crude compound from various solvents, several polymorphs of gliotoxin were obtained. One solution gave excellent crystals and an X-ray study was commenced on them in a too hasty belief that they were one particular polymorph. However, the subsequent Patterson function obtained revealed no sulphur-sulphur vectors; in fact the map was indicative of an all-light-atom structure. On re-examination of the initial unit-cell data, a significant discrepancy was found between the observed and calculated densities; the cell dimensions corresponded very well with those reported for viridin [5]. Subsequent elemental analysis and mass-spectral investigations confirmed the material as authentic viridin. It was easily separated in the solid phase from gliotoxin by flotation in a liquid of suitable density and was found to represent approximately 25% of the solid extract. This

represents a new source of supply of viridin. The structure of the recently isolated metabolite, viridiol, also from Trichoderma viride, is based on that of viridin[6]. There is considerable current interest in the synthesis of ring C aromatic steroids more generally related to viridin [7].

The above preliminary studies and the subsequent data collection were the work of Dr. M.B. Hursthouse while he was a member of the Chemical Crystallography Laboratory, Imperial College, 1965-6.

9.2. Preliminary X-ray Studies.

The crystals were grown from acetone/methyl iodide solution. They are yellowish prisms, with extinction directions parallel to the morphological faces. Oscillation and Weissenberg photographs reveal the Laue symmetry mmm. The systematic absences were hOO, OkO and OOl for h, k, $l \neq 2n$, thus defining the space group uniquely as $P2_12_12_1$. The crystals were stable to X-rays, giving excellent Weissenberg photographs after 15 hours exposure.

Cell dimensions were initially obtained from Weissenberg photographs; precise values were later obtained from axial θ -values measured on a diffractometer.

9.3 Crystal Data.

Viridin: $C_{20}H_{16}O_6$; orthorhombic $P2_12_12_1$ (No.19, D_2^4)

a = 16.090 (1); b = 16.162 (1);

c = 6.195 (1) Å U = 1611.0 Å^3

D_m = 1.44(2) g.cm.$^{-3}$ (by flotation);

D_c = 1.45 g.cm.$^{-3}$ for Z = 4 molecules/cell;

M = 352.0; μ = 6.30 for Cu-K_α (λ_{mean} = 1.54178A);

F (000) = 736.0 electrons.

The layers 0kℓ - 5kℓ were photographed with Ni-filtered Cu-k_α radiation using the equi-inclination Weissenberg method and multi-film packs from a crystal of approximate dimensions 0.6 x 0.5 x 0.5 mm^3; errors due to absorption were considered to be negligible. A total of 1580 reflection intensities were visually estimated and placed on an arbitary common scale by comparison with common data from the h0ℓ and h1ℓ layers. The POLO program was used for this inter-layer scaling. A further 250 reflections were treated as accidental absences and were included in the data list.

The data were put on an absolute scale and normalised structure factors (E's) computed by means of the DATFIX program in the X-RAY'63 system. Table 9.1 lists the relevant

Table 9.1.

	Experimental.	Theoretical values for centric.[8]	Theoretical values for acentric.
$\langle \lvert E \rvert \rangle$	0.835	0.798	0.886
$\langle \lvert E \rvert^2 \rangle$	1.000	1.000	1.000
$\langle \lvert E^2-1 \rvert \rangle$	0.848	0.968	0.736
Percentage of data with $\lvert E \rvert > 1$	33.8	32.0	36.8
$\lvert E \rvert > 2$	2.6	5.0	1.8
$\lvert E \rvert > 3$	0.2	0.3	0.01

statistical averages compared with those calculated for equi-atom structures, with atoms in random positions. The considerable deviations from ideality are doubtless the result of a non-random distribution of atoms in the unit-cell. They indicate the probable presence of hypersymmetry [9] due to the repeated motif of, for example, benzene rings, which produces localised symmetry elements not used by the space group.

9.4. Structure Solution and Refinement.

The structure was solved by statistical methods, based on those of Karle and Karle [10], using the symbolic addition procedure followed by tangent refinement. The origin and

enantiomorph-defining phases, together with three symbolic ones were chosen from the 182 reflections whose $|E|$ values were greater than 1.6. Selection of a particular reflection was made on the basis of as high an $|E|$ value as possible, consistent with a high number of Σ_2 interactions entered into, and large triple-product values for each interaction. The starting set used is detailed in Table 9.2.

Table 9.2.

h			$\|E_h\|$	Phase (in millicycles)
6	13	0	3.23	0)
1	14	0	2.97	250) defines origin
0	17	1	2.24	250)
15	0	1	2.02	250 defines enantiomorph
10	18	0	3.20	a
9	9	3	3.08	b
5	8	4	3.00	c

The symbolic-addition procedure was then carried out on this starting set. A phase indication was only accepted if the value of a triple-product exceeded 13.0. After eight cycles of symbolic-addition, each of which used the symbolic phases uniquely determined in the previous one, twenty-nine phases were uniquely

determined in terms of a, b and c. The history of each symbolic phase shows that the build-up of phases was not at all steady. This reflects the apparent inconsistencies which are only resolved when the symbols are assigned numerical values. These "inconsistencies" suggested that both a and c were close to 0 or 500 millicycles (a, which is an invariant, is of course 0 or 500 by definition), with no preferred value for b. However, in the subsequent tangent refinement no assumption was made concerning any of the three symbols, apart from the invariant condition for a. Both b and c were varied between 0 and 1000 millicycles, in steps of 125 millicycles. Thus, 128 different starting-set combinations were investigated.

The procedure adopted for the tangent refinement was that the first twenty-nine reflections (i.e. those from symbolic-addition) were refined for five cycles, then the top eighty, including these twenty-nine were refined for twenty-five cycles and finally the top one hundred and fifty for fifty cycles. The complete procedure took about forty minutes on the CDC 6600 computer.

The minimum triple-product value (alpha value) for a phase indication to be accepted, was set at 13.0. The minimum consistency was set at 0.4, a somewhat arbitary choice. The starting sets with the best solutions, judged on the basis of the criteria tabulated (these are defined in chapter eight), are shown in Table 9.3.

Table 9.3.

a^1	b^1	c^1	⟨consistency⟩	⟨alpha⟩	R_D	R_K	a^2	b^2	c^2
0	0	625	0.690	121	30	26.4	0	-56	-260
0	0	875	0.670	125	32	24.7	0	57	-232
0	125	750	0.679	125	31	23.7	0	70	-251
0	250	875	0.694	120	30	24.4	0	80	-248
0	750	625	0.687	124	30	24.0	0	-87	-250
500	250	625	0.683	127	31	22.4	0	78	-250
500	750	875	0.668	121	32	26.9	0	-104	-249

[1]Initial value of phases (quoted in millicycles).

[2]Final " " " " " "

All the sets showed some oscillatory behaviour from cycle to cycle except for the (500, 250, 625) one. Table 9.4 details the history of this set through the fifty cycles:- after only thirty-eight cycles all the phases had settled down completely. Table 9.5 shows what has happened to each phase during the refinement. Only a few phases were somewhat unreliable, as judged from their low consistency indices.

An E-map was then computed using these 150 phases as coefficients. (Figure 9.1). It is characterised by a number of peaks of approximately equal height. There were very few spurious peaks with heights significantly above the

Table 9.4. Progress of the phase refinement.

CYCLE	AVERAGE CONSISTENCY	AVERAGE ALPHA	R
1	0.127	2.97	0.00
2	0.127	2.97	0.00
3	0.127	2.97	0.00
4	0.127	2.97	0.00
5	0.127	2.97	0.00
6	0.290	6.99	0.10
7	0.337	18.59	0.27
8	0.381	22.69	0.24
9	0.391	25.33	0.22
10	0.404	27.42	0.19
11	0.409	29.43	0.19
12	0.396	33.06	0.21
13	0.407	35.85	0.19
14	0.412	35.84	0.18
15	0.409	36.14	0.19
16	0.413	35.93	0.18
17	0.409	36.17	0.19
18	0.413	35.95	0.18
19	0.409	36.17	0.19
20	0.413	35.95	0.18
21	0.410	36.17	0.18
22	0.413	35.95	0.18
23	0.410	36.17	0.18
24	0.413	35.95	0.18
25	0.410	36.17	0.18
26	0.694	48.51	0.27
27	0.581	85.45	0.40
28	0.625	94.79	0.36
29	0.644	99.31	0.34
30	0.640	106.09	0.35
31	0.656	108.35	0.34
32	0.669	116.56	0.32
33	0.682	122.25	0.31
34	0.681	124.53	0.31
35	0.677	126.58	0.31
36	0.683	126.14	0.31
37	0.682	126.55	0.31
38	0.683	126.61	0.31
39	0.683	126.62	0.31
40	0.683	126.62	0.31
41	0.683	126.62	0.31
42	0.683	126.62	0.31
43	0.683	126.62	0.31
44	0.683	126.62	0.31
45	0.683	126.62	0.31
46	0.683	126.62	0.31
47	0.683	126.62	0.31
48	0.683	126.62	0.31
49	0.683	126.62	0.31
50	0.683	126.62	0.31

FINAL VALUE FOR RKARLE= 0.224

Table 1.2.

						PHASES AFTER CYCLE NUMBER										
						1	5	10	15	20	25	30	35	40	45	
0	13	-	3.21	174.98	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r
1	14	-	2.67	141.92	23+/-	25+/-	23+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	n/r
-	12	-	3.24	43.65	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	n/r
15	-	1	3.17	72.94	n/r	n/r	n/r	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	n/r
0*	14	1	1.2	1.6.64	5n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r
A	9	1	1.1	1.6.64	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	n/r
A	8	4	3.17	313.47	781.+/-124.4	735.+/-124.4	724.+/-124.4	748.+/-124.4	748.+/-124.4	748.+/-124.4	748.+/-124.4	748.+/-124.4	748.+/-124.4	748.+/-124.4	748.+/-124.4	n/r
0*	7	-	3.59	1.1.57	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r
14	15	-	3.28	62.87	5n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r
15	7	-	3.34	79.22	n/r	n/r	n/r	n/r	272.2	269.7	267.1	269.1	271.1	269.4	271.1	269.7
14	3	1	3.17	44.34	n/r	n/r	n/r	n/r	284.7	111.4	129.9	127.4	129.4	175.1	178.2	184.4
14	11	1	3.11	45.5*	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r
1	8	1	1.64	124.69	n/r	n/r	n/r	n/r	143.7	153.1	139.2	137.4	137.4	136.1	135.4	135.4
0*	8	1	1.61	43.47	n/r	n/r	n/r	n/r	111.1	143.4	134.4	136.4	142.2	135.4	137.4	137.4
12	4	1	1.64	41.42	25+/-	25+/-	25+/-	25+/-	125.4	124.7	124.7	124.7	124.7	124.7	124.7	124.7
A	11	2	1.64	47.64	n/r	n/r	n/r	n/r	124.1	163.7	141.4	119.4	227.2	249.4	234.7	249.4
0*	2	1	1.63	170.72	25+/-	25+/-	25+/-	25+/-	171.4	151.4	141.2	147.7	126.1	151.4	131.4	131.4
A	9	1	1.67	177.89	25+/-	25+/-	25+/-	25+/-	284.1	284.1	282.1	171.7	284.4	284.1	284.1	284.1
A	12	2	1.66	71.69	n/r	n/r	n/r	n/r	275.4	24.1	24.1	24.1	24.1	24.1	24.4	24.4
11	7	1	1.64	73.61	5n/r	n/r	25+/-	25+/-	n/r	177.-	n/r	136.4	n/r	112.4	111.4	111.4
4	7	2	1.77	177.57	n/r	n/r	n/r	n/r	14.4	4.4	2.4	1.4	1.4	1.4	1.4	1.4
*	15	4	1.73	45.61	25+/-	25+/-	25+/-	25+/-	144.4	124.4	124.4	124.4	124.4	124.4	124.4	124.4
9	3	1	1.74	119.44	5n/r	n/r	n/r	n/r	434.4	45.4	434.4	434.7	434.7	434.7	434.7	434.7
3	14	1	1.74	51.61	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-
19	3	2	1.71	57.17	25+/-	25+/-	25+/-	25+/-	107.2	13.7	24.4	12.4	24.4	24.4	24.4	24.4
12	4	4	1.69	42.94	25+/-	12n/-	12n/-	12n/-	37.1	-8.-	-46.4	-61.4	-121.-	-111.-	-117.-	-117.-
8	4	1	1.65	78.26	25+/-	5n/-	25+/-	25+/-	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4
1	11	2	1.82	72.66	n/r	n/r	n/r	n/r	-174.1	-224.4	-221.4	-224.4	-225.4	-177.-	-222.4	-222.4
14	1	1	1.82	42.92	n/r	n/r	n/r	n/r	121.3	84.4	104.4	74.4	41.4	21.2	14.-	14.4
2*	2	1	2.07	143.27	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r
16	7	1	2.23	213.84	n/r	n/r	n/r	n/r	431.4	14.4	4.4	4.4	4.4	4.4	4.4	4.4
13	8	1	2.72	41.83	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r
F	11	4	2.64	170.67	n/r	n/r	n/r	n/r	-477.4	-34.-	-4.4	-120.4	-141.4	-277.4	-264.1	-254.4
17	14	4	2.44	149.33	n/r	n/r	n/r	n/r	571.4	57.7	57.7	57.7	57.7	57.7	57.7	57.7
1	8	4	2.46	58.32	n/r	n/r	n/r	n/r	571.4	57.7	57.7	57.7	57.7	57.7	57.7	57.7
9	15	4	2.44	57.03	n/r	n/r	n/r	n/r	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-
13	1	4	2.41	99.88	n/r	n/r	n/r	n/r	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-
A	5	4	2.38	143.78	n/r	n/r	n/r	n/r	-174.1	-15.4	-161.4	-172.4	-132.4	-88.4	-81.4	-82.7
1A	5	4	2.39	49.53	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r
A	2	2	2.37	85.43	n/r	n/r	n/r	n/r	-256.1	-449.4	-449.4	-449.4	-449.4	-449.4	-449.4	-449.4
A	8	4	2.37	78.63	n/r	n/r	n/r	n/r	226.4	268.4	268.1	274.4	224.4	219.4	221.4	221.4
8*	6	1	2.39	91.37	n/r	n/r	n/r	n/r	482.4	59.1	463.4	482.4	-465.4	-432.4	-445.4	-445.4
1	5C	4	2.37	73.57	n/r	n/r	n/r	n/r	85.4	-16.4	-192.4	-214.4	-268.4	-258.4	-258.4	-258.4
5	6	1	2.3	148.11	n/r	n/r	n/r	n/r	25+/-	25.-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-
A	8	1	2.20	148.07	n/r	n/r	n/r	n/r	284.4	284.4	141.4	284.1	272.4	284.1	284.4	284.4
A	5	1	2.29	89.65	n/r	n/r	n/r	n/r	-15.1	-54.4	-14.-	-54.4	24.4	54.4	-54.1	-54.1
5	9	1	2.26	44.77	n/r	n/r	n/r	n/r	25+/-	25.-	25+/-	25+/-	25+/-	25+/-	25+/-	n/r
14	1	1	2.22	76.46	n/r	n/r	n/r	n/r	57.1	52.4	40.-	46.1	124.2	141.4	144.4	144.4
18	9	1	2.22	85.65	n/r	n/r	n/r	n/r	-455.7	42n.4	-439.1	437.4	-401.4	402.4	405.1	404.4
5	17	4	2.26	99.26	n/r	n/r	n/r	n/r	284.4	284.4	284.4	284.4	284.4	284.4	284.4	284.4
4	15	4	2.24	69.29	n/r	n/r	n/r	n/r	284.4	284.4	284.4	284.4	284.4	n/r	n/r	n/r
1	3	4	2.24	71.66	n/r	n/r	n/r	n/r	-447.4	-27.2	-42.4	-24.1	-49.2	-24.4	-74.7	-74.4
19	11	4	2.23	68.25	n/r	n/r	n/r	n/r	291.1	31.7	382.7	324.4	184.4	51.1	44.7	44.4
17	10	4	2.21	43.44	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	284.4	-284.4	-284.4
14	2	2	2.21	53.38	n/r	n/r	n/r	n/r	21.4	-54.4	-14.4	-47.7	37.4	18.4	17.4	19.4
14	3	2	2.27	179.68	n/r	n/r	n/r	n/r	584.4	584.4	584.4	584.4	584.4	584.4	584.4	n/r
9	2	2	2.17	124.64	n/r	n/r	n/r	n/r	551.4	544.4	544.4	544.4	544.4	508.4	n/r	n/r
19	2	2	2.16	78.36	n/r	n/r	n/r	n/r	-289.5	-241.9	-284.4	-285.2	-284.7	-287.4	-281.7	-281.2
F	4	2	2.15	138.72	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r
17	8	2	2.15	42.68	n/r	n/r	n/r	n/r	584.4	584.4	584.4	584.4	584.4	584.4	584.4	584.4
12	6	2	2.15	59.26	n/r	n/r	n/r	n/r	-271.1	-28.-	-224.4	-254.2	-204.4	-178.4	-157.4	-157.4
89	6	2	2.15	174.07	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r
1	14	1	2.14	73.64	n/r	n/r	n/r	n/r	324.1	284.4	284.7	284.4	423.4	437.1	434.4	434.4
14	3A	4	2.13	51.52	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r
2	12	3	2.09	89.26	n/r	n/r	n/r	n/r	345.1	427.5	427.1	430.4	-134.-	-247.4	-239.3	-239.4
8	9	6	2.09	123.36	n/r	n/r	n/r	n/r	-561.8	-561.4	-564.4	-568.4	564.4	564.4	564.4	564.4
83	7	A	2.08	32.06	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r
3	9	2	2.07	77.78	n/r	n/r	n/r	n/r	n/r	92.4	67.2	52.1	25.4	87.-	69.4	74.4
15	9	1	2.05	44.44	n/r	n/r	n/r	n/r	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-	25+/-
11	13	2	2.04	66.37	n/r	n/r	n/r	n/r	-25+/-	-25+/-	-25+/-	-25+/-	-25+/-	-25+/-	-25+/-	-25+/-
9	9	4	2.14	77.27	n/r	n/r	n/r	n/r	n/r	377.9	354.4	353.4	346.4	484.2	442.4	423.1
8	9	2	2.03	80.14	n/r	n/r	n/r	n/r	459.4	-467.4	492.4	-493.3	-456.7	-481.7	-480.7	-480.5
1E	11	1	2.03	44.81	n/r	n/r	n/r	n/r	-337.4	-327.4	-125.4	-144.7	-264.4	-264.4	-232.4	-232.4
15	3	3	2.07	99.6F	n/r	n/r	n/r	n/r	-25+/-	-25+/-	-25+/-	-25+/-	-25+/-	-25+/-	-25+/-	-25+/-
12	5	1	2.02	91.92	n/r	n/r	n/r	n/r	n/r	1.0	25+/-	25+/-	25+/-	25+/-	-25+/-	-25+/-
10	8	2	2.08	178.76	n/r	n/r	n/r	n/r	5n4.4	484.2	461.2	-494.1	463.7	-493.5	-494.4	-492.2
3	2	1	9.66	143.48	n/r	n/r	n/r	n/r	n/r	324.5	94.9	44.1	n/r	41.9	24.4	24.4
7	3	6	1.99	131.57	n/r	n/r	n/r	n/r	-25+/-	-25+/-	-25+/-	-25+/-	25+/-	25+/-	25+/-	25+/-
7	13	2	1.97	78.44	n/r	n/r	n/r	n/r	n/r	91.1	324.5	154.4	135.4	932.4	942.7	224.4
6	6	4	1.97	106.84	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	21.4	-2.1	-2.4	-1.4
12	16	8	1.96	53.36	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r
86	8	4	1.96	42.99	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	584.4	584.4	584.4
1	15	9	1.95	81.67	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-25+/-	-25+/-	-25+/-
7	2	3	1.96	99.16	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	195.1	114.2	114.4
6	4	1	1.93	141.47	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r
7	2	2	1.92	74.91	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	375.4	144.4	114.4
4	3	9	1.91	173.34	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r
3	15	4	1.91	37.52	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-182.-	-121.4	-114.1
6	3	5	1.91	95.14	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	174.4	124.4	124.7
7	7	2	1.94	89.88	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-282.-	-284.-	-287.4
8	4	1	1.88	87.89	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	231.4	284.4	284.4
11	11	2	1.88	41.93	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	482.2	477.4	477.3
12	14	5	1.88	42.22	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	504.4	504.4	504.4
F	2	8	1.87	83.47	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	504.4	-504.4	-504.4
3	10	8	1.87	125.83	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-254.-	-254.-	-254.4
9	8	2	1.87	72.88	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-422.2	-468.1	-472.4
12	8	1	1.87	71.68	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-1.4	-4.0	-1.4
10	4	3	1.87	81.21	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	252.4	172.4	114.4
18	6	4	1.86	34.38	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-462.-	-464.-	-464.4
5	8	2	1.86	142.45	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	34.3	-44.2	-61.4
12	1	4	1.85	92.13	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-254.-	-254.-	-254.-
4	3	3	1.83	83.58	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-254.-	254.-	254.-
9	12	2	1.84	83.12	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	217.4	214.4	204.7
F	11	8	1.84	94.27	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	504.4	504.4	114.4
9	14	2	1.83	76.36	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-933.-	-242.-	-241.4
5	9	5	1.83	93.36	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-254.-	-254.-	-254.-
6	8	4	1.82	92.38	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-287.-	-214.7	-231.5
7	8	3	1.82	106.42	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-484.2	484.1	484.4
18	2	4	1.81	87.56	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	504.4	504.4	504.4
9	8	4	1.81	100.5C	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	254.4	254.4	254.4
9	5	4	1.81	139.17	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	254.4	254.4	254.4
9	5	2	1.79	191.22	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-189.-	-244.4	-233.5
19	9	3	1.78	74.17	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	254.-	254.-	254.-
18	7	1	1.78	76.87	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-934.-	-734.-	-734.-
11	2	7	1.76	104.61	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	776.-	264.4	264.4
13	9	5	1.78	42.55	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-142.-	-144.7	-102.4
F	1	4	1.78	49.62	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-254.-	-254.-	-254.-
12	8	2	1.77	92.84	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-367.-	-284.-	-284.-
15	9	1	1.77	57.41	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-254.-	-254.-	-254.-
17	1	1	1.77	137.41	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-151.-	-212.-	-212.7
19	1	1	1.76	101.47	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	254.-	254.-	254.-
1E	12	2	1.76	143.31	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-11.8	-8.4	-11.4
A	1E	4	1.78	84.12	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-348.-	-344.3	-175.1
F	8	1	1.76	83.83	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	124.-	114.2	121.7
11	16	1	1.74	58.45	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	1271.-	1.4	1.4
4	12	1	1.77	89.03	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-171.-	-142.2	-144.4
9	15	4	1.75	91.37	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	504.4	504.4	504.4
15	2	4	1.74	63.16	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	219.2	221.4	221.4
13	1	4	1.75	63.45	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	254.-	254.4	254.-
A	9	5	1.74	65.87	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-441.-	-444.4	-444.4
11	4	1	1.74	64.59	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	134.2	134.4	134.4
15	1E	1	1.74	67.81	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	254.-	254.-	254.-
17	5	4	1.72	49.67	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	781.2	784.4	784.4
12	11	2	1.72	56.78	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	81.2	8.7	17.4
16	12	4	1.71	43.76	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	504.4	504.4	504.4
13	5	4	1.71	51.42	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-254.-	-254.-	-254.-
14	11	4	1.71	72.87	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	984.4	114.2	114.2
1	2	4	1.71	74.88	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	81.4	114.4	114.2
2	2	1	1.71	137.3F	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	314.4	112.4	114.4
3	4	5	1.71	59.58	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	412.2	434.4	444.2
A	-7	4	1.71	69.17	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	314.4	284.4	284.4
4	14	-	1.69	30.35	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	954.-	254.-	254.-
4	9	1	1.69	126.11	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-124.7	-241.-	-244.7
17	9	1	1.69	44.19	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	954.-	254.-	254.-
3	6	-	1.69	117.47	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	954.-	254.-	254.-
4	8	2	1.64	111.17	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	-139.4	-60.1	-84.4
7	4	4	1.68	70.8F	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	314.4	284.4	284.4
1	17	2	1.68	71.12	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	92.8	121.1	122.4
4	11	4	1.69	174.75	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	n/r	954.-	254.-	254.-
8	9	4	1.68	56.97	8.4	8.4	8.4	8.4	8.4	8.4	8.4	n/r	84.7	134.4	134.4	134.4

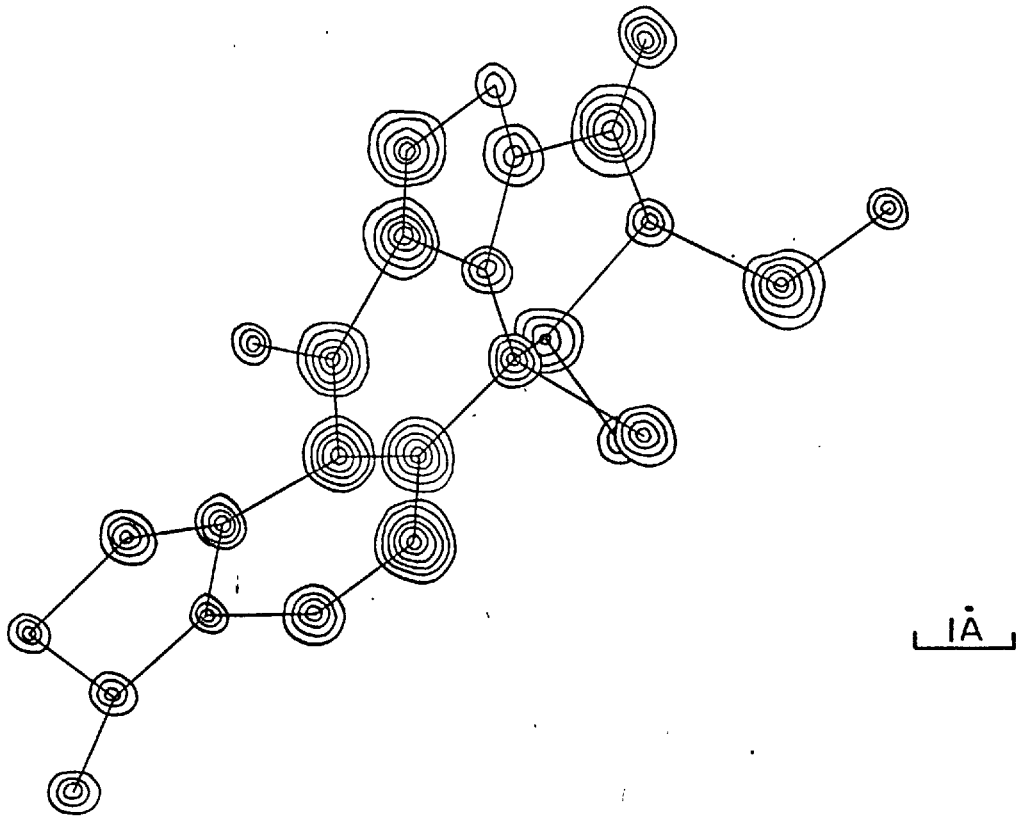


Figure 9.1. E-map for viridin. The contours are at equal, arbitrary intervals.

background level. The eighteen peaks that made chemical sense were selected. These were treated as carbon atoms and given isotropic temperature factors of 3.0 $Å^2$. After a structure-factor calculation and three cycles of ORFLS least-squares, the conventional discrepancy index, R, stood at 0.426. All temperature factors were at a sensible level. A difference Fourier map revealed two more peaks, and the subsequent structure-factor calculation reduced R to 0.387. However, one of these last two peaks had been included at an erroneous position. On correcting this and including three more peaks from the last difference map, R fell to 0.275. A final difference map revealed the last three atoms in their expected positions. Inclusion of these in a structur factor calculation followed by four cycles of BLOKLS block-diagonal least-squares refinement, resulted in an R factor of 0.185.

It had not been possible to distinguish between oxygen and carbon atoms by their peak heights in the E-map, but the six oxygen atoms were readily identified partly by the bond lengths and partly by their somewhat lower temperature factors After three more cycles of refinement using BLOKLS, R fell to 0.148; however, the overall scale factor was showing oscillatory behaviour. Individual layer scale factors were now included and after four more cycles of refinement R was now 0.141. A difference map subsequently revealed all the hydrogen atoms in their expected positions. They were

included in a structure-factor calculation, each being assigned an isotropic temperature factor 0.5$\mathring{A}^2$ greater than the atom to which it was bound. After four cycles R was now 0.131 and all parameter shifts were less than 0.04 of the corresponding standard deviation. The hydrogen parameters were not refined.

The temperature factors were converted into their anisotropic β_{ij} equivalents. Six cycles of least-squares with fixed layer scale factors and a 0.5 damping factor gave an R factor of 0.106. A Hughes-type weighting scheme was suggested for the zero and first layers on the basis of an analysis (DELSIG) of the observed and calculated structure-factors over ranges of $|F_o|$ and $\sin^2\theta$. It took the form $\sqrt{w} = 1$ for $F_o < F^*$ and $\sqrt{w} = F^*/F_o$ for $F_o > F^*$, where w is the weight to be applied in least-squares refinement and F^* is a constant. The latter was set to 410 and 610 for the zero and first layers respectively, on the scale of the structure-factors in Table 9.6. Unit weights were used for the other layers as the analysis indicated this to be the most appropiate scheme.

A few mis-punchings of intensity data were corrected and five low-angle intense reflections with $F_c > F_o$ were removed for suspected extinction. R was now 0.103. The β_{ij}'s were converted back to their isotropic B-factors and these were refined together with the non-hydrogen positional parameters and layer scale-factors for a further six cycles

Table 9.6.

Final observed and calculated structure factors; column headings are h, k, followed by ℓ, $10|F_o|$, $10|F_c|$. The accidental absences are marked (*). The five reflections omitted for suspected extinction were:-

h	k	ℓ	$10\|F_o\|$	$10\|F_c\|$
2	2	0	801	923
1	1	1	751	889
2	0	1	734	908
2	2	1	878	1033
3	2	1	956	1124

0,K,0

1	434	481
2	13*	28
3	84	89
4	81	71
5	94	72
6	76	53
7	201	199
8	356	359
9	61	61
10	64	65

1,K,0

1	483	497
2	133	114
3	322	307
4	361	323
5	244	247
6	46	42
7	380	358
8	291	241
9	264	235
10	392	358
11	106	91
12	70	70
13	26	25
14	375	370
15	219	211
16	70	64
17	70	61
18	34	40
19	73	89
20	29	24

2,K,0

0	429	458
1	111	73
3	226	198
4	545	581
5	306	335
6	291	254
7	342	329
9	52	49
10	168	137
11	267	243
12	216	212
13	47	26
14	136	125
15	195	185
16	117	113
17	13*	3
18	41	42
19	13*	16
20	29	31

3,K,0

1	86	67
2	498	543
3	60	72
4	668	754
5	245	216
6	13*	14
7	246	200
8	13*	1
9	13*	16
10	179	155
11	74	71
12	13*	26
13	180	181
14	116	105
15	267	243
16	47	42
17	142	138
18	41	28
19	13*	4
20	13*	73

4,K,0

0	250	277
1	141	141
2	109	95
3	777	864
4	106	71
5	546	549
6	170	131
7	13*	6
8	72	97
9	180	163
10	116	109
11	283	275
12	45	50
13	165	186
14	182	180
15	238	224
16	13*	12
17	13*	16
18	13*	35
19	13*	10
20	13*	17

5,K,0

1	366	346
2	291	265
3	112	71
4	29	44
5	506	526
6	646	653
7	542	564
8	211	171
9	370	338
10	406	372
11	106	90
12	13*	15
13	144	155
14	13*	5
15	128	130
16	47	56
17	13*	25
18	13*	24
19	113	85
20	13*	18

6,K,0

0	235	215
1	256	235
2	243	198
3	210	179
4	561	607
5	93	82
6	85	91
7	13*	36
8	262	274
9	155	113
10	13*	4
11	204	191
12	87	91
13	355	304
14	119	98
15	95	106
16	64	47
17	102	91
18	52	52
19	13*	1
20	13*	61

7,K,0

1	320	301
2	132	94
3	574	552
4	232	188
5	354	379
6	187	152
7	13*	22
8	100	74
9	13*	36
10	119	125
11	175	148
12	13*	26
13	13*	8
14	181	178
15	81	69
16	43	38
17	29	34

8,K,0

1	223	163
0	503	566
2	341	301
3	13*	24
4	13*	4
5	112	71
6	55	31
7	13*	27
8	218	192
9	334	315
10	65	70
11	244	218
12	138	132
13	119	124
14	117	94
15	92	91
16	41	33
17	13*	0
18	13*	39

9,K,0

1	247	207
2	90	64
3	169	155
4	351	295
5	144	142
6	310	275
7	265	227
8	289	249
9	146	134
10	178	151
11	34	53
12	154	143
13	13*	29
5	187	152
14	32	40
15	13*	40
16	13*	25
17	13*	34
18	13*	24

10,K,0

0	117	90
1	13*	10
2	105	96
3	81	60
4	211	177
5	85	70
3	247	225
7	45	45
8	161	161
9	116	106
10	58	48
11	69	89
12	34	27
13	32	27
14	101	93
15	41	37
16	13*	53
17	13*	12
18	192	178

11,K,0

1	316	305
2	13*	19
3	13*	8
4	124	119
5	118	108
6	164	181
7	175	134
8	358	348
9	217	213
10	13*	19
11	46	56
12	67	59
13	182	167
14	98	65
15	13*	19
16	13*	24
17	13*	21

12,K,0

0	155	137
1	287	270
2	13*	0
3	120	121
4	113	121
5	114	102
6	142	106
7	96	97
8	97	86
9	77	111
10	168	154
11	116	122
12	112	112
13	26	48
14	143	137
15	13*	21
16	126	116

13,K,0

1	338	334
2	13*	56
3	75	44
4	83	81
5	216	197
6	13*	30
7	236	228
8	46	50
9	118	105
10	115	120
11	144	134
12	13*	17
13	119	134
14	50	40
15	93	74
16	89	62

14,K,0

0	118	142
1	181	175
2	46	66
3	68	88
4	166	164
5	13*	31
8	13*	28
7	13*	28
8	13*	33
9	104	115
10	13*	39
11	60	69
12	13*	6
13	144	119
14	136	116
15	138	119

15,K,0

1	195	178
2	116	124
3	217	223
4	13*	0
5	118	122
6	13*	12
7	13*	32
8	182	167
9	13*	21
10	138	149

16,K,0

0	383	421
1	13*	3
2	13*	9
3	212	206
4	13*	12
5	217	207
6	32	48
7	107	106
8	13*	28
9	13*	0
10	92	63
11	13*	44
12	13*	27
13	101	106

17,K,0

1	163	163
2	13*	32
3	126	149
4	13*	42
5	165	170
6	26	53
7	26	45
8	112	134
9	13*	22
10	13*	18
11	47	35

18,K,0

0	165	160
1	13*	7
2	142	134
3	13*	32
4	26	55
5	93	107
6	37	53
7	46	60
8	32	32

19,K,0

1	80	77
2	13*	16
3	97	94
4	13*	14
5	13*	33
6	13*	28
7	13*	6

20,K,0

0	257	258
1	59	54
2	157	139
3	92	77
4	69	64
5	69	54

0,K,P1

1	65	69
2	127	115
3	180	187
4	213	209
5	383	390
6	56	37
7	197	174
8	424	394
9	134	109
10	156	147
11	75	88
12	93	92
13	13*	18
14	13*	21
15	112	127
16	13*	14
17	206	208
18	134	142
19	81	88
20	19	24

1,K,1

0	436	460
2	289	278
3	483	476
4	473	488
5	443	435
6	188	162
7	414	396
8	320	290
9	113	92
10	167	143
11	121	111
12	187	161
13	43	63
14	275	284
15	85	80
16	144	151
17	104	106
18	33	32
19	13*	23
20	45	46

2,K,1

1	661	749
3	577	624
4	83	44
5	200	194
6	370	338
7	367	331
8	112	19
9	348	301
10	61	63
11	45	37
12	249	247
13	239	271
14	91	95
15	129	132
16	13*	46
17	33	52
18	99	110
19	38	44
20	64	63

3,K,1

0	504	570
1	496	562
3	626	667
4	333	306
5	199	192
6	162	149
7	334	305
8	394	363
9	217	186
10	224	207
11	81	94
12	99	104
13	198	200
14	166	164
15	128	141
16	88	92
17	117	123
18	43	54
19	27	23
20	71	64

4,F,1

0	373	383
1	431	429
2	696	744
3	578	579
4	293	256
5	555	531
6	337	296
7	269	244
8	174	152
9	154	132
10	110	96
11	101	101
12	264	283
13	200	186
14	142	185
15	82	92
16	83	86
17	47	59
18	67	76
19	36	41

5,K,1

0	329	297
1	354	343
2	485	479
3	262	230
4	260	240
5	111	96
6	113	81
7	61	59
8	71	59
9	75	65
10	45	56
11	200	186
12	152	139
13	156	150
14	91	92
15	51	55
17	72	77
18	41	45
19	47	51

6,K,1

0	340	312
1	343	283
2	393	372
3	483	450
4	177	142
5	219	184
6	147	161
7	318	274
8	74	73
9	332	312
10	13*	19
11	253	240
12	154	153
13	203	206
14	104	110
15	56	66
16	13*	25
17	30	34
18	27	42
19	23	35

7,K,1

0	237	193
1	82	81
2	392	355
3	501	471
4	165	167
5	176	137
6	228	184
7	222	219
8	100	78
9	124	112
10	13*	27
11	176	185
12	195	184
13	91	75
14	82	99
15	79	77
16	103	95
17	43	49
18	36	40
19	49	53

8,K,1

8	51	41
1	263	221
2	99	85
3	358	307
4	181	157
5	123	103
6	395	339
7	83	69
8	203	171
9	145	125
10	71	81
11	163	144
12	166	160
13	139	122
14	89	78
15	13*	18
16	115	113
17	56	68
18	35	36

9,K,1

0	164	141
1	123	104
2	191	154
3	277	244
4	141	114
5	271	230
6	79	70
7	129	98
8	247	253
9	157	137
10	36	46
11	173	164
12	105	117
13	92	82
14	76	79
15	93	100
16	51	52
17	82	87
18	81	75

10,K,1

0	85	66
1	365	381
2	97	81
3	298	250
4	101	63
5	169	138
6	143	117
7	98	97
8	202	197
9	193	188
10	138	135
11	235	224
12	116	107
13	91	67
14	83	72
15	95	85
16	13*	17
17	33	51

11,K,1

0	165	132
1	166	145
2	123	109
3	141	121
4	160	140
5	98	87
6	264	248
7	36	52
8	36	51
9	64	70
10	66	66
11	202	204
12	88	100
13	121	134
14	13*	28
15	86	92
16	123	126
17	49	45

12,K,1

0	223	199
1	119	97
2	179	167
3	140	122
4	94	97
5	139	118
6	115	101
7	13*	50
8	91	103
9	74	78
10	27	36
11	23	53
12	13*	8
13	56	61
14	83	79
15	53	55
16	61	54

13,K,1

0	193	151
1	89	98
2	162	161
3	224	223
4	127	128
5	181	172
6	129	122
7	98	107
8	91	81
9	89	116
10	86	93
11	23	36
12	45	55
13	99	96
14	45	52
15	23	40

14,K,1

0	138	134
1	287	310
2	234	246
3	196	198
4	27	53
5	91	77
6	123	129
7	172	182
8	102	118
9	49	61
10	89	99
11	45	50
12	59	49
13	89	106
14	45	57

15,K,1

0	229	227
1	161	165
2	95	84
3	74	66
4	151	146
5	91	61
6	134	134
7	23	56
8	23	42
9	53	48
10	23	43
11	13*	40
12	19	54
13	74	83

16,K,1

1	89	91
1	51	55
2	134	133
3	13*	3
4	158	173
5	46	66
6	47	82
7	81	84

16,K,1		
8	10	54
9	56	78
10	38	63
11	36	53
12	51	68

17,K,1		
1	83	98
2	107	118
3	13*	24
4	58	82
5	45	42
6	61	79
7	118	128
8	38	40
9	63	68
10	33	50

18,K,1		
0	76	75
1	75	88
2	143	157
3	43	45
4	30	38
5	38	61
6	27	50
7	56	61
8	64	79

19,K,1		
0	75	79
1	13*	15
2	64	62
3	88	95
4	129	140
5	23	32
6	74	82
7	144	152

20,K,1		
0	71	73
1	13*	34
2	80	79
3	13*	30
4	13*	41
5	13*	41

0,K,2		
0	419	447
1	583	602
2	327	326
3	307	305
4	259	232
5	201	191
6	628	661
7	33	15
8	149	146
9	321	322
10	103	112
11	51	37
12	199	192
13	164	177
14	13*	17
15	79	82
16	91	99
17	13*	11
18	13*	24
19	13*	10
20	61	72

1,K,2		
0	452	424
1	269	232
2	629	632
3	210	184
4	320	303
5	369	342
6	131	116
7	247	225
8	357	337
9	156	165
10	155	152
11	64	86
12	45	47
13	126	117
14	60	55
15	126	133
16	94	104
17	145	153
18	30	45
19	55	62
20	72	77

2,K,2		
0	271	272
1	253	249
2	529	530
3	332	339
4	168	171
5	479	492
6	217	194
7	353	344
8	118	98
9	134	122
10	138	132
11	83	82
12	124	123
13	13*	33
14	147	141
15	157	157
16	114	119
17	13*	[illegible]
18	13*	25
19	13*	14
20	13*	39

3,K,2		
0	235	252
1	208	[illegible]
2	186	157
3	137	125
4	253	243
5	472	449
6	248	213
7	190	187
8	189	182
9	177	151
10	222	215
11	242	227
12	100	101
13	59	76
14	60	47
15	139	72
16	138	123
17	150	138
18	13*	35
19	38	56
20	13*	68

4,K,2		
0	754	900
1	517	492
2	207	176
3	390	396
4	361	338
5	201	185
6	269	253
7	187	143
8	94	74
9	287	248
10	53	58
11	239	234
12	155	150
13	158	157
14	112	101
15	82	88
16	38	63
17	13*	7
18	119	124
19	56	101

5,K,2		
0	340	301
1	241	221
2	168	170
3	255	245
4	352	330
5	463	448
6	442	418
7	138	99
8	110	102
9	50	78
10	251	245
11	274	277
12	243	232
13	103	100
14	198	205
15	58	52
16	13*	13
17	49	50
18	13*	40
19	13*	12

6,K,2		
0	63	63
1	203	149
2	182	157
3	382	348
4	501	469
5	249	221
6	194	195
7	359	359
8	153	157
9	284	276
10	137	129
11	226	238
12	60	80
13	103	114
14	167	149
15	49	71
16	92	95
17	33	60
18	27	48
19	53	56

7,K,2		
0	176	172
1	211	175
2	484	465
3	314	292
4	294	265
5	240	220
6	169	149
7	355	323
8	206	180
9	117	112
10	173	155
11	205	200
12	103	111
13	235	218
14	13*	29
15	13*	48
16	13*	19
17	13*	40
18	13*	17

8,K,2		
0	70	49
1	320	293
2	309	292
3	515	[illegible]
4	171	134
5	147	115
6	72	69

7	219	168
8	181	130
9	297	290
10	198	194
11	169	168
12	208	206
13	13*	15
14	13*	43
15	53	71
16	68	78
17	60	65
18	70	67

9,K,2		
0	208	190
1	249	209
2	161	134
3	191	160
4	215	185
5	104	95
6	161	110
7	166	153
8	152	120
9	131	111
10	179	177
11	180	180
12	168	162
13	41	68
14	13*	9
15	71	81
16	30	64
17	27	41

10,K,2		
0	72	63
1	258	184
2	116	105
3	217	196
4	336	308
5	95	89
6	149	122
7	129	132
8	155	140
9	158	137
10	84	77
11	103	98
12	175	168
13	79	75
14	74	80
15	95	94
16	19	46
17	23	40

11,K,2		
0	267	259
1	109	107
2	208	205
3	193	175
4	149	131
5	41	45
6	82	93
7	155	124
8	158	147
9	60	61
10	103	118
11	58	64
12	56	51
13	13*	9
14	109	122
15	13*	28
16	63	78

12,K,2		
0	314	308
1	13*	30
2	310	297
3	240	244
4	293	314
5	205	207
6	179	171
7	13*	37
8	199	194
9	94	99
10	134	10
11	159	165
12	84	78
13	13*	46
14	13*	35
15	27	36
16	95	97

13,K,2		
0	13*	24
1	94	83
2	13*	27
3	103	89
4	194	187
5	84	91
6	180	164
7	41	53
8	93	97
9	13*	52
10	55	84
11	140	135
12	33	51
13	95	108
14	67	59
15	43	42

14,K,2		
0	13*	21
1	170	153
2	255	233
3	134	125
4	180	174
5	13*	45
6	110	129
7	148	143

9	55	49
10	51	63
11	13*	13
12	13*	14
13	27	33
14	82	92

15,K,2		
0	83	68
1	103	135
2	83	93
3	41	36
4	130	133
5	115	124
6	149	183
7	95	98
8	83	93
9	49	58
10	13*	4
11	96	102

14,K,2		
8	96	117

15,K,2		
12	88	80
13	13*	30

16,K,2		
0	70	69
1	56	58
2	38	60
3	88	100
4	13*	27
5	36	65
6	53	81
7	36	41
8	82	104
9	30	53
10	41	54
11	70	81

17,K,2		
0	183	203
1	74	81
2	104	128
3	72	78
4	36	32
5	13*	22
6	99	131
7	63	64
8	13*	6
9	36	45
10	150	158

18,K,2		
0	13*	0
1	81	93
2	45	54
3	30	55
4	53	67
5	103	103
6	13*	15
7	95	91
8	45	46

19,K,2		
0	47	59
1	13*	10
2	84	81
3	90	95
4	49	56
5	30	39
6	47	53

0,K,3		
1	215	205
2	82	75
3	78	91
4	280	272
5	189	175
6	230	219
7	256	245
8	217	223
9	14*	1
10	98	97
11	49	51
12	65	70
13	14*	24
14	65	74
15	57	41
16	89	95
17	14*	21
18	14*	25
19	62	65

1,K,3		
0	253	245
1	187	151
2	352	311
3	284	256
4	191	181
5	378	362
6	107	81
7	75	69
8	63	66
9	150	130
10	165	169
11	55	64
12	34	36
13	128	124
14	83	97
15	34	46
16	71	12
17	45	73

18	14*	21
19	14*	11

2,K,3		
0	34	37
1	250	238
2	268	250
3	233	222
4	205	189
5	99	83
6	133	105
7	103	102
8	172	176
9	137	136
10	159	158
12	272	280
13	112	123
14	136	125
15	53	53
16	86	103
18	66	76
19	93	84

3,K,3		
0	204	209
1	380	341
2	284	242
3	193	194
4	217	203
5	74	75
6	63	56
7	162	162
8	197	176
9	42	53
10	116	120
11	105	121
12	130	158
13	57	70
14	14*	88
15	99	137
16	34	51
17	31	44
18	53	53

4,K,3		
0	494	503
1	295	283
2	228	207
3	112	96
4	238	215
5	103	65
6	14*	9
7	152	147
8	169	170
9	88	92
10	92	77
11	86	79
12	65	81
13	57	78
14	134	136
15	14*	32
16	84	88
17	60	62
18	75	69

5,K,3		
0	391	369
1	212	195
2	213	196
3	185	164
4	47	45
5	248	217
6	283	266
7	234	223
8	144	147
9	145	134
10	85	69
11	167	159
12	14*	12
13	14*	35
14	108	117
15	14*	29
16	124	128
17	14*	34
18	47	59

6,K,3		
0	338	354
1	252	229
2	202	199
3	261	253
4	111	102
5	103	90
6	146	135
7	292	278
8	89	72
9	276	267
10	199	194
11	149	144
12	132	145
13	43	49
14	37	48
15	49	64
16	73	94
17	47	64

7,K,3		
0	14*	5
1	113	99
2	123	90
3	75	73
4	45	82
5	234	171
6	129	87
7	313	308
8	274	275
9	277	285
10	111	113

11	40	48
12	14*	24
13	14*	34
14	51	79
15	47	72
16	57	68
17	31	42

8,K,3		
0	90	83
1	221	207
2	234	208
3	43	42
4	159	135
5	387	354
6	365	357
7	186	178
8	37	61
9	78	80
10	328	322
11	57	74
12	55	67
13	53	50
14	14*	17
15	14*	19
16	20	28

9,K,3		
0	102	78
1	136	116
2	84	74
3	60	50
4	183	173
5	220	205
6	177	180
7	231	208
8	184	177
9	377	396
10	57	55
11	136	146
12	53	59
13	89	103
14	71	83
15	42	49
16	14*	28

10,K,3		
0	24	23
1	145	123
2	14*	39
3	165	161
4	180	157
5	85	64
6	321	319
7	171	159
8	275	271
9	168	154
10	117	125
11	66	57
12	57	119
13	14*	19
14	14*	20
15	14*	6
16	42	51

11,K,3		
0	14*	22
1	170	162
2	126	132
3	173	161
4	55	70
5	184	176
6	104	125
7	218	220
8	168	169
9	14*	26
10	14*	35
11	14*	16
12	92	97
13	71	70
14	57	61
15	47	72

12,K,3		
0	14*	19
1	184	178
2	14*	37
3	79	83
4	231	241
5	43	55
6	217	226
7	114	137
8	14*	37
9	14*	6
10	14*	3
11	49	44
12	14*	9
13	28	42
14	65	50

13,K,3		
0	132	144
1	97	99
2	144	75
3	14*	35
4	137	137
5	125	134
6	110	119
7	85	91
8	14*	31
9	14*	24
10	49	42
11	45	56
12	[illegible]	49
13	41	49

14,K,3

1	174	112
2	14*	144
3	221	214
4	7A	80
5	100	96
6	14*	14
7	102	102
8	14*	34
9	31	44
10	14*	6
11	12A	116
12	74	A7
13	71	6A

15,K,3

0	174	192
1	14*	52
2	168	172
3	74	7A
4	51	6A
5	14*	11
6	49	72
7	14*	19
8	14*	20
9	42	65
10	14*	27

16,K,3

0	14*	16
1	49	54
2	34	36
3	97	102
4	47	64
5	103	120
6	55	67
7	51	51
8	55	73
9	49	61

17,K,3

0	134	133
1	14*	44
2	45	65
3	31	41
4	14*	7
5	40	51
6	92	100
7	74	77
8	14*	29

18,K,3

0	47	50
1	75	90
2	90	82
3	51	55
4	58	60
5	66	57

0,K,4

0	180	175
1	242	217
2	162	154
3	142	132
4	219	232
5	253	275
6	171	166
7	14*	24
8	67	78
9	14*	36
10	130	119
11	78	87
12	54	59
13	145	164
14	20	24
15	29	26
16	14*	19
17	14*	20

1,K,4

0	107	103
1	184	15A
2	157	135
3	255	247
4	168	155
5	116	113
6	104	99
7	120	111
8	124	153
9	119	137
10	308	331
11	14*	15
12	198	221
13	14*	14
14	130	133
15	29	25
16	20	17
17	56	63

2,K,4

0	190	191
1	102	88
2	42	44
3	315	323
4	191	194
5	65	57
6	183	19A
7	22A	23A
8	29	47
9	48	41
10	128	144
11	32	35
12	46	48
13	14*	21
14	14*	25
15	29	14
16	14*	34
17	14*	32

3,K,4

0	87	73
1	115	93
2	144	144
3	101	100
4	185	176
5	137	137
6	153	155
7	16A	156
8	60	68
9	146	138
10	245	256
11	117	118
12	14*	36
13	71	66
14	70	8A
15	35	45
16	35	57
17	82	81

4,K,4

0	160	185
1	129	104
2	184	143
3	302	2A7
4	166	144
5	229	234
6	241	238
7	15A	147
8	86	76
9	114	114
10	220	225
11	86	82
12	20	25
13	28	30
14	41	46
15	54	56
16	32	34
17	20	32

5,K,4

0	105	A8
1	176	141
2	110	113
3	239	224
4	125	106
5	175	142
6	25A	234
7	219	212
8	417	416
9	46	63
10	56	76
11	123	141
12	14*	18
13	124	113
14	50	62
15	14*	18
16	38	50
17	78	66

6,K,4

0	25	36
1	173	160
2	95	73
3	250	238
4	94	86
5	374	397
6	206	218
7	234	250
8	242	254
9	46	66
10	14*	11
11	14*	23
12	67	66
13	52	76
14	14*	28
15	62	77
16	63	A2

7,K,4

0	38	3
1	127	114
2	29	27
3	88	92
4	259	246
5	127	129
6	75	83
7	190	174
8	32	43
9	65	66
10	103	97
11	91	90
12	14*	40
13	71	74
14	75	77
15	52	56
16	67	66

8,K,4

4	91	89
5	75	79
6	127	127
7	137	132
8	262	273
9	154	14A
10	65	60
11	94	90
12	52	51
13	26	3A
14	50	48
15	84	78

9,K,4

0	62	67
1	43	45
2	205	209
3	197	18A
4	11A	120
5	202	191
6	20	34
7	86	85
8	10A	11A
9	215	21A
10	43	55
11	14*	19
12	26	22
13	52	5A
14	99	97
15	32	37

10,K,4

0	256	293
1	202	204
2	324	350
3	78	82
4	205	189
5	206	199
6	86	92
7	137	101
8	137	140
9	14*	27
10	62	81
11	54	67
12	38	35
13	25	29
14	14*	22

11,K,4

0	134	159
1	65	76
2	160	157
3	173	16A
4	123	123
5	41	45
6	159	156
7	91	101
8	147	14A
9	43	63
10	46	71
11	90	102
12	113	103
13	109	91
14	56	49

12,K,4

0	92	85
1	135	141
2	80	92
3	56	67
4	14*	16
5	102	103
6	168	173
7	75	80
8	41	56
9	91	90
10	75	75
11	171	172
12	29	43

13,K,4

0	102	116
1	46	57
2	90	91
3	127	133
4	43	50
5	75	92
6	74	75
7	41	59
8	52	56
9	78	86
10	129	130
11	108	96
12	14	32

14,K,4

0	62	76
1	43	53
2	74	86
3	74	73
4	29	34
5	141	140
6	20	3A
7	65	60
8	14	34
9	32	44
10	14*	15

15,K,4

2	80	82
3	3A	42
4	127	130
5	9A	97
6	56	66
7	29	34
8	80	86
9	25	45

16,K,4

0	75	89
1	87	83
2	62	72
3	73	77
4	5A	56
5	67	66
6	131	12A
7	35	55

17,K,4

0	78	82
1	41	37
2	71	61
3	4A	48
4	90	65
5	92	99

0,K,5

1	22	20
2	170	164
3	166	179
4	8A	77
5	114	116
6	61	57
7	209	219
8	65	78
9	102	105
10	63	59
11	65	76
12	74	86
13	85	100
14	101	106
15	15*	14
16	54	67

1,K,5

0	31	40
1	70	69
2	97	91
3	167	155
4	165	142
5	61	68
6	85	76
7	67	57
8	129	115
9	61	70
10	97	108
11	15	31
12	77	81
13	41	36
14	31	38
15	22	32

2,K,5

0	47	52
1	89	61
2	119	110
3	265	263
4	227	241
5	238	246
6	178	189
7	52	48
8	211	225
9	115	125
10	164	173
11	162	186
12	57	58
13	76	80
14	52	40
15	31	27

3,K,5

0	44	34
1	52	41
2	122	113
3	242	238
4	259	258
5	304	288
6	164	152
7	15*	12
8	61	77
9	15*	30
10	41	43
11	135	154
12	15*	26
13	92	98
14	47	5

4,K,5

0	47	52
1	115	140
2	9A	71
3	10C	114
4	115	11A
5	177	174
6	169	165
7	35	47
8	8*	98
9	112	129
10	145	159
11	41	48
12	44	46
13	61	53
14	74	84

5,K,5

0	34	23
1	44	35
2	151	148
3	57	71
4	15	18
5	194	193
6	99	92
7	72	75
8	72	65
9	198	204
10	110	112
11	91	96
12	115	116
13	31	36
14	70	78
15	50	45

6,K,5

0	82	78
1	89	7A
2	105	85
3	267	275
4	44	3A
5	27	22
6	77	74
7	99	95
8	89	85
9	59	59
10	117	123
11	83	95
12	15	38
13	96	9A
14	70	80

7,K,5

0	15*	25
1	151	156
2	209	206
3	85	92
4	70	74
5	181	179
6	102	89
7	41	60
8	41	39
9	88	91
10	80	72
11	38	59
12	35	40
13	38	44
14	31	26

8,K,5

0	188	19A
1	119	127
2	199	200
3	109	115
4	59	56
5	202	202
6	15*	9
7	15*	14
8	93	84
9	57	59
10	61	58
11	69	59
12	50	49
13	35	34
14	72	69

9,K,5

0	162	165
1	15*	14
2	83	90
3	22	25
4	15*	14
5	134	136
6	67	47
7	124	120
8	15*	8
9	96	16A
10	44	55
11	69	69
12	85	83
13	22	23

10,K,5

0	15*	0
1	15*	6
2	103	99
3	22	4A
4	15*	23
5	113	121
6	15	23
7	95	66
8	95	94
9	44	40
10	95	85
11	76	66
12	83	54

11,K,5

0	15*	24
1	54	64
2	52	63
3	15*	15
4	101	115
5	15*	29
6	8A	97
7	94	46
8	35	31
9	148	129
10	27	37
11	31	36

12,K,5

0	100	106
1	15	26
2	22	26
3	105	117
4	15*	12
5	52	47
6	143	149
7	133	134
8	15*	25
9	52	40
10	44	48

13,K,5

0	83	78
1	147	148
2	107	107
3	105	104
4	148	121
5	22	30
6	56	66
7	38	38
8	22	44
9	50	54

14,K,5

0	41	39
1	74	73
2	72	74
3	63	60
4	112	110
5	97	96
6	41	41
7	47	45
8	59	67

15,K,5

0	67	65
1	67	58
2	106	103
3	22	28
4	72	70
5	76	71

0,K,6

0	41	56
1	221	226
2	239	234
3	77	88
4	137	164
5	15*	26
6	82	92
7	31	40
8	49	59
9	35	43
10	15*	23
11	35	44
12	15*	36
13	15*	4

1,K,6

0	185	178
1	211	203
2	135	121
3	269	293
4	70	64
5	70	67
6	147	142
7	15*	26
8	92	96
9	15*	18
10	41	53
11	27	35
12	22	37
13	86	77

6,K,7

1	95	92
2	88	94
3	19*	20
4	19*	15
5	59	60
6	14*	137
7	101	94
8	145	142
9	19*	37

1,K,7

1	99	93
2	74	76
3	74	8*
4	19*	23
5	19*	56
6	19*	12
7	2*	45
8	161	161
9	74	72

Table 9.7.

Final positional parameters for the non-hydrogen atoms, as fractions of the unit-cell edges. Standard deviations are in parentheses.

Atom	x	y	z
C(1)	0.50626(39)	-0.01541(44)	-0.09978(109)
C(2)	0.56680(41)	0.05810(46)	-0.10534(131)
C(3)	0.54435(48)	0.13332(44)	0.02935(145)
C(4)	0.49528(43)	0.11424(41)	0.21749(137)
C(5)	0.46106(37)	0.03375(36)	0.24983(109)
C(6)	0.40357(41)	0.03954(38)	0.40735(119)
C(7)	0.34568(40)	-0.02463(41)	0.46805(117)
C(8)	0.34637(36)	-0.09417(37)	0.30985(110)
C(9)	0.40425(35)	-0.10129(38)	0.14162(112)
C(10)	0.48037(34)	-0.04386(37)	0.13018(105)
C(11)	0.39813(40)	-0.16609(42)	-0.00963(126)
C(12)	0.33547(44)	-0.22370(45)	0.00228(145)
C(13)	0.27865(40)	-0.21675(43)	0.16964(128)
C(14)	0.28193(36)	-0.15434(39)	0.32443(112)
C(15)	0.21088(42)	-0.16010(47)	0.48036(136)
C(16)	0.16340(47)	-0.23819(49)	0.41328(156)
C(17)	0.20637(43)	-0.27063(46)	0.21378(145)
C(18)	0.55187(38)	-0.08892(41)	0.24901(117)
O(19)	0.53962(32)	-0.08077(35)	-0.22526(85)

Table 9.7 (continued).

O(20)	0.64604(28)	0.02763(34)	-0.03080(107)
C(21)	0.71692(60)	0.07225(86)	-0.11571(258)
O(22)	0.56654(45)	0.20228(37)	-0.02232(139)
C(23)	0.45750(51)	0.16348(45)	0.36988(159)
O(24)	0.40178(31)	0.11977(28)	0.49161(95)
O(25)	0.30043(34)	-0.01988(33)	0.62742(89)
O(26)	0.18674(37)	-0.33112(40)	0.10811(124)

Table 9.8.

Final anisotropic thermal parameters (β_{ij}'s) for the non-hydrogen atoms, $\times 10^5$. Standard deviations are in parentheses.

Atom	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(1)	160(22)	301(27)	294(187)	-6(21)	123(55)	4(59)
C(2)	175(24)	290(29)	1320(229)	16(22)	35(66)	169(69)
C(3)	265(28)	231(27)	2050(265)	-36(24)	119(81)	195(72)
C(4)	181(23)	197(23)	1746(244)	-31(21)	-19(66)	75(67)
C(5)	145(20)	156(21)	625(178)	19(18)	-110(54)	21(54)
C(6)	200(23)	144(21)	1258(206)	12(19)	23(63)	-235(56)
C(7)	187(23)	222(24)	759(190)	37(21)	-51(58)	15(58)
C(8)	113(19)	158(21)	694(181)	2(17)	-82(50)	-84(49)
C(9)	95(18)	196(22)	714(178)	9(17)	-49(54)	-112(54)
C(10)	108(19)	170(21)	381(167)	23(17)	-125(51)	-63(51)
C(11)	173(23)	236(25)	1123(200)	-20(21)	43(66)	-170(63)
C(12)	240(27)	270(28)	1581(232)	-37(23)	-22(75)	-267(75)
C(13)	152(22)	239(26)	1463(221)	-80(21)	-56(62)	-25(64)
C(14)	92(19)	193(22)	966(189)	6(18)	38(52)	-2(55)
C(15)	179(24)	317(30)	1463(224)	-51(23)	188(68)	-92(74)
C(16)	233(28)	290(30)	2540(296)	-90(25)	160(78)	-11(82)
C(17)	185(26)	272(29)	2156(263)	-82(23)	-91(72)	27(75)
C(18)	150(22)	241(24)	733(194)	67(20)	-157(59)	37(59)
O(19)	269(20)	390(23)	581(142)	-54(20)	200(49)	-182(49)

Table 9.8 (continued).

O(20)	111(16)	375(23)	2706(201)	-21(17)	147(51)	230(64)
C(21)	270(37)	888(77)	6493(647)	-160(46)	770(142)	582(200)
O(22)	589(36)	276(24)	4310(297)	-99(243)	719(96)	294(77)
C(23)	316(31)	190(26)	2699(291)	-27(25)	116(92)	-130(78)
O(24)	278(19)	162(16)	1835(165)	25(16)	143(57)	-274(48)
O(25)	339(22)	311(22)	970(147)	-35(19)	393(54)	-219(51)
O(26)	326(25)	429(28)	3509(255)	-222(23)	-23(73)	-353(78)

Table 9.9.

Observed but unrefined positioned parameters for the hydrogen atoms, as fractions of the cell edge.

	x	y	z
H(1) - C(1)	0.452	0.001	-0.181
H(2) - C(2)	0.570	0.078	-0.267
H(3) - C(11)	0.441	-0.170	-0.134
H(4) - C(12)	0.331	-0.271	-0.110
H(5) - C(15)	0.233	-0.165	0.637
H(6) - C(15)	0.173	-0.109	0.471
H(7) - C(16)	0.165	-0.281	0.537
H(8) - C(16)	0.102	-0.224	0.383
H(9) - C(18)	0.567	-0.154	0.130
H(10)- C(18)	0.587	-0.052	0.270
H(11)- C(18)	0.529	-0.112	0.380
H(12)- O(19)	0.607	-0.118	-0.160
H(13)- C(21)	0.760	0.030	-0.205
H(14)- C(21)	0.709	0.060	-0.240
H(15)- C(21)	0.770	0.070	0.044
H(16)- C(23)	0.470	0.226	0.391

Each hydrogen atom was given a B-factor 0.5Å^2 greater than that of the atom to which it is attached.

Average C - H distance is 1.060Å.

of BLOKLS, by which time the scale factors were judged to have settled down completely. For the final stage of refinement, the B's were reconverted to β_{ij}'s and a final six cycles of least-squares were performed on all non-hydrogen parameters, with the new layer scale factors kept fixed. All parameter shifts were now less than 0.01 of the corresponding standard deviations and refinement was judged to be complete with R now 0.0916. The final structure-factors listed in Table 9.6 were calculated with the final parameters listed in Tables 9.7 and 9.8, together with their estimated standard deviations (σ). The observed hydrogen positions are listed in Table 9.9.

9.5. Discussion of Structure Solution.

A study of the "best" sets of phases (see Table 9.3), shows a marked similarity in the final values of phases a, b and c after tangent refinement regardless of their initially postulated values. There was a similar concordance for the majority of the other phases, but a few seemed to be more sensitive to the starting set and were consequently less consistent. Thus the E-maps derivable from these sets would have had much in common. Only the one E-map was in fact computed.

It can be seen from Table 9.10, which details the initial 150 phases, that there is in general excellent agreement between the true and the selected best set of tangent-refined phases. The average error in the three-dimensional phases is

Table 9.10.

Phase angles for the 150 reflections used in the tangent refinement (ϕ_t), compared with those calculated from the final structure-factor listing, (ϕ_c). Phases are in millicycles.

h	k	ℓ	E	ϕ_c	ϕ_t	$\lvert\phi_t - \phi_c\rvert$
6	13	0	3.23	0	0	0
1	14	0	2.97	250	250	0
0	17	1	2.24	250	250	0
15	0	1	2.02	250	250	0
10	18	0	3.20	0	0	0
9	9	3	3.08	84	78	6
5	8	4	3.00	-247	-250	3
20	2	0	2.52	0	0	0
14	15	0	2.29	500	500	0
19	7	1	2.28	249	291	42
14	3	3	2.12	-120	-189	69
14	13	0	2.03	500	500	0
7	9	3	1.99	356	356	0
12	6	3	1.91	-362	-312	50
12	4	3	1.89	-286	-302	16
5	11	2	1.88	256	255	1
14	2	1	1.87	120	133	13
6	9	3	1.87	309	264	45
8	12	2	1.84	157	108	49
11	7	3	1.84	84	-46	130
6	7	2	1.77	-19	-9	10
1	12	4	1.77	-332	-280	52
7	3	1	1.76	501	447	54
7	14	0	1.74	-250	-250	0
12	3	2	1.71	-238	-47	191
12	6	4	1.69	-166	-100	66

Table 9.10. (Continued).

9	4	0	1.65	-250	-250	0
6	11	2	1.62	-340	-274	66
13	1	5	1.62	-36	15	51
20	0	0	2.87	0	0	0
16	0	0	2.73	500	500	0
11	8	0	2.72	250	250	0
8	10	3	2.69	-263	-254	9
0	16	0	2.49	500	500	0
10	2	4	2.49	-494	-481	13
3	15	0	2.45	-250	-250	0
13	1	0	2.41	250	250	0
6	5	4	2.38	-59	-92	33
16	5	0	2.38	500	500	0
8	3	2	2.37	-392	-484	92
8	8	4	2.37	247	221	26
10	6	3	2.37	-444	-465	21
1	10	4	2.32	-274	-256	18
5	6	0	2.30	250	250	0
8	6	3	2.29	300	259	41
8	5	3	2.29	121	64	57
5	7	0	2.29	250	250	0
14	1	1	2.27	134	165	31
10	8	3	2.27	501	495	6
5	10	0	2.26	250	250	0
4	15	0	2.24	0	0	0
1	3	6	2.24	-108	-79	29
12	11	4	2.23	28	46	18
17	10	0	2.21	-250	-250	0
14	2	2	2.21	6	13	7
16	3	0	2.20	500	500	0
4	0	2	2.17	500	500	0
12	2	2	2.16	-238	-264	26

Table 9.10. (Continued).

0	6	2	2.16	0	0	0
17	0	2	2.15	500	500	0
12	4	2	2.15	-119	-157	38
12	0	2	2.15	0	0	0
1	14	1	2.14	370	405	35
14	14	0	2.13	0	0	0
2	12	3	2.09	-204	-239	35
8	9	0	2.09	500	500	0
13	7	0	2.08	-250	-250	0
3	5	5	2.07	-55	24	79
15	8	0	2.05	250	250	0
11	13	0	2.04	-250	-250	0
9	9	4	2.04	361	422	61
8	9	2	2.03	-392	-481	89
10	11	1	2.03	-344	-238	96
15	3	0	2.02	-250	-250	0
17	5	0	2.02	-250	-250	0
10	4	2	2.00	-453	-482	29
7	3	0	1.99	250	250	0
7	13	2	1.97	210	209	1
6	4	2	1.97	29	-3	32
12	16	0	1.96	0	0	0
10	0	4	1.96	500	500	0
1	15	0	1.95	-250	-250	0
7	7	3	1.94	87	110	23
6	4	0	1.93	0	0	0
7	2	2	1.92	334	335	1
4	3	0	1.91	0	0	0
3	10	4	1.91	-397	-310	87
6	3	5	1.91	179	324	145
7	7	2	1.90	-230	-288	58
8	6	1	1.89	364	202	162

Table 9.10. (Continued).

11	11	1	1.88	757	477	180
12	14	0	1.88	500	500	0
0	2	6	1.87	-500	-500	0
1	10	0	1.87	-250	-250	0
1	8	7	1.87	-426	-472	46
12	1	0	1.87	0	0	0
19	4	1	1.87	239	115	124
16	6	4	1.86	-534	-466	68
5	6	2	1.86	-17	-92	75
17	1	0	1.85	-250	-250	0
4	0	3	1.85	250	250	0
5	12	2	1.84	191	206	15
8	11	0	1.84	500	500	0
5	14	2	1.83	-251	-241	10
5	9	0	1.83	-250	-250	0
6	8	4	1.82	-259	-211	48
7	8	3	1.82	375	489	114
18	2	0	1.81	500	500	0
9	8	0	1.81	250	250	0
5	5	0	1.81	250	250	0
5	5	2	1.79	-329	-258	71
15	0	3	1.79	250	250	0
18	2	1	1.79	-181	-241	60
11	2	2	1.78	299	266	33
11	9	5	1.78	-171	-198	27
0	1	6	1.78	-250	-250	0
12	8	2	1.77	-290	-290	0
11	9	0	1.77	-250	-250	0
10	1	1	1.77	-139	-213	74
15	1	0	1.76	250	250	0
10	12	2	1.76	20	-11	31
4	10	4	1.76	-219	-174	45
6	9	1	1.76	356	324	32

Table 9.10. (Continued).

11	16	1	1.76	-669	-447	222
4	12	1	1.76	-315	-345	30
2	15	0	1.75	500	500	0
15	2	3	1.75	261	303	42
11	1	0	1.75	250	250	0
5	9	5	1.74	-468	-443	25
11	6	1	1.74	331	342	11
15	10	0	1.73	250	250	0
10	5	4	1.72	166	296	130
12	11	2	1.72	34	10	24
16	13	0	1.71	500	500	0
13	5	0	1.71	-250	-250	0
14	11	3	1.71	367	317	50
1	1	6	1.71	123	120	3
3	4	5	1.70	438	445	7
6	7	3	1.70	310	298	12
5	19	0	1.69	250	250	0
4	5	1	1.69	-243	-284	41
17	0	3	1.69	250	250	0
3	4	0	1.69	250	250	0
6	9	2	1.69	-162	-95	67
7	4	4	1.69	-242	-289	47
1	17	2	1.69	113	122	9
4	11	0	1.69	0	0	0
8	5	5	1.68	223	207	16

50 millicycles; this compares very favourably with that for L-arginine dihydrate of about 150 millicycles [11], and that for panamine of about 120 millicycles [12]. This demonstrates the power of the tangent formula in phase refining and is the reason for the well-resolved E-map and its subsequent ease of interpretation. It is interesting to note that we have only used six phases per non-hydrogen atom; increasing this ratio simply increased the sharpness and height of the peaks, as expected. The distribution of the phase errors is shown in figure 9.2. There is a marked decrease in the error $|\phi_T - \phi_c|$ with increasing E value i.e. the probability of a phase being correct becomes greater with increasing E, in agreement with a number of other frequency distributions that have been published.

The use of the symbolic-addition procedure in non-centrosymmetric structures [10] has been criticised by a number of workers [13], [14], [15], principally because of the restrictive nature of the $\sum_2$ formula However, as shown here, if carefully applied the formula can be very valuable in increasing the basic set of starting phases. The use of a severe rejection criterion (a minimum triple product value of 13.0) has meant that only symbolic phases with a relatively high probability of being correct were accepted; this together with the

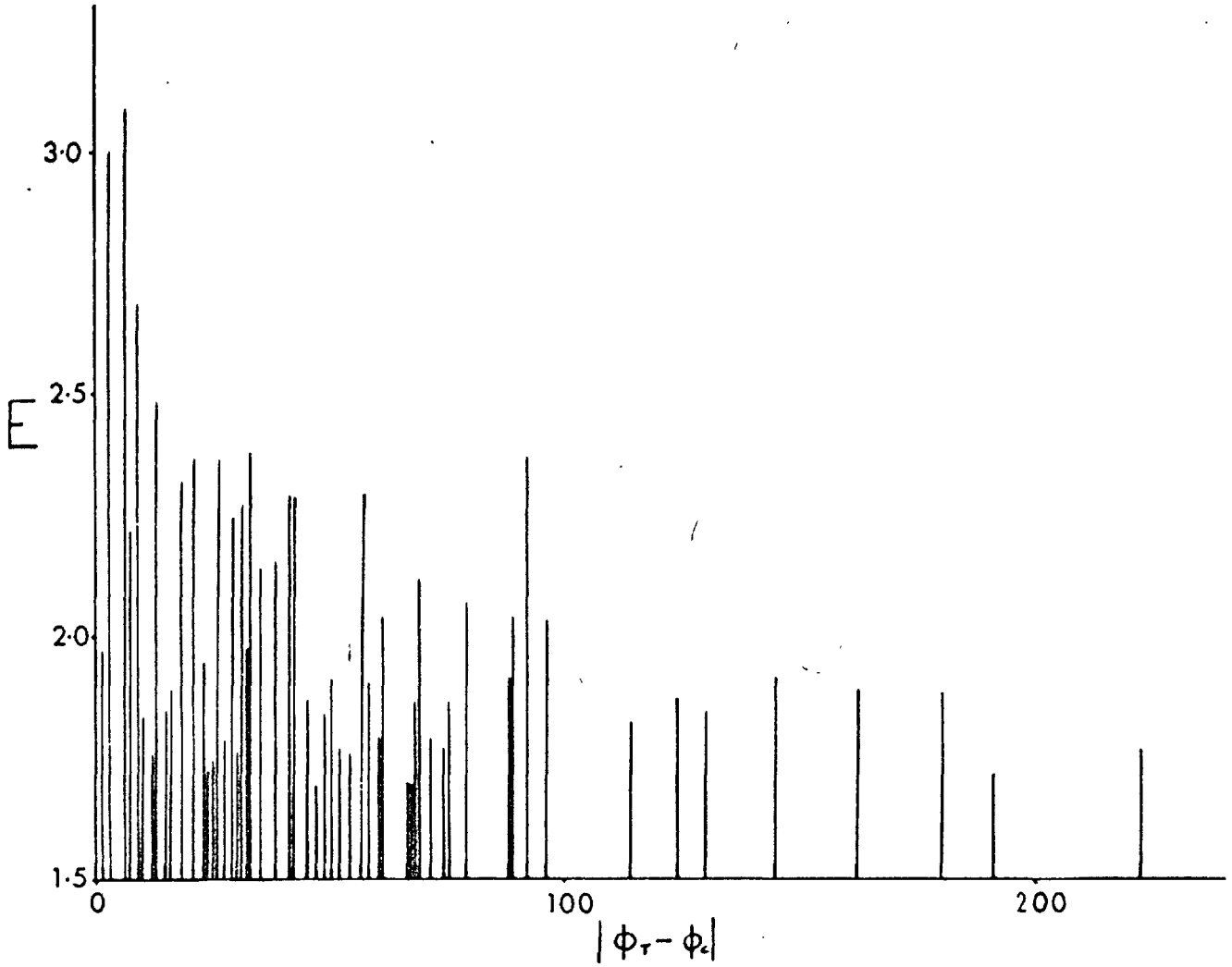


Figure 9.2. Error distribution of the tangent-refined phases.

further care in not accepting any symbolic inconsistencies in successive iterations of the $\sum_2$ formula, has safeguarded our procedure. Table 9.10 shows that none of the two-dimensional reflections was determined incorrectly, and that only one three-dimensional reflection in the initial set of 29, was in serious error compared with its phase after the tangent refinement. In circumstances such as these the tangent formula works very well.

The basis of the criteria used in selecting the "best" set of phases is worth examining. It seems that the value of Karle's reliability index (R_K) is the most sensitive of all the indicators, taken together with the behaviour of the complete set of phases. A set of phases was obtained with a different quartet of origin and enantiomorph-defining reflections; these gave an R_K of 0.235, but with considerable oscillatory behaviour during the tangent refinement, even after 60 cycles. The corresponding E-map made no chemical sense. It was characterised by a few high peaks and very many lower ones, scattered randomly in the unit-cell. Full-matrix least-squares refinement was attempted on peaks corresponding to what were thought to be possible fragments of the structure, but to no avail. Although shifts of up to several Angstrom units in an individual cycle were obtained the results were meaningless, and this first set of phases was abandoned. Thus it seems that smooth and relatively

speedy phase convergence, signifies a valid phase set. It is interesting to note that Drew's reliability index (R_D) was rather insensitive in this problem, for no apparent reason.

It is felt that the success of this method in enabling the complete structure to be resolved clearly in one E-map is due to the fact that tangent refinement was performed on all possible starting sets, with the symbols incremented in relatively fine intervals. This contrasts with the more usual approach of refining only on the more probable starting sets, previously indicated by the symbolic-addition. In that event the best solution often cannot be reached as the tangent formula has only a limited ability to adjust phases.

9.6. Description of the Molecule.

The structure of the molecule is shown in c-axis projection in Figure 9.3. The relative stereochemistry proposed by Grove [2] has been confirmed. Tables 9.11 and 9.12 detail the intramolecular bonded distances and valence angles, together with their estimated standard deviations. The latter are probably low estimates since block-diagonal least-squares refinement was used to optimise the parameters. The bond lengths and angles are also shown in Figures 9.4 and 9.5.

The pure sp^3-sp^3 carbon-carbon single bond lengths for C(1)-C(2), C(1)-C(10), C(10)-C(18) and C(15)-C(16) are in

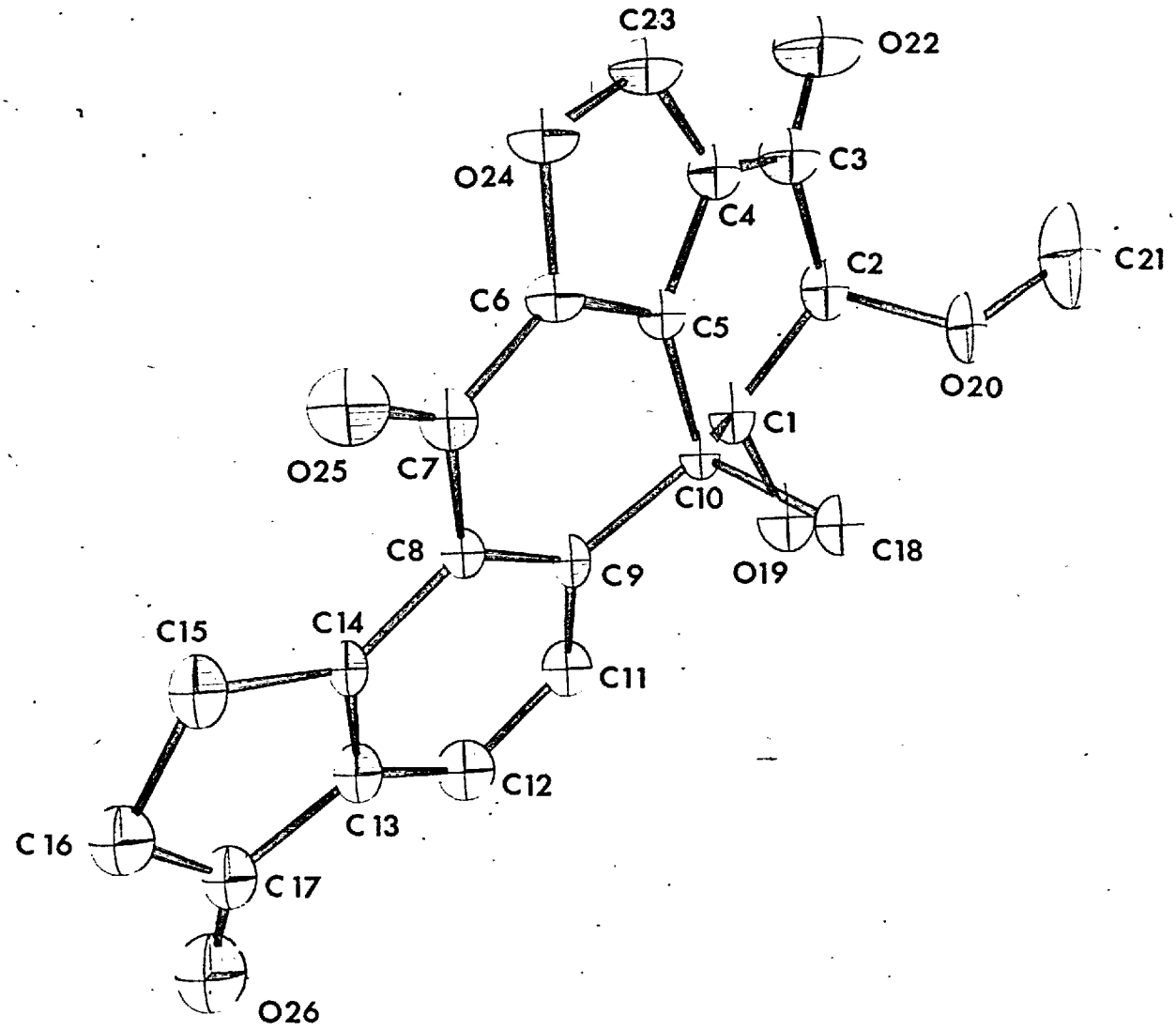


Figure 9.3. The c-axis projection of a single molecule of viridin. The ellipsoids are scaled to include 50% probability.

Table 9.11.

Intramolecular bonded distances (r) and their estimated standard deviations (σ), in parentheses.

Atoms	r(Å)	Atoms	r(Å)
C(1)-C(2)	1.537(10)	C(11)-C(12)	1.374(10)
C(1)-C(10)	1.554(9)	C(12)-C(13)	1.387(11)
C(1)-O(19)	1.417(9)	C(13)-C(14)	1.393(10)
C(2)-C(3)	1.518(11)	C(13)-C(17)	1.478(10)
C(2)-O(20)	1.443(8)	C(14)-C(15)	1.500(10)
C(3)-C(4)	1.441(12)	C(15)-C(16)	1.533(11)
C(3)-O(22)	1.213(10)	C(16)-C(17)	1.510(12)
C(4)-C(5)	1.427(9)	C(17)-O(26)	1.218(10)
C(4)-C(23)	1.376(12)	O(20)-C(21)	1.448(13)
C(5)-C(6)	1.348(9)	C(23)-O(24)	1.368(10)
C(5)-C(10)	1.490(9)		
C(6)-C(7)	1.444(9)		
C(6)-O(24)	1.398(8)		
C(7)-C(8)	1.491(9)		
C(7)-O(25)	1.229(9)		
C(8)-C(9)	1.402(9)		
C(8)-C(14)	1.424(8)		
C(9)-C(10)	1.538(8)		
C(9)-C(11)	1.409(10)		
C(10)-C(18)	1.548(9)		

Table 9.12.

Valence angles in the asymmetric unit, (θ), with their estimated standard deviations in parentheses.

Atoms	θ(°)	Atoms	θ(°)
C(2)-C(1)-C(10)	114.8(6)	C(7)-C(8)-C(9)	123.7(5)
C(2)-C(1)-O(19)	108.9(5)	C(7)-C(8)-C(14)	117.9(6)
C(10)-C(1)-O(19)	112.6(5)	C(9)-C(8)-C(14)	118.3(6)
C(1)-C(2)-C(3)	117.1(6)	C(8)-C(9)-C(10)	120.9(6)
C(1)-C(2)-O(20)	106.8(6)	C(8)-C(9)-C(11)	120.6(6)
C(3)-C(2)-O(20)	107.9(6)	C(10)-C(9)-C(11)	118.3(5)
C(2)-C(3)-C(4)	113.8(6)	C(1)-C(10)-C(5)	105.3(5)
C(2)-C(3)-O(22)	121.4(8)	C(1)-C(10)-C(9)	115.7(5)
C(4)-C(3)-O(22)	124.8(8)	C(1)-C(10)-C(18)	112.1(5)
C(3)-C(4)-C(5)	121.3(7)	C(5)-C(10)-C(9)	108.6(5)
C(3)-C(4)-C(23)	132.3(7)	C(5)-C(10)-C(18)	108.3(5)
C(5)-C(4)-C(23)	105.1(6)	C(9)-C(10)-C(18)	106.6(5)
C(4)-C(5)-C(6)	107.7(6)	C(9)-C(11)-C(12)	121.3(7)
C(4)-C(5)-C(10)	128.1(6)	C(11)-C(12)-C(13)	118.0(7)
C(6)-C(5)-C(10)	124.2(5)	C(12)-C(13)-C(14)	123.3(6)
C(5)-C(6)-C(7)	125.6(6)	C(12)-C(13)-C(17)	127.5(7)
C(5)-C(6)-O(24)	110.4(5)	C(14)-C(13)-C(17)	109.2(6)
C(7)-C(6)-O(24)	123.8(6)	C(8)-C(14)-C(13)	118.6(6)
C(6)-C(7)-C(8)	111.4(6)	C(8)-C(14)-C(15)	129.7(6)
C(6)-C(7)-O(25)	123.1(6)	C(13)-C(14)-C(15)	111.7(6)
C(8)-C(7)-O(25)	125.4(6)	C(14)-C(15)-C(16)	104.9(6)

Table 9.12. (Continued).

Atoms	θ(°)
C(15)-C(16)-C(17)	106.2(6)
C(13)-C(17)-C(16)	107.9(6)
C(13)-C(17)-O(26)	125.3(8)
C(16)-C(17)-O(26)	126.9(7)
C(2)-O(20)-C(21)	114.2(7)
C(4)-C(23)-O(24)	111.7(6)
C(6)-O(24)-C(23)	105.1(6)

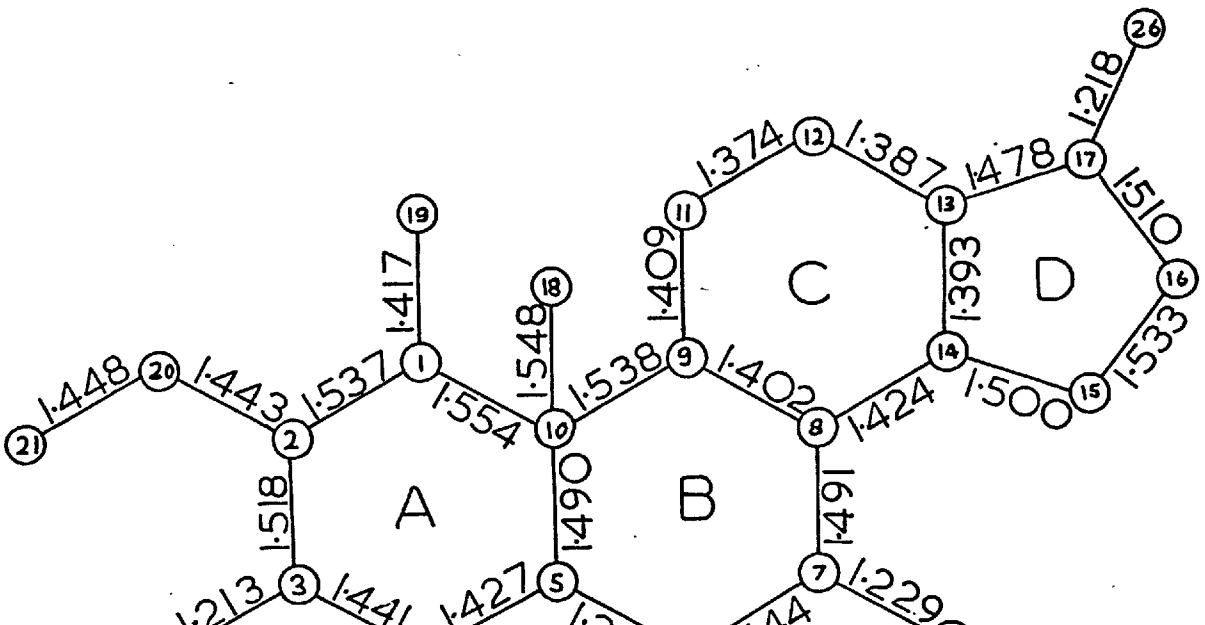


Figure 9.4. Intramolecular bond lengths (in Å).

C(1)-C(10)-C(9) = 115.7°
C(5)-C(10)-C(18)=108.3°

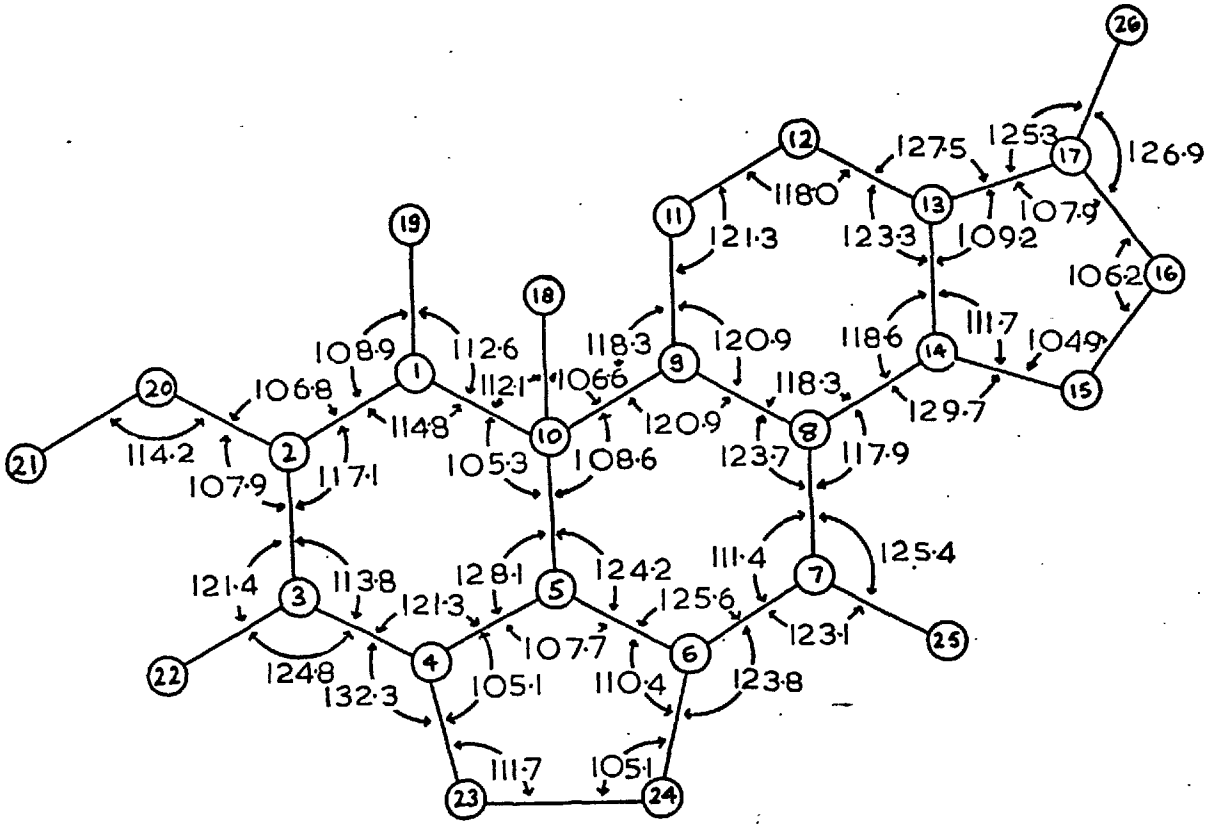


Figure 9.5. Intramolecular bond angles (in degrees).

excellent agreement with the accepted value of 1.537 Å[16]. Ring C is fully aromatic as indicated by its bond lengths and angles, none of which differ significantly from the normal values of 1.394 Å and 120° respectively, and by its complete planarity. Ring D is of some interest; from its bond lengths and angles it is formally almost a cyclopentenone. The short C(13)-C(17) bond (1.478 Å;σ=0.010 Å) is readily explicable in terms of sp^2-sp^2 shortening. Bond C(14)-C(15) is a sp^2-sp^3 type; its length is significantly shorter ($> 3\sigma$) than 1.54 Å. The sp^2 hybrid orbitals at C(13) and C(14) further cause the angles C(14)-C(13)-C(17) and C(13)-C(14)-C(15) to deviate markedly from the corresponding ones in androsterone [17] of 99° and 104° for the normal steroidal cyclopentanone ring D, to ones of 109° and 112° respectively. Bond C(15)-C(16) has fully sp^3-sp^3 character whilst C(16)-C(17) is of type sp^2-sp^3 and is correspondingly shorter. Ring D is virtually planar (Table 9.13), and is roughly coplanar with ring C. In view of the conjugation on C(13) and C(17), this is to be expected. The two rings are at an angle of about one degree to each other. Figure 9.6 shows a projection of the molecule edgewise to the rings C-D [18]. The only other ring C benzenoid steroid whose crystal structure has been determined [19], has no carbonyl at C(17); ring D is in effect a cyclopentene and the

predicted envelope conformation was indeed found. Thus, the determining factor in producing a flattening of ring D in viridin is the presence of the carbonyl moiety. The alkaloid acutumine similarly has an almost planar cyclopentenone ring [20]. Bromogeigerin acetate [21] on the other hand, has the carbon, which is analogous to C(16) here, displaced more than 0.3 A from the plane of the ring. The cyclopentenone is here fused to a cycloheptane ring in the chair form at C(14) and C(15) i.e. it is not fused across the double bond, and thus the former does not have the rather severe steric constraints imposed on it that are important in the viridin situation.

Ring B shows considerable deviation from the usual geometry of a cyclohexadienone; thus, C(5), C(6), C(8) and C(9) are closely coplanar (Table 9.13), whilst C(7) and C(10) are 0.108 Å and 0.304 Å respectively out of this plane. The overall ring geometry can best be described as a slightly flattened boat, a somewhat unusual situation. This marked non-planarity contrasts with the cyclohexadienone system in, for example, 2-bromo-β-santonin [22], which has all six ring atoms reasonably coplanar. Several bonds in the ring here have lengths suggesting some double-bond character (in particular C(5)-C(6), which is almost purely double bond). Bonds C(6)-C(7) and C(7)-C(8) are shortened by the conjugation of the carbonyl group with

Table 9.13.

Deviations from the least-squares planes, in Å. Those in brackets have not been included in the calculation of the plane.

	A1	A2	A3	B1	B2	B3
C(1)	(-0.571)		(-0.540)	(-0.734)	(-0.535)	
C(2)	-0.005	0.000	0.008			
C(3)	0.005	0.002	-0.031		(-0.358)	
C(4)	(0.110)	0.000	0.050	(-0.482)		
C(5)	-0.005		-0.043	-0.095	0.007	
C(6)				-0.047	-0.007	-0.003
C(7)				0.109	(0.108)	0.011
C(8)				-0.027	0.007	-0.003
C(9)				-0.106	-0.007	
C(10)	0.005		0.016	0.165	(0.304)	
C(11)				(-0.314)	(-0.185)	
C(12)						
C(13)						
C(14)				(-0.152)	(-0.153)	
C(15)						
C(16)						
C(17)						
C(18)	(1.146)				(1.182)	
O(19)	(-0.434)					
O(20)	(1.357)					
C(21)						
O(22)	(-0.075)	-0.001				
C(23)						
O(24)				(-0.302)	(-0.283)	
O(25)				(0.291)	(0.233)	-0.004
O(26)						

Table 9.13.(Continued).

	C1	C2D1	D1	D2	E	F
C(1)					(-0.712)	
C(2)					(-0.303)	
C(3)					(-0.284)	0.092
C(4)					-0.011	0.071
C(5)	(0.336)				0.019	0.387
C(6)	(0.363)				-0.020	0.167
C(7)	(0.091)	-0.070			(-0.207)	0.172
C(8)	0.004	-0.001			(-0.594)	0.216
C(9)	0.000	-0.025			(-0.589)	0.418
C(10)	(-0.136)	0.096			(0.034)	
C(11)	-0.004	-0.036				0.368
C(12)	0.004	-0.030				0.115
C(13)	0.000	0.001	0.002	0.000		-0.013
C(14)	-0.005	0.022	-0.015	0.012		-0.030
C(15)	(0.048)	0.000	0.020	-0.023		
C(16)	(0.006)	0.043	-0.019	0.022		
C(17)	(0.017)	0.003	0.011	-0.005		-0.368
C(18)						
O(19)						
O(20)						
C(21)						
O(22)					(-0.489)	-0.521
C(23)					-0.001	-0.286
O(24)					0.013	-0.203
O(25)					(-0.094)	0.107
O(26)		-0.004	(0.018)	-0.006		-0.465

Table 9.14.

Equations of the least-squares planes. These are of the form $lx + my + nz = p$, where x, y, and z are expressed in Å units.

Plane	l	m	n	p
A1	0.128	-0.255	0.363	0.672
A2	0.135	-0.188	0.331	0.718
A3	0.129	-0.294	0.351	0.678
B1	0.965	-0.754	0.403	0.533
B2	0.102	-0.730	0.389	0.542
B3	0.114	-0.732	0.336	0.568
C1	0.934	-0.928	0.358	0.522
C2D1	-0.912	0.925	-0.367	-0.918
D1	-0.922	0.923	-0.364	-0.519
D2	-0.921	0.929	-0.362	-0.519
E	0.115	-0.363	0.409	0.620
F	0.116	-0.762	0.504	0.550

the delocalised rings C and E. Similarly C(5)-C(10) is a sp^2-sp^3 bond and its length of 1.490 Å is as expected. Several of the internal ring angles, especially those at C(5) and C(10), deviate by up to five degrees from the expected values. Bond C(8)-C(9) is an aromatic one and consequently it is considerably longer (~9σ) than the equivalent one in 2-bromo-β-santonin. It is felt that the fact that C(8)-C(9) is not a formal double bond is an important factor in producing the geometry found for ring B. Steric considerations must also play a considerable role, with angular strain at the A-B-E ring junctions producing a situation in which ring B is forced to adopt a boat shape, as attempts at constructing a model of the molecule have shown.

Ring A is a distorted chair form of cyclohexenone. C(3), C(4) and C(5) are sp^2 hybrids, whereas C(1), C(2) and C(10) are sp^3 ones. The bond lengths and geometry accord well with this assignment, provided allowance is made for the angle distortions arising from ring fusion. The atoms C(2), C(3), C(4), C(5) and C(10) are moderately coplanar, whilst C(1) is over 0.5 Å out of this plane. (Plane A3 in Table 9.13). The three ring substituents are seen to be in their predicted [2] conformations; C(18) is axial, O(19) equatorial, and O(20) axial. They are staggered at -53° and 56° to each other respectively (Table 9.15).

The furan system (ring E) shows no significant deviations from the expected geometry. It is planar within experimental error and its bond lengths and angles correspond well with the values found for furan itself by microwave methods [23], and for several natural products having a furan ring in their structures [24], and for furan-α,α'-dicarboxylic acid [25]. The alternation in bond lengths shows that furan has only partial ring delocalisation, in contrast to, say, benzene; it is thus termed a 'non-alternant' molecule.

The crystal structures of several steroids with a 1,3-cyclohexenone-type ring A have recently been accurately determined, with a very similar overall geometry in all cases, which agrees well with that found here. Thus, in 6β, 7β-methylene-17β-hydroxyandrost-4-en-3-one 17-acetate [26], the same five-atom coplanar arrangement is found, with C(1) pointing in the opposite direction to C(18); Braun et al term this ring shape the "sofa" conformation. An identical arrangement occurs in 6β-bromoprogesterone [27]; it is interesting to compare the dihedral angles calculated for ring A in this molecule with those for ring A in viridin. (Table 9.16).

The differences between the two sets of values reflect the increased angular strain in viridin, which is a consequence of the unique A-B-E ring fusion. It is

Table 9.15.

Dihedral angles (ω), in degrees, in the asymmetric unit. Format is atom 1 (atom 2, atom 3) atom 4. This is looking down the 2→3 bond and the angle quoted is that required to rotate the projection of bond 1→2 to coincide with that of bond 3→4. A positive sign denotes clockwise rotation.

Constituent atoms.	ω	Constituent atoms.	ω
C(1)(C(2),C(3))C(4)	28.6	C(4)(C(3),C(2))O(20)	-91.8
C(1)(C(2),C(3))O(22)	-151.1	C(4)(C(5),C(6))C(7)	170.3
C(1)(C(2),O(20))C(21)	152.4	C(4)(C(5),C(6))O(24)	-3.9
C(1)(C(10),C(5))C(4)	-28.9	C(4)(C(5),C(10))C(9)	-28.9
C(1)(C(10),C(5))C(6)	148.4	C(4)(C(5),C(10))C(18)	-153.4
C(1)(C(10),C(9))C(8)	-142.4	C(4)(C(23),O(24))C(6)	91.1
C(1)(C(10),C(9))C(11)	43.4	C(5)(C(4),C(3))O(22)	170.3
C(2)(C(1),C(10))C(5)	45.1	C(5)(C(4),C(23))O(24)	-0.9
C(2)(C(1),C(10))C(9)	165.0	C(5)(C(6),C(7))C(8)	-10.0
C(2)(C(1),C(10))C(18)	-72.4	C(5)(C(6),C(7))O(25)	172.0
C(2)(C(3),C(4))C(5)	-9.4	C(5)(C(6),O(24))C(23)	3.3
C(2)(C(3),C(4))C(23)	-174.5	C(5)(C(10),C(1))O(19)	170.5
C(3)(C(2),C(1))C(10)	-49.8	C(5)(C(10),C(9))C(8)	-24.3
C(3)(C(2),C(1))O(19)	-177.0	C(5)(C(10),C(9))C(11)	161.4
C(3)(C(2),O(20))C(21)	-80.9	C(6)(C(5),C(10))C(9)	23.8
C(3)(C(4),C(5))C(6)	-165.8	C(6)(C(5),C(10))C(18)	-91.6
C(3)(C(4),C(5))C(10)	11.9	C(6)(C(7),C(8))C(9)	8.4
C(3)(C(4),C(23))O(24)	166.0	C(6)(C(7),C(8))C(14)	-168.3

Table 9.15. (Continued).

Constituent atoms.	ω	Constituent atoms.	ω
C(7)(C(6),C(5))C(10)	-7.5	C(11)(C(12),C(13))C(14)	0.3
C(7)(C(6),O(24))C(23)	171.0	C(11)(C(12),C(13))C(17)	179.5
C(7)(C(8),C(9))C(10)	9.7	C(12)(C(13),C(14))C(15)	177.0
C(7)(C(8),C(9))C(11)	-176.2	C(12)(C(13),C(17))C(16)	-180.0
C(7)(C(8),C(14))C(13)	176.0	C(12)(C(13),C(17))O(26)	1.2
C(7)(C(8),C(14))C(15)	-0.7	C(13)(C(14),C(15))C(16)	3.2
C(8)(C(9),C(10))C(18)	92.2	C(13)(C(17),C(16))C(15)	2.6
C(8)(C(9),C(11))C(12)	0.3	C(14)(C(8),C(7))O(25)	9.7
C(8)(C(14),C(13))C(12)	0.5	C(14)(C(13),C(17))C(16)	-0.7
C(8)(C(14),C(13))C(17)	-178.9	C(14)(C(13),C(17))O(26)	-179.5
C(8)(C(14),C(15))C(16)	-180.0	C(14)(C(15),C(16))C(17)	-3.4
C(9)(C(8),C(7))O(25)	-173.7	C(15)(C(14),C(13))C(17)	-1.6
C(9)(C(8),C(14))C(13)	-0.8	C(18)(C(10),C(1))O(19)	52.9
C(9)(C(8),C(14))C(15)	-177.5	O(19)(C(1),C(2))O(20)	-55.9
C(9)(C(10),C(1))O(19)	-69.5	O(20)(C(2),C(3))O(22)	88.4
C(9)(C(11),C(12))C(13)	-0.7	O(22)(C(3),C(4))C(23)	5.2
C(10)(C(1),C(2))O(20)	71.3	O(24)(C(6),C(7))O(25)	-14.6
C(10)(C(5),C(4))C(23)	-179.5		
C(10)(C(5),C(6))O(24)	178.4		
C(10)(C(9),C(8))C(14)	-173.7		
C(10)(C(9),C(11))C(12)	174.6		
C(11)(C(9),C(8))C(14)	0.5		
C(11)(C(9),C(10))C(18)	-82.0		

Table 9.16.

ω(A-B), in degrees, is the dihedral angle about the A-B bond, in which the other two atoms required to define the angle are those attached to either end of the bond, and are in ring A.

Bond	ω for Viridin	ω for 6β-bromoprogesterone
C(1)-C(2)	-49.8	-50.7
C(2)-C(3)	28.6	21.9
C(3)-C(4)	-9.4	3.7
C(4)-C(5)	11.9	0.7
C(5)-C(10)	-28.9	-28.7
C(1)-C(10)	45.1	52.6

significant that a Dreiding model of this part of the molecule cannot be constructed. Figure 9.6 shows the molecule to be largely flat along its length, apart from ring A and the slight curve of rings B and E. The fusion strain is shown by several other features of the geometry, for example, substituents C(13), C(10) and C(7) are considerably displaced from the plane of the furan ring, indicating the bent nature of the sp^2 hybrids at C(4), C(5) and C(6). The large value of the C(3)-C(4)-C(23) angle (132) is another consequence of this fusion. It is noteworthy that the conjugated system which might be thought to extend through the molecule, does not in fact do so, as shown by the marked deviations of a number of the atoms from the best plane through this system (plane F in Table 9.13).

Figure 9.6. A stereoscopic drawing of viridin parallel to the planes of rings C and D.

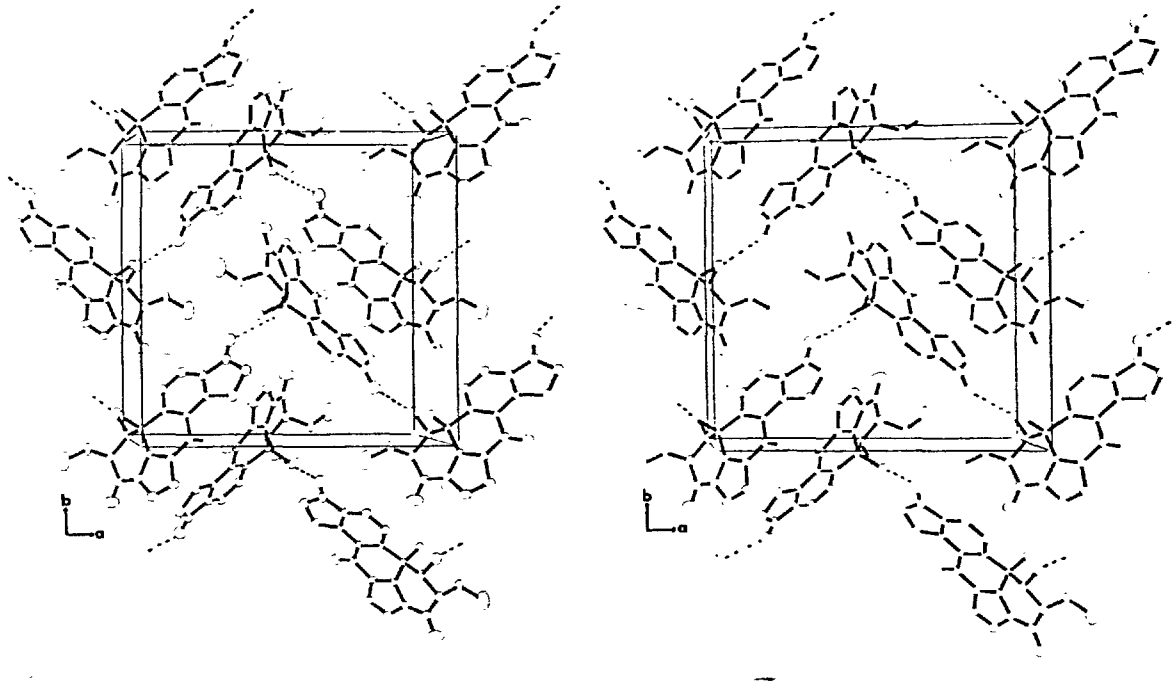


Figure 9.7. A stereodiagram of the unit-cell contents viewed along the c-axis. Dashed lines represent hydrogen bonds.

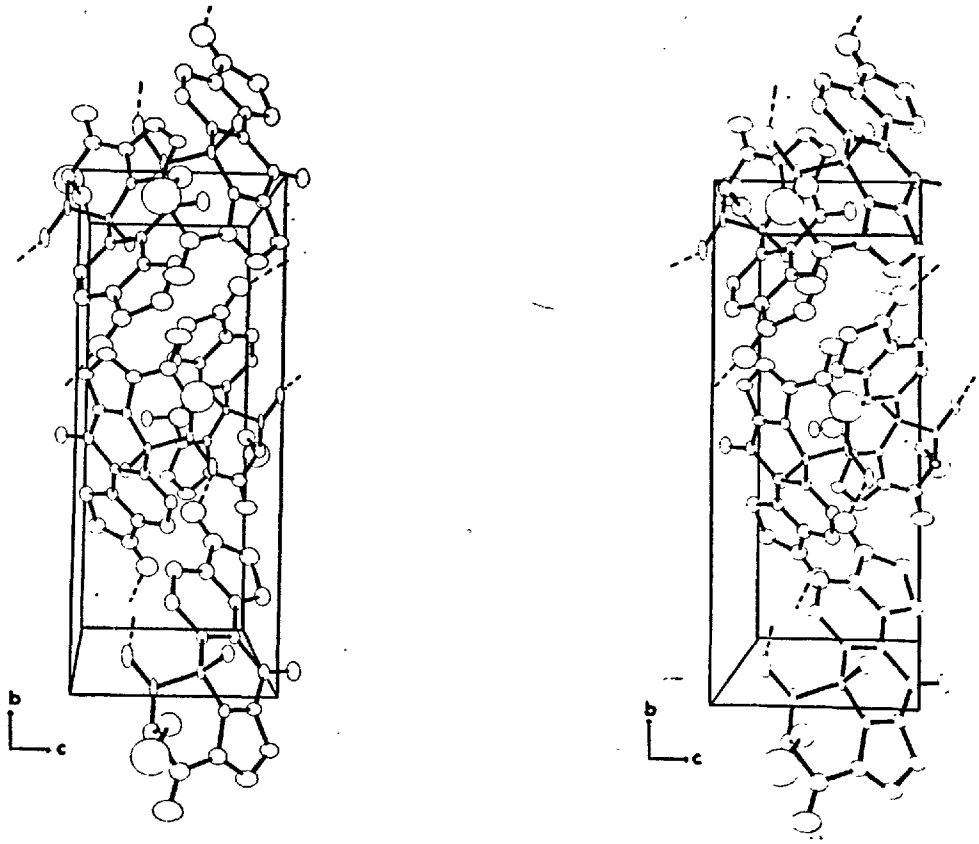


Figure 9.8. A stereodiagram of the unit-cell contents viewed along the a-axis. Dashed lines represent hydrogen bonds.

Table 9.17.

Intermolecular contacts less than 3.6Å (in Å).

C(1)	$C(6)^{I}$	3.58
C(7)	$O(25)^{II}$	3.24
C(8)	$O(25)^{II}$	3.20
C(12).....	$O(22)^{IV}$	3.57
C(13).....	$O(22)^{III}$	3.56
C(14).....	$O(22)^{III}$	3.58
C(14).....	$O(25)^{II}$	3.34
C(15).....	$O(24)^{II}$	3.59
C(16).....	$O(24)^{II}$	3.40
C(17).....	$O(24)^{II}$	3.30
C(18).....	$O(26)^{V}$	3.36
C(18).....	$O(19)^{I}$	3.27
O(19).....	$O(26)^{V}$	2.86 (hydrogen bond)
O(20).....	$O(26)^{V}$	3.28
O(25).....	$O(25)^{II}$	3.56

Roman numerals refer to equivalent positions relative to the reference molecule at x, y, z.

I	$x, y, 1 + z.$
II	$\frac{1}{2} - x, -y, \frac{1}{2} + z.$
III	$1 - x, \frac{1}{2} + y, \frac{1}{2} - z.$
IV	$1 - x, y + \frac{1}{2}, -\frac{1}{2} - z.$
V	$\frac{1}{2} + x, -\frac{1}{2} - y, - z.$

Planarity, or near planarity, is of course, a necessary condition for conjugation [28].

9.7. Intermolecular distances and packing.

The [100] and [001] projections of the crystal structure are shown in Figures 9.7 and 9.8. The intermolecular contact O(19)-O(26) of 2.86Å corresponds to a hydrogen bond of the O-H...O type. The other distances (less than 4Å), are all greater than the sum of the Van der Waals radii for the various atoms; although several of the distances of closest approach are relatively short, this is felt to be a consequence more of the close-packing in the lattice than of the existance of weak C-H...O hydrogen bonds. Table 9.17 details the inter-molecular contacts. The structure may be considered to consist of infinite chains of molecules parallel to the a-axis (i.e. along a screw axis), weakly linked together by the single hydrogen bond. The chains are packed together in a zig-zag manner.

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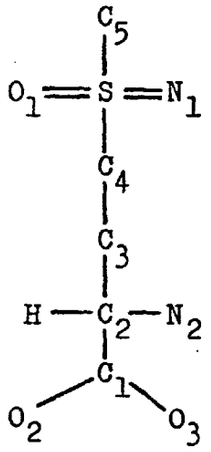
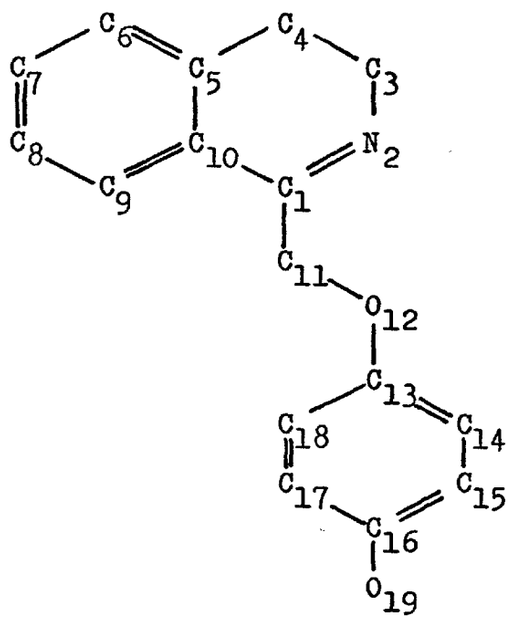
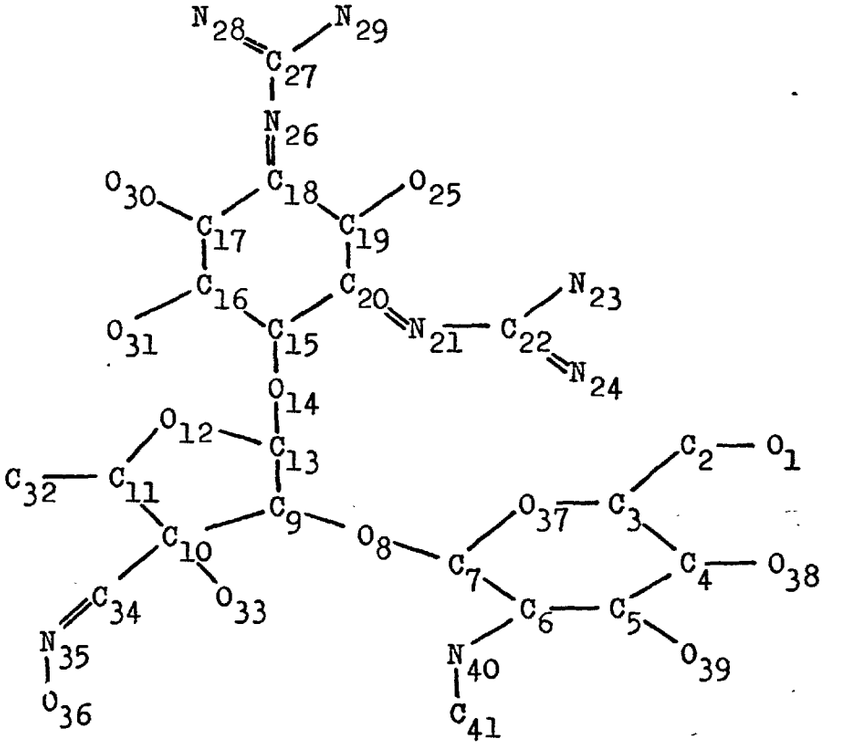


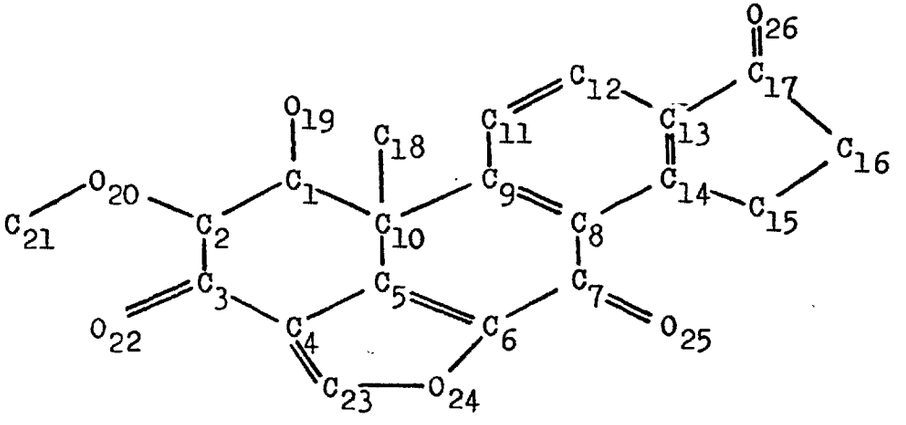
2(S),S(R)-Methionine sulphoximine.



16-Hydroxyphenoxymethyl-3,4-dihydroisoquinoline.



Streptomycin oxime.



Viridin.