'X-Ray Studies of the Structures of Some Biologically Significant Molecules'

A Thesis Presented for the Degree
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by

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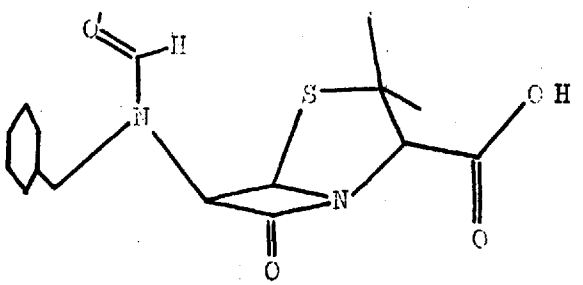
Abstract

The crystal structures of three natural-product derivatives have been examined by three-dimensional X-ray diffraction techniques.

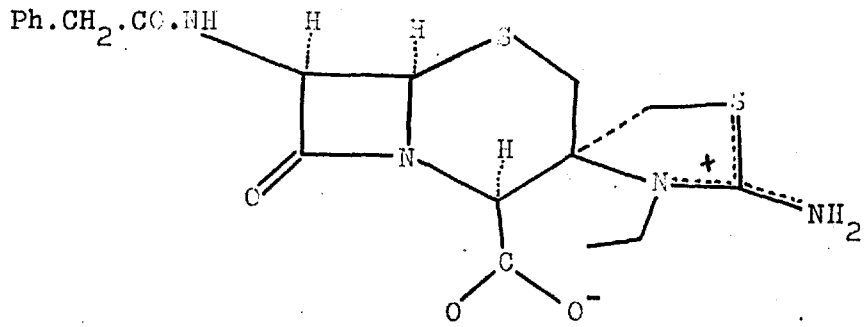
6-(N-Benzylformamido)penicillanic acid ($C_{16}H_{18}O_4N_2S$) is an isomer of the antibiotic, benzylpenicillin, but possesses only one ten-thousandth part of its antibacterial activity. The crystals are monoclinic with a = 19.58 Å, b = 6.427 Å, c = 13.98 Å, $\beta = 108^{\circ}$ and spacegroup C2, with four molecules in the unit cell. The structure, which is shown below was determined with the aid of a graphical Patterson



superposition technique. It is similar to those found by previous X-ray studies on the penicillins except for the position of one of the possible hydrogen-bonding groups (O'). This difference is consistent with current explanations for the antibiotic properties of the penicillins.

The crystal structure of a spiro-cyclic derivative of another antibiotic series, the cephalosporins, has been

determined. This spiro compound ($C_{19}H_{22}N_4O_4S_2 \cdot 3H_2O$) crystallizes in the monoclinic system with a = 8.063 Å, b = 7.184 Å, c = 19.72 Å and $\beta = 100.3^{\circ}$. The spacegroup is $P2_1$ with two molecules per cell. The molecule, represented by



the diagram above, forms a novel tricyclic system and presents many interesting aspects of structure and conformation, which are discussed in some detail.

Attempts have also been made to determine the crystal structure of zeorin acetate ($C_{32}H_{54}O_3$), a member of the triterpene series. The crystals are orthorhombic with a = 8.78 Å, b = 11.59 Å, c = 29.15 Å and belong to the spacegroup $P2_12_12_1$, with four molecules in the unit cell. This compound presents substantial crystallographic problems and efforts have been made to interpret its Patterson function mainly with the aid of a relatively modern Patterson searching technique.

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To

Gloria

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CHAPTER 1

The Phase Problem and an Introduction to the Patterson Function

One of the first stages in the X-ray analysis of a crystal structure is the measurement of the intensities of the spectra that arise from the diffraction of X-radiation by the three-dimensional crystal lattice. Each diffracted beam can be regarded as the 'reflexion' of X-rays by a particular set of parallel crystal planes. Directionally, a diffracted beam will depend only upon the orientation and separation of its reflecting planes with respect to the 'unit cell' of the crystal lattice. The intensity, however, depends upon the type and arrangement of the atoms with regard to these planes.

If there are N atoms in the unit cell, the diffraction pattern of the crystal lattice can be regarded as made up from the patterns of N atomic lattices and the total wave diffracted from the unit cell can be described by the equation

$$F(hkl) = \sum_{1}^{N} f_r \exp^{i\phi_r} \qquad \ldots\ldots (1)$$

where F(hkl) is the 'Structure Factor' for the particular reflexion and f_r is the 'form factor' of the rth. atom. The

form factor describes the power with which atoms of type r 'reflect' X-rays and it depends upon the atomic number and decreases with increasing Bragg angle, θ. ϕ_r is the phase angle of the diffracted beam from the rth. atomic lattice.

Since a crystal is periodic in three dimensions, Bragg (1929) has shown that the electron density (ρ) at any point x, y, z can be represented by a three-dimensional Fourier series of the form

$$\rho(xyz) = \frac{1}{V} \sum_{h,k,l=-\infty}^{\infty} \sum F(hkl) \exp(-2\pi i(hx + ky + lz)) \quad \ldots\ldots(2)$$

where V is the volume of the unit cell and the function is everywhere continuous. A good and convenient approximation to the structure factor is obtained assuming that all the electron density in the crystal occurs in spherically symmetrical atoms. The structure factor (equation 1) can then be written as

$$F(hkl) = \sum_{r=1}^{N} f_r \exp 2\pi i(hx_r + ky_r + lz_r) \quad \ldots\ldots (3)$$

where x_r, y_r, z_r are the coordinates of the rth. atom expressed as fractions of unit-cell edges. Thus the expression (2) for the electron density can be written as

$$\rho(xyz) = \frac{1}{V} \sum_{h,k,l=-\infty}^{\infty} \sum \left\{ \sum_{r=1}^{N} f_r \exp -2\pi i \, (h(x-x_r)+k(y-y_r)+l(z-z_r)) \right\} \ldots\ldots (4)$$

The Fourier series (4) will consist of N resolved, spherically symmetrical peaks at the positions of the atoms x_r, y_r, z_r and

the number of electrons in each peak will be equal to Z_r, the atomic number. (The Fourier in the form of equation (4) is pertinent to the discussion of the Patterson in later sections).

Crystal structure analysis involves substituting the values of the structure factors, F(hkl), into equation (2) in order to obtain a representation of the electron density distribution in the unit cell. However, the structure factors are, in general, complex quantities and the X-ray crystallographer observes intensities, which are proportional to the square of the modulus of the structure factors, $|F(hkl)|^2$. It is the determination of the phases that constitutes the 'phase problem' in X-ray crystallography.

One of the most successful methods proposed to overcome this problem is due to Patterson (1935). He derived a Fourier series with the coefficients, F(hkl), replaced by $|F(hkl)|^2$ and related the series to the inter-atomic vectors in the unit cell. The rest of this chapter is devoted to a description of this 'Patterson series' as it has been used extensively throughout the thesis. Some of the simpler applications are also described here.

Using for simplicity the one-dimensional series of period 'a' the equation

$$\rho(x) = \frac{1}{a} \sum_{h=-\infty}^{\infty} F(h) \exp -2\pi i(hx) \quad \ldots\ldots (5)$$

gives the electron density at any point x. The density distribution about x can also be expressed as a function of a parameter u, i.e. $\rho(x + u)$. The total amount of material between x and x + dx is $\rho(x)dx$, which gives the weighted distribution about x as $\rho(x)\,\rho(x + u)dx$ and the weighted average distribution as

$$A(u) = \int_0^1 \rho(x)\,\rho(x + u)dx \quad \ldots\ldots (6)$$

Thus from equation (5) and utilising the property that

$$\int_0^1 \exp -2\pi i(m + n)x\, dx = 1 \text{ if } n = -m \text{ and is otherwise}$$

zero, equation (6) gives

$$A(u) = a^{-2} \sum_{h=-\infty}^{\infty} |F(h)|^2 \exp 2\pi ihu \quad \ldots\ldots (7)$$

This is the one-dimensional Patterson series and the derivation can be extended to three dimensions to give

$$A(uvw) = V^{-2} \sum_{h,k,l=-\infty}^{\infty} \sum \sum |F(hkl)|^2 \exp 2\pi i(hu + kv + lw) \quad \ldots. (8)$$

where the distribution is now a function of the three parameters u, v, w about the point x, y, z. The general form of the three-dimensional function is usually written as

$$P(uvw) = \frac{1}{V} \sum_{h,k,l=-\infty}^{\infty} \sum \sum |F(hkl)|^2 \cos 2\pi(hu + kv + lw) \ldots (9)$$

Equation (9) differs from equation (8) for A(uvw) by a factor of 1/V and in the use of the cosine. The former factor is merely a result of defining the function as an average value of the electron density product, and the latter because in the absence of fluorescence, $F(hkl) = F^*(\bar{h}\bar{k}\bar{l})$, so that their imaginary components cancel. As the cosine function is even, it follows that the Patterson function is centrosymmetric.

The physical meaning of the Patterson can be readily seen by considering it in the form of equation (6). Thus A(u) will only be large when both $\rho(x)$ and $\rho(x + u)$ are large, so that peaks appear in the Patterson corresponding to peaks in the electron density distribution separated by a vector 'u'. The Patterson function, then, will consist of a large, multiple peak at the origin (ie. the sum of the product $\rho(x)\,\rho(x)$ for all x), and peaks whose vectors from the origin represent inter-atomic vectors in the crystal. Although a Fourier series is a continuous function it is sometimes useful to describe the Patterson as the vector set of the electron density.

Properties of the Patterson

Writing the one-dimensional structure factor in the form of equation (3), it follows that

$$|F(h)|^2 = \sum_{r,s=1}^{N} f_r f_s \exp 2\pi i h(x_r - x_s) \quad \ldots\ldots (10)$$

Substituting equation (10) in (7) gives

$$A(u) = a^{-2} \sum_{h=-\infty}^{\infty} \left\{ \sum_{r,s=1}^{N} f_r f_s \exp 2\pi i h(u - (x_s - x_r)) \right\}$$

$$= a^{-2} \sum_{h=-\infty}^{\infty} \sum_{r=1}^{N} f_r^2 \exp 2\pi i h u + a^{-2} \sum_{h=-\infty}^{\infty} \sum_{\substack{r,s=1 \\ r \neq s}}^{N} f_r f_s \exp 2\pi i h(u-(x_s-x_r)) \ldots (11)$$

The Patterson peaks thus correspond to the Fourier representation of artificial atoms with specific 'form factors'. N of these, represented by the first term on the right hand side of equation (11) coincide at the origin with 'form factors' of f_r^2; the remaining N(N-1) are situated at points $\pm(x_s - x_r)$ with 'form factors' of $f_r f_s$.

Thus the total number of 'electrons' in the origin peak is $\sum_{r=1}^{N} Z_r^2$, and peaks corresponding to a vector between two atoms at x_r and x_s respectively will contain $Z_r Z_s$ 'electrons' and the peak height will be roughly proportional to this product in the three-dimensional case. It can also be noted that the volume of a three-dimensional Patterson peak is roughly eight times that of a corresponding peak in $\rho(xyz)$. Coupled with the fact

that the Patterson contains N^2 peaks in the same volume as the electron density contains N, this means that the resolution of the former is poor compared to that of the latter.

Also, the origin peak can be removed by using as coefficients in the summation, $|F_o(h)|^2$, where

$$|F_o(h)|^2 = |F(h)|^2 - \sum_{r=1}^{N} f_r^2$$

The new series will retain all the information regarding interatomic vectors and may also show peaks that were previously obscured by the large origin peak.

Sharpening

Patterson (1935) suggested a method of improving the resolution of the Patterson series, which in effect attempts to transform the observed $|F(h)|^2$ terms into what they would be if the crystal were composed of point atoms. As the different form-factor curves are, in fact, very similar when scaled to unity, he suggested setting up an average f-factor per electron of

$$\hat{f}(h) = \sum_{r=1}^{N} f_r(h) \; / \; \sum_{r=1}^{N} Z_r$$

and the sharpened synthesis is then computed by dividing each $|F(h)|^2$ term by the appropriate $\hat{f}^2(h)$.

Another factor to be taken into account is the thermal

motion of the atoms. This, if isotropic and uniform for all atoms, tends to reduce the observed values of $|F(h)|$ by a factor of

$$\exp\left(-B \sin^2\theta/\lambda^2\right)$$

where λ is the wavelength of the X-radiation and B a constant for the particular crystal, which can be estimated from the intensity data by the method of Wilson (1942). Thus, division of each $|F(h)|^2$ by

$$\hat{f}^2(h) \exp\left(-2B \sin^2\theta/\lambda^2\right)$$

willgive a Patterson function sharpened to represent point atoms at rest.

An undesirable result of sharpening the Patterson is the appearance of false peaks. Equation (2) indicates that a Fourier series is ideally the sum of an infinite number of terms. In practice the number of terms is limited but the unsharpened series converges rapidly because of the thermal attenuation of the terms. However, if sharpening is applied, the importance of these high-order terms will be greatly increased. The truncation of the data becomes obvious and produces series-termination ripples in the Fourier map.

In recent years, more empirical sharpening functions have been used which attempt to compromise between resolving power and freedom from non-convergence effects. For example, Shoemaker et al. (1953) have used the function

$$(2\sin\theta/\lambda)^4 \exp -\alpha^2(2\sin\theta/\lambda)^2 \quad \text{with} \quad \alpha = 2.2$$

in the structure analysis of DL-serine. The function had a maximum value at $\sin\theta = 0.5\sin\theta_{max}$ and tended to reduce the Fourier ripple.

The Use of Symmetry

Harker (1936) suggested that the use of the symmetry of the spacegroup could lead to a simplification in the interpretation of the Patterson.

For a diad, coincident with the b axis (for example), the coordinates of a pair of atoms related by the symmetry are

$$x \quad y \quad z$$

$$-x \quad y \quad -z$$

The vectors between them will have components

$$\pm (2x \quad 0 \quad 2z)$$

and the 'Harker section' in the Patterson at $v = 0$ will contain peaks corresponding to vectors between all atoms related by this symmetry element. Equation (9) takes the form

$$P(u0w) = \frac{1}{V}\sum_{h,k,l=-\infty}^{\infty}\sum |F(hkl)|^2 \cos 2\pi(hu + lw)$$

$$= \frac{1}{V}\sum_{h,l=-\infty}^{\infty}\sum\left\{\sum_{k=-\infty}^{\infty}|F(hkl)|^2\cos 2\pi(hu + lw)\right\}$$

$$= \frac{1}{V}\sum_{h,l=-\infty}^{\infty}\sum C_{h,l}\cos 2\pi(hu + lw) \qquad \ldots\ldots (12)$$

where $C_{h,l} = \sum_{k=-\infty}^{\infty} |F(hkl)|^2$. Use of equation (12) reduces computation to that of the two-dimensional case but as three-dimensional data are used the resolution of the Harker section will be far better than the corresponding two-dimensional projection. Analogous results can be derived for screw axes but the relevant Harker plane is then displaced along the screw. For mirror and glide planes, the summation reduces to one-dimensional Harker lines normal to the plane.

In any Harker region there are always some extra peaks which arise accidentally and arise from non-equivalent atoms. Such peaks confuse the impression and are not readily distinguishable from true Harker peaks.

Interpretation of the Patterson

The complexity of this problem depends upon the nature of the substance being examined and consequently, the methods of solution provide one of the most diverse and interesting aspects of crystal structure analysis. This thesis is concerned with organic molecules which for convenience can be divided into two groups :

(a) Molecules containing a relatively large number of atoms of low atomic number with only a few heavy scatterers.

(b) Molecules containing only light-atom scatterers.

The interpretation of the Patterson of crystals containing type (a) molecules is reasonably straightforward. The initial step is the determination of the coordinates of the heavy atoms.

The Heavy Atom Method

It has been shown (page 12) that the height of a Patterson peak representing a vector between two atoms of atomic number Z_r and Z_s respectively is roughly proportional to $Z_r Z_s$. If the crystal contains relatively few atoms of high scattering power, the Patterson peaks of these 'heavy' atoms will stand out against a background of smaller peaks. It is then usually a simple matter to determine the coordinates of the heavy atoms, especially by making use of Harker regions. If partial structure factors, $F^H(hkl)$, are then calculated from these positions, the total structure factor is given by

$$F(hkl) = F^H(hkl) + F^L(hkl)$$

where $F^L(hkl)$ is the structure factor contribution from the light atoms. In general, $F^H(hkl)$ will be much greater than $F^L(hkl)$ and the computation of a Fourier series using the phases of $F^H(hkl)$ applied to the observed structure amplitudes should enable the location of light atoms. In the computer program available here, a rejection test can be applied where F(hkl)

is excluded from the Fourier synthesis if

$$\left|F^{H}(hkl)\right| < x\left|F_{o}(hkl)\right|$$

where x is normally 0.25 - 0.33 and $\left|F_{o}(hkl)\right|$ is the observed structure amplitude. In this way, structure factors to which the heavy atoms contribute very little can be excluded from the summation because of uncertainties in phase. In most cases, the first Fourier map is not clear enough to locate all the light atom peaks . The above process must be repeated several times, using at each stage the atomic positions located in the preceding map to phase the structure factors for the next. Lipson and Cochran (1953) have suggested that the method works best when the sum of the squares of the atomic numbers of the heavy and light atoms are approximately equal, i.e. each group contributes approximately equally to the average intensity.

Image Seeking

Clastre and Gay (1950), Garrido (1950), McLachlan (1950) and Beevers and Robertson (1950) simultaneously published methods for the interpretation of the Patterson which are classed as 'superposition' methods since they involve the superposition of one Patterson map on another. These methods can be applied when the proportion of heavy atoms in the crystal is insufficient to determine the phases of a majority of the

structure factors. Since then, superposition methods have been formalised and given a rigorous theoretical treatment as image-seeking methods by M.J.Buerger, whose work from 1950 onwards is presented in his book, Vector Space (1959).

Considering the Patterson as the vector set of the electron density (page 11), solution of the Patterson can be looked upon as identifying its fundamental set. That this can be done for discrete points was first shown by Wrinch (1939), who used the term 'image' to describe, say, the point ab at the end of the vector $\vec{ab}$, as the way point b looks from a. Thus the Patterson can be imagined as the superposition of the images of the structure as seen from each atom in turn, each image being translated to the origin of the Patterson. Searching for these images can be carried out graphically or by using a digital computer.

A single peak in the Patterson that represents a vector between two symmetry-related atoms is first selected, (it may be useful in this respect to predict the expected peak heights based upon the height of the origin peak). For instance, this may be a heavy-atom peak from which the heavy-atom positions can be found (at points r_1, ...r_j). The values of the Patterson are then compared for the set of points (r_1+r, r_2+r,.. ....r_j+r) and $\vec{r}$ is regarded as a vector of the fundamental set only if the Patterson at all these points is large. Buerger

(1959) describes three types of functions for testing the 'vector-end' Patterson values; the product, sum and minimum functions. The product function is defined as the product of the weighted values of the Patterson at the ends of the vectors, and the other two functions are respectively the sum and the minimum of these Patterson values. This multiple comparison is carried out right through the cell by varying the common vector $\vec{r}$. In the above heavy-atom example, the weight of each Patterson value will be the same, but if other atomic species are included as 'image points', the vector-end Patterson values must be weighted according to their scattering powers. Buerger discusses the merits of each image-seeking function and their relation to the electron density. He recommends the minimum function as it is simple to use and the other two tend to give false maxima. Thus a contoured map of, say, the minimum function can be obtained for the unique part of the unit cell, and the peaks will represent possible atomic positions.

If two image points are used in the search, the resulting function is said to be of rank two. Normally, this is not enough to reveal the complete structure and a stepwise procedure has to be employed, whereby functions of higher rank are computed by using as image points, atomic positions that have been determined from lower-ranking functions.

Graphically, the procedure can be carried out by preparing

a duplicate contoured map of the Patterson on tracing paper and laying it on the original map with its origin on the selected single peak of the lower map. The minimum function can then be contoured on a perspex sheet superimposed on both maps. In practice it is difficult to prepare , by this method, minimum functions of rank higher than two.

For structures of type (b) (page 16), containing no heavy atoms, the solution of the Patterson is generally quite complex. The function normally consists of clusters of unresolved peaks, some of which may not be distinguishable from the background. Recognition of single peaks becomes extremely difficult and consequently, normal image-seeking methods are unsuitable. However, in recent years, modified image-seeking methods have been developed for specific use with light-atom structures. A description of these methods is deferred until later (Chapter 7), prior to the account of attempts to solve the crystal structure of zeorin acetate ($C_{32}H_{54}O_3$) by a Patterson searching method.

CHAPTER 2

Data Treatment and General Crystallographic Methods Used

All intensity data were collected photographically by the Weissenberg method using filtered CuKα radiation. Two four-film exposures on Ilford Industrial 'G' were carried out for each layer, the time ratio being approximately 60 : 1. Using the equi-inclination setting for non-zero layers, the bulk of the data was collected about the shortest crystallographic axis, and between one and three layers about a second axis for data correlation. Intensities were estimated visually using calibrated 'wedges', and inter-film scales were calculated by hand. No corrections were applied for absorption. Unless the mass absorption coefficient of the crystal is very high, it is expedient to try to avoid making absorption corrections by choosing a specimen of small enough dimensions. This was done in each case and the pertinent data are given in the relevant chapters. Primary extinction is due to the interference effects of out-of-phase rays that have been 'back-reflected' into the primary and reflected beams by the stack of planes in the particular reflecting position. A large reduction in intensity can occur for strong, low-order reflexions and consequently

any suspected primary extinction was dealt with by omitting the reflexions from the structure refinement.

A series of computer programs were used to obtain a complete set of data on one relative scale. All the programs mentioned form part of a crystallographic system for the Atlas computer. Formulated mainly by M.G.B.Drew (1966), and named ATSYS, this system facilitates the handling of data and enhances continuity by the option of storing both programs and data on magnetic tape. Further details can be obtained from the reference cited and the list of references therein. In each case, the programmer's name is given, and the author, who fully appreciates the exasperation sometimes involved, is grateful to them all.

LSCD.....R.D.Diamand.

The unit cell dimensions are calculated by a least-squares method outlined by Alexander and Klug (1954). The method assumes that any systematic errors are a function of the Bragg angle, θ, so that an error term is added to the observational equations. In this work the data were derived from Weissenberg films.

FIFI..... R.D.Diamand.

Lorentz and polarisation corrections of the form

$$(Lp)^{-1} = 2\xi \cos\theta / (1 + \cos^2 2\theta)$$

are applied to a set of intensity data, where ξ is the radial cylindrical coordinate of the reciprocal lattice point.

POLO..... M.G.B.Drew.

Procedures can be carried out as follows:

(a) Pick out common reflexions from separate lists of data and print out their ratios. Common reflexions will be used to calculate the inter-layer scales (b), and this list usually indicates mis-indexing and other trivial mistakes.

(b) Calculate the inter-layer scales by the least-squares method of Hamilton, Rollett and Sparks (1965), and apply them to the data.

(c) Sort a list into any order and modify parts of the list.

LOLA..... R.A.Sparks, modified by the author.

Inter-layer scales can be calculated by the method of Rollett and Sparks (1960), now superseded by the method in POLO.

Other ATSYS programs used for calculations in this thesis are :-

MATT..... the author.

Wilson's (1942) method is applied to estimate the absolute scale and overall temperature factor, from two or

three-dimensional data. The procedure used is analogous to that outlined by Rogers (1965). The data are output to facilitate the plotting of the graph of $\log_e(\langle I \rangle / \sigma_2)$ against $\sin^2\theta/\lambda^2$, where $\langle I \rangle$ and σ_2 are the average values of $|F(hkl)|^2$ (on a relative scale) and $\sum_{r=1}^{N} f_r^2$ for shells of the reciprocal lattice extending over equal intervals of $\sin^2\theta$. The values of B, the overall isotropic temperature factor and K, the scale to be applied to $|F(hkl)|$ are also calculated by a simple least-squares routine.

BOSS..... M.M.Harding, modified by M.G.B.Drew.

The program is basically for the calculation of Fourier series but includes a routine to calculate structure factors from a set of atomic positions. An agreement analysis can also be prepared, i.e. a list of $\sum F_o(hkl)$ and $\sum F_c(hkl)$ and R for various groups of reflexions where k

$$R = \frac{\sum k \left| |F_o| - |F_c| \right|}{\sum k |F_o|}$$

is the scale factor and F_c the calculated structure factor, (adopting the more convenient notation), and the summations are over the appropriate groups of reflexions.

DIDO..... R.A.Sparks, modified by the author.

Using the eigenvalue method outlined by Rollett (1965)

the best plane through a set of specified atoms is calculated together with the distances of other atoms from this plane.

BABA..... R.D.Diamand, modified by M.G.B.Drew.

The program calculates structure factors from input atomic coordinates and refines, by a block diagonal, least-squares method, positions, temperature factors and an over-all scale. The anisotropic temperature factors are of the form β_{ij} where the contribution to the structure factor is

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

The method is essentially that outlined by Cruickshank (1961). The scale, applied to F_o, is coupled with an overall temperature factor in a 2 x 2 matrix, and 3 x 3 and 6 x 6 matrices are computed for positions and anisotropic vibrations respectively. The function minimised is

$$\sum w\,(k\,|F_o| - |F_c|)^2$$

where $\sqrt{w}$ is the weight applied to each observation. The weighting scheme used is that described by Rollett (1961) where

$$\sqrt{w} = F^+ / F_o \quad \text{if } F_o > F^+$$

$$\sqrt{w} = 1 \quad \text{if } F_o < F^+$$

The value of F^+ can be chosen from the agreement analysis of

BOSS to give roughly constant values of $\sum w (|F_o| - |F_c|)^2$ (i.e. $w\Delta^2$) over ranges of $|F_o|$.

The program also optionally applies a 'fudge factor' for modification of parameter shifts. This is particularly useful to dampen the oscillation of non-centrosymmetric structures whose origin is not defined.

ELSI..... R.D.Diamand and M.G.B.Drew.

Bond distances and angles are calculated and their standard deviations. The variances of the normal equations obtained from BABA are used to calculate the standard deviations, which are thus lower than their true value as most of the off-diagonal terms are ignored. The formula used for the estimation of the standard deviation, σ, of a variable x_r is that of Cruickshank (1959) where

$$\sigma^2 (x_r) = V_{rr} \sum w \Delta^2/(m - n)$$

V_{rr} is the variance of the parameter x_r, m the number of observations and n the number of parameters refined.

A limited number of programs have been used from the 'X-Ray '63' system for the I.B.M. 7090. Thanks are extended to the authors (mentioned in the text) and especially J.Stewart who was chiefly responsible for making the programs available at Imperial College.

CHAPTER 3

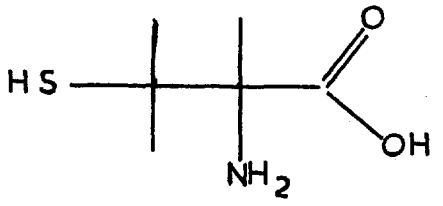
Chemistry of the Penicillins and Cephalosporins.

The Penicillins.

That the antibiotic penicillin is produced from a mould, later identified as a strain of Penicillium notatum, was first observed by Fleming (1929). Penicillin is active against a large number of organisms such as Diplococcus pneumoniae (pneumonia organism), Streptococcus pyrogenes (associated with scarlet and rheumatic fevers) and Staphylococcus aureus (skin infections), but early clinical use was limited by its instability. This also complicated the chemical work on the structure of penicillin and a further difficulty was that certain bacteria secrete an enzyme, penicillinase, which inactivates the antibiotic. Nevertheless, the potentialities of penicillin for full-scale use against many infectious diseases led to some very intense work from 1940–45. During this period of the Second World War, chemical information was restricted and consequently, an historical account of the structure determination is complex. The following outline of the fundamental chemical points, for which no references are given, was taken from 'The Chemistry of Penicillin', edited by Clarke,

Johnson and Robinson (1949).

The first solid forms of penicillin , though stable when dry, were soon found to be inhomogeneous, and it was not until 1943 that a chemically pure compound possessing the properties of penicillin was isolated. Further studies showed that more than one such compound existed, and the chemical differences between them soon became evident. Hydrolytic degradation of the individual penicillins gave, in each case, the same sulphur-containing amino-acid, penicillamine (I), carbon dioxide and a penillo-

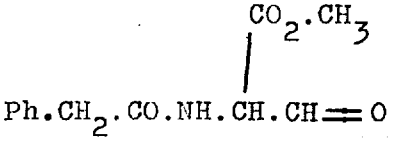


(I)

aldehyde ($R.CO.NH.CH_2.CH:O$). The structure of (I) was confirmed by synthesis, its absolute configuration being D. The chemical differences between the penicillins were attributed to the identity of the group R in the penilloaldehydes. This provided a basis for nomenclature, so that with $R = C_6H_5.CH_2$ the parent penicillin was given the name benzylpenicillin.

Although the penicillins are strong monobasic acids, the action of methanol on benzylpenicillin under mild conditions, gave a monomethyl ester of a dibasic acid, benzylpenicilloic acid.

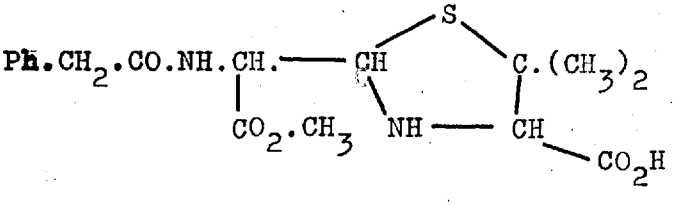
This indicated that the penicillins also contain a labile, but 'masked' carboxyl group, since esterification of free carboxyl groups normally takes place under more vigorous conditions. The monomethyl ester of benzylpenicilloic acid was further degraded to penicillamine (I) and the benzylpenaldic ester (II). Further hydrolysis of the latter compound gave benzylpenaldic acid which



(II)

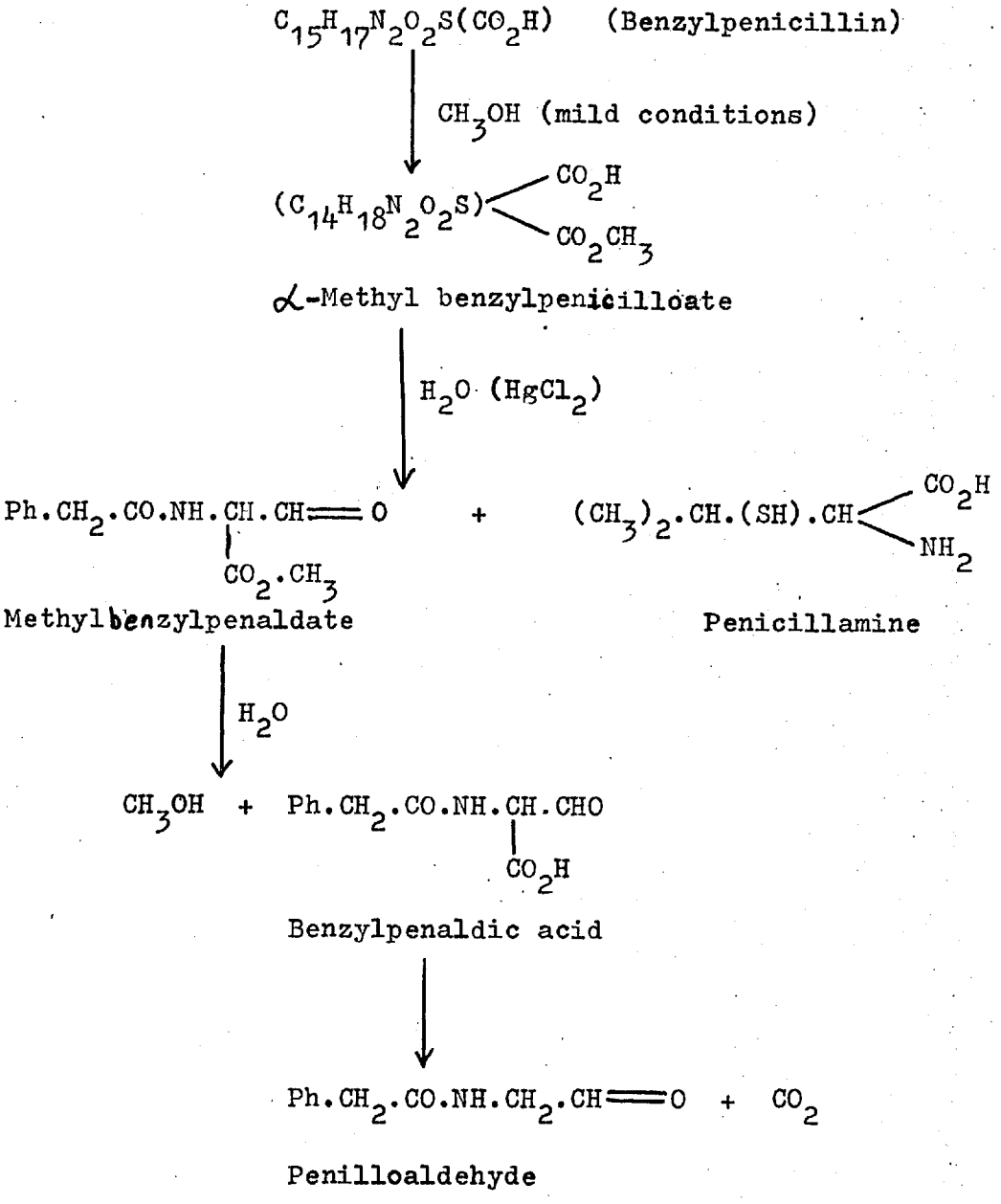
loses carbon dioxide to give benzylpenilloaldehyde.

These reactions are indicated schematically in Figure 1, and showed the free carboxyl group to be associated with the penicillamine residue, and the masked group with the penilloaldehyde. It had also been demonstrated that penicillamine yielded thiazolidines on condensation with carbonyl compounds and consequently, the monomethyl ester of benzylpenicilloic acid was formulated as (III), which was supported by synthesis.

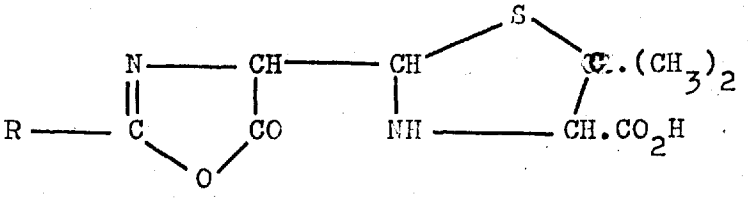


(III)

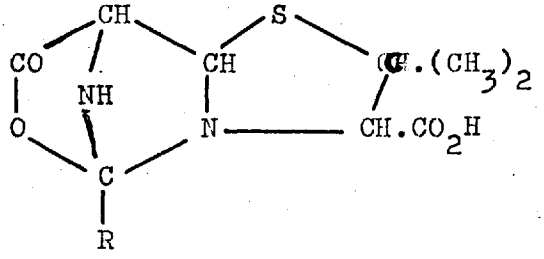
Figure 1.


$C_{15}H_{17}N_2O_2S(CO_2H)$ (Benzylpenicillin)
CH_3OH (mild conditions)
$(C_{14}H_{18}N_2O_2S)$ CO_2H CO_2CH_3
α-Methyl benzylpenicilloate
H_2O ($HgCl_2$)
$Ph.CH_2.CO.NH.CH.CH{=}O$
$CO_2.CH_3$
+
$(CH_3)_2.CH.(SH).CH$ CO_2H NH_2
Methylbenzylpenaldate
Penicillamine
H_2O
CH_3OH + $Ph.CH_2.CO.NH.CH.CHO$
CO_2H
Benzylpenaldic acid
$Ph.CH_2.CO.NH.CH_2.CH{=}O$ + CO_2
Penilloaldehyde

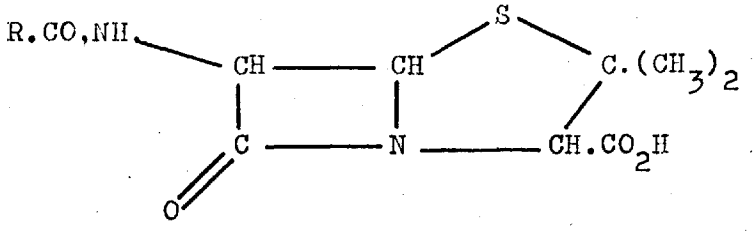
Thus, for the organic chemists, the problem was reduced to postulating a linkage, working from (III), that would explain the masked carboxyl group of the penicillins. However, any postulated structure also had to satisfy other criteria that had arisen during the very exhaustive chemical work. Three main structural formulae were proposed, namely the oxazolone (IV), tricyclic (V) and β-lactam (VI).



(IV) Oxazolone



(V) Tricyclic

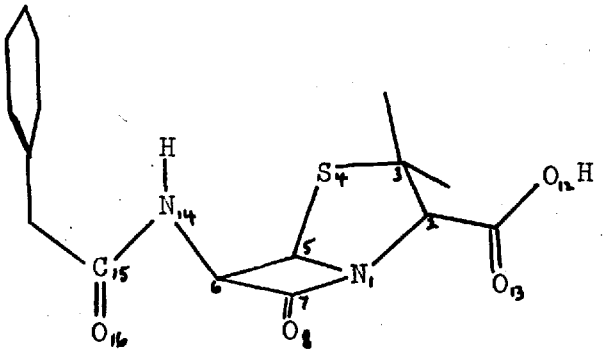


(VI) β-Lactam

The β-lactam structure gradually became the most popular, and was finally confirmed by an X-ray analysis by Crowfoot, Bunn, Rogers-Low and Turner-Jones (1949).

Two-dimensional X-ray investigations were carried out on the sodium, potassium and rubidium salts of benzylpenicillin. The latter two salts are isomorphous and the Patterson series was used to locate approximately, the metal ion positions. Unfortunately, it was found that the heavy atoms contributed to only a limited number of reflexions, so that the Fourier maps were difficult to interpret except that the sulphur atom was located. The sodium salt was examined by optical methods and the derived Fourier maps were little better than those from the isomorphous salts. However, by comparison of the two sets of Fouriers it became clear that the molecule existed as a 'curled' configuration, not extended as had previously been thought, and when the results were viewed without bias towards any of the proposed structures (the oxazolone structure was originally taken as model), the adoption of trial positions and subsequent refinement, led to the β-lactam structure (VI) for benzylpenicillin. The general configuration of the molecule is represented by (VII). The thiazolidine ring is not planar, C_2 lies out of the plane of the other atoms on the opposite side to C_7 and similarly, O_8 projects out of the plane of the β-lactam ring system towards the sulphur atom. The amide group in the phenylacetyl side-chain

is planar and possesses dimensions of a normal amide.

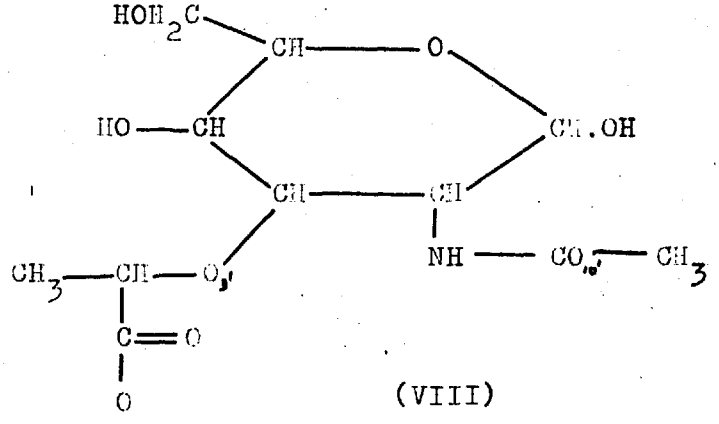


(VII)

The crystal structure of phenoxymethylpenicillin has been determined by Abrahamsson, Crowfoot Hodgkin and Maslen (1963). The molecule shows marked similarities in the general configurations of the thiazolidine and β-lactam rings to those in benzylpenicillin. The main difference is that in benzylpenicillin, the plane of the benzene ring is approximately parallel to the β-lactam-thiazolidine system, whereas in phenoxymethylpenicillin, the benzene ring is considerably inclined to this system. The differences may be largely due to packing considerations in the crystals.

In 6-aminopenicillanic acid (6-APA), as determined by Diamand (1963), the out-of-plane atom in the thiazolidine ring is N_1, which is projected on the same side of the plane as N_{14}. This is in contrast to the situation in the other two penicillins where C_2 deviates most from the best plane.

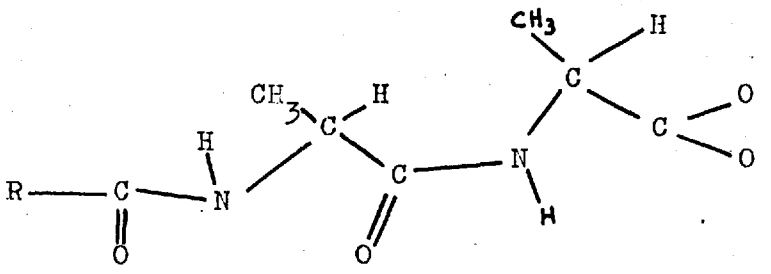
The mechanism by which penicillin kills bacterial cells is still not clear. Evidence has been presented by Park and Strominger (1957) that the antibiotic interferes with the synthesis of the bacterial cell wall, by reacting preferentially with an enzyme binding site that normally takes part in cell-wall synthesis. Collins and Richmond (1962) suggested that penicillin may bind to the enzyme in preference to some derivative of N-acetylmuramic acid (VIII). They point out that



(VIII)

in one of the permissible conformations of N-acetylmuramic acid, the carboxyl group can occupy the same position as that in benzylpenicillin and similarly O_3' and O_{10}' in N-acetylmuramic acid can occupy identical positions to N_1 and O_{16} in benzylpenicillin. They further suggest that the binding of the N-acetylmuramic acid residue to an 'active centre' will depend primarily on ionic and hydrogen-bonding forces, and that the high affinity of penicillin for the active centre could be explained by the fact that the possible hydrogen-bonding group O_8, has no counterpart in N-acetylmuramic acid. Similar considerations can be applied to the cephalosporins.

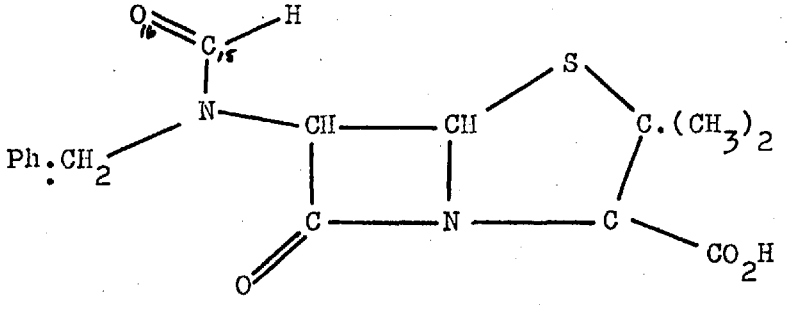
Tipper and Strominger (1965) have suggested that penicillin interferes with cell-wall synthesis by binding to an active centre in preference to an acyl-D-alanyl-D-alanine (IX), and



(IX)

not N-acetylmuramic acid. They show that similar spatial considerations apply as in Collins and Richmond's hypothesis described previously.

6-(N-Benzylformamido)penicillanic acid (6-NBF-PA) represented by (X), is an isomer of benzylpenicillin but Housley

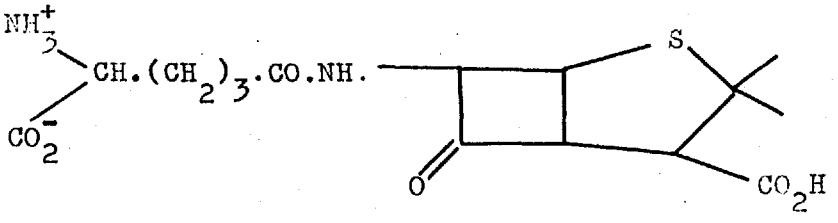


(X)

and Spooner (1965) have measured its antibiotic activity as less than one ten-thousandth of that of benzylpenicillin. A three-dimensional X-ray study (described in Chapter 4) has been undertaken mainly to determine the positions of the hydrogen-bonding groups. These studies have, in fact, shown that the carbonyl group $C_{15} - O_{16}$ is on the 'wrong' side of the molecule, in terms of Collins and Richmond's explanation, to participate in possible hydrogen bonding to an active centre, and hence its low antibiotic activity is consistent with their theory.

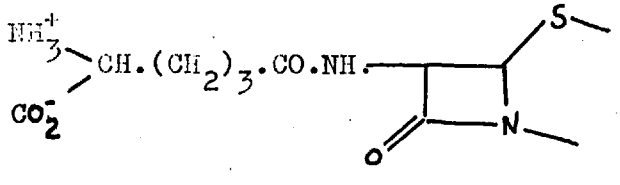
The Cephalosporins

The isolation of cephalosporin C (ceph. C) has been described by Newton and Abraham (1955). A species of the mould 'cephalosporium' produced a mixture of penicillin N (XI), and



(XI)

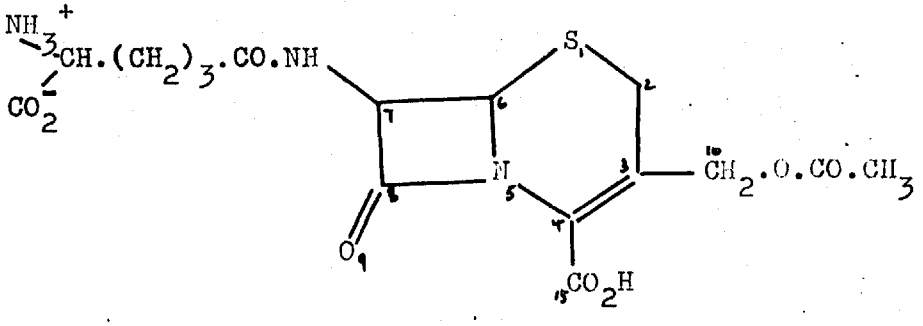
another antibiotic, ceph. C. Chemical evidence given by Abraham and Newton (1961) for the structure of ceph. C indicated a monoamino dicarboxylic acid present as a residue of α-amino-adipic acid linked to the rest of the molecule by its δ-carboxyl group. The infra-red absorption spectrum of the sodium salt showed a maximum at 5.62 μ, which is close to the carbonyl stretching frequency of the β-lactam ring in the penicillins. This fact and the results of hydrogenolysis experiments indicated the presence of the part structure (XII).



(XII)

These initial lines of enquiry were based on the assumption that ceph. C contained the characteristic skeleton of the penicillins, although the ultra-violet absorption spectrum of ceph. C showed a maximum at 260 mμ, which could not be explained in terms of the fused four and five-membered ring system. Further chemical work and nuclear magnetic resonance spectroscopy threw more doubts on this hypothesis, which was thus rejected.

Abraham and Newton then proposed the structure (XIII),



(XIII)

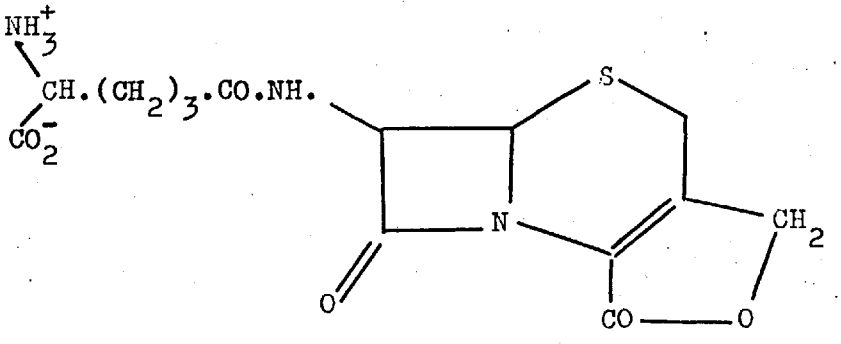
which was consistent with all the chemical reactions, while the 3-4 double bond would account for the U.V. maximum at 260 mμ. However, the possibility of other structures, especially those with a 2-3 double bond were not excluded.

Before any further chemical evidence became available regarding this structure, the X-ray analysis by Crowfoot Hodgkin and Maslen (1961) indicated the presence of a six-membered ring containing sulphur. Thenceforward, the chemical and X-ray crystallographic progress was rapid, and both finally substantiated the structure (XIII) for ceph. C.

The full X-ray analysis was complicated by the low

reflecting power and rapid deterioration of the crystals. Consequently, the accuracy achieved was limited but the analysis showed that the atoms C_2, C_3, C_4, N_5, C_{10} and C_{15} form a plane with S_1 about 0.6 Å above the plane, and C_6 about 0.6 Å below it. The length of the bond $C_3 - C_4$ (1.31 Å) is in agreement with its formulation as a double bond. The carbonyl group of the amide is nearly normal to the β-lactam ring and the protons at C_6, C_7 are cis and in the same configuration as in the penicillins.

Lactonisation of ceph. C by hydrochloric acid at room temperature produces another antibiotic, ceph. Cc, represented by (XIV). The X-ray analysis of ceph. Cc (carried out by

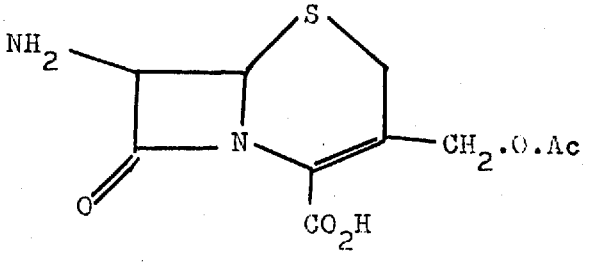


(XIV)

Diamand (1963)) has confirmed the general configuration of the molecule and provided quite accurate bond lengths, the true standard deviations probably being 0.02 - 0.03 Å.

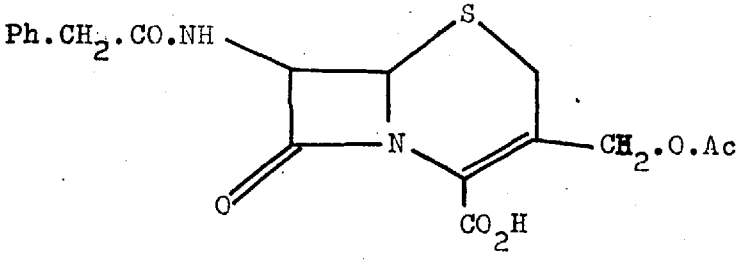
The biological activity of the cephalosporins has been studied by Newton and Abraham (1956). Ceph. C shows only 0.1 %

of the activity of benzylpenicillin against Staph. aureus, but is relatively stable to penicillinase. By analogy with the penicillins Loader, Newton and Abraham (1961) suggested that the substitution of, say, a phenylacetyl group for the α-aminoadipic acid residue in ceph. C might produce more active compounds that would still be stable to penicillinase. Treatment of ceph. C with dilute acid at room temperature produced the ceph. C nucleus, 7-aminocephalosporanic acid (XV), which although



(XV)

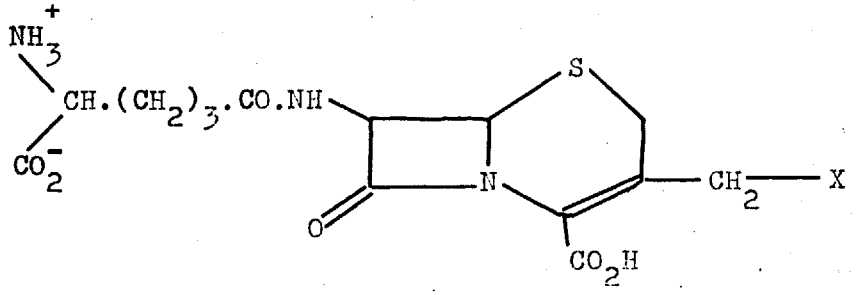
biologically inactive itself, was acylated to give 7-phenylacetamidocephalosporanic acid (XVI). This possesses approximately



(XVI)

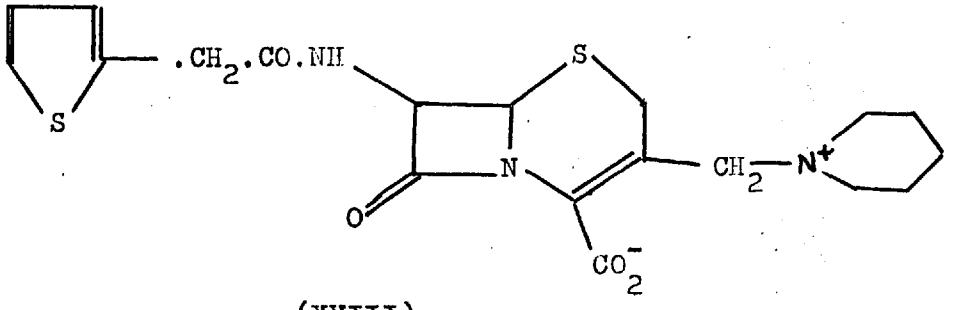
one hundred times the activity of ceph. C against Staph. aureus, i.e. similar to benzyl penicillin but more stable to penicillinase.

Another interesting reaction of ceph. C was observed by Hale, Newton and Abraham (1961). The acetoxy group in ceph. C can be displaced by nucleophiles, especially hetero tertiary bases such as pyridine. The resulting compounds, known as the ceph. C_A family, show increased antibacterial activity and are represented by (XVII), X = pyridine etc.



(XVII)

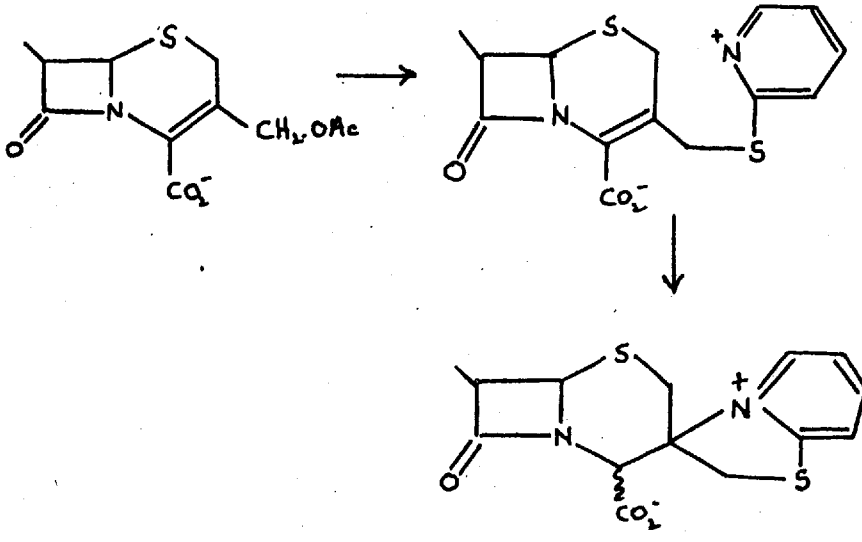
Muggleton, O'Callaghan and Stevens (1964) have prepared many analogues of ceph. C, in which both the α-aminoadipic acid side-chain and the acetoxy group were replaced. Their work led to the production of cephaloridine (XVIII) (Trade name 'Ceporin'



(XVIII)

Glaxo Ltd.), a highly active antibiotic of low toxicity.

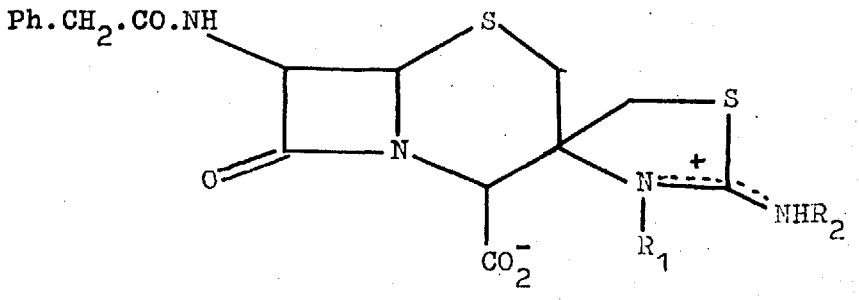
Cocker et al. (1965) have replaced the acetoxy group in ceph. C with sulphur nucleophiles such as thiourea, and Fazakerly et al. (1965) produced spirocyclic compounds by the reaction of certain bidentate nucleophiles with 7-phenylacetamido cephalosporanic acid. Nucleophiles such as pyrid-2-thione and thiouracil give products that lack the U.V. absorption maximum at 260 mμ, and it was suggested that the reaction, which is illustrated below for pyrid-2-thione, is a two-stage process.



(XIX)

First, the acetoxy group is displaced from the cephalosporanic acid derivative by the sulphur end of the nucleophile, followed by internal Michael addition by the nitrogen atom giving compounds of type (XIX), with a spiro atom at the 3 position. The spiro compounds are dextrorotatory and possess weak antibacterial activity. A substance thought to be the thiazolinium

derivative (XX), R_1, R_2 = Et, H or H, Et was formed when

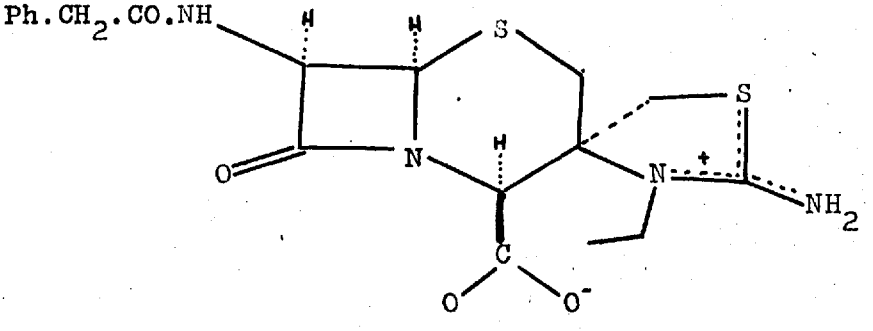


(XX)

7-phenylacetamidocephalosporanic acid and N-ethylthiourea were mixed in aqueous solution at 37° for several days. Electrophoresis experiments, together with an I.R. absorption maximum at 1610 - 1630 cm^{-1} due to an ionized carboxyl group, indicated that it occurred as a zwitter-ion. The compound possesses only one fifteenth of the anti-bacterial activity of 7-phenyl acetamidocephalosporanic acid against Staph. aureus (Long (1966)). If (XX) is correct it contains two new centres of asymmetry.

The prospects of determining the detailed stereochemistry of this molecule by any means other than X-rays seemed poor. Thus a three-dimensional X-ray determination has been carried out to determine the stereochemistry at the asymmetric centres, and to study the conformations of this new tricyclic system. The results, which are reported in Chapter 5, indicate that (XX)

is essentially correct, but should be written more precisely as (XXa).



(XXa)

CHAPTER 4

The Crystal Structure of 6-(N-Benzylformamido)penicillanic Acid

Preliminary Data

The crystals were prepared and the compound characterized by J.R.Housley and D.F.Spooner of the Boots Pure Drug Co. Ltd., Nottingham and supplied to the author by J.F.Collins of the Medical Research Council, Mill Hill, London. After treating 6-benzylaminopenicillanic acid at room temperature with an ethereal solution of acetic formic anhydride, 6-NBF-PA was crystallized from ether/light petroleum (40° - 60°) at 0° C mainly as clusters of ill-formed, monoclinic needles, elongated along [010]. The solution in dioxan is dextrorotatory with $[\alpha]_D^{25} = +351^\circ$. The structural formula and numbering scheme are shown in the pull-out diagram inside the back cover.

Formula $C_{16}H_{18}O_4N_2S$ Molecular Weight (formula) = 335

Mass absorption coefficient for CuKα, $\mu = 18.5$ cm^{-1}

Unit-cell dimensions

$a = 19.58$ (0.01 Å), $b = 6.427$ (0.003) Å, $c = 13.98$ (0.006) Å

$\beta = 108.0^\circ$ (0.1), $V = 1673$ Å^3, $Z = 4$ $F(000) = 704$ electrons.

D_{obs}(by flotation) = 1.33 g.cm^{-3} D_{calc} = 1.33 g.cm^{-3}

Absent Spectra:

only among hkl for h + k = 2n + 1

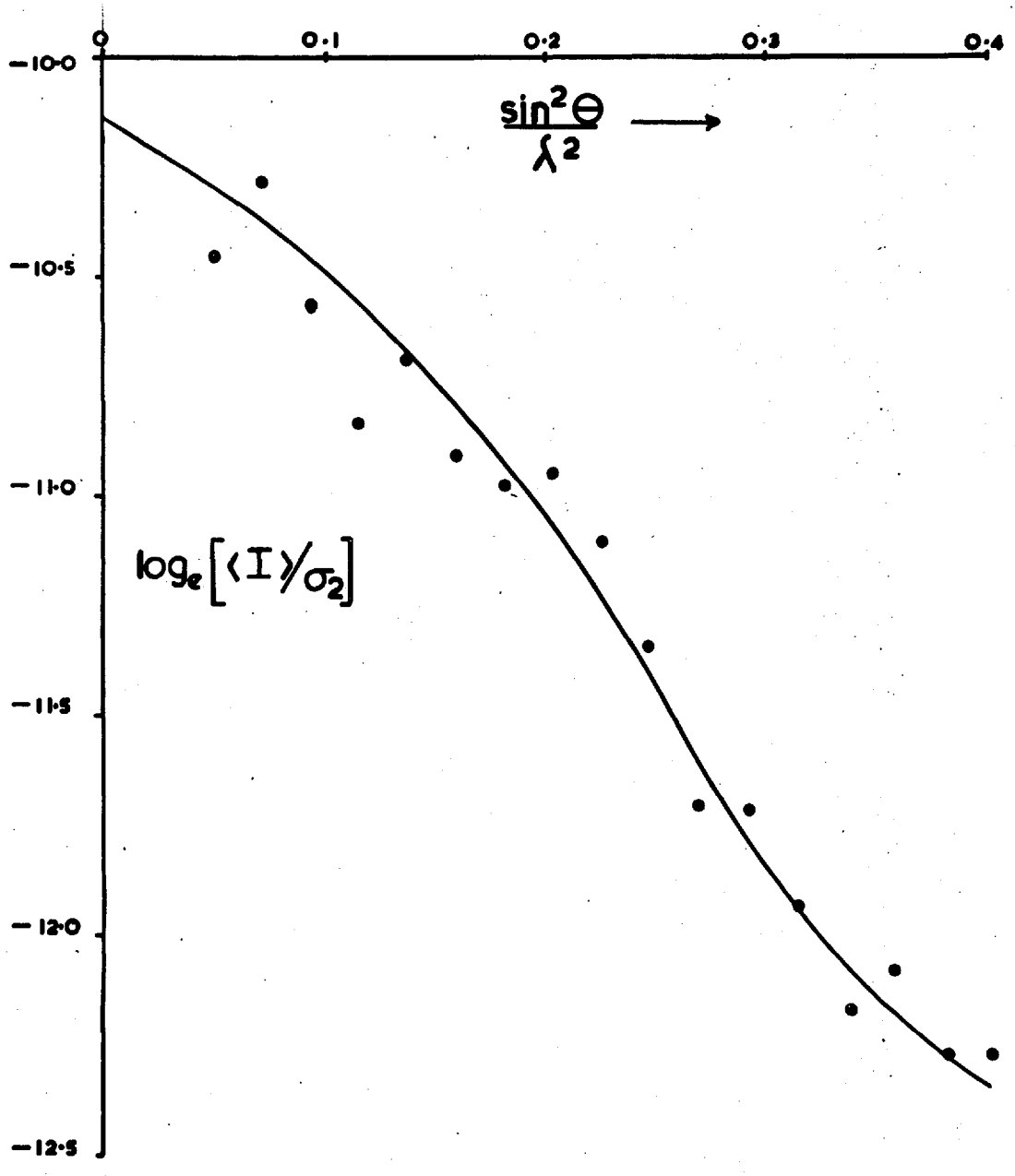
The compound is optically active, so that the spacegroup is uniquely determined as C2. 1580 independent reflexions were estimated visually and correlated from six layers collected about the b axis and reflexions from the zero layer about the c axis. The crystal used for the collection of the main axis data had the approximate dimensions 0.50 x 0.15 x 0.10 mm. and in view of the value of the absorption coefficient(18.5 cm^{-1}) absorption corrections were considered unnecessary.

A Wilson plot based on three-dimensional data was calculated by MATT and is shown in Figure 1. The data were divided into 17 shells, each containing 90 - 110 reflexions. Unobserved reflexions were included as $|F_{unobs}| = 2/3|F_{min}|$, where $|F_{min}|$ is the minimum observable value of $|F|$ at the appropriate point in reciprocal space. The least-squares method gave B = 2.98, K = 145.9 and the results from the graph are B = 2.64, K = 159.2.(After structure refinement the final scale was found to be 131.4.)

Structure Determination

The four sulphur atoms in the unitcell give rise to ± six S - S inter-vectors which overlap to give ± three

Figure 1. 6-NBF-PA Wilson plot based on three-dimensional data.



distinct peaks in the three-dimensional Patterson. In terms of the coordinates x, y, z of one of the sulphur atoms their positions are given by

$$\pm \begin{cases} 2x,\ 0,\ 2z \\ \frac{1}{2},\ \frac{1}{2},\ 0 \\ 2x + \frac{1}{2},\ \frac{1}{2},\ 2z \end{cases}$$

Only the first peak need be considered as the second and third are merely related to the origin and first peaks respectively by the C-face centering of the spacegroup.

The [010] Patterson projection was computed with each $|F|^2$ term modified by its value of $(Lp)^{-1}$ as a crude sharpening function. The position of the S - S peak could not be unambiguously assigned , and of the four most likely peaks, the highest was chosen to give the tentative sulphur position of x = 0.205, y = 0.0, z = 0.208 (Coordinates of the peak were estimated by Booth's (1948) method.).

A solution of the crystal structure directly from the sulphur position was attempted using the FATAL program written by J.Rollett for the Mercury computer. The program was used to calculate structure factors from the above sulphur position, then compute a three-dimensional Fourier map and finally to search the Fourier map for peaks above a certain height. Six additional peaks were recognised possessing geometry reasonably consistent with that of a fused β-lactam-thiazolidine ring system. However, when the additional positions were input to

a second FATAL run, the R value increased from 0.58 to 0.62 and the resulting peaks showed no indication of any further structural details. The method was therefore abandoned (Only two of the additional peaks were near final atomic positions).

In order to check the position of the sulphur atom, a three-dimensional Patterson function was computed using as sharpening function

$$1/\hat{f}^2 \exp\left(3 \sin^2\theta/\lambda^2\right)$$

where $\hat{f}$ is the form factor for sulphur and the resulting Patterson is specifically sharpened for sulphur peaks. The S - S peak was easily located in the Harker section P(u, 0, w) and gave the position of the sulphur atom as x = 0.206, y = 0.0, z = 0.214, which is close to that derived from the [010] Patterson projection. It was thought that the FATAL method may have failed because the sulphur atom was not heavy enough to determine unambiguously enough of the phases, the ratio $\sum Z_H^2$ to $\sum Z_L^2$ being only 0.27. Even though Fourier methods, applied carefully, may have led to the solution of the structure, it was felt that superposition methods would provide a simpler route.

Duplicate copies of the (u,w) sections of the sharpened, three-dimensional Patterson were prepared on tracing paper and contoured at arbitrary but uniform intervals. The origin of the zero section was then laid on the S - S peak of the duplicate

and the minimum function plotted on a perspex sheet by drawing the minimum contours of coincident peaks in the superposed Pattersons. The other sections were paired off with their duplicates using an identical u,w displacement. The resulting minimum function was of rank two as the procedure was equivalent to selecting as image points the two sulphur atoms at x = 0.206, y = 0, z = 0.214 and x = -0.206, y = 0, z = -0.214, i.e. related by a diad. Thus the origin of the minimum function was situated half-way along the u,w displacement.

Figure 2 is a composite diagram derived from all the sections of the minimum function. The mirror plane of the Patterson spacegroup, C2/m, is not eliminated in this function, which contains a pseudo mirror plane through y = 0. Although this rendered the y coordinates ambiguous, the main structural features of the molecule were recognisable, as shown in Figure 2. However, the structure near N_{14} was ambiguous, though the cluster of peaks to the left of this strongly suggested the location of the benzene ring, giving the molecule a curled conformation as in benzylpenicillin. (This was in fact later vindicated).

Three-dimensional structure factor and Fourier calculations were carried out including the fourteen positions marked in Figure 2. The Fourier map, in which the pseudo mirror plane was now eliminated, gave unambiguous peaks for all 23 atoms, and

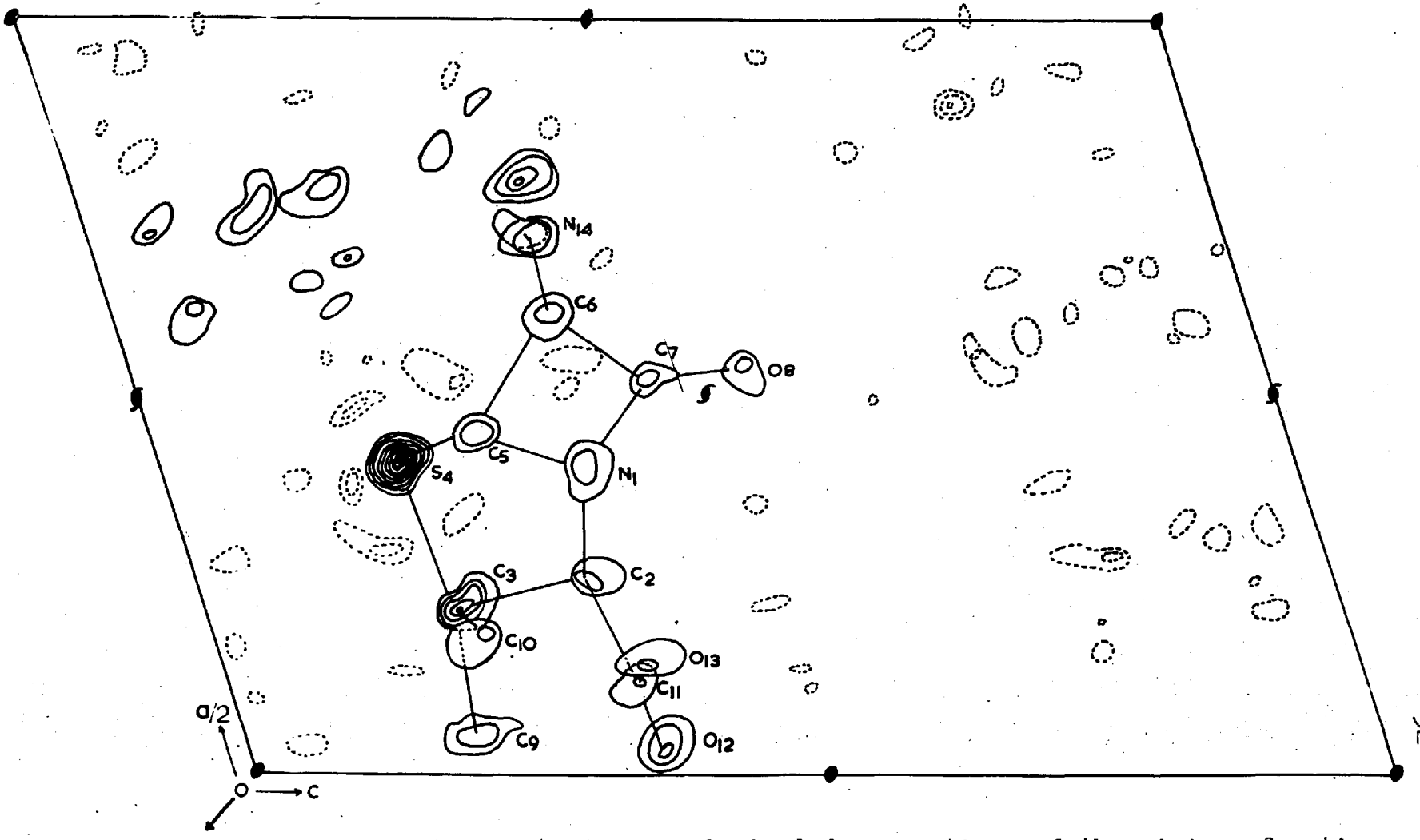


Figure 2. Composite diagram derived from sections of the minimum function.

indicated that C_9 and C_{10} had been misplaced. The details near N_{14} were clarified and showed that C_{15} and O_{16} (diagram inside back cover) were projecting 'down' as seen in Figure 2. The suggested position of the benzene ring was confirmed. Before starting least-squares refinement, a Fourier map phased on all 23 atomic positions was calculated and when deriving the revised atomic positions, double shifts were applied to the observed peak positions as recommended by Donohue (1950).

Refinement

Rollett's Mercury program for the least-squares refinement of parameters was now used to refine the atomic coordinates and isotropic temperature factors for all 23 atoms. The method is essentially that used in BABA and described briefly in Chapter 2. Rollett's weighting scheme 2 was used and checks were made at various stages of refinement with an agreement analysis program written by G.A.Mair and the bond distances and angles program of R.A.Sparks.

The course of the refinement is shown in Table 1. At cycle A6, all the thermal parameters were converted into their anisotropic form but only the sulphur coordinates and thermal parameters refined. The anisotropic refinement was then continued including all atoms and switched to the Atlas program

Table 1. The refinement process for 6-NBF-PA

Cycle	R	$\sum w\Delta^2 \times 10^{-4}$
I1	0.308	16419
I2	0.225	10393
I3	0.188	8184
I4	0.179	7134
I5	0.167	5838
A6	0.154	4622
A7	0.140	3544
A8	0.105	1314
A9	0.099	990
A10	0.127	3177
A11	0.102	2154
A12	0.096	1951
A13	0.095	1912
A14	0.095	1894
A15	0.109	2398
A16	0.106	2197
A17	0.106	2179
A18	0.106	2169
A19	0.103	2038
A20	0.101	1989
A21	0.100	1984
A22	0.100	1982
A23	0.100	1981
A24	0.100	1981
A25	0.100	1980
A26	0.100	1980
A27	0.100	1980

I = isotropic refinememt

A = anisotropic refinement

BABA at A8.(The F^+ value in the weighting scheme was inadvertently decreased at A8 but corrected again at A10). During cycles A12, A13 and A14 it was noted that little convergence was taking place and that the refinement was oscillatory. Thus 164 unobserved reflexions were included at $\frac{2}{3}F_{min}$ and a fudge factor of 0.75 applied. The process was again disturbed at A18 when the calculated positions of the 18 hydrogen atoms were included in the structure factor calculations but not refined. Hydrogen atom positions were calculated on an X-Ray '63 program written by D.F.High, assuming staggered conformations for the C_9 and C_{10} methyl groups. An earlier difference Fourier showed peaks at or near the calculated hydrogen atom positions varying in height from 0.1 to 0.6 $e/A^{\circ 3}$ but the difference map exhibited ripple as high as 0.5 $e/A^{\circ 3}$ and cannot be taken as conclusive evidence for the hydrogen atom positions - the positions of the methyl hydrogens were especially ill-defined.

Subsequent refinement was extremely slow, and at A27 the coordinate shifts were less than one tenth of their standard deviations, so that refinement was ceased.

Results and Discussion

The final atomic coordinates are shown in Table 2, together with their standard deviations, and the anisotropic

Table 2. Atomic coordinates and standard deviations for 6-(N-benzylformamido) penicillanic acid (fractional values).

	X	Y	Z	σ_x	σ_y	σ_z
N1	0.19398	0.19816	0.36500	0.00027	0.00135	0.00042
C2	0.12891	0.06769	0.34228	0.00035	0.00140	0.00056
C3	0.11559	-0.01477	0.23186	0.00035	0.00200	0.00056
S4	0.20502	-0.00849	0.21308	0.00009	0.00058	0.00014
C5	0.23075	0.21859	0.28823	0.00036	0.00160	0.00054
C6	0.30337	0.22048	0.37611	0.00034	0.00161	0.00054
C7	0.25648	0.16794	0.44425	0.00033	0.00163	0.00055
O8	0.26642	0.12197	0.52951	0.00027	0.00123	0.00038
C9	0.08592	-0.22774	0.22020	0.00057	0.00203	0.00087
C10	0.06584	0.13802	0.15406	0.00051	0.00232	0.00066
C11	0.06760	0.19723	0.35731	0.00034	0.00166	0.00057
O12	0.01415	0.07078	0.36209	0.00030	0.00118	0.00058
O13	0.06669	0.38004	0.36511	0.00031	0.00127	0.00056
N14	0.35902	0.07255	0.37699	0.00029	0.00116	0.00047
C15	0.35506	-0.12767	0.39730	0.00041	0.00166	0.00064
O16	0.39630	-0.26675	0.38916	0.00030	0.00122	0.00050
C17	0.41454	0.14853	0.33327	0.00036	0.00157	0.00060
C18	0.38742	0.15183	0.21828	0.00043	0.00177	0.00070
C19	0.38852	-0.02767	0.16578	0.00066	0.00256	0.00079
C20	0.36315	-0.03048	0.06097	0.00089	0.00299	0.00085

Table 2 continued

	X	Y	Z	σ_x	σ_y	σ_z
C21	0.33591	0.15432	0.01082	0.00090	0.00327	0.00096
C22	0.33318	0.33570	0.06531	0.00077	0.00299	0.00100
C23	0.36263	0.33517	0.16991	0.00064	0.00219	0.00090

Table 3. Anisotropic temperature factor coefficients for 6-NBF-PA.

	β_{11}	β_{22}	β_{33}	β_{23}	β_{13}	β_{12}
N1	0.00076	0.02892	0.00363	-0.00141	0.00102	0.00000
C2	0.00126	0.01857	0.00476	-0.00145	0.00197	-0.00075
C3	0.00141	0.02953	0.00482	-0.00397	0.00060	0.00063
S4	0.00172	0.03483	0.00469	-0.00725	0.00226	-0.00134
C5	0.00152	0.02347	0.00402	0.00054	0.00170	-0.00120
C6	0.00120	0.02536	0.00431	-0.00098	0.00205	-0.00169
C7	0.00098	0.02762	0.00436	-0.00276	0.00128	-0.00011
O8	0.00184	0.03298	0.00399	-0.00110	0.00168	0.00392
C9	0.00359	0.02473	0.00924	-0.01337	0.00471	-0.00704
C10	0.00269	0.04859	0.00430	-0.00059	-0.00052	0.00300
C11	0.00106	0.02594	0.00489	-0.00133	0.00132	-0.00227
O12	0.00162	0.02382	0.01028	-0.00654	0.00458	-0.00344
O13	0.00176	0.02437	0.00946	-0.00154	0.00332	0.00069
N14	0.00124	0.01738	0.00475	-0.00050	0.00186	-0.00105
C15	0.00172	0.02048	0.00591	0.00442	0.00222	-0.00121
O16	0.00218	0.02204	0.00804	0.00320	0.00501	0.00384
C17	0.00130	0.02296	0.00553	-0.00036	0.00255	-0.00156
C18	0.00217	0.02514	0.00696	0.00902	0.00442	0.00236
C19	0.00529	0.03373	0.00658	0.00307	0.00461	0.00438
C20	0.00867	0.04386	0.00607	0.00664	0.00731	-0.00130

Table 3 continued

	β_{11}	β_{22}	β_{33}	β_{23}	β_{13}	β_{12}
C21	0.00713	0.06227	0.00677	0.00839	0.00559	-0.00320
C22	0.00495	0.06718	0.00829	0.02674	0.00268	0.00124
C23	0.00408	0.03213	0.00831	0.01258	0.00387	0.00236

All temperature factors in this Table are positive-definite.

Table 4. 6-NBF-PA observed and calculated structure factors (x 100) in the order

* h k

l $|F_o|$ $|F_c|$ α (radians)

Unobserved reflexions are marked † , and the reflexions not inluded are :-

0 0 1
0 0 2
1 1 $\bar{1}$
1 1 1
1 3 $\bar{1}$
1 3 1
2 0 $\bar{2}$
2 0 $\bar{1}$
2 0 1
2 0 2

as they were all obscured by the backstop.

•	0	0	
3	3318	3263	0.000
4	4295	4473	3.142
5	6885	7072	0.000
6	1524	1411	0.000
7	4924	4660	3.142
8	1602	1455	0.000
9	114	130	0.000 †
10	606	557	3.142
11	609	484	0.000
12	1548	1446	3.142
13	1172	1077	0.000
14	1113	999	0.000
15	114	114	3.142 †
16	492	475	3.142
17	66	49	3.142 †
•	0	2	
0	3603	4349	1.737
1	3168	3777	-0.974
2	1422	1463	2.813
3	2657	2687	2.536
4	1559	1621	-0.301
5	786	8[illegible]4	-0.496
6	1826	1983	1.611
7	1507	1662	-2.912
8	995	1067	2.404
9	1347	1412	0.668
10	823	7[illegible]1	-1.129
11	1020	1070	2.319
12	410	411	-1.637
13	93	74	1.298 †
14	341	299	0.607
15	271	328	0.[illegible]61
16	66	333	-2.913 †
•	0	4	
0	1136	1019	-1.347
1	992	989	0.096
2	1841	1736	2.988
3	623	525	-3.104
4	1027	984	-0.564
5	1022	927	-0.134
6	1018	981	2.708
7	775	682	-2.913
8	308	195	-1.782
9	400	320	-1.032
10	573	528	0.300
11	513	446	2.964
12	609	615	2.855
13	237	243	-1.469
14	465	518	-0.497
•	0	6	
0	613	5[illegible]3	0.996
•	0	8	
0	501	324	-1.227
•	1	1	
-16	174	153	-1.683
-15	383	315	-0.203
-14	758	815	0.851
-13	1314	1349	-2.413
-12	869	787	1.947
-11	286	251	0.243
-1[illegible]	1084	116[illegible]	-0.768
-9	522	443	-1.[illegible]20
-8	1257	1294	2.271
-7	1233	11[illegible]4	2.802
-6	4216	3646	0.386
-5	1747	14[illegible]4	-1.635
-4	2467	2228	-2.170
-3	4157	3425	2.167
-2	2957	2707	-2.716
[illegible]	1313	1407	1.[illegible]80
3	1037	997	1.151
4	4591	5538	-1.255
5	2355	2364	1.415
6	2938	2973	-3.[illegible]03
7	522	5[illegible]3	-2.[illegible]26
8	1426	1235	[illegible].630
9	1569	1644	-[illegible].872
10	1136	117[illegible]	3.[illegible]49
11	920	873	2.2[illegible]5
12	854	869	-1.4[illegible]8
13	961	1026	[illegible].654
14	383	335	-1.282
15	474	49[illegible]	-2.899
16	394	42[illegible]	3.111

•	1	3	
-16	66	192	1.980 †
-15	335	430	-0.282
-14	348	337	-3.012
-13	147	126	2.536 †
-12	869	920	2.686
-11	329	323	-2.119
-10	689	585	-0.506
-9	726	729	-0.382
-8	708	662	-2.740
-7	595	601	-1.899
-6	1350	1348	1.141
-5	2001	1744	-0.701
-4	465	346	-1.907
-3	483	1043	-2.675
-2	1815	1770	-0.343
0	1203	1095	1.044
2	3123	3004	-3.067
3	1668	1712	-0.025
4	1956	2037	-0.254
5	961	896	1.871
6	1308	1396	-3.039
7	915	921	3.118
8	1153	1238	0.045
9	1323	1417	0.217
10	735	700	-1.111
11	775	683	2.903
12	400	469	-0.255
13	301	290	-1.206
14	114	308	0.647 †
15	93	170	2.330 †
•	1	5	
-13	348	456	-2.897
-12	354	439	2.229
-11	580	575	0.794
-10	513	494	-1.203
-9	114	171	1.375 †
-8	599	534	-2.796
-7	410	345	-2.520
-6	469	342	0.425
-5	1221	1207	-0.432
-4	864	709	2.868
-3	554	684	2.205
-2	714	707	-1.977
-1	955	906	-0.569
0	879	1014	0.546
1	1600	1605	2.636
2	708	756	2.066
3	752	636	-0.113
4	839	697	-1.037
5	335	360	1.068
6	995	856	2.271
7	758	621	-3.055
8	1115	1121	-0.496
9	114	53	-0.253 †
10	446	377	1.286
11	492	483	-2.594
12	360	350	-3.113
13	469	568	-0.433
•	2	0	
-17	66	20	0.000 †
-16	93	140	3.142 †
-15	641	613	0.000
-14	637	502	3.142
-13	1163	1193	3.142
-12	1867	1797	0.000
-11	279	199	3.142
-10	186	194	0.000
-9	301	313	0.000
-8	2186	1886	3.142
-7	197	230	0.000
-6	620	556	0.000
-5	732	712	3.142
-4	5065	4696	3.142
-3	3693	3089	3.142
0	4330	3833	3.142
3	6347	6822	0.000
4	3506	3604	3.142
5	255	281	3.142
6	2180	1863	0.000
7	114	78	0.000 †
8	3550	3161	0.000
9	1554	1343	3.142

10	1981	1809	3.142
11	131	62	3.142 †
12	1088	853	0.000
13	348	303	0.000
14	322	297	3.142
15	834	843	3.142
16	360	376	0.000
•	2	2	
-16	534	681	-0.396
-15	218	248	2.865
-14	354	317	-2.740
-13	93	84	1.172 †
-12	517	527	1.854
-11	1793	1939	-0.783
-10	780	782	-1.256
-9	1402	1363	-2.927
-8	818	686	1.804
-7	882	889	0.519
-6	1639	1478	-0.546
-5	680	781	-3.089
-4	2280	2783	-2.638
-3	2304	2565	0.851
-2	617	587	-0.208
-1	1790	2139	1.694
0	588	667	-1.789
1	2402	3052	2.611
2	4246	4184	-0.913
3	2509	2789	-0.288
4	1056	1054	-2.027
5	1606	1992	2.617
6	975	1019	2.475
7	1682	1789	-0.576
8	1123	1248	-0.635
9	813	823	3.064
10	1249	1455	2.802
11	271	362	-0.219
12	634	724	0.841
13	255	214	-1.939
14	348	380	2.048
15	410	488	-2.847
16	44	133	-0.203 †
•	2	4	
-15	308	372	-0.520
-14	451	541	-3.053
-13	378	365	1.648
-12	474	433	-0.794
-11	542	509	0.170
-10	699	673	0.715
-9	769	711	-2.929
-8	810	789	3.113
-7	732	727	0.424
-6	617	514	-0.695
-5	606	496	1.281
-4	263	291	2.665
-3	1855	1956	3.003
-2	1955	2053	0.001
-1	857	925	-0.833
0	1388	1376	2.232
1	1411	1483	-2.762
2	1264	1092	-0.312
3	1146	1077	0.602
4	1049	885	-0.221
5	1347	1296	3.046
6	908	830	-3.018
7	1010	920	-0.632
8	918	913	0.404
9	546	502	1.079
10	970	1003	-2.756
11	114	245	2.051 †
12	526	592	-0.654
13	228	202	-0.298
14	451	599	2.300
•	2	6	
0	647	589	3.067
•	2	8	
0	378	198	2.014
•	3	1	
-17	341	154	-0.708
-16	394	196	0.134
-15	263	304	2.050
-14	573	598	-2.714
-13	1266	1328	0.648
-12	114	193	-2.674 †
-11	492	446	-2.067
-10	1043	988	-2.965

-9	1306	1271	2.006	11	383	469	-0.073	-1	1135	1304	2.212
-8	1526	1585	-0.934	12	389	479	-0.944	0	478	220	-1.506
-7	2228	1902	0.343	*	4	0		1	1514	1556	-0.220
-6	689	654	2.026	-17	93	233	0.000 +	2	791	781	2.911
-5	1669	1465	-2.160	-16	335	326	3.142	3	660	527	0.307
-4	1728	1617	2.467	-15	538	496	3.142	4	872	849	3.000
-3	3203	3245	-0.077	-14	959	865	0.000	5	93	127	-0.167 +
-2	3012	3653	0.392	-13	131	51	3.142 +	6	964	951	-0.396
-1	1194	1837	1.184	-12	1052	935	3.142	7	271	274	-2.988
0	2370	3016	1.388	-11	197	129	3.142	8	431	374	1.938
1	2277	2849	2.615	-10	1172	973	3.142	9	372	307	3.021
2	2777	3053	-0.076	-9	2616	2323	0.000	10	509	505	-0.046
3	702	801	-0.831	-8	1337	1184	-0.000	11	263	358	-0.819
4	2343	2311	-3.036	-7	591	765	0.000	12	329	410	1.995
5	2281	2346	1.431	-6	904	746	0.000	13	400	488	2.849
6	1078	1051	0.586	-5	5537	4891	3.142	*	4	6	
7	1782	1806	-1.303	-4	4632	4339	0.000	8	147	88	-0.484
8	1449	1308	1.184	-3	2826	2813	0.000	*	5	1	
9	2222	2154	-2.797	-1	2665	2690	3.142	-17	329	491	-2.974
10	329	321	-1.212	0	5494	5375	3.142	-16	271	216	-2.607
11	1230	1231	0.513	1	3374	3243	3.142	-15	263	288	-0.633
12	562	598	0.139	2	1989	1973	0.000	-14	1052	964	0.428
13	426	455	-3.037	3	5490	4831	3.142	-13	802	836	-1.839
14	383	475	2.534	4	1276	1088	0.000	-12	657	629	2.507
15	301	329	-1.553	5	1298	1133	3.142	-11	813	778	-2.487
16	378	459	0.236	6	308	223	3.142	-10	1086	1167	-0.020
*	3	3		7	1581	1331	0.000	-9	844	928	2.032
-16	93	120	0.190 +	8	2079	1928	3.142	-8	294	236	-2.904
-15	509	701	-3.090	9	335	207	3.142	-7	2164	2083	-2.619
-14	416	439	1.006	10	1084	994	0.000	-6	939	923	-3.076
-13	147	119	2.264 +	11	1388	1364	0.000	-5	2840	2705	-0.681
-12	904	953	-0.571	12	988	943	3.142	-4	2616	2377	2.074
-11	904	937	1.541	13	513	470	3.142	-3	1674	1650	2.888
-10	1555	1592	-2.996	14	114	109	3.142 +	-2	5458	5832	-1.601
-9	1274	1277	2.679	15	322	339	0.000	-1	1924	1861	1.803
-8	1003	977	0.155	16	66	13	0.000 +	0	3602	3818	-0.108
-7	847	844	-1.659	*	4	2		1	1222	1284	-0.421
-6	877	841	1.809	-17	66	153	1.714 +	2	3797	4198	-2.993
-5	1960	1727	-3.123	-16	405	434	-3.119	3	1411	1459	-0.611
-4	1422	1262	-2.553	-15	228	206	2.481	4	821	816	1.911
-3	2478	1841	0.101	-14	93	157	1.376 +	5	1987	1884	0.235
-2	2408	1936	-1.428	-13	577	566	0.222	6	1080	972	0.549
-1	1872	1738	1.738	-12	93	204	-2.124 +	7	1963	1985	3.099
0	1843	1728	2.357	-11	813	964	2.768	8	1807	1802	-2.171
1	3610	2397	-1.188	-10	197	182	2.834	9	1197	1123	0.603
2	2774	2275	0.664	-9	1041	1057	-0.638	10	341	278	-0.087
3	546	524	-3.100	-8	1432	1509	0.058	11	294	260	1.020
4	1987	2062	-3.006	-7	1347	1340	2.919	12	884	904	-2.887
5	1165	1240	2.399	-6	1410	1523	-2.625	13	147	168	0.086
6	988	976	-0.835	-5	970	949	-0.405	14	394	415	0.126
7	823	916	-0.313	-4	1872	1949	-0.431	15	66	152	-1.510 +
8	1504	1585	2.397	-3	2547	2681	-1.125	*	5	3	
9	1170	1401	-2.851	-2	3291	3992	2.161	-16	366	343	1.854
10	329	385	1.461	-1	2469	2987	-2.242	-15	315	271	-0.140
11	800	917	-0.159	0	1891	2045	1.620	-14	366	337	-1.088
12	147	95	-1.961 +	1	1583	1745	-0.090	-13	505	455	-0.063
13	400	484	1.817	2	1566	1697	0.006	-12	808	743	-2.653
14	372	536	3.112	3	1714	1853	-2.817	-11	1094	989	-0.188
15	301	401	-0.929	4	1570	1390	2.747	-10	961	958	0.670
*	3	5		5	1533	1653	-0.136	-9	746	661	-1.129
-14	66	179	-2.985 +	6	1123	1159	0.167	-8	1261	1193	2.128
-13	131	123	0.488	7	647	611	1.890	-7	1062	1015	-2.688
-12	426	462	-0.068	8	1078	1155	-2.127	-6	772	758	0.499
-11	400	350	-2.578	9	764	782	-1.531	-5	1773	1591	0.405
-10	421	325	1.991	10	966	1134	-0.379	-4	1627	1460	-1.549
-9	114	88	-0.964 +	11	405	470	1.424	-3	2130	2286	2.561
-8	546	566	1.777	12	366	361	-2.790	-2	1334	1406	2.264
-7	546	478	-0.812	13	410	483	2.706	-1	1047	998	-1.996
-6	641	649	-1.314	14	426	473	-1.976	0	1131	1231	0.036
-5	1217	1046	2.580	15	410	485	-0.114	1	1623	1484	-0.514
-4	517	379	-0.515	*	4	4		2	823	675	-0.162
-3	372	426	1.585	-15	208	262	2.566	3	1615	1617	2.728
-2	834	857	-0.162	-14	263	217	-0.184	4	1140	1138	-1.091
-1	1443	1429	-1.751	-13	255	226	-0.772	5	1264	1263	0.664
0	973	1064	-3.055	-12	93	166	-2.505 +	6	617	528	-1.900
1	1010	739	0.428	-11	542	476	-2.838	7	228	247	0.345
2	218	168	-0.427	-10	821	684	0.029	8	228	151	-2.158
3	538	374	0.519	-9	660	508	-0.177	9	329	318	-1.659
4	573	515	-2.377	-8	218	216	-3.138	10	405	370	-0.141
5	550	471	2.810	-7	474	417	-1.229	11	630	773	2.612
6	335	233	-1.586	-6	839	828	2.749	12	206	307	2.978
7	623	513	-0.166	-5	813	809	0.258	13	114	163	-2.117 +
8	651	588	1.740	-4	591	571	0.016	14	335	396	-0.556
9	174	188	2.993	-3	492	482	-0.399	*	5	5	
10	360	296	-2.954	-2	769	870	-3.021	-14	218	209	-0.374

-13	263	261	3.108	
-12	218	207	-2.329	
-11	465	378	1.307	
-10	301	224	-1.560	
-9	627	564	-0.651	
-8	752	639	2.754	
-7	526	540	1.703	
-6	389	390	-0.781	
-5	93	239	2.626	†
-4	651	591	0.475	
-3	538	594	-2.198	
-2	1561	1692	2.411	
-1	1086	1296	-0.207	
0	558	382	-1.128	
1	455	412	2.485	
2	580	579	-0.843	
3	1022	986	2.861	
4	802	812	0.160	
5	651	607	-1.321	
6	246	136	2.201	
7	301	185	-3.102	
8	294	292	-3.054	
9	114	103	-0.069	†
10	335	356	0.357	
11	186	280	2.659	
12	66	206	-3.001	†
*	6	0		
-17	279	325	3.142	
-16	591	567	0.000	
-15	859	746	0.000	
-14	354	259	0.000	
-13	1434	1214	3.142	
-12	651	523	3.142	
-11	1701	1538	0.000	
-10	530	412	3.142	
-9	952	935	3.142	
-8	279	90	3.142	
-7	389	306	3.142	
-6	2800	2906	0.000	
-5	218	276	0.000	
-4	3922	3683	3.142	
-3	3744	3657	0.000	
-1	8206	9736	0.000	
0	2936	2535	3.142	
1	4289	3948	3.142	
2	2217	1910	0.000	
4	1369	1085	0.000	
5	1784	1622	0.000	
6	2882	2736	3.142	
7	680	657	3.142	
8	1027	808	0.000	
9	577	577	3.142	
10	1106	1110	0.000	
11	995	894	3.142	
12	341	314	0.000	
13	565	591	0.000	
14	93	105	3.142	†
15	372	413	3.142	
*	6	2		
-17	66	226	2.248	†
-16	348	397	0.813	
-15	271	268	-0.850	
-14	446	415	0.964	
-13	899	838	-2.555	
-12	641	749	-2.869	
-11	1219	1143	1.024	
-10	501	478	-0.200	
-9	816	711	2.431	
-8	1166	1160	2.150	
-7	829	832	-1.806	
-6	1084	965	0.288	
-5	1547	1567	0.728	
-4	2064	2358	-2.711	
-3	1967	2187	1.357	
-2	1249	1270	-0.932	
-1	1138	1096	2.555	
0	2726	2714	-0.504	
1	1824	1811	3.139	
2	2490	2199	3.127	
3	1749	1788	1.031	
4	988	975	-0.525	
5	780	838	1.846	
6	1222	1340	2.905	
7	664	662	-2.314	
8	1106	1197	1.988	
9	925	872	-0.497	
10	558	603	1.727	
11	542	592	-3.013	
12	93	114	-1.548	†
13	348	365	0.033	
14	93	209	-0.061	†
*	6	4		
-15	517	608	-0.947	
-14	322	285	2.286	
-13	554	609	2.802	
-12	114	133	2.818	†
-11	634	537	0.935	
-10	929	824	-1.799	
-9	394	353	-2.538	
-8	657	658	2.305	
-7	389	421	-1.944	
-6	943	933	0.080	
-5	315	264	3.101	
-4	1072	1216	-1.559	
-3	1252	1276	2.341	
-2	961	902	-0.237	
-1	1217	1328	-1.646	
0	1288	1412	1.542	
1	720	633	-2.874	
2	487	414	-2.133	
3	400	361	0.566	
4	405	289	-1.150	
5	586	568	3.138	
6	271	360	0.820	
7	465	410	2.887	
8	465	408	-1.420	
9	738	769	0.063	
10	573	627	-3.059	
11	93	110	-2.775	†
12	263	221	-3.084	
13	271	316	-0.370	
*	6	6		
0	405	382	2.192	
*	7	1		
-17	474	513	0.387	
-16	360	307	-0.520	
-15	348	333	1.375	
-14	999	955	-2.775	
-13	641	633	2.930	
-12	975	996	-0.204	
-11	1694	1763	-0.073	
-10	1852	1774	-2.590	
-9	2341	2084	1.387	
-8	329	453	-1.556	
-7	1212	1344	-1.291	
-6	2276	1568	0.720	
-5	2924	2760	-2.633	
-4	2006	2045	2.545	
-3	2518	2499	0.696	
-2	595	628	-1.212	
-1	2364	2655	3.099	
0	1519	1501	-2.380	
1	2391	2235	-2.205	
2	2493	2203	0.827	
3	2276	1486	-0.940	
4	918	1024	1.847	
5	1559	1581	2.844	
6	1912	1888	-1.300	
7	562	515	0.638	
8	372	372	-0.728	
9	237	136	-2.782	
10	966	1027	2.114	
11	702	735	-1.821	
12	595	683	0.405	
13	93	110	-3.080	†
14	474	537	-2.244	
*	7	3		
-16	546	636	-0.980	
-15	416	319	2.210	
-14	366	352	2.887	
-13	711	681	-2.949	
-12	660	462	0.430	
-11	617	574	-0.862	
-10	791	817	-0.901	
-9	1435	1370	2.915	
-8	465	394	-0.794	
-7	1533	1481	0.439	
-6	984	1010	-1.526	
-5	595	492	-0.556	
-4	667	732	2.702	
-3	641	579	0.455	
-2	1072	1048	0.963	
-1	1074	1009	-1.488	
0	1519	1360	2.626	
1	492	437	-2.930	
2	729	666	-1.344	
3	1592	1538	0.452	
4	620	587	2.946	
5	1233	1334	-2.378	
6	1076	1113	1.507	
7	726	692	-0.689	
8	908	1013	-0.338	
9	329	336	1.130	
10	637	688	-3.118	
11	131	162	-0.580	†
12	114	230	0.490	†
13	366	370	-0.743	
*	7	5		
-14	522	658	2.423	
-13	93	79	0.195	†
-12	627	533	0.039	
-11	114	98	-0.707	†
-10	246	281	-1.807	
-9	744	621	2.813	
-8	114	37	1.256	†
-7	1005	955	-0.761	
-6	735	736	1.074	
-5	789	775	2.825	
-4	588	505	-1.819	
-3	93	45	0.954	†
-2	884	810	-1.119	
-1	1113	1168	1.287	
0	1153	1157	2.779	
1	93	178	-1.410	†
2	1074	990	-0.159	
3	465	392	-2.524	
4	538	469	2.156	
5	550	445	0.725	
6	925	858	-2.905	
7	891	971	-0.215	
8	237	260	0.483	
9	394	479	2.838	
10	294	315	-1.525	
11	228	240	-3.004	
*	8	0		
-17	469	449	0.000	
-16	114	1	0.000	†
-15	606	504	3.142	
-14	1001	907	3.142	
-13	1183	1062	0.000	
-12	1226	1147	0.000	
-11	1497	1255	3.142	
-10	329	239	3.142	
-9	670	545	3.142	
-8	161	19	3.142	
-7	1256	1214	0.000	
-6	1984	2837	3.142	
-5	3281	3222	3.142	
-4	2245	2404	0.000	
-3	286	306	3.142	
-2	975	807	3.142	
-1	3765	3723	3.142	
0	800	552	0.000	
1	1319	1092	0.000	
2	2604	1741	0.000	
3	1918	1741	3.142	
4	114	44	0.000	†
5	834	753	0.000	
6	366	288	0.000	
7	1238	1022	0.000	
8	1155	1059	3.142	
9	360	178	3.142	
10	208	130	3.142	
11	818	690	0.000	
12	114	68	3.142	†
13	348	361	3.142	
14	394	461	3.142	
*	8	2		
-17	441	535	-0.520	
-16	66	39	-2.521	†
-15	378	322	3.039	
-14	93	96	2.779	†
-13	534	443	0.005	
-12	1203	1227	-0.753	
-11	378	309	-1.066	

-10	1673	1822	2.893
-9	366	361	2.035
-8	2029	2054	-0.103
-7	1489	1512	-1.740
-6	1039	1093	2.396
-5	800	750	-2.618
-4	995	979	2.539
-3	1827	2228	0.089
-2	766	525	0.206
-1	1533	1550	3.094
0	2117	1697	0.563
1	2939	2838	-1.539
2	565	493	1.531
3	1420	1483	0.252
4	2352	2516	3.041
5	474	410	0.117
6	1699	1942	-0.570
7	1104	1125	-2.802
8	93	167	0.799 †
9	1003	1098	2.203
10	478	529	-2.233
11	758	833	0.495
12	174	208	-1.974
13	66	142	-2.961 †
*	8	4	
-15	513	543	2.894
-14	93	116	1.454 †
-13	761	733	-0.164
-12	588	527	0.084
-11	588	448	1.532
-10	1348	1386	-2.994
-9	279	238	2.277
-8	1276	1426	0.610
-7	1098	1044	-1.345
-6	920	845	1.926
-5	1148	1255	2.731
-4	1070	1213	-2.134
-3	2338	2545	0.378
-2	906	829	-2.791
-1	864	892	-1.885
0	1111	1079	2.064
1	1266	1217	-0.640
2	854	858	-0.152
3	1564	1615	2.164
4	1475	1512	-1.825
5	279	183	2.152
6	899	858	1.192
7	1027	989	-0.721
8	849	901	2.158
9	627	640	-2.886
10	538	535	-0.763
11	208	271	1.618
12	308	410	-0.259
*	8	6	
0	341	443	-2.823
*	9	1	
-17	246	250	-1.202
-16	329	341	2.374
-15	554	608	2.877
-14	660	627	0.195
-13	246	154	-1.033
-12	341	264	2.674
-11	984	970	-3.124
-10	1360	1399	1.804
-9	2657	2485	-0.864
-8	2107	1960	0.162
-7	2705	2604	2.714
-6	1348	1118	-1.615
-5	1799	1602	1.059
-4	2020	2467	-0.370
-3	3786	3996	0.054
-2	4056	4399	2.687
-1	1142	1071	2.653
0	1386	914	-0.289
1	1212	899	1.597
2	1394	1293	-1.348
3	2312	2211	1.865
4	1360	1306	2.626
5	1848	2024	-0.681
6	389	321	2.211
7	372	398	2.445
8	565	489	-2.289
9	294	322	0.619
10	755	764	-0.801
11	218	128	0.577
12	383	418	-3.059
13	246	260	-2.784
14	110	118	-0.270 †
*	9	3	
-16	534	608	3.022
-15	114	135	-2.618 †
-14	620	614	0.846
-13	834	905	-0.867
-12	1066	956	1.954
-11	1261	1265	2.958
-10	1368	1230	-2.582
-9	2129	2235	0.486
-8	746	669	-1.742
-7	1217	1298	-2.612
-6	1618	1583	1.847
-5	1681	1737	-2.198
-4	2139	2139	0.075
-3	772	731	1.882
-2	1793	1773	-2.567
-1	959	786	1.336
0	1699	1580	-0.508
1	263	180	3.123
2	939	840	1.683
3	1779	1607	3.088
4	809	825	-1.784
5	1631	1697	0.438
6	400	443	-2.061
7	995	1018	3.088
8	405	394	2.138
9	147	206	-1.734 †
10	301	318	-0.090
11	114	230	1.016 †
12	161	258	-3.073
*	9	5	
-14	66	28	-1.509 †
-13	667	577	0.092
-12	623	586	-2.273
-11	735	647	2.643
-10	654	513	0.192
-9	114	78	2.482 †
-8	522	439	-0.767
-7	670	621	-0.861
-6	1617	1942	2.667
-5	988	926	-0.378
-4	1144	1109	-0.287
-3	896	768	2.777
-2	1121	1001	-1.265
-1	1031	966	2.890
0	920	855	1.448
1	1772	1866	-0.780
2	1192	1085	2.332
3	573	473	-3.139
4	839	750	-1.056
5	714	717	2.438
6	794	816	-0.198
7	394	341	2.183
8	465	506	2.703
9	360	413	-1.071
10	66	265	-0.350 †
*	10	0	
-17	599	591	3.142
-16	329	208	3.142
-15	263	244	0.000
-14	131	123	0.000 †
-13	513	453	0.000
-12	1161	1016	3.142
-11	455	434	3.142
-10	1776	1598	0.000
-9	1281	1177	0.000
-8	562	538	3.142
-7	1868	1842	3.142
-6	1720	1505	3.142
-5	4202	4194	0.000
-4	1648	2182	0.000
-3	1762	2522	3.142
-2	4456	3856	3.142
-1	446	426	0.000
0	1602	1493	0.000
1	114	242	0.000 †
2	2786	2424	3.142
3	711	627	0.000
4	410	375	3.142
5	660	399	0.000
6	341	304	0.000
7	2352	2138	3.142
8	1024	1006	0.000
9	948	996	0.000
10	197	123	0.000
11	673	694	3.142
12	348	250	3.142
13	66	127	3.142 †
*	10	2	
-17	255	258	-2.744
-16	492	562	2.978
-15	416	379	0.489
-14	778	844	-0.579
-13	550	485	2.666
-12	271	297	2.216
-11	509	459	3.121
-10	975	915	-0.070
-9	1631	1745	-0.043
-8	2425	2569	-3.072
-7	378	425	3.118
-6	1473	1499	-0.111
-5	301	221	-2.934
-4	1276	1438	-0.718
-3	2316	2705	2.933
-2	2218	2590	-2.315
-1	2551	2495	0.143
0	726	627	1.099
1	749	712	-1.489
2	1037	956	2.581
3	1413	1371	2.589
4	2231	2442	-0.553
5	831	885	0.977
6	821	815	2.873
7	943	1027	-1.519
8	348	418	1.287
9	580	564	-0.693
10	341	346	2.047
11	246	260	-2.435
12	66	125	-2.993 †
*	10	4	
-15	286	250	1.074
-14	372	332	-0.277
-13	569	637	-1.481
-12	752	637	2.549
-11	509	438	-0.933
-10	677	641	-0.139
-9	699	666	1.901
-8	1226	1236	-1.032
-7	1453	1423	2.518
-6	487	416	1.861
-5	1681	1919	-0.615
-4	975	945	2.285
-3	1076	1100	2.690
-2	383	339	-2.740
-1	602	474	1.446
0	2064	2184	-0.374
1	961	1030	-3.102
2	1012	988	2.086
3	677	772	-1.742
4	329	254	0.585
5	1068	1137	0.038
6	654	643	-2.764
7	501	451	2.237
8	208	218	-1.529
9	228	215	-0.634
10	66	175	0.494 †
11	322	392	2.098
*	10	6	
0	526	315	-0.745
*	11	1	
-17	279	262	2.811
-16	294	222	0.362
-15	699	726	-0.237
-14	394	414	-1.666
-13	534	448	2.745
-12	1108	1057	-2.818
-11	1288	1381	0.804
-10	546	516	-0.620
-9	741	766	-2.576
-8	1475	1588	-3.048
-7	766	673	-2.625
-6	1588	1765	0.247
-5	662	872	0.946
-4	1773	1862	-2.626
-3	2170	2221	-2.133
-2	1944	1882	0.763
-1	2399	2231	-0.661

0	946	857	0.068
1	3472	3295	-3.048
2	1115	992	0.764
3	1428	1191	-0.086
4	667	584	-0.471
5	286	268	1.615
6	1972	1841	-3.063
7	114	196	2.503 †
8	1554	1614	0.277
9	228	252	-3.115
10	301	317	-2.800
11	263	247	-2.784
12	66	117	-2.539 †
*	11	3	
-16	335	378	0.470
-15	431	429	-0.559
-14	492	440	-1.237
-13	617	584	2.280
-12	606	498	-1.213
-11	617	549	1.248
-10	813	827	0.102
-9	689	661	-2.643
-8	780	854	2.705
-7	651	579	0.099
-6	847	920	-0.214
-5	131	76	0.259 †
-4	1259	1481	2.672
-3	530	427	2.864
-2	746	758	-1.705
-1	2167	2085	-0.196
0	538	499	1.963
1	869	885	-3.070
2	973	858	2.887
3	1049	1025	-0.468
4	1001	988	0.359
5	522	479	-3.018
6	237	234	2.633
7	329	328	-1.521
8	383	290	0.366
9	131	82	0.294 †
10	400	433	2.860
11	93	151	2.379 †
*	11	5	
-13	378	369	3.038
-12	573	587	1.981
-11	487	428	-1.093
-10	114	175	-0.081 †
-9	114	42	2.201 †
-8	460	442	2.405
-7	522	399	2.549
-6	696	630	-0.528
-5	389	368	-0.304
-4	644	539	-2.754
-3	1199	1157	2.142
-2	874	744	-0.521
-1	844	698	-0.381
0	469	392	2.922
1	348	185	-2.245
2	744	648	2.509
3	627	446	-0.183
4	764	823	-0.970
5	595	644	1.882
6	436	415	3.060
7	348	336	-1.432
8	66	169	1.667 †
*	12	0	
-17	446	380	0.000
-16	505	449	0.000
-15	186	295	3.142
-14	558	495	3.142
-13	505	466	3.142
-12	913	1012	0.000
-11	465	365	0.000
-10	131	131	3.142 †
-9	831	711	3.142
-8	534	416	3.142
-7	1300	1209	0.000
-6	1819	1619	0.000
-5	1587	1480	3.142
-4	580	665	3.142
-3	831	889	0.000
-2	2746	2709	0.000
-1	1080	998	3.142
0	1117	969	3.142
1	114	163	3.142 †

2	1895	1599	0.000
3	957	901	0.000
4	197	124	3.142
5	1759	1593	3.142
6	657	555	3.142
7	1734	1609	0.000
8	131	54	3.142 †
9	131	27	3.142 †
10	764	778	3.142
11	263	280	0.000
12	228	305	0.000
*	12	2	
-17	360	397	1.320
-16	421	510	-0.222
-15	329	334	-2.181
-14	558	520	2.822
-13	664	613	-2.621
-12	1066	979	1.551
-11	1513	1540	-0.142
-10	752	698	-2.497
-9	1257	1391	2.603
-8	714	734	-0.619
-7	315	266	0.635
-6	1642	1893	0.697
-5	1425	1532	-2.404
-4	1368	1542	2.267
-3	941	1037	0.060
-2	692	668	-1.279
-1	1016	991	-0.397
0	1981	2019	2.917
1	1651	1684	3.107
2	2166	2231	0.380
3	896	849	-0.684
4	884	880	-3.025
5	927	855	2.852
6	538	495	-2.526
7	1305	1334	0.508
8	416	424	-0.069
9	692	784	2.858
10	174	178	-1.898
11	66	53	-0.309 †
*	12	4	
-15	147	185	-0.838
-14	431	561	2.471
-13	426	418	2.602
-12	360	402	-1.080
-11	93	93	2.706 †
-10	487	458	-0.983
-9	114	236	2.710 †
-8	1027	1039	2.660
-7	1417	1444	-0.565
-6	114	136	2.509 †
-5	451	430	-1.762
-4	573	511	2.261
-3	522	463	-3.049
-2	1337	1322	-0.701
-1	1228	1092	1.305
0	1240	1195	3.115
1	562	486	-1.480
2	366	326	1.993
3	823	684	-0.731
4	279	264	1.144
5	906	935	2.252
6	634	633	-1.676
7	246	273	-0.488
8	501	562	0.123
9	513	557	-2.812
10	308	422	2.074
*	12	6	
0	114	237	-2.587
*	13	1	
-17	335	309	-0.174
-16	66	121	2.680 †
-15	720	763	-2.759
-14	348	273	0.812
-13	372	348	0.052
-12	1076	1153	-0.189
-11	591	590	-2.428
-10	1094	1187	2.050
-9	977	953	-1.873
-8	483	400	0.280
-7	1306	1455	0.506
-6	1443	1511	-2.225
-5	1697	1841	2.386
-4	294	321	0.615

-3	255	337	0.219
-2	509	620	-2.359
-1	680	453	-1.911
0	1552	1603	-3.039
1	2371	2054	0.501
2	1391	1303	-1.059
3	613	588	2.382
4	966	893	-2.906
5	950	806	-1.930
6	1052	994	0.252
7	580	558	-0.009
8	634	624	2.780
9	546	598	3.096
10	93	176	-0.991 †
11	354	430	-0.083
*	13	3	
-16	66	37	-2.394 †
-15	93	176	-2.999 †
-14	591	592	2.761
-13	465	415	-0.287
-12	496	441	-0.638
-11	400	371	-0.610
-10	1119	1107	3.009
-9	161	71	2.572 †
-8	1183	1183	0.229
-7	1022	962	-1.486
-6	147	98	1.586 †
-5	1403	1370	2.378
-4	580	489	-1.920
-3	1276	1326	0.051
-2	1201	1114	-0.424
-1	2077	1833	2.887
0	469	345	-3.089
1	228	178	-0.993
2	1464	1314	0.003
3	161	121	-2.908 †
4	1194	1135	-3.122
5	329	300	1.641
6	808	787	-0.712
7	637	649	0.243
8	431	539	2.849
9	372	460	2.775
10	301	246	-0.575
*	13	5	
-13	66	453	-0.217 †
-12	66	11	0.542 †
-11	93	73	2.760 †
-10	255	204	-2.357
-9	460	388	2.532
-8	602	562	-0.219
-7	421	408	0.589
-6	546	478	3.134
-5	577	465	-2.043
-4	492	360	2.167
-3	673	604	-0.475
-2	839	721	1.308
-1	816	719	-2.667
0	114	187	-0.061 †
1	114	118	1.090 †
2	478	412	-1.124
3	400	349	1.561
4	383	357	2.182
5	383	408	-2.208
6	538	540	0.088
7	66	122	0.408 †
*	14	0	
-17	66	106	0.000 †
-16	620	626	3.142
-15	114	193	3.142 †
-14	651	730	0.000
-13	131	46	0.000 †
-12	686	693	3.142
-11	591	573	0.000
-10	1231	1288	3.142
-9	286	295	0.000
-8	1313	1413	0.000
-7	1503	1497	3.142
-6	717	474	3.142
-5	1305	1208	0.000
-4	1467	1270	3.142
-3	1131	966	0.000
-2	1106	1004	3.142
-1	1523	1300	3.142
0	1744	1599	0.000
1	834	729	0.000

2	1064	926	3.142	
3	354	271	0.000	
4	1142	960	3.142	
5	421	339	0.000	
6	1648	1466	0.000	
7	657	640	3.142	
8	455	417	3.142	
9	114	148	3.142	†
10	613	722	0.000	
11	246	341	3.142	
*	14	2		
-16	131	153	-2.633	
-15	197	206	2.851	
-14	348	307	0.418	
-13	670	690	-0.544	
-12	421	461	-2.603	
-11	1010	1291	2.397	
-10	879	961	-1.662	
-9	831	870	0.061	
-8	647	645	-0.170	
-7	821	871	-2.617	
-6	1172	1221	2.549	
-5	294	288	-0.256	
-4	913	966	-0.808	
-3	1642	1739	0.285	
-2	936	951	-2.878	
-1	1305	1388	2.870	
0	1041	1078	-0.492	
1	766	685	0.442	
2	550	489	0.016	
3	1188	1160	2.926	
4	599	500	-2.872	
5	1416	1436	-0.676	
6	492	398	1.497	
7	487	517	2.666	
8	341	424	2.789	
9	114	226	-2.106	†
10	416	552	0.963	
*	14	4		
-14	580	645	0.176	
-13	66	93	1.269	†
-12	308	291	3.140	
-11	554	578	2.862	
-10	93	141	1.694	†
-9	906	830	0.234	
-8	366	342	-1.479	
-7	400	394	-2.819	
-6	680	620	2.607	
-5	738	721	2.727	
-4	1644	1959	-0.289	
-3	372	379	2.427	
-2	738	745	-2.562	
-1	400	318	-0.035	
0	630	611	2.149	
1	696	763	-0.717	
2	400	387	0.979	
3	844	791	-2.968	
4	93	120	-0.246	†
5	436	435	0.915	
6	301	357	-0.952	
7	174	144	2.332	
8	558	717	2.767	
*	14	6		
9	93	181	1.905	†
*	15	1		
-16	446	415	-2.925	
-15	410	354	0.101	
-14	329	286	0.321	
-13	354	347	2.868	
-12	469	487	2.512	
-11	451	371	0.347	
-10	1208	1247	-1.576	
-9	1569	1613	0.655	
-8	922	882	2.768	
-7	968	1211	-2.627	
-6	1499	1653	0.280	
-5	758	821	-1.446	
-4	1286	1284	0.509	
-3	1054	872	2.994	
-2	1692	1633	2.927	
-1	847	812	-0.661	
0	1710	1619	0.574	
1	372	380	-2.611	
2	509	443	2.666	
3	1197	1139	3.018	
4	887	892	-0.919	
5	786	776	0.450	
6	558	575	1.648	
7	623	653	-2.231	
8	237	223	-2.755	
9	702	787	0.455	
10	315	462	-1.576	
*	15	3		
-15	416	356	0.564	
-14	416	472	0.177	
-13	660	662	-3.068	
-12	301	352	2.849	
-11	147	188	2.401	†
-10	823	821	-0.212	
-9	147	139	0.973	†
-8	660	602	-2.754	
-7	469	468	3.097	
-6	660	639	1.981	
-5	988	1110	-0.495	
-4	522	437	-2.356	
-3	522	532	1.675	
-2	657	649	-2.756	
-1	522	480	-0.540	
0	894	849	1.319	
1	813	755	-1.356	
2	872	865	2.628	
3	147	95	2.664	†
4	708	745	-0.408	
5	301	170	0.376	
6	441	431	-3.034	
7	354	407	2.667	
8	93	82	1.468	†
*	15	5		
-12	66	393	2.104	†
-11	301	277	0.960	
-10	496	529	-1.162	
-9	255	308	1.483	
-8	228	207	-1.745	
-7	483	420	-2.757	
-6	400	386	1.503	
-5	849	827	-0.342	
-4	237	191	-2.768	
-3	580	493	2.816	
-2	410	308	-1.503	
-1	620	577	1.689	
0	952	812	-0.991	
1	354	291	1.210	
2	644	662	3.016	
3	378	376	-0.979	
4	341	387	1.867	
5	542	471	-0.603	
*	16	0		
-16	436	410	0.000	
-15	93	150	0.000	†
-14	550	481	0.000	
-13	1226	1212	3.142	
-12	131	193	0.000	†
-11	943	998	0.000	
-10	360	214	3.142	
-9	294	125	3.142	
-8	906	860	3.142	
-7	874	810	3.142	
-6	2503	2709	0.000	
-5	131	20	3.142	†
-4	2157	2123	3.142	
-3	131	38	0.000	†
-2	573	359	0.000	
-1	577	493	0.000	
0	1012	1070	0.000	
1	1885	1685	3.142	
2	513	472	3.142	
3	934	773	0.000	
4	131	213	3.142	†
5	279	329	0.000	
6	623	573	3.142	
7	383	417	3.142	
8	859	940	0.000	
9	174	206	0.000	
*	16	2		
-15	714	766	-0.118	
-14	431	480	2.616	
-13	378	414	-2.901	
-12	451	469	0.391	
-11	93	213	-1.185	†
-10	378	393	0.040	
-9	513	563	-3.132	
-8	492	648	2.779	
-7	775	809	-0.496	
-6	436	396	1.163	
-5	436	417	-2.938	
-4	573	539	-1.454	
-3	839	893	-3.110	
-2	308	280	-0.114	
-1	831	885	-0.023	
0	538	524	1.673	
1	620	564	-2.497	
2	580	503	3.102	
3	664	644	-0.292	
4	534	534	-0.064	
5	400	435	2.063	
6	294	318	-2.441	
7	66	96	2.685	†
8	66	125	-1.029	†
*	16	4		
-13	66	103	-0.397	†
-12	496	446	1.750	
-11	569	576	-0.702	
-10	246	206	1.092	
-9	263	195	-2.947	
-8	378	365	-2.599	
-7	421	394	1.039	
-6	534	528	-0.185	
-5	455	352	-2.144	
-4	1027	992	2.262	
-3	366	293	-0.964	
-2	322	191	-0.543	
-1	599	514	-0.011	
0	436	363	-2.637	
1	580	501	2.527	
2	263	218	-0.989	
3	246	259	0.822	
4	441	462	-0.287	
5	237	268	-2.057	
6	378	429	2.455	
*	16	6		
0	366	292	1.352	
*	17	1		
-16	465	488	-0.166	
-15	478	473	-1.897	
-14	778	832	2.362	
-13	505	495	-2.325	
-12	986	998	0.322	
-11	114	119	1.606	†
-10	1243	1151	-2.236	
-9	929	887	2.112	
-8	114	109	-0.915	†
-7	904	921	-0.687	
-6	1486	1592	1.265	
-5	1901	1872	-2.300	
-4	1140	1141	-2.354	
-3	2767	2809	0.807	
-2	2316	2412	-1.762	
-1	1170	1174	1.196	
0	738	719	2.372	
1	1242	1257	-2.260	
2	1206	1123	0.479	
3	573	529	-0.509	
4	874	848	-2.798	
5	657	710	1.620	
6	237	306	-2.525	
7	271	332	-0.132	
8	509	610	0.634	
*	17	3		
-14	400	429	-3.053	
-13	161	233	-0.309	
-12	455	454	1.231	
-11	573	511	-0.589	
-10	478	387	-2.635	
-9	441	483	2.383	
-8	389	366	-0.621	
-7	400	429	0.346	
-6	147	236	2.129	†
-5	465	364	-2.723	
-4	465	347	-0.435	
-3	657	639	3.026	
-2	1037	1120	-0.207	
-1	613	576	2.331	
0	496	539	-2.980	
1	147	183	-1.200	†
2	308	216	0.023	

3	465	446	0.861	
4	436	457	-1.471	
5	400	409	2.588	
6	161	199	2.005	
7	66	348	-0.931	†
•	17	5		
-10	66	412	2.957	†
-9	218	191	2.411	
-8	93	110	1.038	†
-7	588	543	-1.415	
-6	569	480	0.599	
-5	667	588	3.098	
-4	335	227	2.084	
-3	580	511	-0.008	
-2	421	366	-1.636	
-1	93	73	1.250	†
0	577	484	2.068	
1	550	486	-2.127	
2	460	463	0.494	
3	66	164	-1.511	†
•	18	0		
-15	93	116	3.142	†
-14	93	71	3.142	†
-13	702	602	0.000	
-12	664	555	0.000	
-11	699	531	3.142	
-10	1174	1170	3.142	
-9	1144	1166	0.000	
-8	416	284	0.000	
-7	513	466	0.000	
-6	360	272	3.142	
-5	2184	2279	3.142	
-4	2184	2182	0.000	
-3	887	865	0.000	
-2	1895	1895	3.142	
-1	1604	1478	0.000	
0	964	944	3.142	
1	131	171	0.000	†
2	1522	1485	0.000	
3	1484	1410	3.142	
4	114	17	0.000	†
5	341	383	0.000	
6	93	64	3.142	†
7	436	473	0.000	
8	66	389	3.142	†
•	18	2		
-15	802	815	2.573	
-14	66	114	-2.450	†
-13	522	592	0.253	
-12	542	536	-0.100	
-11	711	729	-2.496	
-10	744	712	2.630	
-9	741	681	0.581	
-8	522	659	-1.038	
-7	702	766	1.254	
-6	595	614	-2.652	
-5	271	268	-2.468	
-4	872	994	1.269	
-3	818	874	-1.045	
-2	308	393	-2.183	
-1	1082	1211	2.288	
0	322	353	-1.631	
1	591	580	-0.015	
2	246	245	0.293	
3	599	625	-2.262	
4	446	484	2.602	
5	286	341	1.267	
6	301	389	-0.241	
7	66	60	2.338	†
•	18	4		
-12	492	457	-0.053	
-11	400	299	-3.001	
-10	246	241	-2.987	
-9	372	493	2.334	
-8	723	754	-0.756	
-7	322	266	0.355	
-6	569	495	2.766	
-5	301	244	-2.479	
-4	335	297	-0.945	
-3	474	452	0.512	
-2	208	125	1.493	
-1	764	648	3.039	
0	237	200	-1.599	
1	329	293	-0.647	
2	348	334	1.101	

3	66	114	0.371	†
4	394	357	3.030	
•	19	1		
-15	714	673	1.220	
-14	761	687	-1.236	
-13	657	660	0.226	
-12	538	454	-2.902	
-11	966	905	3.075	
-10	492	445	0.554	
-9	723	785	-0.356	
-8	114	144	0.434	†
-7	501	468	-2.856	
-6	966	937	2.881	
-5	478	411	0.583	
-4	877	942	-0.056	
-3	1027	1083	-2.566	
-2	602	618	1.293	
-1	526	484	-2.926	
0	696	730	0.284	
1	677	616	-1.149	
2	554	563	2.816	
3	208	229	3.123	
4	301	238	-0.865	
5	360	397	0.245	
6	66	131	-0.175	†
•	19	3		
-13	348	351	-0.457	
-12	441	424	-3.048	
-11	550	532	2.760	
-10	114	151	-0.670	†
-9	595	691	0.067	
-8	286	350	-1.269	
-7	791	733	2.972	
-6	711	681	2.035	
-5	894	932	-1.106	
-4	720	716	0.267	
-3	483	453	0.555	
-2	1630	1617	-3.040	
-1	469	482	0.293	
0	469	329	-0.247	
1	436	424	-0.558	
2	366	403	1.541	
3	711	822	-2.719	
4	93	173	0.497	†
5	66	378	0.155	†
•	19	5		
-8	255	146	0.008	
-7	341	487	2.328	
-6	329	607	-2.168	
-5	66	488	0.843	†
-4	66	205	-0.103	†
-3	66	136	-2.041	†
-2	66	216	2.977	†
-1	66	94	1.603	†
0	301	233	-1.346	
•	20	0		
-14	441	321	0.000	
-13	93	38	0.000	†
-12	680	617	3.142	
-11	522	400	3.142	
-10	1066	913	0.000	
-9	271	203	0.000	
-8	335	292	3.142	
-7	769	746	3.142	
-6	400	348	0.000	
-5	755	710	0.000	
-4	451	452	3.142	
-3	197	152	3.142	
-2	772	712	3.142	
-1	920	811	0.000	
0	580	593	0.000	
1	1121	1105	3.142	
2	301	318	0.000	
3	487	419	3.142	
4	294	206	0.000	
5	1196	1084	0.000	
6	546	889	3.142	
•	20	2		
-13	670	649	3.111	
-12	627	613	3.081	
-11	354	330	-0.401	
-10	492	602	-0.046	
-9	378	410	-0.331	
-8	899	991	2.964	
-7	647	626	2.993	

-6	869	899	-0.307	
-5	455	407	-0.538	
-4	558	580	0.291	
-3	1041	1198	2.910	
-2	530	486	-3.068	
-1	1197	1219	-0.051	
0	93	92	-0.845	†
1	218	207	3.000	
2	478	534	2.752	
3	186	231	-1.654	
4	630	722	-0.288	
•	20	4		
-10	474	460	-0.640	
-9	174	162	-1.073	
-8	478	442	2.469	
-7	627	557	-3.008	
-6	441	392	0.885	
-5	1078	1074	-0.477	
-4	431	436	2.747	
-3	509	530	3.097	
-2	294	242	-0.940	
-1	255	248	1.937	
0	492	516	-0.517	
1	66	124	1.486	†
2	383	534	2.953	
•	21	1		
-13	1070	943	-2.670	
-12	228	218	1.907	
-11	829	788	-0.421	
-10	542	388	0.935	
-9	378	462	2.369	
-8	934	884	-2.506	
-7	400	371	0.354	
-6	908	899	-0.062	
-5	93	156	0.531	†
-4	975	1021	-3.016	
-3	460	478	2.339	
-2	602	612	-0.484	
-1	1076	1091	0.383	
0	813	857	-2.809	
1	329	340	2.643	
2	348	286	-2.928	
3	366	465	-0.413	
4	705	761	0.606	
•	21	3		
-11	389	408	-0.165	
-10	329	318	-0.769	
-9	538	577	2.822	
-8	483	491	2.848	
-7	436	388	0.182	
-6	606	697	-0.710	
-5	315	245	2.547	
-4	416	431	2.402	
-3	627	603	-2.887	
-2	686	747	-0.113	
-1	237	271	0.746	
0	279	304	-1.911	
1	641	702	2.547	
2	66	290	-0.781	†
•	22	0		
-13	378	248	3.142	
-12	990	979	0.000	
-11	93	62	3.142	†
-10	218	238	3.142	
-9	460	429	3.142	
-8	651	560	3.142	
-7	1632	1638	0.000	
-6	114	59	3.142	†
-5	1480	1468	3.142	
-4	501	403	0.000	
-3	301	272	0.000	
-2	831	790	0.000	
-1	400	241	3.142	
0	752	898	3.142	
1	93	38	3.142	†
2	341	429	0.000	
3	606	531	0.000	
•	22	2		
-11	647	686	0.192	
-10	599	657	-2.883	
-9	446	520	2.870	
-8	441	509	0.107	
-7	66	192	-1.887	†
-6	383	349	0.720	
-5	501	471	-2.036	

-4	1024	1122	3.120
-3	872	897	0.343
-2	651	723	-0.242
-1	329	422	3.078
0	416	457	2.904
1	394	390	-2.165
2	558	607	0.031
*	22	4	
-5	686	712	1.923
-4	565	501	-1.801
*	23	1	
-11	186	176	-2.190
-10	831	820	-3.102
-9	421	397	0.654
-8	723	741	-0.266
-7	405	403	2.254
-6	394	420	-2.070
-5	797	789	-2.756
-4	1007	1103	0.964
-3	1058	1188	-0.897
-2	469	530	2.202
-1	595	676	2.911
0	410	419	-1.575
1	197	249	0.270
*	23	3	
-8	308	254	-0.495
-7	496	526	-1.024
-6	492	553	1.698
-5	758	578	-2.559
-4	308	202	2.788
-3	869	899	0.192
-2	335	329	-2.470
*	24	0	
-10	66	147	0.000 †
-9	1339	1201	0.000
-8	908	725	3.142
-7	93	163	3.142 †
-6	400	352	3.142
-5	93	59	0.000 †
-4	1166	996	0.000
-3	400	405	3.142
-2	496	431	3.142
0	208	256	3.142
*	24	2	
-8	522	495	-0.995
-7	228	309	2.804
-6	394	376	2.354
-5	186	150	2.937
-4	602	652	-0.479
-3	308	318	0.691

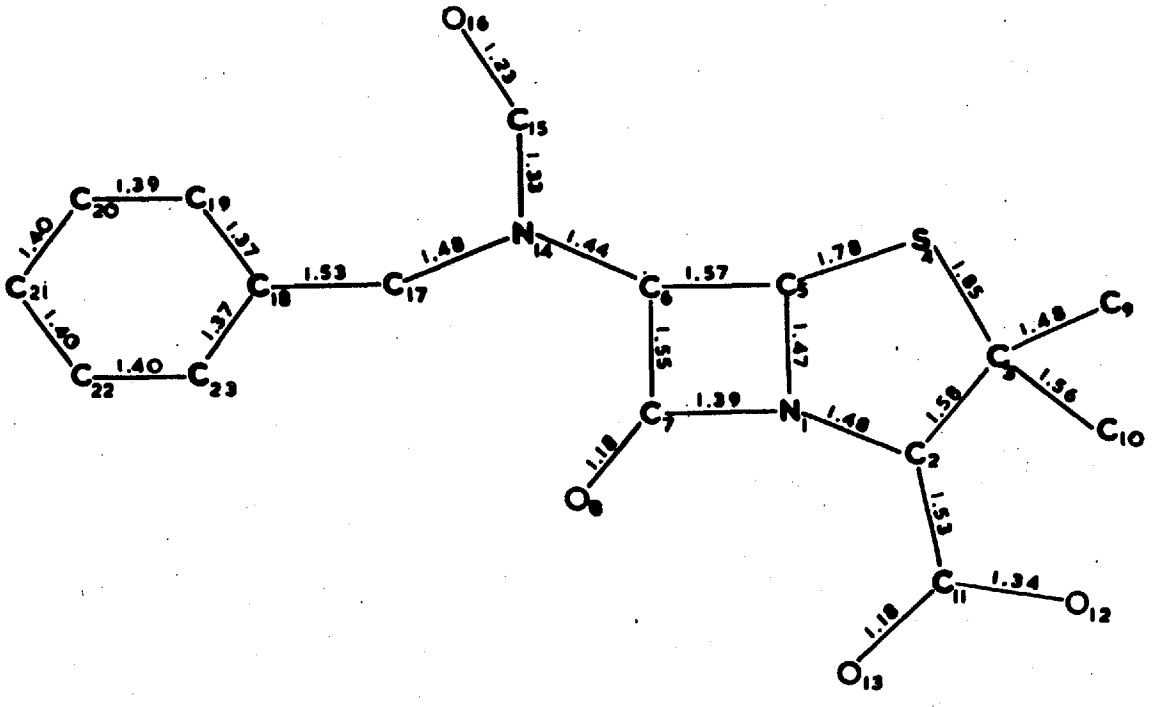
Table 5. 6-NBF-PA intra-molecular bond distances and their estimated standard deviations in Angstrom units.

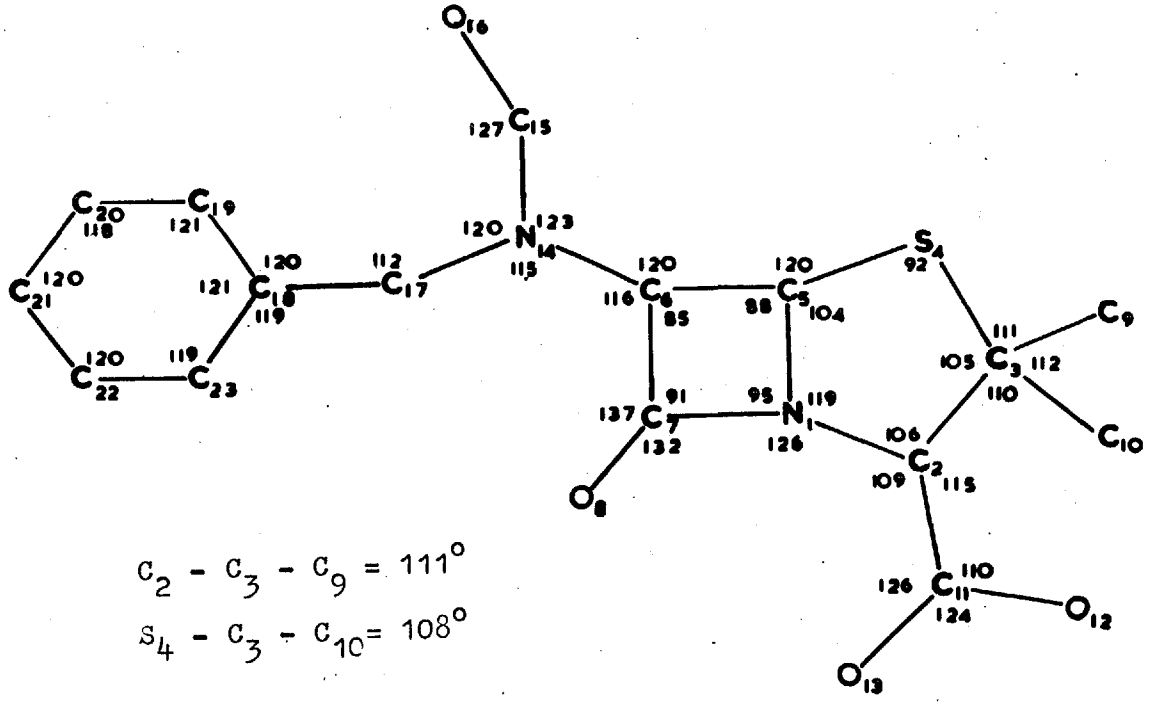
Bond		Bond	
N1 - C2	1.476 (0.009)	N14 - C17	1.485 (0.008)
N1 - C5	1.471 (0.008)	C15 - O16	1.233 (0.011)
N1 - C7	1.387 (0.008)	C17 - C18	1.529 (0.012)
C2 - C3	1.576 (0.011)	C18 - C19	1.371 (0.018)
C2 - C11	1.529 (0.009)	C18 - C23	1.371 (0.017)
C3 - S4	1.850 (0.005)	C19 - C20	1.394 (0.015)
C3 - C9	1.476 (0.017)	C20 - C21	1.398 (0.026)
C3 - C10	1.563 (0.014)	C21 - C22	1.403 (0.026)
S4 - C5	1.778 (0.010)	C22 - C23	1.396 (0.018)
C5 - C6	1.566 (0.009)		
C6 - C7	1.551 (0.008)		
C6 - N14	1.443 (0.009)		
C7 - O8	1.184 (0.009)		
C11 - O12	1.342 (0.009)		
C11 - O13	1.180 (0.013)		
N14 - C15	1.325 (0.013)		

Table 6. 6-NBF-PA intra-molecular bond angles and their standard deviations in degrees.

C2 - N1 - C5	118.6 (0.6)	C6 - N14 - C15	123.2 (1.1)
C2 - N1 - C7	125.6 (0.7)	C6 - N14 - C17	115.3 (0.5)
C5 - N1 - C7	94.9 (0.5)	C15 - N14 - C17	120.4 (0.9)
N1 - C2 - C3	105.8 (0.7)	N14 - C15 - O16	126.9 (1.5)
N1 - C2 - C11	108.9 (0.5)	C2 - C11 - O12	109.5 (0.5)
C3 - C2 - C11	114.9 (0.8)	C2 - C11 - O13	126.1 (1.3)
C2 - C3 - S4	104.7 (0.5)	O12 - C11 - O13	124.4 (1.4)
C2 - C3 - C9	111.1 (1.1)	N14 - C17 - C18	112.1 (0.8)
C2 - C3 - C10	110.5 (0.8)	C17 - C18 - C19	119.6 (1.3)
S4 - C3 - C9	111.2 (0.7)	C17 - C18 - C23	119.1 (1.3)
S4 - C3 - C10	107.5 (0.6)	C19 - C18 - C23	121.4 (1.7)
C9 - C3 - C10	111.5 (1.4)	C18 - C19 - C20	120.9 (1.7)
C3 - S4 - C5	92.4 (0.4)	C19 - C20 - C21	118.2 (1.7)
N1 - C5 - S4	104.2 (0.6)	C20 - C21 - C22	120.4 (2.6)
N1 - C5 - C6	87.6 (0.4)	C21 - C22 - C23	119.6 (1.9)
S4 - C5 - C6	119.5 (0.8)	C18 - C23 - C22	119.2 (1.7)
C5 - C6 - C7	85.1 (0.5)	N1 - C7 - C6	91.3 (0.4)
C5 - C6 - N14	120.1 (0.7)	N1 - C7 - O8	132.0 (1.1)
C7 - C6 - N14	116.1 (0.6)	C6 - C7 - O8	136.7 (1.1)

Figure 3. 6-NBF-PA bond distances and angles.





$C_2 - C_3 - C_9 = 111^\circ$

$S_4 - C_3 - C_{10} = 108^\circ$

temperature factor coefficients are shown in Table 3. The observed and calculated structure factors are listed in Table 4, the final R value being 0.100. Bond distances and angles and their estimated standard deviations were calculated by ELSI and are shown in Tables 5 and 6, and in Figure 3.

Configuration of the Molecule

Figure 4 is a projection of the 6-(N-benzylformamido)-penicillanic acid molecule down the b axis, as determined by the analysis. The projection down the c axis is shown in Figure 5. With the exception of the N-benzylformamido side-chain, the structure of the molecule is similar to those found for the other penicillins mentioned in Chapter 3.

The benzyl group in the side-chain is curled round towards the fused ring system as in benzyl penicillin, but its position in 6-NBF-PA is slightly different. It can be expressed as an imaginary rotation , of approximately 90°, of the benzyl group about the bond N14 - C17 in the direction of the sulphur atom. The least-squares planes through the groups of atoms in the molecule have been calculated with DIDO and are shown in Table 7. The amide group in the side-chain is planar, although the deviations are not as low as would be expected (Table 7a), and orientated approximately normal to the plane of the β-lactam ring. The dimensions of the group are consistent with those

Figure 4. 6-NBF-PA projected down the b axis

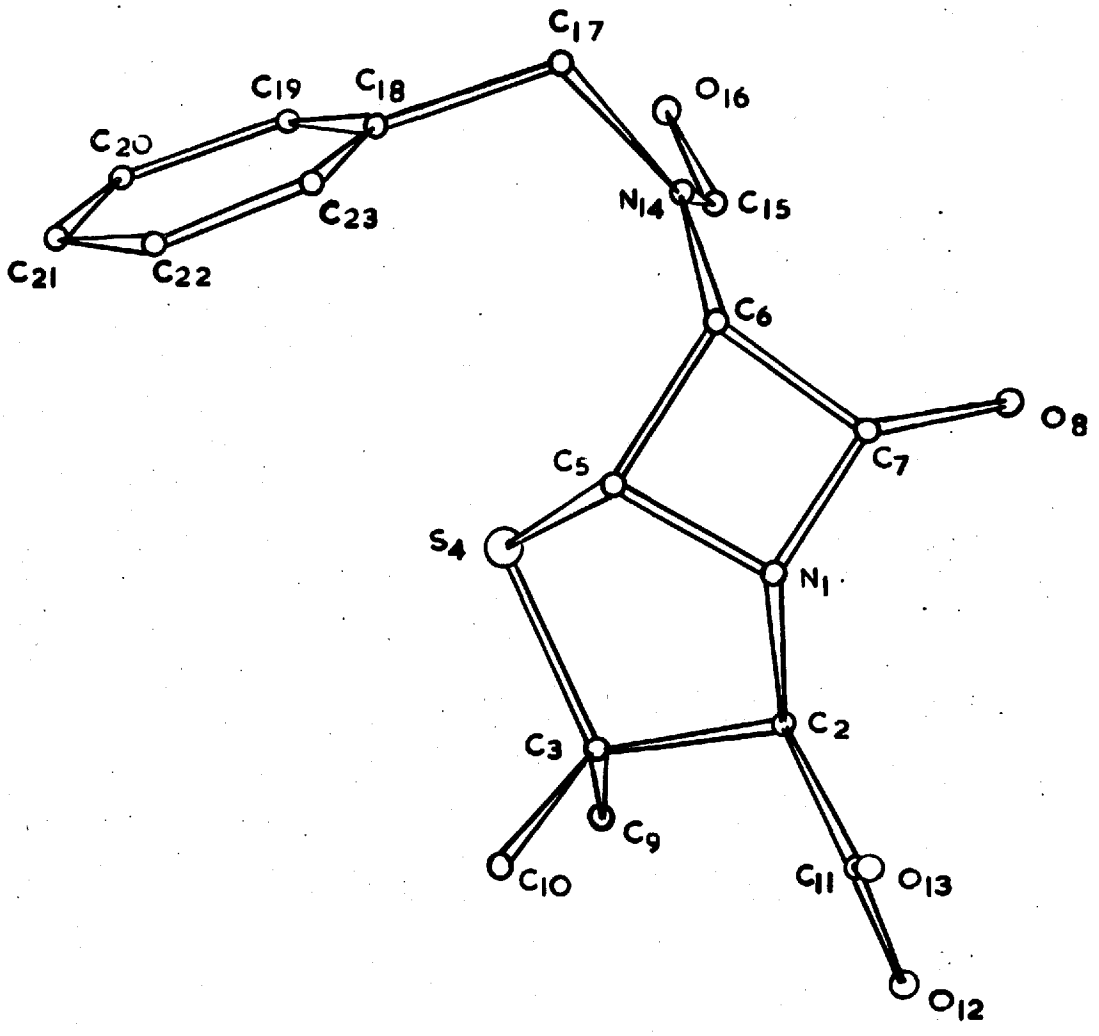


Figure 5. 6-NBF-PA projected down the c axis.

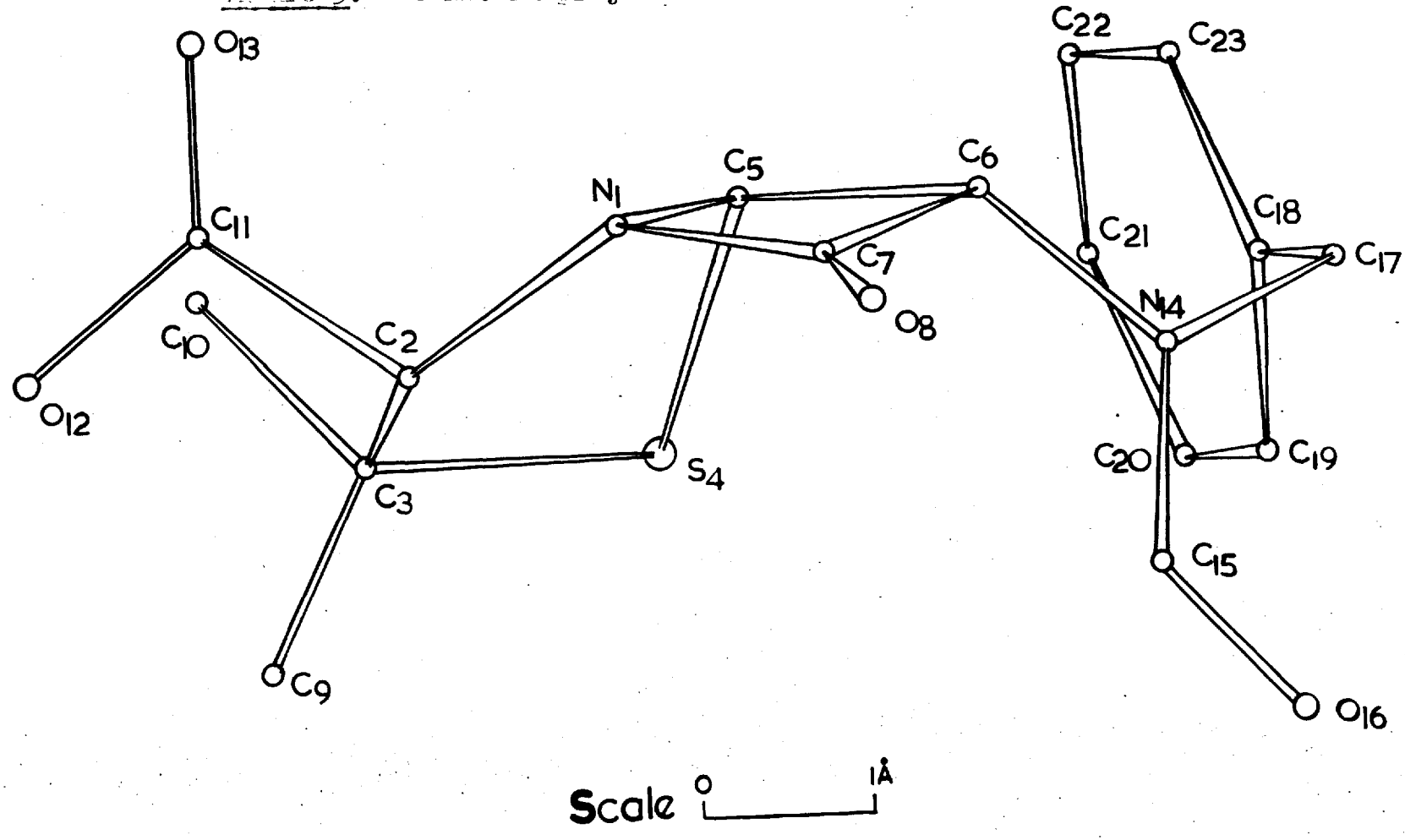


Table 7. Deviations (Å) of the atoms in 6-NBF-PA from the calculated least-squares planes. Atoms marked with $ were not included in the calculation of the plane. $\sum d^2$ is the sum of the squares of the deviations from the plane (in Å^2)

a. Amide

$\sum d^2 = 0.00943$

Atom	Deviation
C6	-0.043
C15	0.017
O16	-0.033
N14	0.075
C17	-0.015

b. Benzene Ring

$\sum d^2 = 0.00297$

Atom	Deviation
C17	-0.020
C18	0.007
C19	0.002
C20	0.005
C21	0.000
C22	-0.032
C23	0.040

c. Thiazolidine Ring

$\sum d^2 = 0.00014$

Atom	Deviation
C3	-0.004
C2	0.007
N1	-0.008
C5	0.004
S4 $	0.729

d. β-lactam

$\sum d^2 = 0.01858$

Atom	Deviation
N1	0.072
C5	-0.089
C6	0.057
C7	0.008
O8	-0.047

e. β-lactam

$\sum d^2 = 0.00002$

Atom	Deviation
N1	-0.001
C6	-0.001
C7	0.004
O8	-0.002
C5 $	0.209

of a normal resonating amide. O16 is cis to the benzyl group and C15 is trans to the proton at C6, which is opposite to the analogous position in the other penicillins.

In terms of Collins and Richmond's explanation of the biological activity of the penicillins, O16 is on the 'wrong' side of the molecule with respect to the other polar groups (N1, O13, O8) to form a possible hydrogen-bonding system with a protein substrate similar to that postulated for N-acetyl-muramic acid and the penicillins. As pointed out by Hunt and Rogers (1964), O16 can only be brought into the required position by rotation about C6 - N14 followed by cis-trans isomerization of the amide group. This combination seems improbable even in physiological solutions; the rotation would be hindered by the size of the benzyl group and the isomerization would require energy equal to the resonance energy of the amide. (Wheland (1955) estimates this value as 17 - 60 K cal./mole but with a large degree of uncertainty). Hence, the low antibacterial activity of 6-NBF-PA is consistent with Collins and Richmond's theory, and, it should be noted, with Tipper and Strominger's view that penicillin binds to the active centre in preference to a substituted D-alanyl-D-alanine.

Packing

The unitcell contents and the adjacent molecules in the 6-NBF-PA crystal are shown projected down the b axis in

Figure 6 and down the c axis in Figure 7. Some inter-molecular distances are shown in both diagrams and distances less than 3.25 Å are shown in Table 8. Atoms related to those in Table 2 by the symmetry of the spacegroup are referred to by terms in parentheses following the atom name.

The distances between the atoms O16 and O12 ($x + \frac{1}{2}$, $y - \frac{1}{2}$, z) of 2.665 Å strongly suggests a hydrogen bond between these two atoms. This is supported by the bond lengths in the carboxyl group, C11 - O12 (1.342 Å) and C11 - O13 (1.180 Å) which agree quite well with the values of 1.358 Å and 1.233 Å given by Sutton (1965) for the unionized form. The result is that the molecules related by centering form an approximately linear hydrogen-bonding system roughly along the ab diagonals. That this is a 'criss-cross' arrangement can be seen from Figure 7.

The general packing arrangement is similar to that described for potassium benzylpenicillin by Crowfoot et al. (1949) and for phenoxymethylpenicillin by Abrahamsson et al. (1963). The polar groups are on one side of the molecule and the hydrocarbon parts on the other, so that the molecules make contact with one another in alternate polar and non-polar layers, which in 6-NBF-PA are parallel to the a axis.

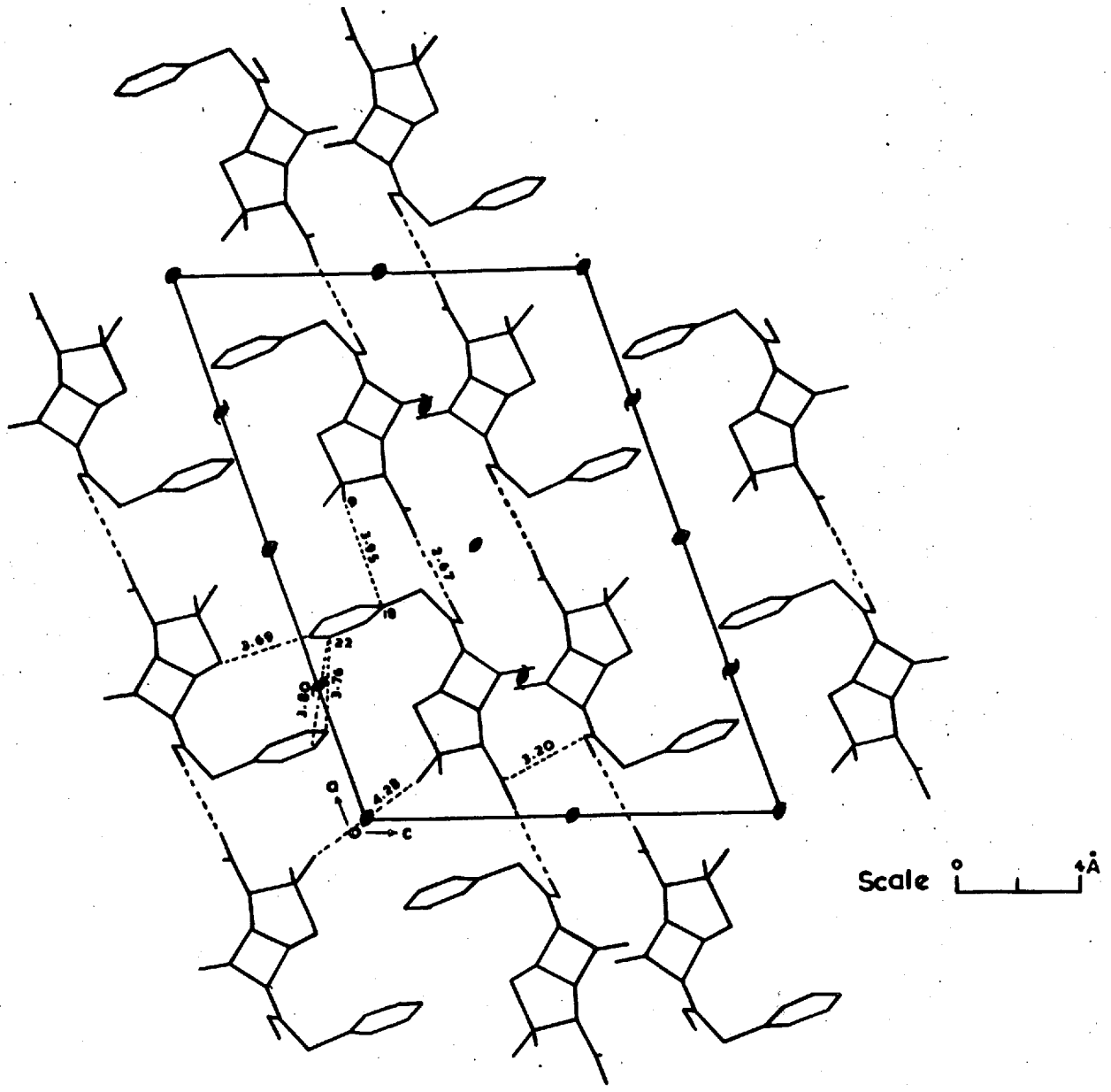


Figure 6. Crystal structure of 6-NBF-PA projected down the b axis

Figure 7. The crystal structure of 6-NBF-PA projected down the c axis.

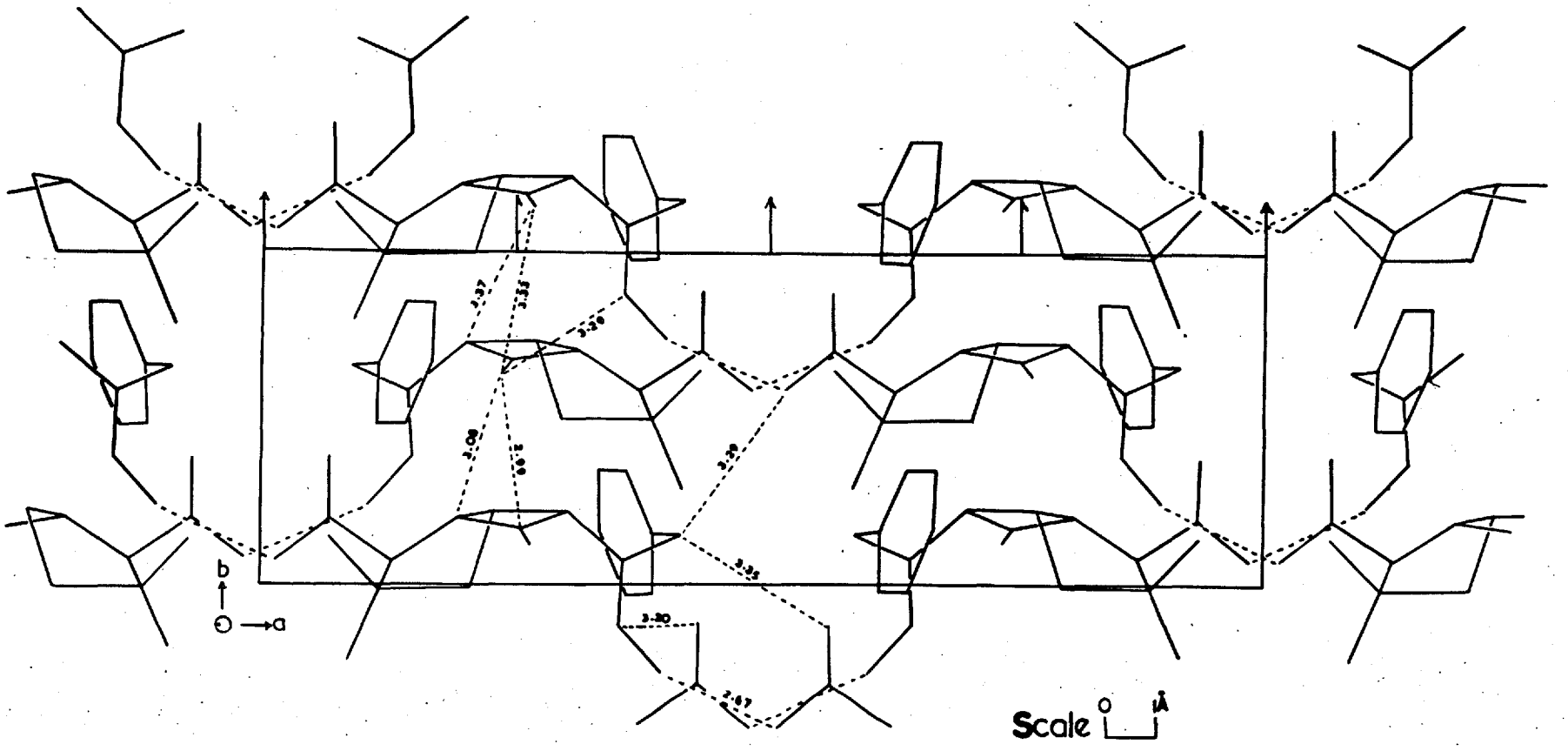


Table 8. Inter-molecular distances in 6-NBF-PA in Å.

The atomic positions are related to those in Table 2 by the terms in parentheses which are :-

(1) = $\frac{1}{2}$ - x, y + $\frac{1}{2}$, 1-z
(2) = x + $\frac{1}{2}$, y - $\frac{1}{2}$, z
(3) = x + $\frac{1}{2}$, y + $\frac{1}{2}$, z

N1 - O8 (1)	3.080	C11 - O16 (1)	3.398
C6 - O8 (1)	3.374	O13 - C15 (1)	3.200
C7 - O8 (1)	2.992	O13 - O16 (1)	3.417
O8 - O8 (1)	3.331	O16 - O12 (2)	2.665
O8 - C15(1)	3.286	C17 - O12 (3)	3.293
C11- C15(1)	3.479	C17 - O13 (2)	3.351

The Thiazolidine and β-Lactam Rings

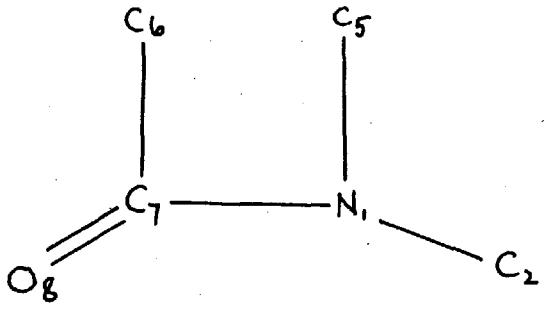
Diamand (1963) has tabulated the bond lengths and angles of the penicillin nucleus from the determination of the crystal structures of 6-APA, potassium benzylpenicillin refined by Rollett and Vaciago, phenoxymethylpenicillin and p-bromo-phenoxymethylpenicillin by Watson. Most of the values are in good agreement with those listed in Tables 5 and 6 for 6-NBF-PA. The shortening of the bond C3 - C9 (1.476 (0.017 Å)) is probably not significant in terms of the real standard deviations, and there is no apparent reason for the distortion of the angle C3 - C2 - C11 (114.9°) from the tetrahedral value of 109° 28'.

The thiazolidine ring has four atoms, N1, C2, C3 and C5 in a plane (Table 7c) with S4 0.729 Å below this plane viewing the molecule down the b axis as in Figure 4. This conformation is different to that of the thiazolidine rings in the other penicillins. As mentioned in Chapter 3, in benzylpenicillin and phenoxymethylpenicillin C2 is the out-of-plane atom, in the latter case by 0.51 Å on the opposite side of the plane to C7. In 6-APA, N1 deviates from the best plane through the other four atoms by 0.42 Å, on the same side of the plane as N14.. The smaller value for the angle C3 - S4 - C5 (92.4°) compared with 96° - 97° in the other penicillins probably results from this conformational difference.

Although the bond S4 - C5 (1.778 Å) is only 4 e.s.d.'s lower than the average value of 1.817 Å, given by Sutton (1965) for a single C - S bond, S4 - C3 (1.850 Å) is 7 e.s.d.'s higher. This agrees with the values for the other penicillins except potassium benzylpenicillin for which Rollett and Vaciago give S4 - C5 (1.85 Å) and S4 - C3 (1.82 Å). The lengthening of the bond S4 - C3 has been discussed by Diamand (1963). By considering the mean value for this bond length and its true standard deviation from previous determinations, he tentatively suggests S4 - C3 has a bond length in the range 1.84 Å to 1.87 Å. The value in 6-NBF-PA lies in this range.

The non-planarity of the β-lactam ring can be seen from Table 7d, the deviation of C5 from the calculated plane being as high as 0.089 Å. Table 7e shows that by excluding C5 from the least-squares calculation, the 'cyclic amide' atoms N1, C6, C7 and O8 lie in a plane well within the limits of experimental accuracy, with C5 0.204 Å out of the plane on the same side as S4. This is similar to the arrangement in 6-APA, phenoxymethylpenicillin, ceph. Cc and the 3-spiro-thiazolinium derivative (Chapter 5) where the deviations of the out-of-plane atom vary from approximately 0.14 Å to 0.25 Å. In each case it is the atom bonded to the sulphur atom that lies out of the plane.

As Diamand (1963) has mentioned, the tendency of the group



to be planar, so that resonance can occur in the cyclic amide, is probably opposed to some extent by the tendency of the five- (or six) membered ring to adopt its most stable conformation. This will result in C5 being pulled out of the plane of the β-lactam ring. That resonance occurs to some extent in 6-NBF-PA is indicated by the length of the bond N1 - C7 (1.387 Å), which is nearer to the value of the C - N bond length in amides (1.333 Å) than the accepted value of 1.472 Å for a C - N single bond. The angle C2 - N1 - C5 (118.6°) suggests that N1 is tending to planarity.

CHAPTER 5

The Crystal Structure of (3S,4S,6R,7R)-2'-Amino-3'-ethyl-2'-thiazolinium-4'-spiro-3-(7-phenylacetamidocepham-4-carboxylate) trihydrate

Preliminary Data

The crystals, which were supplied by Dr.A.G.Long of Glaxo Ltd., and prepared as indicated on page 44, were colourless monoclinic needles. The crystallographically unique axis, b, was parallel to the needle axis and the crystal selected for data collection had the approximate dimensions 0.6 x 0.2 x 0.13 mm. The structural formula and numbering scheme are shown in the diagram inside the back cover.

Formula $C_{19}H_{28}N_4O_7S_2$ Molecular Weight (formula) = 488

Mass absorption coefficient for CuKα, μ = 25.5 cm^{-1}

Unit-cell dimensions

a = 8.063 (0.008) Å, b = 7.184 (0.008) Å, c = 19.72 (0.02) Å

β = 100.3° (0.2) V = 1123 $Å^3$, Z = 2, F(000) = 516 electrons

D_{obs}(by flotation) = 1.45 $g.cm^{-3}$ D_{calc} = 1.44 $g.cm^{-3}$.

Absent Spectra: only among 0k0 for k = 2n + 1

As the compound is dextrorotatory (page 43), the space-group is uniquely determined as $P2_1$. 2269 independent reflexions were estimated visually using three intensity wedges to accommodate spot-shape changes from layer to layer. The data were correlated from six layers collected about the b axis and three about the a axis, using LOLA for the determination of the inter-layer scale factors. It was considered that the value of μ and the crystal dimensions rendered absorption corrections unnecessary (for data collected about the a axis, a crystal was sliced normal to the needle axis to give a rough cube of side 0.3 mm).

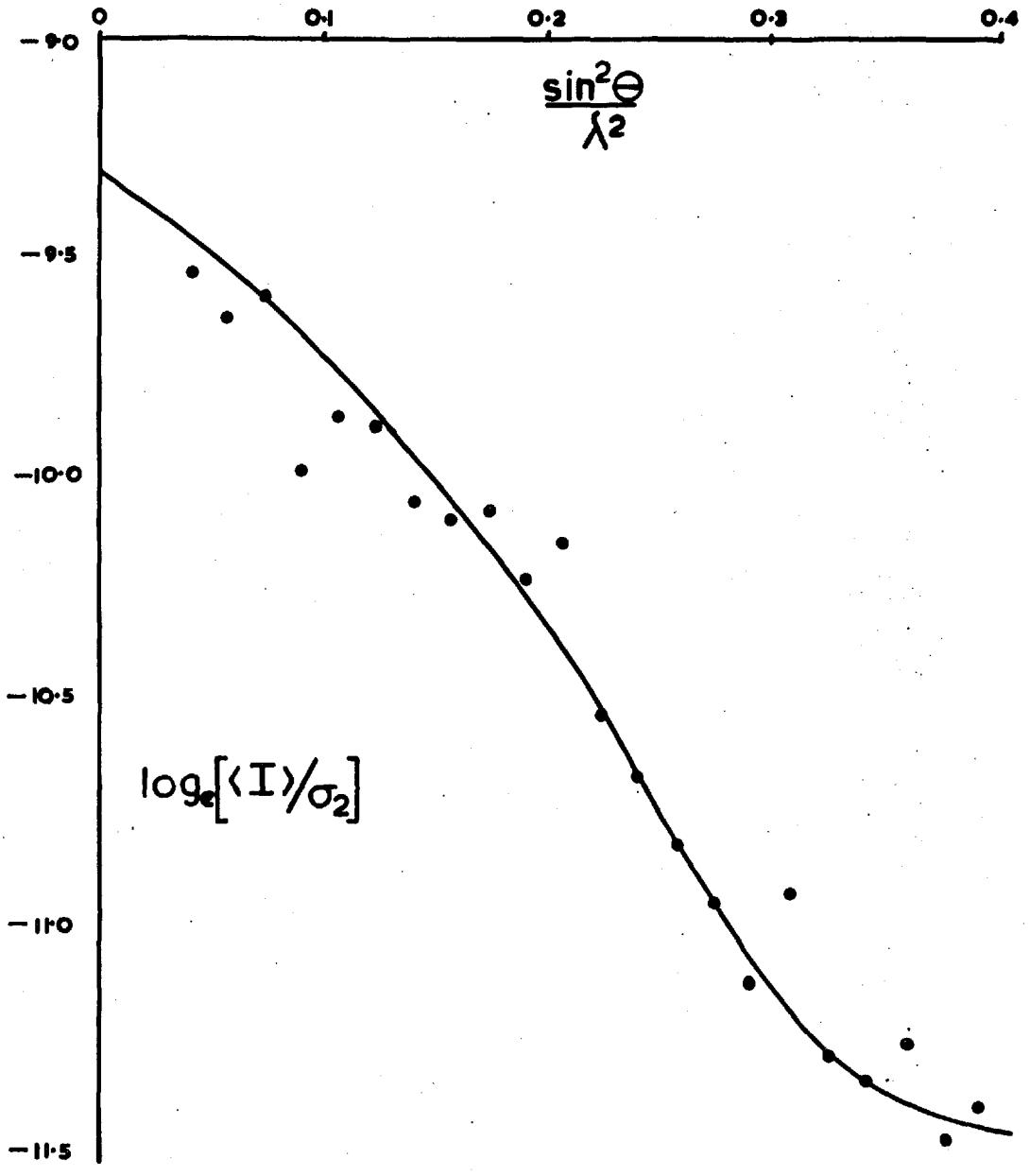
Figure 1 shows the Wilson plot calculated for three-dimensional data including the unobserved reflexions at $|F_{unobs}| = \frac{2}{3}|F_{min}|$. The data were divided into 22 shells each containing 80 - 120 reflexions. The least-squares method gave B = 2.95, K = 100.4. From the graph, the best line through the points in the middle of the $\sin^2\theta/\lambda^2$ range gave B = 2.91, and the intercept gave K = 104.6 (The final scale after structure refinement was 101.2)

Structure Determination

1. Location of the Sulphur Atoms

The four sulphur atoms in the unit cell give rise to

Figure 1. 3-Spiro-thiazolinium derivative Wilson plot based on three-dimensional data.



six centrosymmetrically related pairs of S - S inter vectors. In each six, two correspond to peaks in the three-dimensional Patterson, occuring in the Harker section at $v = \frac{1}{2}$, and the remaining four correspond to two peaks of double weight at

$$x_1 - x_2,\ y_1 - y_2,\ z_1 - z_2$$

$$x_1 + x_2,\ y_1 - y_2 + \tfrac{1}{2},\ z_1 + z_2$$

where x_1, y_1, z_1 and x_2, y_2, z_2 are the positions of the two independent sulphur atoms. A three-dimensional Patterson was computed with coefficients sharpened by the function

$$1/\hat{f}^2 \cdot \exp(3\sin^2\theta/\lambda^2)$$

where $\hat{f}$ is the formfactor for sulphur. Four peaks were recognised which complied with the conditions described above and gave the following fractional coordinates for the sulphur atoms :

x	y	z
0.155	0	0.069
0.395	0.182	0.293

The distance between these two positions, 4.7 Å, seemed reasonable assuming the approximate geometry of ceph. Cc as determined by Diamand (1963)

2. Location of the Light Atoms

The ratio of the sum of the squares of the scattering powers of the sulphur atoms to that of the light atoms is 0.39

and Fourier methods were used to locate the positions of the light atoms. The first Fourier map, phased on the coordinates of the sulphur atoms revealed five additional peaks, one of which was subsequently found to have been misplaced. Three further Fouriers enabled the assignment of all thirty-two positions and the calculated and observed structure factors showed an overall agreement factor, R, of 0.29. During the course of the analysis, reflexions for which

$$|F_o| < 0.25\ k\ |F_c| \quad \text{where } k = \frac{\sum |F_o|}{\sum |F_c|}$$

were omitted from the Fourier calculations, and double shifts were applied to the estimated coordinates before input to the next stage, as recommended by Donohue (1950)

Refinement

Four cycles of isotropic, block-diagonal, least-squares refinement reduced R to 0.114 and a maximum coordinate shift of 0.03 Å. At this stage a bond-distance calculation indicated sensible bond lengths and a difference Fourier confirmed that the postulated structure was substantially correct. An agreement analysis was used to check the validity of the inter-layer scaling and that the choice of $F^+ = 1500$ in the weighting scheme produced fairly constant values of $\sum w\Delta^2$ over ranges

of $|F_o|$.

The progress of the structure refinement is shown in Table 1. It should be noted that the R value associated with a particular cycle was calculated from atomic coordinates before refinement. Agreement analyses and molecular geometry calculations were used to check the course of the refinement after cycles I8, A10 and A16.

Four further cycles of isotropic least squares (I5 to I8) produced very little convergence and so for input to A9, the individual isotropic temperature factors were converted to the equivalent anisotropic form and refinement continued on all 32 atoms, applying a fudge factor of 0.8 to all parameter shifts. At cycle A11, two reflexions (1 0 $\bar{2}$ and 1 0 $\bar{1}$) were omitted for suspected extinction and 214 unobserved reflexions included at $\frac{2}{3}F_{min}$, where F_{min} is the minimum observed value of F at the relevant $\sin^2\theta$. After cycle A16, a difference Fourier was calculated and suggested the positions of all hydrogen atoms except one bonded to C6, two bonded to C15, the three methyl-group hydrogens at C23 and the six of the three water molecules. The location of two peaks near N29 in approximately the correct orientation suggested the presence of the group $=NH_2^+$. However, the peak heights in the difference map varied from 0.13 to 0.3 $e\text{A}^{-3}$, and as the difference Fourier still exhibited ripple of the order 0.2 $e\text{A}^{-3}$ these positions were not taken as

Table 1. The refinement process for the 3-spiro-thiazolinium-7-PAC derivative. I = isotropic, A = anisotropic refinement.

Cycle Number	Agreement Factor, R	$\sum w\Delta^2 \times 10^{-4}$	Maximum coordinate shift (Å)
I1	0.289	35503	0.15
I2	0.175	14427	0.20
I3	0.133	7340	0.05
I4	0.114	5991	0.03
I5	0.113	5485	0.01
I6	0.112	5370	0.01
I7	0.112	5357	0.005
I8	0.112	5353	0.002
A9	0.112	5366	
A10	0.089	3487	0.014
A11	0.082	3034	0.008
A12	0.083	2634	0.014
A13	0.081	2441	0.007
A14	0.081	2420	0.004
A15	0.081	2411	0.002
A16	0.081	2408	0.002
A17	0.077	2073	0.009
A18	0.075	1963	0.003
A19	0.075	1955	0.0015
A20	0.075	1950	0.0008

conclusive proof of hydrogen atom positions.

Using a program written by D.F.High for the I.B.M. 7090, hydrogen positions were calculated assuming the staggered conformation for the methyl group at C23. The observed and calculated positions are shown in Table 2, excluding those of the water molecules. They show good agreement except for the atom bonded to C20. All the calculated hydrogen positions listed in Table 2 were included in subsequent structure-factor calculations with isotropic temperature factors 1.5 times those to which the hydrogen atoms are bonded. The hydrogens of the water molecules were not included as it was considered that their positions could not be well enough defined by stereochemical considerations.

Refinement was considered complete after cycle A20 when the position shifts were about one tenth of their standard deviations. Structure factors were calculated from the positions output from A20 to give a final R value of 0.075. The calculated and observed structure factors are listed in Table 3a.

A difference Fourier was calculated from the structure factors listed in Table 3a, in an attempt to locate the hydrogen atoms of the water molecules. The map exhibited a ripple up to about 0.17 eA^{-3} and the areas around the oxygen atom positions gave no indication of the hydrogen atoms.

Table 2. Observed and calculated hydrogen positions(in fractional coordinates) for the 3-spiro-thiazolinium-7-PAC derivative.

Atom.	Bonded to-	Calculated			Observed		
		x	y	z	x	y	z
H1	C2	0.475	0.632	0.201	0.491	0.625	0.200
H2	C2	0.453	0.872	0.178	0.438	0.883	0.167
H3	C4	0.052	0.674	0.235	0.054	0.675	0.233
H4	C6	0.271	0.608	0.363			
H5	C7	0.460	0.331	0.374	0.417	0.350	0.383
H6	C9	0.167	1.012	0.186	0.167	1.000	0.183
H7	C9	-0.017	0.882	0.152	0.000	0.875	0.150
H8	N13	0.674	0.586	0.311	0.646	0.600	0.308
H9	C15	0.979	0.488	0.317			
H10	C15	1.050	0.368	0.397			
H11	C17	1.090	0.540	0.485	1.083	0.500	0.500
H12	C18	1.329	0.387	0.567	1.375	0.450	0.550
H13	C19	1.444	0.090	0.539	1.438	0.000	0.542
H14	C20	1.319	-0.073	0.428	1.542	-0.150	0.400
H15	C21	1.089	0.080	0.346	1.125	0.133	0.367
H16	C22	0.199	0.407	0.033	0.167	0.350	0.067
H17	C22	0.248	0.364	0.124	0.250	0.350	0.117
H18	C23	0.495	0.284	0.067			
H19	C23	0.484	0.449	0.038			
H20	C23	0.544	0.537	0.123			
H21	N29	0.248	0.612	-0.041	0.229	0.600	-0.027
H22	N29	0.191	0.849	-0.054	0.188	0.850	-0.050

Table 3a. The 3-spiro-thiazolinium-7-PAC derivative observed and calculated structure factors (x 100) in the order

*	h	k			
l			$\lvert F_o \rvert$	$\lvert F_c \rvert$	α (radians)

Unobserved reflexions are marked † , and 0 0 1 and 0 0 2 were obscured by the backstop. The two reflexions omitted for suspected extinction were

h	k	l	$\lvert F_o \rvert$	$\lvert F_c \rvert$	α
1	0	$\bar{2}$	6003	8419	0.000
1	0	$\bar{1}$	5789	8266	0.000

•	0	0	
3	581	753	0.000
4	1998	2057	3.142
5	3643	3929	3.142
6	131	107	0.000 †
7	564.	5453	3.142
8	4696	5061	3.142
9	2269	2307	3.142
1	3419	3258	0.000
11	1214	1092	0.000
12	910	854	0.000
13	1251	1218	0.000
14	1420	1293	0.000
15	615	459	0.000
16	506	389	3.142
17	615	558	3.142
18	554	449	3.142
19	175	177	3.142 †
2.	175	99	3.142 †
21	1001	893	3.142
22	1222	1190	3.142
23	143	51	3.142 †
24	777	709	0.000
•	0	1	
1	3369	4338	-1.582
2	4261	4704	2.872
3	2615	2926	0.455
4	5303	5082	-2.876
5	3762	3672	1.710
6	7,28	7210	0.218
7	2505	2568	2.761
8	3403	2982	1.862
9	3642	3778	-0.745
1	2170	1999	-1.102
11	1214	1247	2.918
12	2626	2515	-0.957
13	3401	3414	-1.162
14	158.	1580	2.281
15	1231	1045	2.095
16	1717	1660	-0.094
17	1.22	886	1.212
18	1705	1581	1.241
19	764	826	-0.031
2.	905	941	-0.810
21	1167	1178	2.429
22	143	156	2.540 †
23	671	643	0.097
24	101	181	-1.016 †
•	0	2	
0	5124	5636	1.563
1	2232	2581	0.064
2	2467	2568	-0.569
3	3229	3489	1.690
4	3566	3505	3.115
5	6445	6675	-2.365
6	2754	2719	2.948
7	3011	2860	2.549
8	2223	2107	-2.109
9	3.35	2890	-1.042
1	3475	3429	-2.645
11	1387	1298	0.814
12	2798	2782	0.329
13	1140	1086	1.296
14	1342	1241	1.485
15	2445	2415	0.056
16	1376	1421	-0.417
17	1590	1512	2.156
18	1162	1221	-1.212
19	563	547	-1.081
2	1056	1054	-2.914
21	554	607	2.677
22	563	573	-2.366
23	545	524	2.762
24	429	490	1.728
•	0	3	
1	3070	3537	-1.199
2	2911	3251	2.259
3	2144	2343	1.601
•	2219	2153	-1.198
5	1 42	455	1.360
6	2899	2970	1.743
7	2 85	2801	-0.311
8	1871	1804	-1.592
9	365	291	-0.865
1	2038	1861	-2.714
10	2038	1861	-2.714
12	736	661	1.211
13	506	387	2.045
14	1056	1049	-2.029
15	392	311	-2.473
16	757	708	2.134
17	858	759	1.738
18	175	116	-2.685 †
19	365	379	2.766
20	852	933	2.148
21	496	502	-1.435
22	392	302	2.824
23	1037	1024	2.307
•	0	4	
0	1462	1574	-0.992
1	3515	3566	0.323
2	2480	2688	1.404
3	336	263	-2.572
4	2186	2274	-1.073
5	1350	1444	0.492
6	1108	1013	-2.856
7	2223	2126	-1.824
8	2120	2071	2.103
9	441	466	-2.456
10	2381	2388	-1.954
11	572	493	2.889
12	2457	2544	1.556
13	1299	1303	1.434
14	632	562	-1.124
15	970	893	0.580
16	777	760	-0.890
17	1334	1330	-1.164
18	858	913	0.894
19	143	144	2.489 †
20	1017	1138	-2.187
21	750	814	-2.964
22	708	771	2.724
•	0	5	
1	1637	1765	0.686
2	1574	1429	-1.268
3	722	664	2.096
4	3993	3927	1.247
5	1941	2022	1.192
6	1299	1180	3.053
7	1380	1297	0.778
8	991	961	-2.160
9	1930	1895	-2.425
10	656	611	-2.178
11	1197	1062	1.497
12	1650	1777	-1.564
13	1541	1535	-1.151
14	1567	1590	0.384
15	1295	1328	0.437
16	336	247	-0.058
17	598	546	1.621
18	694	691	0.915
19	429	417	0.961
20	864	803	-3.050
•	0	6	
0	1740	1625	0.827
1	248	235	2.985
2	581	518	-0.994
3	1702	1810	0.427
4	590	379	2.656
5	2794	2741	-2.871
6	2214	2040	3.057
7	226	395	-0.591
8	1729	1571	-1.448
9	2285	2226	-2.731
10	922	759	1.267
11	905	789	0.026
12	202	341	0.566
13	202	388	0.244
14	722	698	0.053
15	202	239	2.745
16	624	663	0.189
17	1171	1176	0.263
18	405	323	-2.172
19	581	678	-2.724
•	0	7	
1	226	319	1.637
2	422	849	1.224
3	1427	1491	1.160
4	1357	1345	2.563
5	910	809	-2.945
5	910	809	-2.945
7	882	802	1.709
8	770	522	-1.955
9	1405	1375	-1.322
10	1027	1108	-1.722
11	1547	1649	-2.469
12	864	948	-1.492
13	679	660	0.109
14	102	171	-2.666
15	192	266	2.232
16	1027	975	0.547
•	0	8	
0	797	849	1.505
1	1327	1334	0.064
2	1365	1300	-0.673
3	498	495	2.104
4	764	740	1.366
5	474	259	1.010
6	922	991	-3.072
7	938	1040	-3.024
8	598	567	-1.117
9	392	334	-1.861
10	729	813	-2.688
11	143	58	1.061
12	701	627	0.234
•	0	9	
1	632	490	0.623
2	1380	1269	2.082
3	1567	1569	2.246
4	679	630	1.364
5	526	547	1.699
•	1	0	
-25	554	453	0.000
-24	441	410	3.142
-23	598	715	3.142
-22	405	412	3.142
-21	464	428	3.142
-20	350	328	0.000
-19	268	276	0.000
-18	365	426	3.142
-17	350	182	0.000
-16	784	819	0.000
-15	581	477	0.000
-14	700	706	3.142
-13	1153	1148	3.142
-12	1357	1263	3.142
-11	2066	1891	3.142
-10	2953	2770	3.142
-9	4661	4552	3.142
-8	2311	2285	0.000
-7	379	161	0.000
-6	1247	1293	3.142
-5	8392	10458	0.000
-4	4258	4573	3.142
-3	3872	4151	3.142
0	1288	1195	0.000
1	2340	3145	3.142
2	656	680	3.142
3	3639	4243	3.142
4	4479	4906	3.142
5	1975	1868	3.142
6	1874	1749	0.000
7	1705	1694	0.000
8	101	174	3.142 †
9	516	536	0.000
10	101	10	0.000 †
11	3058	2977	0.000
12	2316	2146	0.000
13	516	420	0.000 †
14	1311	1294	3.142
15	143	44	0.000
16	516	402	3.142
17	1452	1494	0.000
18	452	345	0.000
19	1126	1187	0.000
20	474	497	3.142
21	844	817	3.142
22	485	431	0.000
23	248	269	0.000
24	882	914	3.142
•	1	1	
-25	101	231	2.254 †
-24	101	180	3.001 †
-23	960	1179	-0.975
-22	648	671	-1.274

-21	743	738	2.937	
-20	353	322	2.183	
-19	1431	1444	-1.268	
-18	1494	1509	2.226	
-17	1288	1184	2.197	
-16	1903	1886	-0.756	
-15	922	814	-0.290	
-14	1871	1718	-1.435	
-13	526	471	-0.933	
-12	1628	1456	0.837	
-11	3273	3185	2.662	
-10	2711	2350	-0.803	
-9	2615	2406	0.039	
-8	722	754	-2.987	
-7	2375	2135	3.060	
-6	2872	2601	3.026	
-5	3595	2973	-2.830	
-4	7912	8655	-1.121	
-3	3684	3869	-3.049	
-2	5301	5691	0.601	
-1	5107	5857	-3.130	
0	3567	3537	-2.722	
1	2907	2700	-2.739	
2	2033	1997	1.611	
3	3308	3706	2.382	
4	2160	2268	-0.975	
5	3804	3683	3.013	
6	3520	3925	2.724	
7	1720	1578	1.038	
8	2902	3030	-0.416	
9	1653	1567	-2.637	
10	1735	1784	-2.369	
11	3523	3818	-1.400	
12	2805	2894	-0.821	
13	960	836	1.276	
14	949	930	1.381	
15	1455	1376	-0.273	
16	2193	2344	1.776	
17	1807	1726	1.321	
18	1612	1546	0.693	
19	790	793	-2.704	
20	910	973	-3.002	
21	526	448	-1.419	
22	464	565	-0.708	
23	417	468	-1.203	
24	392	440	-2.160 †	
*	1	2		
-24	417	396	-1.790	
-23	743	778	-1.232	
-22	1292	1403	2.468	
-21	722	686	2.915	
-20	632	613	-0.149	
-19	1032	1032	0.475	
-18	708	697	0.630	
-17	2472	2511	0.128	
-16	1714	1717	0.071	
-15	464	440	-1.507	
-14	2207	2077	-0.472	
-13	1829	1755	-0.614	
-12	2160	2056	2.997	
-11	1668	1687	2.908	
-10	2402	2324	-2.409	
-9	764	756	1.460	
-8	2842	2780	1.489	
-7	1158	967	-3.001	
-6	3126	2553	2.004	
-5	3844	3911	1.123	
-4	3608	3765	-0.452	
-3	4197	4839	-1.270	
-2	2823	2893	2.323	
-1	1898	1871	-0.905	
0	2655	2856	-0.146	
1	1708	1860	-0.479	
2	526	459	-3.133	
3	4154	4824	-2.968	
4	1827	1634	2.823	
5	3851	4433	2.841	
6	2879	2922	2.155	
7	648	499	3.095	
8	2329	2253	-1.191	
9	1732	1859	-2.200	
10	2761	2787	-0.227	
11	2.23	1887	-0.623	
12	1288	1346	2.857	
13	722	769	0.825	
14	1469	1455	0.294	
15	1061	918	-2.968	
16	1534	1597	2.869	
17	1193	1184	-3.011	
18	694	619	-1.932	
19	816	812	1.972	
20	441	131	-2.000	
21	615	681	-2.394	
22	581	695	2.591	
23	365	390	2.120	
*	1	3		
-23	101	79	1.383 †	
-22	598	529	-1.686	
-21	392	322	-0.843	
-20	441	439	-2.256	
-19	876	913	2.751	
-18	1090	1098	-1.654	
-17	976	859	-0.980	
-16	1180	962	0.696	
-15	663	618	-2.526	
-14	1251	1240	-2.767	
-13	1810	1771	1.489	
-12	2098	1952	1.077	
-11	1534	1394	0.425	
-10	1259	1177	1.555	
-9	3737	3823	2.331	
-8	922	818	-1.206	
-7	3364	3466	-0.932	
-6	2445	2432	-2.164	
-5	3131	3048	-2.043	
-4	3773	3909	-1.363	
-3	840	747	-0.797	
-2	5167	5449	0.494	
-1	2911	2900	0.038	
0	3762	3715	1.090	
1	3789	4184	1.759	
2	2690	2986	0.872	
3	944	891	0.105	
4	729	647	-1.668	
5	598	583	-2.970	
6	1037	1137	-2.482	
7	899	737	-0.270	
8	1717	1602	-1.286	
9	1838	1674	-1.378	
10	1222	1188	-2.064	
11	663	654	0.502	
12	701	717	1.752	
13	175	165	-0.539 †	
14	392	287	-0.741	
15	1749	1717	1.852	
16	750	765	2.990	
17	474	393	2.351	
18	1205	1219	1.832	
19	441	416	2.741	
20	1239	1338	-1.435	
21	722	740	-3.033	
22	563	591	-3.091	
23	722	890	-0.754	
*	1	4		
-22	965	984	-2.393	
-21	452	515	-2.827	
-20	671	716	1.784	
-19	101	219	3.041 †	
-18	464	427	-0.635	
-17	1214	1125	0.566	
-16	970	922	-0.155	
-15	708	635	-0.970	
-14	581	493	2.451	
-13	2155	2186	2.235	
-12	1037	977	-1.539	
-11	970	1017	-3.078	
-10	2273	2374	-2.579	
-9	2785	2688	-2.536	
-8	2033	2013	-2.497	
-7	1574	1458	1.301	
-6	1749	1468	0.284	
-5	1007	925	-1.484	
-4	840	750	0.306	
-3	1511	1459	1.080	
-2	3226	3410	0.707	
-1	2834	2915	-0.034	
0	1066	1219	-0.163	
1	933	931	-1.301	
2	3201	3397	-1.748	
3	202	131	-2.032	
4	1509	1626	2.341	
5	2883	3269	-2.878	
6	2675	2647	3.044	
7	2071	2068	1.710	
8	1448	1443	1.448	
9	1554	1541	-1.703	
10	1625	1638	0.433	
11	2366	2400	0.397	
12	1735	1777	-0.481	
13	1413	1349	0.286	
14	777	715	1.482	
15	1222	1272	-1.629	
16	679	708	-0.213	
17	624	580	2.226	
18	143	189	-1.729 †	
19	441	448	-2.910	
20	736	749	2.838	
21	101	216	1.926 †	
*	1	5		
-21	379	351	0.253	
-20	474	456	-2.266	
-19	598	551	-1.431	
-18	350	343	-0.077	
-17	143	140	0.708 †	
-16	545	470	-2.605	
-15	722	636	2.295	
-14	1376	1346	1.471	
-13	679	761	1.929	
-12	1108	1057	-2.642	
-11	1726	1600	0.404	
-10	1445	1494	1.235	
-9	101	158	-1.401 †	
-8	1099	1073	-1.626	
-7	1868	1704	-1.712	
-6	2432	2318	-2.203	
-5	2545	2502	-1.781	
-4	303	315	-1.503	
-3	1136	1015	-0.965	
-2	640	604	-2.346	
-1	3431	3260	1.366	
0	2011	2101	1.789	
1	1099	1077	3.113	
2	648	527	2.094	
3	1761	1669	2.007	
4	1995	2187	2.852	
5	1557	1508	-3.020	
6	1218	1123	2.601	
7	2242	2290	-1.457	
8	3341	3438	-1.284	
9	1775	1693	0.556	
10	474	344	2.397	
11	1268	1212	-0.865	
12	615	452	-1.657	
13	750	710	0.187	
14	840	760	0.644	
15	1307	1336	2.673	
16	828	887	1.732	
17	828	980	0.819	
18	624	606	-2.769	
19	464	386	-2.870	
20	379	424	0.384	
*	1	6		
-18	1066	1076	0.328	
-17	417	323	0.783	
-16	816	767	0.252	
-15	1022	1061	0.270	
-14	175	115	-3.121 †	
-13	175	376	-0.335 †	
-12	1272	1145	0.362	
-11	1153	1081	2.354	
-10	1222	1175	-2.623	
-9	1380	1423	-1.747	
-8	202	303	-2.167	
-7	2579	2518	-2.611	
-6	1012	988	-2.937	
-5	2515	2657	0.894	
-4	1941	1820	1.185	
-3	615	705	1.237	
-2	1409	1411	0.307	
-1	1315	1271	0.713	
0	701	595	0.061	
1	607	575	-0.406	
2	496	416	-2.164	
3	2033	2060	-1.691	
4	2436	2499	-2.489	

5	1427	1418	2.722
6	1012	862	-2.400
7	715	680	2.538
8	1514	1512	-3.095
9	1459	1445	0.132
10	922	888	0.016
11	202	336	-1.668
12	656	737	0.691
13	202	70	0.691
14	417	486	-1.911
15	671	708	0.310
16	202	89	-0.458 †
17	743	844	-2.677
18	545	636	-3.078
*	1	7	
-16	417	485	0.349
-15	598	622	1.979
-14	899	985	2.572
-13	834	862	1.627
-12	694	683	0.636
-11	417	470	-2.561
-10	764	815	1.260
-9	648	679	-0.054
-8	1099	1065	-2.318
-7	1012	970	-1.685
-6	764	687	-0.713
-5	846	628	-0.282
-4	701	500	0.014
-3	222	190	1.174
-2	496	428	0.523
-1	496	472	-1.885
	864	829	1.232
1	1095	1089	0.802
2	1151	1145	1.518
3	1307	1381	-3.088
4	1042	1010	-1.772
5	910	867	1.223
6	954	911	1.878
7	996	949	-0.876
8	1295	1352	-0.539
9	1085	1125	-1.668
10	516	473	-2.034
11	175	147	0.270 †
12	175	86	-0.057 †
13	686	675	2.849
14	526	503	1.301
15	729	814	0.685
*	1	8	
-11	694	717	-2.483
-10	590	633	-2.519
-9	485	355	-2.560
-8	770	834	-2.782
-7	535	494	-1.885
-6	485	418	-0.275
-5	852	914	2.650
-4	175	165	1.111
-3	1323	1364	-0.263
-2	1117	1063	0.550
-1	790	732	0.525
	797	780	-0.601
1	303	314	-2.390
2	417	319	2.936
3	770	801	0.792
4	175	203	-2.788
5	1551	1563	-2.998
6	893	915	2.613
7	572	590	-0.118
8	417	384	-1.907
9	452	498	2.976
10	496	510	0.227
11	485	511	0.407
*	1	9	
-5	452	503	-1.074
-4	1101	977	-0.860
-3	452	461	-2.007
-2	1131	1069	2.828
-1	663	573	1.471
	581	512	0.694
1	954	1037	2.055
2	938	954	2.271
*	2	0	
-25	101	435	3.142 †
-24	101	46	3.142 †
-23	161	262	3.142 †
-22	143	24	0.388 †
-21	143	160	0.000 †
-20	175	97	0.000 †
-19	175	68	3.142 †
-18	175	205	0.000 †
-17	1452	1524	0.000
-16	1383	1398	0.000
-15	679	676	0.000
-14	1705	1629	3.142
-13	1222	1024	0.000
-12	2965	2743	3.142
-11	4295	4457	3.142
-10	2191	2188	3.142
-9	1188	1101	0.000
-8	2246	2141	3.142
-7	2579	2624	0.000
-6	6076	6398	0.000
-5	3037	2860	0.000
-4	4476	4272	3.142
-3	5372	5938	0.000
-2	2003	2309	3.142
-1	3703	3996	3.142
0	6470	7279	3.142
1	1990	1854	0.000
2	5779	6670	3.142
3	6923	6772	3.142
4	1303	1125	0.000
5	648	542	0.000
6	1022	1072	3.142
7	1784	1789	0.000
8	101	160	3.142 †
9	101	133	0.000 †
10	2353	2448	0.000
11	1843	1868	0.000
12	1131	1195	0.000
13	1531	1469	3.142
14	1383	1369	0.000
15	496	449	3.142
16	1587	1563	3.142
17	1239	1213	0.000
18	770	757	0.000
19	679	546	3.142
20	175	58	0.000 †
21	526	514	0.000
22	303	271	3.142
23	554	513	3.142
*	2	1	
-24	1007	1040	-1.280
-23	722	734	-2.135
-22	516	440	-2.955
-21	1090	1219	-0.564
-20	976	1076	-0.784
-19	986	945	2.220
-18	572	481	1.334
-17	1755	1792	0.054
-16	1521	1508	1.063
-15	1197	1170	1.484
-14	405	243	0.736
-13	1239	1117	2.953
-12	1527	1513	2.523
-11	1631	1389	0.422
-10	3869	4145	-0.380
-9	2883	2803	-1.199
-8	1327	1349	-2.376
-7	2226	2217	-0.119
-6	2003	1593	-0.698
-5	1772	1666	-2.574
-4	7133	6204	-1.823
-3	3554	3824	1.000
-2	5267	5919	2.703
-1	7852	8880	2.153
0	3497	3513	1.583
1	3863	4280	2.263
2	1007	1038	0.972
3	4731	5289	-0.550
4	2688	2529	-1.994
5	1659	1715	-2.819
6	3902	4286	-1.679
7	4980	5644	-0.730
8	2300	2469	3.027
9	1832	1919	3.051
10	1749	1831	-0.478
11	1801	1739	2.612
12	2521	2792	2.504
13	2000	2118	0.610
14	757	693	2.870
15	1113	1044	1.804
16	1080	1004	2.072
17	777	729	-0.380
18	1099	1112	-2.690
19	715	678	-2.181
20	648	615	-1.058
21	701	774	-1.596
22	640	603	-2.985
23	101	51	-2.953 †
*	2	2	
-24	464	324	-0.651
-23	640	620	2.963
-22	379	322	1.585
-21	809	836	-0.637
-20	563	536	1.655
-19	663	628	0.825
-18	1423	1385	-0.065
-17	960	829	-0.903
-16	671	320	1.812
-15	736	580	-1.377
-14	954	911	-2.039
-13	1954	1803	2.573
-12	1380	1380	-2.601
-11	1445	1346	-1.874
-10	2813	2632	2.084
-9	3023	2804	2.620
-8	2759	2720	-1.161
-7	2974	2915	0.212
-6	4117	4083	0.182
-5	3312	3145	0.600
-4	2816	2656	-1.604
-3	3419	3420	2.188
-2	3507	3379	0.111
-1	5285	5769	-0.663
0	3484	3776	1.491
1	4288	4609	2.486
2	3668	3558	-1.103
3	4825	5182	-0.705
4	2953	3013	-2.918
5	2151	2358	2.429
6	2248	2045	0.913
7	1898	1677	3.098
8	2515	2721	0.033
9	2073	2175	-0.684
10	417	311	-0.837
11	1938	1902	2.005
12	1299	1254	0.812
13	2051	2120	-0.533
14	922	886	-3.024
15	1987	2241	-3.019
16	1500	1543	-2.902
17	607	592	-2.828
18	840	870	1.832
19	797	736	-1.146
20	365	255	-2.595
21	876	986	2.140
22	350	321	-1.992
23	736	769	-1.016
*	2	3	
-23	563	565	-1.450
-22	581	601	-1.299
-21	143	87	-0.319 †
-20	927	915	2.912
-19	572	561	-1.756
-18	545	448	0.551
-17	1051	974	1.384
-16	803	766	-2.482
-15	876	790	-2.523
-14	3315	3517	2.270
-13	1094	1032	2.896
-12	1042	1021	-1.322
-11	1099	1124	-2.315
-10	2291	2211	1.875
-9	1887	1878	-0.811
-8	2630	2518	-1.254
-7	991	946	0.973
-6	2651	2467	-1.041
-5	4385	4336	-1.256
-4	2543	2576	2.536
-3	1319	1236	2.192
-2	2443	2308	0.679
-1	1210	1241	1.452
0	3448	3515	1.636
1	1871	1711	1.923
2	1793	1673	-2.229

3	965	793	-0.402
4	2778	3179	-0.054
5	1956	2161	-1.664
6	757	737	0.123
7	679	521	2.976
8	1483	1463	-1.409
9	1162	1219	-0.372
10	1807	1737	1.408
11	777	677	3.090
12	1156	957	-1.900
13	1684	1660	1.462
14	1151	1018	2.271
15	1214	1150	-1.231
16	899	922	1.189
17	1690	1762	1.286
18	846	864	-0.336
19	777	783	-1.009
20	598	617	-2.782
21	615	615	-1.328
22	846	934	-0.905
*	2	4	
-22	474	480	0.651
-21	581	586	1.291
-20	516	525	-0.785
-19	632	610	0.558
-18	933	856	1.064
-17	598	523	-0.441
-16	648	528	-1.400
-15	1113	1160	-2.975
-14	981	945	2.007
-13	563	471	-2.148
-12	970	967	-2.766
-11	1868	1795	2.963
-10	1737	1708	-1.511
-9	694	589	0.790
-8	1175	1046	0.901
-7	143	207	2.526 †
-6	2031	1952	-1.588
-5	1061	862	-1.036
-4	3459	3384	0.877
-3	1032	890	-2.583
-2	1849	1913	-2.543
-1	1943	1877	2.026
0	598	424	-1.557
1	2807	2721	-1.664
2	1338	1167	1.194
3	1807	1845	2.837
4	4249	4484	-2.729
5	1136	1063	2.614
6	3347	3565	1.406
7	1721	1745	-2.365
8	1723	1658	-1.967
9	1511	1477	0.950
10	2329	2426	-0.271
11	2335	2597	-0.475
12	441	377	1.487
13	143	139	0.964 †
14	392	360	-1.012
15	694	648	1.693
16	656	748	1.684
17	474	429	-2.744
18	643	663	-2.684
19	506	430	2.145
20	101	261	0.429 †
21	632	694	-0.940
*	2	5	
-21	392	427	-1.461
-20	516	534	-1.915
-19	417	429	1.270
-18	35	337	2.046
-17	101	271	2.220 †
-16	1251	1329	0.557
-15	1211	1239	0.719
-14	803	733	-2.906
-13	474	411	3.116
-12	1365	1403	-0.223
-11	1235	1277	0.790
-10	1446	961	-1.414
-9	32	255	-1.225
-8	35	331	1.785
-7	2207	2235	-2.161
-6	729	773	-2.189
-5	852	826	0.517
-4	572	495	-1.944
-3	474	453	2.498
-2	3011	2936	1.254
-1	1145	969	1.818
0	1243	1087	-2.307
1	1729	1701	1.447
2	1956	2002	1.751
3	2424	2608	-2.114
4	736	668	-3.105
5	1665	1762	3.026
6	2056	2065	-1.144
7	1914	1997	-1.507
8	1615	1645	-0.162
9	1593	1559	-0.104
10	1075	1034	-2.380
11	143	207	2.614 †
12	797	781	1.171
13	1319	1481	-2.889
14	816	819	3.015
15	1158	1259	1.482
16	671	734	1.868
17	949	917	-2.930
18	101	130	1.165 †
19	535	482	-0.116
*	2	6	
-19	464	647	0.266
-18	286	185	0.105
-17	694	652	-0.180
-16	679	671	0.410
-15	790	793	-2.001
-14	1027	1040	-2.001
-13	624	614	1.246
-12	1243	1261	2.975
-11	1804	1802	-3.117
-10	1108	957	1.214
-9	202	207	-2.721
-8	2068	1972	-2.618
-7	899	881	-0.064
-6	2787	2773	0.536
-5	1982	2012	0.749
-4	1222	1184	-0.167
-3	1434	1408	0.083
-2	828	704	-0.613
-1	202	329	-2.490
0	954	1016	2.377
1	474	504	-0.223
2	828	701	-2.345
3	2174	2318	-2.740
4	202	275	-1.152
5	764	633	1.454
6	1075	1018	3.073
7	1977	1951	0.661
8	1113	881	0.081
9	1280	1314	-1.068
10	790	736	-0.860
11	938	923	0.607
12	192	191	-1.747
13	563	533	3.030
14	743	778	1.583
15	336	338	3.073
16	949	1011	-2.990
17	485	579	1.991
*	2	7	
-16	516	536	3.056
-15	1061	1035	2.280
-14	981	992	1.024
-13	160	246	2.059 †
-12	954	988	-2.242
-11	485	453	-2.447
-10	506	452	-0.248
-9	743	664	-1.394
-8	624	588	-1.963
-7	1099	917	-0.822
-6	1405	1387	-1.645
-5	797	631	-3.094
-4	1037	1006	0.671
-3	876	829	0.218
-2	1517	1466	2.936
-1	202	300	0.286
0	1784	1784	0.816
1	1280	1321	1.964
2	202	324	-2.980 †
3	656	527	-0.296
4	1122	1060	-2.954
5	784	657	3.130
6	764	812	-1.049
7	1524	1611	-0.484
8	590	592	-1.033
9	175	107	-0.296 †
10	656	666	0.215
11	175	314	1.817 †
12	922	927	2.216
13	893	961	1.132
*	2	8	
-12	822	847	3.139
-11	607	579	-2.765
-10	960	940	3.013
-9	496	503	-2.499
-8	581	621	-0.435
-7	392	327	1.130
-6	157	383	2.748 †
-5	910	884	-0.378
-4	1368	1416	-0.047
-3	474	452	0.383
-2	822	864	1.206
-1	169	251	-1.251 †
0	1099	1192	-2.408
1	1188	1237	3.057
2	157	260	-2.995 †
3	991	1070	-2.725
4	1564	1701	3.080
5	157	75	3.000 †
6	1042	1053	-0.757
7	143	323	3.080 †
8	663	724	1.574
9	1046	1203	0.308
10	986	860	-0.038
*	2	9	
-3	1197	1198	2.234
-2	581	544	1.979
-1	392	430	1.471
0	797	812	2.102
1	392	379	1.664
*	3	0	
-24	101	180	0.000 †
-23	101	269	3.142 †
-22	882	930	0.000
-21	960	986	0.000
-20	143	108	0.000 †
-19	441	413	3.142
-18	864	932	0.000
-17	143	121	0.000 †
-16	679	592	0.000
-15	1452	1459	0.000
-14	175	156	3.142 †
-13	2603	2894	3.142
-12	1606	1597	3.142
-11	3226	3420	0.000
-10	876	788	0.000
-9	632	676	3.142
-8	976	854	0.000
-7	1307	1243	0.000
-6	4777	5054	-0.000
-5	2743	2688	0.000
-4	101	188	0.000 †
-3	4181	4150	3.142
-2	6003	7205	3.142
-1	101	144	0.000 †
0	101	269	0.000 †
1	6261	7299	3.142
2	1167	1054	3.142
3	101	112	3.142 †
4	1580	1440	0.000
5	101	188	3.142 †
6	464	295	3.142
7	1483	1392	3.142
8	506	334	3.142
9	2264	2058	0.000
10	1438	1297	0.000
11	2053	1998	3.142
12	2282	2410	0.000
13	840	701	0.000
14	581	429	3.142
15	2669	2683	3.142
17	1231	1135	3.142
18	828	726	3.142
19	175	214	0.000 †
20	143	149	0.000 †
21	516	388	3.142
22	226	286	0.000
*	3	1	
-24	640	623	-2.818

-23	101	170	2.083	†
-22	922	908	-0.467	
-21	506	445	-3.015	
-20	1046	1035	2.486	
-19	965	1011	0.460	
-18	1017	1019	0.301	
-17	572	475	1.426	
-16	350	336	1.562	
-15	981	986	-0.637	
-14	1061	1046	3.079	
-13	607	499	1.082	
-12	1380	1480	-1.305	
-11	1383	1357	-1.584	
-10	910	805	2.878	
-9	1879	1709	-0.967	
-8	2729	2732	0.983	
-7	1995	1850	2.763	
-6	2355	2335	-3.129	
-5	4711	4673	-0.648	
-4	905	929	1.223	
-3	3090	2925	0.132	
-2	2228	1770	-1.313	
-1	4094	4139	2.027	
0	4658	5172	1.229	
1	3194	3259	0.368	
2	3453	3966	-0.887	
3	944	1049	-2.438	
4	4510	4926	-2.352	
5	2144	2318	-0.576	
6	1051	1016	-1.616	
7	1117	1217	-2.304	
8	1951	2279	-0.182	
9	429	526	-0.756	
10	1650	1735	2.169	
11	2565	2791	1.880	
12	1717	2014	0.677	
13	607	664	-2.883	
14	1193	1286	-3.079	
15	506	549	-3.069	
16	905	920	-2.514	
17	976	959	-3.067	
18	405	314	-1.474	
19	708	761	-1.003	
20	268	342	-2.371	
21	615	532	-3.134	
22	624	580	-0.402	
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-24	268	284	1.535	
-23	545	488	0.554	
-22	701	723	-0.193	
-21	143	183	1.905	†
-20	175	114	-0.514	†
-19	736	664	-1.016	
-18	175	147	2.174	†
-17	864	833	3.032	
-16	1772	1673	-1.971	
-15	2158	1917	2.702	
-14	1784	1759	2.788	
-13	175	169	-2.506	†
-12	572	532	2.408	
-11	2355	2462	2.631	
-10	1494	1327	-2.676	
-9	1218	1020	-1.256	
-8	1142	979	1.191	
-7	4203	4223	0.043	
-6	5934	6561	-1.047	
-5	1821	1627	1.775	
-4	1459	1318	2.370	
-3	2235	2296	0.301	
-2	2078	1936	-0.190	
-1	4966	5175	2.606	
0	1772	1640	3.075	
1	2267	2281	0.028	
2	2543	2440	-2.888	
3	2239	2111	-0.992	
4	2375	2450	-1.560	
5	1831	1565	0.009	
6	2073	2054	0.917	
7	797	798	0.612	
8	632	674	-0.188	
9	870	758	0.085	
10	1113	1062	1.949	
11	1761	1754	0.715	
12	686	753	0.916	
13	640	691	-2.076	
14	1042	1106	-2.478	
15	864	857	-2.198	
16	822	936	2.749	
17	202	263	2.099	†
18	846	674	-0.604	
19	175	240	0.259	†
20	545	509	0.825	
21	905	819	-0.563	
22	496	488	-1.433	
•	3	3		
-23	563	585	-1.530	
-22	545	471	2.189	
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-20	417	433	-1.774	
-19	1368	1412	1.526	
-18	607	575	2.137	
-17	392	341	0.071	
-16	970	1021	1.232	
-15	2366	2447	1.563	
-14	474	407	2.981	
-13	1795	1722	-1.607	
-12	1737	1578	3.045	
-11	1761	1664	-1.468	
-10	3626	3631	-1.277	
-9	452	361	-1.866	
-8	927	813	-2.577	
-7	303	187	-1.091	
-6	722	468	-1.643	
-5	1840	1503	2.384	
-4	1784	1732	0.995	
-3	2359	2204	1.488	
-2	2451	2406	2.216	
-1	1490	1356	1.965	
0	1770	1575	-2.315	
1	722	520	-2.964	
2	858	896	-1.581	
3	2081	2138	-1.917	
4	2165	2244	-1.936	
5	1801	1768	-3.050	
6	1815	1713	-1.476	
7	1201	1114	-1.957	
8	2083	2183	1.614	
9	1732	1670	1.227	
10	1323	1348	-0.310	
11	1184	1093	1.024	
12	1821	1966	1.444	
13	927	1023	-2.695	
14	1080	1102	-1.925	
15	671	659	0.455	
16	743	794	0.601	
17	828	827	-0.653	
18	648	562	-2.019	
19	405	298	2.337	
20	666	615	-0.702	
21	1117	1158	-0.624	
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-22	417	481	0.661	
-21	777	842	-0.586	
-20	350	360	0.404	
-19	976	1035	1.254	
-18	545	557	0.299	
-17	143	238	-1.854	†
-16	882	930	2.564	
-15	1483	1642	-1.500	
-14	496	279	2.844	
-13	545	419	2.619	
-12	870	899	-2.642	
-11	1099	1047	-1.251	
-10	1684	1665	0.484	
-9	1705	1656	1.290	
-8	686	572	2.007	
-7	1180	1070	-0.583	
-6	2543	2430	0.285	
-5	2110	1932	-0.127	
-4	1587	1357	-1.955	
-3	2038	1951	2.061	
-2	1948	1765	2.678	
-1	3253	3447	-2.612	
0	1681	1619	-2.616	
1	1662	1555	1.945	
2	1634	1666	-2.765	
3	1547	1420	-2.299	
4	764	693	2.193	
5	1090	965	0.166	
6	336	908	-1.077	
7	607	543	0.160	
8	1865	1863	0.722	
9	1860	1884	-0.449	
10	1032	1027	-1.808	
11	922	925	2.745	
12	392	377	-2.791	
13	496	496	-1.616	
14	1149	1123	2.677	
15	777	805	2.480	
16	1145	1222	-2.424	
17	111	220	2.500	
18	506	459	1.762	
19	590	502	-1.224	
20	268	302	0.116	
•	3	5		
-20	365	325	-1.650	
-19	736	707	3.125	
-18	750	741	1.193	
-17	1272	1418	0.569	
-16	770	900	1.497	
-15	485	460	2.955	
-14	452	458	-1.437	
-13	581	557	0.482	
-12	770	745	-1.752	
-11	701	740	-2.611	
-10	893	838	-0.317	
-9	1784	1739	-1.383	
-8	1583	1377	-1.674	
-7	1455	1277	1.030	
-6	1099	1006	1.375	
-5	485	404	2.058	
-4	2061	2105	0.943	
-3	1802	1762	1.904	
-2	1145	976	2.699	
-1	991	895	1.834	
0	2262	2234	1.642	
1	1668	1709	-0.508	
2	2417	2490	-1.355	
3	1231	1145	-0.982	
4	516	469	0.394	
5	2093	2236	-1.818	
6	1612	1556	-0.832	
7	1171	1225	-0.321	
8	405	353	1.696	
9	797	775	2.145	
10	1473	1670	1.588	
11	1007	1100	2.279	
12	986	1137	-2.797	
13	615	607	2.005	
14	590	575	1.396	
15	303	316	-2.178	
16	834	769	-2.639	
17	474	365	-0.663	
18	632	593	-0.519	
•	4	0		
-23	1247	1238	0.000	
-22	1012	997	0.000	
-21	143	154	3.142	†
-20	143	124	0.000	†
-19	143	184	3.142	†
-18	816	736	3.142	
-17	864	806	3.142	
-16	175	285	0.000	†
-15	1380	1386	3.142	
-14	1251	1187	3.142	
-13	175	105	3.142	†
-12	2451	2238	0.000	
-11	2763	2658	0.000	
-10	1001	1076	0.000	
-9	1149	1023	0.000	
-8	2794	2733	0.000	
-7	1402	1363	3.142	
-6	1108	944	3.142	
-5	2599	2453	0.000	
-4	2992	2992	3.142	
-3	2276	2124	3.142	
-2	405	538	3.142	
-1	3220	3375	3.142	
0	1402	1399	3.142	
1	5135	5079	0.000	
2	4704	4612	0.000	
3	965	750	0.000	
4	143	124	3.142	†
5	2134	2226	0.000	
6	1901	1755	0.000	

7	701	642	0.000
8	143	248	3.142 †
9	1911	1780	3.142
10	3444	3563	3.142
11	2480	2276	0.000
12	1032	1040	0.000
13	1427	1237	3.142
14	1514	1432	3.142
15	671	671	3.142
16	485	427	3.142
17	694	681	3.142
18	175	42	0.000 †
19	379	283	0.000
20	834	741	3.142
21	663	529	0.000
•	4	1	
-24	679	715	0.186
-23	535	516	-0.181
-22	516	603	-2.089
-21	648	701	2.831
-20	615	657	0.205
-19	143	142	0.583 †
-18	1483	1587	2.225
-17	722	644	2.871
-16	671	693	-2.837
-15	870	912	2.898
-14	175	278	1.682 †
-13	1534	1573	-0.715
-12	1012	994	-2.236
-11	2346	2321	-2.173
-10	3221	3234	-1.303
-9	554	428	0.038
-8	3167	3152	1.991
-7	933	1000	2.525
-6	2013	2228	1.931
-5	686	527	0.857
-4	1919	1838	1.568
-3	1027	901	0.853
-2	2820	2867	0.514
-1	976	1149	2.898
0	1702	1819	-0.427
1	3868	4177	-0.669
2	2066	2082	-1.091
3	1046	1058	-1.979
4	2840	3126	-0.870
5	2163	2274	-2.895
6	1372	1422	2.076
7	2865	2558	0.492
8	852	831	0.099
9	2507	2652	2.386
10	1376	1474	1.431
11	1514	1678	0.965
12	949	909	1.002
13	1507	1401	-2.719
14	1681	1863	-1.034
15	1311	1348	-2.058
16	581	565	-2.611
17	1027	1016	-0.361
18	350	382	-0.160
19	607	541	2.435
20	336	268	0.432
21	350	362	0.243
•	4	2	
-24	656	723	0.176
-23	694	657	0.394
-22	365	352	1.624
-21	464	395	-1.591
-20	143	140	-1.810 †
-19	656	658	-2.670
-18	840	841	-3.103
-17	1171	1115	-2.691
-16	1323	1322	2.928
-15	954	926	2.796
-14	1161	1156	-0.386
-13	202	412	-2.140 †
-12	1205	1189	2.065
-11	976	872	-8.092
-10	615	595	0.656
-9	648	628	1.956
-8	1292	1163	1.053
-7	3956	3813	-1.074
-6	1911	1839	2.928
-5	893	896	1.948
-4	2697	2653	-1.197
-3	1887	1810	-2.528

-2	3065	3212	2.811
-1	1511	1398	-2.949
0	1117	1013	-2.145
1	1717	1701	2.645
2	2328	2245	3.018
3	1235	1229	-0.475
4	2888	3206	0.342
5	1398	1412	1.282
6	554	538	-2.878
7	722	756	-0.720
8	535	532	-2.856
9	392	287	1.049
10	743	707	-0.206
11	1108	1100	-2.527
12	496	430	2.637
13	864	840	-0.843
14	202	129	0.109 †
15	729	685	2.139
16	452	466	0.671
17	175	218	-0.345 †
18	663	567	1.069
19	572	560	0.526
20	1323	1159	-0.617
•	4	3	
-23	417	415	0.289
-22	764	736	-0.774
-21	101	202	-0.111 †
-20	996	1011	1.946
-19	927	903	2.357
-18	870	918	-0.557
-17	1413	1388	1.195
-16	882	861	1.412
-15	686	611	-2.382
-14	1117	1055	-1.655
-13	777	776	0.429
-12	1752	1714	-1.071
-11	2728	2749	-0.968
-10	1331	1100	-1.931
-9	1452	1247	1.409
-8	1775	1555	0.526
-7	701	486	3.723
-6	2353	2433	2.650
-5	2396	2283	1.818
-4	143	271	-0.546 †
-3	2278	2089	2.171
-2	1122	975	3.086
-1	2935	2906	-2.044
0	2181	2173	2.811
1	1416	1279	-0.062
2	2624	2634	-0.680
3	770	642	3.118
4	822	700	-2.907
5	1865	1844	-1.910
6	1956	1883	-2.343
7	1603	1700	1.581
8	656	623	1.264
9	905	903	-1.626
10	1445	1592	1.316
11	1402	1449	1.786
12	485	420	-2.569
13	870	847	-2.016
14	598	601	0.810
15	175	248	-1.072 †
16	1413	1348	-1.354
17	572	561	-2.183
18	632	552	2.460
19	320	293	-1.188
•	4	4	
-20	365	381	-2.142
-19	365	450	-1.771
-18	101	189	1.780 †
-17	350	342	2.583
-16	679	708	-1.657
-15	581	659	-0.836
-14	496	470	3.103
-13	392	399	-0.415
-12	1259	1125	0.187
-11	1729	1722	1.097
-10	1292	1112	1.889
-9	1634	1444	-2.486
-8	586	408	0.323
-7	2329	2318	0.649
-6	1319	1285	-0.921
-5	944	848	-1.286
-4	1856	944	-0.381

-3	143	234	2.609 †
-2	1153	1048	-3.084
-1	1874	1722	2.831
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1	764	770	1.729
2	2255	2233	-1.577
3	1987	2048	1.673
4	1365	1263	-0.195
5	1987	1971	-1.189
6	1001	938	-0.201
7	2177	2504	-0.310
8	1149	1170	-0.509
9	1243	1342	3.141
10	1327	1342	1.895
11	803	815	-2.200
12	686	624	-2.171
13	1153	1160	2.134
14	899	922	-3.058
15	916	923	-2.364
16	336	324	2.590
17	365	393	-2.750
18	607	562	-2.186
•	4	5	
-20	336	315	2.224
-19	268	253	1.344
-18	656	646	0.929
-17	248	244	-2.898
-16	777	755	3.126
-15	101	151	0.081 †
-14	797	883	-1.552
-13	1394	1715	-2.173
-12	822	906	3.130
-11	417	359	1.115
-10	870	874	-1.533
-9	888	793	-0.950
-8	694	488	0.486
-7	1504	1519	0.914
-6	1136	1072	1.608
-5	2103	2053	1.603
-4	757	736	0.926
-3	2226	2325	-1.978
-2	1201	1099	-2.987
-1	2141	1918	1.562
0	1117	1057	-1.897
1	1887	1896	-0.952
2	2160	2350	-0.586
3	1126	1063	-0.054
4	526	519	-2.394
5	1214	1240	-0.237
6	764	739	1.299
7	545	439	-2.359
8	615	630	1.972
9	1625	1774	1.764
10	986	1089	2.025
11	441	438	2.752
12	379	411	1.400
13	750	784	0.233
14	1099	976	-2.076
15	365	282	-2.346
16	876	803	0.142
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-22	320	343	3.142
-21	286	243	3.142
-20	392	347	3.142
-19	632	554	3.142
-18	143	80	3.142 †
-17	143	185	0.000 †
-16	686	708	3.142
-15	1473	1507	3.142
-14	581	521	0.000
-13	1222	1229	0.000
-12	175	42	3.142 †
-11	598	507	3.142
-10	1346	1259	-0.000
-9	2273	2311	3.142
-8	1153	1020	3.142
-7	1235	1095	0.000
-6	1583	1439	3.142
-5	143	196	0.000 †
-4	3737	3807	0.000
-3	945	821	0.000
-2	2735	2540	3.142
-1	594	388	0.000
0	1085	896	0.000
1	1959	1873	0.000

2	2053	1849	3.142	-1	1383	1277	-2.920	0	1898	2005	-0.229
3	1801	1643	0.000	0	3420	3612	2.763	1	526	396	-0.680
4	1171	996	0.000	1	2298	2275	-2.609	2	1184	1135	-0.054
5	143	247	0.000 †	2	2531	2598	-0.417	3	1272	1194	-0.930
6	3799	3095	0.000	3	1264	1305	0.434	4	1527	1468	-0.597
7	1810	1731	0.000	4	175	200	-2.958 †	5	2223	2301	0.648
8	1259	1118	3.142	5	464	373	0.883	6	1303	1308	-0.004
9	516	466	3.142	6	392	386	2.755	7	1001	1071	-2.069
10	1131	952	3.142	7	624	620	1.031	8	1714	1861	2.318
11	905	750	0.000	8	1175	1270	-0.527	9	1541	1721	2.310
12	1531	1485	3.142	9	1438	1568	2.595	10	485	501	-2.477
13	572	593	3.142	10	1531	1688	-3.116	11	500	574	2.739
14	175	42	0.000 †	11	405	339	-2.618	12	485	419	2.384
15	1342	996	3.142	12	632	664	-1.075	13	986	1010	-1.614
16	429	365	3.142	13	777	712	-2.302	14	590	540	-0.219
17	1387	1255	0.000	14	202	192	1.657 †	15	474	495	0.528
18	379	360	0.000	15	816	718	-0.210	16	615	577	-1.793
19	143	30	3.142 †	16	452	347	-1.977	•	5	5	
•	5	1		17	526	491	1.471	-19	320	395	2.842
-23	864	965	2.515	18	405	343	0.131	-18	607	622	-2.418
-22	101	326	1.134 †	19	350	367	-1.282	-17	202	249	3.123
-21	590	643	0.277	•	5	3		-16	757	706	0.100
-20	679	773	2.723	-22	417	549	1.636	-15	1022	1166	-1.351
-19	764	847	2.551	-21	858	938	2.018	-14	671	739	-2.177
-18	722	773	-1.231	-20	797	674	2.840	-13	392	423	1.559
-17	663	633	2.871	-19	101	206	-1.080 †	-12	485	492	-0.163
-16	803	799	2.916	-18	1149	1125	0.843	-11	809	772	-2.237
-15	1080	1330	-1.093	-17	581	556	-1.925	-10	506	528	2.830
-14	441	503	-0.823	-16	1544	1557	-1.217	-9	1051	1079	1.458
-13	938	929	3.043	-15	954	982	-0.956	-8	852	757	-2.576
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-11	834	837	-1.622	-13	1153	1189	-0.875	-6	1434	1508	1.492
-10	1307	1351	-2.638	-12	1476	1499	-0.719	-5	916	793	-2.357
-9	1699	1581	2.001	-11	474	387	2.984	-4	1056	1149	-2.045
-8	1075	1067	1.110	-10	1746	1759	1.456	-3	1131	1198	-0.364
-7	2103	2086	1.231	-9	598	562	-0.177	-2	933	858	-1.477
-6	2599	2753	1.997	-8	3284	3560	2.268	-1	1239	1186	-2.258
-5	1122	1069	2.201	-7	1977	1947	1.654	0	1824	1965	-0.757
-4	1452	1436	-0.791	-6	1085	1016	-0.233	1	615	624	0.860
-3	777	769	-1.570	-5	1956	1916	1.280	2	464	374	-2.220
-2	996	959	-2.425	-4	429	377	-3.106	3	464	365	-0.489
-1	2177	2104	-0.984	-3	3048	3032	-1.474	4	648	612	1.909
0	3382	3115	-2.389	-2	1292	1262	-2.581	5	1251	1330	2.516
1	1818	1841	-2.157	-1	790	792	-1.573	6	1022	1078	2.913
2	2174	2156	-0.584	0	2085	1960	-0.719	7	1104	1068	2.401
3	624	539	-1.747	1	1998	1993	-0.860	8	686	683	1.262
4	2083	2288	2.025	2	2300	2307	1.662	9	143	183	-1.628 †
5	1668	1757	1.851	3	1046	903	0.729	10	452	465	1.065
6	976	973	0.913	4	991	1046	-1.259	11	981	1040	0.586
7	970	902	1.480	5	1080	1064	2.251	12	770	838	-0.627
8	1662	1851	3.089	6	1342	1380	1.704	13	1022	929	-1.686
9	1531	1703	0.030	7	175	169	-0.901 †	14	392	331	0.406
10	572	606	1.266	8	175	337	-2.322 †	15	572	634	-0.266
11	1551	1733	2.431	9	743	734	1.191	•	6	0	
12	1361	1415	-1.288	10	485	476	1.930	-20	736	743	3.142
13	1184	1219	-0.829	11	1284	1258	-1.940	-19	615	652	3.142
14	474	514	-1.710	12	169	137	-2.413	-18	143	107	3.142 †
15	954	1025	-0.873	13	722	680	1.556	-17	803	813	3.142
16	750	821	-0.329	14	590	652	-1.561	-16	429	455	3.142
17	464	429	0.840	15	1303	1251	-1.449	-15	679	602	0.000
18	286	273	2.365	16	336	328	-2.679	-14	581	485	3.142
19	893	841	0.599	17	944	822	2.986	-13	175	146	0.000 †
•	5	2		18	143	141	-3.124 †	-12	1644	1719	-0.000
-23	379	363	-1.425	•	5	4		-11	816	800	0.000
-22	535	461	-1.089	-21	648	718	-1.805	-10	722	694	3.142
-21	365	348	-1.808	-20	405	559	-1.486	-9	1007	877	3.142
-20	809	821	2.686	-19	572	735	1.931	-8	1056	1003	3.142
-19	350	239	0.917	-18	535	693	2.999	-7	2577	2407	3.142
-18	535	427	2.123	-17	743	864	-2.836	-6	175	44	0.000 †
-17	554	534	2.068	-16	572	562	2.199	-5	485	356	0.000
-16	1637	1717	1.107	-15	417	424	1.903	-4	175	100	3.142 †
-15	648	525	-0.867	-14	922	1049	-1.773	-3	1821	1712	3.142
-14	648	680	2.997	-13	590	529	0.326	-2	1587	1417	0.000
-13	1284	1157	-0.454	-12	1264	1307	1.003	-1	1517	1430	0.000
-12	3604	3712	-0.810	-11	893	938	-1.605	0	905	775	3.142
-11	485	361	0.992	-10	143	319	-1.186 †	1	485	243	0.000
-10	2003	2009	1.787	-9	1487	1384	0.469	2	656	629	0.000
-9	2151	2022	-1.388	-8	1007	850	-1.932	3	965	1060	0.000
-8	828	824	-0.577	-7	1017	915	-3.086	4	175	252	0.000 †
-7	991	942	2.073	-6	526	417	0.640	5	1251	1077	3.142
-6	1551	1406	1.809	-5	1243	1169	1.111	6	1462	1300	0.000
-5	496	455	2.375	-4	429	461	2.308	7	175	143	3.142 †
-4	2443	2274	2.236	-3	2667	2850	-2.297	8	1787	1698	3.142
-3	944	856	2.466	-2	2278	2244	2.590	9	452	334	3.142
-2	2756	2831	-1.544	-1	1353	1395	0.750	10	922	749	3.142

11	1708	1664	3.142	
12	1222	1121	0.000	
13	852	786	0.000	
14	516	433	3.142	
15	1334	1111	0.000	
16	1887	1613	0.000	
17	143	4	0.000	†
•	6	1		
-21	729	801	2.700	
-20	708	741	-1.033	
-19	595	713	-0.304	
-18	632	661	-1.448	
-17	708	787	-1.753	
-16	846	935	-0.842	
-15	563	536	0.059	
-14	840	740	1.207	
-13	441	379	1.643	
-12	441	489	-2.535	
-11	1497	1562	2.531	
-1	1544	1582	1.400	
-9	1380	1375	0.646	
-8	882	722	-0.495	
-7	875	875	3.042	
-6	535	450	1.170	
-5	1259	1376	-0.687	
-4	944	979	-1.141	
-3	1227	1024	-1.638	
-2	2496	2619	-2.263	
-1	1708	1514	-2.599	
	2463	2253	2.576	
1	336	311	-1.471	
2	417	412	1.493	
3	2223	2388	2.336	
4	965	1034	1.472	
5	1331	1343	0.917	
6	2490	2330	2.354	
7	2146	2430	-1.489	
8	1746	1791	-0.870	
9	175	195	2.672	†
1	965	849	-2.935	
11	417	353	-0.922	
12	175	90	0.709	‡
13	175	192	-2.199	‡
14	405	439	-0.037	
15	485	536	0.310	
16	92	74	-2.069	†
17	563	519	2.604	
•	6	2		
-22	365	407	-2.145	
-21	545	454	1.731	
-2	111	227	-1.908	†
-19	101	282	2.994	†
-18	545	473	0.951	
-17	933	912	-0.288	
-16	379	425	-2.781	
-15	554	489	2.767	
-14	944	953	-0.994	
-13	1577	1598	-0.272	
-12	1387	1334	0.181	
-11	1323	1192	1.238	
-1	1175	1065	-0.143	
-9	202	306	0.809	†
-8	1323	1259	2.289	
-7	1485	1435	-2.522	
-6	1137	990	-1.839	
-5	1721	1726	1.961	
-4	1161	911	3.120	
-3	1596	1522	-1.592	
-2	474	371	-1.796	
-1	1372	1473	-2.924	
	1609	1543	-0.082	
1	2422	2434	0.689	
2	1122	1084	1.453	
3	1151	1037	0.145	
4	572	499	-0.856	
5	996	984	-1.447	
6	581	558	0.041	
7	202	133	-0.012	†
8	1625	1742	-2.617	
9	1199	1172	2.566	
1	615	516	-1.739	
11	803	843	-2.831	
12	882	962	2.690	
13	632	561	0.729	
14	954	888	-0.482	

15	648	470	0.352	
16	286	302	3.093	
17	441	403	-1.730	
•	6	3		
-21	365	394	-1.858	
-20	535	551	-0.122	
-19	101	235	-0.484	†
-18	379	431	-2.266	
-17	1307	1382	-1.261	
-16	701	690	-1.314	
-15	452	266	2.280	
-14	535	461	-0.996	
-13	1372	1271	2.602	
-12	1596	1571	2.005	
-11	392	320	-1.941	
-10	686	564	2.035	
-9	1656	1559	2.742	
-8	624	527	2.964	
-7	1210	1179	0.478	
-6	1104	1045	0.392	
-5	1714	1608	-0.361	
-4	2066	1945	-0.812	
-3	1094	1062	2.969	
-2	905	820	-2.569	
-1	1596	1587	-0.594	
0	954	909	-1.523	
1	1104	1081	1.036	
2	624	633	1.960	
3	686	806	-2.089	
4	888	884	0.951	
5	1795	1903	0.958	
6	882	859	-0.462	
7	485	334	1.035	
8	671	667	1.755	
9	175	193	-2.824	†
10	1153	1166	-1.322	
11	350	361	1.933	
12	834	807	2.411	
13	563	549	-1.546	
14	708	607	-1.583	
15	143	70	1.138	†
•	6	4		
-19	496	459	-2.736	
-18	496	446	-2.979	
-17	554	525	1.853	
-16	516	556	-2.255	
-15	474	551	-2.066	
-14	640	770	1.043	
-13	143	241	-1.528	†
-12	143	245	2.981	†
-11	722	733	-0.316	
-10	545	426	1.372	
-9	143	77	1.020	†
-8	777	765	-2.593	
-7	632	613	2.764	
-6	840	924	1.494	
-5	1117	1028	-2.378	
-4	2202	2227	-2.553	
-3	143	265	-1.692	†
-2	590	530	-0.023	
-1	1857	1708	-0.988	
0	671	672	1.543	
1	590	520	0.566	
2	870	823	-0.260	
3	708	685	0.770	
4	1315	1432	1.383	
5	496	418	-0.913	
6	615	645	-2.260	
7	1046	1062	2.258	
8	506	571	-2.909	
9	770	862	-2.737	
10	846	908	2.821	
11	120	231	-1.172	
12	888	812	-0.983	
13	715	660	0.228	
14	704	776	0.397	
•	6	5		
-17	365	290	-0.151	
-16	581	610	-1.604	
-15	303	317	-1.660	
-14	736	826	0.642	
-13	333	294	-0.830	
-12	581	661	-3.138	
-11	679	771	1.602	
-10	545	560	0.225	

-9	441	427	-2.046	
-8	485	484	1.352	
-7	1046	1133	2.159	
-6	809	728	3.003	
-5	954	968	-0.544	
-4	840	829	-0.462	
-3	736	710	-1.941	
-2	916	946	-2.117	
-1	679	632	-2.246	
0	496	481	-2.821	
1	535	484	-2.343	
2	405	306	2.450	
3	770	787	1.673	
4	516	517	2.205	
5	770	891	2.654	
6	464	457	1.758	
7	365	417	1.063	
8	303	302	-2.740	
9	143	102	-0.850	†
10	701	701	0.043	
11	1108	1112	-1.723	
12	803	739	-2.194	
•	7	0		
-19	320	258	0.000	
-18	101	142	3.142	†
-17	572	489	0.000	
-16	648	620	0.000	
-15	143	224	3.142	†
-14	429	367	-0.000	
-13	1749	1793	0.000	
-12	1117	1096	3.142	
-11	365	354	3.142	
-10	1473	1413	0.000	
-9	175	44	3.142	†
-8	2115	2061	3.142	
-7	1007	946	3.142	
-6	175	64	3.142	†
-5	777	697	3.142	
-4	933	913	0.000	
-3	933	842	0.000	
-2	452	368	3.142	
-1	1699	1519	3.142	
0	736	623	0.000	
1	2160	1948	0.000	
2	526	465	0.000	
3	2669	2689	3.142	
4	1218	1151	3.142	
5	175	174	0.000	†
6	960	968	3.142	
7	350	281	3.142	
8	777	416	3.142	
9	1483	1443	3.142	
10	175	228	0.000	†
11	1066	1041	0.000	
12	330	275	3.142	
13	506	407	0.000	
14	1409	1181	0.000	
15	1051	956	0.000	
•	7	1		
-21	526	562	-0.900	
-20	663	701	-0.039	
-19	392	395	2.763	
-18	101	152	-1.409	†
-17	624	654	-0.820	
-16	101	245	-2.044	†
-15	607	549	0.874	
-14	715	696	0.435	
-13	143	174	-0.454	†
-12	615	542	2.129	
-11	1071	1115	0.991	
-10	1346	1399	-0.092	
-9	441	410	-2.346	
-8	516	547	1.981	
-7	624	620	-1.174	
-6	803	815	-1.946	
-5	840	818	2.355	
-4	624	610	-0.801	
-3	1162	1123	-1.514	
-2	1455	1315	2.589	
-1	1130	1122	-1.576	
0	1046	968	-0.238	
1	1507	1558	1.895	
2	175	336	-0.634	†
3	1323	1338	0.055	
4	1342	1417	2.057	

5	1334	1445	2.596	-15	506	565	0.879	12	143	70	0.000 †
6	554	561	-0.662	-14	452	476	-0.312		8	1	
7	694	830	-1.014	-13	111	199	-1.352 †	-18	286	309	-0.676
8	175	213	-3.097 †	-12	506	445	1.029	-17	101	149	-3.082
9	828	884	-2.025	-11	563	556	0.923	-16	506	539	2.326
10	535	476	-1.062	-10	764	800	-2.057	-15	101	85	0.563 †
11	175	69	-2.365 †	-9	1158	1424	-1.914	-14	464	435	-2.888
12	535	491	2.721	-8	729	739	1.896	-13	809	852	1.419
13	642	539	-0.176	-7	828	810	2.516	-12	949	1131	0.930
14	753	703	-0.275	-6	1259	1282	-2.484	-11	143	126	1.022
15	764	737	3.024	-5	722	682	2.575	-10	624	677	-2.633
	7	2		-4	485	412	-0.487	-9	590	591	-1.540
-20	320	322	-2.606	-3	996	1137	-0.412	-8	1288	1302	-1.286
-19	303	285	-0.113	-2	572	574	0.043	-7	143	123	-2.909 †
-18	572	641	-0.561	-1	750	693	1.479	-6	607	556	-1.755
-17	101	91	0.207 †	0	128	259	-1.437 †	-5	175	129	-2.414 †
-16	417	380	3.037	1	607	606	-1.111	-4	350	273	2.779
-15	615	635	-1.129	2	1175	1244	2.016	-3	175	114	1.903 †
-14	648	655	-0.642	3	976	1035	2.343	-2	1080	1085	0.357
-13	944	1004	1.599	4	1037	1069	-2.431	-1	986	957	1.205
-12	679	675	1.192	5	686	781	-2.956	0	981	841	1.887
-11	694	711	-0.385	6	464	425	-2.167	1	729	684	-0.212
-10	701	789	2.010	7	516	583	-2.077	2	797	1029	-0.677
-9	1726	1768	-3.108	8	336	385	-2.940	3	784	774	1.756
-8	1846	1868	-2.234	9	474	473	1.645	4	175	187	2.009 †
-7	202	230	2.586 †	10	452	366	-0.922	5	590	639	-0.820
-6	1659	1481	2.132	11	701	649	-1.119	6	526	562	-0.735
-5	1323	1289	-1.156		7	5		7	286	208	2.457
-4	816	801	-0.249	-15	679	806	0.457	8	581	537	-0.911
-3	764	750	-2.936	-14	303	365	-1.816	9	648	740	-0.669
-2	1500	1624	0.335	-13	350	328	2.224	10	722	713	3.111
-1	2239	2432	-0.029	-12	1323	1506	1.450	11	417	371	2.731
	1117	1047	0.030	-11	286	293	0.677	12	336	290	-0.138
1	1387	1454	1.200	-10	286	231	-0.082		8	2	
2	764	696	0.518	-9	101	248	-0.289 †	-15	101	290	2.381 †
3	954	956	-2.002	-8	563	534	-2.647	-14	590	548	2.721
4	809	796	2.234	-7	545	543	-2.743	-13	336	323	-1.453
5	1162	1295	-1.342	-6	516	546	0.217	-12	143	187	1.319 †
6	554	567	-1.987	-5	554	562	-0.385	-11	1099	1121	2.598
7	715	737	2.670	-4	834	846	-1.194	-10	1188	1265	-2.567
8	526	520	1.577	-3	526	505	-1.634	-9	876	807	-1.917
9	708	736	-0.651	-2	496	460	1.813	-8	464	362	-2.721
10	632	633	-3.132	-1	452	376	3.019	-7	663	679	1.277
11	545	581	2.596	0	840	958	2.663	-6	474	464	1.246
12	663	646	0.148	1	828	844	1.332	-5	474	421	-1.815
13	656	677	0.539	2	392	495	0.922	-4	954	865	1.336
14	563	452	0.991	3	248	345	2.692	-3	1405	1424	-0.272
	7	3		4	101	81	1.739 †	-2	1066	1099	-0.629
-19	686	617	-1.639	5	750	721	0.691	-1	175	278	0.964 †
-18	545	558	-1.938	6	268	251	-1.885	0	175	329	0.062 †
-17	554	540	3.062	7	417	372	-1.639	1	598	582	-0.898
-16	708	760	-2.383	8	202	182	-0.334 †	2	526	490	-2.685
-15	329	282	2.404	9	722	734	-1.256	3	1188	1241	3.051 †
-14	1251	1307	1.616		8	0		4	175	258	-2.357
-13	1337	1113	1.612	-19	535	534	0.000	5	485	416	-2.557
-12	143	259	-0.106 †	-18	1153	1211	0.000	6	743	719	1.874
-11	1194	1062	2.553	-17	106	69	0.000 †	7	590	630	0.571
-1	464	494	-2.606	-16	320	242	-3.142	8	175	220	0.569 †
-9	949	933	-0.908	-15	809	793	0.000	9	175	167	2.628 †
-8	175	151	-0.733 †	-14	101	186	0.000 †	10	143	189	-1.080 †
-7	736	648	-2.973	-13	1051	1012	3.142	11	663	630	-0.547
-6	1101	822	-1.135	-12	143	259	3.142 †		8	3	
-5	1247	1253	-1.843	-11	1094	925	0.000	-16	545	478	1.629
-4	175	212	0.507 †	-10	143	128	0.000 †	-15	656	567	1.164
-3	799	711	1.878	-9	429	268	3.142	-14	268	283	1.556
-2	1452	1554	-1.474	-8	143	58	0.000 †	-13	506	530	2.339
-1	736	736	1.052	-7	143	103	3.142 †	-12	101	204	2.752 †
	922	874	1.333	-6	563	424	3.142	-11	320	309	-0.203
1	784	722	-1.062	-5	1051	933	0.000	-10	816	891	-0.792
2	175	289	-1.541 †	-4	1323	1258	0.000	-9	417	407	-1.336
3	1022	960	1.815	-3	441	406	3.142	-8	607	589	2.341
4	893	839	1.623	-2	1323	1187	3.142	-7	1007	973	-1.457
5	590	621	-0.274	-1	175	60	3.142 †	-6	441	450	-0.645
6	581	446	2.584	0	1405	1325	0.000	-5	441	359	3.109
7	794	846	2.204	1	175	70	0.000 †	-4	441	421	-2.617
8	963	900	-1.764	2	960	824	3.142	-3	365	275	-1.781
9	640	646	-2.003	3	910	773	3.142	-2	679	593	1.243
10	175	293	1.844 †	4	893	831	3.142	-1	563	509	1.931
11	379	326	-0.776	5	803	748	3.142	0	350	347	-1.788
12	441	377	-1.070	6	226	227	0.000	1	991	997	-2.848
13	441	384	0.799	7	202	248	0.000	2	1188	1196	2.162
	7	4		8	572	491	0.000	3	175	113	0.919 †
-18	286	421	1.171	9	563	506	0.000	4	175	100	1.235 †
-17	268	244	1.092	10	143	23	3.142 †	5	379	394	1.972
-16	320	309	0.378	11	535	430	3.142	6	535	577	3.014

7	864	789	-1.582
8	143	192	-2.014 †
9	268	243	-0.941
*	8	4	
-15	392	421	-0.207
-14	656	705	-1.487
-13	615	611	1.752
-12	268	361	0.975
-11	268	347	-1.554
-1	115	2:6	0.687 †
-9	392	338	-2.570
-8	1012	1096	-2.653
-7	485	445	2.545
-6	615	633	1.319
-5	526	563	0.843
-4	840	892	-0.587
-3	124	261	-0.045 †
-2	417	465	0.900
-1	554	570	-0.431
	111	215	0.873 †
1	624	714	2.324
2	648	718	3.068
3	506	461	-2.793
4	526	579	2.810
5	336	403	-2.365
6	581	634	-1.888
7	76	200	2.843 †
8	554	580	0.452
*	8	5	
-1.	268	251	2.788
-9	226	278	-1.465
-8	268	335	-1.964
-7	202	195	0.960 †
-6	516	456	-0.673
-5	286	260	-2.200
-4	809	773	0.937
-3	485	505	-0.377
-2	320	313	-1.969
-1	516	550	1.993
	624	792	1.395
1	379	385	1.609
2	248	246	-1.218
3	452	408	-0.724
4	441	485	-0.198
*	9	0	
-16	764	698	0.000
-15	78	154	3.142 †
-14	1113	980	3.142
-13	101	65	3.142 †
-12	708	708	0.000
-11	474	472	3.142
-1	777	761	3.142
-9	365	356	0.000
-8	365	323	3.142
-7	143	227	3.142 †
-6	976	873	0.000
-5	876	805	0.000
-4	143	146	3.142 †
-3	143	260	3.142 †
-2	784	679	0.000
-1	715	615	0.000
	143	116	3.142 †
1	624	519	0.000
2	452	247	3.142
3	797	726	3.142
4	640	488	3.142
5	736	489	3.142
6	679	622	0.000
7	1099	1026	0.000
8	1037	911	0.000
*	9	1	
-12	496	567	2.855
-11	485	601	-3.139
-1:	101	206	-1.226 †
-9	632	677	-1.698
-8	679	740	-2.114
-7	143	98	-3.076 †
-6	417	460	0.403
-5	764	797	2.318
-4	143	201	-0.842 †
-3	790	809	-0.218
-2	554	574	1.396
-1	927	977	1.368
	496	507	-0.103
1	598	661	-1.779

2	545	543	3.033
3	143	175	-1.863 †
4	965	1135	-0.633
5	336	210	2.162
6	429	447	2.272
7	572	591	-0.595
*	9	2	
-14	320	279	-3.009
-13	526	579	2.538
-12	516	587	3.122
-11	581	612	-2.054
-10	764	843	-1.504
-9	429	412	1.837
-8	379	327	-0.073
-7	496	531	-0.598
-6	143	217	-0.461 †
-5	916	947	1.082
-4	392	422	0.017
-3	143	155	-1.544 †
-2	640	732	2.783
-1	392	356	-0.895
0	535	548	-1.185
1	1046	1256	2.848
2	922	1042	3.077
3	392	413	-2.247
4	365	409	-1.913
5	474	561	2.485
6	143	172	1.641 †
7	143	202	0.875 †
*	9	3	
-12	607	645	-0.275
-11	1001	957	-0.737
-10	598	241	0.783
-9	248	237	2.617
-8	535	429	-1.985
-7	101	46	0.561 †
-6	392	358	2.319
-5	405	360	-1.664
-4	143	69	2.218 †
-3	694	651	1.627
-2	392	380	1.163
-1	143	144	-1.451 †
0	379	410	2.218
1	797	816	2.565
2	554	466	-1.623
3	143	84	2.613 †
4	320	278	2.057
5	101	620	-1.534 †
*	9	4	
-9	516	609	-1.569
-8	303	405	-0.512
-7	648	797	1.027
-6	379	386	0.454
-5	392	372	-0.078
-4	679	779	1.085
-3	268	271	0.248
-2	429	368	-1.822
-1	563	594	2.608
0	405	376	2.221
1	336	331	-1.095
*	10	0	
-10	590	548	0.000
-9	78	91	0.000
-8	303	259	3.142
-7	750	687	0.000
-6	101	284	0.000 †
-5	101	33	3.142 †
-4	101	138	0.000 †
-3	852	703	3.142
-2	615	549	3.142
-1	563	544	0.000
0	101	127	0.000 †
1	379	346	0.000
*	10	1	
-10	303	352	-2.942
-9	429	441	-1.781
-8	516	534	0.284
-7	90	28	0.567
-6	365	313	2.416
-5	101	95	2.236 †
-4	945	486	-1.101
-3	365	347	1.093
-2	607	553	1.405
-1	535	454	-0.914
0	816	803	3.033

1	899	1010	2.648
*	10	2	
-8	365	432	0.092
-7	506	578	1.185
-6	417	446	-0.642
-5	350	340	-0.667
-4	464	611	3.116
-3	656	882	-3.107
-2	101	128	-2.224 †
-1	392	477	-1.877

Results and Discussion

The atomic coordinates of the non-hydrogen atoms and their standard deviations are shown in Table 3, and Table 4 lists the anisotropic temperature factor coefficients. ELSI was used to calculate the intra-molecular bond distances and angles and their standard deviations shown in Tables 5 and 6. The intra-molecular bond distances and angles are also shown in Figure 2. That the estimated standard deviations are too low can be seen from the dimensions of the benzene ring, in which the C - C bond lengths range from 1.34 Å to 1.43 Å, indicating that the e.s.d.'s in Tables 5 and 6 have been underestimated by perhaps a factor of three. This is, no doubt due to the block-diagonal approximation of the least-squares program.

The Configuration of the Molecule

The view of the molecule as shown in Figure 3 was derived from the final atomic coordinates by redefining their values with respect to the plane of the atoms S1, C6 and C4. Figure 3 shows that in the crystal at least, the molecule exists in a rather extended configuration as opposed to the curled forms of benzylpenicillin and 6-NBF-PA. The asymmetric centres at C6 and C7 are both R, (using the terms for chirality proposed and defined by Cahn, Ingold and Prelog (1966)), which is the

Table 3b. Atomic coordinates and standard deviations output from cycle A20 for the 3-spiro-thiazolinium-7-PAC derivative.

(fractional values)	X	Y	Z	σ_x	σ_y	σ_z
S_1	0.40182	0.81764	0.29429	0.00019	0.00027	0.00007
C_2	0.39849	0.75748	0.20363	0.00066	0.00092	0.00028
C_3	0.21655	0.71990	0.16468	0.00060	0.00088	0.00026
C_4	0.12234	0.58335	0.20692	0.00062	0.00084	0.00026
N_5	0.24289	0.48782	0.26052	0.00052	0.00077	0.00021
C_6	0.33625	0.58921	0.32018	0.00073	0.00098	0.00026
C_7	0.46553	0.42010	0.33023	0.00070	0.00098	0.00028
C_8	0.36282	0.35330	0.25995	0.00066	0.00092	0.00027
C_9	0.11601	0.90107	0.15082	0.00071	0.00091	0.00030
S_{10}	0.15464	0.99421	0.06962	0.00021	0.00027	0.00008
C_{11}	0.20262	0.77005	0.04416	0.00063	0.00096	0.00027
N_{12}	0.23123	0.64606	0.09555	0.00056	0.00070	0.00021
N_{13}	0.64054	0.46150	0.33197	0.00058	0.00090	0.00025
C_{14}	0.76834	0.34013	0.35927	0.00078	0.00111	0.00034
C_{15}	0.93905	0.40895	0.35974	0.00083	0.00156	0.00048
C_{16}	1.07545	0.31144	0.41139	0.00081	0.00131	0.00033
C_{17}	1.14044	0.40453	0.47206	0.00099	0.00140	0.00040
C_{18}	1.27654	0.31844	0.51807	0.00106	0.00179	0.00036
C_{19}	1.33923	0.15357	0.50272	0.00110	0.00164	0.00038
C_{20}	1.27030	0.06257	0.44115	0.00107	0.00154	0.00042
C_{21}	1.14061	0.14745	0.39573	0.00093	0.00130	0.00037

Table 3b continued

	X	Y	Z	σ_x	σ_y	σ_z
C_{22}	0.27546	0.45226	0.08176	0.00067	0.00084	0.00028
C_{23}	0.46137	0.42789	0.07739	0.00077	0.00116	0.00033
C_{24}	-0.01397	0.45690	0.16453	0.00062	0.00088	0.00028
O_{25}	-0.10451	0.53246	0.11447	0.00050	0.00068	0.00020
O_{26}	-0.02332	0.29427	0.18508	0.00054	0.00072	0.00024
O_{27}	0.38446	0.23180	0.21984	0.00051	0.00068	0.00020
O_{28}	0.72824	0.18588	0.38048	0.00065	0.00099	0.00036
N_{29}	0.21564	0.73742	-0.01975	0.00059	0.00082	0.00022
O_{30}	0.70759	0.08559	0.19846	0.00062	0.00082	0.00029
O_{31}	0.69064	-0.12790	0.08104	0.00069	0.00087	0.00024
O_{32}	0.83998	-0.17257	0.30179	0.00080	0.00108	0.00036

Table 4. 3-Spiro-thiazolinium-7-PAC derivative anisotropic temperature factor coefficients

	β_{11}	β_{22}	β_{33}	β_{23}	β_{13}	β_{12}
S_1	0.01324	0.01220	0.00187	-0.00206	0.00064	-0.00504
C_2	0.00903	0.01125	0.00197	0.00026	0.00086	-0.00217
C_3	0.00734	0.01075	0.00164	-0.00223	0.00090	-0.00045
C_4	0.00786	0.00808	0.00158	-0.00056	0.00030	0.00101
N_5	0.00926	0.00950	0.00148	-0.00042	0.00068	-0.00296
C_6	0.01284	0.01448	0.00127	-0.00205	0.00099	-0.00650
C_7	0.01100	0.01362	0.00160	0.00103	0.00022	-0.00552
C_8	0.00975	0.01222	0.00166	0.00068	0.00067	-0.00319
C_9	0.01071	0.00948	0.00217	-0.00085	0.00101	0.00188
S_{10}	0.01699	0.00784	0.00258	0.00162	0.00185	0.00242
C_{11}	0.00803	0.01461	0.00178	0.00190	-0.00030	-0.00442
N_{12}	0.01089	0.00809	0.00134	-0.00034	0.00134	0.00126
N_{13}	0.00968	0.01821	0.00186	0.00190	0.00021	-0.00072
C_{14}	0.01317	0.01506	0.00264	0.00395	0.00071	0.00166
C_{15}	0.00995	0.03357	0.00476	0.01460	0.00175	0.00231
C_{16}	0.01310	0.02097	0.00245	0.00450	0.00145	-0.00224
C_{17}	0.01874	0.02357	0.00293	-0.00231	0.00341	0.00332
C_{18}	0.02237	0.03500	0.00215	-0.00224	0.00032	0.00438
C_{19}	0.02146	0.03230	0.00236	0.00161	0.00001	0.00536
C_{20}	0.02163	0.02569	0.00304	0.00097	0.00220	0.01332
C_{21}	0.01679	0.02045	0.00264	-0.00189	0.00030	0.00571

Table 4 continued

	β_{11}	β_{22}	β_{33}	β_{23}	β_{13}	β_{12}
C_{22}	0.01081	0.00798	0.00185	-0.00108	0.00220	0.00277
C_{23}	0.01271	0.01931	0.00236	-0.00047	0.00400	0.00858
C_{24}	0.00816	0.01076	0.00194	-0.00154	0.00022	-0.00327
O_{25}	0.01222	0.01287	0.00195	-0.00138	-0.00012	-0.00492
O_{26}	0.01310	0.01081	0.00302	0.00097	0.00058	-0.00694
O_{27}	0.01206	0.01117	0.00211	-0.00107	0.00071	0.00150
O_{28}	0.01386	0.02159	0.00582	0.01169	0.00399	0.00008
N_{29}	0.01151	0.01258	0.00150	0.00179	0.00099	-0.00395
O_{30}	0.01459	0.01452	0.00415	-0.00168	0.00483	-0.00280
O_{31}	0.02132	0.01960	0.00238	-0.00145	0.00261	0.00092
O_{32}	0.02324	0.02141	0.00524	0.00452	0.00542	-0.00097

All temperature factors in this table are positive definite.

Table 5. 3-Spiro-thiazolinium-7-PAC derivative intra-molecular bond distances and estimated standard deviations in Ångstroms.

Bond		Bond	
S1 - C2	1.835 (.005)	C14 - O28	1.233 (.010)
S1 - C6	1.825 (.007)	C15 - C16	1.529 (.011)
C2 - C3	1.553 (.007)	C16 - C17	1.389 (.010)
C3 - C4	1.568 (.007)	C16 - C21	1.348 (.012)
C3 - C9	1.532 (.009)	C17 - C18	1.433 (.012)
C3 - N12	1.487 (.006)	C18 - C19	1.343 (.016)
C4 - N5	1.472 (.007)	C19 - C20	1.403 (.011)
C4 - C24	1.550 (.008)	C20 - C21	1.390 (.011)
N5 - C6	1.471 (.007)	C22 - C23	1.527 (.008)
N5 - C8	1.369 (.008)	C24 - O25	1.243 (.006)
C6 - C7	1.590 (.009)	C24 - O26	1.243 (.008)
C7 - C8	1.558 (.007)		
C7 - N13	1.437 (.007)		
C8 - O27	1.211 (.007)		
C9 - S10	1.813 (.005)		
S10 - C11	1.751 (.007)		
C11 - N12	1.338 (.007)		
C11 - N29	1.306 (.006)		
N12 - C22	1.475 (.008)		
N13 - C14	1.351 (.009)		
C14 - C15	1.507 (.010)		

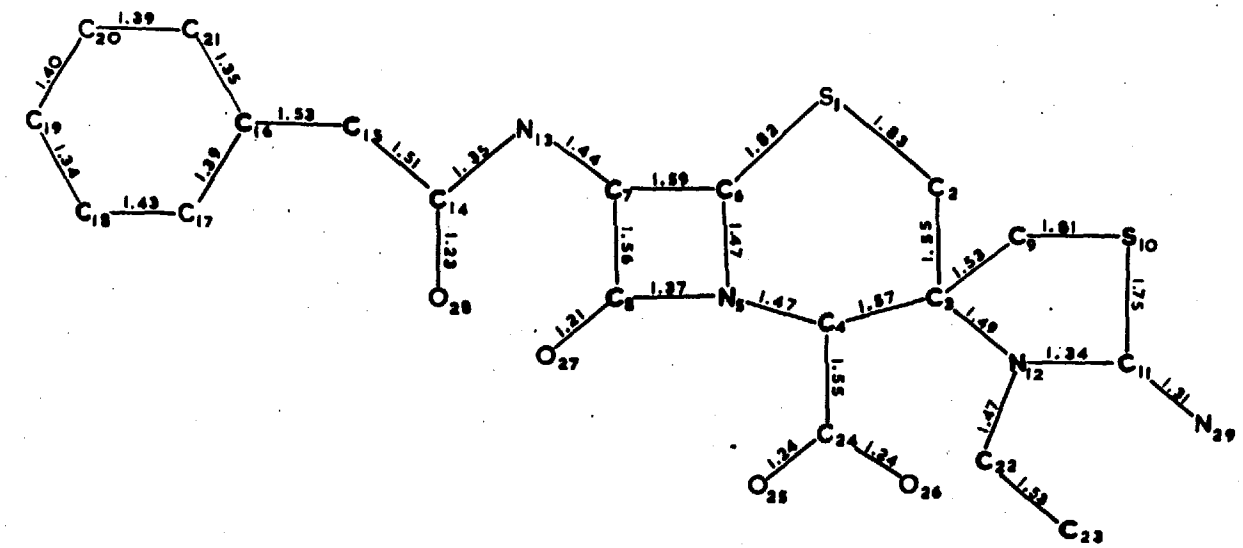
Table 6. 3-Spiro-thiazolinium-7-PAC derivative intra-molecular bond angles and standard deviations in degrees.

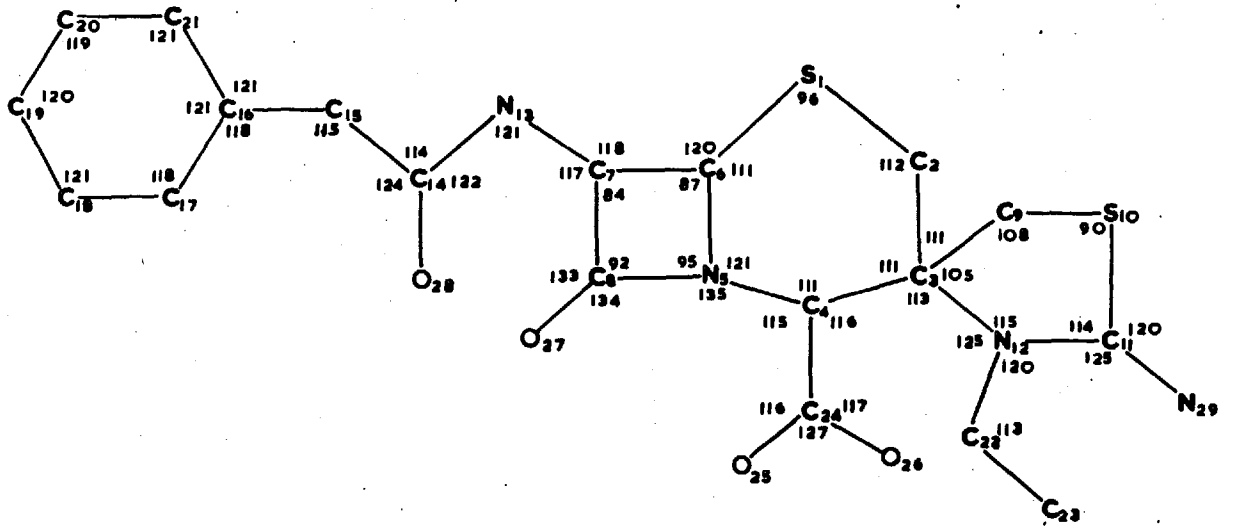
C2 - S1 - C6	96.1 (0.3)	C9 - S10 - C11	89.7 (0.3)
S1 - C2 - C3	111.8 (0.4)	S10 - C11 - N12	114.3 (0.7)
C2 - C3 - C4	110.5 (0.5)	S10 - C11 - N29	120.5 (0.6)
C2 - C3 - C9	111.3 (0.6)	N12 - C11 - N29	125.1 (0.7)
C2 - C3 - N12	107.0 (0.5)	C3 - N12 - C22	124.6 (0.6)
C4 - C3 - C9	109.6 (0.6)	C3 - N12 - C11	115.2 (0.5)
C4 - C3 - N12	112.9 (0.5)	C11 - N12 - C22	120.2 (0.7)
C9 - C3 - N12	105.4 (0.5)	C7 - N13 - C14	121.1 (0.7)
C3 - C4 - N5	110.7 (0.5)	N13 - C14 - C15	114.2 (0.7)
C3 - C4 - C24	116.4 (0.6)	N13 - C14 - O28	121.7 (1.1)
N5 - C4 - C24	115.3 (0.5)	C15 - C14 - O28	124.1 (1.1)
C4 - N5 - C6	121.4 (0.5)	C14 - C15 - C16	114.9 (0.7)
C4 - N5 - C8	134.5 (0.8)	C15 - C16 - C17	118.0 (0.8)
C6 - N5 - C8	95.5 (0.5)	C15 - C16 - C21	120.8 (1.1)
C6 - C7 - C8	83.8 (0.4)	C17 - C16 - C21	121.0 (1.1)
C6 - C7 - N13	117.7 (0.7)	C16 - C17 - C18	117.8 (0.9)
C8 - C7 - N13	116.7 (0.6)	C17 - C18 - C19	120.9 (1.4)
N5 - C8 - C7	92.3 (0.4)	C18 - C19 - C20	119.9 (1.3)
N5 - C8 - O27	134.3 (1.0)	C19 - C20 - C21	119.3 (1.0)
C7 - C8 - O27	133.4 (0.8)	C16 - C21 - C20	121.0 (1.2)
S10 - C9 - C3	107.6 (0.5)	N12 - C22 - C23	113.0 (0.6)

Table 6 continued

C4 - C24 - 025	115.6 (0.6)
C4 - C24 - 026	117.0 (0.7)
025 - C24 - 026	127.5 (0.9)

Figure 2. The 3-spiro-thiazolinium-7-PAC derivative bond lengths and angles.

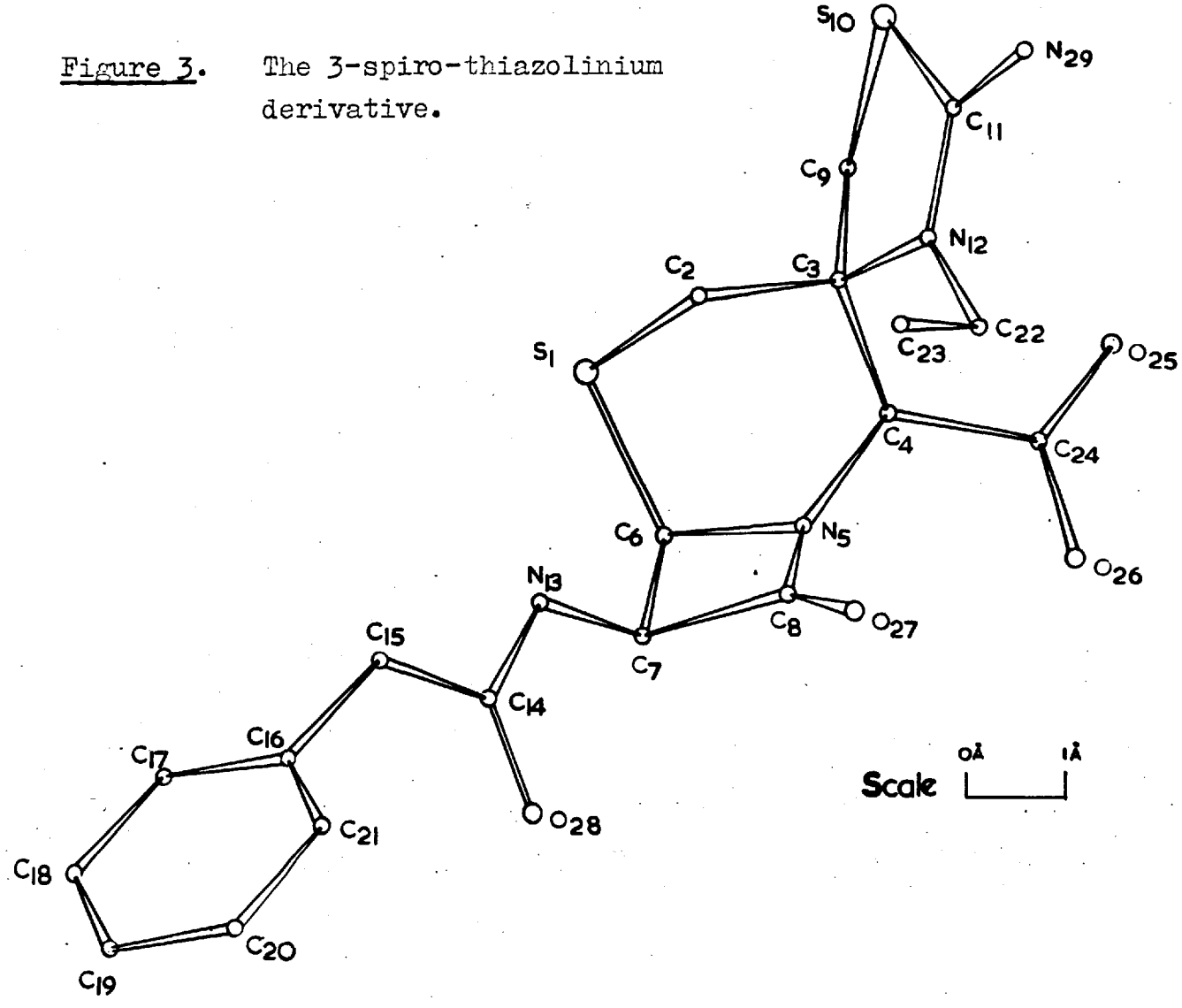




$C_2 - C_3 - N_{12} = 107^\circ$

$C_4 - C_3 - C_9 = 110^\circ$

Figure 3. The 3-spiro-thiazolinium derivative.



same as in all previously determined cephalosporins and penicillins. The asymmetric centre at C4 is S, whereas the corresponding centres in benzylpenicillin, phenoxymethylpenicillin and 6-NBF-PA possess the opposite chirality, R. Using the same notation, the spiro atom at C3 is S. It was the difficulty of describing the configuration at C3 that led the author, with the assistance of Dr.A.G.Long (1966), to adopt the Cahn-Ingold-Prelog notation which gives the name (3S,4S,6R,7R)-2'-amino-3'-ethyl-2'-thiazolinium-4'-spiro-3-(7-phenylacetamidocepham-4-carboxylate) trihydrate, where the 'cepham' system, suggested by Morin et al. (1962) is shown in Figure 4.

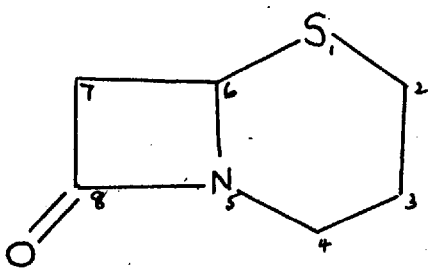


Figure 4. The cepham system

The analysis has shown that the ethyl group (C22, C23) is bonded to the cyclic nitrogen atom (N12) of the thiazolinium group.

The Thiazolinium Ring

DIDO was used to calculate the least-squares planes through sets of atoms in the molecule, and the results are shown in Table 7.

The atoms C3, S10, C11, N12, C22 and N29 form a plane (Table 7a), the average deviation from the plane being 0.01 Å, and C9 lies 0.45 Å below the plane looking at the molecule as seen in Figure 3. The bond C9 - S10 (1.813 Å) is in good agreement with the average value of 1.817 Å for a single C - S bond given by Sutton (1965). However, S10 - C11 (1.751 Å) is approximately 9 e.s.d.'s shorter than this and nearer to the value of 1.718 Å in the conjugated, heterocyclic compound thiophen. Sutton gives the average C - N bond length in conjugated heterocyclics as 1.339 Å, with which the bond C11 - N12 (1.338 Å) is in good agreement, and C11 - N29 (1.306 Å) is approximately 5 e.s.d.'s shorter.

These results suggest that the thiazolinium system can be represented as in Figure 5, N29 being probably hydrogen

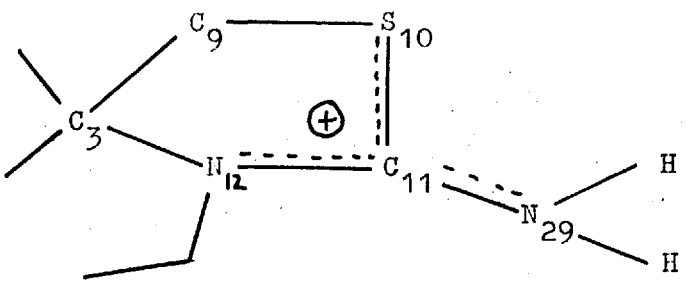


Figure 5. The thiazolinium ring.

bonded (see page 122). The bond lengths of the carboxyl group, C24 - 025 (1.243 Å) and C24 - 026 (1.243 Å) indicate that it is present in the ionized form (C - O = 1.26 Å (Sutton)), so that the analysis substantiates the electrophoresis and infra-red data that suggested the compound occurs as a zwitter-ion. The carboxylate ion is also planar (Table 7e).

The bond angle C9 - S10 - C11 (89.7°) agrees quite well with the C - S - C angle in thiophen (91.3°), although the comparison is questionable as thiophen is completely conjugated. The angle N12 - C22 - C23 (113.0°) differs significantly from the equilibrium value of 109° 28'. This can be explained by repulsion between the C23 methyl group and the π electrons at N12, the distance N12 - C23 being 2.503 Å.

The Tetrahydrothiazine Ring

The atoms S1, C2, C3, C4, N5 and C6 form a distorted boat conformation with C2 and N5 at the prow and stern. The boat is distorted in that the bond S1 - C6 is far from parallel to C3 - C4. This can be seen in Figure 3; for an undistorted boat, the atoms S1, C3, C4 and C6 would be in a plane, whereas C3 actually lies 0.656 Å above the plane through the other three atoms.

The reasons for the preferred boat conformation in the crystal structure are difficult to assess. One explanation

may be that the chair form would separate the charges of the thiazolinium system and the carboxylate ion. Whether this is the case was difficult to decide by constructing models, especially as it is not known where the charge is concentrated in the thiazolinium group. The shortest inter-atomic distances relevant to this point are given below in Å

C11 - O25	3.492
C11 - C24	3.902
N12 - C24	2.927
N12 - O25	2.915

Changing the model from the boat to the chair form moved O25 away from N12 but did not noticeably affect the N12 - C24 distance, though it did bring the exocyclic ethyl group into crowding with the β-lactam-tetrahydrothiazine ring system. Even in the boat conformation, the ethyl group is quite close to C2 and O27 as shown by the distances C2 - C23 (3.537 Å), C2 - C22 (3.273 Å), C22 - O27 (3.137 Å) and C23 - O27 (3.299 Å).

Another close contact that may affect the conformation of the six-membered ring is that between the group $\begin{matrix} H \\ H \end{matrix}\!>\!C22$ and the carboxylate ion. The distance C22 - C24 is 3.080 Å, and one of the C22 hydrogen atoms points towards the carboxylate ion. This probably accounts for the fact that the angles C3 - C4 - C24 (116.4°) and N5 - C4 - C24 (115.3°) are both approximately 10 e.s.d.'s from the tetrahedral value and the

distortion may be favoured in that it results in a partial staggering of the bonds C3 - N12 and C4 - C24. It is also possible that the role of the water molecules in the crystal could contribute to the stability of a particular conformer. As will be described later, the water molecules enter into a hydrogen-bonding scheme that includes N29, O25 and O26.

The high degree of overcrowding that has been described may result in restricted rotation of the carboxylate ion around the bond C4 - C24 and possibly account for the low anti-bacterial activity of the spiro compound, in that it would be difficult for either O25 or O26 to acquire a 'penicillin-like' position. Rotation would be especially hindered by the close contacts between O25 and C22 (3.293 Å), O26 and O27 (3.267 Å) and C9 and O25 (3.199 Å).

The rest of the dimensions in the tetrahydrothiazine ring are unremarkable. The bonds S1 - C2 (1.835 Å) and S1 - C6 (1.825 Å) are not significantly different from the value given by Sutton (1.817 Å) for a C - S single bond and the value of 1.80 Å determined by Diamand (1963) for ceph. Cc. The dimensions of the ionized carboxyl group agree well with those listed by Sutton, for example in the tartaric acid ion and the zwitter-ion, L-threonine.

The β-Lactam Ring

The bond lengths and angles of the β-lactam ring in the 3-spiro-thiazolinium-7-PAC derivative are similar to those determined for 6-NBF-PA. The length of the bond C6 - C7 (1.590 Å) is possibly not significant in terms of the real standard deviations; previous crystallographic work has not indicated a bond significantly longer than the accepted value of 1.539 Å.

The least-squares plane through the atoms of the β-lactam ring including O27 shows deviations between 0.02 and 0.10 Å (Table 7b). However, the atoms N5, C7, C8 and O27 form a plane well within the experimental accuracy, with C6 projecting 0.245 Å out of the plane on the same side as S1. This arrangement agrees with that found for 6-NBF-PA.

That resonance takes place to a certain extent in the cyclic amide is shown by the bond length N5 - C8 (1.369 Å) and the angle C4 - N5 - C6 (121.4°) indicates the corresponding tendency for N5 to be planar. However, the nitrogen atom in the 3-spiro-thiazolinium-7-PAC derivative has achieved a greater degree of planarity than the corresponding atom in 6-NBF-PA, as shown by the sum of the angles round both atoms (351° and 340° respectively). This is probably because the six-membered ring in the spiro cephalosporin possesses more degrees of freedom than the thiazolidine ring, so that planarity at N5 can be achieved without exerting a great deal of strain at C6.

Table 7. DEviations (Å) of the atoms from the calculated least-squares planes. Atoms marked with * were not included in the calculation of the plane. $\sum d^2$ is the sum of the squares of the deviations (Å)^2 from the plane.

(a) Thiazolinium Ring

$\sum d^2 = 0.00089$

Atom	Deviation
S10	-0.001
C11	-0.020
N12	-0.008
N29	0.017
C3	0.013
C22	-0.002
C9 *	-0.451

(b) β-Lactam Ring

$\sum d^2 = 0.02569$

Atom	Deviation
N5	-0.080
C6	0.104
C7	-0.063
C8	-0.023
O27	0.062

(c) β-Lactam Ring

$\sum d^2 = 0.00010$

Atom	Deviation
N5	0.002
C7	0.002
C8	-0.009
O27	0.004
C6 *	0.245

(d) Benzene Ring

$\sum d^2 = 0.00040$

Atom	Deviation
C16	-0.008
C17	0.000
C18	0.003
C19	0.003
C20	-0.011
C21	0.014
C15 *	0.091

Table 7 continued

(e) Carboxylate Ion

$\sum d^2 = 0.00011$

Atom	Deviation
O25	-0.003
C24	0.009
O26	-0.003
C4	-0.002

(f) Amide Group

$\sum d^2 = 0.00107$

Atom	Deviation
N13	0.025
C14	-0.002
O28	0.006
C15	-0.011
C7	-0.017

The Phenylacetamido Side Chain

The amide group is planar (Table 7f) and the bond lengths are consistent with those of a normal resonating amide. The angle N13 - C14 - C15 (114.2°) is 8 e.s.d.'s from the expected value of 120°, and C14 - C15 - C16 (114.9°) is 7 e.s.d.'s above the tetrahedral value. These results can be explained by repulsive forces between the carbonyl electrons and the π electrons of the benzene ring, the distance C16 - O28 being 2.900 Å. The extended form of the side chain which results in this close contact is probably stabilized by packing conditions in the crystal.

Packing and Hydrogen Bonding

In the following discussion, atoms that are related to those of Table 3b by the symmetry of the spacegroup are referred to by terms in parentheses following the atom symbol.

Some close-contact distances are shown in Figure 8 which is a projection of adjacent unit cells down the b axis. Inter-molecular distances less than 3.5 Å are listed in Table 8, including those between the oxygen atoms (O30, O31, O32) of the three water molecules.

The molecules of the spiro compound lie diagonally across the cell; the arrangements around the screw axes at

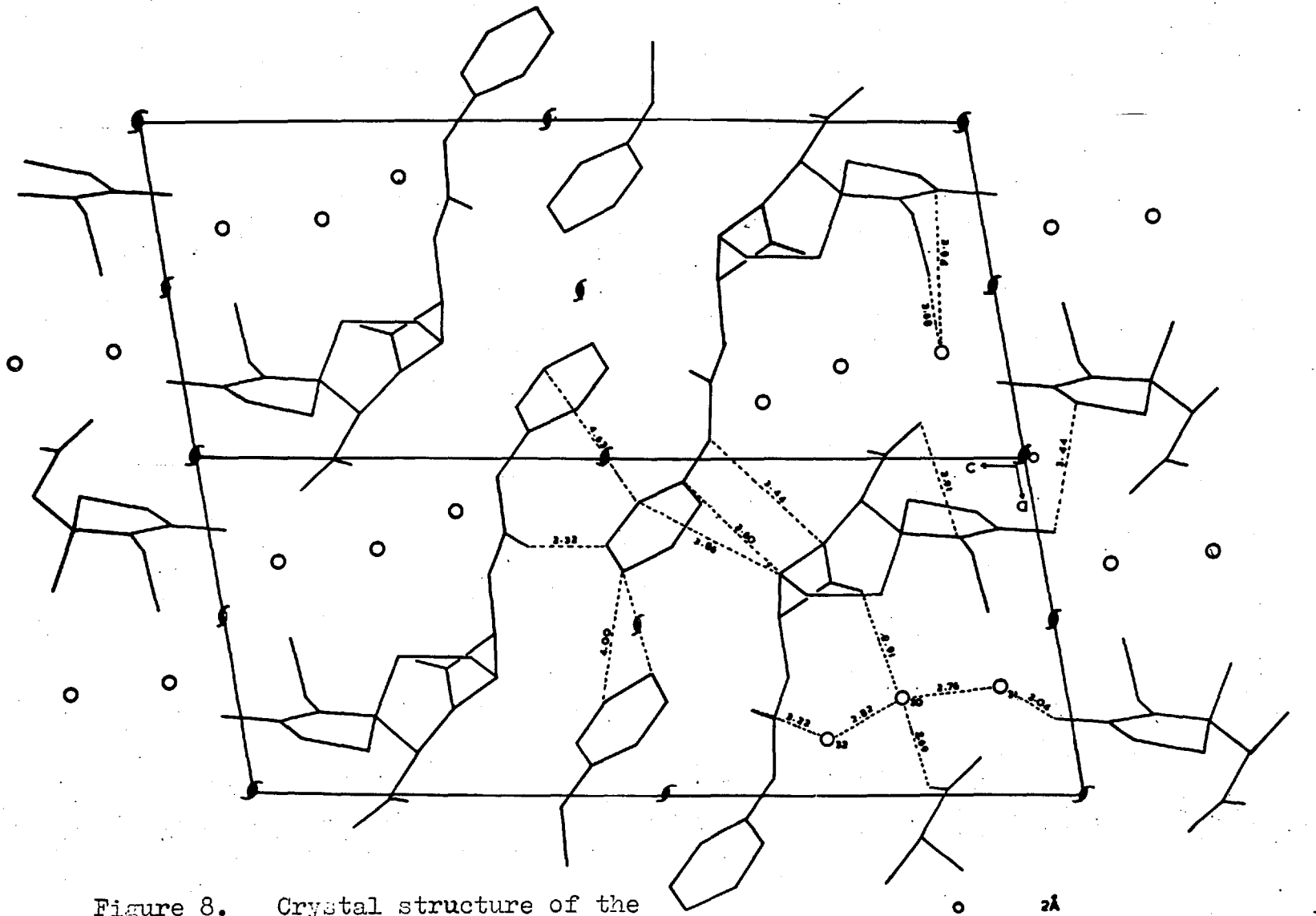


Figure 8. Crystal structure of the spiro compound projected down b.

Table 8. Inter-molecular distances (Å) less than 3.5 Å in the crystal of the 3-spiro-thiazolinium-7-PAC derivative. The terms in parentheses following the atom name relate the position of the atom to the values listed in Table 3b. The first term describes the symmetry relation where

0/ = x, y, z
1/ = -x, y - ½, -z

and the other terms give the number of cell translations in the directions x, y and z respectively.

Vector	Symmetry	Distance	Vector	Symmetry	Distance
S1 - O27	(0/0 1 0)	3.310	C24 - N29	(1/0 0 0)	3.412
C2 - O27	(0/0 1 0)	3.426	O25 - O31	(0/-1 1 0)	2.954
C2 - O30	(0/0 1 0)	3.445	O25 - N29	(1/0 0 0)	2.866
N5 - C15	(0/-1 0 0)	3.445	O26 - O30	(0/-1 0 0)	2.689
C9 - O26	(0/0 1 0)	3.157	O26 - N29	(1/0 0 0)	3.376
C9 - O27	(0/0 1 0)	3.336	O27 - O30		2.909
C9 - O31	(0/-1 1 0)	3.466	O28 - O32		3.217
S10 - C22	(0/0 1 0)	3.428	O30 - O31		2.760
S10 - N29	(1/0 1 0)	3.444	O30 - O32		2.820
N13 - O32	(0/0 1 0)	3.193	O31 - N29	(1/1 0 0)	3.035
C15 - O32	(0/0 1 0)	3.264	C18 - O28	(1/2 1 1)	3.317

O 8

z = 0, 1 etc. concern the polar groups, whereas the screw axes at z = 1/2, 3/2 etc. are surrounded mainly by the non-polar benzyl groups. The close contacts around the screw axis at $x = \frac{1}{2}$, $z = \frac{1}{2}$ are between the benzene rings and the β -lactam rings though the distances are quite large as shown in Figure 8.

The oxygen atoms of the three water molecules are located (Figure 8) such that the cephalosporin molecules in adjacent unit cells are linked in all three crystallographic directions by hydrogen bonds. The network so formed can be seen in Figure 9, which is a projection down the a axis. The positions of the atoms shown in Figure 9 are related to those in Table 3b as follows :

CB11 = C11 (1 - x, y - $\frac{1}{2}$, -z)

CC11 = C11 (1 - x, y + $\frac{1}{2}$, -z)

CA24 = C24 (1 + x, y, z)

OA25 = O25 (1 + x, y, z)

OA26 = O26 (1 + x, y, z)

NB29 = N29 (1 - x, y - $\frac{1}{2}$, -z)

NC29 = N29 (1 - x, y + $\frac{1}{2}$, -z)

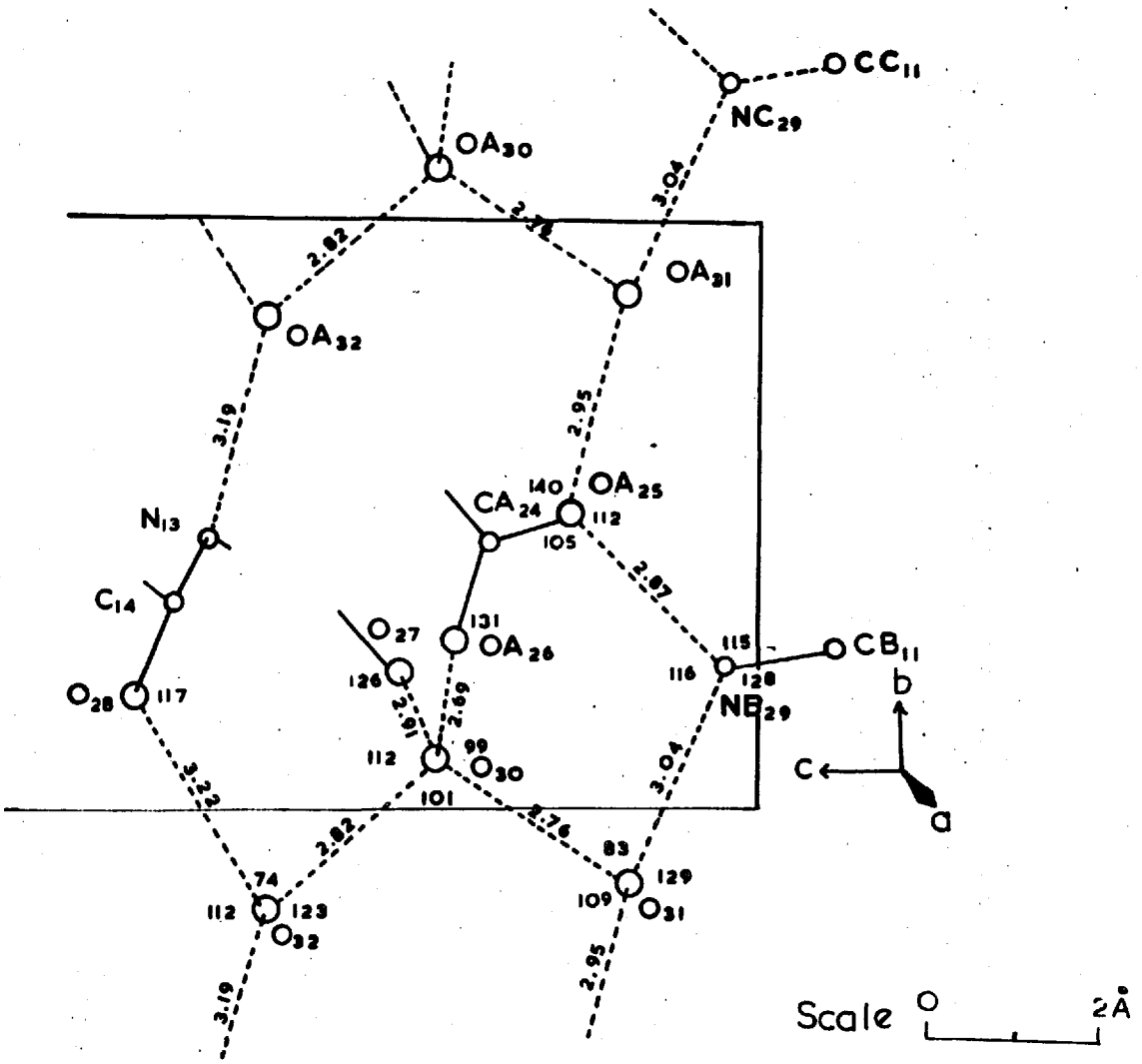
OA30 = O30 (x, y + 1, z)

OA31 = O31 (x, y + 1, z)

OA32 = O32 (x, y + 1, z)

O30 appears to be tetrahedrally bonded to O32 (2.820 Å), O31 (2.760 Å), O26 (1 +x, y, z) (2.689 Å) and O27 (2.909 Å).

Figure 9. The 3-spiro-thiazolinium-7-PAC derivative hydrogen bonding system projected down the a axis



$OA_{26}—O_{30}—O_{27} = 125$

$O_{27}—O_{30}—O_{31} = 114$

$OA_{26}—O_{30}—O_{32} = 103$

The angles around O30 are shown in Figure 9. Pimental and McClellan (1960) have tabulated the known data for the O - H .....O distances and the O.....O.....O angles for water in organic crystals. The values vary over a wide range; the distances from 2.65 Å to 2.99 Å, and the angles from 83° to 120°, and the values associated with O30 are in good agreement with this range. If the hydrogen bonding is as suggested, the hydrogen atoms bonded to O30 must lie approximately in the directions of O27 and O26 (1 + x, y, z).

The atoms surrounding O31 are O25 (1 + x, y - 1, z) (2.954 Å), N29 (1 - x, y - $\frac{1}{2}$, -z) (3.035 Å) and O30 (2.760 Å). N12 (1 + x, y, z) is toofar from O31 (4.101 Å) to take part in any hydrogen bonding. The location of two possible hydrogen atoms bonded to N29 has been described on page 89. For the calculation of the position of H21 (1 - x, y - $\frac{1}{2}$, -z) (Table 2), it was assumed that a linear hydrogen bond existed between O31 and N29 (1 - x, y - $\frac{1}{2}$, -z). The assumption for the calculation of H22 was that the atoms O31, C11(1 - x, y - $\frac{1}{2}$, -z) and the hydrogen atom would form a trigonal arrangement around N29 (1 - x, y - $\frac{1}{2}$, -z). The observed and calculated positions agree well, and the observed position of H21 (1 - x, y - $\frac{1}{2}$, -z) lies 19° off the line N29 (1 - x, y - $\frac{1}{2}$, -z) - O31 and that of H22 (1 - x, y - $\frac{1}{2}$, -z) is situated 7° from the line N29 (1 - x, y - $\frac{1}{2}$, -z) - O25 (1 + x, y, z). Thus, the hydrogen atoms bonded to O31 probably lie in the directions of O30 and

025 (1 + x, y, z).

The distances 032 - 028 (3.217 Å) and 032 - N13 (x, y - 1, z) (3.193 Å) suggest that weak hydrogen bonding may exist between these atoms. The angles around 032 are shown in Figure 9 and only 030 - 032 - 028 (74°) lies out of the range given by Pimentel and McClellan, and the two lone pair orbitals of 028 will not be pointing well towards 032, resulting in only a weak hydrogen bond. The calculated and observed positions of the hydrogen bonded to N13 (x, y - 1, z) are situated respectively 28° and 17° off the line 032 - N13 (x, y - 1, z), which indicates that the hydrogen atoms bonded to 032 are orientated towards 030 and 028.

The hydrogen-bonding network that has been described must contribute substantially to the stability of the crystal, and as mentioned on page 118 possibly plays an important part in stabilising the observed boat conformation of the tetrahydrothiazine ring.

CHAPTER 6

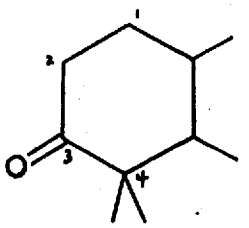
The Chemistry of Zeorin

As mentioned at the end of Chapter 1, attempts have been made to solve the crystal structure of zeorin acetate, a compound containing only carbon, hydrogen and oxygen ($C_{32}H_{54}O_3$) and derived from the natural product zeorin, a member of the triterpenoid series. The chemical work, which is briefly described in this chapter has established the structure of zeorin fairly well and shown that it is derived from a unique, saturated hydrocarbon zeorinane. The proposed structure of zeorin fits in well with the theory of squalene as the biogenetic precursor of the triterpenoids.

That zeorin is a component of foliaceous lichens was recognised by Hesse (1906) and Zopf (1909) but its chemistry received little attention until Asahina and Akagi (1938) demonstrated that it belongs to the triterpenoid series and has the formula $C_{30}H_{52}O_2$. Mild acetylation produced the mono-acetate, zeorin acetate, and Asahina and Yosioka (1940) showed that more vigorous acetylation gave anhydrozeorin acetate, by elimination of the elements of water from zeorin acetate. This indicated that zeorin contained a tertiary - OH group.

Zeorin, when heated with alcoholic hydrochloric acid gave a dehydration product, zeorinin, which also formed a monoacetate and when oxidised with dichromate gave a ketone, zeorininone ($C_{30}H_{48}O$), indicating the presence in zeorin of a secondary - OH group.

These conclusions were substantiated and extended by Barton and Bruun (1952). Infra-red data for zeorinone (produced by chromic acid oxidation of zeorin) indicated a cyclohexanone part-structure, but the oxygen atom could not be at the C_3 position, which is most common in the triterpenoids and shown in (I), as zeorinone is unreactive to normal carbonyl reagents



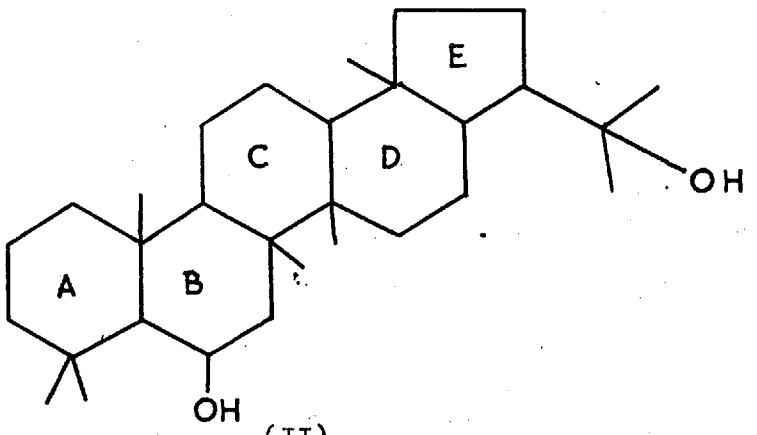
(I)

and reduction. Dehydration of zeorin acetate gave zeorinin acetate or an isomer, isozeorinin acetate, depending upon the reaction conditions. The infra-red absorption spectrum of isozeorinin acetate (probably equivalent to the anhydrozeorin acetate of Asahina and Yosioka) indicated the presence of a methylene group as $>C=CH_2$, hence the tertiary - OH of zeorin as $>CMe - OH$. That zeorin is not a simple derivative of a known triterpene was demonstrated by the preparation of

zeorinane, the unique and fully saturated hydrocarbon.

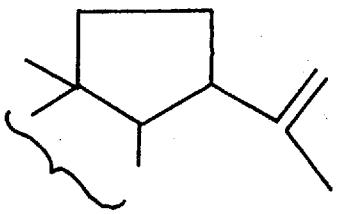
The above and other important reactions of zeorin and its derivatives are shown in Figure 1.

After further work, Barton, de Mayo and Orr (1958) proposed the structure (II) for zeorin.



(II)

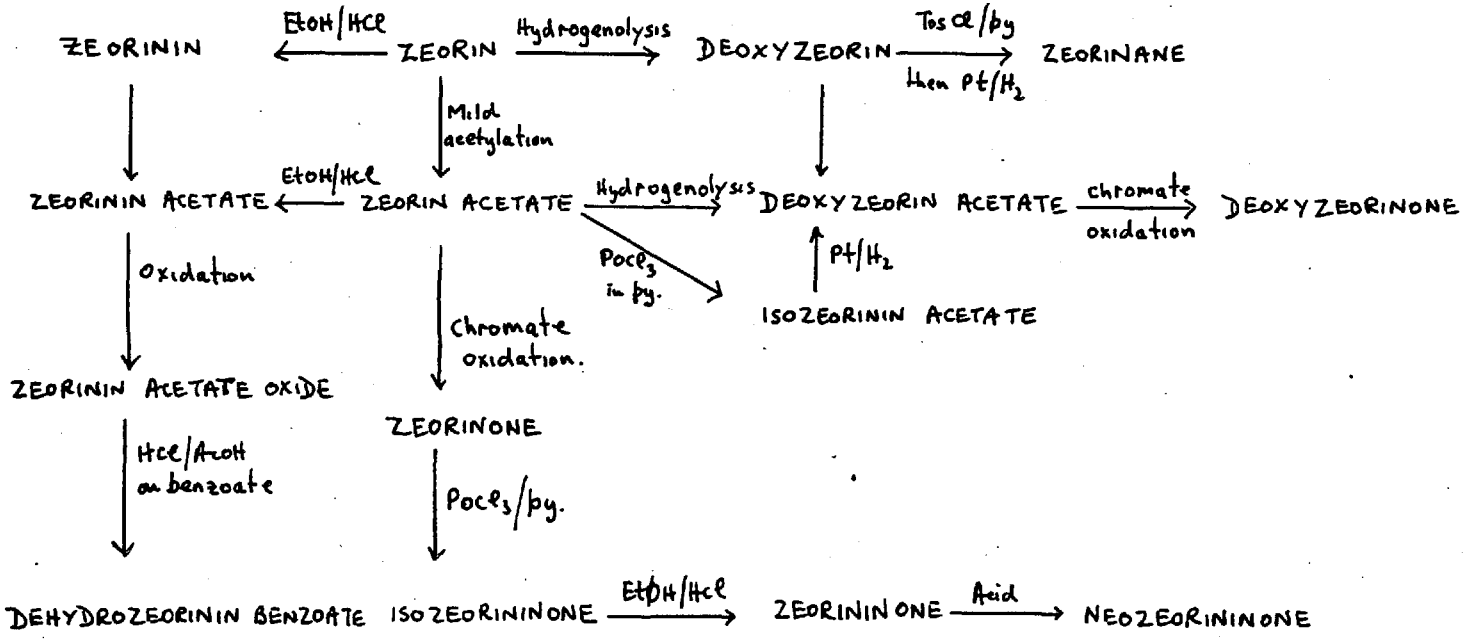
Ozonolysis of crude isozeorinin acetate gave a methyl ketone with the loss of one carbon atom, indicating the part-structure (III) in isozeorinin acetate (that the E ring is five-membered



(III)

is shown in the following section). The ozonolysis also produced acetone which suggested the presence of the

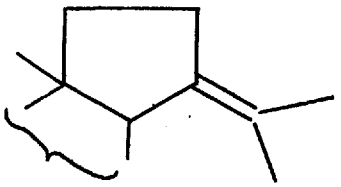
Figure 1. Some reactions of zeorin.



TosCl = p - $C_6H_4Me.SO_2Cl$

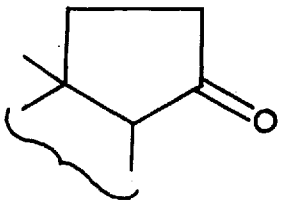
Py = pyridine.

isopropylidene isomer (IV) in the crude starting material.



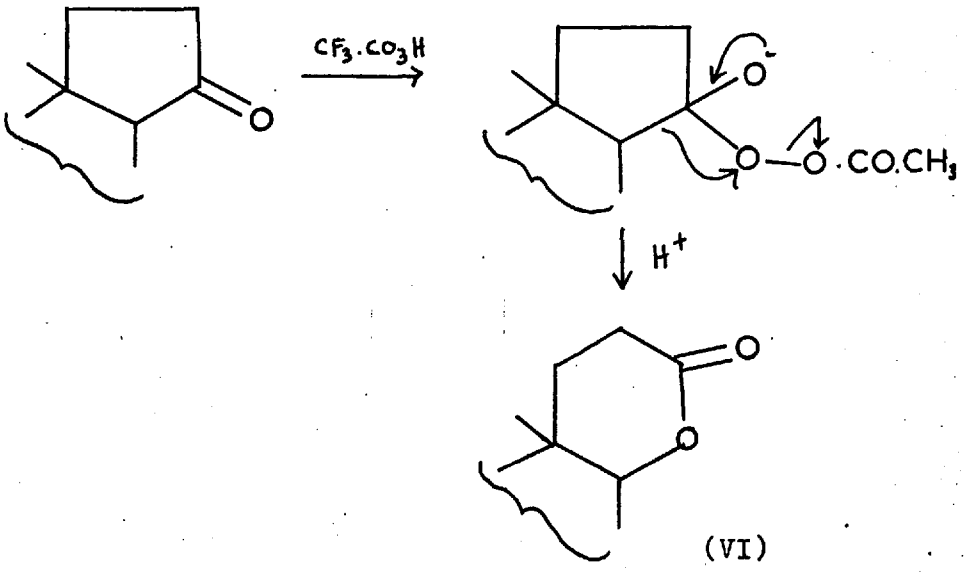
(IV)

Recognition of the ketone (V) was made by further oxidation



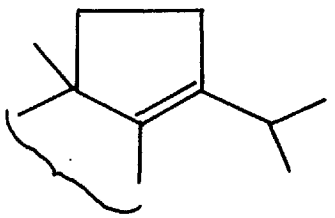
(V)

of the product with trifluoro peracetic acid to give the δ-lactone (VI) by the following mechanism.



(VI)

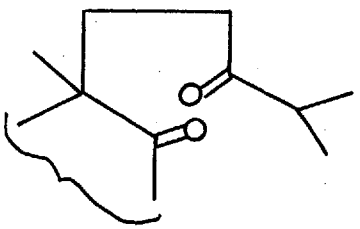
(VI) was recognised as a δ-lactone from its infra-red spectrum, and its formation indicated that the E ring must be five-membered. Hence zeorinin was assigned the part-structure (VII)



(VII)

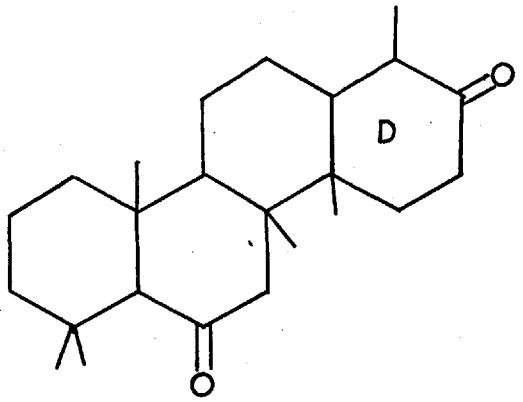
where the double bond of isozeorinin,(III), has migrated into the E ring.

Other oxidative experiments on zeorinin acetate using osmium tetroxide and lead tetra-acetate, resulted in the 1:5 diketone (VIII). Barton et al. predicted that (VIII) should



(VIII)

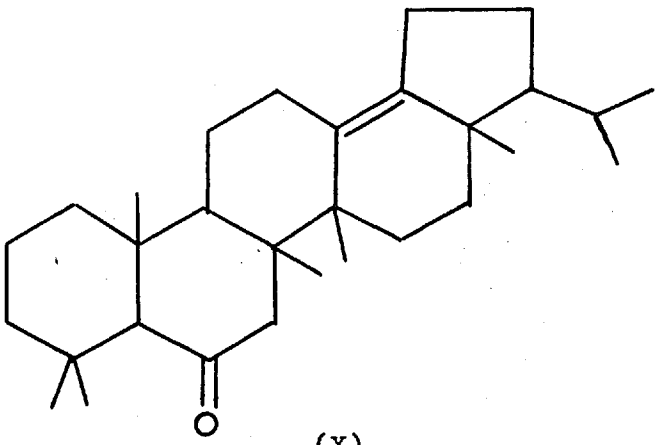
undergo a reverse Michael addition, to give after oxidation, the diketone (IX). The product from the action of alkali in



(IX)

ethylene glycol on (VIII) followed by oxidation showed a single band in the I.R. spectrum at 1703 cm^{-1}, characteristic of a cyclohexanone structure and confirming that the D ring must be six-membered,as in (IX).

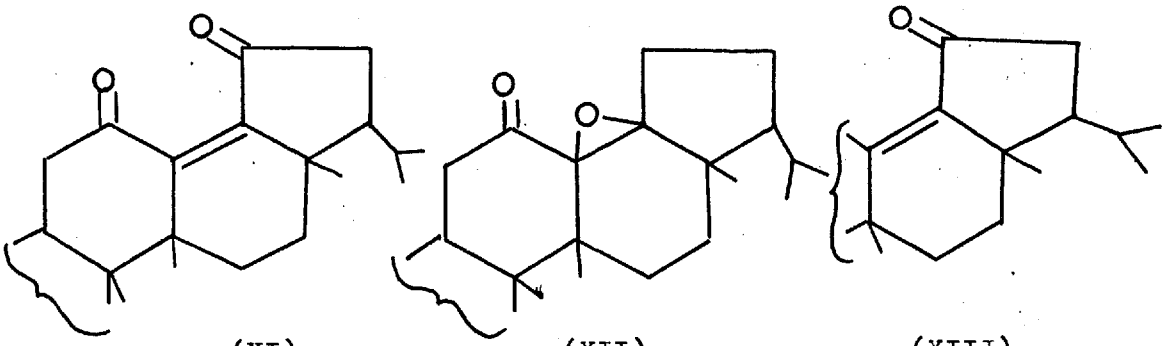
The probable identity of the C ring was determined by studies on neozeorininone, obtained from acid isomerisation of zeorininone. By analogy with other rearrangements in the triterpenoid series, neozeorininone was assigned the structure (X).



(X)

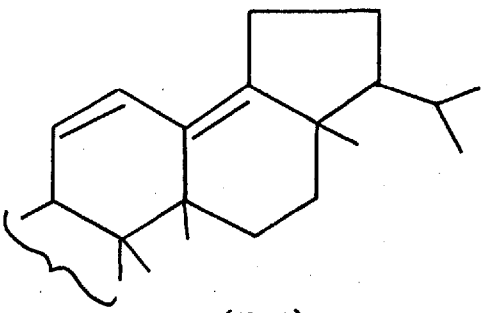
This was supported by oxidation of neozeorininone with chromic

acid to give three products whose spectral properties could be explained in terms of the part-structures (XI), (XII) and (XIII).



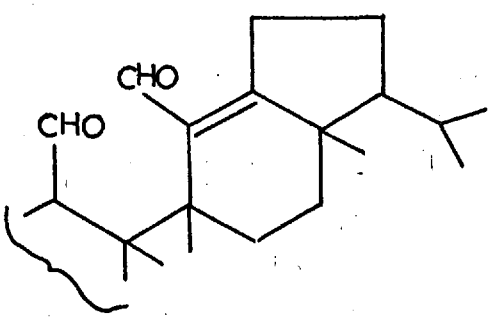
(XI) (XII) (XIII)

Selenium dioxide oxidation of neozeorinone afforded a conjugated, heteroannular diene (XIV), which was converted into an aldol



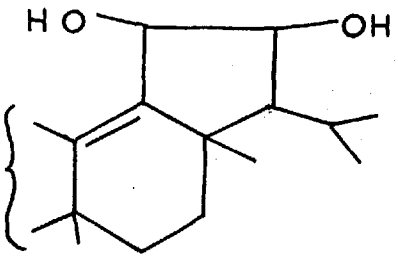
(XIV)

condensation product via the dialdehyde (XV). The formation



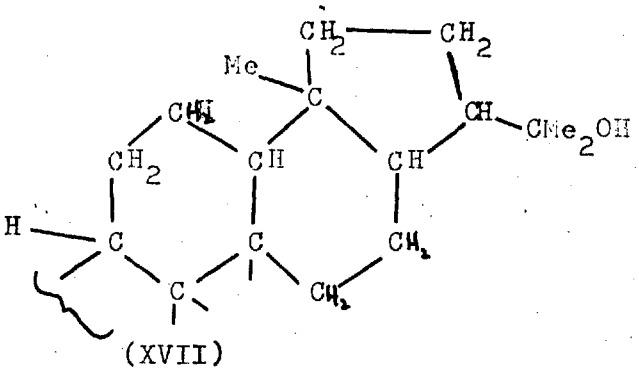
(XV)

of an aldol condensation product and its probable structure, deduced from spectral properties, excluded the partial structure (XVI) for the parent diol, and together with the reactions so



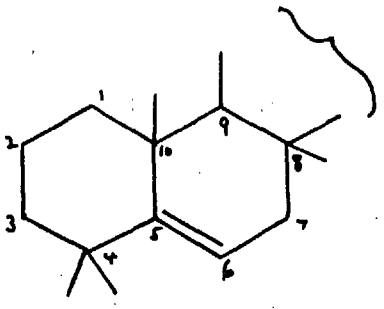
(XVI)

far, suggested the part-structure (XVII) for the C, D and E rings in zeorin.



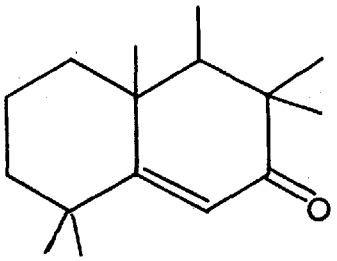
(XVII)

The secondary - OH group is located at the C6 position in the B ring. This was demonstrated by dehydration of deoxy-zeorin (obtained by hydrogenolysis of zeorin) to give, as main product, a compound represented by the part-structure (XVIII).



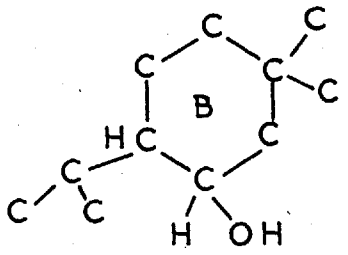
(XVIII)

Chromic acid oxidation of this unsaturated hydrocarbon gave an $\alpha\beta$-unsaturated ketone (XIX), which was stable to the action



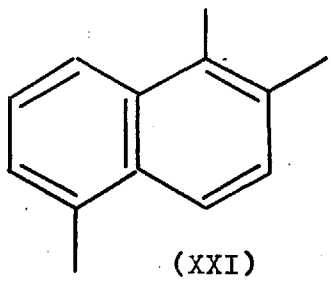
(XIX)

of bromine and selenium dioxide, in agreement with the absence in (XIX) of a replaceable α-hydrogen atom, and suggesting that the B ring possesses the part-structure (XX).



(XX)

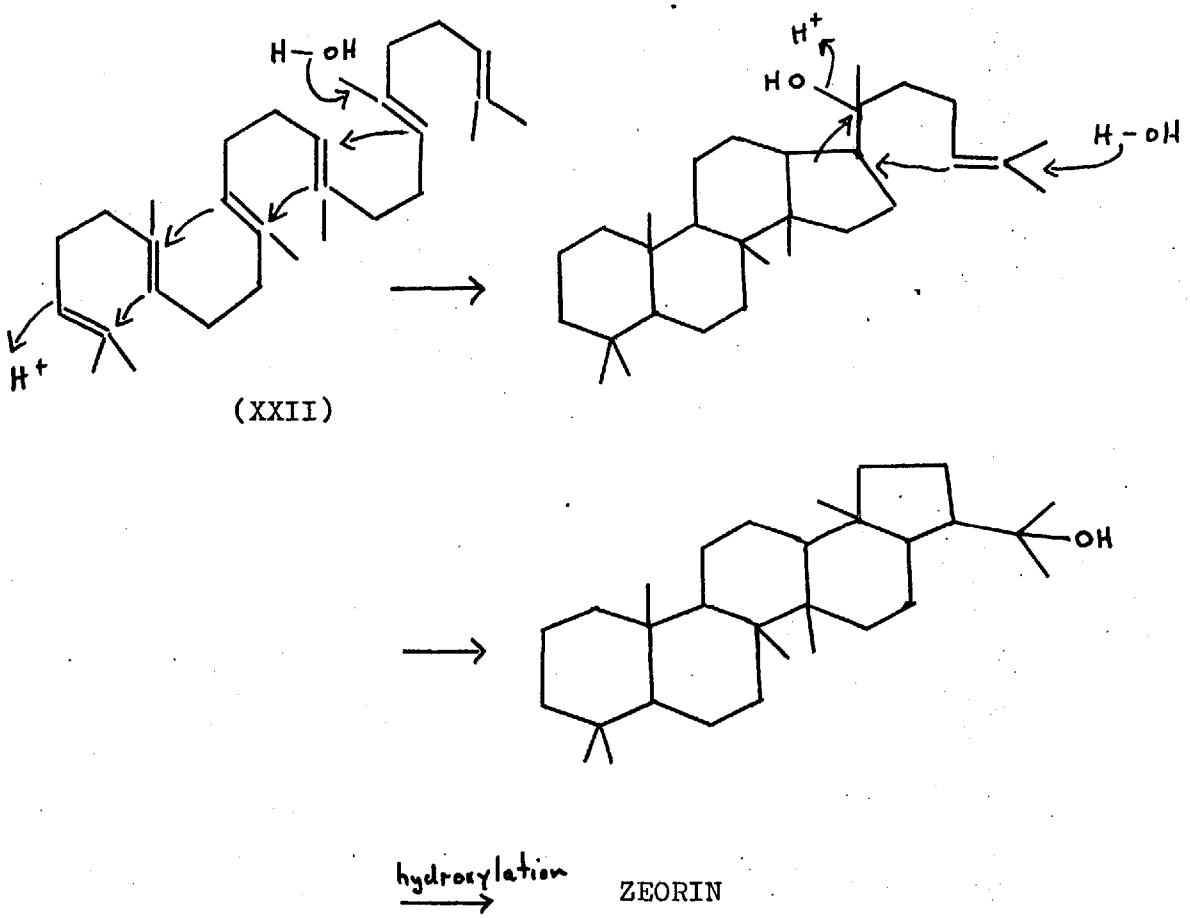
The structure of the A and B rings was further supported by the fact that Asahina and Yosioka obtained 1 : 2 : 5 trimethyl naphthalene (XXI) from zeorin. It is not likely that the C



(XXI)

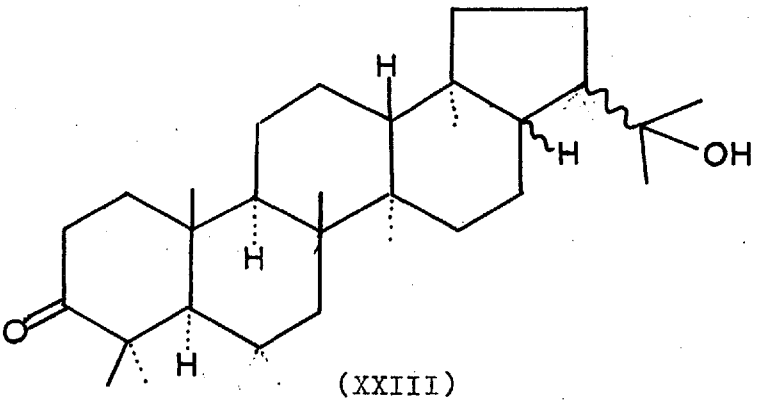
and D rings would have given this product.

Barton, de Mayo and Orr (1958) then suggest that it is 'attractive' to place the part-structure (XVI) and the A and B rings together as implied in structure (II) so that the biogenesis of zeorin, with squalene as precursor,(XXII), can be represented by the cyclisation of squalene followed by rearrangement and hydroxylation, as follows :



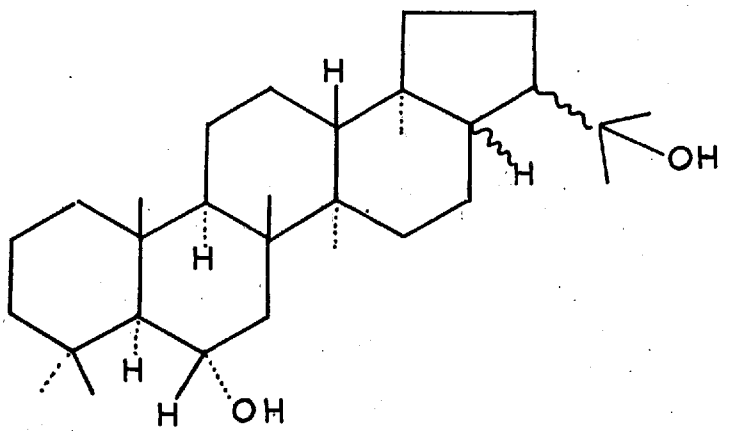
(XXII)

Structure (II) has also received support from the elucidation of the structure of hydroxyhopanone (XXIII), a



(XXIII)

triterpenoid obtained from dammar resin. This compound, studied by Dunstan et al. (1957), Schaffner et al. (1957) and Fazakerley, Halsall and Jones (1959) forms a series of compounds analogous to zeorinone. By consideration of the molecular rotation changes between the derivatives, it is suggested that seven of the nine asymmetric centres in zeorin can be assigned as in hydroxyhopanone, only the stereochemistry at C17 and C21 being ambiguous. Thus zeorin can be written as in (XXIV), the



(XXIV)

- OH at C6 being α-equatorial, as suggested by the ease of acetylation. Similarities between the zeorin and hydroxyhopane series have also been pointed out by Huneck (1961). Wolff - Kishner reduction of zeorinone produced 6-deoxyzeorin, which was shown by physical methods to be probably identical to hydroxyhopane. Huneck (1963) used nuclear magnetic resonance spectroscopy on zeorin acetate to deduce that the proton at C6 is β-axial, and that the protons at C17 and C21 are both β-axial.

Thus, the chemical work that has been described establishes the structure of zeorin fairly well, but it was felt that a confirmation of the structural details was desirable. Two crystalline derivatives of zeorin were initially supplied by Professor D.H.R.Barton of this department, with a view to determining the crystal structure by the Heavy-Atom method. However, both zeorin iodoacetate and zeorin-m-iodobenzoate deteriorated very rapidly when exposed to X-rays.

Crystals of zeorin acetate, again supplied by Professor Barton, did not deteriorate noticeably when exposed to X-rays for long periods. Although lack of a heavy atom in the crystal would cause substantial crystallographic problems, it was felt that the postulated structure and stereochemistry of the molecule would provide a useful starting point in an X-ray

analysis by Patterson searching techniques. It was considered that if the method had proved successful, it might have been applicable to similar compounds in the triterpene series and in the steroids.

Another possible means of determining the crystal structure of zeorin acetate was by one of the 'Direct Methods' of phase determination (e.g. Harker and Kasper (1947), Sayre (1952), Hauptman and Karle (1953)) but these methods are difficult to apply to non-centrosymmetric crystals. Zeorin acetate is in fact noncentrosymmetric (spacegroup $P2_12_12_1$) and of the two possible centrosymmetric projections, down $\underline{a}$ (8.78 Å) and $\underline{b}$ (11.59 Å) preliminary investigations (Chapter 8) indicated that the former was an inconvenient 'edge-on' view of the molecule and a considerable degree of overlap would occur in the latter.

CHAPTER 7

Interpretation of the Patterson for Complex Structures

In recent years there have been basically two different approaches towards interpreting the Patterson of molecules containing a large number of similarly weighted atoms. All the methods are dependent upon the use of a high-speed digital computer.

If no reasonable assumptions concerning the structure of the molecule can be made, it may be possible to achieve a solution by the use of an image-seeking function to compare for each point in the unit cell (x, y, z), values of the Patterson corresponding to all vectors between x, y, z and its symmetry-related points. Mighell and Jacobson (1963) have used this method which they call Vector Verification; if all the vectors have corresponding peaks in the Patterson, then the point may define an approximate atomic location. The validity of all suggested positions can then be tested by computing vectors between them and searching the Patterson for the corresponding peaks.

Simpson et al. (1963) made use of the Symmetry Minimum Function (SMF) in the solution of the structure of iso - $B_{18}H_{22}$.

The method is essentially the same as that of Vector Verification and was used first to locate trial atomic positions, and then, in conjunction with normal image-seeking procedures to locate the rest of the molecule. Methods along similar lines have been developed by other authors (Alden et al. (1964), Hamilton (1965)).

The second approach to the problem of Patterson interpretation is to presuppose that the molecule contains a fragment of approximately known internal geometry. Such a method has been described by Nordman and Nakatsu (1963), Nordman and Kumra (1965) and more recently by Nordman (1966).

The orientation of the molecular fragment in the unit cell can be expressed in terms of three Eulerian angles ϕ, θ, ψ with respect to a Cartesian system, fixed in relation to the crystallographic axes. The position (x_o, y_o, z_o) of one atom of the fragment is then required to define uniquely the location of the entire group in the unit cell. The vector set of the molecular fragment is computed over ranges of ϕ, θ, ψ, and at each angular combination the Patterson values at the ends of the vectors can be examined by an image-seeking function. Nordman and Nakatsu chose the minimum function

$$M_p(\phi, \theta, \psi) = \text{Min.}(P_1, P_2, \ldots\ldots P_p)$$

where p is the number of vectors in the vector set and P_p is

the value of the Patterson at the end of the p'th. vector. The values of ϕ, θ, ψ in this'orientation search' that give peaks in the three-dimensional minimum function may then define possible orientations of the molecular fragment in the unit cell.

To find possible values of the positional parameter (x_o, y_o, z_o), a second search (location search) of the Patterson is carried out, but this time computing vectors between atoms in the suitably orientated fragment and their symmetry-related positions. Vectors within a particular fragment are excluded as they have already been considered in the orientation search, so the vector set used will comprise Harker and general vectors. Selecting any arbitrary values as starting point (unless there is evidence of a limited range) the search ranges are successive values of x, y, z along the three-Cartesian axes. The search is less than three-dimensional whenever the origin of the unit cell is arbitrary in one or two dimensions. If tentative values of x_o, y_o, z_o can be found, the rest of the molecule can be identified by normal image-seeking techniques or by Fourier methods. Nordman (1966) has described improvements to the method that include the assignment of vector weights, and a vector refinement procedure to maximise and differentiate between different 'fits' that may be obtained in practice.

A similar method which presupposes a known molecular

fragment has been developed by Hoppe (1957) and is termed the Convolution Molecule Method. It is possible to calculate the 'partial Pattersons' corresponding to both vectors within a molecular fragment and those between symmetry-related fragments. The resulting 'convolution molecules' are termed 'even-indexed' and 'mixed-indexed' respectively as they are calculated by Fourier summations using as coefficients $F_i F_i^*$ and $F_i F_j^*$ where F_i, F_j are 'molecular structure factors'.

The even-indexed convolution molecule may then be used in an orientation search and the mixed-indexed function in a translational search as described for the previous method. In theory, the degree of fit at each orientation and translation could be judged by subtracting the convolution molecule from the Patterson value (both on the same scale) at all points in Patterson space and then summing separately, the positive and negative differences. A large negative sum is unacceptable but a large positive value merely indicates that additional peaks are present in the Patterson due to vectors not accounted for in the convolution molecule. However, this is impracticable in terms of computing time (automated by Huber (1965)) and so the procedure is limited to the maxima of the convolution molecules. The weight of each vector is naturally taken into account by virtue of using the peak heights in this way.

The Computer Programs Used

In view of the structure proposed for zeorin as a result of chemical evidence (Chapter 6), it seemed reasonable to attempt an interpretation of the Patterson of zeorin acetate by an approach in which this information could be utilized. The A, B, C, D, and E rings most probably form a rather rigid system, parts of which would be suitable to define a molecular fragment. The automated version of the Convolution Molecule method was not available but Dr. Nordman of the University of Michigan kindly supplied the listings of computer programs similar to those used by Nordman and Nakatsu (1963). Slight modifications have been made and the programs are now available for use on the I.B.M. 7090 at Imperial College. The three distinct programs are written mainly in MAP (Macro Assembly Programming Language) with several FORTRAN IV subroutines for input and output.

Orientation Search

A flow diagram is shown in Figure 1.

1. The relative coordinates of the atoms in the molecular fragment are input to the program as unweighted Cartesian vector components ($\mathring{A}$), together with three Eulerian-angle search ranges (for convenience the angles ϕ, θ, ψ will be written

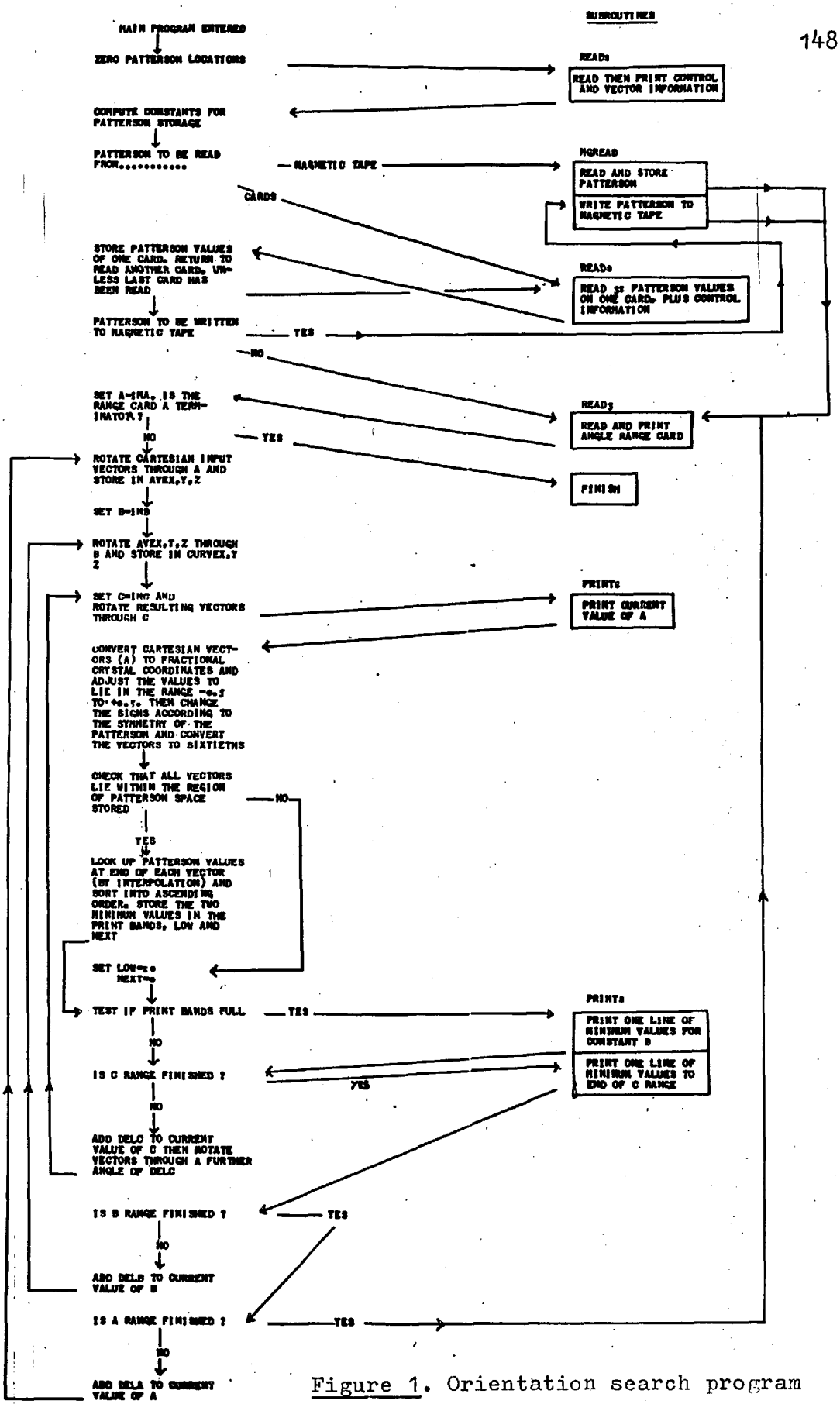


Figure 1. Orientation search program

as A, B, C) and the precomputed Patterson (section 5, page 151), which is stored in the computer in a packed format.

2. The search ranges are input as nine angles :-

(i) The values through which the vectors are initially rotated to the starting point of the search (INA, INB, INC).

(ii) The values by which A, B, and C are to be successively incremented (DELA, DELB, DELC)

(iii) The final values of the three angles (FINA, FINB, FINC).

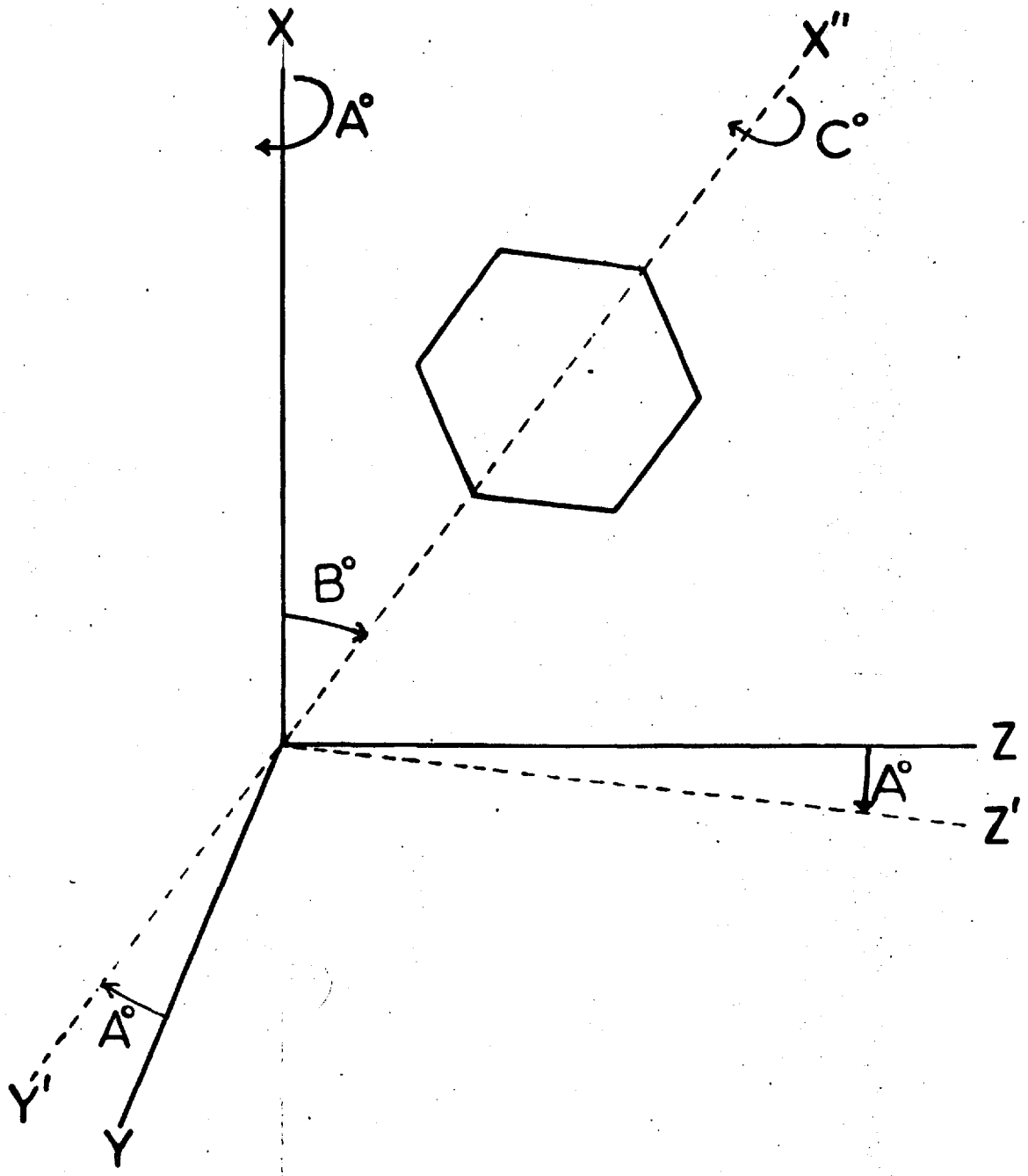
The system is shown in Figure 2, where X, Y and Z are the Cartesian axes of Patterson space. The input vectors are first rotated about the X axis through INA. This rotation also moves the Y and Z axes to Y' and Z' in the YZ plane, making angles of INA with the original axes. Then follows a rotation of INB about Y', which also rotates the X axis through INB to give the X'' axis. The third rotation of INC is about X" to give the vectors their starting orientation for the search.

3. Further rotations are best described by the three nested loops shown below, which also include the starting orientation.

Rotate about the X axis, by INA(DELA)FINA to give a Y' axis
..... Y'..... INB(DELB)FINB ..... X"....
..... X"..... INC(DELC)FINC .....

Calculation
REPEAT
Reset C = INC
REPEAT
Reset B = INB
REPEAT

Figure 2. The Eulerian angles.



4. At each orientation, the appropriately transformed Cartesian vector components are converted to fractional crystal coordinates, which are adjusted to lie in the range $-\frac{1}{2}$ to $+\frac{1}{2}$ by successive addition or subtraction of unity, and then their signs are changed according to the Patterson symmetry, i.e. 2/m for monoclinic and mmm for orthorhombic spacegroups. The vectors are then expressed in sixtieths, as this, at the moment, is the only permissible grid interval. The Patterson values at the ends of the vectors are then evaluated by linear interpolation between four neighbouring grid points, and the two minimum values are stored in the print bands LOW and NEXT, which are subsequently printed out to give the rotational minimum function.

5. The routines for reading and packing the Patterson function in core storage require that the Patterson be initially input on punched cards in a strict format. This is rather unsatisfactory in that it limits each Patterson value to two digits (otherwise the number of cards becomes excessive). A better approach would have been to revise the input routines to accept off I.B.M. magnetic tape, a Patterson with a larger range of values. Unfortunately, no satisfactory Fourier program was available and so it was found expedient to calculate and store the Patterson on I.C.T. magnetic tape using BOSS, and then punch the required cards by a simple Atlas program, 'Patterson Search Punch' (PSP).

Location Search

A flow diagram is shown in Figure 3.

1. A set of Cartesian coordinates (Å) of the molecular fragment are input to the program together with one or more possible sets of Eulerian angles A, B and C, obtained from the orientation search. Up to three symmetry-element matrices are input for the generation of the equivalent positions of the fragment, and also three search ranges as follows :-

(i) The initial values in fractional coordinates to be added to the input positions (INX, INY, INZ).

(ii) The values by which the positions are to be incremented along the crystallographic axes (DELX, DELY, DELZ).

(iii) The final values (FINX, FINY, FINZ).

After input of the Patterson and rotation of the fragment through the Eulerian angles, the Cartesian coordinates are converted to fractional values and the fragment then moved to some arbitrary starting point by addition of INX, INY, INZ to its atomic positions.

2. One or more symmetry-related fragments are then generated and the unique vectors between them and the original fragment are computed. The vectors are adjusted and vector-end Patterson values interpolated and printed as in the orientation search.

3. The fragment is then moved in the crystallographic

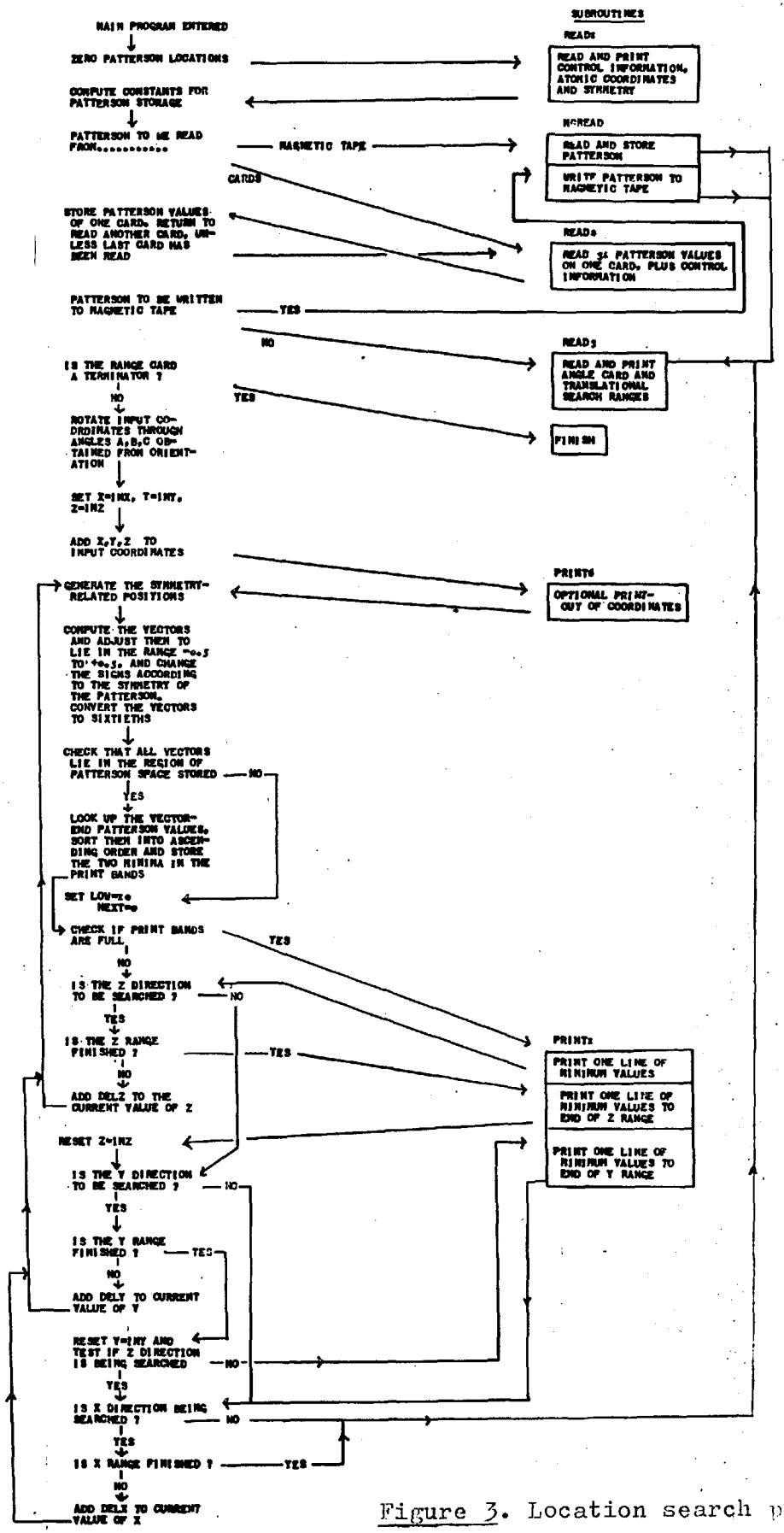


Figure 3. Location search program.

directions through the specified search ranges and (2) is repeated at every stage. The process can be described by three nested loops as follows :-

```
INX(DELX)FINX
INY(DELY)FINY
INZ(DELZ)FINZ
Calculation
REPEAT
Reset Z = INZ
REPEAT
Reset Y = INY
REPEAT
```

Superposition

A flow diagram is shown in Figure 4.

1. The positions of the known molecular fragment (plus the symmetry-related positions if required) are input as fractional coordinates together with the search ranges INX, Y, Z; DELX, Y, Z; FINX, Y, Z and the Patterson function.

2. Vectors from input positions to the point INX, INY, INZ are computed, then adjusted and the Patterson values interpolated and printed as in the previous two programs.

3. The process is repeated by computing vectors from the input positions to all the points generated by the search ranges

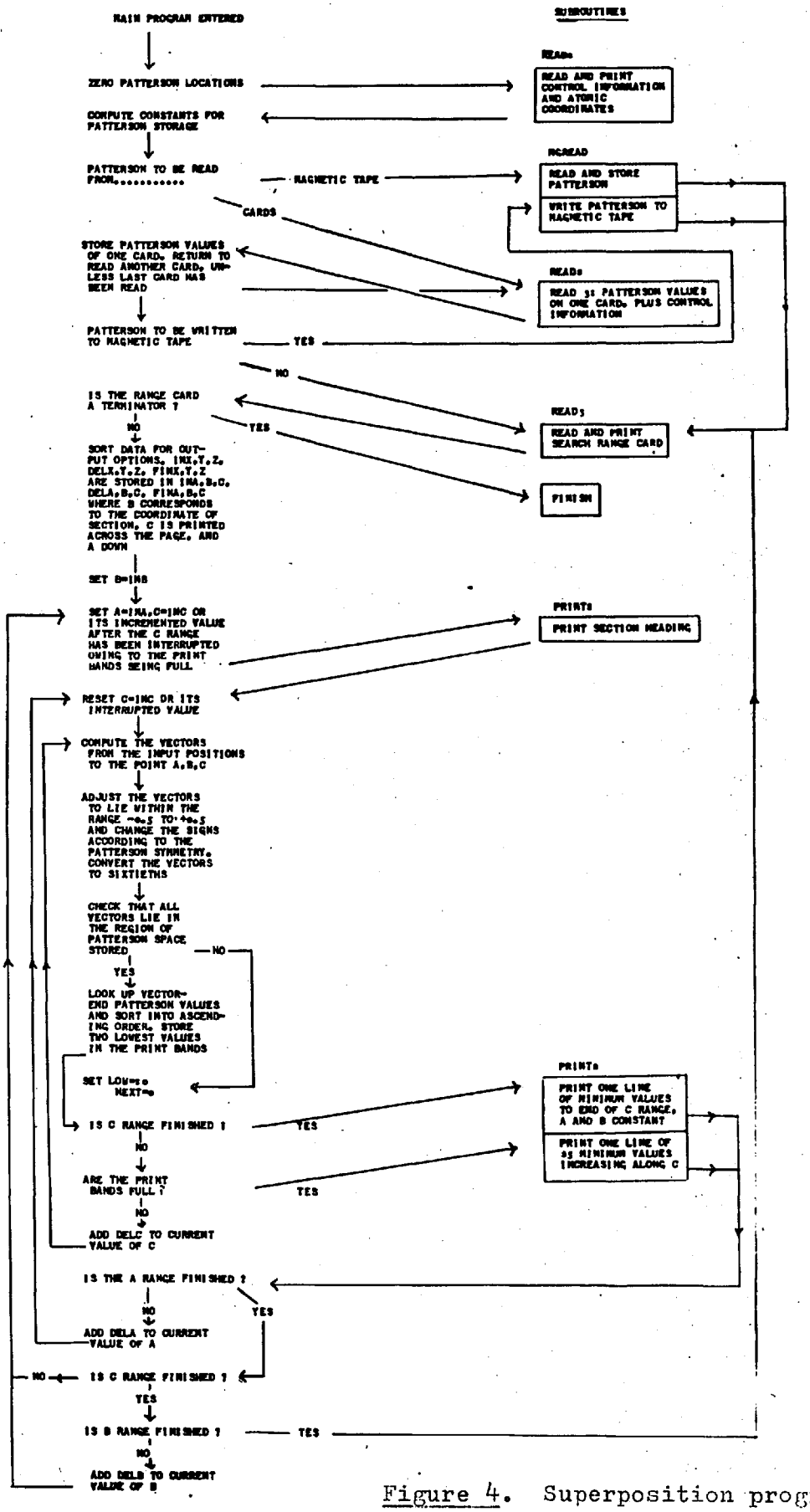
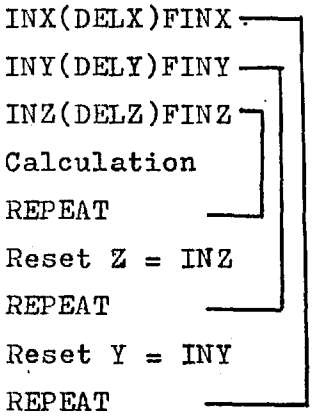


Figure 4. Superposition program.

as follows :-



4. The minimum function is printed as parallel sections up an optional crystallographic axis, in a form suitable for contouring.

Further details of the programs are described in "Patterson Search Programs" by Hunt (1966). Many difficulties were initially experienced in attempting to generalise and make the programs available for any I.B.M. 7090/7094 installation. It soon became evident that the programs had been written mainly for one spacegroup, P2/m, and consequently many routines were, in the author's opinion, illogical and unnecessary when applied to other symmetries. Changes have been made in a way to make most use of what was already given, and the result (as the flow diagrams indicate), is a rather inefficient sequence of operations. Nevertheless, the computing times on the I.B.M. 7090 are quite realistic, for example an orientation

search with 66 vectors (i.e. all the vectors within a molecular fragment of 12 atoms) over 7000 angular combinations took approximately 25 minutes, though increasing the number of vectors above this value leads to excessive computing times. The location search and the superposition programs are both approximately as fast as in the example given.

CHAPTER 8

An X-Ray Investigation of the Crystal Structure of Zeorin Acetate.

Preliminary Data

The crystals were colourless orthorhombic laths, elongated along [100] with (001) prominent. The dimensions of the crystal selected for data collection were approximately 0.40 x 0.15 x 0.12 mm. The structural formula proposed by Barton, de Mayo and Orr (1958) is shown in Figure 1.

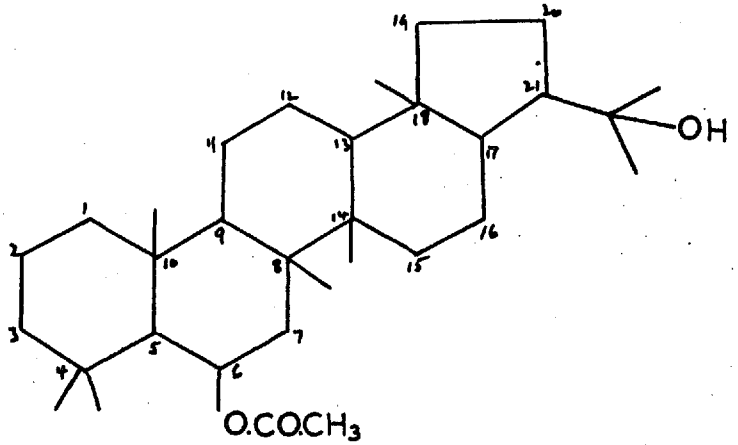


Figure 1. The proposed structure of zeorin acetate.

Formula $C_{32}H_{54}O_3$ Molecular Weight (formula) = 486

Unitcell Dimensions : a = 8.78 (0.02) Å, b = 11.59 (0.02) Å, c = 29.15 (0.04) Å. V = 2967 Å^3, Z = 4, F(000) = 1080 e.

Mass absorption coefficient for CuKα, μ = 5.2 cm^{-1}

D_{obs} (flotation) = 1.09 $g.cm^{-3}$ D_{calc} = 1.09 $g.cm^{-3}$

Absent spectra only among

hOO for h = 2n + 1

OkO for k = 2n + 1

OOl for l = 2n + 1

so that the spacegroup is uniquely determined as $P2_12_12_1$. Intensity data for the layers Okl to 7kl were estimated visually using three graduated wedges to take account of spot-shape changes on the higher layers. In view of the dimensions of the crystal used and the low value of the absorption coefficient, corrections for absorption were considered unnecessary. The data were correlated by comparison with reflexions common to hOl, giving a total of 2490 independent reflexions.

The Wilson plot based on three-dimensional data is shown in Figure 2. The data were divided into 25 shells each containing 110 - 140 reflexions, and unobserved reflexions were included as zero. The abnormality in the curve is not surprising in view of the structure proposed for zeorin acetate. It is evident from the Patterson (Figure 3) that many inter-atomic vectors accidentally coincide so that at certain $\sin^2\theta$ values the intensity averages will be abnormally high. The least-squares method gave B = 3.2, K = 23.7 and

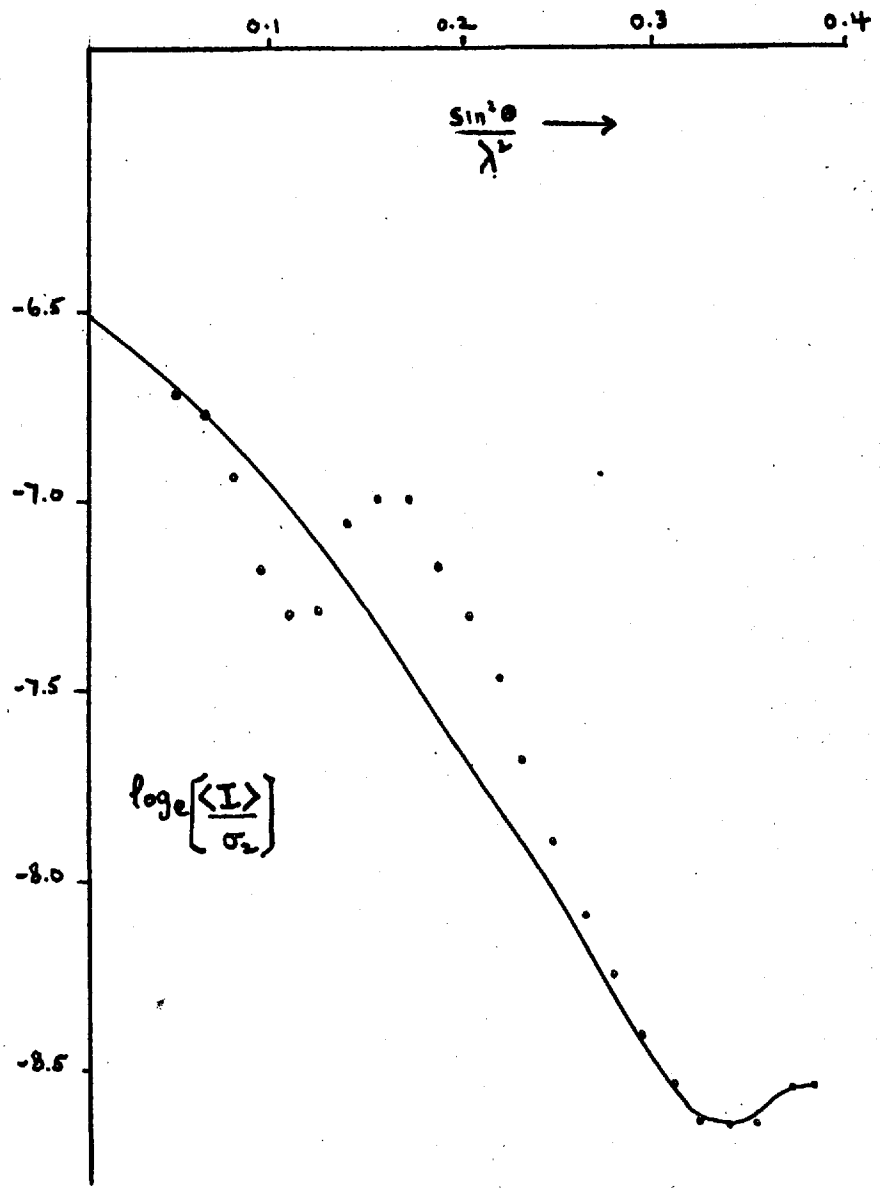


Figure 2. Zeorin acetate Wilson plot based on three-dimensional data.

the graph B = 3.8, K = 25.8.

The approximate length of the triterpenoid nucleus (i.e. the A, B, C, D and E rings) is 12 Å and the lengths of the a and b axes (8.78 Å and 11.59 Å) are too short to accommodate this dimension including the side chain. This strongly suggests that the length of the molecule lies in the c direction. A brief examination of the crystals with a polarizing microscope and quartz wedge showed $n_c > n_a > n_b$, (where n is the refractive index along the axial direction denoted by the suffix) but the birefringencies were not large. This confirmed that the molecules have their longest dimension parallel to c and suggested that their planes are roughly parallel to (010). The latter conclusion was corroborated by the observation that the two strongest reflexions are from (020) and (021).

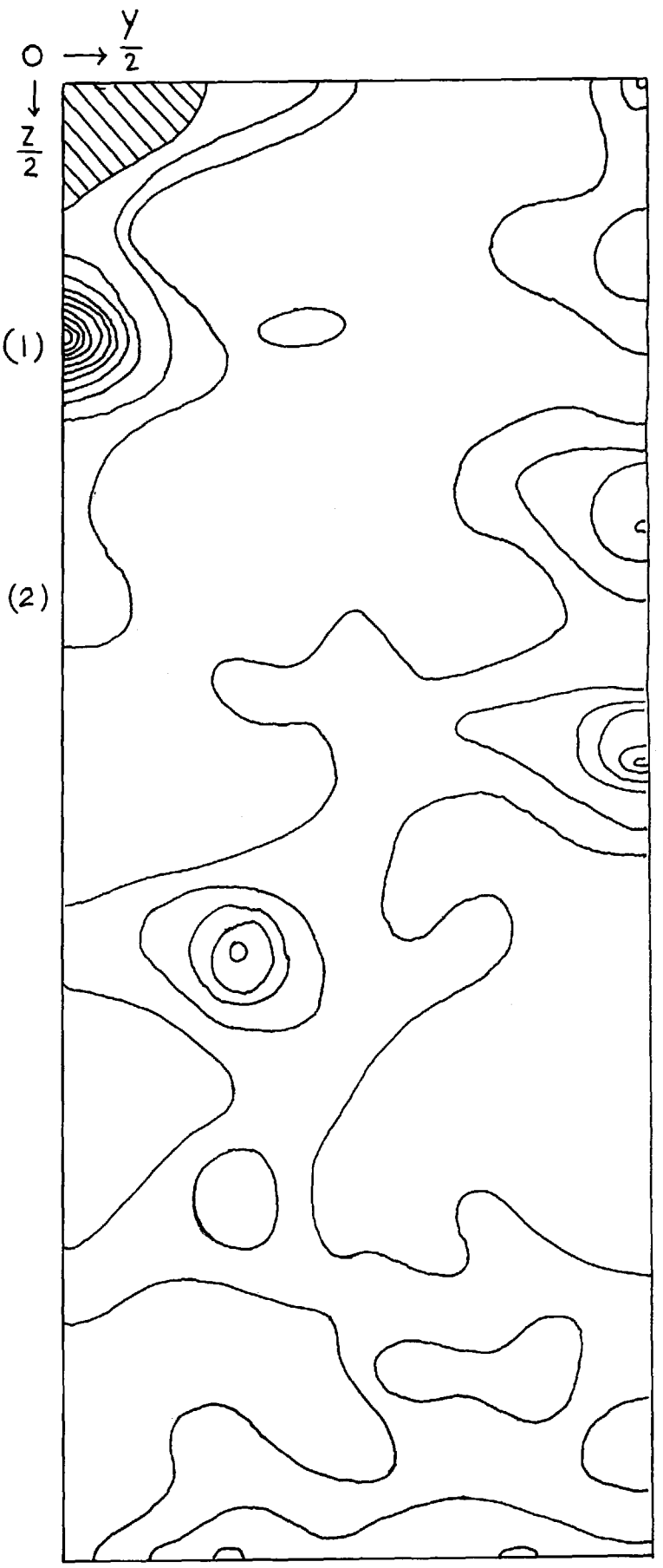
Visual Examination of the Patterson

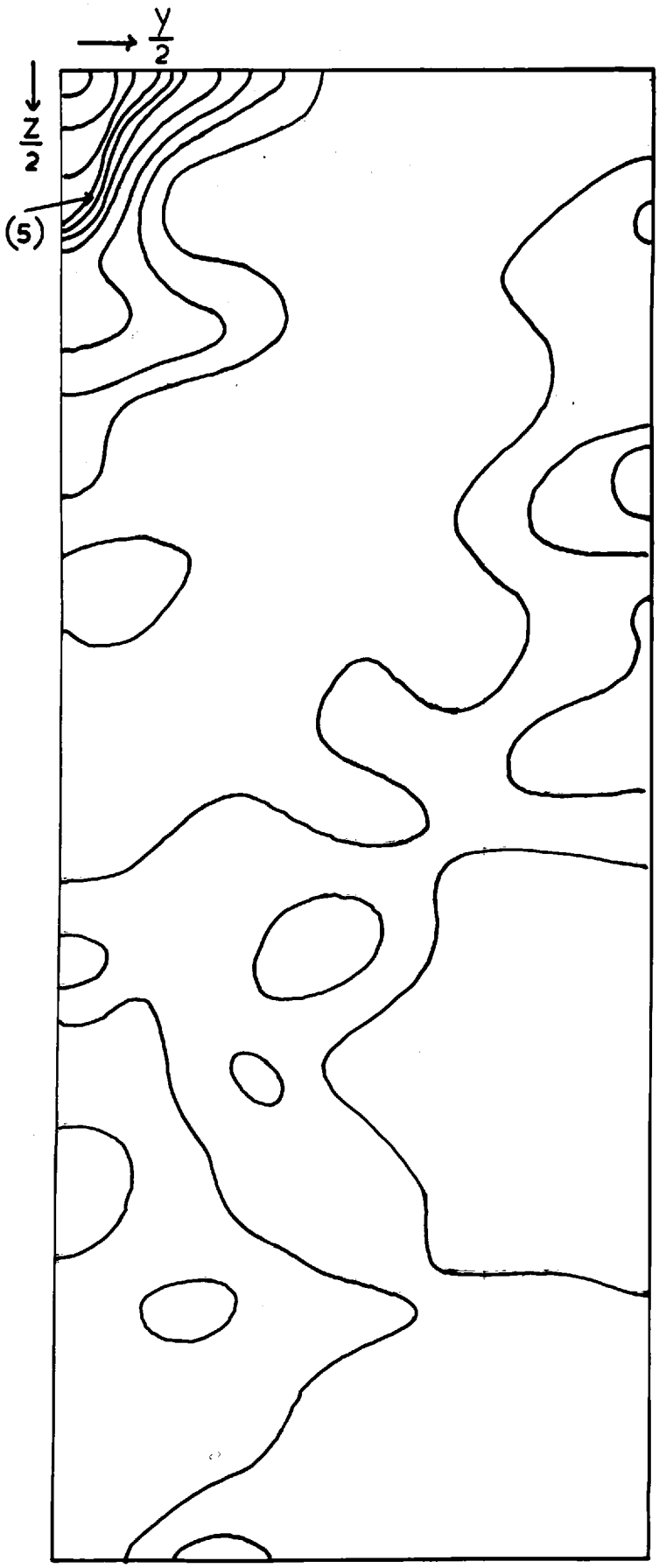
The unsharpened, three-dimensional Patterson function is shown in Figure 3 on pages 162 to 170, computed as sections normal to the a axis (the axes have been labelled x, y and z). The function is on an arbitrary scale and contoured at levels of 50 starting at 0. The computation did not include the term $F(000)^2$ and the negative parts of the Patterson have not been contoured, though to avoid ambiguity the areas within the

Figure 3.

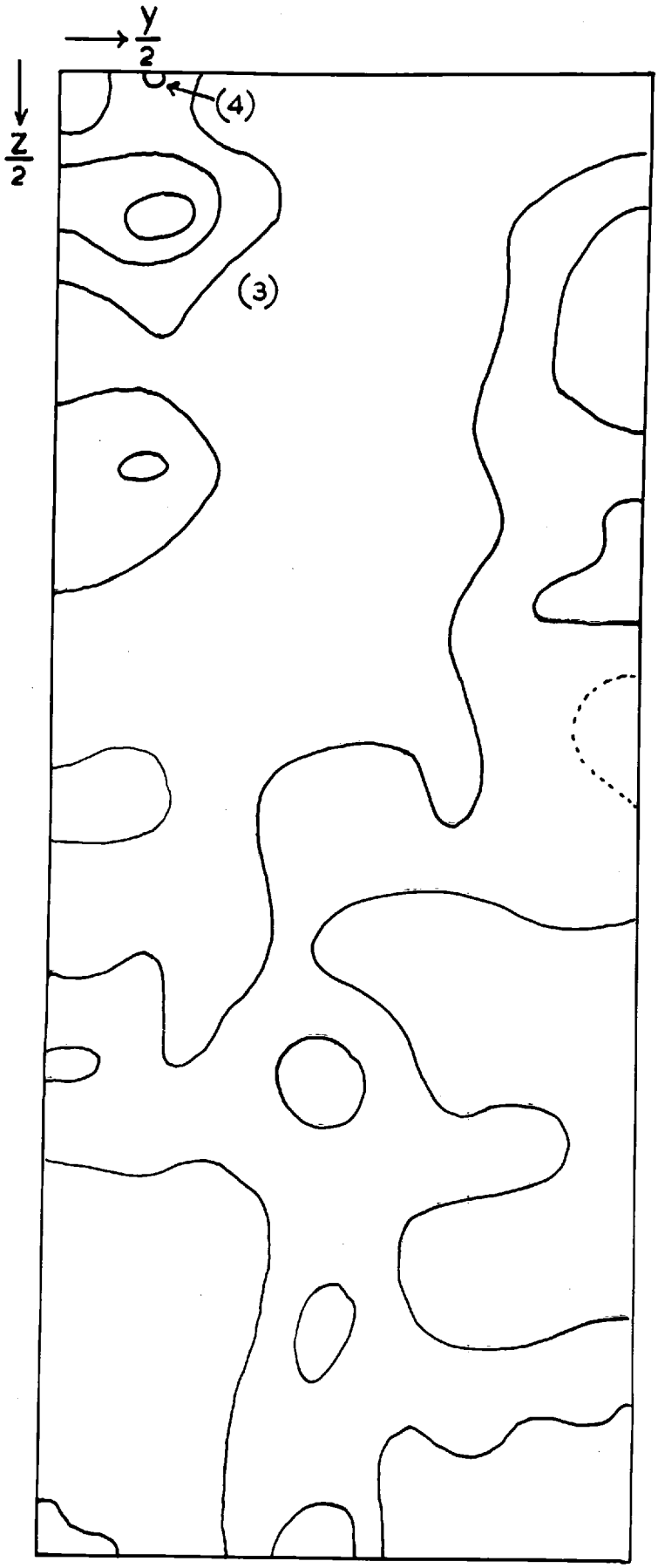
Zeorin acetate

unsharpened Patterson.

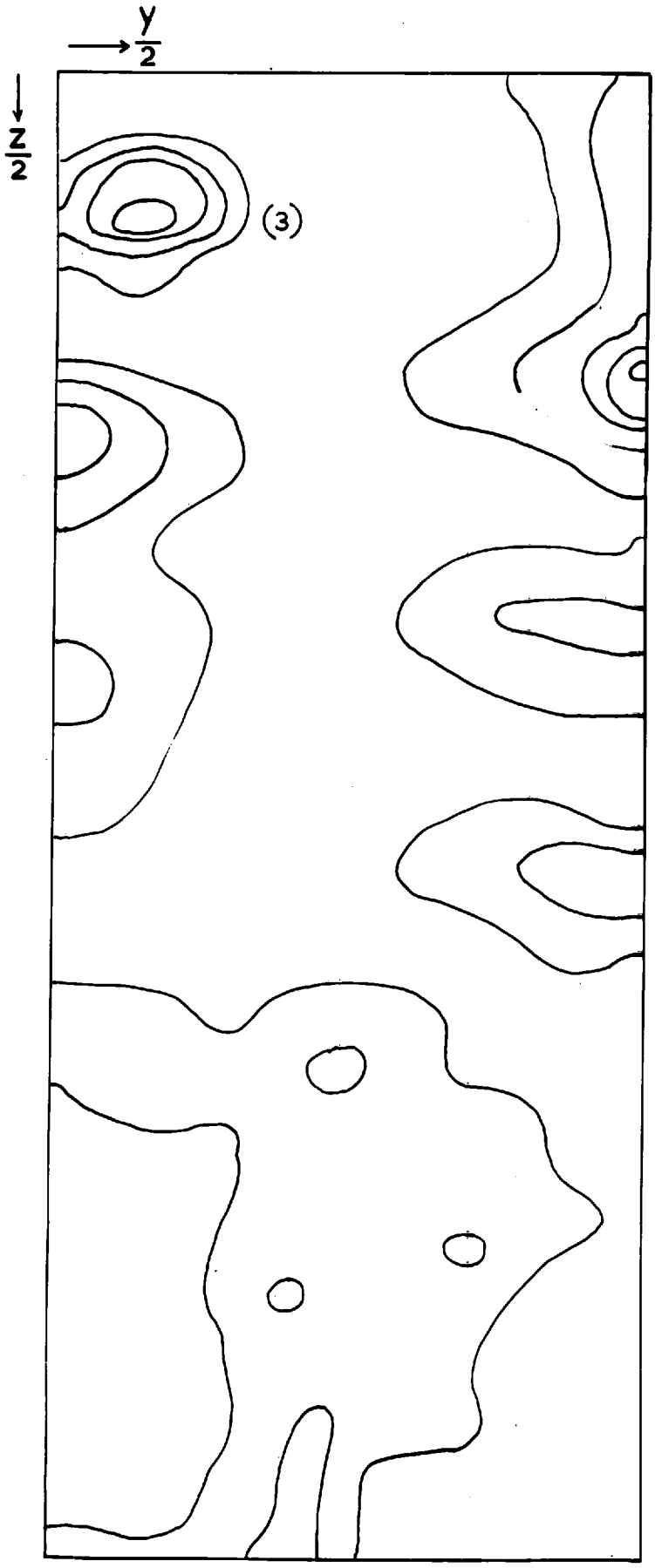

O → y/2
z/2
(1)
(2)
X = 0/12



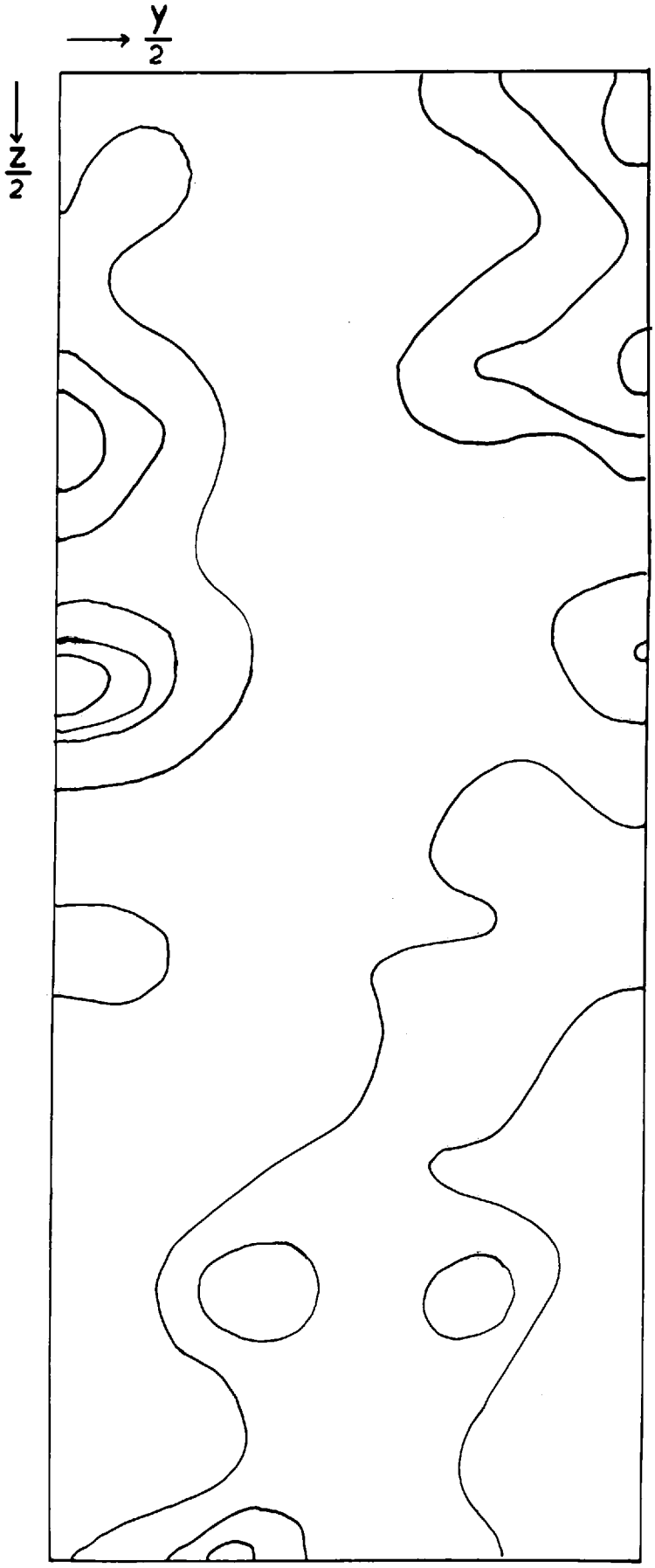
$x = \frac{1}{12}$



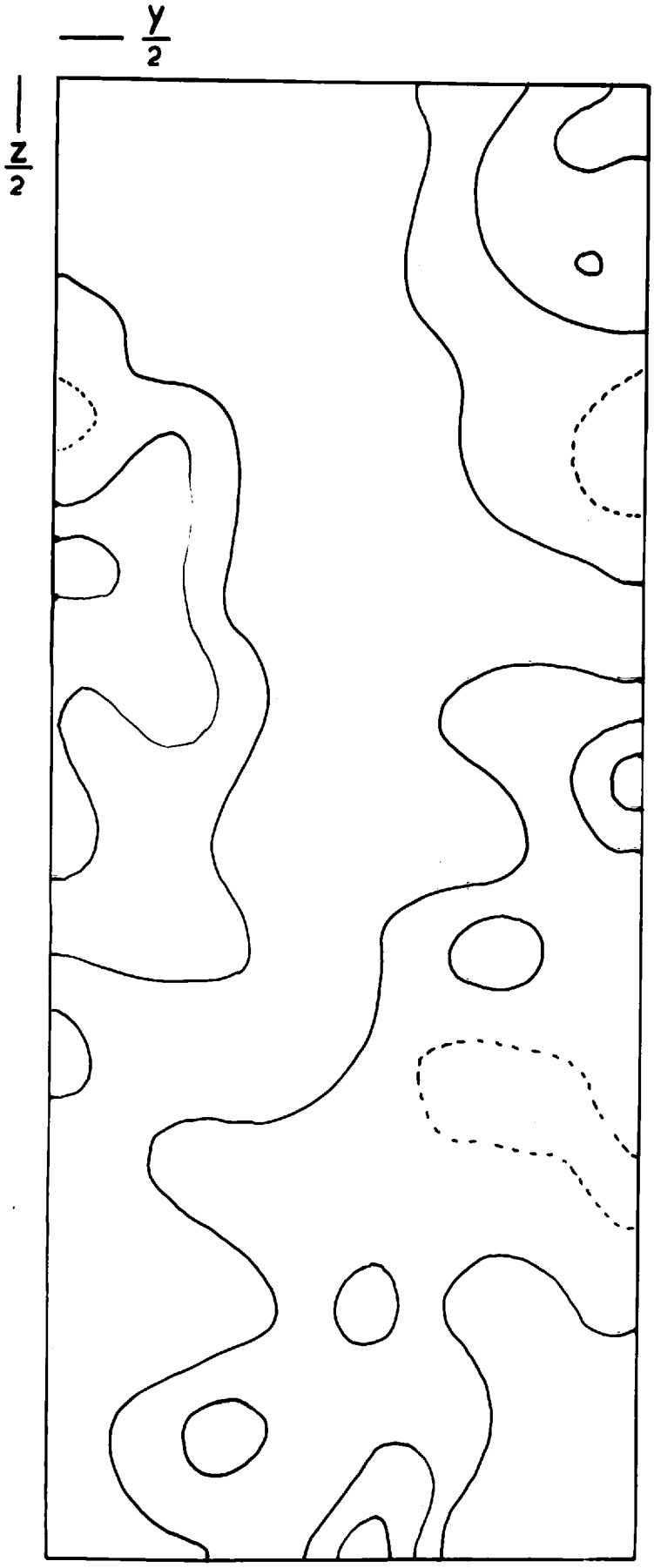
$x = \frac{2}{12}$



$x = \frac{3}{12}$



$x = \frac{4}{12}$


y/2
z/2
x = 5/12


y/2
z/2
x = 6/12

broken contours are negative,

The height of the origin peak on an arbitrary scale is 2000 and this is proportional to $\sum_{i=1}^{N} f_i^2 = 5592$. Thus, the peak heights corresponding to single inter-atomic vectors should be approximately :-

Vector	Peak Height
C - C	14
C - O	17
O - O	24

These peaks will be indistinguishable in the negative areas of the Patterson, and are dwarfed by multiple peaks.

The set of points corresponding approximately to the postulated structure of the A, B, C and D rings is shown in Figure 4a. The system is puckered and the points marked (●) are regarded as above the plane of the paper. Figure 4b is the vector set of the points in Figure 4a; it forms three parallel 'sheets' and in broadside view the points make up an equilateral triangular mesh. Those in the central sheet are 'strongest' and form the larger mesh of side ≈ 2.4 Å. The points in the other two sheets are mirror images, offset by roughly 0.5 Å and approximately 1.5 Å apart . Peaks in the Patterson corresponding to the outer sheets will probably not be as easily distinguished as those in the central layer.

The Patterson does, in fact, exhibit a broad, flattish region of high density around the origin . It is fairly well

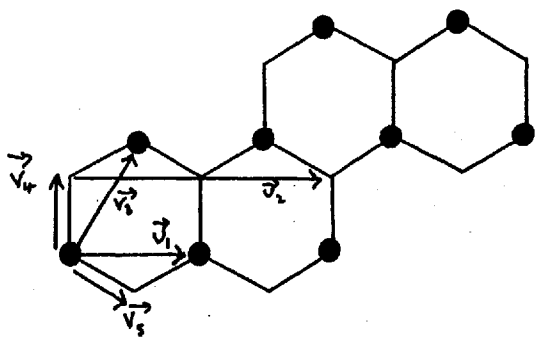


Figure 4a. The postulated structure of the A, B, C and D rings. The system is puckered, atoms marked (●) are regarded as being above the plane of the paper.

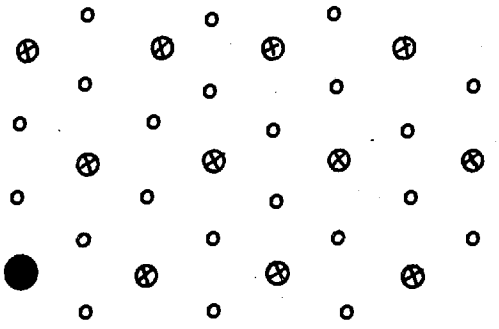
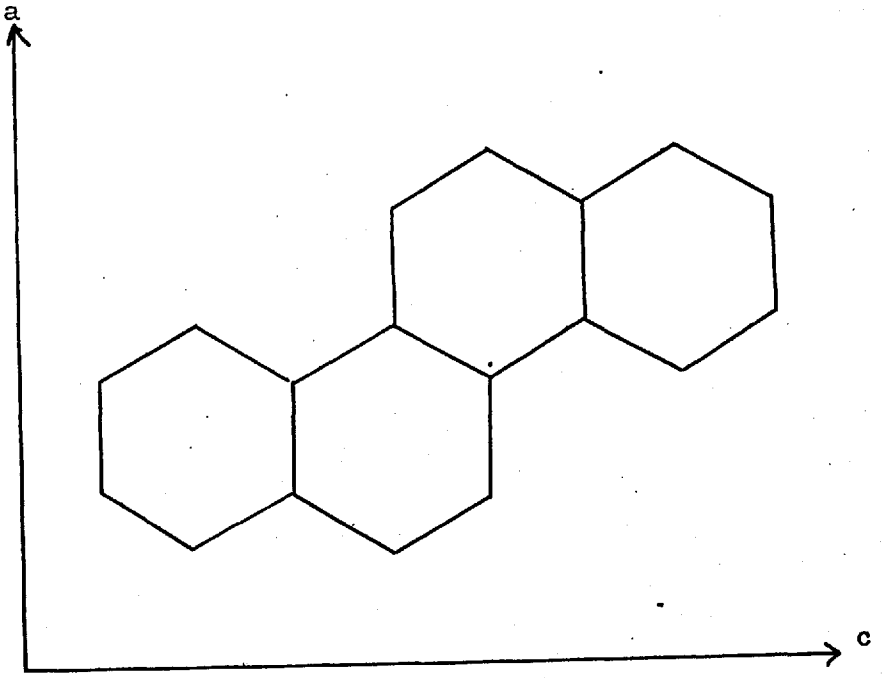


Figure 4b. Part of the vector set of the points in Figure 4a. Points marked (⊗) lie in the central sheet Points marked (o) represent superimposed points athwart the central sheet.

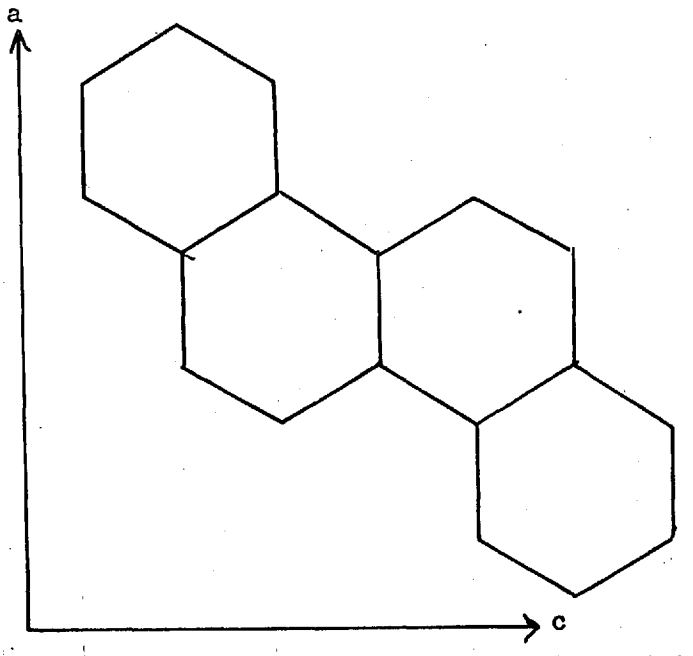
confined to the (u, θ, w) plane, so all the molecules in the cell must be roughly normal to b. The peak clusters around (u, $\frac{1}{2}$, w) and ($\frac{1}{2}$, v, w), presumably between the b and a screw-related molecules also confirm the general alignment.

In the u = 0 section of the Patterson (page 163), the strong peak labelled (1) was attributed to the accumulation of vectors of the type $\vec{v}_1$ in Figure 4a and peak (2) to those of type $\vec{v}_2$. The length of the vectors from the origin to these peaks (2.4 Å and 4.9 Å respectively) agrees with the calculated distances across the cyclohexane rings. The strong peak (3) (pages 165 and 166) was attributed to vectors of the type $\vec{v}_3$ in Figure 4a, its distance from the origin being 2.5 Å. Thus, the origin peak and peaks (1) and (3) form the equilateral triangular mesh associated with the points in the central layer in Figure 4b. Their positions suggest two possible orientations for a zeorin acetate molecule as shown in Figures 5a and 5b.

The projection of the vector from the origin to peak (3) on to (001) makes an angle with (010) of approximately, $\alpha = 30^{\circ}$. With the aid of Dreiding models it was apparent that this peak could arise from two possible orientations of the molecule seen 'end-on' as in Figures 6a and 6b (the vectors marked are as in Figure 4a). If the orientation is similar to that shown in Figure 6b, then there should be a peak in the Patterson

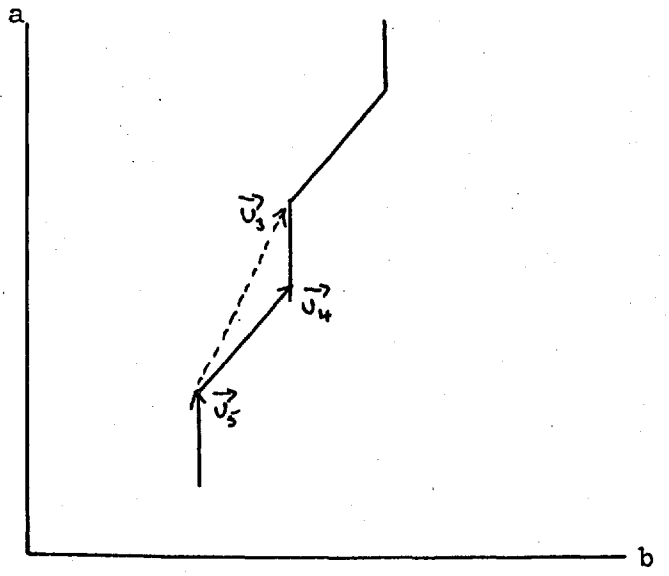


(a)

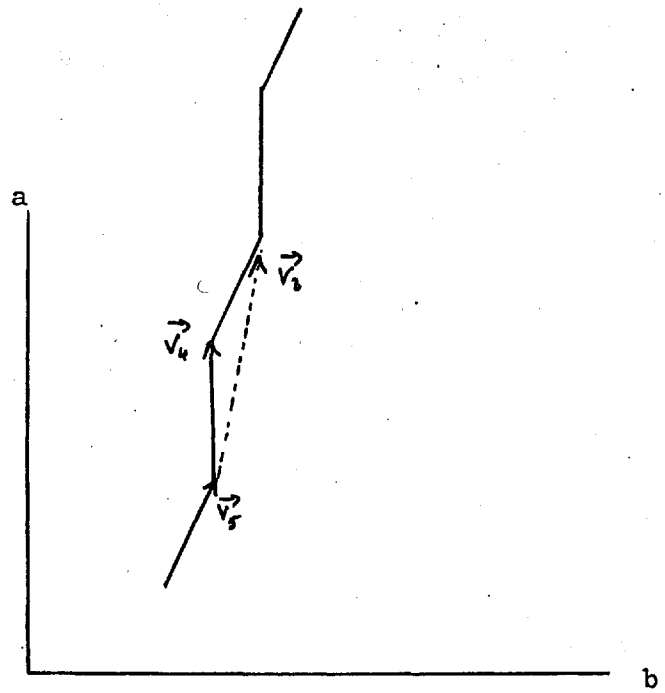


(b)

Figure 5. Two suggested orientations for zeorin acetate in 'broadside' view.



(a)



(b)

Figure 6. Idealized 'end-on' view of the A, B, C and D rings.

approximately 1.5 Å from the origin, on or close to the a axis and corresponding to $\vec{v}_4$. No such peak was discernible. However, assuming an orientation similar to that in Figure 6a, it was possible to locate Patterson peaks corresponding to the vectors $\vec{v}_4$ and $\vec{v}_5$. These peaks, (4) and (5) are shown in Figure 3 on pages 165 and 164, their distances from the origin being approximately 1.6 Å and 1.5 Å respectively.

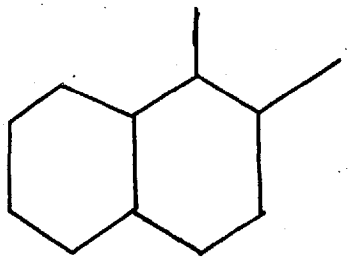
The Use of Patterson-Searching Techniques

The method and computer programs used have been described in Chapter 7.

As shown in Figures 4a and 4b, the vector set of a single molecule (postulated) consists of three parallel sheets (considering the ring system only). The peaks in the Patterson corresponding to the inmost sheet are the strongest and there should be several less than 3.4 Å (i.e. they will be clear of inter-molecular vectors). Although the longer vectors are more sensitive to angular changes, they may run into the region of inter-molecular vectors and be difficult to disentangle. It therefore seemed advisable at first to deal only with the shorter and medium-length vectors.

Considering only the rings A, B, C and D, they form

an approximately centrosymmetric group with vectors too numerous and too long to use in initial searches. Computing time becomes excessive with more than about 78 vectors (i.e. all the vectors between 13 atoms). A cyclohexane ring is too small a unit and occurs too often either as complete or incomplete rings. A decalin ring is possibly acceptable but it was decided to use the group



as the computing times would still be reasonable and it would possibly be more selective than the ten-atom group; it can occur four times in the molecule and possibly more considering the five-membered ring and other substituent groups.

As the unit cell contains four symmetry-related molecules, the region round the origin in the Patterson contains sets of peaks corresponding to four overlapping sets of intra-molecular vectors. However, this is implicit in the mmm symmetry of the Patterson so that only one quadrant of space need be searched. Any multiple fits are then due to repetition of the search group within one molecule.

The structure of methyl melaleucate iodoacetate (Figure 7)

determined by Hall and Maslen (1965), was used to define the

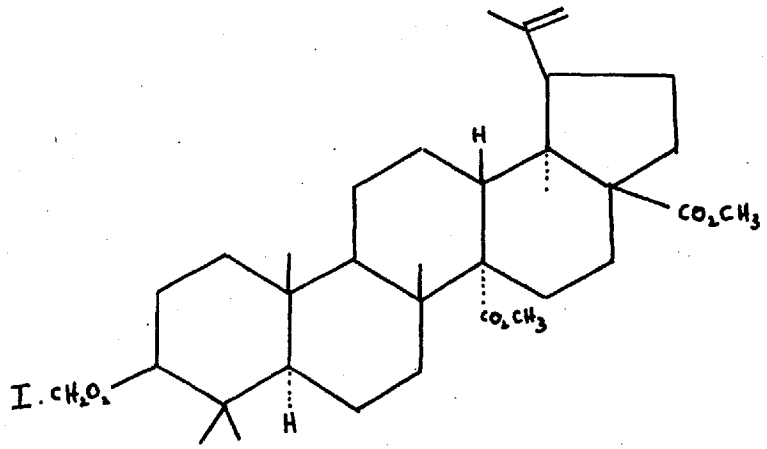
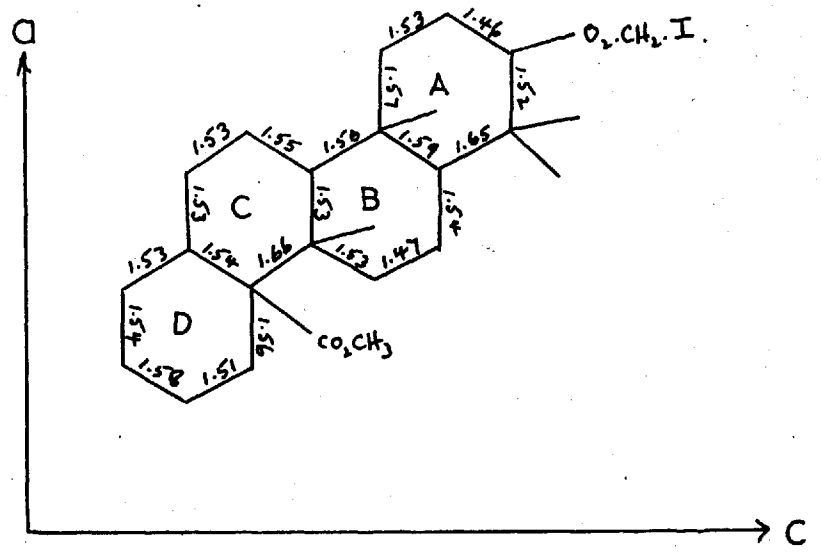
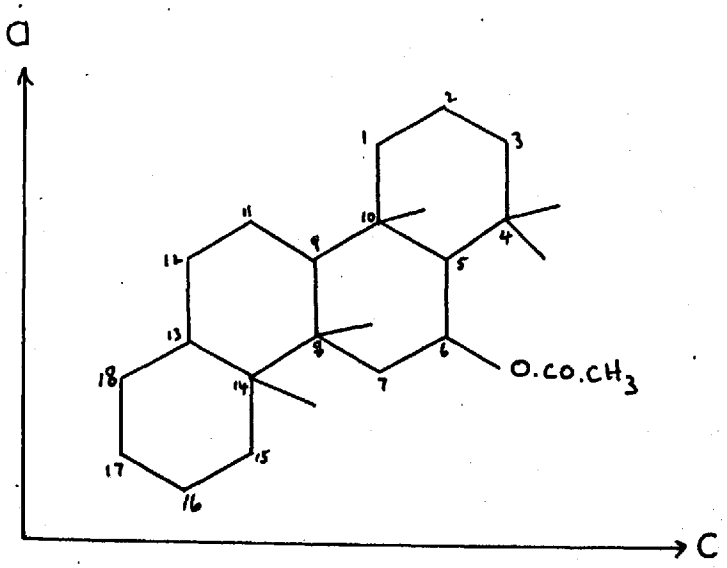


Figure 7. Structural formula of methyl melaleucate iodoacetate.

geometry of a molecular fragment. The bond distances of the A, B, C and D rings are shown in Figure 8a, which also gives the approximate orientation of the molecule projected down [010]. The naming of the axes in Figure 8a is not the same as that given by Hall and Maslen. The a and c axes have been interchanged so that the orientation is similar to one postulated for zeorin acetate. Hall and Maslen noted that the degree of substitution at carbon atoms bears a direct relationship to the lengthening of the C - C bonds. Long range steric effects may also account for some of the abnormal bond lengths and angles. As the substitution in zeorin acetate (Figure 8b) is probably very similar to that in methyl melaleucate iodoacetate, except at C_3, it is reasonable to assume that the geometry of the A, B, C and D rings of the two compounds will not differ to a great extent.



(a)



(b)

Figure 8. (a) Bond lengths in methyl melaleucate iodoacetate. (b) The A, B, C and D rings in the postulated structure of zeorin acetate.

Work with the Unsharpened Patterson

Orientation Search

As the unsharpened Patterson had given an approximate orientation of the molecule, it was decided to proceed with the unsharpened function for the search. This was computed on a 31 x 31 x 31 grid and punched on cards with PSP. As mentioned in Chapter 7, input format requires not more than two digits for each Patterson grid point. Consequently, the Patterson as shown in Figure 3 was scaled down so that most values fell within the range -48 to +48. Each value was then adjusted by addition of 48 to give the range 0 to 96. Any negatives were punched as 0 and those above 99 (mainly in the origin peak) as 99. The origin peak was not removed, which would reduce the power of the search in that the Patterson values at the ends of the vectors between adjacent atoms would be obscured by the origin peak in some orientations. In Figure 3, only peak (5) (of those visible) was affected in this way and it was considered that this would have little effect on the position of the minimum, especially as it is the coordinates of the longer vectors that are more susceptible to angular changes and hence define the position of the minimum more accurately.

The 66 vectors between the atoms C1 - C11, C14 of methyl melaleucate iodoacetate (Figure 8a) were input to the orient-

ation search program (although they form rather a deformed group, especially about C3 and C5). Increments of 10° were initially employed to make computing time practical.

The highest peak in the rotational minimum function had LOW = 41, (0 in Figure 3 corresponds to 48 in the Patterson used for the search), NEXT = 43 with A = 0°, B = 60° and C = -10°, and corresponds approximately to a clockwise rotation, by 60° about the b axis, of the A and B rings (as shown in Figure 8a), to give the orientation shown in Figure 9. This

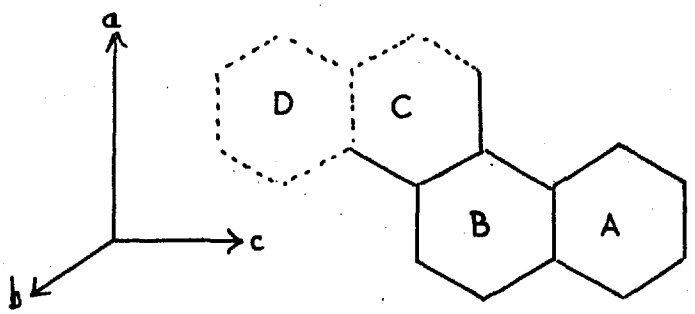


Figure 9.

corresponds quite closely with a possible position suggested by the preliminary investigations of the Patterson. Other peaks in the minimum function with LOW $\approx$ 36 occurred in the Euler-angle ranges

$$A = -30^{\circ} \text{ to } +20^{\circ} \begin{cases} B = -70^{\circ} \text{ to } -60^{\circ} & C = 10^{\circ} \\ B = -30^{\circ} \text{ to } -20^{\circ} & C = 20^{\circ} \\ B = 85^{\circ} \text{ to } 95^{\circ} & C = 30^{\circ} \end{cases}$$

The first peak may be accounted for by a fit with the B/C rings

as shown in Figure 9.

The increment was reduced to 5° and the Patterson searched over ranges that included all four peaks. The minimum values for the latter three still remained at approximately 36, but for the first peak were LOW = 42, NEXT = 43. Decreasing the increment to 1° showed that this peak was in fact triplet with

A°	B°	C°	LOW/NEXT
0	58	-14	43/43
-1	61	-13	43/43
-1	52	-14	41/41

Two of these peaks could be accounted for as representing the fit of the molecular fragment with the A/B and C/D rings respectively, suggesting that the C and D rings are as shown in Figure 9. The third peak which is fractionally weaker may represent the partial fit with a single ring and its appendages. However, the boundaries between the three peaks are ill-defined and the differences between the assumed and actual geometry must be a factor that contributes to this effect.

The orientation of the molecular fragment with A = 0°, B = 58° and C = -14° is shown projected down the b axis in Figure 10a, and down the c axis in Figure 10b.

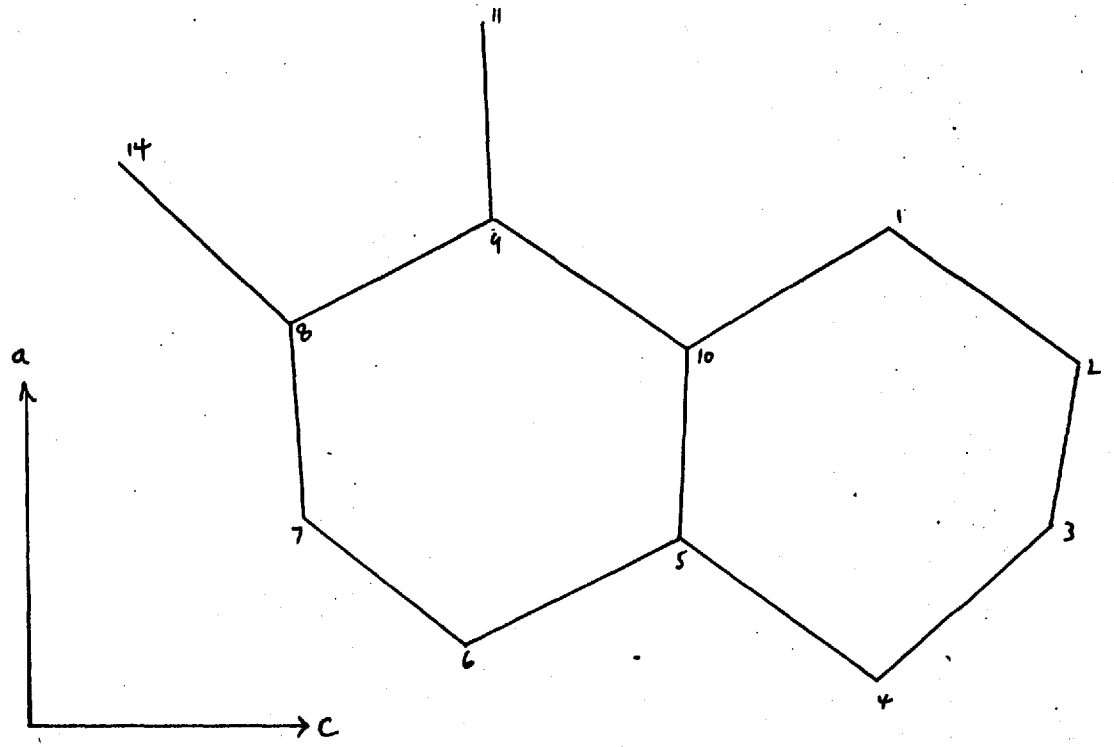


Figure 10a. The molecular fragment projected down b

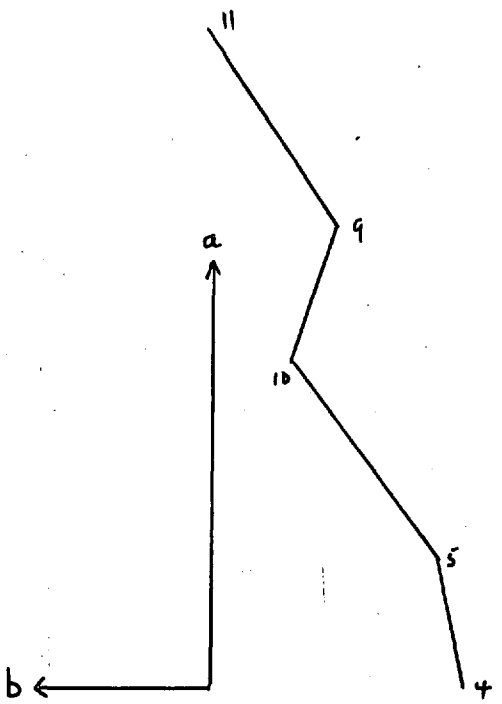


Figure 10b. The atoms C_4, C_5, C_{10}, C_9 and C_{11} projected down c

Location Search

For the spacegroup $P2_12_12_1$, this process can be carried out by two separate two-dimensional searches. With the molecular fragment in the orientation A = 0^o, B = 58^o and C = -14^o, the Patterson function was searched along the X and Y directions, computing vectors between the fragments related by the c screw. The appropriate ranges were :-

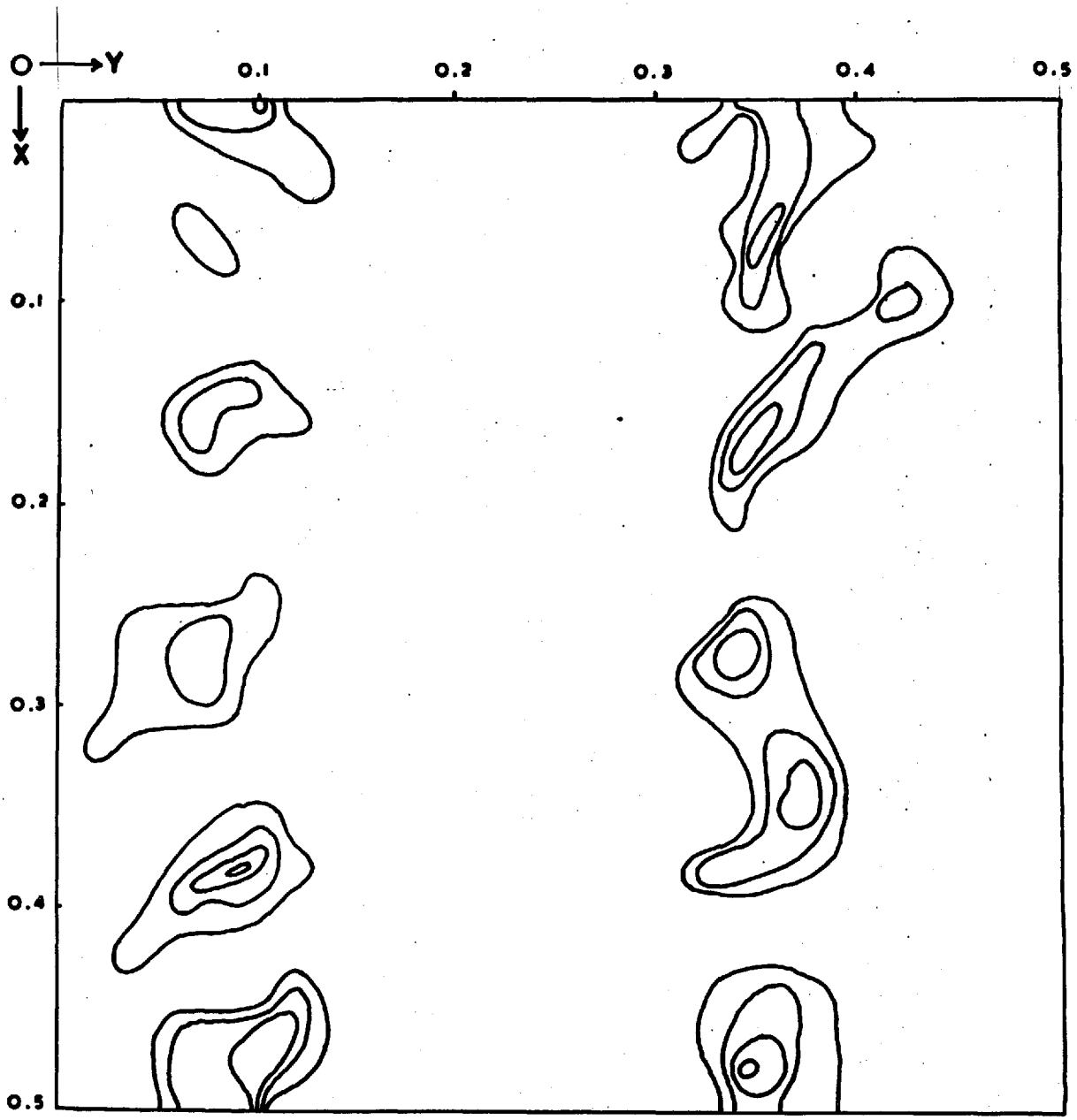
INX, Y = 0.0 DELX, Y = 0.01 FINX, Y = 0.50

The increments are approximately 0.1 Å and the searching was carried out in the unique part of the unit cell.

The translational minimum function is shown in Figure 11. Only the values of LOW are contoured at the levels 38, 40, 42 and 44. The background level was generally between 25 and 32. The peak heights agree quite well with those of rotational minimum function but as can be seen, the function is poorly resolved especially in the X direction, although the highest values are confined to two narrow ranges in the Y direction, from 0.34 to 0.37 and 0.07 to 0.10.

The translational search computing vectors across the a screw tended to confirm the ranges for the Y values shown in Figure 11. Outside these ranges the minimum function was generally of the order 25 to 32 although there were, in places, peaks as high as 39. The function also suggested that

Figure 11. Translational minimum function in the Y and Z directions.



two different Z ranges, 0.40 to 0.44 and 0.36 to 0.38, are associated with the two peaks at Y = 0.07 to 0.10 and Y = 0.34 to 0.37 respectively. A third search computing vectors across the b screw did little to define the minimum more clearly and gave a minimum function in which the peak heights were generally lower than in the other two searches.

In an attempt to find unambiguously, the translational parameters (x_o, y_o, z_o) (page 144), two three-dimensional searches were carried out. Both were over the complete X range (0.0 to 0.5) but with different Y and Z ranges corresponding to those suggested by the two-dimensional minimum functions. The resulting peak heights (background level = 30 to 34) were generally lower than those in the two-dimensional maps. The four highest peaks are shown in Table 1.

Table 1. The highest peaks in the three-dimensional, translational minimum function.

Peak	X	Y	Z	LOW/NEXT
A	0.14	0.34	0.37	39/39
B	0.14	0.07	0.43	39/40
C	0.08	0.33	0.39	39/39
D	0.23	0.08	0.43	38/39

Table 1 shows that the differences between the heights of the four peaks are very small and thus from the results so far it was felt that the original Patterson was not sharp

enough to define, unambiguously, the translational parameters x_o, y_o, z_o. However, two three-dimensional Fourier maps were computed using the coordinates of the two highest peaks in Table 1 (A and B) as values of x_o, y_o, z_o for the structure factor calculations. The agreement between the observed and calculated structure factors (scale of 20.0 applied to Fo) was 0.55 in each case. It was difficult to interpret the Fourier map derived from the coordinates of peak A in a way consistent with the expected geometry of the zeorin acetate molecule. The Fourier derived from peak B was a little more encouraging and gave tentative positions for 18 atoms as shown schematically in Figure 12. The peaks corresponding to the atoms marked 12 and 16 in Figure 12 were extremely weak. An attempt was

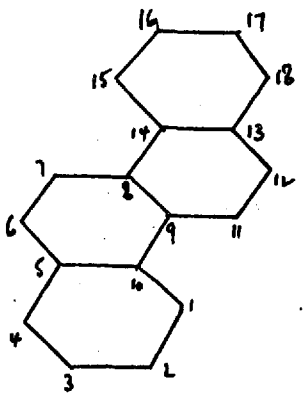


Figure 12.

made to refine these coordinates by the Fourier Refinement program of the X-Ray '63 System. This program, written by J.R.Holden calculates the 27-point block Fourier around each

atomic positions, estimates the coordinates of the point of maximum electron density within the block and then repeats the process with these peak positions as new atomic coordinates. The method, when applied to the 18 positions in Figure 12, reduced the R value to only 0.52 and some of the inter-atomic bond lengths attained unacceptable values. The shifts of the atomic positions were unsystematic and did not indicate that the group as a whole ought to be moved in any particular direction. A difference Fourier derived from the original positions was similarly uninformative.

2. Work with the Sharpened Patterson

In view of the ambiguity in the determination of the translational parameters, x_o, y_o, z_o that has been described, the method was repeated using the Patterson function sharpened to point atoms at rest by the modification

$$1/\hat{f}^2 \cdot \exp\,(6 \sin^2 \theta/\lambda^2)$$

where $\hat{f}$ is the formfactor for carbon. The resulting Patterson did not exhibit any serious diffraction ripple except in the neighbourhood of the origin peak and the zeroth section is shown in Figure 13 which can be compared with the corresponding section of the unsharpened Patterson on page 163. A sharpening

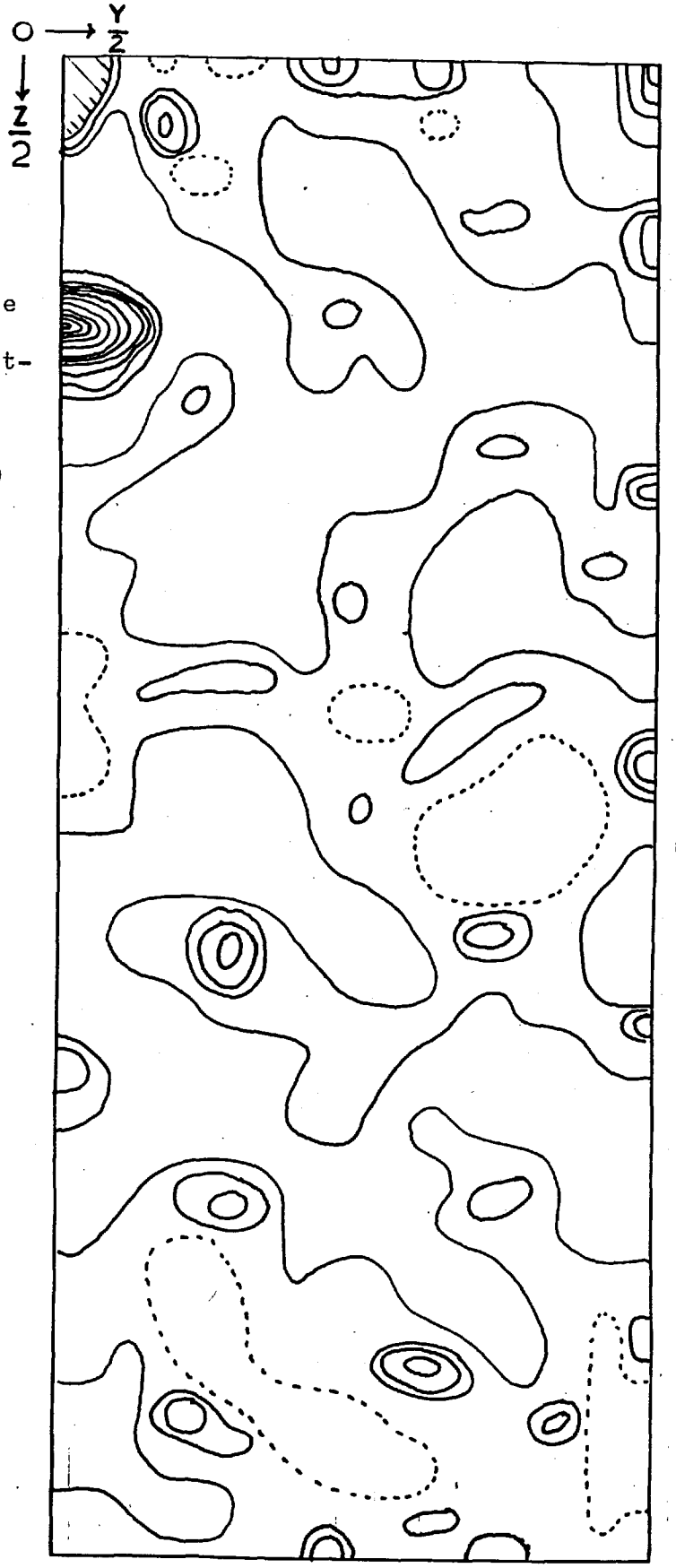


Figure 13.

Zeorin acetate sharpened Patterson.

Section x = 0

function of the type

$$\exp (6\sin^2 \theta / \lambda 2)$$

similar to that used by Nordman and Nakatsu (1963), did not produced any marked differences between the sharpened and unsharpened Pattersons.

Orientation Search

During the course of the work with the unsharpened Patterson, there were indications that the distortion of the molecular fragment may have contributed to the poor resolution of the minimum functions. Consequently, the ten atoms comprising the B and C rings of Hall and Maslens's (1965) determination of methyl melaleucate iodoacetate were selected as molecular fragment. In the orientation search, vectors between adjacent atoms were excluded to avoid the effects of the ripple near the origin peak. The approximate orientation of the molecule had been fairly well defined, as described in the preceeding sections and so the ranges of the rotational search were limited to A = -30° to $+30^\circ$, B = -90° to $+90^\circ$ and C = -50° to $+50^\circ$.

The minimum function showed four strong peaks for which LOW and NEXT were approximately 36 and 37 respectively compared with the background level of 10 - 25 (0 in Figure 13 was adjusted

to 36 for the search). After decreasing the angular increments the values shown in Table 2 were obtained for the four peaks,

Table 2. Rotational parameters of the four highest peaks in the minimum function.

Peak	A°	B°	C°	LOW/NEXT
1	-5	-70	10	36/37
2	+7	-59	23	37/39
3	-2	47	-35	39/40
4	1	57	-19	37/38

although the angles could not be fixed within $\pm$ 2°. The orientation of the molecular fragment defined by the parameters of peak 2 in Table 2 is shown projected down the b axis in Figure 14a and down the c axis in Figure 14b and roughly agrees with the positions deduced in the earlier work. The values of the angles $\alpha_1 = 39°$ and $\alpha_2 = 28°$ can be compared with the corresponding angle of 30° obtained from peak (3) in the unsharpened Patterson.

Table 2 shows that the peaks occur in two pairs 1,2 and 3,4 related to each other by a rotation of approximately 120° about the b axis. The differences between peaks 1 and 2 can be explained by the fits of the molecular fragment with the A/B and C/D rings respectively, similar to the situation described for the unsharpened search (there were also three peaks with LOW $\approx$ 32 in the ranges A = -5° to +5°, B = -10° to

$+10^{\circ}$, C = -10° to $+25^{\circ}$ which may have been due to fits with the B/C rings and the A and D rings plus appendages, but they were not investigated further). However, the differences between peaks 1 and 2 are in this case quite marked and strongly suggest that the molecule is situated in the unit cell as shown in Figure 15. This is different from that suggested by the Fourier

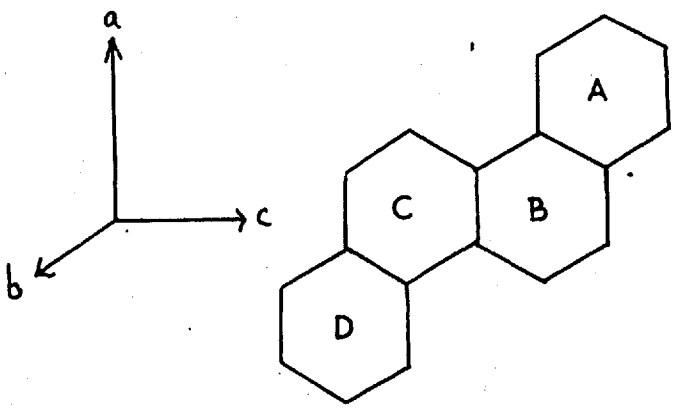


Figure 15.

maps derived from the unsharpened Patterson.

Using Dreiding models it was seen that with the molecular fragment in theorientation corresponding to peak 2 (Figures 14a and 14b), the position of peak 4 is equivalent to an approximate rotation of the group through 180° about the c axis. The relationship between peaks 2 and 4 occurs because the ten atoms of the molecular fragment approximately form a centro-symmetric set. Rotating the vectors through 180° about a

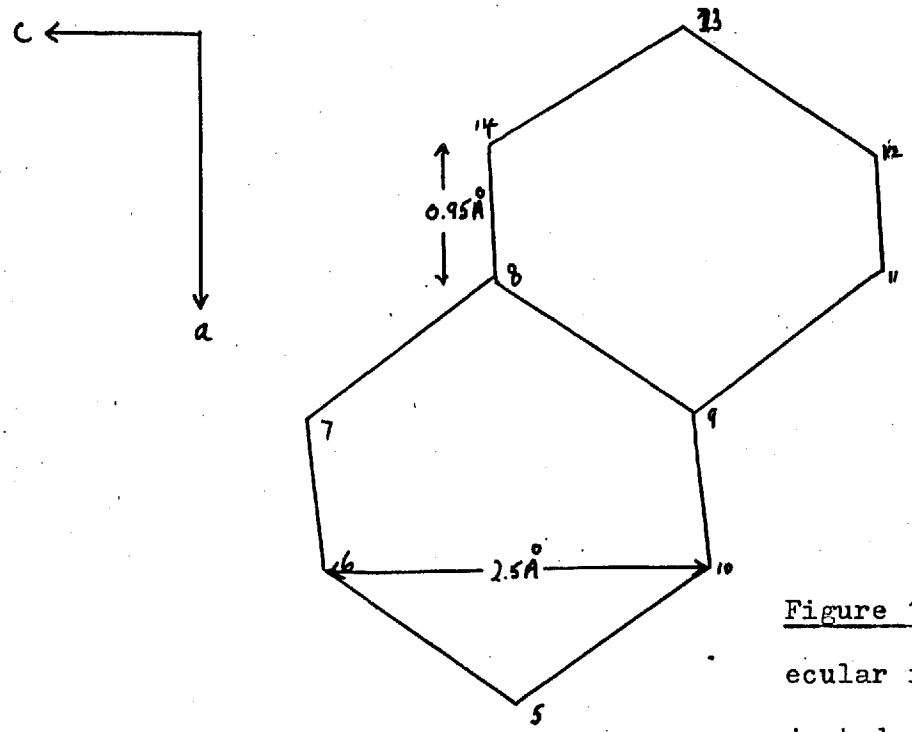


Figure 14a. The molecular fragment projected down the b axis.

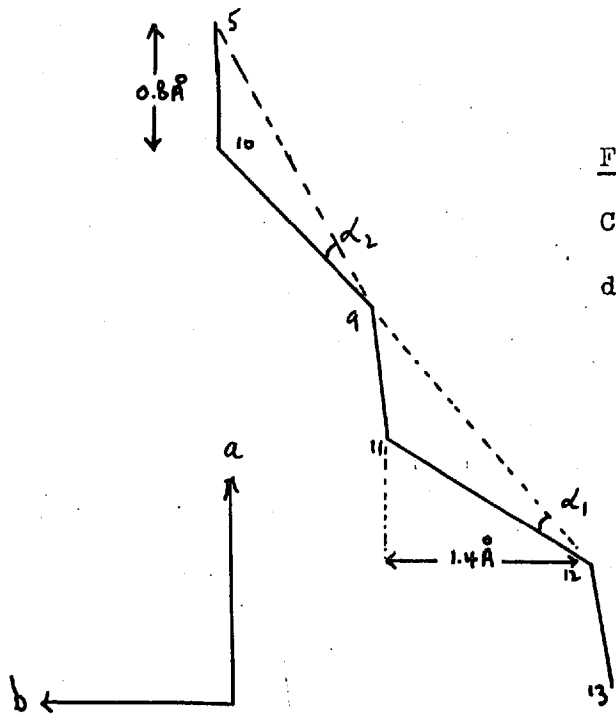


Figure 14b. Atoms C_5, C_9, C_{10}, C_{11}, C_{12} and C_{13} projected down c.

crystallographic axis and making all their signs positive to deal with the symmetry of the spacegroup, will give a set of vectors nearly, but not quite, equivalent to the original set. Peak 1 must be related to peak 3 in the same manner.

Location Search

The translational minimum function derived from the search along the Y and Z directions with the molecular fragment in the orientation A = 7°, B = -59° and C = 23° contained a large number of peaks with LOW = 32 to 39 compared with the background level of 15 - 25. However, the number of peaks was far too large to enable any unambiguous assignment of values to the translational parameters y_o, z_o. It was noticed that the peaks occurred at very regular intervals, 0.7 Å in Y and 1.2 Å in Z. The search along X and Z showed a similar phenomenon the intervals being 0.4 Å in X and 1.2 Å in Z. These repeat distances are approximately one half of the projected interatomic distances in the molecular fragment shown in Figures 14a and 14b. In order to try and reduce this effect, the positions were calculated for a molecular fragment comprising two trans decalin rings both in the orientation A = 7°, B = 59° and C = 23°, and joined at C8 - C9 as in Figure 15. In the resulting translational minimum functions the 'periodicity' was not reduced to any marked extent.

Attempts were made to interpret these results by postulating partial fits between the molecule fragment and the postulated structure of zeorin acetate, as the former was translated along the crystallographic directions in the orientation shown in Figures 14a and 14b, and taking into account packing considerations. These methods proved abortive and as it was becoming almost as difficult to interpret the translational minimum function as it would be to visually interpret the Patterson, the method was abandoned.

Conclusion

In retrospect, there are several factors that may have contributed to the failure of this Patterson search technique as a method for the determination of the crystal structure of zeorin acetate.

Certainly for structures that have been solved by this method, the geometry of the molecular fragment has been well defined. The two alkaloid structures analysed by Nordman and Nakatsu (1963) and Nordman and Kumra (1965) contained planar oxindole and indole groups respectively and the solution of a pyrethosin derivative by Nordman and Gabe (1965) was attained using an isoxazolone group and three other atoms as molecular

fragment. In both the sharpened and unsharpened work on zeorin acetate the rotational parameters A, B and C were not well defined, which must have resulted in part from the differences between the geometry of the trans decalin rings in methyl melaleucate iodoacetate and zeorin acetate. The vector refinement program described by Nordman (1966) would probably be useful in this respect but was not available for this work.

The 'periodicity' that was noted in the translational minimum function during the work on the sharpened Patterson suggests that more serious difficulties are present. Although it was not recognised at the time, this effect was also present in the unsharpened work, but to a less marked extent. It was felt that the effect was due to the fact that the molecular fragment must consist of at least a trans decalin ring system, as this is the most well-defined feature of the molecule. The structure postulated from the chemical evidence contains three such systems of the A/B, B/C and C/D rings and a similar system in the part structure defined by the D/E rings. It was conceivable that the 'periodicity' could be reduced by using a molecular fragment comprising a larger number of atoms, though the results in this respect were disappointing. In the previous structure determinations to which this method has been applied, the compounds have all possessed only one, unique part structure that was used as molecular fragment and their unit cells con-

tained only two molecules (i.e. spacegroup $P2_1$ in each case).

The Convolution Molecule method that has been developed by Hoppe (1957) and described briefly on page 145 offers an alternative approach to the determination of the structure of zeorin acetate that would possibly not involve some of the difficulties encountered here. Attention is, in fact, now being turned towards this method.

It does seem that this method is not seriously dependent upon an accurate knowledge of the geometry of a molecular fragment. Huber and Hoppe (1965) have described the structure determination of the steroid, ecdysone, in which a ring junction initially assumed to be trans fused was later shown to be cis. However, it is not clear whether the 'periodicity' effect would be a problem. The Convolution Molecule method would also overcome a defect in the Patterson search technique used here, that of applying vector weights. However, it should be a simple modification to incorporate a scheme similar to that described by Nordman (1966) into the Patterson search programs.

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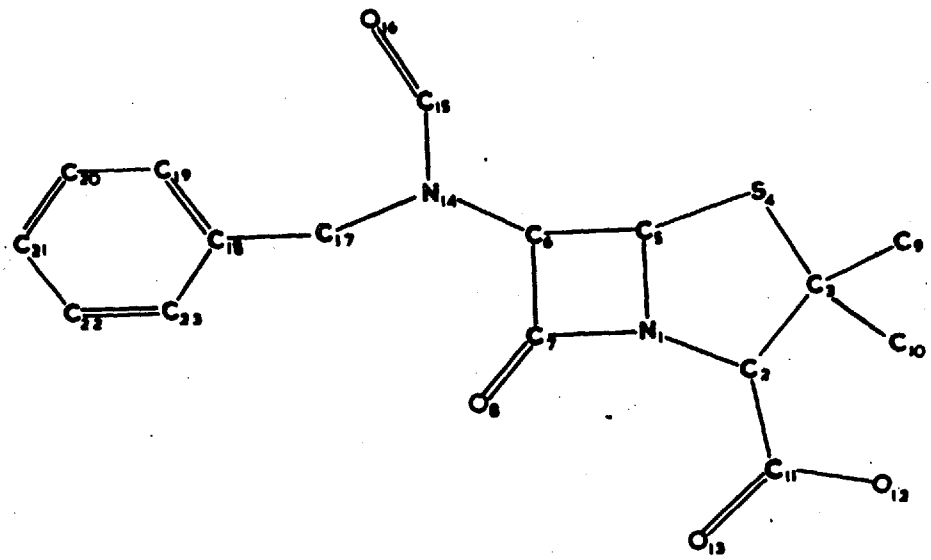
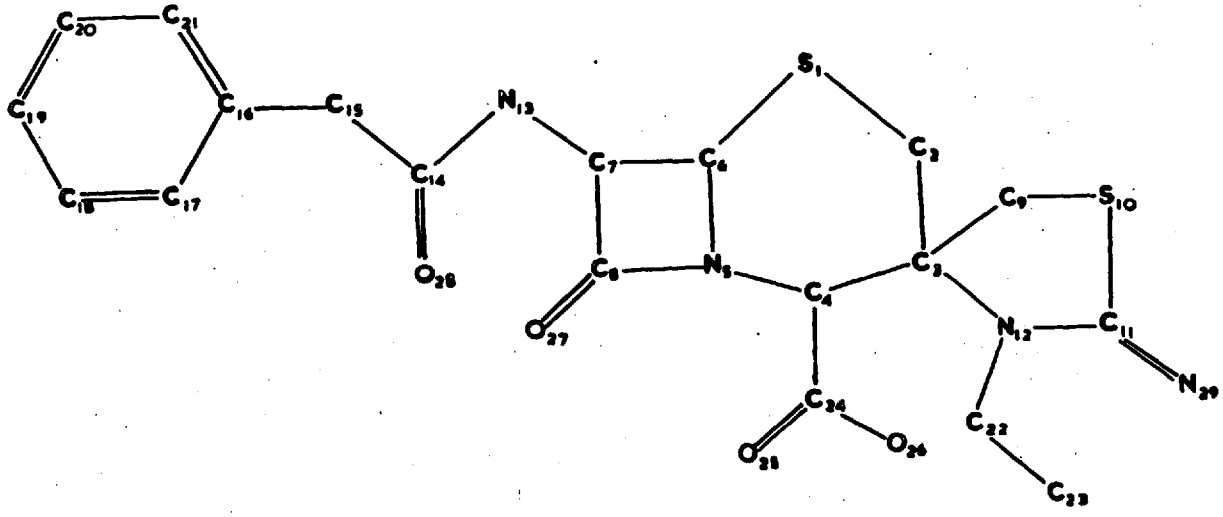


Structural formula for 6-NBF-PA.



Structural formula for the 3-spiro-thiazolinium-7-PAC derivative.