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THE CRYSTAL AND MOLECULAR STRUCTURE OF NOVOBIOCIN

BY

DAVID JOHN TAYLOR

A THESIS SUBMITTED FOR THE

DEGREE OF

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NATIONAL ACADEMIC AWARDS

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SCHOOL OF

MATHEMATICAL SCIENCES

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DECLARATION

The work described in this thesis was carried out in the School of Mathematical Sciences, Plymouth Polytechnic, under the supervision of Dr. M. O. Boles.

This is to certify that the work described in this thesis has been performed by Mr. D. J. Taylor under my supervision.

M. O. Boles

.....

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ABSTRACT

Previous studies on the biological activity of the antibiotic novobiocin have indicated that this unique molecule is required intact for full antibacterial activity. Structure-activity investigations performed by other workers have not been able to ascribe its antibacterial activity to any one feature of the chemical structure. Single crystal X-ray techniques have therefore been used to determine the structure in the solid state in the hope that this will contribute to a better understanding of the mode of action of the drug.

The thesis is divided into four chapters.

The known chemistry of the antibiotic is presented in detail in the first section. The syntheses of novobiocin and its analogues and its relationship to other antibiotics are then discussed and the section concludes with a review of structure-activity relationships in novobiocins and the mode of action of the drug.

The single crystal X-ray technique is then described with special emphasis on the direct methods of phase determination used in this study.

The determination of the crystal structure from photographic data is presented in chapter 3. The method of phase determination is discussed in detail and amplified in appendix B. The gross structural features and the hydrogen bonding scheme are also described.

Further refinement of the crystal structure from diffractometer data is presented in the final section together with the treatment of disorder within the structure. The molecular parameters obtained are discussed in terms of their relationships to those found in analogous chemical compounds.

The final part of this section concentrates on particular features in the molecule with reference to previous structural studies made using other spectral techniques.

Computer programs written for the initial structure determination are listed in appendix A and the paper published in Acta Crystallographica is included in appendix D.

CHAPTER 1

THE ANTIBIOTIC NOVOBIOCIN

1.1 Discovery and Use

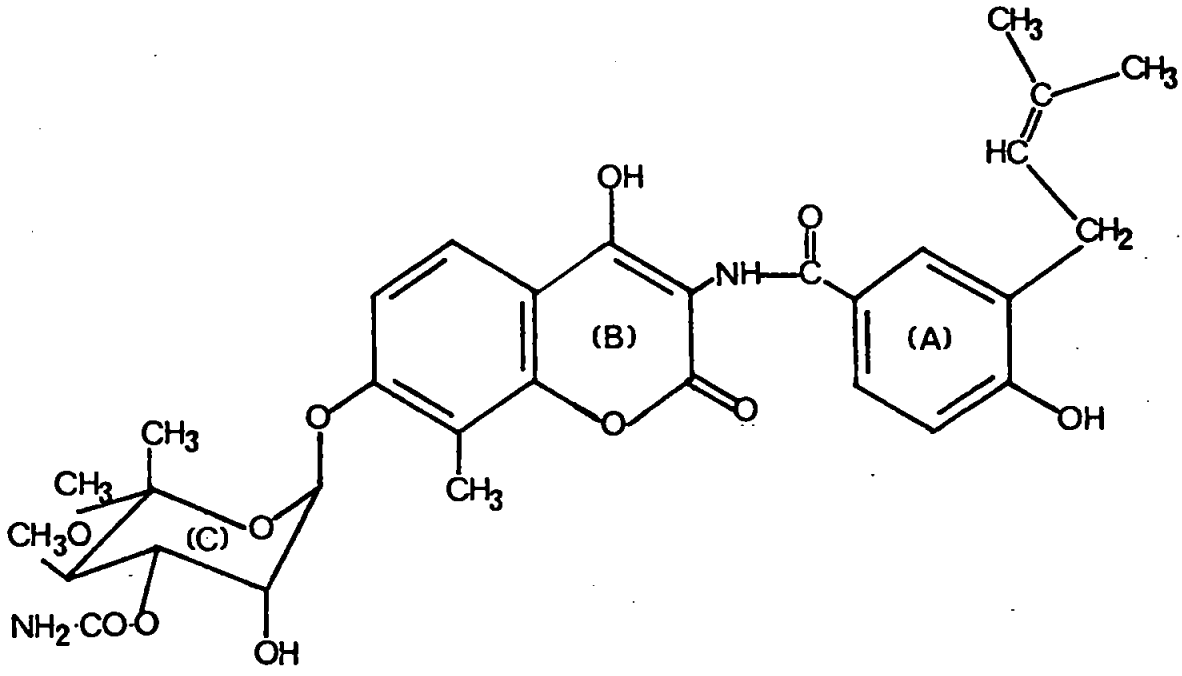
In the mid 1950's a number of research groups [1-5] independently discovered an antibiotic produced by the organisms Streptomyces niveus [6] and Streptomyces spheroides [7] which were isolated from soil samples. The antibiotic was described as having a wide range of antibacterial activity mainly against Gram-positive bacteria [8]. Most strains of Staphylococcus aureus are particularly susceptible having a minimum inhibitory concentration of 0.1-5.0 μg/ml [8]. It was soon recognised that these independent research efforts had produced the same compound [3,5]. The antibiotic was given the name novobiocin [80].

Novobiocin is not cross-resistant with commonly used antibiotics [9]. It is normally reserved for use against penicillin-resistant staphylococal infections [10]. A complete account of the range of antibacterial activity and the clinical applications of novobiocin has been presented in the review articles by Finland and Nichols [11] and by Macey and Spooner [12].

Recent studies by Brand and Toribara [13,14] have shown that novobiocin forms complexes with serum albumins. They have also shown that the circular dichroic spectra of these complexes are unique to the serum albumin of a particular species. Novobiocin may therefore be used as a reagent to distinguish serum albumins from different species.

1.2 The Chemical Structure of Novobiocin

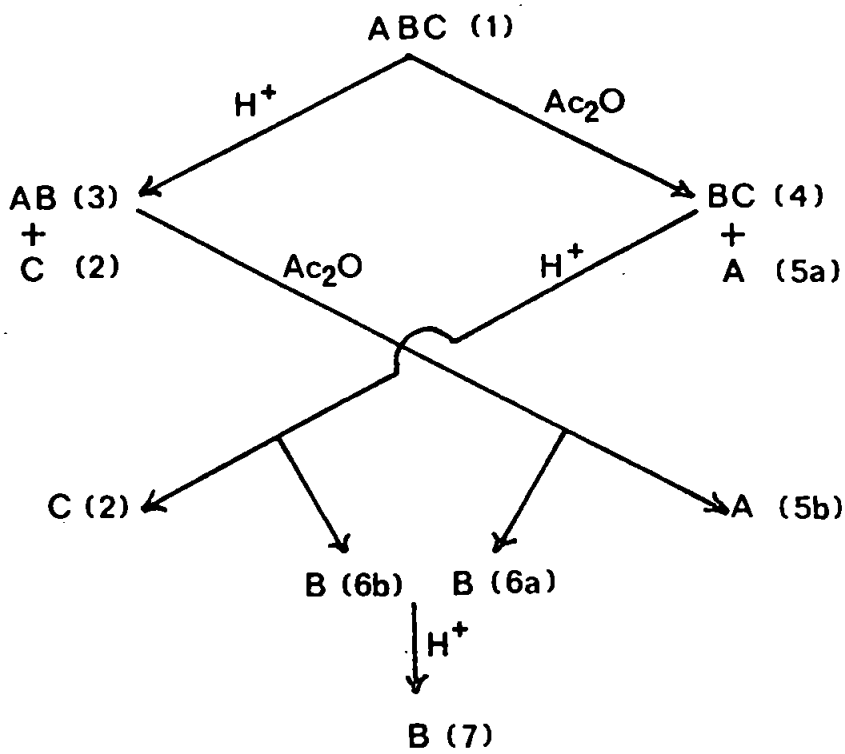
The chemical name of novobiocin (1) as illustrated below is 7 - [3 - O - carbamyl - 5, 5 - dimethyl - 4 - O - methyl - α - L - lyxosyl] - 4 - hydroxy - 3 - [4 - hydroxy - 3 - (3 - methylbut - 2 - enyl) benzamido] - 8 - methylcoumarin [10].



1 NOVOBIOCIN

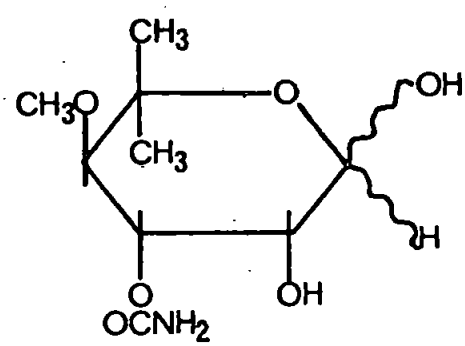
Independent studies in two laboratories [17,23,25-29,32] have elucidated the structure of novobiocin and its dihydro derivative. The molecule consists of three distinct moieties; namely : a substituted benzoic acid (A), an aminohydroxy coumarin (B), and a branched chain sugar (C) containing the rarely occurring carbamate. (A) is joined to (B) by an amide bond and (B) is joined to (C) via a glycoside link. These are the points at which cleavage reactions can occur freeing the individual moieties for structure determination The application of the cleavage reactions may be summarised by the

use of the ABC nomenclature [9]. (See below and in Figure 1.1).



Full details of the chemical characterisation of A, B and C are given in the review article by Hoeksema and Smith [9] and the references cited therein.

The stereochemistry of the sugar ring (C) has been the subject of several investigations. The early studies suggested that 3 - carbamyl - 4 - O - methyl noviose (8) had the L - lyxose configuration.



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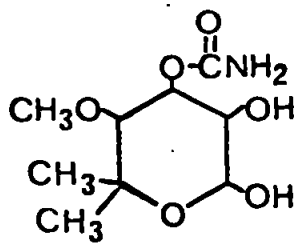

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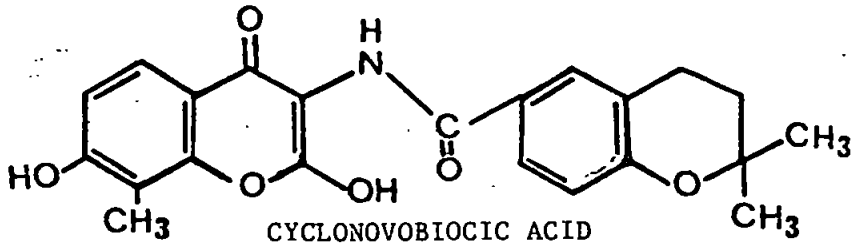
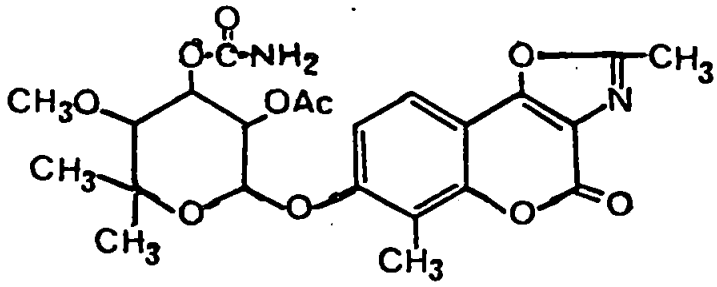


3. CYCLONOVOBIOCIC ACID



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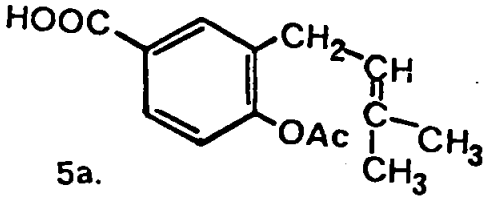
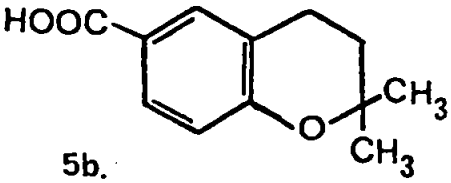
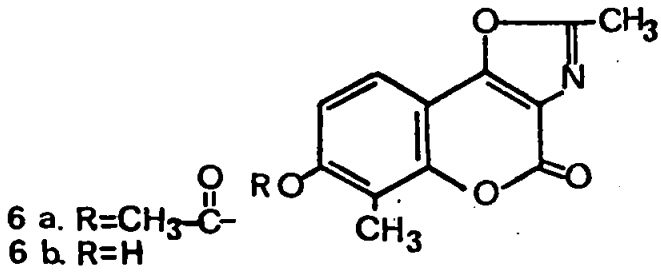


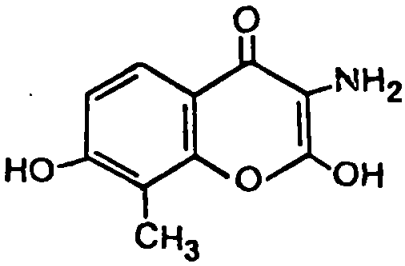
5a.



5b.



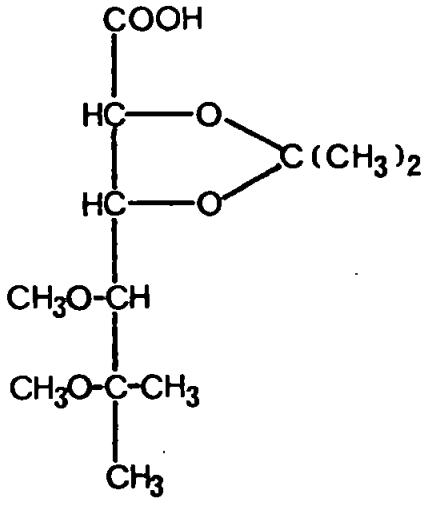
6 a. R=CH_3-C(=O)-
6 b. R=H



7.

Figure 1.1 Chemical structures of the A B C moieties produced by the cleavage reactions.

this arrangement was confirmed by synthetic studies [30]. It was found that the compound 5, 5 - dimethyl - 4, 5 - di - O - methyl - 2, 3 - isopropylidene - L - lyxonic acid (9) could be derived from the novobiocin sugar



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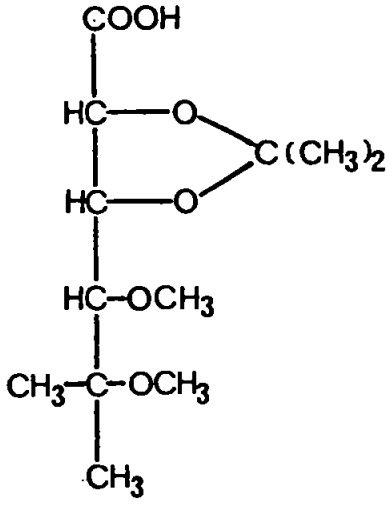

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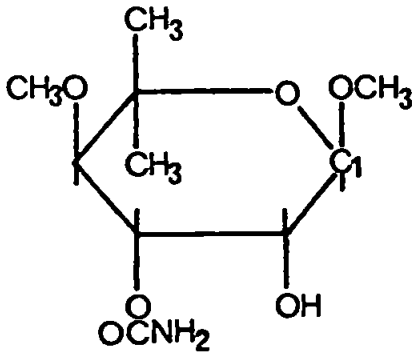
This compound was then synthesised in stereospecific fashion from methyl 2, 3 isopropylidene - L - rhamno furanoside which has the L - lyxose configuration. The identity of the crystalline benzhydryl ammonium salts of the acids produced by the two routes confirmed the assignment of configuration. A second stereospecific synthesis from a D - ribose derivative gave (10) which was shown to be markedly different from (9).

The conformation of the sugar ring and the configuration of the anomeric carbon remained to be determined. Walton et al [31] allocated to the two anomeric methyl 3 - O - carbamyl noviosides $[\alpha]_D^{25} = -28^o$ and $[\alpha]_D^{26} = +130^o$ (c = 1.0 in methanol)

the α and β configurations respectively at C_1



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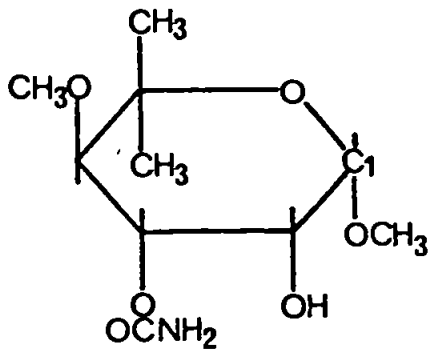


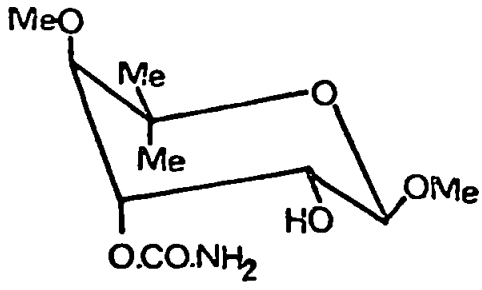
12

Using the values of molecular rotation for (11), (12) and novobiocin (1), they applied Hudson's rules of isorotation [33] and showed that the glycoside link in novobiocin is of the α - L - configuration.

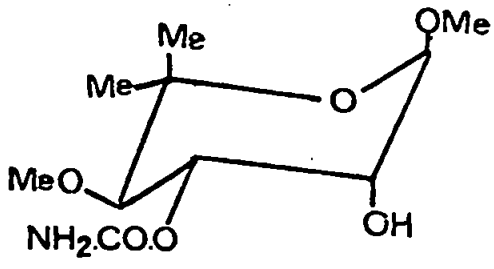
Proton magnetic resonance studies were made on the conformation of the sugar ring by two groups of workers, Barker et al [34] and Golding and Rickards [35]. Both sets of workers drew on the conclusions of Lemieux et al [36] that the chemical shifts for acetoxy and methoxy groups in six membered rings are characteristic of their axial or equatorial orientations.

Barker et al showed that the chemical shifts attributed to the protons in the 1 and 4 methoxy groups indicated that for the α anomer the Cl conformation was favoured and for the β anomer the 1C conformation (Figure 1.2).

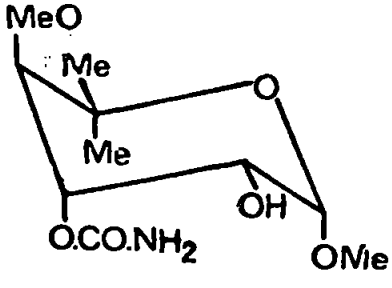
Golding and Rickards however showed that the assignments of these chemical shifts were incorrect and should be interchanged. Further they found that the magnitudes of the coupling constants between the hydrogens attached to the ring carbons provided



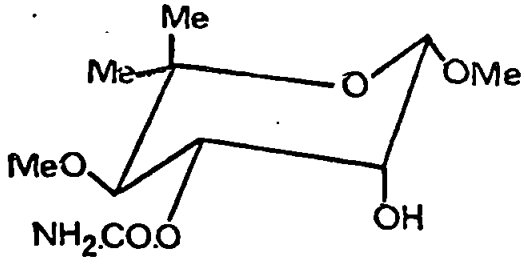
α C1



α 1C



β C1



β 1C

Figure 1.2 The anomers and conformers of L-methyl noviosides

unequivocal evidence that α and β noviosides and novobiocin exist in the 1C conformation. The diamagnetic shift of the H_1 proton in going from the α to the β methyl noviosides agreed with its equatorial and axial orientations in the α1C and β1C conformations.

These studies completed the stereochemistry of the sugar ring and of novobiocin. The molecule therefore has the chemical structure (1) as shown at the beginning of this section.

1.3 Salts and Derivatives

The annual production of novobiocin in the U.S.A. alone in 1961 was about 15,000 kg [9]. The salt of the greatest commercial importance at that time was the acid sodium salt which has been isolated in at least three polymorphic forms [9]. The mono calcium acid salt was also of considerable commercial significance [17]. Many other Group I and Group II metal salts of novobiocin involving one or both acidic functions have also been prepared [15,20].

Amine acid salts of primary, secondary and tertiary amines are easily made, though they are frequently difficult to crystallise [15]. Esters of novobiocin have been made firstly on the sugar then on the phenolic hydroxyl and lastly with difficulty, on the enolic hydroxyl. All of these materials are reported to be active in vitro and in vivo [21,22]. Ethers have also been prepared by reaction with diazomethane [17,23].

Hydrogenation of novobiocin in ethanolic solution using a Pt or Ni catalyst results in the uptake of one mole of H_2 in the side chain attached to ring (A) and the formation of dihydronovobiocin. $C_{31}H_{38}N_2O_{11}$ [23] with antibacterial properties nearly identical with the parent compound. Crystalline salts of dihydronovobiocin are

formed when reacted with alkali [17]. Novobiocin and its dihydro derivative may be separated from each other by a paper chromatographic technique devised by Wolf and Nescot [24].

1.4 The Chemistry of Novobiocin

Novobiocin is a dibasic acid with a molecular formula of $C_{31}H_{36}N_2O_{11}$. It has been crystallised in two polymorphic forms [15], the less common form [16], referred to as 'form 1' melts at 174-178°C while the more prevalent 'form 2' melts at 152-156°C. The two polymorphs are interconvertible via an amorphous form. Form 2 crystallises from such solvent systems as acetone and water, ethanol or ethyl acetate. Form 1 may be obtained from a solvent mixture of acetone and hexane [9]. The two polymorphs exhibit distinct infra-red spectra [15].

Ultraviolet spectra of novobiocin in solution show a bathochromic shift of the principal absorption from 308 mμ to 333 mμ when the solvent is changed from an alkaline ethanolic solution to an acidic one [15,17]. Brand and Toribara [13] have related this shift to a change in the conformation of the molecule. This shift will be examined more closely in Chapter 4 in the light of the determined crystal structure. Novobiocin is levorotatory, the rotation varying with the pH of the solvent.

e.g. $[\alpha]_D^{23-28^o} = -63.0^o$ (c = 1%, 95% ethanol, 2 decimetres [15,18])

Novobiocin is stable in either acid crystal form at 24°C in the absence of light, to which it is slightly sensitive [15].

The acid forms are soluble in aqueous solution above pH 7.5 and insoluble in more acid solutions. Novobiocin is also soluble in

acetone, ethyl or amyl acetate, methanol, ethanol and pyridine [15].

The pKa values of the two acid functions in water are 4.3 for the enolic and 9.1 for the phenolic group [15]. The stability of the antibiotic under acidic and basic conditions is very different. Mildly acidic conditions, pH 3-6, cause little rundown. Vigorous acid treatment degrades the antibiotic rapidly but this is not important in fermentation or extraction processes. Mildly alkaline conditions produce considerable degradation which is a serious problem in novobiocin processing. In a buffer solution at pH 10 a rapid decrease in antibacterial activity is observed which levels off after about two hours when 65-70% of the initial activity remains [9].

Hinman et al [19] showed that the above loss of activity was due to the formation of an equilibrium between novobiocin and an inactive material. On separation by countercurrent distribution the inactive substance, named isonovobiocin, was shown to have identical physical properties to novobiocin. If isonovobiocin was subjected to the basic equilibrating conditions 55-60% re-conversion to novobiocin occurred. The rate of isonovobiocin formation increases with temperature and decreases with a drop in pH. More vigorous alkaline treatment of isonovobiocin leads to a second degradation product $C_{31}H_{35}NO_{10}$ called descarbamyl novobiocin [19] having negligible antibacterial activity.

Hinman et al [19] showed that isonovobiocin has the same structure as novobiocin except for a migration of the carbamyl group from carbon 3 to carbon 2 in the noviose ring (C). Descarbamyl-novobiocin has hydroxyl groups on both carbons 2 and 3 of this

sugar ring.

1.5 Synthetic and Biosynthetic Studies of Novobiocin and Novobiocin Analogues

1.5. i Synthetic Studies

Novobiocin has been modified in an effort to obtain analogues which would be chemotherapeutically superior to the parent compound. To date none has been found but several have been detected in the same range of antibacterial activity as novobiocin itself [37].

Although the total synthesis of novobiocin and analogues by chemical means has been achieved it is a long and difficult process [38]. The complete synthesis of the aglycon portions of various novobiocins, e.g. cyclonovobiocic acid (3) was reported in 1958 [39]. Transformation of descarbamyl novobiocins to mixtures of novobiocins and isonovobiocins using carbamyl chloride appeared in the patent literature in 1959 [40].

Synthesis of the sugar moiety is the most difficult stage and the sugar was often obtained in early studies from novobiocin itself for use in the preparation of analogues [41]. Condensation of the various novobiocic acids with noviosyl chlorides has produced analogues with antimicrobial properties, e.g. desmethyl novobiocin and cyclonovobiocin [41,42].

1.5. ii Biochemically Produced Analogues

Several groups of workers have made analogues of novobiocin by biochemical methods. Walton et al [43] fed various substituted benzoic acids, (ring (A)), to the novobiocin producing organism Streptomyces spheroides and obtained 19 novobiocin analogues. Their studies indicated that:

1. New analogues with para-substituents on ring (A) may be obtained biosynthetically; all acceptable precursors had an oxygen or nitrogen atom attached to the benzene ring.
2. New analogues with meta-substituents on ring (A) were obtained; all acceptable precursors contained various alkyl, aryl, or substituted alkyl groups and required a carbon atom attached to the benzene ring.

Kominek et al [37] considered substitution of ring (A) by hydrolysing the amide bond between rings (A) and (B), isolating the BC portion and acylating it with various acids. The cell free extractions of a bacterium were isolated and found to cause hydrolysis of the amide bond to yield the desired noviosylaminocoumarin. This compound, named novenamine was crystallised pure as the hydrochloride. It has very weak antibacterial properties. Chemical N - acylation of novenamine produced analogues of varying antibacterial activity, none as active as novobiocin in vivo against Staphylococcus aureus.

Modification of the aminocoumarin moiety was achieved by Birch [44] and Lemaux and Sebek [45]. A chloro analogue of the coumarin (Cl replaces CH_3) was prepared chemically and incubated with mutants of Streptomyces niveus which do not synthesise the coumarin portion of the antibiotic. A new chlorinated novobiocin analogue was produced with a spectrum of activity similar to that of novobiocin.

1.5. iii <u>The Biosynthesis of Novobiocin</u>

The recent paper by Kominek and Sebek [37] provides a comprehensive survey of previous biosynthetic studies and discusses the biochemical implications of their recent research. Their conclusions are best summarised in the form of the two charts shown in Figures 1.3 and 1.4.

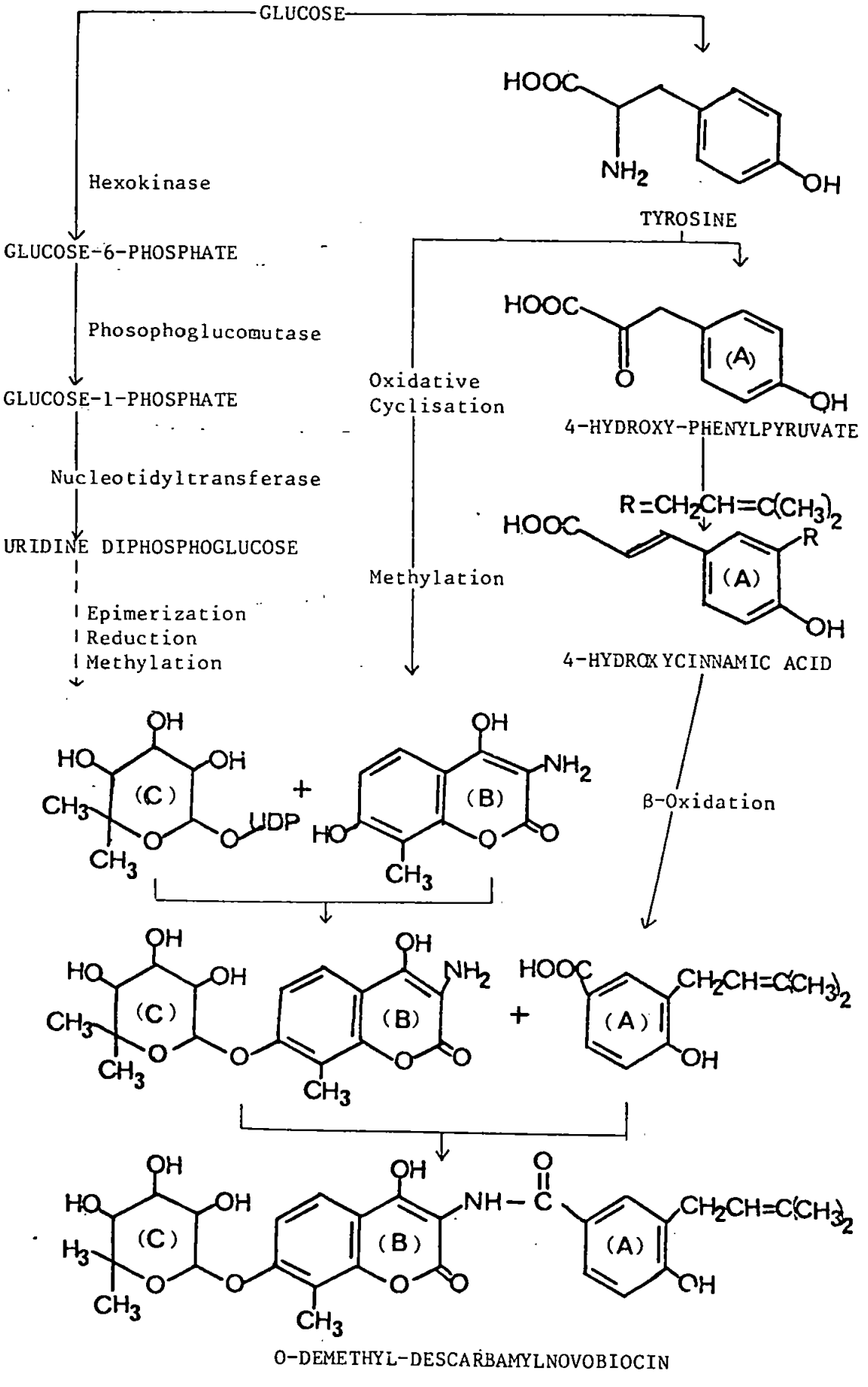


Figure 1.3 Tentative pathway for the synthesis of the individual ring systems of novobiocin and their linkage

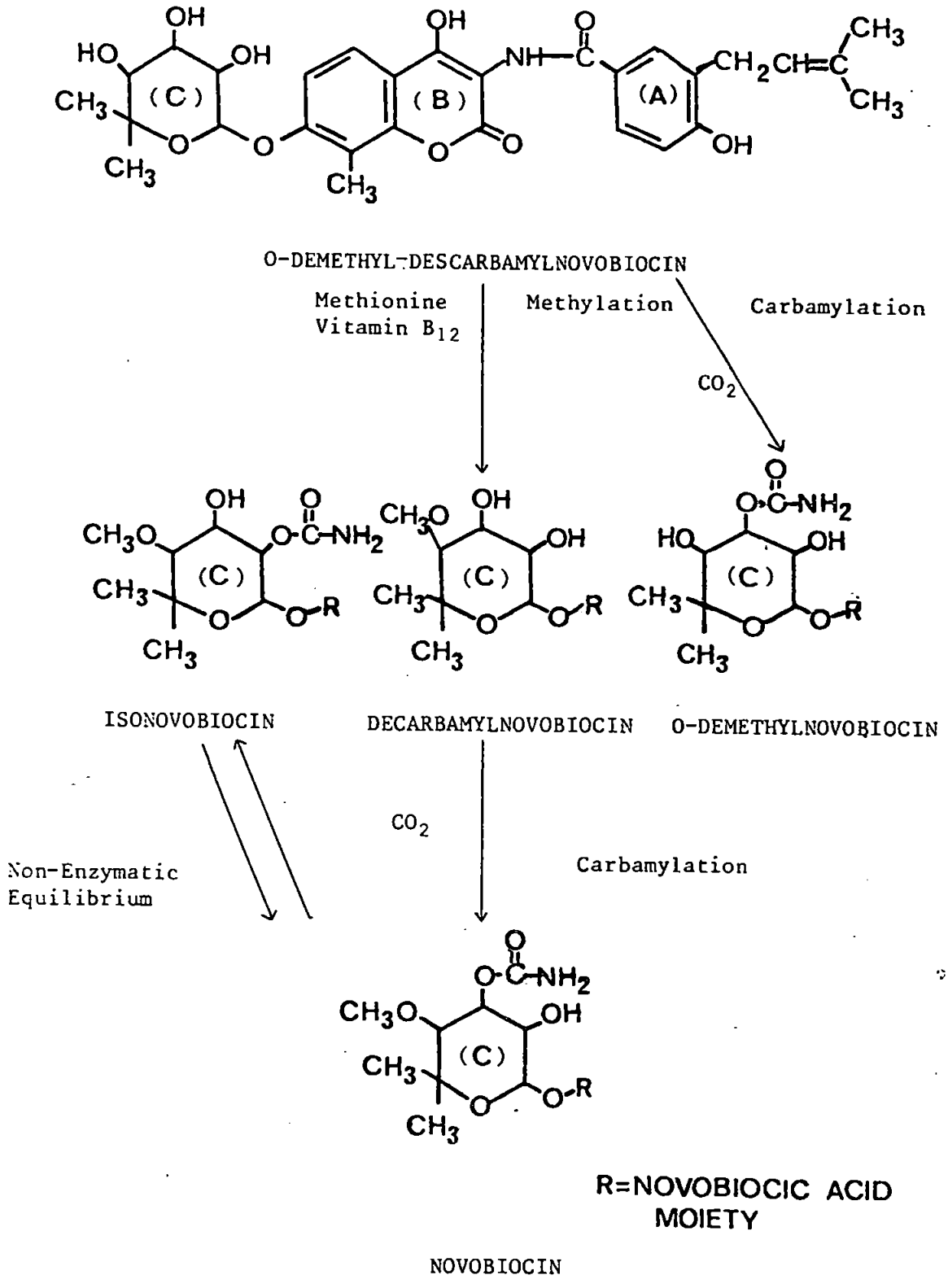
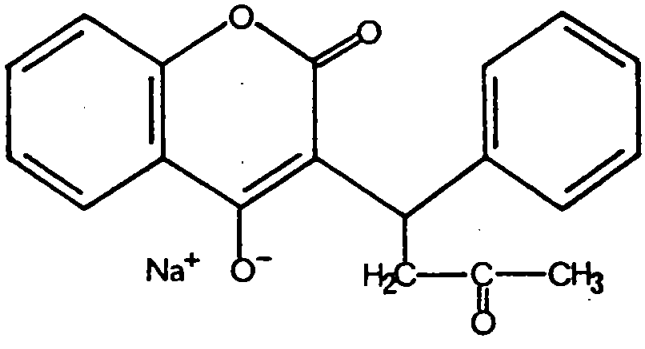


Figure 1.4 Carbamylation and methylation in the formation of novobiocin

1.6 Relationship to Other Antibiotics

Novobiocin does not belong to any particular chemically defined group of antibiotics [10]. There are, however, some groupings in the molecule that are similar to those in other antibiotics. The coumarin nucleus, ring (B), occurs in many antibacterial substances [53,54] and in particular in synthetic 3 - acylamino - 4 - hydroxycoumarins [55]. It also occurs in drugs such as warfarin (13)



13 WARFARIN SODIUM

Ring (A) has a phenolic group and phenols are known to interfere with membrane integrity [56,57]. However the behaviour of novobiocin is not similar to that of phenols [58,59].

Novobiocin has marked chemical similarities to the antibiotics of the coumermycin family. The structure of these antibiotics are compared in Figure 1.5. Keil et al [60], while searching for a more soluble coumermycin derivative, produced a number of close analogues of novobiocin.

e.g. [PNB] - NH - CO - C_6H_5-3-(R_1)-4-OH

with R_1 = CH_3, n - C_4H_9, $C(CH_3) = C(CH_3)_2$, $CH(CH_3)\ CH(CH_3)_2$ or n - C_6H_{13}.

All these showed high antibacterial activity particularly n - C_4H_9 and

n - C_6H_{13}, but suffered from high serum binding in vivo.

Recently the new antibiotic chlorobiocin (see Figure 1.5) has been isolated by Mancy et al [61] and Ninet et al [62]. This is produced by three species of Streptomyces. It contains a chlorine atom in the 8 position of the aminocoumarin in place of the methyl group and the carbamyl group of the noviose sugar is replaced by a methyl pyrrole.

Morris and Russell [10] comment on reports concerning cross resistance with other antibiotics [2,63-65]. These reports indicate that an organism resistant to novobiocin is usually sensitive to other commonly used antibiotics and that organisms resistant to particular antibiotics are sensitive to novobiocin. These studies involved the use of bacitracin, chloramphenicol, penicillin, tetracycline, polymyxin and streptomycin and suggest [10] that the effects produced by novobiocin are different from those produced by these other antibiotics.

1.7 Structure Activity Relationships in Novobiocins

The preparation of derivatives and analogues of novobiocin has been dealt with in previous sections of this chapter. This section collates this data from the viewpoint of the correlations between structure and activity in novobiocin and its analogues (see Figure 1.6).

Alteration of the acyl moiety, ring (A), does give rise to analogues with antibacterial activity but none were as active as novobiocin in vivo [37,43,44]. Figure 1.6 and section 1.5. ii indicate the restrictions on the substituents of ring (A) that retain significant activity.

The aminocoumarin moiety, ring (B), was modified by Birch et al [44] to give a chloro derivative, X = Cl (see also 1.5. ii). Desmethyl-

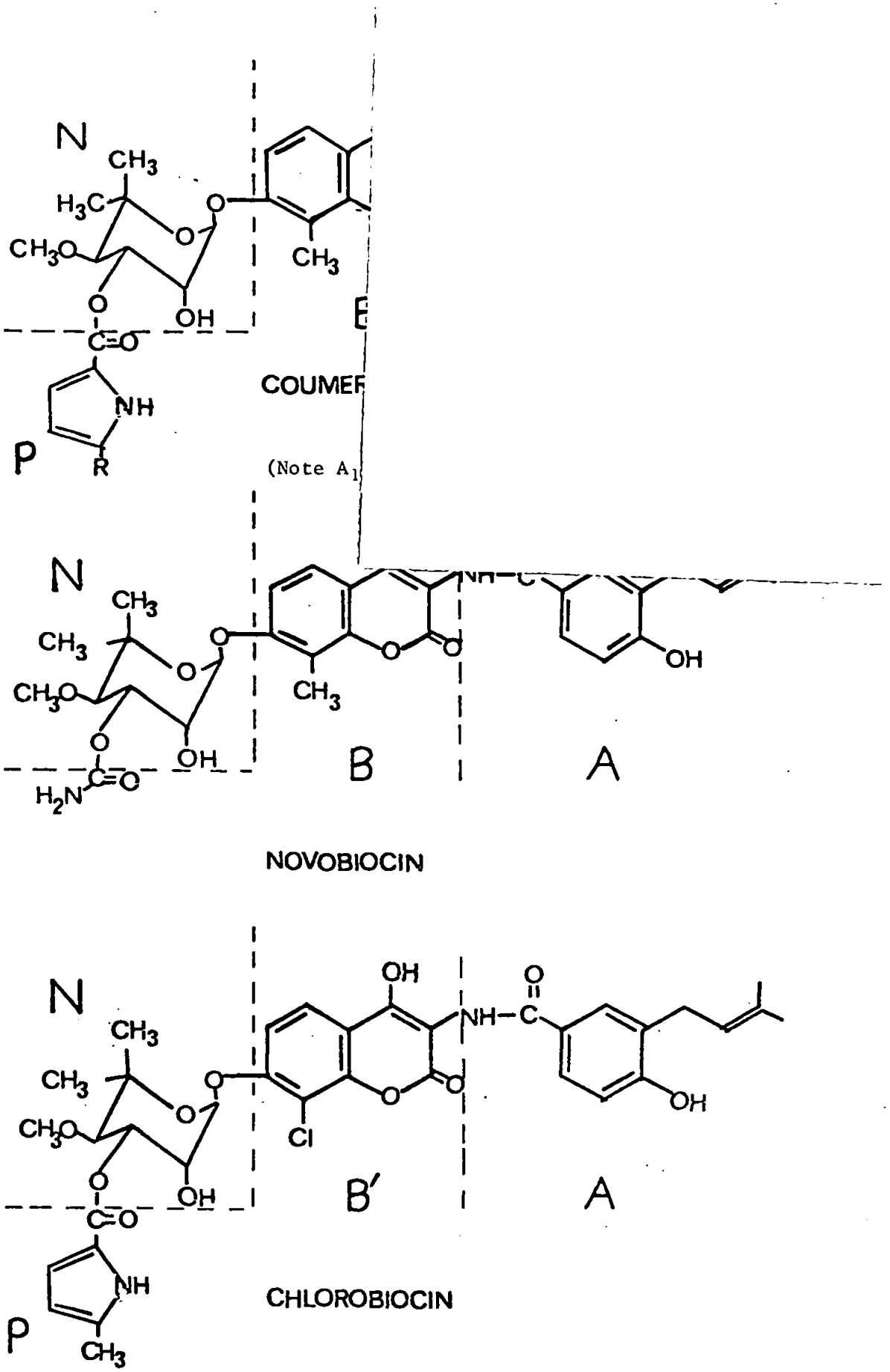
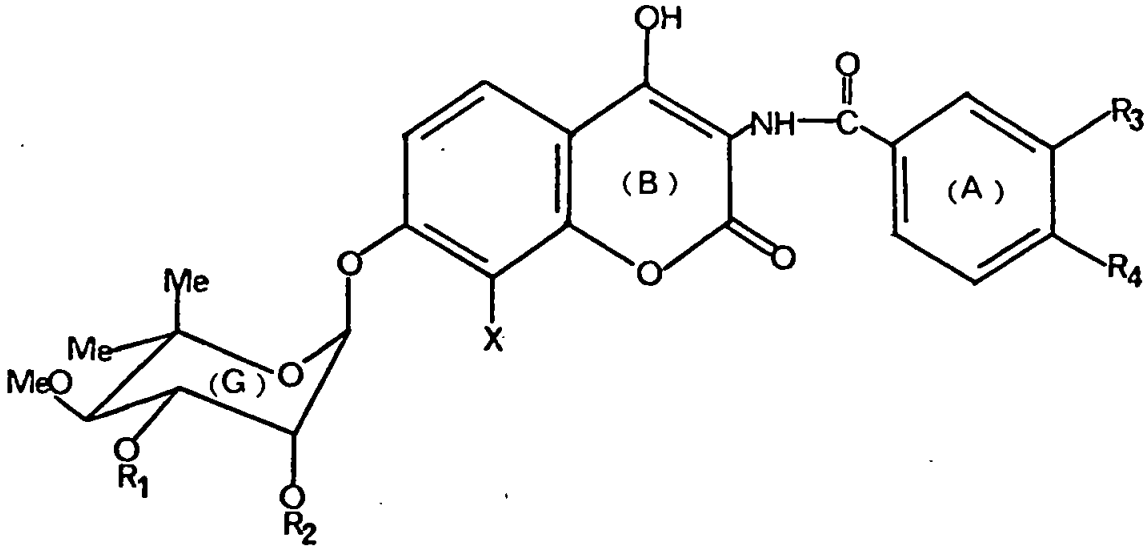


Figure 1.5 Comparison of the chemical structures of novobiocins and coumermycins



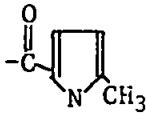
	X	R_1	R_2	R_3	R_4	Activity
Novobiocin	Me	$-\overset{O}{\overset{\|\|}{C}}-NH_2$	H	$-CH_2-CH=C(CH_3)_2$	OH	Active
Dihydro-Novobiocin	Me	$-\overset{O}{\overset{\|\|}{C}}-NH_2$	H	$-CH_2-CH_2-CH(CH_3)_2$	OH	Active
Iso-Novobiocin	Me	-H	$-\overset{O}{\overset{\|\|}{C}}-NH_2$	$-CH_2-CH=C(CH_3)_2$	OH	Inactive
Descarbamyl-Novobiocin	Me	-H	H	$-CH_2-CH=C(CH_3)_2$	OH	Inactive
Desmethyl-Novobiocins	H	$-\overset{O}{\overset{\|\|}{C}}-NH$	H	$-CH_2-CH=C(CH_3)_2$	OH	Active
Walton's analogues (para)	Me	$-\overset{O}{\overset{\|\|}{C}}-NH_2$	H	$-CH_2-CH=C(CH_3)_2$	OR,NR	Active
Walton's analogues (meta)	Me	$-\overset{O}{\overset{\|\|}{C}}-NH_2$	H	-R,-Ar,-RX	OH	Active
Birch's Chloro analogue	Cl	$-\overset{O}{\overset{\|\|}{C}}-NH_2$	H	$-CH_2-CH=C(CH_3)_2$	OH	Active
Chloro-biocin	CL	$-\overset{O}{\overset{\|\|}{C}}$ 	H	$-CH_2-CH=C(CH_3)_2$	OH	Active

Figure 1.6 Structure activity relationships in novobiocins

novobiocins are also known (see also 1.5. i). Both of these analogues are active substances.

The most sensitive moiety is the noviose ring (C). Any movement of the carbamyl group (isonovobiocin) or its removal (descarbamylnovobiocin) results in a complete loss of activity. This indicates that the presence and positioning of this group are of major importance in conferring activity on the molecule. Replacement of this group with 5 - methyl - pyrrolyl - 2-carbonyl produces active substances of the coumermycin family with a wide variety of acyl moieties (rings (A)). Chlorobiocin (see 1.6) also indicates the active nature of the pyrrole group. The spectra of activity of these pyrrole 'analogues' do differ however from those of the novobiocin series.

Many simple derivatives of novobiocin have been made (see 1.3). The salts, esters and ethers are all active substances similar in activity to the free acid. Dihydronovobiocin is almost identical in activity to the parent compound.

The free acid exists in two crystalline polymorphs and an amorphous form (1.4). Both Form 1 and the amorphous form show antibacterial activity in vitro and in vivo. Form 2 shows comparable in vitro activity in solution. However when taken orally this form does not protect mice infected with Staphylococcus aureus nor does it produce significant blood levels in humans [9,66]. The alkali salts do not suffer from these anomalies. These observations suggest that small changes in conformation have marked effects on the antibacterial activity.

It has been demonstrated that the activity of novobiocin is very dependent on the presence and position of the carbamyl group in ring (C).

This suggests, therefore, that the activity of novobiocin is not directly related to the presence of the coumarin moiety (referred to in section 1.6). Morris and Russell (1971) [10] concluded that it is not possible to attribute the major effects of novobiocin to any particular part of its unique chemical structure, the intact molecule being necessary for full antibacterial activity.

1.8 The Mode of Action of Novobiocin

In the integrated cell, inhibition of one process may lead to the inhibition (or stimulation) of other processes through the alteration of feedback systems or other control mechanisms. Firm conclusions cannot therefore be drawn from a study of the effect of a drug on a single process. It is therefore necessary to study the temporal changes in a wide variety of processes following the administration of the drug to identify the earliest processes that are affected. The target of an antibiotic is that element or elements within the cell with which the antibiotic combines so as to alter the functioning of the cell. This target may be a specific substance such as a protein, enzyme or nucleic acid or a less specific element such as a phospholipid or a divalent cation [69].

Studies by Brock (1962)[70] indicated that novobiocin forms a charge transfer complex with Mg^{2+} ions. He also showed in a later publication (1967) [69] that many of the effects upon bacteria induced by novobiocin are similar to those induced by a magnesium deficiency. Other workers [71,72] have shown that such a complex is not formed but the conditions of these experiments are not identical with those used by Brock. In particular the concentrations of drug and Mg^{2+} ions are

different and Brock's experiments were conducted on the free acid whereas the later workers used monosodium novobiocin. Brock also showed that no complex formed between descarbamylnovobiocin and Mg^{2+} which suggests that the carbamyl group may interact with the enolic or some other electron-donating part of the molecule. (This will be discussed later in Chapter 3). In addition to the conflicting evidence of complex formation Morris and Russell [10] listed the results of more recent biological studies which indicated that induction of a magnesium deficiency does not provide a complete explanation of the mode of action.

After considering the evidence available at the time (1971), Morris and Russell arrived at the following conclusions concerning the mode of action of novobiocin.

a) The effects of novobiocin on cell wall, RNA and protein syntheses and on enzyme systems may be considered to be secondary effects of the antibiotic.

b) The theory that novobiocin induces an intracellular deficiency of Mg^{2+} ions is far from convincing.

c) Recent findings [73-75] show that novobiocin has an immediate and pronounced effect on DNA synthesis which may be of primary importance.

d) Novobiocin interferes at an early stage with membrane integrity [73,78].

e) Membrane and chromosome in the bacterial cell are closely associated and therefore conclusions (c) and (d) need further research to clarify the situation [76-77].

This study was initiated in the anticipation that a full three-dimensional structure determination will contribute to a better

understanding of the mode of action of novobiocin and to the reasons why this unique molecule appears to be required intact for full antibacterial activity.

CHAPTER 2

SINGLE CRYSTAL X-RAY TECHNIQUES

2.1 X-Ray Diffraction and the Reciprocal Lattice

A crystal is a three-dimensional periodic array of atoms. It is characterised by a parallelepiped-shaped structural sub-unit, the unit cell, which is repeated in three dimensions to form the complete crystal. The unit cell is defined by three vectors a, b and c, the unit cell edges, and the arrangement of atoms within this defines the complete crystal structure.

The atoms within the unit cell may be replaced by a representative 'lattice' point and the collection of points so formed is termed the direct lattice of the crystal. A crystal acts as a three-dimensional diffraction grating for X-rays and the angles of diffraction depend upon the dimensions of the lattice and the wavelength of the X-rays. The lattice points may be arranged arbitrarily in a number of ways in parallel, equidistant lattice planes. The set of planes intersecting the axes at $\frac{a}{h}$, $\frac{b}{k}$, $\frac{c}{l}$ is denoted by the Miller indices hkl.

X-ray diffraction from a crystal was described by Bragg (1913) [81] in terms of specular reflection from a set of lattice planes containing scatterers. He showed that for a diffraction maximum to be obtained:

$$\lambda = 2d_{hkl} \sin\theta \qquad (2.1)$$

where λ is the wavelength of the incident X-rays.

θ is the angle of incidence of the beam to the plane and d_{hkl} is the interplanar spacing.

For diffraction from a three-dimensional grating, described by

the vectors $\underline{a}$, $\underline{b}$ and $\underline{c}$, the Miller indices, hkl, can be associated with each diffracted beam and indicate that scatterers separated by $\underline{a}$ scatter with a phase difference of $2\pi h$, $\underline{b}$ with a phase difference of $2\pi k$ and $\underline{c}$ with a phase difference of $2\pi l$. The inverse relationship between $2\sin\theta$ and d_{hkl} leads to the introduction of the concept of a reciprocal lattice. This is defined by the vectors $\underline{a}^*$, $\underline{b}^*$, $\underline{c}^*$, related to the direct lattice by the equations:

$$\begin{array}{lllllll} \underline{a}.\underline{a}^* & = & 1 & \underline{a}.\underline{b}^* & = & 0 & \underline{a}.\underline{c}^* = 0 \\ \underline{b}.\underline{a}^* & = & 0 & \underline{b}.\underline{b}^* & = & 1 & \underline{b}.\underline{c}^* = 0 \\ \underline{c}.\underline{a}^* & = & 0 & \underline{c}.\underline{b}^* & = & 0 & \underline{c}.\underline{c}^* = 1 \end{array} \qquad (2.2)$$

i.e. $\underline{a}^*$ is perpendicular to the plane defined by $\underline{b}$ and $\underline{c}$ (100) and has magnitude such that its scalar product with $\underline{a}$ is unity.

e.g. $|\underline{a}^*| = 1/d_{100}$

The reciprocal-space vector $\underline{S}_{hkl}$ is associated with the hkl reflection by the equation:

$$\underline{S}_{hkl} = h\underline{a}^* + k\underline{b}^* + l\underline{c}^* \qquad (2.3)$$

where $|\underline{S}_{hkl}| = 2\sin\theta/\lambda = 1/d_{hkl}$

The quantity $hx + ky + lz$ appears in many later formulae. If we represent a point in the unit cell by the vector:

$$\underline{r} = x\underline{a} + y\underline{b} + z\underline{c} \qquad (2.4)$$

and a point in reciprocal space by the vector:

$$\underline{h} = h\underline{a}^* + k\underline{b}^* + l\underline{c}^* \qquad (2.5)$$

Then from the definition of the reciprocal lattice:

$$hx + ky + lz = \underline{h}.\underline{r} \qquad (2.6)$$

X-rays are scattered coherently with a phase change of π by the electron clouds of the atoms. If the electron distribution is assumed to be spherically symmetric then the ratio of the amplitude

of coherent scattering from the atom to that from a point electron situated at the atomic centre is given by:

$$f(\underline{h}) = 4\pi \int_{r=0}^{\infty} \rho(\underline{r}) r^2 \frac{\sin 2\pi \underline{h}.\underline{r}}{2\pi \underline{h}.\underline{r}} \, dr \tag{2.7}$$

where $\rho(\underline{r})$ is the electron density at a distance $|\underline{r}|$ from the atomic centre. $f(\underline{h})$ is the scattering factor for the atom at rest. The factor $\frac{\sin 2\pi \underline{h}.\underline{r}}{2\pi \underline{h}.\underline{r}}$ shows that destructive interference between different regions of the electron cloud increases with increasing θ so reducing the magnitude of $f(\underline{h})$.

For a scattering process the scattering pattern is the Fourier transform (F.T.) of the scattering object. An X-ray diffraction pattern is therefore the F.T. of the electron density. Therefore:

$$F(\underline{t}) = \int_{\substack{\text{unit}\\\text{cell}}} \rho(\underline{r}) \exp(2\pi i \underline{t}.\underline{r}) dV \tag{2.8}$$

$$\rho(\underline{r}) = \int_{V*} F(\underline{t}) \exp(-2\pi i \underline{t}.\underline{r}) dV* \tag{2.9}$$

Where $F(\underline{t})$ is the diffraction pattern of a single unit cell of the crystal. The crystal itself is the convolution of a single unit cell with the crystal lattice. The convolution theorem leads therefore to the following table of inter-relationships [82]:

unit cell	*	crystal lattice	=	crystal
↕ F.T.		↕ F.T.		↕ F.T.
$F(\underline{t})$	X	reciprocal lattice	=	X-ray diffraction pattern

Since $F(\underline{h})$ is observed only at reciprocal lattice points, equations

2.8 and 2.9 become the familiar equations 2.10 and 2.11 for structure factors and electron density:

$$F_{\underline{h}} = \int_{\substack{\text{unit}\\ \text{cell}}} \rho(\underline{r})\exp(2\pi i\underline{h}.\underline{r})dV \tag{2.10}$$

$$\rho(\underline{r}) = \frac{1}{V}\sum F_{\underline{h}}\exp(-2\pi i\underline{h}.\underline{r}) \tag{2.11}$$

The bonding between atoms in a molecule changes the electron configurations around them only slightly. The integral in equation 2.10 may therefore be split up into a sum of integrals, one for each atom and over a volume including the complete electron content of the atom. Therefore from 2.7 and 2.10 we obtain equation 2.12:

$$F_{\underline{h}} = \sum_{j=1}^{N} f_j\exp(2\pi i\underline{h}.\underline{r}_j) \tag{2.12}$$

Where N is the number of atoms in the unit cell, $\underline{r}_j$ is the vector position and f_j is the scattering factor for the j th atom.

The structure factor $F_{\underline{h}}$ is in general complex and may be written as in equation 2.13:

$$F_{\underline{h}} = |F_{\underline{h}}| \exp(i\,\phi_{\underline{h}}) \tag{2.13}$$

The amplitude, $|F_{\underline{h}}|$, is the ratio of the scattering of the unit cell to that from a single point electron and $\phi_{\underline{h}}$ gives the phase relative to that of a point electron at the origin of the unit cell.

The intensity of an X-ray reflection from a crystal is proportional to $|F_{\underline{h}}|^2$ which implies that only the moduli of the structure factors may be deduced from the diffraction data. To solve a structure it is necessary to determine the phases of a large number of X-ray reflections and then compute the electron density function from equation 2.11. Some of the methods used for phase determination

are described in sections 2.6 and 2.7.

2.2 Space Groups

If the space lattice for a crystal is chosen so that it reflects the fundamental symmetry then it will conform to one of the 14 Bravais lattices. It may also be placed into one of the 32 'point groups' which represent the unique combinations of crystallographic symmetry elements. When lattice and point group are combined and translation operators are included there result 230 space groups which describe the only ways in which identical objects may be arranged in an infinite lattice. A space group is defined by the symmetry elements present in the unit cell or by the arrangement of points within the cell that possess these symmetry elements. The collection of arbitrary points such that the operation of any of the symmetry elements present upon any of the points does not generate a new point is termed a set of general equivalent positions.

The presence of translational symmetry elements in a space group gives rise to systematic absences in the X-ray diffraction pattern. Thus it is often possible to infer the space group of a crystal from the systematic absences observed. Two other results usually fix the space group uniquely.

Firstly, the number of molecules, N, in the unit cell is given by:

$$N = \rho \frac{VN_o}{M} \qquad (2.14)$$

where ρ = density of crystal

V = volume of unit cell

N_o = Avogadro's Number

M = molecular weight

N may then be compared with values permitted by the space group.

Secondly, examination of the intensity statistics will often indicate the presence or absence of a centre of symmetry in the structure,[93] or non-translational symmetry elements e.g. mirror - planes (Howell, Phillips and Rogers [83]).

2.3 Measurement of Intensities

The methods for recording and measuring intensities fall into two main categories.

Film Techniques

An X-ray photon has sufficient energy to sensitize a silver halide grain in a photographic emulsion to development. The optical density of a developed film log (I/I_o) is therefore a linear function of the exposure provided that no grain receives more than one quantum. In practice only a limited range of intensities may be recorded. The range provided by a crystal is much larger and this is overcome by the use of the multiple film technique of Robertson [84]. In this method several films are exposed simultaneously, each film acting as a filter to reduce the intensity that falls on the one beneath.

Estimation of the blackening produced may be made visually

or photometrically. As a result of the natural integrating properties of the human eye it is possible to obtain integrated intensities by visual comparison with a calibrated optical density scale prepared by controlled diffraction of a suitable reflection from the crystal studied. More accurate integrated intensities may be obtained photometrically using optical densitometers. The instrumental integration may be performed by the diffraction camera, the densitometer or both.

Counter Techniques

Intensities may be measured by direct counting of the diffracted photons using proportional or scintillation counters. Automated instruments, 'diffractometers' incorporating these detectors have greatly improved the speed and accuracy of data collection. A 'four circle' diffractometer, a common type, has four arcs which are adjusted automatically to bring any desired plane from the crystal into the reflecting position with zero-level Weissenberg geometry of measurement. The photons are counted as the crystal is rotated through the diffracting position, a measured background count is subtracted and the remaining counts are then proportional to the integrated intensity. Since $\sigma_{count} = \sqrt{count}$, weak intensities providing a small number of counts are difficult to measure accurately. The film technique is more applicable in this intensity range.

Two principle scanning procedures may be adopted. In a 'ω' scan the crystal moves while the counter is stationary. It is equivalent to a trace parallel to the centre line of a zero-level Weissenberg photograph. For a '2θ' scan the counter also moves such

that $\Delta 2\theta = 2\Delta\omega$. This corresponds to a diagonal trace through reflections on a common lattice row i.e. along the Laue streaks. A 2θ scan is preferred as high irregular backgrounds are readily detected.

2.4 Factors affecting Observed Intensities

Polarisation

The X-ray beam from a tube is unpolarised. Reflection from a crystal plane is more efficient parallel to the plane than perpendicular to it. The diffracted beam is therefore partially polarised. For an unpolarised incident beam the polarisation factor, p, is given by:

$$P = \frac{1 + \cos^2 2\theta}{2} \tag{2.15}$$

The Lorentz-Factor

This factor arises because the time taken for a reciprocal lattice point to pass through the sphere of reflection varies with its position in reciprocal space and the direction in which it approaches the sphere. For the equi-inclination Weissenberg technique used in this work the factor needed is:

$$L = \frac{\sin\theta}{\sin 2\theta \sqrt{\sin^2\theta - \sin^2\mu}} \tag{2.16}$$

where μ is the equi-inclination setting angle.

Spot Shape Corrections

It is observed that on upper level equi-inclination Weissenberg photographs, reflections close to the centre line are

extended on one half of the film and contracted on the other. Buerger [85] has shown that the effect is due to the divergence of the X-ray beam. Phillips has demonstrated that a correction may be made for the extended upper level reflections [86,87]. It is convenient to combine this correction with the Lorentz and polarisation factors to give a factor W/2LP [88] that varies more slowly with θ and μ than either of its parts and is therefore less sensitive to errors in cell parameters.

Absorption

When a beam of X-rays passes through a crystal it is attenuated such that the emergent beam has intensity, I, related to the original intensity, I_o, by the equation:

$$I = I_o e^{-\mu_\lambda t} \tag{2.17a}$$

where t is the thickness of the crystal and μ_λ is the linear absorption coefficient. μ_λ is given by the equation:

$$\mu_\lambda = \rho \sum \frac{Pn}{100} \cdot \left(\frac{\mu}{\rho}\right)_{\lambda, E_n} \tag{2.17b}$$

where ρ is the density, $\left(\frac{\mu}{\rho}\right)_{\lambda, E_n}$ is the mass absorption coefficient for a given λ and E_n, and the compound is composed of P_n% of element E_n. Systematic errors are therefore introduced into the observed intensities by the different average path lengths of X-ray beams in the crystal for different reflections. Correction factors may be calculated if the crystal shape is known exactly but are very difficult to compute except for the simplest shapes.

Extinction

Additional attenuation of the diffracted beam occurs when the crystal is set at the Bragg angle for a reflection. Darwin [89] termed it extinction and found two types.

Primary Extinction

When a crystal is in the diffracting position the reflected rays are at the proper angle for a second reflection. There is a phase change of $\pi/2$ on reflection [90] therefore any ray multiply reflected n times will interfere destructively with one reflected (n-2) times. This causes the intensity to be proportional to $|F|$ and such a crystal is termed ideally perfect. Most real crystals are composed of small mosaic blocks so primary extinction may be neglected and $I \propto |F|^2$.

Secondary Extinction

For intense reflections a sizable fraction of the incident radiation is reflected by the first planes encountered by the beam. Deeper planes thus receive less radiation and contribute with reduced power to the diffracted beam. The effect is most pronounced for large crystals of small mosaic spread at low $\sin\theta/\lambda$. A crystal where secondary extinction is negligible is termed ideally imperfect.

Temperature Factors

Atoms in a crystal vibrate about their rest positions - the vibration increasing with temperature. The electron cloud is therefore spread over a larger volume and the scattering power decreases with Bragg angle faster than for the ideal stationary model. Debye [138] + Waller [92] showed that for an atom vibrating isotropically the change in scattering power is given by $e^{-B\sin^2\theta/\lambda^2}$. B is termed the

temperature factor and is related to the mean square displacement, $\overline{U^2}$, of the atoms in a direction normal to the reflecting plane by the equation:

$$B = 8 \pi^2 \overline{U^2} \tag{2.18}$$

B therefore has units of $\mathring{A}^2$

The proper scattering factor for an isotropically vibrating atom is therefore:

$$f = f_o e^{-B \sin^2\theta/\lambda^2} \tag{2.19}$$

If the atom is assumed to vibrate anisotropically then six parameters are used to define the vibration.

The general expression is:

$$\exp [- 1/4 \ (B_{11}h^2a^{*2} + B_{22}k^2b^{*2} + B_{33}l^2c^{*2} + 2B_{12}hka^*b^* + 2B_{13}hla^*c^* + 2B_{23}klb^*c^*)] \tag{2.20}$$

where B_{ij} have the same units as B

or

$$\exp [- 2\pi^2 \ (U_{11}h^2a^{*2} + U_{22}k^2b^{*2} + U_{33}l^2c^{*2} + 2U_{12}hka^*b^* + 2U_{13}hla^*c^* + 2U_{23}klb^*c^*)] \tag{2.21}$$

where U_{ij} are mean-square amplitudes of vibration in $\mathring{A}^2$.

2.5 The Wilson Plot

The relative intensity, I_{hkl}, may be written as:

$$I_{hkl} = K \ |(F_{hkl})_R|^2 \exp(-2B\sin^2\theta/\lambda^2) \tag{2.22}$$

where $(F_{hkl})_R$ is the structure factor for the atoms at rest and B is assumed to be the same for each. It was shown by A. J. C. Wilson [93] that K and B may be obtained from the relative intensities. Since

$$<|F_R|^2> = \sum_{j=1}^{N} f_{o,j}^2$$ where the $f_{o,j}$ are scattering factors

for atoms at rest, it follows that if a small number of intensities within a narrow range of sinθ are considered then:

$$\overline{I_\theta}\ (\text{rel}) = K \sum_{j=1}^{N} f_{o_{j,\theta}}^2 \exp\ (-\ 2B\sin^2\theta/\lambda^2) \tag{2.23}$$

where $\overline{I_\theta}$ (rel) is the mean intensity for the range and $f_{o_{j,\theta}}$ are the at rest scattering factors for the mean value of θ.

Re-arranging and taking natural logarithms of both sides gives:

$$\ln \frac{\overline{I}_\theta\ (\text{rel})}{\sum_j f_{o_{j,\theta}}{}^2} = \ln K - \frac{2B\sin^2\theta}{\lambda^2} \tag{2.24}$$

Thus if the data is divided into zones of equal $\sin^2\theta/\lambda^2$ and $\ln\ (\overline{I}_\theta/\sum_j f_{o_{j,\theta}}^2)$ is plotted against $\sin^2\theta/\lambda^2$ a straight line is obtained of slope − 2B and intercept lnK. The graph is termed a Wilson plot and though the accuracy is not high the results are adequate for preliminary working values of K and the overall temperature factor B.

2.6 The Patterson Function - Heavy Atom Technique

A. L. Patterson [109] defined the function:

$$P(\underline{r}) = P(-\underline{r}) = V \int_V \rho(\underline{u})\rho(\underline{u} + \underline{r})d\underline{v} \tag{2.25}$$

and showed that it may be re-written as:

$$P(r) = \frac{1}{V} \sum |F_{\underline{h}}|^2 \exp\ (-\ 2\pi\ \ i\ \underline{h}.\underline{r}) \tag{2.26}$$

This function has peaks of weight $Z_i\ Z_j$ at $\underline{r}_i - \underline{r}_j$ where i, j take all values from 1 to N the number of atoms in the unit cell, and

may be computed without knowledge of the phases of $F_{\underline{h}}$. A Patterson map of a unit cell contains N(N-1) non origin peaks. The map is therefore very crowded and only vectors between heavy atoms or repeated vectors are commonly resolved. In practice a Patterson map is often used to locate the co-ordinates of a small number of heavy atoms in the structure.

If a crystal contains an atom of high atomic number then equation 2.12 for a structure factor may be re-written as:

$$F_{\underline{h}} = f_H \exp 2\pi i (\underline{h}.\underline{r}_H) + \sum_{j=1}^{N-1} f_{L_j} \exp 2\pi i (\underline{h}.\underline{r}_{L_j}) \qquad (2.27)$$

where f_H, f_{L_j} are the scattering factors for heavy and light atoms respectively. If the position of the heavy atom is known then a Fourier synthesis computed with phases derived from the heavy atom alone will usually reveal new atomic positions.

2.7 Direct Methods

In these techniques the phases of structure factors are derived directly by mathematical means from the measured intensities. In a crystal it may be assumed that:

a) The electron density is everywhere positive.

b) The electron density consists of discrete spherically symmetric atoms.

When properly used this information contains enough power to solve crystal structures and is the physical basis of direct methods.

2.7. i Unitary and Normalized Structure Factors

For discrete atoms, observed structure factors may be converted to unitary or normalized structure factors that take account of the

decline in $|F_{\underline{h}}|$ with $\sin\theta$. The unitary structure factor $U_{\underline{h}}$ is defined by:

$$U_{\underline{h}} = \frac{F_{\underline{h}}}{\sum_{j=1}^{N} f_j} \tag{2.28}$$

where $f_j = f_{o,j} e^{-B\sin^2\theta/\lambda^2}$

Since $\sum_{j=1}^{N} f_j$ is the maximum possible value of $F_{\underline{h}}$, $|U_{\underline{h}}|$ ranges from -1 to +1. By analogy with equation 2.12 $U_{\underline{h}}$ may be written as:

$$U_{\underline{h}} = \sum_{j=1}^{N} n_j \exp 2\pi i \underline{h}.\underline{x}_j \tag{2.29}$$

where n_j, the unitary scattering factor is defined as:

$$n_j = f_j / \sum_{j=1}^{N} f_j \tag{2.30}$$

The normalized structure factor $E_{\underline{h}}$ is given by:

$$|E_{\underline{h}}|^2 = |F_{\underline{h}}|^2 / \sum_{j=1}^{N} f_j^2 \tag{2.31}$$

Since from Wilson's statistics [93] $\sum_{j=1}^{N} f_j^2$ is the expected intensity of a reflection, $E_{\underline{h}}$ is a structure factor expressed as a fraction of its expected amplitude. Corresponding to 2.12 we have:

$$E_{\underline{h}} = \frac{1}{\left[\sum_{j=1}^{N} f_j^2\right]^{\frac{1}{2}}} \sum_{j=1}^{N} f_j \exp 2\pi i \underline{h}.\underline{x}_j \tag{2.32}$$

In practice the general form of equation 2.31 is:

$$|E_{\underline{h}}|^2 = |F_{\underline{h}}|^2/\varepsilon\sum_{j=1}^{N} f_j^2 \qquad (2.33)$$

where ε is a factor which takes account of the effect of space group symmetry on the values of $|F_{\underline{h}}|^2$.

The distribution of $|\underline{E}|$ values is, in principle, independent of the size and content of the unit cell. It is however affected by the presence or absence of a centre of symmetry. Both distributions, however, have $\overline{|\underline{E}|^2} = 1.000$ and this may be used to place reflections on an absolute scale when an estimate of B is available from a Wilson plot. All formulae in direct methods are more powerful when expressed in terms of E's rather than F's.

2.7. ii Inequalities and Probability Relationships

Harker and Kasper [94] made use of the positivity of electron density, Cauchy's inequality and the presence of symmetry elements to derive inequality relationships between structure factors that occasionally yielded definite phase information. Inequalities were further developed by Karle and Hauptman [95] who expressed the condition that the sum of a Fourier series should be everywhere non-negative in the inequality:

$$\begin{vmatrix} U_o & U_{\bar{\underline{h}}_1} & U_{\bar{\underline{h}}_2} & \cdots\cdots\cdots & U_{\bar{\underline{h}}_n} \\ U_{\underline{h}_1} & U_o & U_{\underline{h}_1-\underline{h}_2} & \cdots\cdots\cdots & U_{\underline{h}_1-\underline{h}_n} \\ U_{\underline{h}_2} & U_{\underline{h}_2-\underline{h}_1} & U_o & \cdots\cdots\cdots & U_{\underline{h}_2-\underline{h}_n} \\ \cdot & \cdot & \cdot & & \cdot \\ \cdot & \cdot & \cdot & & \cdot \\ \cdot & \cdot & \cdot & & \cdot \\ U_{\underline{h}_n} & U_{\underline{h}_n-\underline{h}_1} & U_{\underline{h}_n-\underline{h}_2} & \cdots\cdots\cdots & U_o \end{vmatrix} \geqslant 0 \qquad (2.34)$$

which may be of any order. Harker-Kasper inequalities may be derived from particular Karle-Hauptman determinant inequalities. These formulae require reflections of large $|U_{\underline{h}}|$ to indicate signs unequivocally. Since $\overline{U}_{rms} \approx 1/\sqrt{N}$ inequalities are rarely applicable to complex structures. For such structures relationships that are probably true have been devised and are in current use.

Karle and Hauptman [96,97] introduced the Σ_1 formula, a relationship that can indicate a positive or negative sign for $E_{2\underline{h}}$. In non-centrosymmetric space groups the formula may be applied to restricted phases e.g. for $P2_1 2_1 2_1$ the relevant formulae are:

$$S(E_{2h\ 2k\ 0}) \approx S\sum_{l} (-1)^{l+h}(|E_{hkl}|^2 - 1) \qquad (2.35a)$$

$$S(E_{0\ 2k\ 2l}) \approx S\sum_{h} (-1)^{h+k}(|E_{hkl}|^2 - 1) \qquad (2.35b)$$

$$S(E_{2h\ 0\ 2l}) \approx S\sum_{k} (-1)^{k+l}(|E_{hkl}|^2 - 1) \qquad (2.35c)$$

The probability of the Σ_1 formula was given by Karle and Hauptman [97] and Cochran and Woolfson [98] as:

$$P_+(E_{2\underline{h}}) = \tfrac{1}{2} + \tfrac{1}{2} \tanh \sigma_3 |E_{2\underline{h}}| \Sigma_1 / 2\sigma_2^{3/2} \tag{2.36}$$

where $$\sigma_n = \sum_{j=1}^{N} Z_j^n \tag{2.37}$$

It was shown by Sayre [99] that for a structure containing equal, resolved atoms the structure factors, $F_{\underline{h}}$, are connected by precise equations. Since the squared structure $\rho^2(\underline{r})$ contains equal, squared atoms of known shape at atomic sites with structure factor $G_{\underline{h}}$, he obtained from the convolution theorem:

$$\frac{1}{V} G_{\underline{h}} = \sum_{\underline{h}'} \frac{1}{V} F_{\underline{h}'} \frac{1}{V} F_{\underline{h}-\underline{h}'} \tag{2.38}$$

As $F_{\underline{h}}$ and $G_{\underline{h}}$ are related by the equation:

$$F_{\underline{h}} = \frac{f_{\underline{h}} G_{\underline{h}}}{g_{\underline{h}}} \tag{2.39}$$

it follows that:

$$F_{\underline{h}} = \frac{f_{\underline{h}}}{g_{\underline{h}} V} \sum_{\underline{h}'} F_{\underline{h}'} F_{\underline{h}-\underline{h}'} \tag{2.40}$$

which is known as Sayre's equation. This relationship applies generally and for non-centrosymmetric structure factors may be re-written as:

$$|F_{\underline{h}}| \exp(i\phi_{\underline{h}}) = \frac{\theta_{\underline{h}}}{V} \sum_{\underline{h}'} |F_{\underline{h}'}| |F_{\underline{h}-\underline{h}'}| \exp(i\phi_{\underline{h}'}) \exp(i\phi_{\underline{h}-\underline{h}'})$$

<u>or</u> $$|F_{\underline{h}}| \exp(i\phi_{\underline{h}}) = \frac{\theta_{\underline{h}}}{V} \sum_{\underline{h}'} |F_{\underline{h}'}| |F_{\underline{h}-\underline{h}'}| \exp\{i(\phi_{\underline{h}'} + \phi_{\underline{h}-\underline{h}'})\} \tag{2.41}$$

For U's or E's the summations must be over an infinite number of terms because there is no fall off of scattering factor with sin θ. However, if only a few terms, even one, are known on the R.H.S., then the most probable value of $\phi_{\underline{h}}$, $\langle\phi_{\underline{h}}\rangle$, is that given by those terms i.e.,

$$\langle\phi_{\underline{h}}\rangle = \phi_{\underline{h}'} + \phi_{\underline{h}-\underline{h}'} \tag{2.42}$$

If a number of terms are known then $\langle\tan\phi_{\underline{h}}\rangle$ is given by the ratio of imaginary to real components of the R.H.S. i.e.,

$$\langle\tan\phi_{\underline{h}}\rangle = \frac{\sum_{\underline{h}'} |E_{\underline{h}'}||E_{\underline{h}-\underline{h}'}|\sin(\phi_{\underline{h}'} + \phi_{\underline{h}-\underline{h}'})}{\sum_{\underline{h}'} |E_{\underline{h}'}||E_{\underline{h}-\underline{h}'}|\cos(\phi_{\underline{h}'} + \phi_{\underline{h}-\underline{h}'})} \tag{2.43}$$

This equation is known as the tangent formula and is used extensively in phase development procedures. The larger the magnitude of the R.H.S. the better the estimate of $\phi_{\underline{h}}$. The probability distribution of $\phi_{\underline{h}}$ was investigated by Cochran [100] who showed that the variance of $\phi_{\underline{h}}$ decreases with increasing:

$$2N^{-\frac{1}{2}} E_{\underline{h}} \sum_{\underline{h}'} E_{\underline{h}'}E_{\underline{h}-\underline{h}'} \tag{2.44}$$

For centrosymmetric structure Sayre's equation yields by analogous reasoning for $s(\underline{h})$, the sign of a structure factor:

$$s(\underline{h}) \approx s(\underline{h}')\, s(\underline{h} - \underline{h}') \tag{2.45}$$

and $$s(\underline{h}) \approx s(\sum_{\underline{h}'} E_{\underline{h}'}E_{\underline{h}-\underline{h}'}) \tag{2.46}$$

The largest $E_{\underline{h}}$'s in the structure are therefore used in methods employing the above relationships to maximise the probabilities involved.

2.7. iii Origin and Enantiomorph Definition

In general for every space group there will be a choice of permissible origins that have identical point group symmetry and fully reflect the fundamental symmetry of the space group. It is necessary to assign signs to a small number of reflections to select one particular origin. A suitable set of reflections may be chosen by the following procedure due to Main [82].

a) The reflections are separated into categories according to the effect upon their phases of moving the origin to all the space group allowed origins.

b) These categories are plotted on a 'reduced reciprocal lattice' (Figure 2.1).

c) A set of linearly independent vectors is chosen that define a primitive unit cell in the reduced reciprocal lattice.

d) These vectors indicate combinations of reflections that may be used to define the origin.

e.g. for $P2_12_12_1$ There are eight permissible origins:

(0 0 0, 0 0 ½, 0 ½ 0, ½ 0 0, 0 ½ ½, ½ 0 ½, ½ ½ 0, ½ ½ ½)

Applying (a) to the reflections yields eight categories corresponding to the eight parity groups:

(e e e, e e o, e o e, o e e, e o o, o e o, o o e, o o o)

The corresponding reduced reciprocal lattice is shown in Figure 2.1. It is clear that three reflections from the parity group e o e, e e o, and o e o fix the origin by defining a primitive unit cell.

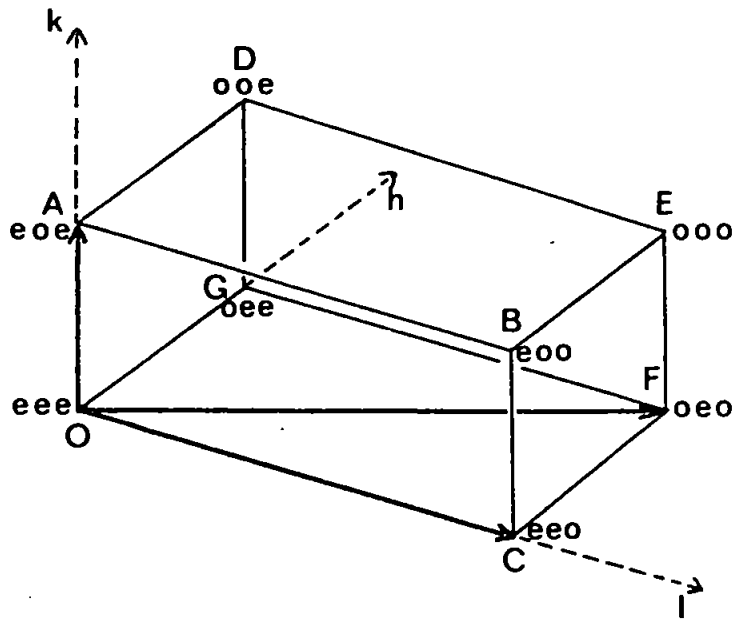


Figure 2.1 The vectors OA,OF,OC define the parallelepiped OABCDEFG

For non-centrosymmetric space groups the enantiomorph must also be specified. Changing the enantiomorph reverses the signs of all phases. This in turn will reverse the signs of the phases of all structure invariants. The enantiomorph may therefore be defined by specifying the sign of the phase of a particular structure invariant to lie between 0 and π rather than π and 2π. For some space groups e.g. $P2_12_12_1$ it is advantageous to use special reflections, whose phase values are restricted to specify origin and enantiomorph.

Reflections selected to define origin and enantiomorph together with those indicated to have high probability by the Σ_1 formulae, (equations 2.35) are used as a starting set for phase development procedures based on equations 2.42 and 2.43.

As a result of the assumptions made in their derivations direct methods are most readily applied to organic structures containing

carbon, nitrogen and oxygen atoms which may be assumed to be of equal size.

2.8 Structure Completion

Completion of the structure is achieved by the use of two types of Fourier syntheses (equation 2.11).

F_o Synthesis

In this procedure the observed structure amplitudes are combined with calculated phases and used as Fourier coefficients in equation 2.11. Atoms included in the model will reappear with increased peak heights; new atoms, not included, will appear as smaller peaks.

If the calculated phases input to the synthesis have been computed by direct methods the coefficients $E_{\underline{h}}$ are often used to produce an 'E map'. The peaks are sharp and some spurious peaks will be observed particularly for aromatic systems.

A disadvantage of F_o syntheses is that series termination effects can cause bad ripples in the map and perturb atom positions especially if a restricted set of amplitudes is used.

Difference Synthesis

This synthesis is given by:

$$\Delta\rho = \frac{1}{V} \sum_{\underline{h}} (|F_o|-|F_c|)\exp(i\alpha_c)\exp(-2\pi i\underline{h}.\underline{r}_j) \qquad (2.47)$$

where α_c is the phase of $|F_c|$.

Difference syntheses are virtually free of series termination effects [101-103]. Correctly placed atoms do not appear, but missing atoms appear as distinct, positive peaks. Spurious atoms will appear as 'holes'

(negative peaks) but care must be taken when rejecting an atom as the use of partial data, inaccurate temperature factors or mis-identification of atom types can produce significant holes.

It is possible to optimize the information obtained from the synthesis, particularly by selecting those reflections where

$|F_c| >> |F_o|$

When all non-hydrogen atoms have been located the positions and temperature factors may be refined by two methods.

2.9 Fourier Refinement

Errors in atomic co-ordinates used in the model result in gradients in the difference synthesis, the correction being to a more positive region. Errors in thermal parameters may also be detected but accurate scaling of F_o's to F_c's is essential for meaningful inferences to be drawn. The presence of thermal anisotropy may also be detected. Quantitative corrections may be obtained but the second technique is more suited for this purpose.

2.10 Least-Squares Refinement

It is impossible to find an exact fit between the observed and calculated structure amplitudes since the observed amplitudes are subject to considerable error. The principle of least squares states that the best values for the parameters are those which minimize the sums of the squares of the properly weighted differences between the observed and calculated values of a function for all observational points. In this case the quantity normally minimized is:

$$D = \sum_{\underline{h}} w_{\underline{h}} (|F_o| - |kF_c|)^2 \tag{2.48}$$

Minimization is achieved by taking the derivative with respect to each of the parameters in turn and equating to zero. Since a structure factor is a transcendental function it is represented by a truncated Taylor Series. Solution of the 'normal equations' in matrix form yield better, though still approximate values for the various parameters. Convergence to the best set of values is indicated by the static nature of the residual, R, through successive cycles of refinement where:

$$R = \frac{\sum_{\underline{h}} w_{\underline{h}} \left| |F_o| - |kF_c| \right|}{\sum_{\underline{h}} w_{\underline{h}} |F_o|} \tag{2.49}$$

2.11 Accuracy of Bond Lengths and Angles

The standard deviations, σ_{p_i}, in the values of the parameters, p_i, varied in a least-squares refinement are given by the general equation:

$$\sigma_{p_i} = \sqrt{b_{ii} \left(\sum_{r=1}^{m} w_r \, \Delta F_r^{\,2}\right)/(m-n)} \tag{2.50}$$

where b_{ii} is the ith diagonal element of the inverse matrix, w_r the weight of the rth ΔF, m the number of observations and n the number of parameters [88,101,104].

Bond lengths and angles are functions of the atomic co-ordinates which are parameters, p_i, of the least-squares refinement. The general equation for the error in a function f related to n uncorrelated

variables $x_1\ x_2\ .\ .\ .\ x_n$ and derived by calculation from them is:

$$\sigma_f = \sqrt{\sum_{j=1}^{n} \frac{\partial f}{\partial x_j}\ \sigma_j^{\,2}} \tag{2.51}$$

where σ_j is the standard deviation in x_j [101,105].

For orthogonal axes the length of a bond between two atoms is given by:

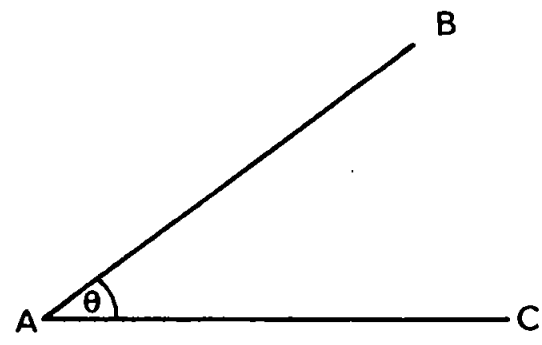
$$l = \sqrt{(\Delta xa)^2 + (\Delta yb)^2 + (\Delta zc)^2} \tag{2.52}$$

When the atoms are uncorrelated the standard deviation in l is given by:

$$\sigma_l = [(\sigma^2_{x_1} + \sigma^2_{x_2})(\Delta xa/l)^2 + (\sigma^2_{y_1} + \sigma^2_{y_2})(\Delta yb/l)^2 + (\sigma^2_{z_1} + \sigma^2_{z_2})(\Delta zc/l)^2]^{\frac{1}{2}} \tag{2.53}$$

where σ_{x_1}, σ_{x_2} are the standard deviations of atoms 1 and 2 in the x direction etc. and, $\Delta x = (x_2 - x_1)\ .\ .\ .$ etc.

Bond angles may be computed from the cosine formula e.g. for the angle θ below.



$$\theta^{o} = \cos^{-1}\left[\frac{(AB)^2 + (AC)^2 - (BC)^2}{2\ AB\ AC}\right] \qquad (2.54)$$

If the errors are isotropic and the positions of the atoms A, B and C are uncorrelated the standard deviation in the bond angle, σ_θ, is given by:

$$\sigma_\theta(\text{radians}) = \left[\frac{\sigma_B^2}{(AB)^2} + \frac{\sigma_A^2(BC)^2}{(AB)^2(AC)^2} + \frac{\sigma_C^2}{(AC)^2}\right]^{\frac{1}{2}} \qquad (2.55)$$

where σ_A, σ_B, σ_C are the standard deviations in the positions of the atoms A, B, C [101].

These formulae do not make allowances for the effect of errors in the cell parameters [106] nor do they take account of systematic errors in the data. Values for the standard deviations of bond lengths and angles must therefore be treated with caution as they are probably under-estimated by the above formulae.

CHAPTER 3

DETERMINATION OF THE CRYSTAL STRUCTURE FROM PHOTOGRAPHIC DATA

3.1 Previous Crystallographic Studies

Powder diffraction studies were performed by Hoeksema, Bergy et al on both crystalline forms of the free acid of novobiocin [15,18]. Ni filtered $CuK\alpha_1$ radiation and a Picker-Waite diffraction unit were used in the study.

The characteristic strongest peaks are listed in Table I.

Form 2 was shown to possess the crystallographic properties listed in Table II.

3.2 Preparation and Crystallisation of Novobiocin

Possible 'heavy-atom' derivatives of novobiocin were considered but are in general unsuitable for an X-ray structural study. All the Group I and Group II neutral salts are amorphous [9]. The calcium acid salt is insoluble in water; it has been crystallised but the solvated crystals effloresce [15] making X-ray work impossible. One expects that the other Group II acid salts might be similar. The Group I acid salts are more tractable but the sodium acid salt has at least three polymorphic forms distinguished by different solubilities and X-ray powder patterns. The acid salts of rubidium and caesium would be possible heavy atom-derivatives but may well be polymorphic [9,15].

It was therefore decided to carry out the structure determination on novobiocin free acid using Direct methods to solve the Phase Problem.

Novobiocin was obtained from the Boots Pure Drug Company as

TABLE 1

FORM I $2\theta^{o}$	INTERPLANAR SPACING (Å)	FORM 2 $2\theta^{o}$	INTERPLANAR SPACING (Å)
6.16	14.34	7.27	12.16
9.02	9.79	12.27	7.21
11.52	7.67	14.63	6.05
13.18	6.71	21.14	4.20
14.33	6.17	23.65	3.76
15.88	5.58	25.95	3.43
23.10	3.85	28.21	3.16
25.90	3.44		
42.28	2.14		
49.05	1.86		

TABLE II

Crystal System	:	Orthorhombic
Crystal Class	:	Rhombic Dipyramidal
Forms Present	:	Macro Prism {210} Brachy Dome {013}
Interfacial Angles	:	$210 \wedge 2\bar{1}0 = 54.5^{o}$, $013 \wedge 0\bar{1}3 = 144^{o}$
Axial Ratio	:	0.514 : 1 : 0.324
Unit Cell Dimensions	:	a = 13.56Å, b = 26.38Å, c = 8.54Å
Optic Sign	:	Negative
Dispersion	:	Extreme, r > v
Common Inclination	:	Inclined Acute Bisectrix
Refractive Indices	:	$\alpha = 1.608$, $\beta = 1.638$, $\gamma = 1.654$
Optic Axial Angle	:	$2V = 71^{o}$
Optic Orientation	:	a = x, b = y
Molar Refraction	:	$3\sqrt{\alpha\beta\gamma} = 1.633$
Density	:	1.3448
Molecular weight	:	~618

the calcium acid salt. This was converted to the free acid by hydrolysis in very dilute hydrochloric acid. Hinman, Caron et al [17] suggest the formula $C_{31}H_{35}N_2O_{11}.Ca^{2+}.2H_2O$ for calcium acid novobiocin giving a molecular weight of 687. Assuming a chemical reaction of the form:

$$C_{31}H_{35}N_2O_{11}.Ca^{2+}.2H_2O + 2HCl \rightarrow CaCl_2 + C_{31}H_{36}N_2O_{11}.xH_2O + H_2O$$

0.1 g of calcium acid novobiocin requires 2.91 ml of 0.1 M HCl for complete neutralisation. Initial experiments were performed on small quantities of the starting material as the supply was limited.

In a typical experiment, 0.25 g of calcium acid novobiocin were suspended in 10 ml of distilled water to which was added 10 ml of 0.1 M HCl. The suspension was shaken in a stoppered flask for several hours to ensure good mixing of the reagents. An equal volume of ethyl acetate was then added and the flask again shaken for several hours, before being left to stand. When the liquid layers had separated the ethyl acetate layer was transferred by pipette to a small conical flask. This was then placed in a darkened cupboard and the solvent allowed to evaporate slowly.

After several days crystals were observed to have formed on the sides of the flask and later on the base and in the body of the liquid. When sufficient crystals had formed the suspension of novobiocin crystals in ethyl acetate was filtered and the crystals washed with a little cold solvent.

Novobiocin free acid crystals prepared as above were examined under crossed polarised light. Very few fragments were observed to be single crystals and these were usually very small. A common crystal habit was a flat plate with two perpendicular extinction

directions unrelated to the sides of the plate. X-ray powder and oscillation photographs of small crystals indicated the cell dimensions of form 2 of the free acid. The melting points of crystals were determined using a Köfler hot-stage microscope. They were several degrees lower than the range 152-156°C given by Hoeksema [15] indicating an impure material.

Re-crystallisations of the acid crystals from ethyl acetate and solvent systems such as acetone - water did improve the optical quality of the crystals. Eventually irregular, plate-like, single crystals were grown of sufficient size for X-ray intensity measurements. Melting points for these crystals were higher than those measured for the early crystallisations but never exceeded 150°C. One notes, however, that the literature values were only obtained after numerous re-crystallisations and were also described as 'decomposition points' [15,16].

It was reported in the review by Hoeksema and Smith [9] that the higher melting point crystals, form 1, could be obtained if novobiocin were crystallised from a solvent mixture of acetone-hexane. All experiments with different proportions of the two solvents have failed to produce crystals of this alternative polymorph. It is worth noting that Kaczka et al [16] reported that form 1 was the rarer crystal form.

3.3 Determination of Unit Cell Parameters and Space Group

All X-ray photographs were taken using a Phillips 1010 generator run at 36 kV, 20 mA. Ni filtered CuKα radiation from a Phillips fine focus tube was used throughout. Diffraction patterns

were recorded using a Stoe Weissenberg camera. Crystals were mounted on glass fibres embedded in plasticene, using a nail varnish as a convenient slow setting glue.

Approximate values for the cell dimensions of novobiocin were obtained from oscillation and zero level Weissenberg photographs (Table III).

Table III

a	= 8.62 ± 0.05 Å	
b	= 13.65 ± 0.10 Å	$\alpha = \beta = \gamma = 90^{o}$
c	= 26.65 ± 0.20 Å	

(the convention a < b < c was adopted in this work).

They were in agreement with the values found by Hoeksema, Bergy et al for form 2 of the free acid (see Table II). Zero level Weissenberg photographs about two axes showed the following systematic absences (Table IV).

Table IV

h 0 0	,	h = 2n + 1
0 k 0	,	k = 2n + 1
0 0 l	,	l = 2n + 1
hk0, h0l, 0kl	,	no absences
hkl	,	no absences

These absences together with the orthorhombic cell showed that the space group is $P2_12_12_1$.

A small crystal of novobiocin was examined on a Hilger and Watts four circle diffractometer at Sussex University using MoKα radiation. Insufficient intensities were measurable for a structure determination to be attempted because of the poor crystal quality. However, refinement of the cell parameters in the orientation matrix

gave the following values (Table V).

Table V

$$a = 8.577 \text{ Å}$$
$$b = 13.610 \text{ Å} \qquad \alpha = \beta = \gamma = 90^{\circ}$$
$$c = 26.357 \text{ Å}$$

These values were used for the photographic study of novobiocin.

The density of novobiocin crystals was measured by flotation in a mixture of dichloromethane and chloroform. A value of $\rho = 1.332 \text{ g cm}^{-3}$ was obtained. Assuming one molecule of water per molecule of novobiocin the number of molecules per unit cell may be calculated from equation 2.14.

i.e. $N = \rho V \frac{No}{M} = 3.92$

This agrees well with the value 4 permitted by the space group. The calculated density for N = 4 is 1.360 g cm^{-3} (c.f. the value of 1.3448 quoted in Table II).

3.4 Data Collection and Preliminary Treatment

The linear absorption coefficient μ_λ for CuKα radiation computed using equation 2.17b was $\mu_\lambda = 8.78 \text{ cm}^{-1}$. In view of the small value of μ_λ and the non standard shape of the crystal no absorption correction was applied to the intensity measurements.

Intensities were recorded photographically using the equi-inclination Weissenberg technique [85,107]. The crystal used for these measurements was a large, irregular flat plate approximately 0.7 x 0.5 x 0.1 mm. The b axis of the crystal was about 20° from the plane of the plate as illustrated in Figure 3.1.

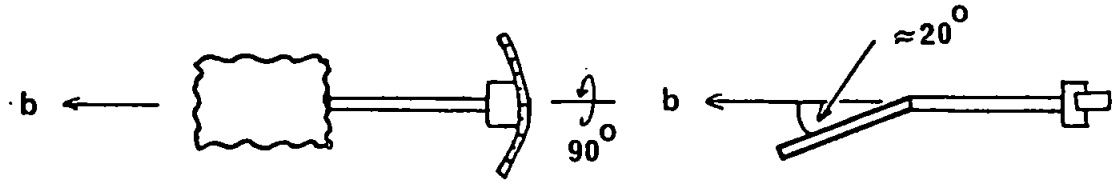


Figure 3.1 Crystal Orientation

The data was therefore collected with the crystal rotating around the b axis.

For $P2_12_12_1$ there is a unique octant of data and for novobiocin there are 3987 unique reflections within the CuKα sphere. 3030 of these were examined up to a k index of 10. A total of 2487 reflections were of measurable intensity.

The intensities were recorded on Ilford Industrial G X-ray film using the multiple film technique [84]. Constant exposure conditions were used for each layer and the processing procedures were also standardised.

The intensities were measured by visual comparison with a pre-calibrated density scale. The scale was prepared by a series of 4° Weissenberg exposures of the 2 0 5 reflection on the zero level b axis film. Steps of $\sqrt{2}$ times the previous exposure were found to produce a sufficiently fine scale for routine measurement. The scale was found to be linear up to 64 times the minimum exposure used. Intensities were measured on a light box viewing each reflection through any background on the scale strip. At the same time

the scale spot was placed on top of representative background on the film. Symmetry related appearances of a reflection on a given film were measured and an average recorded, interpolated between two scale values as necessary.

Scaling of Intensities

The film factor is the amount by which the intensity of a diffracted beam is reduced on passing through one film and one sheet of black paper. Its value was determined by calculating the ratio of the intensities of measurable spots on adjacent films on a zero level Weissenberg. The average value obtained was 2.8 ± 0.2. The factor was then used to bring reflections on the lower films to a common scale. Relative intensities for each layer were then obtained by averaging these values.

A zero level Weissenberg for the same crystal rotating about the 'a' axis was obtained and the reflections measured using a $\sqrt{2}$ density scale prepared from the 0 6 0 reflection. The intensities measured on this photograph were used to scale together the intensities measured on the different layers about the b axis.

3.5 Structure Determination

Some calculations not requiring a large computer core store were performed on an IBM 1130 computer with programs written by the author. The source code, in FORTRAN, of these programs is listed in Appendix A. The major computations were carried out using the X-ray 70 system on the ICL 1906A at the Atlas Computer Laboratory, Chilton. The scattering factor tables given in International Tables for Crystallography (1962) [88] were used in all the relevant calculations.

3.5. i Determination of Absolute Scale and Temperature Factor

The relative intensities were corrected for the Lorentz factor, polarisation and spot shape applying the factor W/2LP [88]. The corrected intensities were placed on an absolute scale, using a ten point Wilson plot [93] (program WPLOT Appendix A.1). The following values were obtained:

Overall temperature factor $B = 4.3\ \text{Å}^2$

F_{rel} scale factor $K = 4.2$

Normalised structure factors, $E_{\underline{h}}$, were then computed from equation 2.33. The inter-layer scale factors were refined by normalising to $\overline{|E|^2} = 1.000$ for each k layer assuming the B value of $4.3\ \text{Å}^2$ obtained from the Wilson plot.

3.5. ii Selection of Starting Set

All interactions of the form:

$$\underline{h}_1 + \underline{h}_2 + \underline{h}_3 = 0 \qquad (3.1)$$

known as Σ_2 relationships, were computed for a list of reflections with $|E_{\underline{h}}| \geq 1.80$ using the program SIGMA2 (Appendix A.2). Three origin defining reflections and one to fix the enantiomorph are required in $P2_12_12_1$ [110,111,112]. One reflection, the 0 6 10, was phased from the Σ_1 relationship (equation 2.35b). Due regard was paid to the ability of starting sets to interact to form Σ_2 relationships with other reflections within the $|E_{\underline{h}}| \geq 1.80$ list. Possible starting sets were also expanded by the sum of angles formula (equation 2.42) to check on the rate of phase determination and to test for inconsistencies at an early state in the phase development process.

Details of the chosen starting set are given in Table VI. The phase expansion process for this set using equation 2.42 is shown in

detail in Appendix B.

Table VI

The starting set for phase determination

	Reflection h k l	|E|	Phase	Number of Σ_2 interactions within E ≥ 1.80 list
Origin-fixing	6 5 0	2.14	0	11
reflections	6 0 11	2.05	$-\pi/2$	11
	1 0 29	2.19	$-\pi/2$	6
Enantiomorph-defining reflection	0 3 6	3.61	$-\pi/2$	24
Σ_1 reflection	0 6 10	2.21	π	15

3.5. iii Phase Development

Phase estimates for all $|E_{\underline{h}}| \geq 1.40$ (355 reflections) were obtained using the weighted tangent formula of Germain, Main and Woolfson [108].

$$\tan\phi_{\underline{h}} = \frac{\sum_{\underline{h}'} \omega_{\underline{h}'} \, |E_{\underline{h}'} \, E_{\underline{h}-\underline{h}'}| \, \sin(\phi_{\underline{h}} + \phi_{\underline{h}-\underline{h}'})}{\sum_{\underline{h}'} \omega_{\underline{h}'} \, |E_{\underline{h}'} \, E_{\underline{h}-\underline{h}'}| \, \cos(\phi_{\underline{h}} + \phi_{\underline{h}-\underline{h}'})} = \frac{T_{\underline{h}}}{B_{\underline{h}}} \tag{3.2}$$

where

$$\omega_{\underline{h}'} = 0.5 + 0.5 \tanh [0.5 \, . \, EC(\underline{h}) \, . \, EC(\underline{h}') . \, EC(\underline{h} - \underline{h}') \times \sigma_3/\sigma_2^{1.5}] \tag{3.3}$$

$$EC(\underline{h}) = (T_{\underline{h}}^2 + B_{\underline{h}}^2)^{\frac{1}{2}} \tag{3.4}$$

and

$$\sigma_r = \sum_{j=1}^{N} Z_j^r \qquad \text{(see 2.37)}$$

The program TANGEN, of the X-RAY 70 package, incorporates this formula and was used to develop phases from the starting set. Tangent refinement was carried out in a series of seven cycles, the $|E_{\underline{h}}|$ limit being lowered after each cycle. Each cycle was iterated until self consistency was obtained among the phases before the $|E_{\underline{h}}|$ limit was lowered. The stages of refinement are shown in Table VII.

Table VII Tangent Formula Refinement

Cycle	$\|E\|$ limit	Number of $\|E\|$s > limit	Number of Unique Σ_2 relationships	Number of Iterations
1	2.0	69	124	20
2	1.8	112	347	20
3	1.6	206	1875	20
4	1.55	247	3034	20
5	1.50	274	3918	20
6	1.45	312	5687	20
7	1.40	355	7827	20

A comparison of phases derived by the tangent formula with those from the final structure is shown in Figure 3.2.

An E map was then prepared using the 355 $|E_{\underline{h}}| \geq 1.40$ and the phase values obtained from TANGEN (program EDENS Appendix A.3). The map showed clearly the 24 atoms of the coumarin and benzene ring systems joined by the peptide bond (see Figure 3.3). Most of the remaining non-hydrogen atoms could have been located from this map; however some atoms in the isobutenyl side chain and in the sugar system were not clearly defined.

NOVOBIOCIN

Comparison of tangent formula phases with phases from final structure.

Total reflections 312

84 restricted phases (0/180 or $\pm\pi/2$), 81 determined correctly.

Distribution of remaining 228:

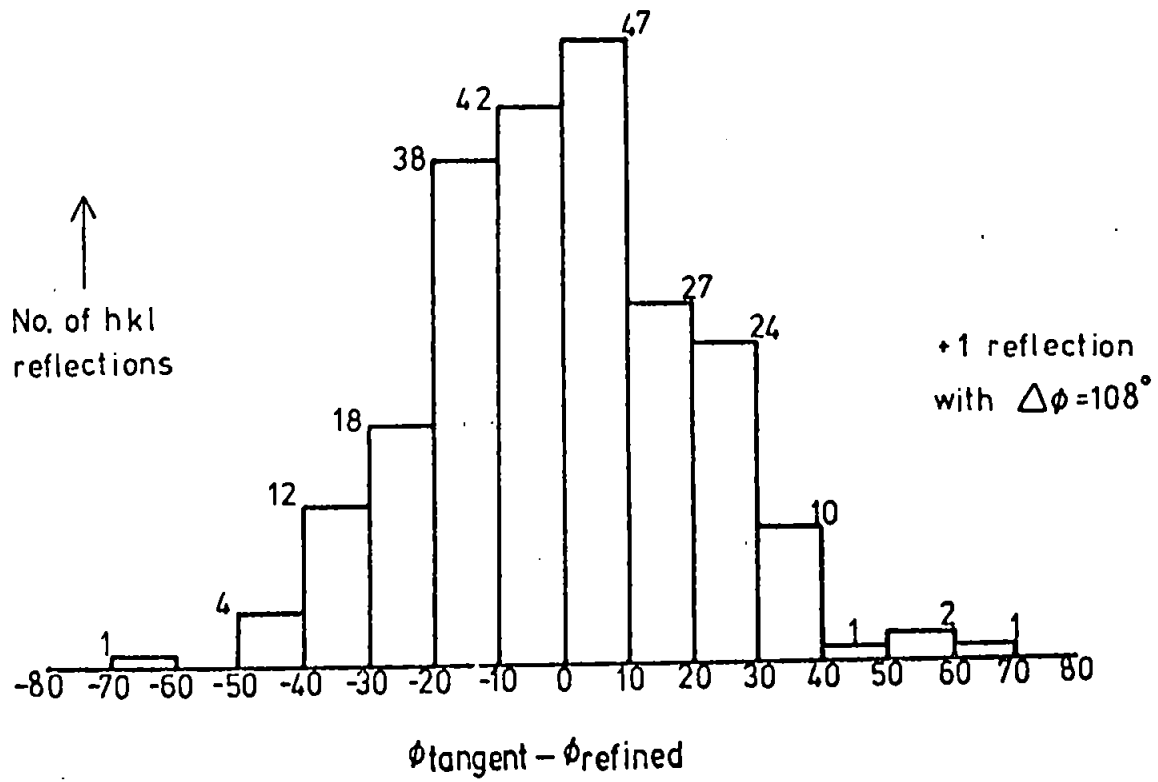


Figure 3.2 Comparison of tangent formula phases with those from the refined structure

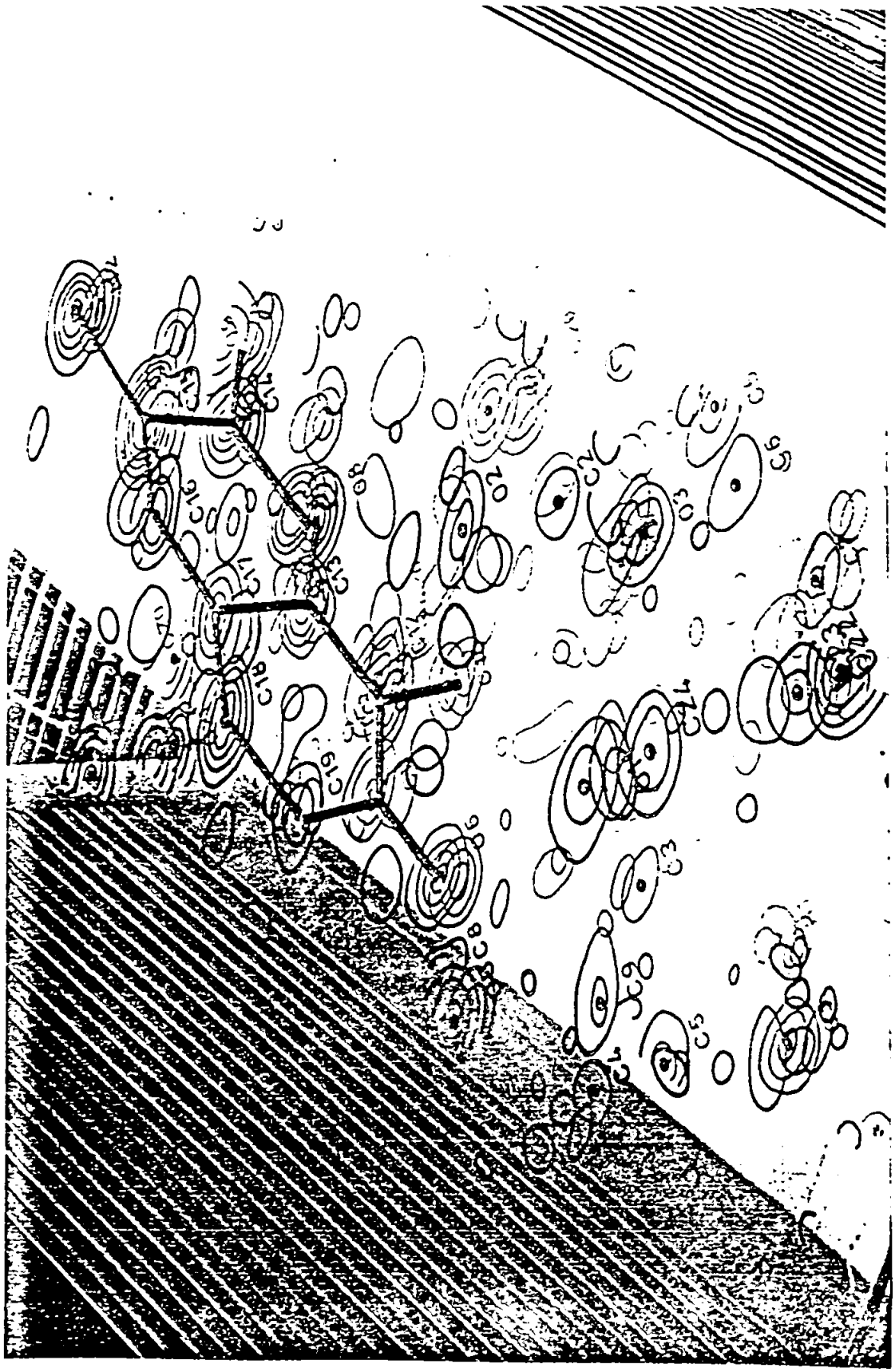


Figure 3.3 Detail from the E map (355 E's) for novobiocin. The coumarin system has been outlined with the black lines.

3.5. iv Structure Completion

A second E map using 534 reflections with phases computed from the 24 known positions yielded 13 more atoms in the noviose moiety.

An F_o Fourier synthesis using all reflections for which $|F_{calc}| > 0.2\ |F_{obs}|$, revealed the position of the 7 remaining non-hydrogen atoms in the molecule as well as a further peak assumed to be the oxygen atom of a water molecule of crystallisation (program EDENS Appendix A.3.).

Electron density maps were interpreted with the help of contours drawn on stacks of transparent perspex sheets. The approximate positions of atoms were determined by hand interpolation from the relevant sections.

3.6 Structure Refinement

The least squares program CRYLSQ and Fourier programs of the X-RAY 70 system were used in the refinement process.

The approximate co-ordinates of the 45 non-hydrogen atoms located on the electron density maps resulted in an R value of 0.26 where $R = \Sigma\big||F_o|-k|F_c|\big|/\Sigma F_o$. Five cycles of full matrix least squares refinement minimizing the function $\Sigma\, w[|F_o|-k|F_c|]^2$ with individual isotropic temperature factors, unit weights and including refinement of the inter-layer scale factors resulted in an R value of 0.15.

An F_o-F_c synthesis (equation 2.47) showed an excess of electron density in the region of the double bond C(28)-C(29) of the isobutenyl group corresponding to approximately 2.0 $e\text{Å}^{-3}$. The possibility of disorder within the crystal structure with the isobutenyl side chain in some other configuration was considered. It

was not found possible, however, to fit an alternative configuration to the observed excess electron density in this region of the difference map (Refinement of the structure using diffractometer data has indicated quite clearly that disorder is present and this will be discussed in detail in Chapter 4.).

Atomic absorption spectrophotometric analysis of novobiocin crystals in tetrahydrofuran indicated that the crystals contained some calcium. It was therefore erroneously assumed that Ca^{2+} ions are held in this region. Refinement was continued with the excess density represented with a fixed temperature factor of 5.0 $Å^2$ and the site occupancy allowed to refine. An occupancy corresponding to 1 Ca^{2+} ion per 35 novobiocin molecules was obtained.

A weighting function $w = 1/|\Delta F|^2$ was used in the final refinement process where $|\Delta F| = 0.055\ |F_{obs}| + 1.17$ obtained from the plot of $\overline{|\Delta F|}$ vs $\overline{|F_{obs}|}$ for 15 ranges of $|F_{obs}|$ (Figure 3.4). The final R value was 0.14 using only the observed reflections, individual isotropic temperature factors and omitting all hydrogen atoms. The largest shift/error was 0.1009.

An F_o-F_c synthesis at this point indicated significant anisotropic thermal vibrations in some atoms. Least squares refinement using anisotropic thermal parameters was not pursued because of the method of data collection and the large number of parameters involved.

3.7 Discussion of the Structure

The final positional and thermal parameters are given in Table VIII. A list of observed and calculated structure factors and phase angles for all reflections is given in Appendix D. The bond

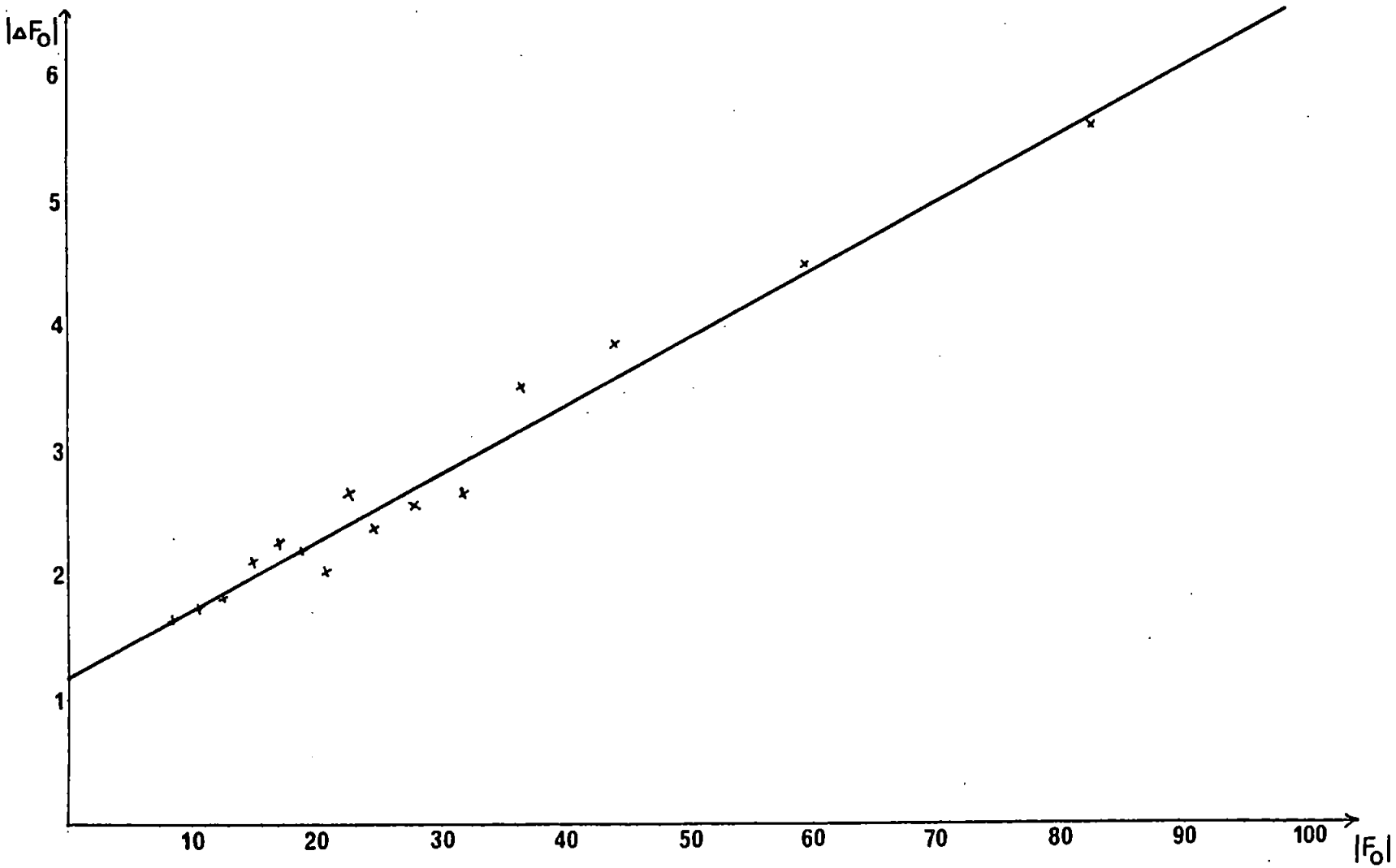


Figure 3.4 A plot of $|\overline{\Delta F}_o|$ v $|\overline{F}_o|$ for 15 ranges of $|F_o|$

TABLE VIII

Photographic data: Positional and thermal parameters

Positional parameters are given as fractions of cell edges x 10^4 . Temperature factors are of the form exp ($-B \sin^2\theta / \lambda^2$) and are given in $Å^2$. Standard deviations in parentheses are with respect to the last figures given .

ATOM	x/a	y/b	z/c	B
C(1)	6006 (17)	4830 (12)	8932 (5)	4.93 (27)
C(2)	7865 (14)	4397 (11)	7749 (4)	3.68 (20)
C(3)	3235 (16)	6578 (12)	7976 (5)	4.83 (26)
C(4)	1831 (16)	5467 (12)	8578 (5)	4.46 (25)
C(5)	4333 (13)	4858 (10)	8201 (4)	3.40 (19)
C(6)	5095 (12)	4615 (10)	7711 (4)	3.17 (18)
C(7)	3977 (12)	4085 (10)	7363 (4)	3.16 (18)
C(8)	2616 (13)	4709 (10)	7262 (4)	3.76 (21)
C(9)	2837 (13)	5497 (10)	8125 (4)	3.39 (19)
C(10)	4435 (13)	6765 (10)	6241 (4)	3.20 (18)
C(11)	2091 (13)	6110 (10)	6708 (4)	3.31 (18)
C(12)	2695 (12)	6707 (9)	6347 (3)	2.87 (17)
C(13)	1619 (11)	7237 (9)	6058 (3)	2.49 (15)
C(14)	1275 (12)	8328 (9)	5368 (4)	3.11 (18)
C(15)	9691 (12)	8374 (9)	5448 (3)	2.92 (17)
C(16)	9034 (12)	7813 (10)	5831 (4)	3.11 (18)
C(17)	0068 (12)	7233 (9)	6150 (3)	2.87 (17)
C(18)	9470 (12)	6670 (9)	6551 (4)	3.08 (17)
C(19)	0486 (13)	6083 (10)	6826 (4)	3.46 (20)
C(20)	7400 (12)	9156 (9)	5021 (4)	3.04 (18)
C(21)	6936 (13)	9876 (10)	4641 (4)	3.45 (20)
C(22)	5343 (12)	9941 (9)	4526 (3)	2.84 (17)
C(23)	4824 (14)	0653 (11)	4190 (4)	4.01 (22)
C(24)	5879 (13)	1221 (10)	3951 (4)	3.45 (20)
C(25)	7474 (16)	1229 (12)	4042 (5)	4.68 (26)
C(26)	7977 (14)	0509 (11)	4404 (4)	4.03 (22)
C(27)	2989 (18)	0624 (12)	4045 (5)	5.01 (28)
C(28)	2122 (28)	1554 (19)	4157 (8)	8.68 (53)
C(29)	1610 (22)	1840 (15)	4614 (6)	6.58 (38)
C(30)	0842 (23)	2818 (17)	4615 (7)	7.57 (45)
C(31)	1725 (30)	1377 (20)	5060 (9)	9.44 (60)

ATOM	x/a	y/b	z/c	B
N(1)	8954 (14)	3736 (10)	7853 (4)	5.01 (23)
N(2)	8931 (11)	9013 (8)	5120 (3)	3.42 (17)
'Ca^{2+}'	2192 (45)	0867 (33)	4580 (13)	5.00 *
O(1)	5338 (9)	5416 (7)	8522 (3)	3.55 (14)
O(2)	8059 (10)	5220 (8)	7603 (3)	4.57 (18)
O(3)	6420 (9)	3987 (7)	7807 (3)	4.00 (16)
O(4)	3511 (11)	3195 (8)	7597 (3)	4.66 (18)
O(5)	1871 (8)	5028 (6)	7731 (3)	3.36 (14)
O(6)	3136 (8)	5517 (6)	6973 (2)	3.16 (13)
O(7)	7548 (9)	7835 (7)	5973 (3)	3.91 (15)
O(8)	2226 (9)	7804 (6)	5676 (3)	3.34 (13)
O(9)	1956 (11)	8799 (7)	5019 (3)	4.37 (17)
O(10)	6360 (11)	8624 (8)	5234 (3)	5.01 (19)
O(11)	5320 (13)	1914 (9)	3587 (4)	5.68 (22)
O(12)	7448 (12)	6861 (8)	8154 (4)	5.67 (22)

* Held constant

distances and angles are listed with their standard deviations in Tables IX and X and are shown in context in Figure 3.5.

The coumarin and substituted benzene, with the peptide bond, all lie approximately in one plane with the isobutenyl side chain on the same side of the main plane as the sugar ring. The temperature factors of the carbon atoms of the isobutenyl side chain are significantly higher than average and are consistent with a lack of rigid bonding of this group. The bond distances of 1.620 Å for C(23)-C(27) and 1.337 Å for C(29)-C(31) are significantly different from an expected value of 1.54 Å for carbon - carbon single bonds. These results were considered to be due to the proximity of the assumed Ca^{2+} ion in some molecules (This point is discussed at length in Chapter 4 following further refinement of the structure from diffractometer data.). The distance of 2.446 Å between O(7) and O(10) suggests the presence of an intra-molecular hydrogen bond between these atoms.

Figure 3.6 shows the structure viewed along the 'a' axis illustrating the back-to-back arrangement of molecules related by the twofold screw axis (2_1 axis) along this direction. Figure 3.7 shows part of the structure viewed normal to the plane containing the coumarin and substituted benzene system (in the direction of the arrow in Figure 3.6). From this it can be seen that the molecule extends '2a' in the 'x' direction and molecules related by the 2_1 axis parallel to the 'x' axis (represented by full and dashed lines in Figure 3.7) almost overlap in certain regions. In particular the benzene ring of the coumarin system of molecule (I) nearly overlaps with the substituted benzene ring of molecule (II) and the benzene ring of the coumarin system of molecule (II) almost overlaps

TABLE IX

Photographic data:

Bond distances and their standard deviations (Å) after weighted least-squares refinement

ATOMS	DISTANCE	ATOMS	DISTANCE
Noviose moiety, ring (C)			
C(5) - C(6)	1.484 (14)	C(5) - O(1)	1.427 (14)
C(6) - C(7)	1.511 (15)	O(1) - C(1)	1.461 (16)
C(7) - C(8)	1.468 (17)		
C(8) - O(5)	1.458 (13)	C(6) - O(3)	1.444 (14)
O(5) - C(9)	1.474 (13)	O(3) - C(2)	1.368 (15)
C(9) - C(5)	1.563 (17)	C(2) - N(1)	1.326 (18)
		C(2) - O(2)	1.195 (17)
C(8) - O(6)	1.410 (15)		
		C(9) - C(3)	1.560 (21)
C(7) - O(4)	1.417 (16)	C(9) - C(4)	1.474 (16)
Aminohydroxycoumarin, ring (B)			
C(19) - C(18)	1.386 (16)	C(17) - C(16)	1.456 (15)
C(18) - C(17)	1.402 (15)	C(16) - C(15)	1.385 (15)
C(17) - C(13)	1.352 (13)	C(15) - C(14)	1.377 (15)
C(13) - C(12)	1.398 (14)	C(14) - O(8)	1.354 (13)
C(12) - C(11)	1.354 (15)	O(8) - C(13)	1.371 (12)
C(11) - C(19)	1.412 (16)		
		C(16) - O(7)	1.329 (13)
C(12) - C(10)	1.520 (15)	C(15) - N(2)	1.390 (15)
C(11) - O(6)	1.393 (14)	C(14) - O(9)	1.264 (14)
Substituted benzoic acid, ring (A)			
C(21) - C(22)	1.402 (15)	C(21) - C(20)	1.456 (16)
C(22) - C(23)	1.388 (17)	C(20) - O(10)	1.280 (15)
C(23) - C(24)	1.346 (18)	C(20) - N(2)	1.345 (14)
C(24) - C(25)	1.389 (18)		
C(25) - C(26)	1.433 (20)	C(24) - O(11)	1.428 (16)
C(26) - C(21)	1.390 (18)		

ATOMS	DISTANCE	ATOMS	DISTANCE
Isobutenyl side-chain			
C(23) - C(27)	1.620 (19)	'Ca^{2+}' - C(27)	1.602 (38)
C(27) - C(28)	1.498 (30)	'Ca^{2+}' - C(28)	1.455 (45)
C(28) - C(29)	1.340 (28)	'Ca^{2+}' - C(29)	1.417 (49)
C(29) - C(30)	1.485 (30)	'Ca^{2+}' - C(31)	1.497 (44)
C(29) - C(31)	1.337 (30)		

TABLE X

Photographic data:

Bond angles and their standard deviations (°)

ATOMS	ANGLE	ATOMS	ANGLE
Noviose moiety, ring (C)			
C(9)—C(5)—C(6)	112.0 (0.9)	O(5)—C(9)—C(5)	118.1 (0.9)
C(9)—C(5)—O(1)	106.0 (1.0)	O(5)—C(9)—C(4)	103.3 (0.9)
C(6)—C(5)—O(1)	111.6 (0.9)	O(5)—C(9)—C(3)	110.7 (0.9)
		C(5)—C(9)—C(3)	112.2 (1.0)
C(5)—C(6)—C(7)	110.9 (0.9)	C(4)—C(9)—C(3)	111.0 (1.1)
C(5)—C(6)—O(3)	109.1 (0.8)	C(4)—C(9)—C(5)	111.2 (1.0)
C(7)—C(6)—O(3)	108.9 (1.0)		
		C(5)—O(1)—C(1)	112.6 (1.0)
C(6)—C(7)—C(8)	109.8 (1.0)		
C(6)—C(7)—O(4)	108.8 (0.9)	C(6)—O(3)—C(2)	116.9 (1.0)
C(8)—C(7)—O(4)	110.4 (0.9)		
		O(3)—C(2)—N(1)	109.8 (1.2)
C(7)—C(8)—O(5)	111.5 (0.9)	O(3)—C(2)—O(2)	113.0 (1.1)
C(7)—C(8)—O(6)	107.3 (0.9)	N(1)—C(2)—O(2)	127.2 (1.2)
O(5)—C(8)—O(6)	111.4 (1.0)		
		C(8)—O(6)—C(11)	121.2 (0.8)
C(8)—O(5)—C(9)	118.7 (0.8)		
Aminohydroxycoumarin, ring (B)			
C(11)-C(19)-C(18)	118.9 (1.0)		
C(19)-C(18)-C(17)	118.6 (1.0)	C(13)-C(12)-C(11)	116.1 (0.9)
		C(13)-C(12)-C(10)	121.4 (0.9)
C(18)-C(17)-C(13)	119.9 (1.0)	C(10)-C(12)-C(11)	122.4 (1.0)
C(18)-C(17)-C(16)	120.6 (0.9)		
C(16)-C(17)-C(13)	119.5 (0.9)	C(12)-C(11)-C(19)	122.9 (1.1)
		C(12)-C(11)-O(6)	117.0 (0.9)
C(17)-C(13)-C(12)	123.3 (0.9)	O(6)--C(11)-C(19)	120.2 (1.0)
C(17)-C(13)-O(8)	120.6 (0.9)		
O(8)—C(13)-C(12)	116.1 (0.8)		

ATOMS	ANGLE	ATOMS	ANGLE
Aminohydroxycoumarin, ring (B) (continued)			
C(17)-C(16)-C(15)	118.1 (0.9)		
C(17)-C(16)-O(7)	115.7 (0.9)	C(15)-C(14)-O(8)	121.7 (1.0)
O(7)—C(16)-C(15)	125.7 (1.0)	C(15)-C(14)-O(9)	123.1 (1.0)
		C(9)—C(14)-O(8)	115.2 (0.9)
C(16)-C(15)-C(14)	119.3 (1.0)		
C(16)-C(15)-N(2)	127.4 (0.9)	C(14)-O(8)—C(13)	120.6 (0.8)
N(2)—C(15)-C(14)	113.3 (0.9)		
Substituted benzoic acid, ring (A)			
C(26)-C(21)-C(22)	119.4 (1.1)		
C(26)-C(21)-C(20)	123.4 (1.0)	C(24)-C(25)-C(26)	114.0 (1.2)
C(20)-C(21)-C(22)	117.2 (1.0)		
		C(25)-C(26)-C(21)	121.9 (1.1)
C(21)-C(22)-C(23)	119.6 (1.0)		
		C(15)-N(2)—C(20)	131.8 (1.0)
C(22)-C(23)-C(24)	118.9 (1.1)		
		N(2)—C(20)-O(10)	120.6 (1.1)
C(23)-C(24)-C(25)	125.9 (1.2)	N(2)—C(20)-C(21)	119.7 (1.0)
C(23)-C(24)-O(11)	117.9 (1.1)	C(21)-C(20)-O(10)	119.5 (1.0)
O(11)-C(24)-C(25)	116.2 (1.1)		
Isobutenyl side-chain			
C(22)-C(23)-C(27)	116.5 (1.1)		
C(24)-C(23)-C(27)	123.9 (1.1)	C(28)-C(29)-C(30)	114.0 (1.8)
		C(28)-C(29)-C(31)	129.0 (2.2)
C(23)-C(27)-C(28)	114.5 (1.4)	C(30)-C(29)-C(31)	117.0 (1.8)
C(27)-C(28)-C(29)	125.9 (1.9)		

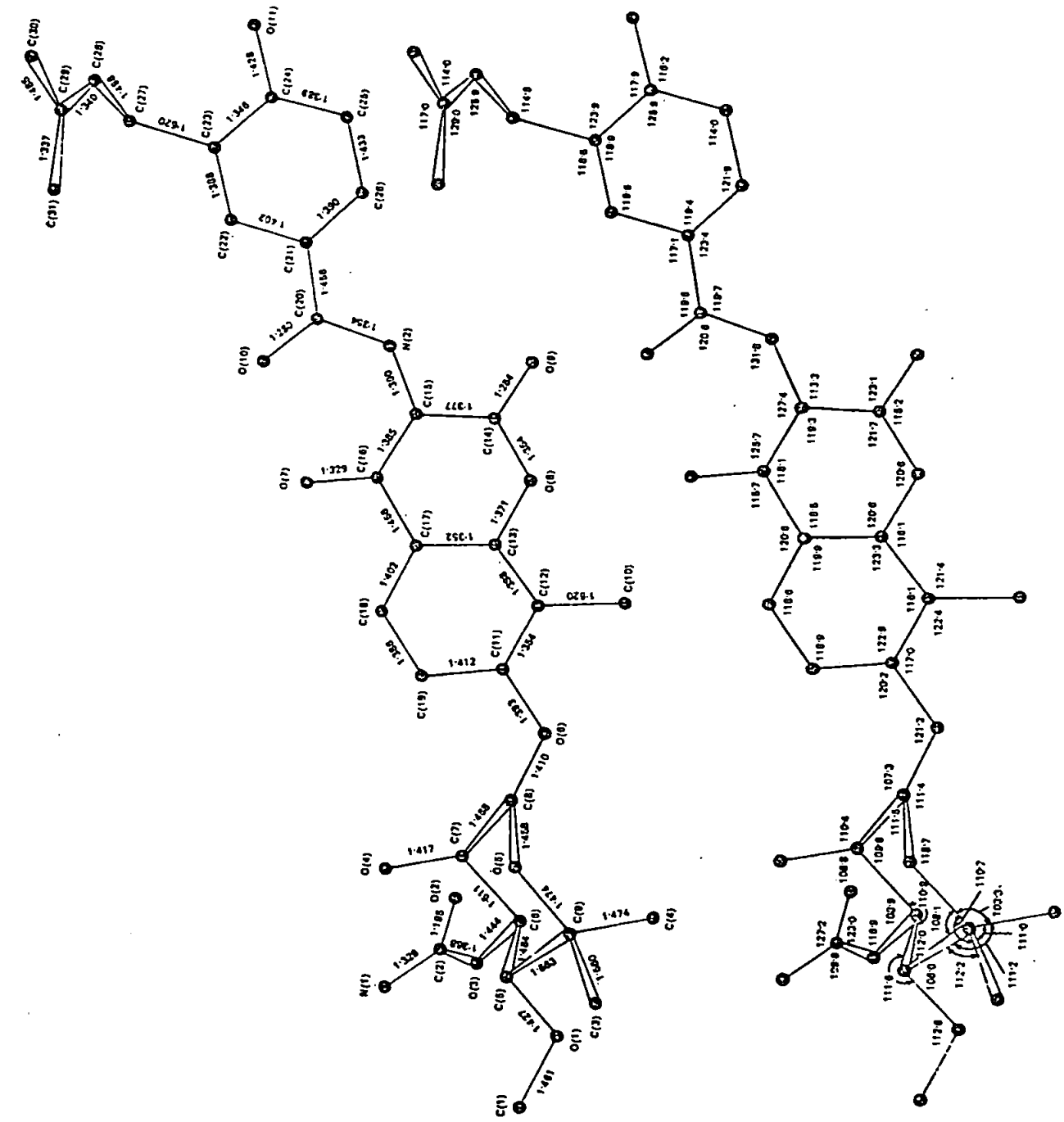


Figure 3.5 Interatomic distances and interbond angles in the novobiocin molecule following refinement using the photographic data.
(The molecule is shown in perspective except for the sugar ring which has been rotated through approximately 80° about C(8).)

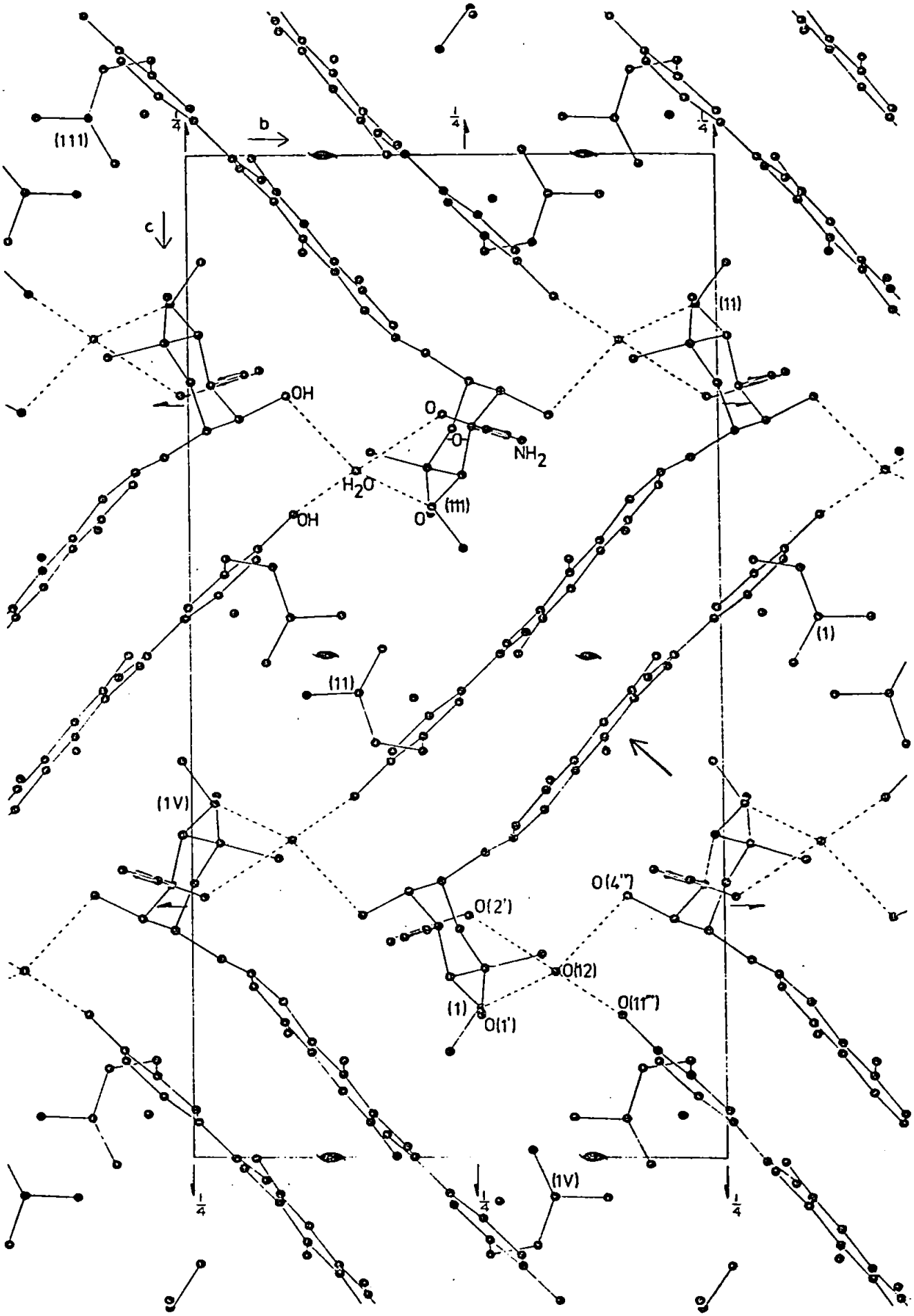


Figure 3.6 The novobiocin crystal structure viewed along the x axis (hydrogen bonds to the water molecule are shown as dashed lines)

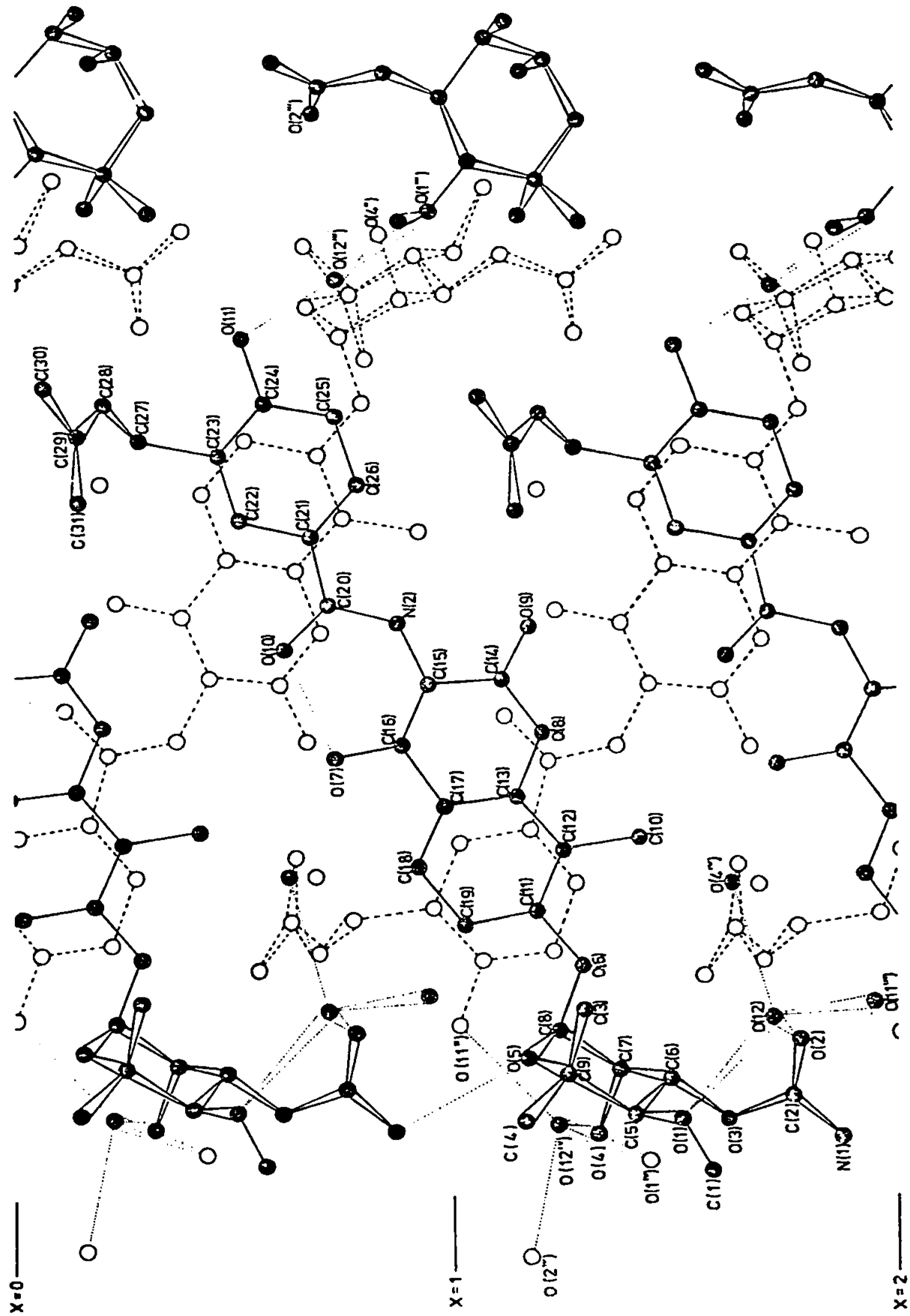


Figure 3.7 Part of the novobiocin structure viewed normal to the main plane of the molecule (in direction of the arrow in Figure 3.6) showing the near overlapping of some rings in symmetry-related molecules and the hydrogen bonds to the water molecule (dotted lines).

the substituted benzene ring of molecule (I) in the next unit cell. The separation of these rings is approximately 3.4 Å and hence they are attracted by Van der Waals forces. Only molecules related by the 2_1 axis along the 'x' direction are affected by the attraction of the overlapping rings.

There is one water molecule per asymmetric unit shown as O (12) in Figure 3.7. The water molecule is in a position where hydrogen bonding can be assumed to take place between itself and three novobiocin molecules. Figure 3.6 shows that two of these are related by the 2_1 axis along 'x' (effectively holding opposite ends of the molecules together). The remaining hydrogen bonds are to O(1) and O(2) on a symmetry related noviose ring, resulting in a continuous linkage of the sugar rings in the 'y' direction via hydrogen bonds.

The separation of 3.083 Å between O(5) and the carbamyl nitrogen N(1) on adjacent molecules (Figure 3.7) suggests the presence of an additional intermolecular hydrogen bond linking the sugar rings of adjacent molecules in the 'x' direction. The hydrogen bond distances involved between the donor and acceptor atoms are shown in Table XI.

Table XI

Distances and their standard deviations (Å) between donor and acceptor atoms involved in hydrogen bonding

Donor	Acceptor	Type of Bond	Distance
O(4)	O(12)	OH .... H_2O	2.814 (14)
O(11)	O(12)	OH .... H_2O	2.771 (14)
O(12)	O(2)	H_2O .... C=O	2.712 (15)
O(12)	O(1)	H_2O .... -O-	2.854 (14)
O(7)	O(10)	OH .... C=O	2.446 (12)
N(1)	O(5)	NH_2 .... -O-	3.075 (15)

The distance of 4.481 Å between N(1) and the enolic oxygen O(7) on the coumarin system eliminates the possibility of interaction between these groups [69].

The results of this photographic study were presented in the paper bound in Appendix D [137].

A more detailed discussion of the geometry of the molecule is given in Chapter 4 following further refinement of the structure from diffractometer data.

CHAPTER 4

REFINEMENT OF THE CRYSTAL STRUCTURE FROM DIFFRACTOMETER DATA

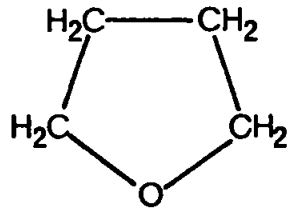
4.1 Introduction

This further study of the crystal structure of novobiocin was undertaken to resolve questions left unanswered by the refinement of the structure using the photographic data. In particular, it was not possible to characterize fully the extensive hydrogen bonding involved in the structure without atomic positions for the relevant hydrogen atoms. In addition it was hoped to improve crystal quality and thus remove the suspected Ca^{2+} ions from the structure.

It was therefore decided to devise an improved process for crystallising novobiocin free acid and to collect data for the crystals on a diffractometer.

4.2 Crystal Preparation

The calcium acid salt of novobiocin is a rather hydrophobic powder and requires exhaustive agitation with dilute HCl for complete displacement of free novobiocin (section 3.2). It was found that a better wetting of the powder could be achieved by dissolving it in a mixture of water and tetrahydrofuran (14) in which it is readily soluble.



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A large quantity (20g) of the calcium acid salt was obtained from the Boots Pure Drug Company and novobiocin free acid prepared in the following way. A concentrated solution of the calcium salt (20g) in 10ml of tetrahydrofuran-water was added to ice cold 0.2m HCl (1.5 equivalents) in a tap funnel liberating novobiocin free acid as a fine dispersion. This was extracted quickly by shaking with three portions of ethyl acetate (300ml) and washed with distilled water to a wash water pH of 7.0. The extract was then washed with brine and dried over anhydrous sodium sulphate. Evaporation of the filtered extract yielded novobiocin free acid as a yellow solid.

Crystals were obtained from several solvent systems. All crystals were of form 2 of the free acid even from an acetone-hexane system (see 3.2). The best crystals were obtained from an acetone-water solvent system and one from this batch, of approximate dimensions 0.5 x 0.5 x 0.5 mm, was used for the data collection by diffractometer.

4.3 Data Collection and Preliminary Treatment

Data collection was performed on a Hilger and Watts 4-circle diffractometer at the University of Sussex using MoKα radiation,

λ = 0.71069Å, from a graphite monochromator. A θ-2θ step scan of 60 x 0.01° steps, each of 0.5 second, was employed with two 15 second background counts measured at each end of the integrated scan. The intensities were recorded in two shells of 1.5° - 20° and 20° - 25° in θ. Three standard intensities were monitored and showed no signs of crystal or beam instability during irradiation.

The observed intensity, I (obs), was obtained from equation 4.1:

$$I(obs) = Ct - (B1 + B2) \qquad (4.1)$$

where Ct, B1 and B2 are the raw integrated count and background counts respectively. Estimated standard deviations, $\sigma(I)$ were calculated from equation 4.2:

$$\sigma(I) = [Ct + B1 + B2 + (q \times I(obs))^2]^{\frac{1}{2}} \qquad (4.2)$$

with q = 0.05 giving a suitable weighting scheme in this case [113]. Lp corrections were then applied to the data. The linear absorption coefficient, μ_λ, for MoKα radiation is only 0.65 cm^{-1} therefore no absorption correction was applied to the data. 2654 reflections were measured of which 1913 were found to be greater than three times their standard deviation. The reflections were placed on an absolute scale by a Wilson plot. The cell dimensions obtained from refinement of the orientation matrix on the diffractometer are given in Table XII.

Table XII

a	= 8.593 Å	
b	= 13.618 Å	$\alpha = \beta = \gamma = 90.0°$
c	= 26.399 Å	

4.4 Refinement of the Structure

4.4. i Initial Refinement

The programs of the X-Ray 70 package were used to process the

data. The final co-ordinates from the photographic structure determination were used as initial co-ordinates for further least-squares refinement. Non-hydrogen atoms were refined anisotropically only where they were shown to undergo anisotropic thermal motion in the F_o-F_c synthesis. Hydrogen atoms were included in the refinement when they were located from F_o-F_c syntheses. The co-ordinates of the hydrogen atoms were refined but their thermal parameters were held constant at a value of U = 0.0443$Å^2$. This resulted in the anisotropic refinement of 14 non-hydrogen atoms and the location and refinement of all hydrogen atom co-ordinates.

Difference Fourier syntheses indicated a requirement for a reduced electron content at C(28), C(30) and C(31) and the need to insert further atomic positions at a low electron level close to the positions of C(30) and C(31). The final difference map showed clearly that there exist two alternative positions for C(30) and C(31) and that the atomic position previously interpreted as that of a Ca^{2+} ion of very low site occupancy, is the alternative position of C(28) which makes the alternative C(30) and C(31) positions tenable. The isobutenyl side chain C(27)-C(31) is therefore disordered with two alternative configurations. The positions of C(27) and C(29) are nearly the same in both configurations and were therefore refined anisotropically as single atoms to take account of small variations in position in the two configurations.

Refinement of the site occupancy factors for the alternative positions of C(28) indicated an occupancy of approximately 0.70 in the original position and 0.30 in the alternative configuration. In consequence the site occupancies for the alternative positions of

C(30) and C(31), (C(30)' and C(31)'), were inserted at 0.30 and not refined. The hydrogen atoms associated with C(30)' and C(31)' were not included in the refinement.

The final R factor for the 1913 reflections > $3\sigma(F)$ was 0.073. 380 variables were used in this block diagonal least squares refinement grouped into two large blocks. The co-ordinates and thermal parameters for the atoms from this X-Ray 70 least squares refinement, using the diffractometer data, are shown in Tables XIII and XIV.

4.4. ii <u>The Disordered Isobutenyl Group</u>

The bond lengths and angles for the two configurations of the isobutenyl side chain discussed in the previous section (4.4. i) are shown in Figure 4.1. Some of the lengths and angles differ significantly from the expected values for such a side chain. The anisotropic thermal parameters obtained for C(27) and C(29) indicated pronounced anisotropy in the y and z directions respectively (see Table XVI). In an attempt to improve the model for the disorder it was decided to derive possible positions for C(27), C(27)' and C(29), C(29)' by geometrical constructions based on the chemical constraints that these atoms should satisfy.

The atomic positions of the atoms C(28), C(28)', C(30), C(30)', C(31) and C(31)' are sufficiently separated from one another for the least squares procedure to refine the co-ordinates to meaningful values. The atoms of this side chain are chemically constrained to lie in a plane as a result of the sp^2 hybridisation of the atoms C(28) and C(29) at either end of the carbon-carbon double bond. It is therefore reasonable to expect that C(27) and C(29) will lie in the same plane as that defined by the atoms C(28), C(30) and C(31) and

TABLE XIII

Atomic positions and temperature factors for non-hydrogen atoms following X-ray '70 refinement of diffractometer data

Anisotropic Atoms

Atom	x/a	y/b	z/c	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
C(1)	5999 (12)	4844 (8)	8923 (4)	807 (72)	1025 (80)	623 (60)	40 (68)	-203 (54)	72 (60)
C(27)	3046 (11)	0678 (9)	4057 (3)	502 (57)	1269 (89)	618 (56)	230 (64)	-26 (49)	283 (61)
C(29)	1582 (12)	1815 (7)	4609 (5)	583 (62)	505 (57)	1631 (107)	63 (51)	-411 (72)	-137 (73)
N(1)	8955 (8)	3752 (5)	7851 (3)	399 (42)	710 (48)	1141 (60)	154 (39)	70 (43)	265 (48)
O(1)	5328 (6)	5404 (4)	8521 (2)	584 (36)	620 (36)	529 (32)	-40 (32)	-173 (29)	82 (29)
O(2)	8045 (6)	5220 (4)	7593 (2)	475 (32)	572 (37)	1107 (46)	-52 (31)	109 (34)	256 (34)
O(4)	3503 (7)	3195 (4)	7606 (2)	856 (43)	478 (32)	732 (37)	-86 (33)	-86 (36)	56 (30)
O(5)	1894(5)	5026 (4)	7728 (2)	362 (28)	634 (33)	568 (31)	17 (29)	8 (26)	76 (29)
O(6)	3149 (5)	5532 (4)	6972 (2)	388 (29)	583(32)	481 (29)	-67 (28)	-96(25)	146 (27)
O(7)	7553 (5)	7846 (4)	5961 (2)	212 (26)	971 (43)	658 (33)	-17(31)	141 (24)	129 (33)
O(8)	2251 (5)	7795 (4)	5665 (2)	299 (27)	527 (30)	550 (29)	-16 (27)	47 (25)	70 (27)
O(9)	1956 (6)	8783 (4)	5013 (2)	300 (29)	765 (39)	782 (38)	19 (30)	75 (28)	275 (34)
O(10)	6376 (6)	8654 (4)	5228 (2)	393 (32)	795 (43)	1003 (45)	-0 (34)	-29 (33)	312 (37)
O(11)	5300 (7)	1924 (5)	3593 (2)	584 (38)	1020 (49)	758 (42)	149 (38)	27 (34)	362 (39)

Isotropic Atoms

Atom	x/a	y/b	z/c	U
C(2)	7850 (10)	4397 (6)	7739 (3)	573 (23)
C(3)	3209 (10)	6552 (6)	7968 (3)	596 (24)
C(4)	1796 (11)	5443 (7)	8583 (3)	718 (27)
C(5)	4313 (8)	4852 (5)	8205 (3)	409 (19)
C(6)	5098 (9)	4617 (5)	7712 (3)	433 (20)
C(7)	4010 (9)	4079 (5)	7358 (3)	480 (21)
C(8)	2595 (9)	4734 (6)	7265 (3)	517 (22)
C(9)	2848 (9)	5481 (6)	8113 (3)	498 (21)
C(10)	4419 (10)	6734 (6)	6242 (3)	521 (22)
C(11)	2088 (9)	6099 (5)	6707 (3)	429 (19)
C(12)	2694 (8)	6700 (5)	6332 (3)	403 (19)
C(13)	1649 (8)	7251 (5)	6055 (3)	405 (19)
C(14)	1293 (9)	8333 (5)	5347 (3)	491 (21)
C(15)	9668 (9)	8357 (5)	5456 (3)	409 (19)
C(16)	9032 (8)	7846 (5)	5830 (3)	445 (19)
C(17)	0043 (8)	7237 (5)	6144 (3)	389 (18)
C(18)	9473 (9)	6662 (6)	6542 (3)	482 (21)
C(19)	0490 (9)	6087 (6)	6826 (3)	498 (21)
C(20)	7377 (9)	9143 (5)	5011 (3)	455 (20)
C(21)	6952 (9)	9891 (5)	4635 (3)	461 (20)
C(22)	5354 (9)	9962 (5)	4529 (3)	438 (20)
C(23)	4794 (9)	0633 (6)	4178 (3)	504 (22)
C(24)	5859 (10)	1253 (6)	3941 (3)	546 (23)
C(25)	7457 (10)	1214 (6)	4042 (3)	543 (22)
C(26)	7980 (9)	0514 (5)	4392 (3)	482 (20)
N(2)	8928 (7)	9009 (5)	5119 (2)	511 (17)
O(3)	6423 (6)	3978 (4)	7804 (2)	507 (14)
O(12)	7454 (7)	6863 (5)	8164 (2)	803 (18)

Atom	x/a	y/b	z/c	U	Site occupation factors
C(28)	2194 (18)	1515 (11)	4137 (6)	530 (66)	0.56 (3)
C(30)	0765 (16)	2769 (10)	4615 (5)	746 (37)	0.70 fixed
C(31)	1722 (25)	1312 (14)	5065 (7)	1168 (62)	0.70 fixed
C(28)'	2222 (28)	0945 (16)	4581 (8)	378 (99)	0.29 (2)
C(30)'	1605 (88)	2786 (49)	4554 (29)	1823 (278)	0.30 fixed
C(31)'	0732 (52)	1753 (31)	5259 (17)	915 (141)	0.30 fixed

Co-ordinates are given as fractions of a cell edge x 10^4. Thermal parameters are of the form U or U_{ij} x 10^4 ($Å^2$). Standard deviations are given in parentheses with respect to the last figures given.

TABLE XIV

Atomic positions for hydrogen atoms following X-ray '70 refinement of diffractometer data

Co-ordinates are given as fractions of a cell edge x 10^4. Standard deviations are given in parentheses. The thermal parameter for all hydrogen atoms was fixed at U = 0.0443 $Å^2$

Atom	x/a	y/b	z/c
H1(C1)	6839 (98)	5290 (55)	9101 (28)
H2(C1)	7046 (92)	4541 (55)	8730 (28)
H3(C1)	5443 (98)	4957 (53)	9116 (29)
H1(C3)	3524 (96)	6845 (58)	8254 (28)
H2(C3)	2045 (90)	6836 (54)	7911 (27)
H3(C3)	3993 (87)	6608 (53)	7629 (28)
H1(C4)	2328 (94)	5796 (56)	8871 (30)
H2(C4)	1477 (93)	4745 (57)	8626 (28)
H3(C4)	1842 (101)	5830 (59)	8568 (30)
H(C5)	4053 (89)	4133 (55)	8378 (27)
H(C6)	5494 (85)	5230 (51)	7554 (29)
H(C7)	4493 (90)	3886 (55)	7021 (28)
H(C8)	2053 (88)	4353 (52)	7019 (27)
H1(C10)	4793 (92)	6929 (56)	5879 (29)
H2(C10)	4636 (94)	6123 (58)	6200 (28)
H3(C10)	4820 (90)	7224 (55)	6439 (28)
H(C18)	8465 (96)	6537 (53)	6559 (27)
H(C19)	0251 (91)	5599 (55)	7124 (28)
H(C22)	4627 (91)	9451 (56)	4742 (28)
H(C25)	8274 (96)	1689 (53)	3875 (27)
H(C26)	9187 (86)	0517 (54)	4500 (27)
H1(N1)	8583 (93)	3141 (53)	7963 (27)
H2(N1)	9909 (91)	3908 (54)	7818 (26)
H (N2)	9961 (73)	9278 (55)	4878 (28)
H1(C27)	2900 (92)	0660 (54)	3797 (27)
H2(C27)	2176 (92)	0110 (53)	4312 (29)

Atom	x/a	y/b	z/c	Site occupation factors
H(C28)	1894 (137)	2001 (81)	3875 (40)	0.7 fixed
H1(C30)	1306 (160)	3156 (92)	4803 (49)	0.7 fixed
H2(C30)	0229 (184)	2840 (124)	4396 (59)	0.7 fixed
H3(C30)	0843 (199)	3007 (126)	4294 (56)	0.7 fixed
H1(C31)	0221 (154)	1089 (96)	5184 (49)	0.7 fixed
H2(C31)	0682 (177)	1483 (114)	5280 (50)	0.7 fixed
H3(C31)	1796 (143)	1324 (81)	5028 (40)	0.7 fixed
H(O4)	3302 (91)	2873 (52)	7443 (27)	
H(O7)	7269 (93)	7705 (54)	6148 (30)	
H(O11)	6004 (91)	2238 (56)	3429 (28)	
H1(O12)	6577 (96)	6675 (56)	8304 (28)	
H2(O12)	8055 (93)	6674 (54)	7938 (28)	

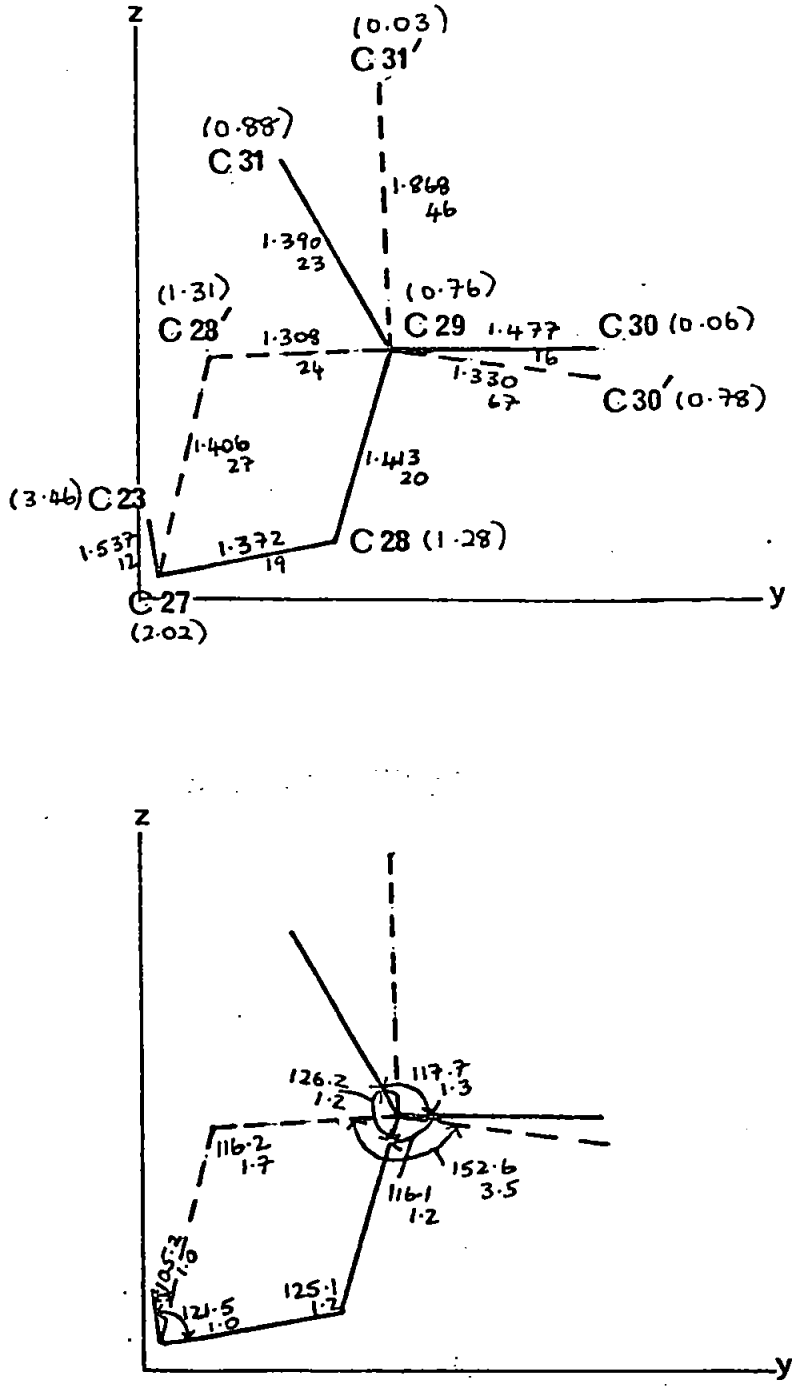


Figure 4.1 Bond lengths (Å) and angles (°) for the two isobutenyl side chains following X-Ray '70 refinement projected down the a axis, relative heights in parentheses (Å)

that C(27)' and C(29)' will lie in the plane defined by C(28)', C(30)' and C(31)'.

Trial co-ordinates for C(29) were calculated in the following way. A point was selected within the triangle defined by C(28), C(30) and C(31) such that the angles and distances from the point to the vertices were in agreement with expected values for the known chemistry.

(Figure 4.2) i.e. $d_1 \approx 1.33$ Å, $d_2 = d_3 \approx 1.5$ Å, $\alpha = \beta = \gamma \approx 120^{\circ}$

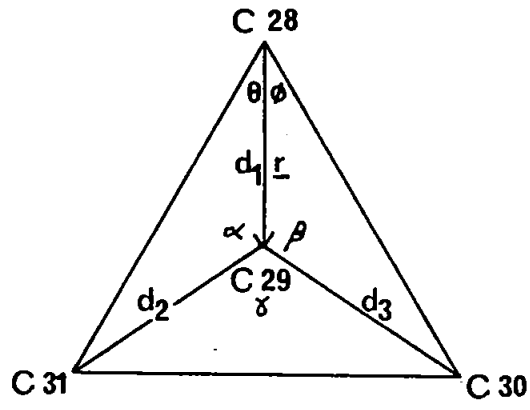


Figure 4.2 The C(28), C(30), C(31) triangle

The co-ordinates of this point within the unit cell were then determined from three simultaneous equations (4.3) for the components of the vector r from an origin at C(28) (Figure 4.3).

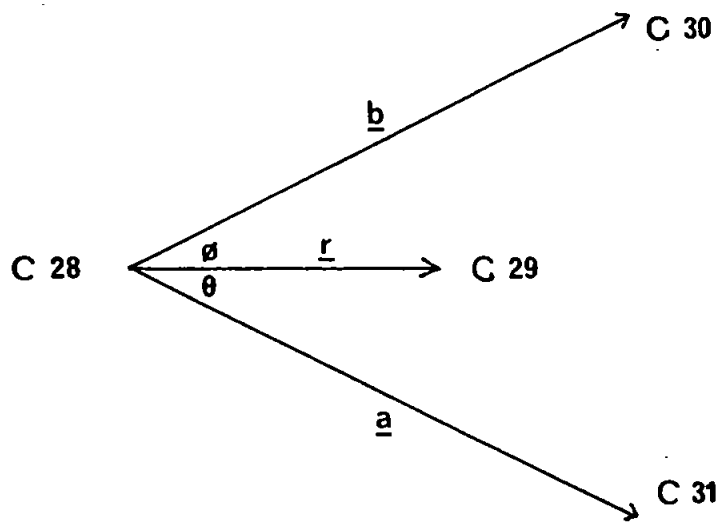


Figure 4.3 Location of co-ordinates for C(29)

$$\underline{r} \cdot \underline{a} \times \underline{b} = 0 \qquad (4.3a)$$

(This equation expresses the condition that $\underline{r}$ must lie in the plane defined by $\underline{a}$ and $\underline{b}$)

$$\underline{r} \cdot \underline{a} = |\underline{r}|\ |\underline{a}| \cos \theta \qquad (4.3b)$$

$$\underline{r} \cdot \underline{b} = |\underline{r}|\ |\underline{b}| \cos \emptyset \qquad (4.3c)$$

Trial co-ordinates for C(29)' were obtained in an analogous manner.

The position of C(27) was obtained by imposing two chemical constraints on the atom. Firstly the distances C(23)-C(27) and C(27)-C(28) were required to be equal to 1.52 Å and make the angle C(23) - C(27) - C(28) $\approx 110^{\circ}$. Secondly C(27) was constrained to lie in the plane defined by C(28), C(30) and C(31). These constraints give rise to three equations (4.4) for the components of a vector $\underline{t}$ defining the position of C(27) with respect to an origin at the mid-point of the C(23)-C(28) line (Figure 4.4).

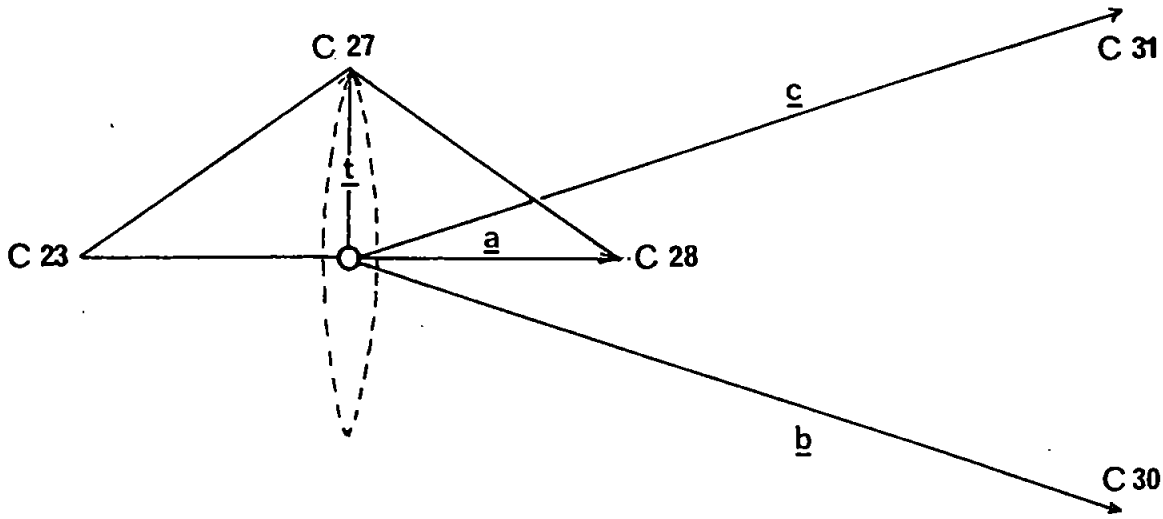


Figure 4.4 Location of co-ordinates for C(27)

$$(\underline{t} - \underline{a}).(\underline{b} - \underline{a}) \times (\underline{c} - \underline{a}) = 0$$

$$(\text{or } \underline{t}.(\underline{b} - \underline{a}) \times (\underline{c} - \underline{a}) = \underline{a}.(\underline{b} \times \underline{c})) \qquad (4.4a)$$

$$|\underline{t}_x|^2 + |\underline{t}_y|^2 + |\underline{t}_z| = |\underline{t}|^2 \qquad (4.4b)$$

$$a_1\underline{t}_x + a_2\underline{t}_y + a_3\underline{t}_z = 0 \qquad (4.4c)$$

where $\underline{a} = a_1\underline{i} + a_2\underline{j} + a_3\underline{k}$ such that $a_1^2 + a_2^2 + a_3^2 = 1$

Equation 4.4a expresses the condition that $\underline{t}$ lies in a plane defined by the ends of the vectors $\underline{a}$, $\underline{b}$ and $\underline{c}$. The position of C(27) also lies on a circle perpendicular to the vector $\underline{a}$ through the origin. This circle is defined by a sphere of radius $|\underline{t}|$ centred at O (equation 4.4b) intersected by a plane normal to the vector $\underline{a}$ through the origin (equation 4.4c).

These three equations are satisfied by two solutions which were compared by the values obtained for the angles C(27) - C(28) - C(29), C(22) - C(23) - C(27) and C(24) - C(23) - C(27). The solution giving the more chemically sensible angles was recorded.

The position of C(27)' was obtained by an analogous procedure.

The bond lengths and angles in the two configurations resulting from the use of the calculated positions for C(27), C(27)', C(29) and C(29)' are listed in Table XV and shown in context in Figure 4.5.

The suggested atomic positions for C(27), C(27)' differ mainly in the y co-ordinate and the positions for C(29), C(29)' mainly in the z co-ordinate. This is in good agreement with the large anisotropic thermal motions observed in those directions following the least squares refinement in which the atoms were assumed to be coincident (Table XVI).

Table XVI

Anisotropic Thermal parameters for C(27) and C(29) as, $U_{ij} \times 10^4$ (Å)^2.

Standard deviations in parentheses.

	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
C(27)	502(57)	1269(89)	618(56)	230(64)	-26(49)	283(61)
C(29)	583(62)	505(57)	1631(107)	63(51)	-411(72)	-137(73)

4.4 iii Final Refinement

The above model was tested in a further refinement of the structure using the least squares program of the SHELX - 76 system. The final co-ordinates from the photographic study were used as initial co-ordinates for the least squares program except for the isobutenyl group where the co-ordinates for the two configurations listed in Table XV were used with fixed site occupancies of 0.7 and 0.3 respectively. 5 cycles of full matrix least squares refinement using the diffractometer data and assuming unit weights resulted in an R factor of 0.1786. All 2654 reflections were used in the refinement in order to provide as many observations per parameter as possible.

TABLE XV

Atomic positions, Bond distances and angles, for the two isobutenyl configurations using the calculated positions for C27, C27', C29, C29'

Positional parameters as fractions of a cell edge x 10^4

atom	x/a	y/b	z/c	atom	x/a	y/b	z/c
C(27)	3091	0578	4025	C(27)'	3110	0922	4083
C(28)	2194	1515	4137	C(28)'	2222	0945	4581
C(29)	1617	1827	4573	C(29)'	1599	1763	4767
C(30)	0765	2769	4615	C(30)'	1605	2786	4554
C(31)	1722	1312	5065	C(31)'	0732	1753	5259

Bond Distances (Å)

atoms	distance	atoms	distance
C(23)-C(27)	1.520	C(23)-C(27)'	1.520
C(27)-C(28)	1.521	C(27)'-C(28)'	1.520
C(28)-C(29)	1.323	C(28)'-C(29)'	1.330
C(29)-C(30)	1.480	C(29)'-C(30)'	1.502
C(29)-C(31)	1.479	C(29)'-C(31)'	1.498

Bond Angles (^{o})

atoms	angle	atoms	angle
C(22)-C(23)-C(27)	118.6	C(22)-C(23)-C(27)'	127.6
C(24)-C(23)-C(27)	122.9	C(24)-C(23)-C(27)'	113.2
C(23)-C(27)-C(28)	113.3	C(23)-C(27)'-C(28)'	110.0
C(27)-C(28)-C(29)	128.9	C(27)'-C(28)'-C(29)'	122.6
C(28)-C(29)-C(30)	121.9	C(28)'-C(29)'-C(30)'	129.6
C(28)-C(29)-C(31)	126.1	C(28)'-C(29)'-C(31)'	120.8
C(30)-C(29)-C(31)	112.0	C(30)'-C(29)'-C(31)'	109.6

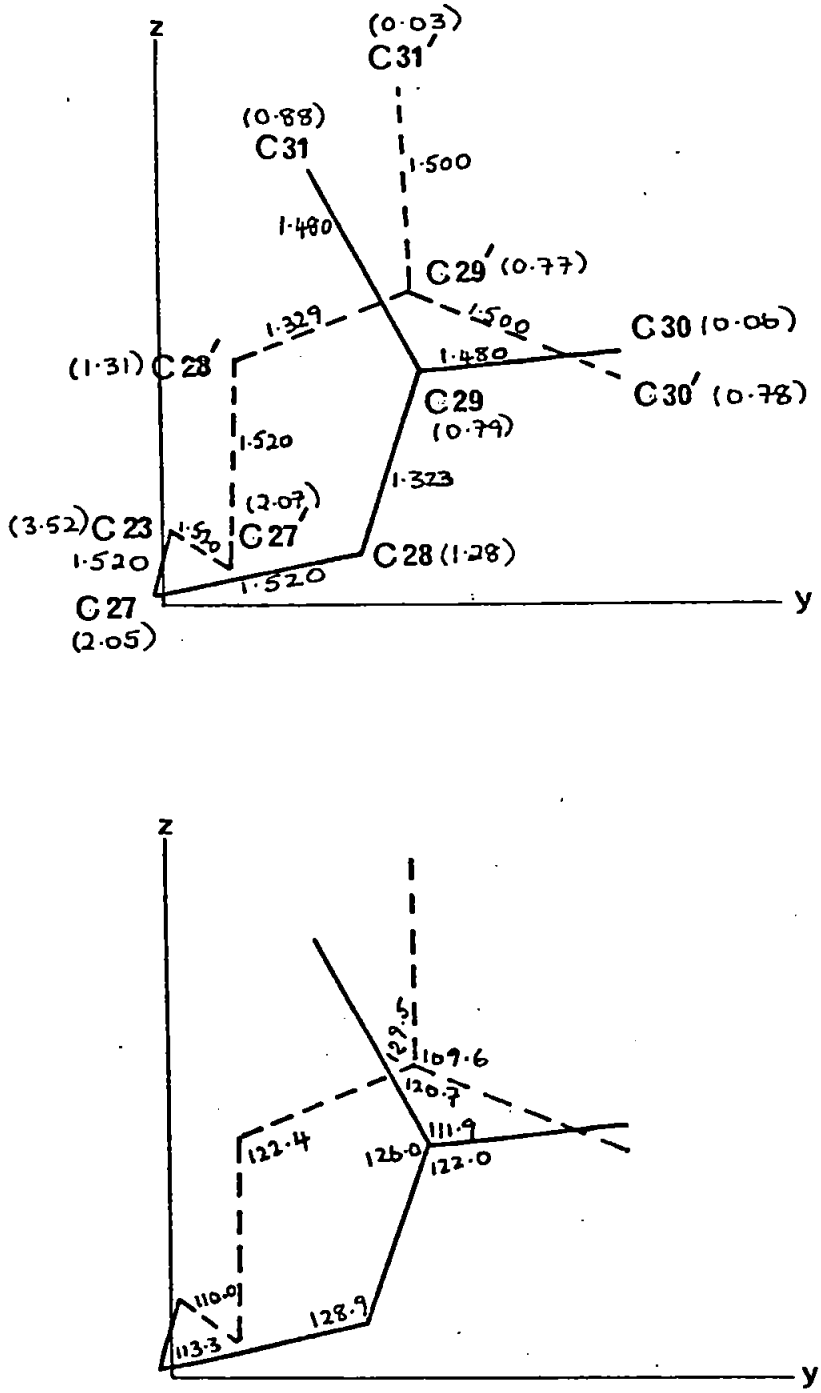


Figure 4.5 Bond lengths (Å) and angles (°) for the two isobutenyl side chains using the calculated positions for C(27), C(27)', C(29) and C(29)' projected down the a axis, relative heights in parentheses (Å)

All non-hydrogen atoms were then refined anisotropically except for the disordered side chain assuming a weighting scheme of the form $a/(\sigma^2(F) + 0.001\ |F|^2)$ where a is initially unity and is refined after each cycle. The data was split into two large blocks and refined by a block diagonal least squares routine. Six cycles of refinement, the variables in each block refining in alternate cycles, resulted in a weighted R factor of 0.1208.

The atoms were then split up into three blocks, each block being associated with one of the main ring systems (A), (B), and (C) (Section 1.2). Hydrogen atoms attached to carbon and nitrogen atoms in the structure were introduced by geometrical construction and given fixed temperature factors corresponding to the isotropic temperature factor of the attached carbon or nitrogen atom. During refinement, the co-ordinates of these hydrogen atoms were linked to the attached non-hydrogen atom in a 'riding' model. Methyl groups were set up in a staggered manner with fixed C-H and H-H distances. They were refined to the correct torsional conformation by treating the methyl group as a rigid group. Hydrogen atoms associated with the major configuration in the disordered side chain were also included with a fixed site occupation factor of 0.7. Six cycles of refinement with the parameters in a given block refining every third cycle resulted in a weighted R factor of 0.1124. At this point the representation of the disorder was changed in order to refine the site occupation factors for the two configurations.

The temperature factors for corresponding atoms in the two configurations were made equal and fixed at these values for the remaining cycles of refinement. The site occupancies for all atoms in

the major configuration were tied to a free variable, x, set initially to a value of 0.7. The atoms of the minor component were given occupancies of 1 - x. Six cycles of refinement resulted in an R factor of 0.1038.

At this stage a difference map was computed, optimised for the location of hydrogen atoms by a weighting scheme depending exponentially on $\sin^2\theta$, which gives greater weights to the low angle reflections. A function was used which gives a weight to a reflection with a $\sin\theta/\lambda$ value of 0.3, one half that of the weight given to a reflection at $\theta = 0$. The positions of all hydrogen atoms associated with oxygen atoms in the structure were easily detected in this difference synthesis. These atoms were included with fixed temperature factors and their co-ordinates allowed to refine. The hydrogen atoms attached to the oxygen atom, O(12), of the water molecule were constrained to bond lengths of 1.00 ± 0.04 Å and a bond angle of 110 ± 10° but were otherwise free to move. A further 15 cycles of refinement resulted in a weighted R factor of 0.0995.

When reflections for which $|F| < 3\sigma$ (F) were omitted, 9 cycles of block diagonal refinement with the same three blocks resulted in a weighted R factor of 0.0637 for 441 variables. This may be compared with the value of 0.073 obtained with the X-Ray 70 programs for this data using a somewhat different model with 380 variables. A Hamilton test [136] on the R factors indicates that the improvement in R factor is significant at the 0.5% level.

The final co-ordinates and temperature factors following this SHELX-76 refinement for all the atoms are listed in Table XVII. A list of observed and calculated structure factors is given in Appendix C.

TABLE XVII

Positions and thermal parameters for all atoms following SHELX refinement

	x/a	y/b	z/c	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C(1)	6002 (12)	4869 (7)	8935 (3)	1023 (73)	827 (62)	568 (50)	181 (48)	-326 (50)	-71 (61)
C(2)	7844 (9)	4385 (6)	7739 (3)	514 (52)	658 (55)	585 (49)	151 (45)	108 (42)	-38 (48)
C(3)	3209 (10)	6550 (5)	7973 (3)	723 (55)	496 (47)	660 (49)	17 (41)	-63 (46)	181 (45)
C(4)	1806 (10)	5458 (7)	8588 (3)	654 (56)	826 (60)	604 (49)	21 (49)	51 (44)	181 (53)
C(5)	4297 (8)	4866 (5)	8207 (3)	458 (40)	432 (40)	463 (40)	41 (34)	-110 (35)	-53 (35)
C(6)	5112 (8)	4618 (5)	7708 (3)	370 (37)	389 (38)	530 (43)	150 (35)	-81 (35)	51 (33)
C(7)	3981 (9)	4068 (5)	7359 (3)	571 (47)	460 (43)	480 (41)	-2 (37)	50 (38)	35 (40)
C(8)	2585 (8)	4741 (5)	7266 (3)	463 (43)	585 (48)	479 (42)	41(36)	-86 (38)	-102 (39)
C(9)	2834 (8)	5490 (6)	8117 (3)	359 (39)	693 (50)	549 (44)	26 (41)	-61 (38)	23 (39)
C(10)	4415 (7)	6731 (6)	6240 (3)	281 (37)	691 (53)	554 (44)	122 (40)	-57 (33)	-21 (37)
C(11)	2062 (8)	6096 (5)	6715 (2)	311 (37)	621 (47)	461 (40)	-4 (38)	-31 (34)	-63 (38)
C(12)	2686 (8)	6699 (5)	6332 (3)	467 (43)	366 (39)	468 (39)	-95 (33)	-47 (37)	-7 (36)
C(13)	1639 (8)	7245 (5)	6054 (2)	422 (40)	401 (39)	412 (38)	-36 (36)	21 (34)	-64 (36)
C(14)	1300 (9)	8331 (5)	5352 (3)	460 (44)	473 (44)	486 (42)	14 (39)	2 (40)	-14 (38)
C(15)	9665 (7)	8359 (5)	5456 (3)	259 (37)	370 (38)	513 (40)	-25 (35)	19 (32)	-61 (30)
C(16)	9047 (7)	7852 (5)	5833 (3)	342 (38)	468 (42)	468 (41)	55 (37)	-2 (35)	-25 (36)
C(17)	0042 (7)	7236 (5)	6142 (3)	365 (37)	433 (41)	426 (38)	-128 (36)	71 (33)	-82 (36)
C(18)	9484 (8)	6657 (6)	6541 (3)	359 (40)	730 (54)	565 (46)	-37 (44)	75 (38)	-131 (41)
C(19)	0498 (8)	6081 (5)	6823 (3)	375 (40)	582 (47)	518 (42)	110 (39)	-27 (36)	-27 (38)
C(20)	7377 (9)	9141 (5)	5009 (3)	350 (42)	544 (46)	627 (47)	-110 (40)	-36 (38)	18 (38)

	x/a	y/b	z/c	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C(21)	6939 (9)	9898 (5)	4629 (3)	619 (49)	390 (38)	403 (36)	-9 (35)	-42 (37)	87 (39)
C(22)	5355 (7)	9972 (5)	4525 (3)	323 (39)	561 (45)	491 (42)	-84 (39)	21 (33)	114 (36)
C(23)	4791 (8)	0642 (6)	4175 (3)	424 (44)	676 (51)	508 (45)	-56 (43)	50 (39)	147 (43)
C(24)	5856 (9)	1254 (6)	3936 (3)	504 (50)	657 (52)	498 (46)	50 (44)	-47 (39)	165 (43)
C(25)	7446 (9)	1208 (6)	4046 (3)	564 (52)	619 (50)	524 (44)	24 (41)	3 (39)	-114 (43)
C(26)	7976 (8)	0512 (5)	4386 (3)	344 (37)	466 (40)	591 (43)	57 (39)	-25 (36)	2 (37)
N(1)	8966 (8)	3742 (5)	7856 (3)	435 (39)	671 (47)	1084 (56)	188 (47)	94 (41)	157 (40)
N(2)	8926 (7)	9015 (4)	5113 (2)	404 (36)	473 (35)	585 (36)	80 (30)	5 (30)	23 (30)
O(1)	5336 (6)	5397 (4)	8521 (2)	593 (33)	605 (33)	532 (30)	32 (28)	-132 (27)	-68 (29)
O(2)	8056 (6)	5208 (4)	7594 (2)	469 (31)	639 (38)	1091 (46)	295 (35)	131 (31)	-77 (30)
O(3)	6430 (6)	3983 (3)	7806 (2)	459 (29)	436 (27)	712 (34)	121 (26)	16 (27)	23 (25)
O(4)	3496 (7)	3198 (4)	7605 (2)	884 (42)	423 (30)	766 (38)	34 (28)	-137 (34)	-115 (30)
O(5)	1899 (5)	5025 (4)	7728 (2)	339 (25)	644 (31)	533 (29)	92 (26)	-5 (23)	-6 (25)
O(6)	3147 (5)	5531 (3)	6971 (2)	390 (26)	571 (29)	526 (27)	127 (25)	-84 (23)	-49 (25)
O(7)	7562 (5)	7826 (4)	5973 (2)	307 (28)	882 (40)	658 (33)	147 (30)	56 (23)	111 (28)
O(8)	2250 (5)	7795 (3)	5664 (2)	359 (25)	543 (27)	505 (25)	97 (25)	41 (23)	-9 (25)
O(9)	1952 (6)	8778 (4)	5015 (2)	414 (29)	769 (36)	770 (35)	282 (32)	73 (28)	34 (29)
O(10)	6376 (7)	8647 (2)	5225 (2)	425 (31)	877 (41)	968 (42)	329 (36)	-0 (31)	-20 (33)
O(11)	5305 (7)	1930 (5)	3594 (2)	606 (36)	1109 (50)	714 (39)	357 (37)	-11 (31)	138 (37)
O(12)	7455 (7)	6857 (4)	8163 (2)	784 (42)	552 (36)	891 (41)	12 (30)	-35 (34)	-48 (32)

	x/a	y/b	z/c	U (fixed)	Site occupation factor (x)
C(27)	3004 (20)	0576 (11)	4028 (7)	650	0.6534 (80)
C(28)	2195 (16)	1523 (10)	4140 (5)	700	"
C(29)	1620 (17)	1857 (10)	4553 (5)	600	"
C(30)	0679 (18)	2763 (12)	4617 (6)	800	"
C(31)	1741 (19)	1318 (11)	5054 (6)	800	"
				(fixed)	(1 - x)
C(27)'	3111 (39)	0873 (18)	4089 (13)	650	0.3466 (80)
C(28)'	2222 (29)	0981 (18)	4585 (9)	700	"
C(29)'	1452 (33)	1743 (20)	4778 (10)	600	"
C(30)'	1383 (35)	2803 (24)	4544 (11)	800	"
C(31)'	0673 (34)	1650 (19)	5271 (11)	800	"
				(fixed)	
H1(C1)	6553 (12)	5359 (7)	9202 (3)	750)	refined with C(1) as a rigid group
H2(C1)	6881 (12)	4518 (7)	8705 (3)	750)	
H3(C1)	5349 (12)	4317 (7)	9138 (3)	750)	
H1(C3)	3756 (10)	6967 (5)	8272 (3)	600)	refined with C(3) as a rigid group
H2(C3)	2063 (10)	6843 (5)	7900 (3)	600)	
H3(C3)	3905 (10)	6599 (5)	7634 (3)	600)	
H1(C4)	2336 (10)	5809 (7)	8911 (3)	750)	refined with C(4) as a rigid group
H2(C4)	1698 (10)	4680 (7)	8659 (3)	750)	
H3(C4)	0666 (10)	5768 (7)	8524 (3)	750)	
H(C5)	3996 (8)	4167 (5)	8374 (3)	500	riding on C(5)
H(C6)	5490 (8)	5301 (5)	7541 (3)	450	riding on C(6)
H(C7)	4546 (9)	3899 (5)	7005 (3)	550	riding on C(7)
H(C8)	1675 (8)	4351 (5)	7068 (3)	600	riding on C(8)
H1(C10)	4669 (7)	7041 (6)	5873 (3)	600)	refined with C(10) as a rigid group
H2(C10)	4869 (7)	5992 (6)	6260 (3)	600)	
H3(C10)	4951 (7)	7174 (6)	6531 (3)	600)	

	x/a	y/b	z/c	U (fixed)		
H(C18)	8254 (8)	6501 (6)	6571 (3)	500		riding on C(18)
H(C19)	9768 (8)	5705 (5)	7094 (3)	550		riding on C(19)
H(C22)	4628 (7)	9469 (5)	4735 (3)	450		riding on C(22)
H(C25)	8239 (9)	1711 (6)	3863 (3)	600		riding on C(25)
H(C26)	9170 (8)	0498 (5)	4500 (3)	550		riding on C(26)
H1(N1)	8700 (85)	3045 (22)	7920 (26)	700	)	N(1)-H1(N1), N(1)-
H2(N1)	0061 (35)	3944 (52)	7814 (26)	700	)	H2(N1) constrained to
					)	1.00 ± 0.02.
H(N2)	9816 (7)	9378 (4)	4956 (2)	550		riding on N(2)
Site occupation factor 0.6534 (80)						
H1(C27)	3353 (20)	0389 (11)	3647 (7)	650		riding on C(27)
H2(C27)	2790 (20)	9912 (11)	4240 (7)	650		riding on C(27)
H(C28)	1756 (16)	1849 (10)	3795 (5)	700		riding on C(28)
H1(C30)	1167 (18)	3396 (12)	4805 (6)	800	)	
H2(C30)	9628 (18)	2539 (12)	4810 (6)	800	)	refined with C(30)
H3(C30)	0410 (18)	2947 (12)	4229 (6)	800	)	as a rigid group
H1(C31)	2423 (19)	0655 (11)	5017 (6)	800	)	
H2(C31)	0635 (19)	1145 (11)	5226 (6)	800	)	refined with C(31)
H3(C31)	2358 (19)	1839 (11)	5287 (6)	800	)	as a rigid group
Site occupation factor 0.3466 (80)						
H1(C27)'	3580 (39)	0734 (18)	4462 (13)	650		riding on C(27)'
H2(C27)'	4017 (39)	1159 (18)	3847 (13)	650		riding on C(27)'
H(C28)'	2278 (29)	0260 (18)	4741 (9)	700		riding on C(28)'
H1(C30)'	2296 (35)	2504 (24)	4309 (11)	800	)	
H2(C30)'	1891 (35)	3150 (24)	4873 (11)	800	)	refined with C(30)'
H3(C30)'	0730 (35)	3339 (24)	4330 (11)	800	)	as a rigid group

	x/a	y/b	z/c	U (fixed)	
H1(C31)'	9563(34)	1796 (19)	5447 (11)	800)	refined with C(31)' as a rigid group
H2(C31)'	0968 (34)	2248 (19)	5020 (11)	800)	
H3(C31)'	1556 (34)	1581 (19)	5560 (11)	800)	
H(O4)	3099 (89)	2726 (52)	7421 (28)	650	
H(O7)	7005 (83)	8203 (52)	5774 (27)	650	
H(O11)	6161 (93)	2255 (57)	3407 (28)	750	
H1(O12)	6699 (69)	6445 (47)	8339 (25)	750)	
H2(O12)	8167 (71)	6540 (50)	7925 (23)	750)	

H1(O12)-O(12) and H2(O12)-O(12) constrained to 1.00 ± 0.04

H1(O12)-H2(O12) constrained to 1.57 ± 0.10

Co-ordinates are given as fractions of a cell edge x 10^4. Thermal parameters are listed as U or U_{ij} x 10^4 $(Å)^2$. Standard deviations are given in parentheses.

4.5 Molecular Geometry

The bond lengths and angles obtained from the SHELX refinement using 1913 reflections ($|F| > 3\sigma |F|, R_w = 0.0637$) are given in Tables XVIII and XIX and are shown in context in Figure 4.6. All carbon-hydrogen bond lengths were fixed at 1.08 Å and those for nitrogen-hydrogen at 1.00 Å. Oxygen-hydrogen bond lengths are given in Table XX and discussed in section 4.6 which deals with the hydrogen bonding in the structure.

Apart from the disordered isobutenyl side chains the main features of the novobiocin structure are as discussed in section 3.7 and the overall views of the structure down the a axis (Figure 3.6) and perpendicular to the main plane (Figure 3.7) are basically unaltered.

In order to compare the detailed molecular geometry with other related compounds it is convenient to examine the three main moieties in turn.

4.5. i The Substituted Benzoic Acid, ring (A)

The bond lengths and angles for this part of the molecule are shown in detail in Figure 4.7. The bond lengths in the phenyl ring are very similar with a mean value of 1.388(6) Å. Details of the least squares plane through the six atoms are given in Table XXI.

TABLE XVIII

Bond distances and their standard deviations (Å) after SHELX refinement

ATOMS	DISTANCE		ATOMS	DISTANCE	
Noviose moiety, ring (C)					
C(5) - C(6)	1.528	(9)	C(5) - O(1)	1.417	(8)
C(6) - C(7)	1.534	(10)	O(1) - C(1)	1.428	(10)
C(7) - C(8)	1.529	(10)			
C(8) - O(5)	1.412	(8)	C(6) - O(3)	1.449	(8)
O(5) - C(9)	1.455	(8)	O(3) - C(2)	1.345	(9)
C(9) - C(5)	1.536	(10)	C(2) - N(1)	1.339	(11)
			C(2) - O(2)	1.198	(10)
C(8) - O(6)	1.413	(8)			
			C(9) - C(3)	1.526	(10)
C(7) - O(4)	1.413	(9)	C(9) - C(4)	1.524	(11)
Amino hydroxy coumarin, ring (B)					
C(19) - C(18)	1.389	(10)	C(17) - C(16)	1.449	(9)
C(18) - C(17)	1.401	(10)	C(16) - C(15)	1.322	(9)
C(17) - C(13)	1.392	(9)	C(15) - C(14)	1.432	(9)
C(13) - C(12)	1.380	(9)	C(14) - O(8)	1.371	(8)
C(12) - C(11)	1.408	(10)	O(8) - C(13)	1.376	(8)
C(11) - C(19)	1.375	(9)			
			C(16) - O(7)	1.329	(8)
C(12) - C(10)	1.506	(9)	C(15) - N(2)	1.421	(8)
C(11) - O(6)	1.385	(8)	C(14) - O(9)	1.215	(9)
Substituted benzoic acid, ring (A)					
C(21) - C(22)	1.393	(10)	C(21) - C(20)	1.486	(10)
C(22) - C(23)	1.386	(10)	C(20) - O(10)	1.231	(9)
C(23) - C(24)	1.389	(11)	C(20) - N(2)	1.371	(9)
C(24) - C(25)	1.399	(11)			
C(25) - C(26)	1.382	(10)	C(24) - O(11)	1.374	(10)
C(26) - C(21)	1.380	(10)			

ATOMS	DISTANCE		ATOMS	DISTANCE	
C(23) - C(27)	1.587	(18)	C(23) - C(27)'	1.495	(34)
C(27) - C(28)	1.494	(20)	C(27)'-C(28)'	1.523	(42)
C(28) - C(29)	1.280	(19)	C(28)'-C(29)'	1.333	(37)
C(29) - C(30)	1.484	(22)	C(29)'-C(30)'	1.570	(42)
C(29) - C(31)	1.516	(20)	C(29)'-C(31)'	1.469	(39)

All C-H bonds 1.08 Å

N-H bonds 1.00 Å

TABLE XIX

Bond angles and their standard deviations (°) after SHELX refinement

ATOMS	ANGLE		ATOMS	ANGLE	
Noviose Moiety, ring (C)					
C(9)-C(5)-C(6)	111.4	(0.5)	O(5)-C(9)-C(5)	108.9	(0.6)
C(9)-C(5)-O(1)	108.8	(0.6)	O(5)-C(9)-C(4)	103.8	(0.6)
C(6)-C(5)-O(1)	109.2	(0.5)	O(5)-C(9)-C(3)	110.7	(0.6)
			C(5)-C(9)-C(3)	112.9	(0.6)
C(5)-C(6)-C(7)	109.6	(0.5)	C(4)-C(9)-C(3)	110.7	(0.7)
C(5)-C(6)-O(3)	109.7	(0.5)	C(4)-C(9)-C(5)	109.5	(0.6)
C(7)-C(6)-O(3)	108.1	(0.5)			
			O(5)-O(1)-C(1)	116.3	(0.6)
C(6)-C(7)-C(8)	107.5	(0.5)			
C(6)-C(7)-O(4)	108.6	(0.6)	C(6)-O(3)-C(2)	116.1	(0.6)
C(8)-C(7)-O(4)	110.2	(0.6)			
			O(3)-C(2)-N(1)	110.7	(0.7)
C(7)-C(8)-O(5)	110.9	(0.6)	O(3)-C(2)-O(2)	124.1	(0.7)
C(7)-C(8)-O(6)	106.1	(0.5)	N(1)-C(2)-O(2)	125.2	(0.8)
O(5)-C(8)-O(6)	114.4	(0.5)			
			C(8)-O(6)-C(11)	117.5	(0.5)
C(8)-O(5)-C(9)	119.5	(0.5)			
Isobutenyl side chains					
C(22)-C(23)-C(27)	117.6	(0.8)	C(22)-C(23)-C(27)'	125.2	(1.3)
C(24)-C(23)-C(27)	124.1	(0.9)	C(24)-C(23)-C(27)'	116.2	(1.3)
C(23)-C(27)-C(28)	110.7	(1.1)	C(23)-C(27)'-C(28)'	112.0	(2.3)
C(27-C(28)-C(29)	130.9	(1.4)	C(27)'-C(28)'-C(29)'	130.8	(2.4)
C(28)-C(29)-C(30)	127.1	(1.3)	C(28)'-C(29)'-C(30)'	125.8	(2.4)
C(28)-C(29)-C(31)	122.9	(1.3)	C(28)'-C(29)'-C(31)'	119.8	(2.4)
C(30)-C(29)-C(31)	109.9	(1.2)	C(30)'-C(29)'-C(31)'	114.2	(2.3)

ATOMS	ANGLE		ATOMS	ANGLE	
Aminohydroxycoumarin, ring (B)					
C(11)-C(19)-C(18)	119.6	(0.7)	C(17)-C(16)-C(15)	119.3	(0.6)
			C(17)-C(16)-O(7)	113.2	(0.6)
C(19)-C(18)-C(17)	120.4	(0.6)	O(7)-C(16)-C(15)	127.5	(0.6)
C(18)-C(17)-C(13)	117.9	(0.6)	C(16)-C(15)-C(14)	121.6	(0.6)
C(18)-C(17)-C(16)	123.2	(0.6)	C(16)-C(15)-N(2)	128.9	(0.6)
C(16)-C(17)-C(13)	118.8	(o.6)	N(2)-C(15)-C(14)	109.5	(0.6)
C(17)-C(13)-C(12)	123.3	(0.6)	C(15)-C(14)-O(8)	118.9	(0.6)
C(17)-C(13)-O(8)	120.4	(0.6)	C(15)-C(14)-O(9)	125.4	(0.7)
O(8)-C(13)-C(12)	116.3	(0.6)	O(9)-C(14)-O(8)	115.7	(0.6)
C(13)-C(12)-C(11)	116.7	(0.6)	C(14)-O(8)-C(13)	120.8	(0.5)
C(13)-C(12)-C(10)	122.8	(0.6)			
C(10)-C(12)-C(11)	120.5	(0.6)			
C(12)-C(11)-C(19)	122.0	(0.6)			
C(12)-C(11)-O(6)	114.7	(0.6)			
O(6)-C(11)-C(19)	123.3	(0.6)			
Substituted Benzoic Acid, ring (A)					
C(24)-C(25)-C(26)	119.2	(0.7)	C(26)-C(21)-C(22)	119.7	(0.6)
			C(26)-C(21)-C(20)	124.8	(0.7)
C(25)-C(26)-C(21)	120.3	(0.7)	C(20)-C(21)-C(22)	115.6	(0.6)
C(15)-N(2)-C(20)	129.8	(0.6)	C(21)-C(22)-C(23)	121.4	(0.6)
N(2)-C(20)-O(10)	121.1	(0.7)	C(22)-C(23)-C(24)	117.9	(0.7)
N(2)-C(20)-C(21)	117.9	(0.6)			
C(21)-C(20)-O(10)	120.9	(0.7)	C(23)-C(24)-C(25)	121.5	(0.7)
			C(23)-C(24)-O(11)	118.2	(0.7)
			O(11)-C(24)-C(25)	120.3	(0.7)

TABLE XX

Oxygen-Hydrogen Bond Lengths and Angles

Bond lengths (Å)

Atoms	Distance
O(4)-H(O4)	0.877 (73)
O(7)-H(O7)	0.876 (71)
O(11)-H(O11)	0.991 (78)
H1(O12)-O(12)	0.977 (62)
H2(O12)-O(12)	0.978 (62)

(restricted to 1.00 ± .04)

Bond Angles (°)

Atoms	Distance
C(7)-O(4)-H(O4)	118.3 (4.8)
C(16)-O(7)-H(O7)	110.0 (4.7)
C(24)-O(11)-H(O11)	111.7 (4.5)
H1(O12)-O(12)-H2(O12)	117.9 (5.4)

(restricted by H1(O12)-H2(O12) constraint of 1.57 ± 0.10)

Refined value H1(O12) ... H2(O12) 1.675

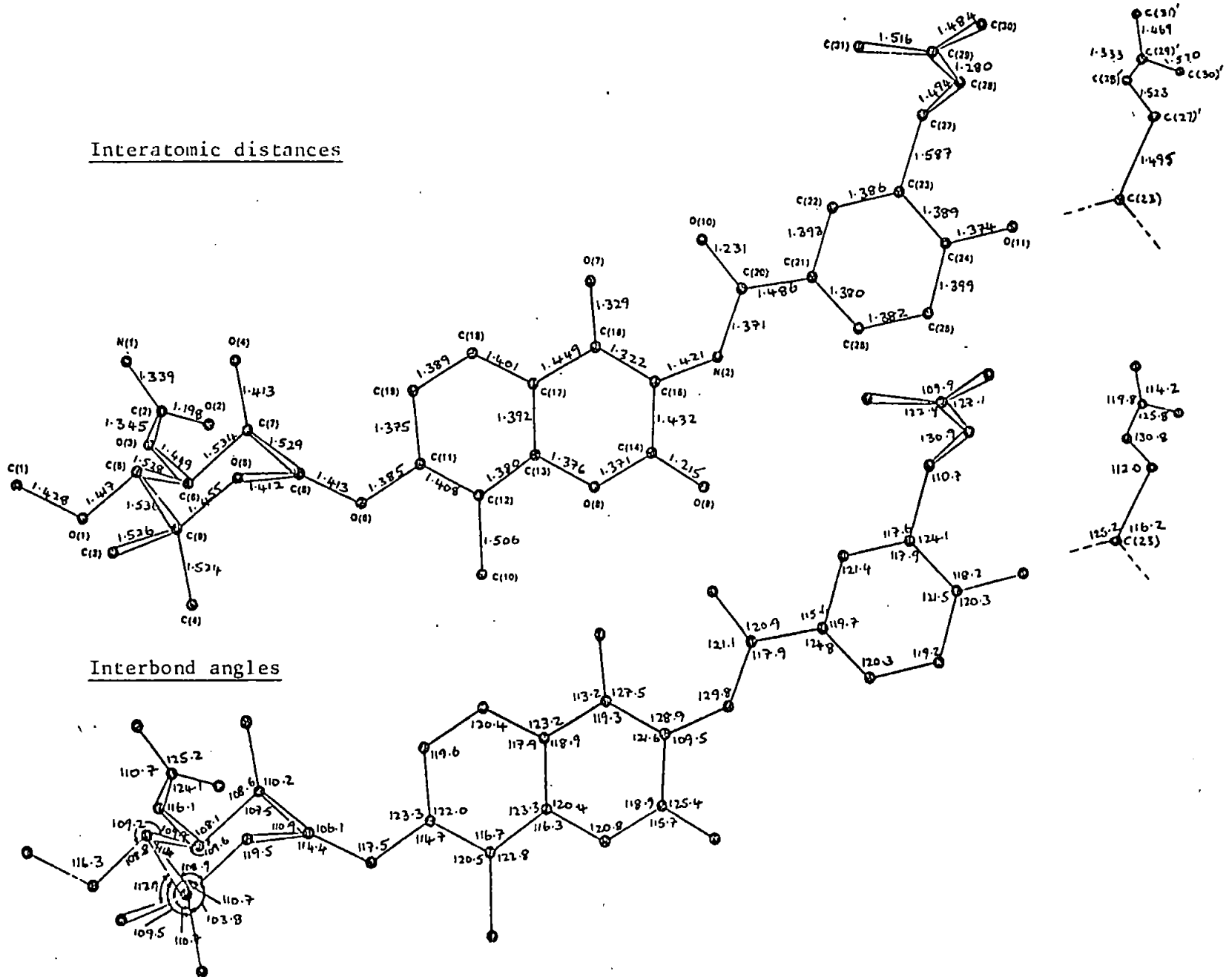
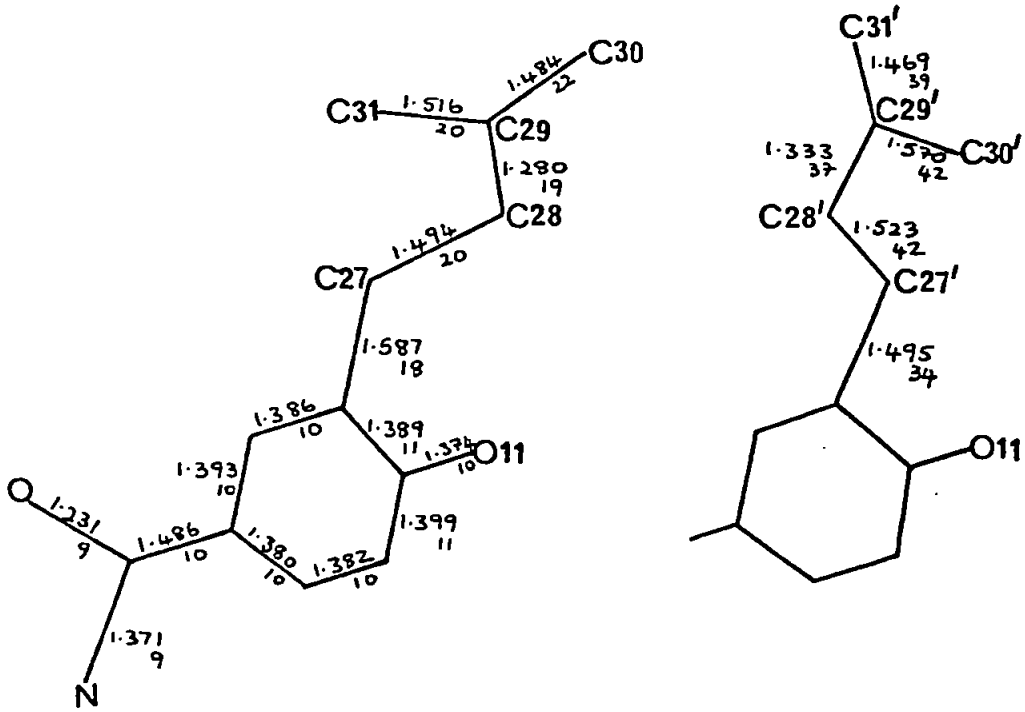
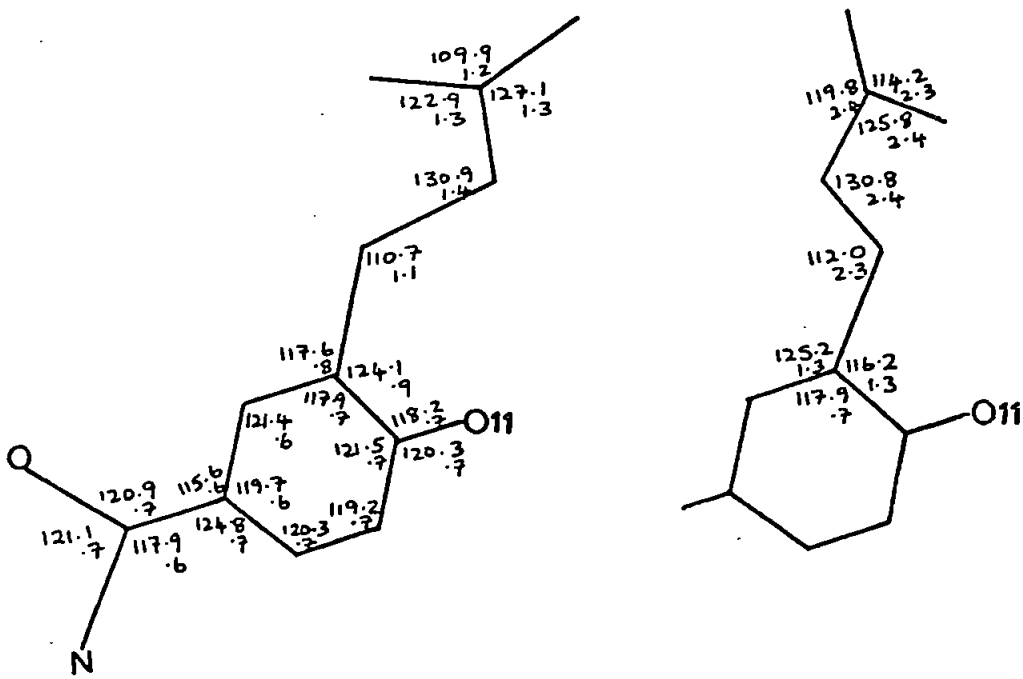


Figure 4.6 Interatomic distances and interbond angles in the novobiocin molecule



a) Interatomic distances



b) Interbond angles

Site occupation factors 0.65 0.35

Figure 4.7 Interatomic distances and interbond angles in the benzoic acid moiety of novobiocin showing the alternative configurations of the isobutenyl group

Table XXI

Least Squares Plane for Phenyl Ring (A)

Direction cosines of the plane		-0.10876	0.67014	0.73422
Origin to plane distance		17.3599 Å		
	atom	deviation Å	atom	deviation Å
Atoms defining the plane	C(21)	-0.003	C(24)	-0.009
	C(22)	0.010	C(25)	0.016
	C(23)	-0.004	C(26)	0.010
Atom to plane distances	C(20)	0.001	C(27)	-0.182
	O(10)	0.062	C(27)'	0.197
	N(2)	-0.056	O(11)	-0.002

None of the atoms is significantly displaced from the plane and the phenolic oxygen, O(11), is clearly coplanar with the benzene ring. The atoms of the peptide bond, C(20), N(2) and O(10), are also nearly coplanar with this ring. The atomic positions for C(27) and C(27)' are displaced by ~ 0.2 Å on either side of the benzene plane.

The bond lengths and angles for the disordered isobutenyl group, shown in Figure 4.7, may be compared with the idealised starting values, Figure 4.5 , and the results following the X-Ray 70 refinement of this data, Figure 4.1. Although the bond lengths and angles obtained from the SHELX refinement (Figure 4.7) do deviate from ideal values, this model appears to be valid and provide a reasonable picture of the two alternative configurations. The final site occupancy factors of 0.65 and 0.35 indicate a ratio of approximately 2 : 1 in favour of the majority component. Details of the least squares planes for the two configurations are given in Table XXII.

Table XXII

Least Squares Planes for the two Isobutenyl Groups

Major Component

Direction cosines of the plane	0.85731	0.48328	0.17735	
Origin to plane distance	11.0971 Å			
	atom	deviation Å	atom	deviation Å
Atoms defining the plane	C(27)	-0.038	C(30)	-0.036
	C(28)	0.042	C(31)	0.000
	C(29)	0.031		
Atom to plane distance	C(23)	1.391		

Minor Component

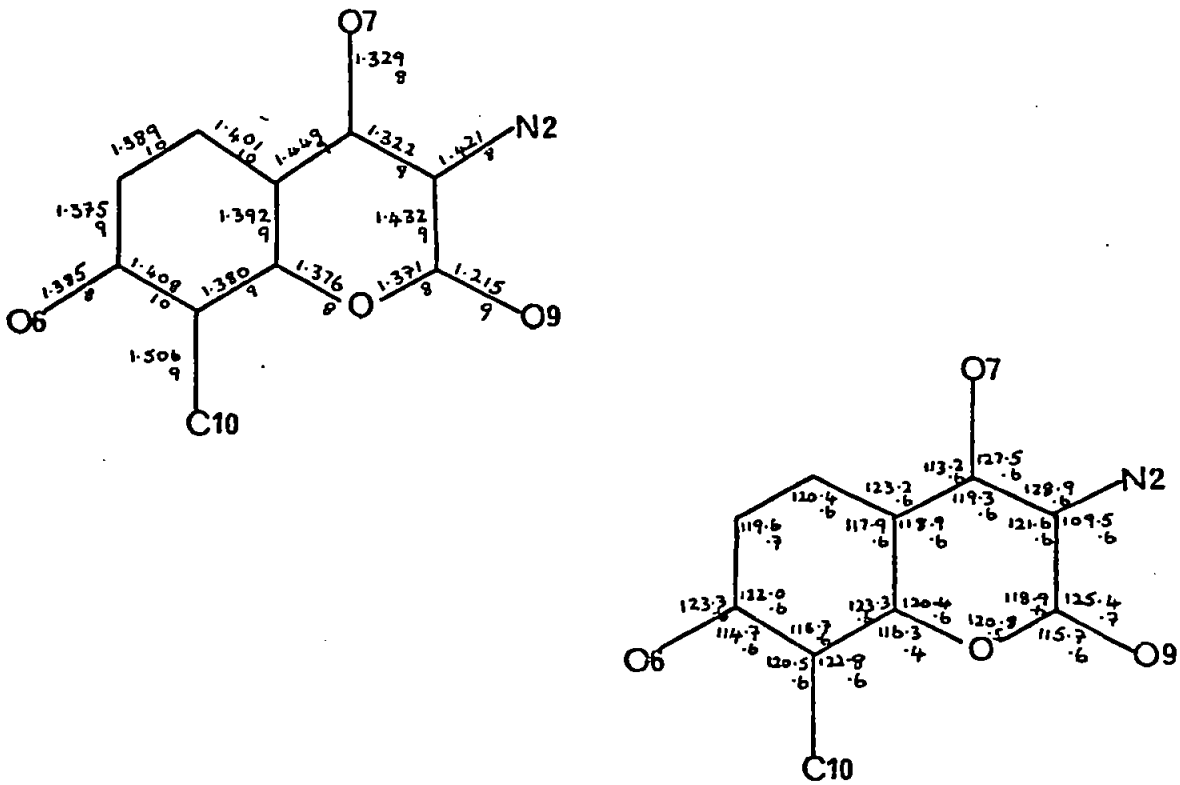
Direction cosines of the plane	0.83887	0.26070	0.47784	
Origin to plane distance	11.2662 Å			
	atom	deviation Å	atom	deviation Å
Atoms defining the plane	C(27)'	-0.005	C(30)'	0.008
	C(28)'	0.017	C(31)'	0.004
	C(29)'	-0.024		
Atom to plane distance	C(23)	1.232		

major-minor interplanar angle = 21.58°

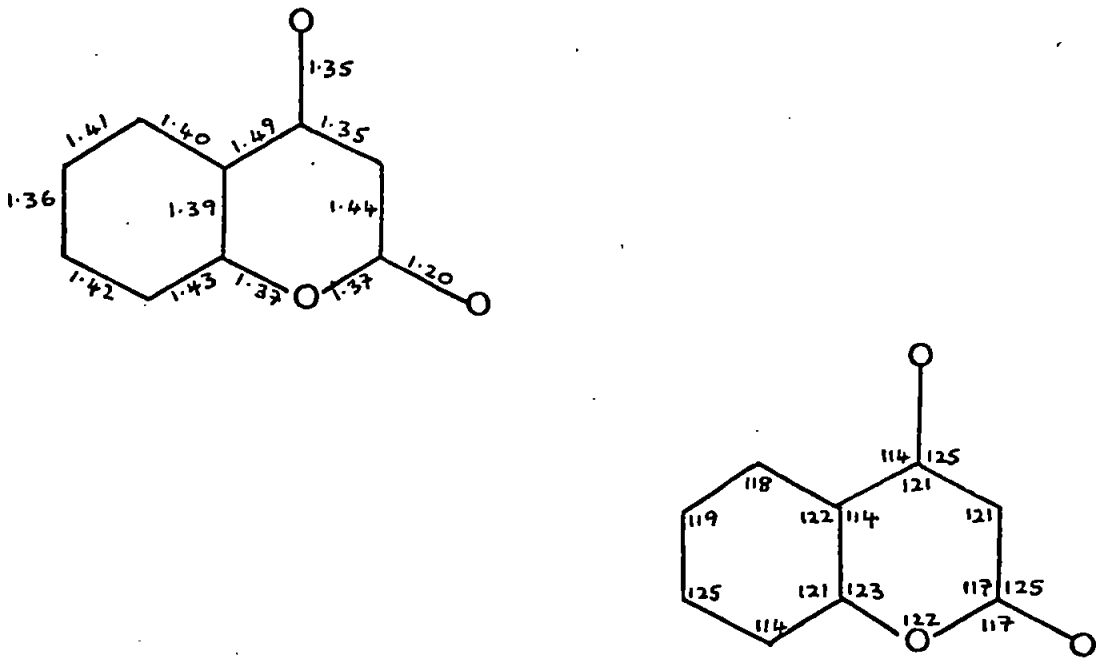
Both isobutenyl groups are planar with no significant deviations from the planes. The interplanar angle between the two configurations is 21.6°.

4.5. ii The Aminohydroxycoumarin, ring (B)

The bond lengths and angles of this moiety are shown in detail in Figure 4.8. They compare well with the values obtained by Gaultier and Hauw, for 4 - hydroxy - coumarin monohydrate [114] shown for comparison in the same figure. The atoms O(7) - C(16) - C(15) - C(14) - O(9) form a conjugated system over which the electrons are delocalised.



a) Novobiocin

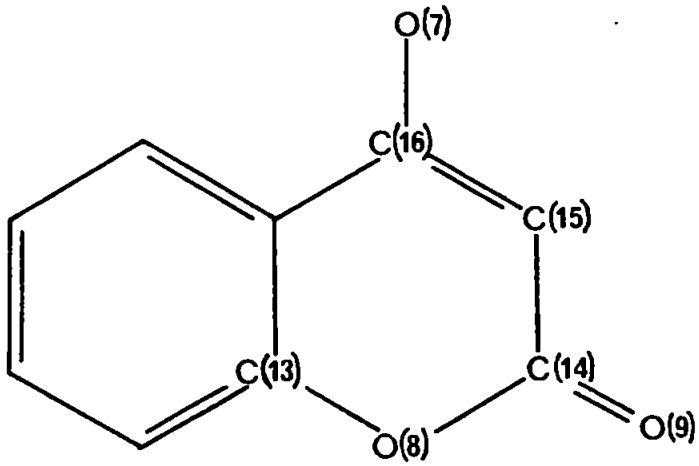


b) 4-hydroxy-coumarin monohydrate

Figure 4.8 Comparison of interatomic distances and interbond angles for novobiocin (a) and 4-hydroxy-coumarin monohydrate (b)

TABLE XXIII

Comparison of Coumarin Bond Lengths for Several Structures



Compound	C(13)-O(8)	O(8)-C(14)	C(14)-O(9)	C(14)-C(15)	C(15)-C(16)	C(16)-O(7)
Dibromo coumarol [115]	1.36	1.36	1.22	1.42	1.38	1.34
Dicoumarol [116]	1.40	1.30	1.22	1.51	1.30	1.32
	1.41	1.33	1.21	1.46	1.31	1.33
4-Hydroxycoumarin [114]	1.37	1.37	1.20	1.44	1.35	1.35
3-Bromo-4-hydroxycoumarin [117]	1.43	1.31	1.26	1.43	1.41	1.34
3-(1-Naphthyl) 4-hydroxycoumarin [118]	1.38	1.36	1.23	1.43	1.37	1.33
Phenprocoumon [119]	1.38	1.37	1.23	1.42	1.37	1.34
Racemic warfarin [120]	1.38	1.38	1.21	1.42	1.36	1.35
(-)-(S)- Warfarin [121]	1.38	1.38	1.22	1.44	1.35	1.35
Coumarin [122]	1.38	1.37	1.20	1.45	1.34	-
Coumarin [123]	1.38	1.37	1.22	1.44	1.34	-
Novobiocin (14%) [137] photographic data	1.371(12)	1.354(13)	1.264(14)	1.377(15)	1.385(15)	1.329(13)
Novobiocin (6.4%) diffractometer data	1.376(8)	1.371(8)	1.215(9)	1.432(9)	1.322(9)	1.329(8)

The length of the bonds C(16) - O(7) at 1.329(8) Å and C(15) - C(14) at 1.432(9) Å are therefore shortened from normal single bond values. The lengths C(13) - O(8) and C(14) - O(8) are nearly equal at ~ 1.374 Å and reflect the general delocalisation in the heterocyclic portion of the coumarin system. The C(17) - C(16) bond length of 1.449(9) Å is also shortened by this effect. Bond lengths and angles for a number of coumarin containing structures are compared with those from novobiocin in Table XXIII.

Details of the least squares plane for the coumarin system are given in Table XXIV.

Table XXIV

Least Squares Plane for Coumarin System, ring (B)

Direction cosines of the plane	0.13399	0.76825	0.62597	
Origin to plane distance	18.8866 Å			
	atom	deviation Å	atom	deviation Å
Atoms defining the plane	C(11)	-0.023	C(17)	-0.011
	C(12)	0.047	C(18)	-0.020
	C(13)	0.037	C(19)	-0.040
	C(14)	-0.026	O(7)	0.041
	C(15)	-0.013	O(8)	0.039
	C(16)	0.008	O(9)	-0.039
Atom to plane distances	O(6)	-0.067	C(20)	-0.197
	C(10)	0.127	O(10)	-0.472
	N(2)	0.023		

None of the atoms contributing to the plane deviates significantly from it. The methyl carbon, C(10), is about 0.13 Å out of plane. The glycoside link to O(6) is also out of plane by 0.07 Å. The

interplanar angle between the coumarin least squares plane and the plane of the glycoside link (Table XXV), defined by C(8) - O(6) - C(11), is 17.9°.

Table XXV

Plane of Glycoside Link

Direction cosines of the plane	-0.07245	0.60657	0.79172	
Origin to plane distance		18.3198 Å		
	atom	deviation Å	atom	deviation Å
Atoms defining the plane	C(11)	-	C(8)	-
	O(6)	-		
Atom to plane distances	C(7)	-0.448	C(19)	0.311
	O(5)	1.243	C(12)	-0.341

The nitrogen N(2) of the peptide bond lies in the coumarin plane but the other atoms in this link, C(20) and O(10), are out of plane by 0.20 Å and 0.47 Å respectively. Details of the least squares plane for the peptide bond are given in Table XXVI.

Table XXVI

Least Squares plane for the Peptide Bond

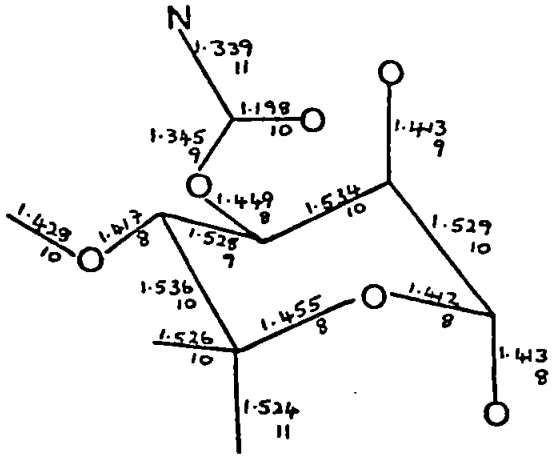
Direction cosines of the plane	-0.06085	0.68647	0.72461	
Origin to plane distance		17.7436 Å		
	atom	deviation Å	atom	deviation Å
Atoms defining the plane	C(21)	0.002	N(2)	-0.001
	C(20)	-0.003	C(15)	0.002
	O(10)	0.001		
Atom to plane distances	C(14)	-0.309	C(26)	0.057
	C(16)	0.280	O(7)	0.602
	C(22)	-0.047	O(9)	-0.569

None of the atoms defining the peptide plane deviates by more than 0.003 Å from the plane which makes interplanar angles of 13.4° to the coumarin plane and 3.0° to the plane of the phenyl group. The torsion angle along the C(15) - N(2) bond, C(16) - C(15) - N(2) - C(20), is -15.6°.

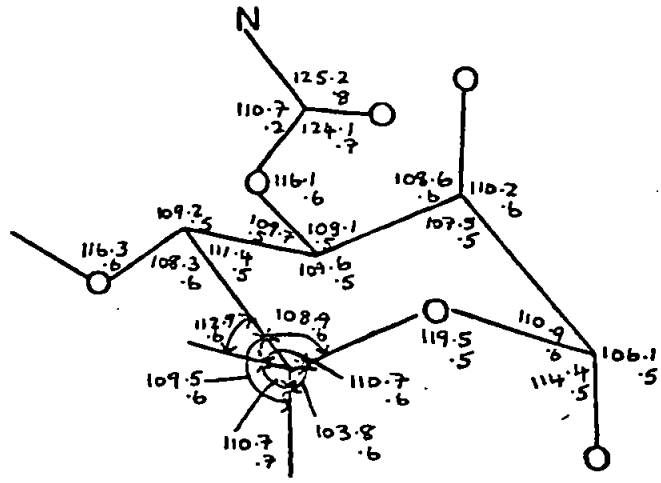
4.5. iii <u>The Noviose Moiety, ring (C)</u>

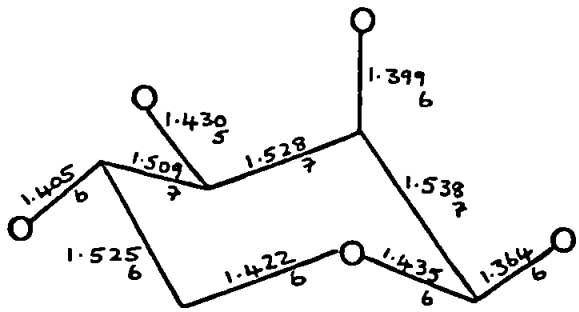
The bond lengths and angles in this moiety are shown in detail in Figure 4.9. They may be compared with the values obtained for β - lyxose by Hordvik [124] which are shown in the same figure for comparison. β - lyxose appears to be the sugar most closely related to noviose for which crystal data is available. Both noviose and β - lyxose exhibit the 1C conformation differing only in the orientation of the oxygen attached to the anomeric carbon. The bond lengths are comparable except for the C(8) - O(6) bond of 1.413(8) Å in noviose which is much longer than the corresponding bond in β - lyxose of 1.364(6) Å. This may be due to the different orientation for the glycosidic oxygen, O(6), in novobiocin affecting the hybridisation at C(8). In addition the p orbitals of C(6) are constrained to interact with the coumarin system to form a π-bond, giving a short O(6) - C(11) bond of 1.385(8) Å. The bond angles found for the two sugars are similar except for the pyranose angle, C(9) - O(5) - C(8), of 119.5(0.5)° found in novobiocin which is several degrees larger than the value of ~ 113° found in other pyranose sugars [124,125,126,127]. A value of 120° was reported for α - rhamnose monohydrate [128] but the accuracy of the data is not high [124].

The noviose ring exists in the chair form which is to be expected for a six membered sugar ring by analogy with cyclohexane.



a) Novobiocin





b) β-lyxose

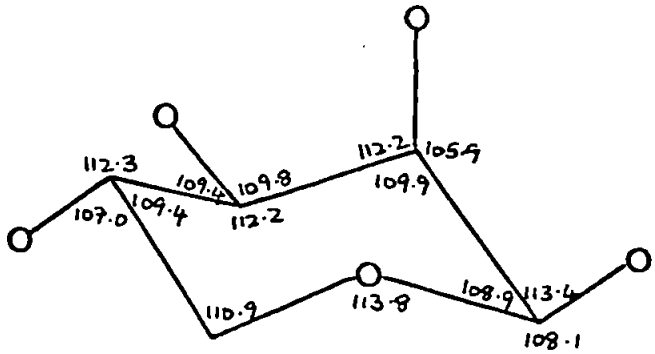


Figure 4.9 Comparison of interatomic distances and interbond angles for the sugar rings in novobiocin (a) and β-lyxose (b)

The torsion angles around the noviose ring are given in Table XXVII.

Table XXVII

Torsion Angles around the Noviose Ring

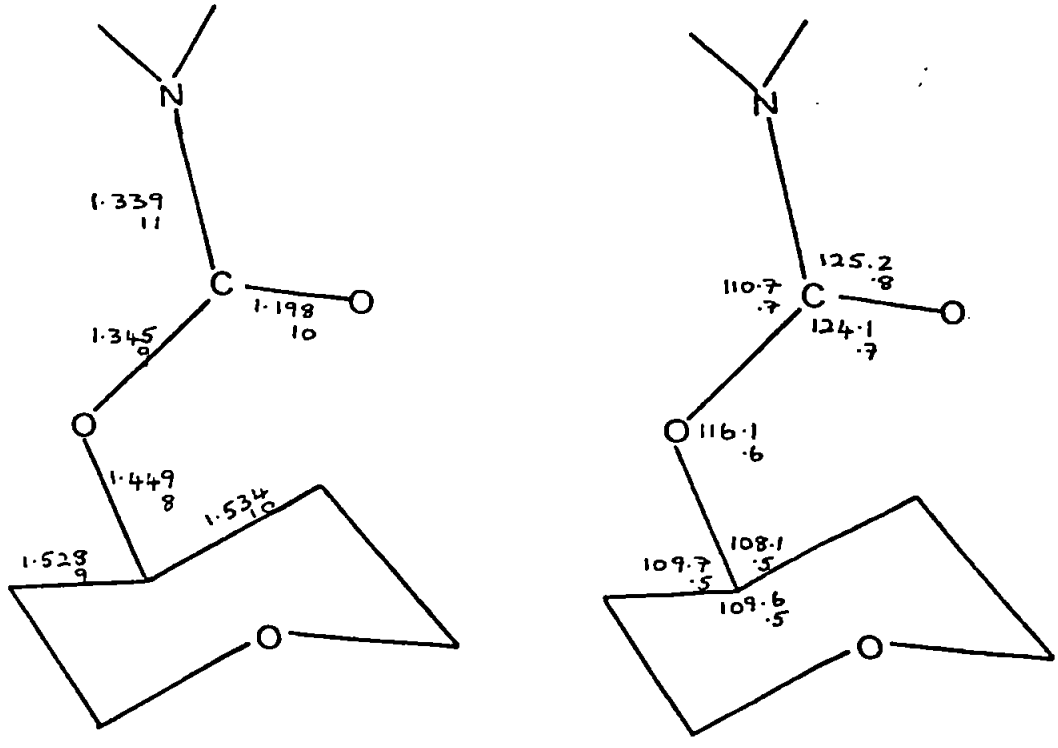
C(8) - O(5) - C(9) - C(5)	- 50.4°
O(5) - C(9) - C(5) - C(6)	+ 49.9°
C(9) - C(5) - C(6) - C(7)	- 58.2°
C(5) - C(6) - C(7) - C(8)	+ 59.7°
C(6) - C(7) - C(8) - O(5)	- 56.6°
C(7) - C(8) - O(5) - C(9)	+ 55.0°

The most unusual substituent of the noviose sugar is the rarely occurring carbamate group attached to C(6). The bond lengths and angles in this side chain are shown in context in Figure 4.10. The bond lengths and angles found for ethyl carbamate (urethane) by Bracher and Small [129] are also shown in this figure for comparison. The carbamate group has very similar geometry in the two compounds. Details of the least squares plane through the atoms of the carbamate group in novobiocin are given in Table XXVIII.

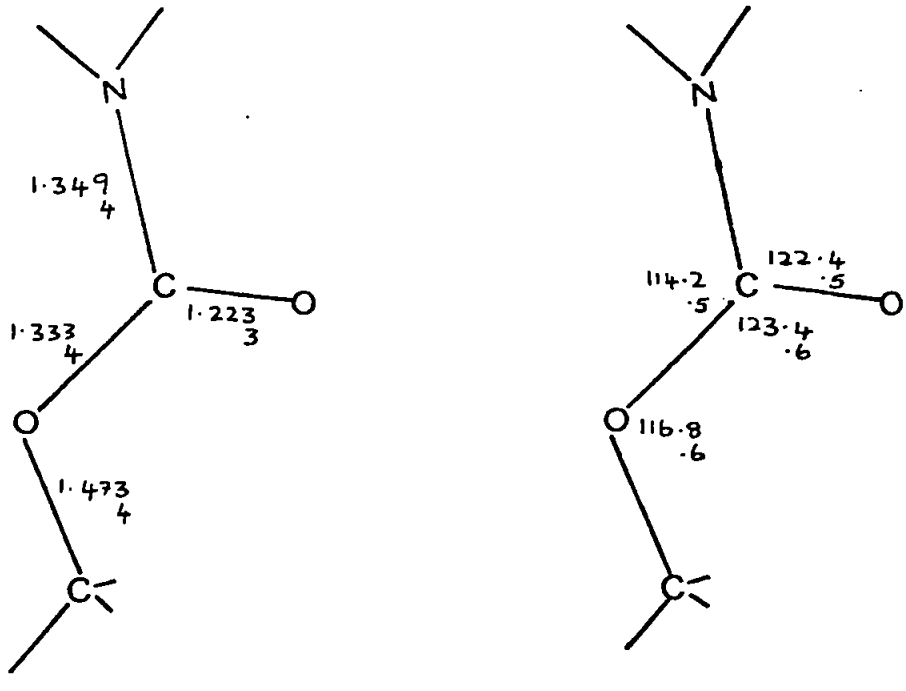
Table XXVIII

Least Squares plane for the Carbamate Group

Direction cosines of the plane	0.00404	0.32312	0.94635	
Origin to plane distance		21.3291 Å		
	atom	deviation Å	atom	deviation Å
Atoms defining the plane	N(1)	0.011	O(3)	-0.017
	O(2)	-0.003	C(6)	0.013
	C(2)	-0.004		
Atom to plane distances	C(5)	1.364	C(7)	-1.105



a) Novobiocin



b) Ethyl carbamate

Figure 4.10 Comparison of interatomic distances and interbond angles for the carbamate group in novobiocin (a) and ethyl carbamate (b)

The plane of this group is well defined which allows delocalisation of electrons into the C(2) - O(3) bond by bringing the p orbitals of O(3) into a favourable position for a π-bond to form. The interplanar angle between the plane of the carbamate group and the coumarin plane is 32.7°.

4.6 Hydrogen Bonding

4.6. i Intramolecular

The hydrogen atom attached to O(7) is in a good position to form an intramolecular hydrogen bond with O(10). The bond is illustrated in Figure 4.11 and gives an angle of 15.9(4.7)° between the O(7) - H(O7) bond and the direction of the hydrogen bond defined by O(7) - O(10).

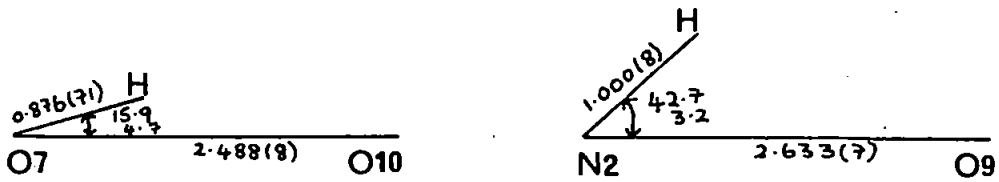


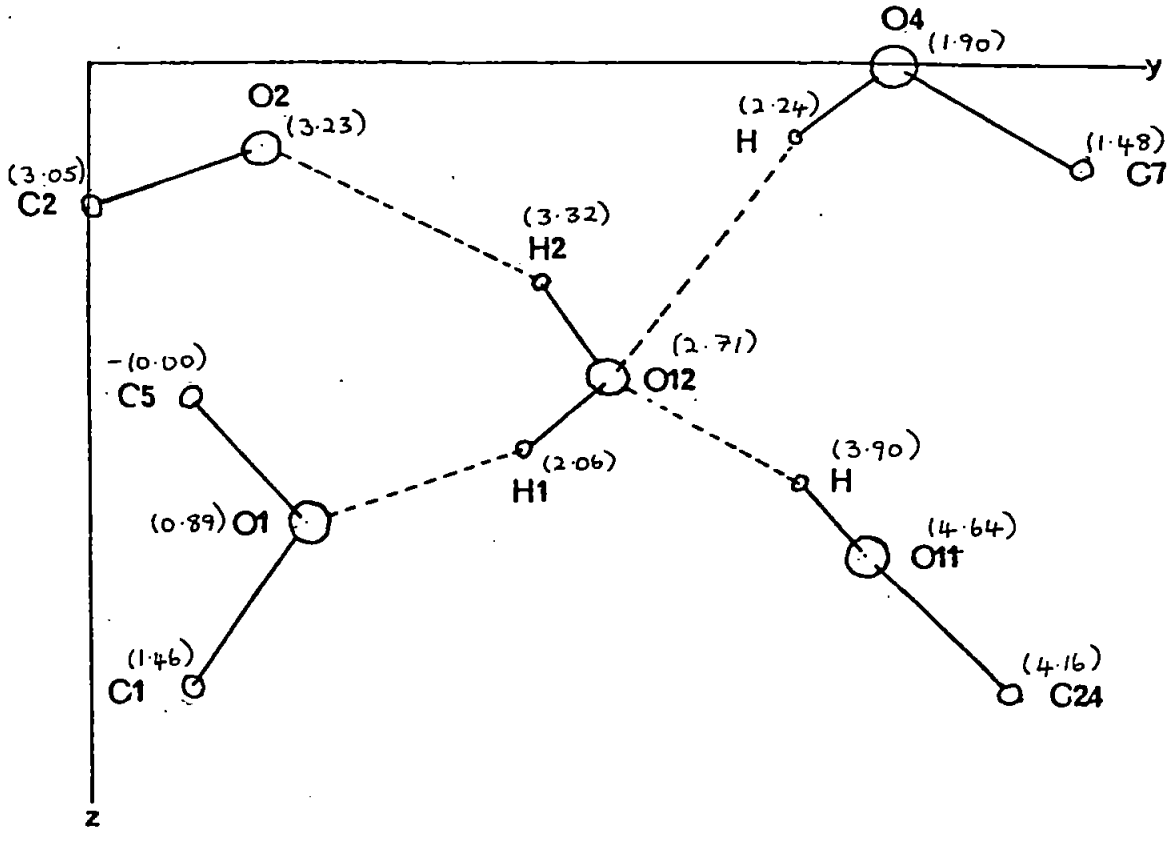
Figure 4.11 Intramolecular hydrogen bonds

The distance of 2.488(8) Å between O(7) and O(10) is comparable to the intramolecular hydrogen bond in maleic acid which has an oxygen-oxygen distance of 2.46(5) Å [130]. The distance of 2.633(7) Å between N(2) and O(9) is comparable to that of 2.57(5) Å for the intramolecular N - H .... O bond in nitroguanidine [131]. The angle between the N(2) - H(N2) bond and the N(2) - O(9) direction is 42.7(3.2)°, which is greater than expected for a hydrogen bond [132] (Figure 4.11).

4.6. ii Intermolecular

As mentioned in section 3.7 the main intermolecular hydrogen bonding in the structure is associated with the water molecule. The atomic positions found for the four hydrogens involved confirm the donor-acceptor pattern given in Table XI. The overall geometry is that of a distorted tetrahedron as shown in Figure 4.12. The water molecule donates its two hydrogen atoms to the oxygens O(1) and O(2) on one novobiocin molecule. O(2) is the carbonyl oxygen of the carbamate group while O(1) is the ether oxygen of the - OMe side chain attached to the carbon C(5) of the noviose ring. Hydrogen bonds to ether oxygens are not common but the distances involved are strong evidence in favour of such a bond. One of the angles between the oxygen-hydrogen bonds and the directions of the two hydrogen bonds is somewhat large [132], see Figure 4.13. However the angle O(1) - O(12) - O(2) of 74.4(0.2)^{o} will prevent the hydrogen bonds to O(1) and O(2) from being linear.

The other two hydrogens involved in the hydrogen bonding of the water molecule are donated from the aliphatic hydroxyl group O(4), on C(7) of the noviose ring and from the phenolic hydroxyl, O(11), on C(24) of the benzoic acid moiety. These two bonds hold the opposite ends of two novobiocin molecules together and join these to a third molecule via the hydrogen bonding. The hydrogen bonds from O(4) and O(11) are very linear as shown in Figure 4.13.



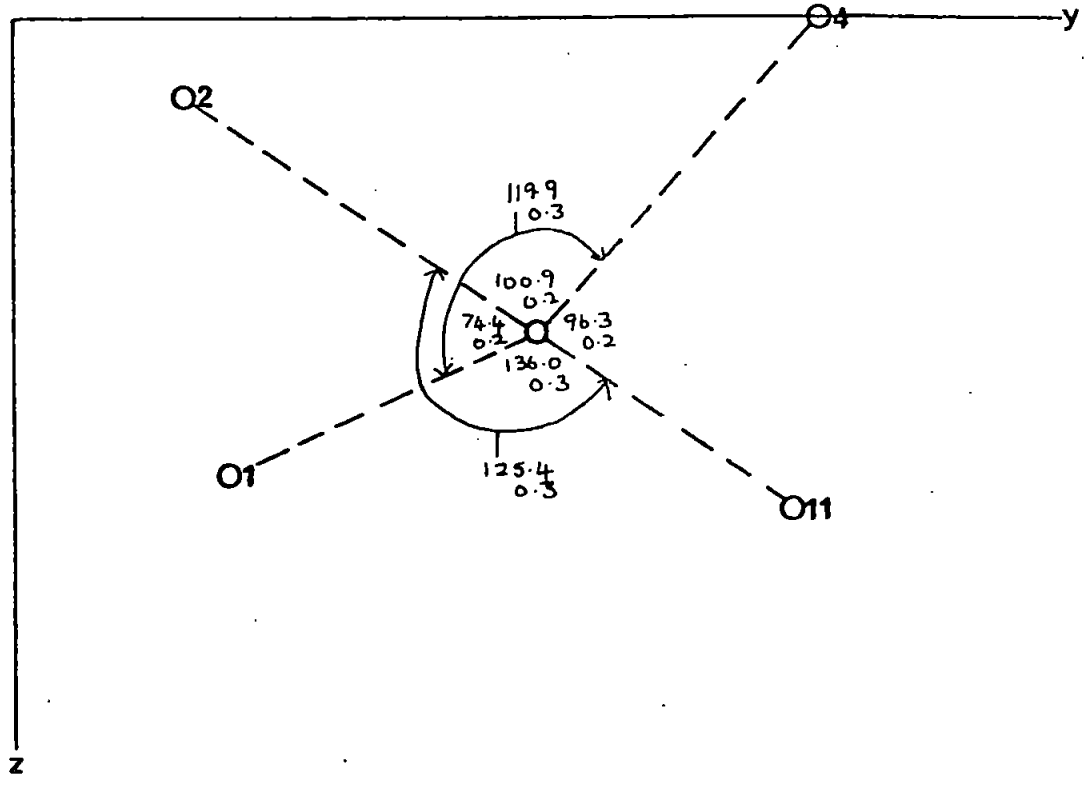


Figure 4.12 Overall geometry of the hydrogen bonding involving the water molecule projected down the a axis , relative heights in parentheses (Å)

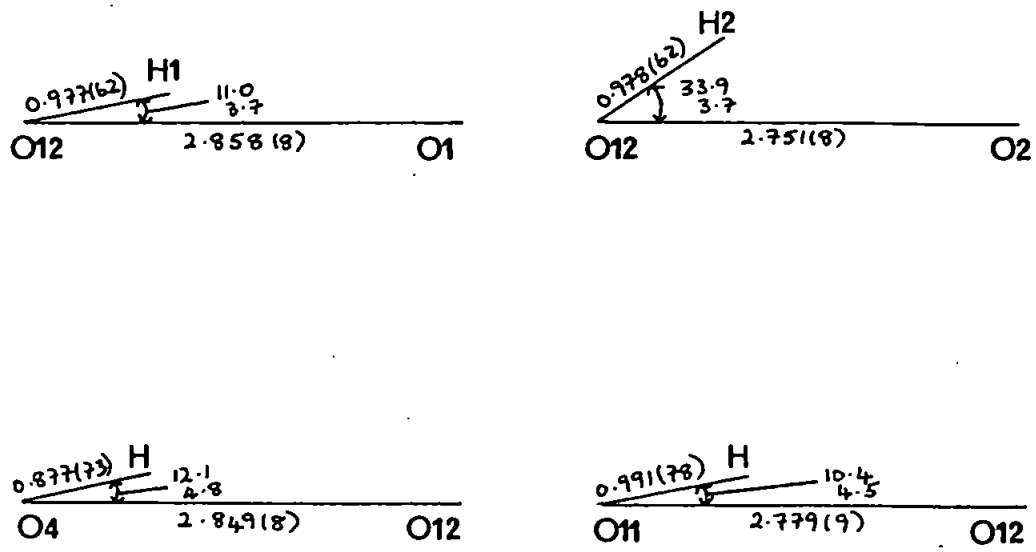


Figure 4.13 Intermolecular hydrogen bonds involving the water molecule

The distances between the oxygen atoms involved in these bonds are consistent with values expected for intermolecular oxygen-water hydrogen bonds [132].

The other intermolecular hydrogen bond between N(1) and O(5) on adjacent molecules in the x direction is confirmed. The geometry of the bond is shown in Figure 4.14.

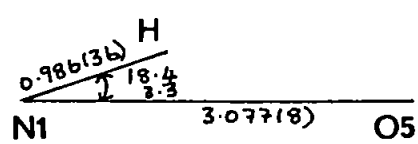


Figure 4.14 The intermolecular hydrogen bond N(1)-H ... O(5)

The angle of 18.4(3.3)^{o} between N(1) - H2(N1) and the direction of the hydrogen bond is acceptable. The N(1) - O(5) distance of 3.077(8) Å compares well with the value of 2.93(10) Å for N - H ... O in amides [133]. The acceptor atom, O(5), is a saturated ring oxygen which is again somewhat unusual.

A summary of donor-acceptor distances is given in Table XXIX.

Table XXIX

Distances and their standard deviations (Å) between donor and acceptor atoms involved in hydrogen bonding (SHELX refinement)

Donor	Acceptor	Type of H-bond	Distance
O(4)	O(12)	ROH .... H_2O	2.849(8)
O(11)	O(12)	ArOH .... H_2O	2.779(9)
O(12)	O(1)	H_2O .... -O-	2.858(8)
O(12)	O(2)	H_2O .... RC=O	2.751(8)
O(7)	O(10)	ArOH .... RC=O	2.488(8)
N(2)	O(9)	NH .... ArC=O	2.633(7)
N(1)	O(5)	NH_2 .... [O]	3.077(8)

4.7 Molecular Packing in the Crystal

Novobiocin molecules related by the 2_1 axes in the x direction lie back to back at the Van der Waals distance with the ring systems overlapping to a considerable extent. The interplanar angle between the coumarin ring of one molecule and the phenyl ring of the related one is quite small, 8.2^{o}. The other pair of molecules similarly related are approximately at right angles to the first pair. The least squares planes of the noviose rings from one pair are very approximately parallel to the extended planar areas of the other pair.

The remaining space in the structure is filled by the isobutenyl

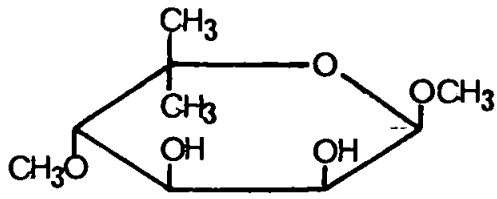
side chains, which point towards each other on either side of the 2_1 axes parallel to the x direction, and the water molecules with their associated hydrogen bonding (see Figure 3.6).

The molecular packing is therefore compact except for the hydrophobic isobutenyl groups which take one of two alternative configurations.

4.8 Stereochemistry of the Noviose Ring

The determined crystal structure shows quite clearly that the noviose moiety of novobiocin exists in the αlC configuration (see Figure 4.9). However the absolute configuration has not been investigated in this study and it has therefore been assumed that the assignment of the - L - configuration to novobiocin by Walton et al [30] is correct. The observed conformation confirms the predictions of Golding and Rickards [35] from their nmr studies on novobiocin and related compounds (see Section 1.2).

In 1975 Achmatowicz et al [134] published the results of a stereo-selective synthesis of methyl-β-DL-novioside (15) from a substituted furfuryl alcohol.

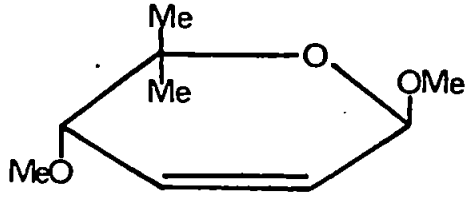


15

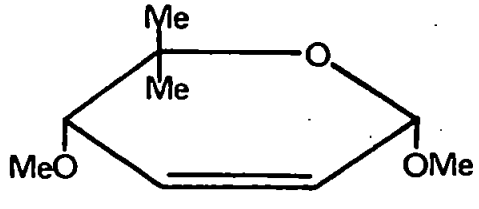
They compared methyl-β-DL-novioside with natural methyl noviosides by ^{1}H nmr, thin layer and gas-liquid chromatography and discovered that the synthetic methyl-β-DL-novioside had the same relative configuration as natural-L-methyl novioside which had previously been assigned α-configuration [31]. This led Achmatowicz to conclude that the glycoside link of novobiocin should have β-L-configuration.

Golding has recently commented [135] that his own interpretations of the ^{1}H nmr's of methyl noviosides [35] are supported by the mass spectra obtained from the two anomers. (Figures 4.15 and 4.16.) The α-anomer gives a small molecular ion and a large M - 31 (- OMe) fragment whereas the β-anomer gives a relatively large molecular ion and a small M - 31 fragment. The assignment of configuration is in agreement with the generalisation that the more sterically hindered of a pair of epimers gives the less intense molecular ion and the more intense fragment, from a process which relieves steric congestion.

However, Golding also concludes that the arguments made by Achmatowicz are difficult to fault and that the latter's nmr analysis of two crucial intermediates involved in the synthesis (16) and (17)



16



17

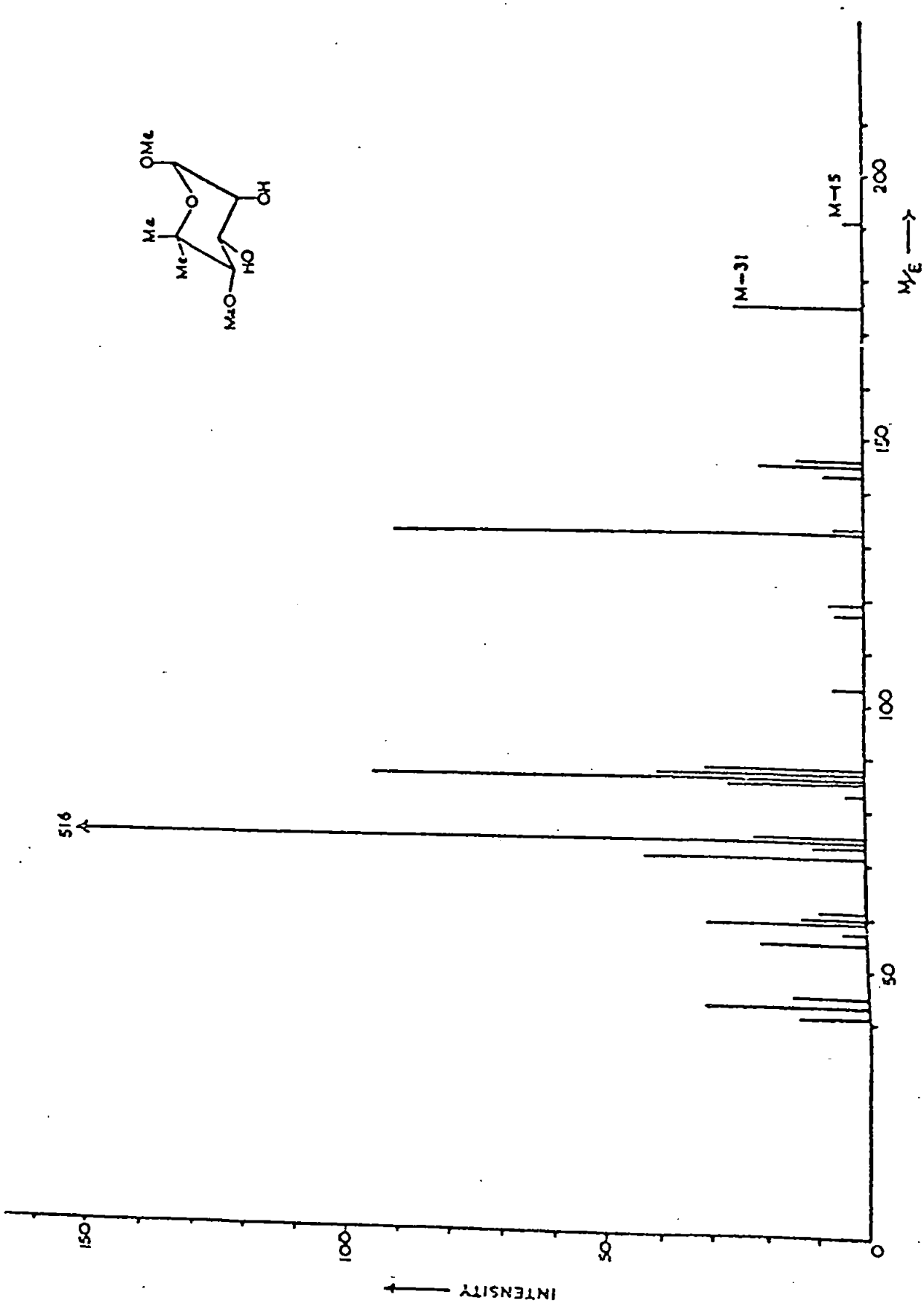


Figure 4.15 Mass spectrum for methyl-α-novioside

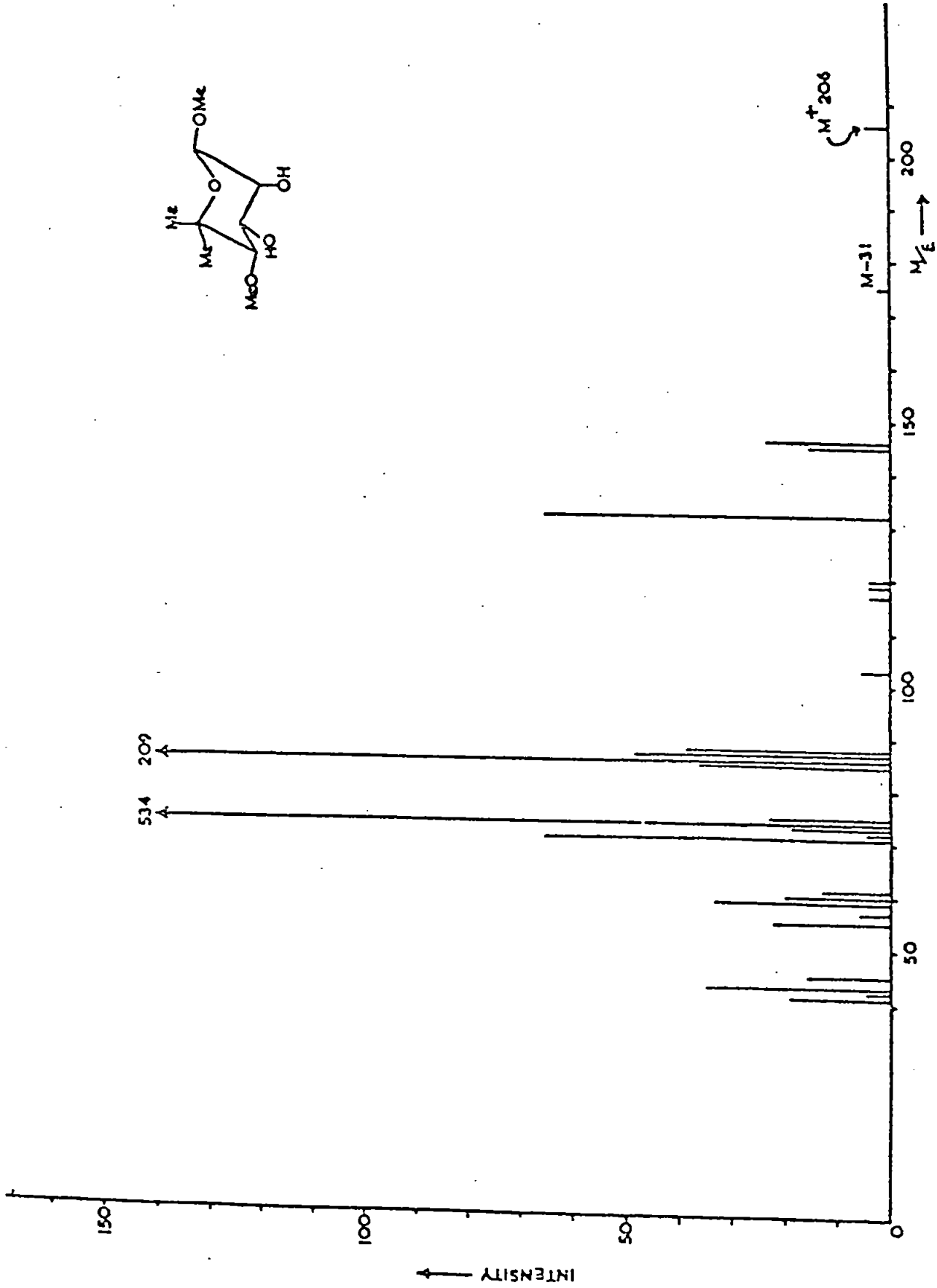


Figure 4.16 Mass spectrum for methyl-β-novioside

can only be incorrect if the conformations of these two molecules are other than perfect half-chairs as he assumes.

The best way to resolve this dilemma would seem to be a study by X-ray crystallography of methyl α or β-novioside or a suitable derivative.

4.9 Conformation of the Aromatic Ring Systems

It was mentioned in Section 1.4 that Brand and Toribara had concluded that the conformation of novobiocin in solution changes with pH [13]. Molecular models of novobiocin made with Corey-Pauling-Koltun space filling models suggest that only with extreme steric crowding can the two aromatic systems lie on the same plane. It was also postulated that the amide bond would be nonplanar [13]. In a neutral aqueous solution the observed UV and circular dichroic spectra suggested that the two aromatic systems would be perpendicular to each other. On changing to an acid pH the observed spectral changes were interpreted as a conformational change bringing the ring systems into partial conjugation with each other.

The determined crystal structure illustrates that in the solid crystal the two ring systems are partially conjugated to each other with an interplanar angle of 16.3^{o}. The amide bond is planar and almost coplanar with the benzene ring. The bond angles in this part of the molecule (Figure 4.17) show that the predicted steric crowding is relieved by a distortion of the bond angles in this region e.g.:

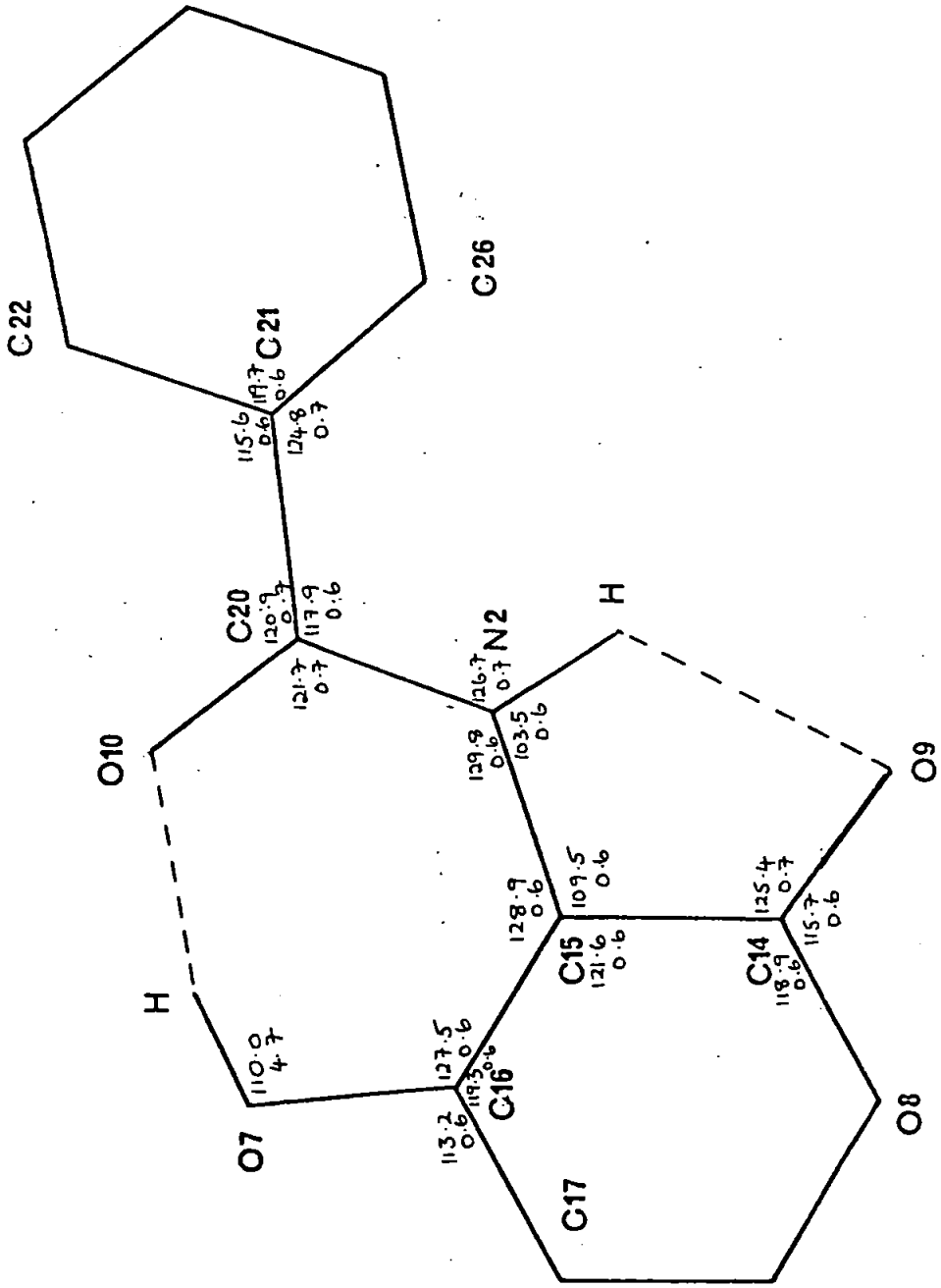


Figure 4.17 The distorted angles of the peptide bond shown projected in the direction of the arrow in figure 3.6

O(7)	-	C(16)	-	C(15)	127.5 (0.6)
C(16)	-	C(15)	-	N(2)	128.9 (0.6)
C(15)	-	N(2)	-	C(20)	129.8 (0.6)

which should be close to 120°. The molecule is held in this position by the hydrogen bond O(7) - H .... O(10), and the possible hydrogen bond between N(2) and O(9).

The crystals used in the diffractometer study were grown from a neutral medium. If the conformation of novobiocin in solution at neutral pH has the aromatic systems perpendicular to each other, then a rotation into the same plane must occur on crystallisation to facilitate packing of the molecules in the crystal.

4.10 Conclusions

It was mentioned in section 1.7 that the anti-bacterial activity of novobiocin is very dependent on the presence and position of the carbamyl group in ring (C). Brock suggested [69] that the carbamyl group might interact with an enolic or other electron donating part of the molecule but the large distance of ~ 4.5 Å between N(1) and the enolic oxygen O(7) eliminates an intramolecular interaction of this kind (see section 3.7). The carbamyl group, however, is heavily involved in the intermolecular hydrogen bonding which stabilises the crystal structure. O(2) is hydrogen bonded to the water molecule and N(1) is linked to the O(5) of the sugar ring of the novobiocin molecule in the next unit cell in the x direction. (see Figure 3.7) It is possible therefore that this is the reason for the importance of this group in conferring antibacterial activity to the antibiotic.

The stereochemistry of the noviose moiety in novobiocin has been the subject of at least three detailed investigations by n.m.r. [34,35,134] (see sections 1.2 and 4.7). Three different conformations for this moiety were predicted from these n.m.r. studies. The determined crystal structure illustrates that the noviose sugar ring has the α-1C conformation which has resolved the dilemma and shown that the predictions of Golding and Rickards [35] were correct.

The amide bond is definitely planarand keeps the coumarin system and the benzoic acid moiety in partial conjugation with each other despite the severe steric crowding and distorted bond angles that result from this arrangement (see sections 1.4 and 4.9).

It is hoped that the determined crystal structure will help in the understanding of the antibacterial action of novobiocin.

APPENDIX A

A.1 WPLOT Program

```
C       DIMENSION FCARB (15), FNITR (15), FOXYG (15), FHYDR (15),
        DIMENSION NUM (10), SIGMA (10), SUMF (10), SCALE (11),
        READ (2,110) SCALE.
110     FORMAT (11(F6.2,1X))
        DO 26 I = 1,10
        NUM (I) = 0
        SUMF (I) = 0.0
26      SIGMA (I) = 0.0
        W = 1.5418
        XC = 124.0
        XN = 8.0
        XO = 44.0
        XH = 144.0
        READ (2,103) FCARB, FNITR, FOXYG, FHYDR
103     FORMAT (4(15F4.2,/,))
        SS = 0.05
        DO 30 I = 1,10
        S = SQRT (SS)
        SPIN = S/W
        N = 1
        S = 0.0
31      SPAM = SPIN - S
        IF (SPAM) 32,33,34
34      S = S + 0.05
        N = N + 1
        GO TO 31
33      FC = FCARB (N)
        FN = FNITR (N)
        FO = FOXYG (N)
        FH = FHYDR (N)
        GO TO 49
32      FC = - 20 * SPAM * FCARB(N-1)-FCARB(N)) + FCARB(N)
        FN = - 20 * SPAM * (FNITR (N-1)-FNITR(N)) + FNITR(N)
        FO = - 20 * SPAM * (FOXYG(N-1)-FOXYG(N)) + FOXYG(N)
```

```
      FH = - 20 * SPAM * (FHYDR(N-1)-FHYDR(N)) + FHYDR(N)
49    SS = SS + 0.1
30    SUMF (I) = (XC * FC * FC + XN*FN*FN* + XO*FO*FO + XH*FH*FH)
61    READ (2,101)I,J,K.L,SIN,CORR,CORTO,ELP,ELPA,RELI
101   FORMAT (I5,I3,I3,I3,F9,4,F7.2,F8.3,F8.2)
      RELI = RELI/SCALE (K + 1)
      RELI = (RELI * CORTO)
      IF (I - 4000) 62,62,63
62    XNUM = 1
      IF (J) 1,1,2
1     XNUM = XNUM*0.5
2     IF (K) 3,3,4
3     XNUM = XNUM*0.5
4     IF (L) 5,5,6
5     XNUM = XNUM*0.5
6     RELI = RELI* XNUM
      SIN = SIN*SIN
      Z = 0.1
      DO 80 I = 1,10
      IF (SIN - Z) 81,81,80
80    Z = Z + 0.1
81    NRANG = I
60    NUM (NRANG) = NUM (NRANG) + 1
      SIGMA (NRANG) = SIGMA(NRANG) + RELI
      GO TO 61
63    DO 40 I = 1,10
      SIGMA (I) = SIGMA (I)/NUM (I)
      SIGMA (I) = SIGMA (I)/SUMF (I)
40    WRITE (3,104)NUM (I), SIGMA (I)
104   FORMAT (I9, F9.6)
      CALL EXIT
      END
```

A.2. SIGMA 2 Program

```
C       PROGRAM TO COMPUTE SIGMA-2 RELATIONSHIPS.
        DIMENSION INI (2000), MTOT (500)
        NET = 99
        NUM = 0
        N = 1
1       READ (2,101) I, J, K, L, E
        WRITE (3,101) I, J, K, L, E
101     FORMAT (I5, 3I3, F8.2)
        IF (I - 4000) 2,2,3
2       NUM = NUM + 1
        INI (N) = J
        INI (N + 1) = K
        INI (N + 2) = L
        INI (N + 3) = IFIX (100.0*E)
        N = N + 4
        GO TO 1
3       MAX = N
        DO 30 I = 1, NUM
30      MTOT (I) = 0
        N = 1
        M = 1
        IO = 5
15      J = INI(N)
        K = INI (N + 1)
        L = INI (N + 2)
14      JPLUS = J + INI(M)
        KPLUS = K + INI(M + 1)
        LPLUS = L + INI(M + 2)
        JMINU = IABS (J - INI(M))
        KMINU = IABS (K - INI(M + 1))
        LMINU = IABS (L - INI(M + 2))
        IOP = 0
7       IF (INI(IO) - JPLUS) 27,9,27
9       IF (INI(IO + 1) - KPLUS) 27,10,27
```

```
10      IF (INI(IO + 2) - LPLUS) 27,11,27
8       IO = IO + 4
        IOP = 0
        IF(MAX - IO) 12,12,7
12      M + M + 4
        IO = M + 4
        IF (MAX - M) 13,13,14
13      N = N + 4
        M = N
        IO = N + 4
        IF (MAX - N) 16,16,15
11      A = 0.25*(N - 1) + 2.01
        NA = IFIX (A)
        A = 0.25* (M - 1) + 2.01
        MA = IFIX (A)
        A = 0.25*(IO-1) + 2.01
        IOA = IFIX (A)
        IF (NET - IOA) 114,27,114
114     NET = OA
        WRITE (3,102)NA, MA, IOA
102     FORMAT (3I5)
        MTOT (NA) = MTOT(NA) + 1
        NTOT (MA) = MTOT(MA) + 1
        MTOT (IOA) = MTOT(IOA) + 1
27      IOP = IOP + 1
        IF (IOP - 1) 31,31,32
32      IF (IOP - 2) 33,33,34
34      IF (IOP - 3) 35,35,56
36      IF (IOP - 4) 37,37,38
38      IF (IOP - 5) 39,39,40
40      IF (IOP - 6) 41,41,42
42      IF (IOP - 7) 43,43,8
31      IF (INI(IO) - JPLUS) 27,50,27
50      IF (INI(IO + 1) - KPLUS) 27,51,27
```

```
51    IF (INI (IO + 2) - LMINU) 27,11,27
33    IF (INI (IO) - JPLUS) 27,44, 27
44    IF (INI (IO + 1) - KMINU( 27,45,27
45    IF (INI (IO + 2) - LMINU) 27,11,27
35    IF (INI (IO) - JPLUS) 27,46,27
46    IF (INI (IO + 1) - KMINU) 27,47,27
47    IF (INI (IO + 2) - LPLUS) 27,11,27
37    IF (INI (IO) - JMINU) 27,48,27
48    IF (INI (IO + 1) - KMINU) 27,49,27
49    IF (INI (IO + 2) - LPLUS) 27,11,27
39    IF (INI (IO) - JMINU) 27,60,27
60    IF (INI (IO + 1) - KMINU) 27,61,27
61    IF (INI (IO + 2) - LMINU) 27,11,27
41    IF (INI (IO) - JMINU) 27,62,27
62    IF (INI (IO + 1) - KPLUS) 27,63,27
63    IF (INI (IO + 2) - LPLUS) 27,11,27
43    IF (INI (IO) - JMINU) 27,64,27
64    IF (INI (IO + 1) - KPLUS) 27,65,27
65    IF (INI (IO + 2) - LMINU) 27,11,27
16    DO 82 I = 1, NUM
      N = I + 1
82    WRITE (3, 103) N, MTOT (N)
103   FORMAT (2I6)
      CALL EXIT
      END
```

A.3 EDENS Program

```
C       ELECTRON DENSITY PROGRAM P2₁2₁2₁
        DIMENSION NK(218),NL(218),NH(12),NUMH(12),NPAR(219),A(218),B(218)
        DIMENSION AZ(218),BZ(218),NAR(218),PUD(21),SPUD(21),FUD(21)
        DIMENSION COSRA(33,41),SINRA(33,41),GRIDR(25,21)
        DIMENSION NGRID(21),SFUD(21),GRID(25,21),COSRX(11,25),SINRX(11,25)
        DEFINE FILE 1(1025,42,U,NRFC).
        LAYER = 1
        DO 200 I = 1, 11
        DO 201 J = 1, 25
        FI = I - 1
        FJ = J - 1
        COSRX(I,J) = COS(0.2618*FI*FJ)
        SINRX(I,J) = SIN(0.2618*FI*FJ)
201     CONTINUE
200     CONTINUE
        DO 30 I = 1,33
        DO 31 J = 1,41
        FI = I-1
        FJ = J-1
        COSRA(I,J) = COS(0.0785375*FI*FJ)
        SINRA(I,J) = SIN(0.0785375*FI*FJ)
31      CONTINUE
30      CONTINUE
313     DO 110 I = 1,21
        NGRID(I) = 0
        FUD(I) = 0
        SFUD(I) = 0
        PUD(I) = 0
        SPUD(I) = 0
110     CONTINUE
        DO 111 I = 1,218
        AZ (I) = 0
        BZ (I) = 0
        NAR (I) = 0
```

```
      NK (I) = 0
      NL (I) = 0
      NPAR (I) = 0
      A (I) = 0
      B (I) = 0
111   CONTINUE
      DO 230 I = 1,25
      DO 231 J = 1,21
      GRID (I,J) = 0
      GRIDR (I,J) = 0
231   CONTINUE
230   CONTINUE
      DO 112 I = 1,12
      NH(I) = 0
      NUMH(I) = 0
112   CONTINUE
      I = 1
      IJ = 1
      NINI = 999
      TONE = 0
      TTWO = 0
18    READ (2,19) NIB,NTWO,NK(IJ),NL(IJ),E,MANGL,WEIT
19    FORMAT (15,313,F8,2,16,F8.2)
      WRITE(3,19)NIB,NTWO,NK(IJ),NL(IJ),E,MANGL,WEIT
      E = 0.01*E
      ANGL = MANGL
      JKSUM = NTWO + NK(IJ)
      KLSUM = NK(IJ) + NL(IJ)
8     IF(JKSUM) 4,5,6
5     NPAR(IJ) = 1
      GO TO 7
4     NPAR(IJ) = 3
      GO TO 7
6     JKSUM = JKSUM-2
      GO TO 8
7     IF(KLSUM)9,10,11
```

```
9       NPAR(IJ) = NPAR(IJ) + 1
        GO TO 10
11      KLSUM = KLSUM-2
        GO TO 7
10      IF(NPAR(IJ)-2)170,171,172
170     NAR(IJ) = 1
        GO TO 180
171     NAR(IJ) =-1
        GO TO 180
172     IF(NPAR(IJ)-3)171,171,170
180     CONTINUE
        NK(IJ)=IFIX(2*NK(IJ)+1.1)
        NL(IJ)=NL(IJ)+1
        IF(NIB-4000)20,20,305
20      IF(NTWO-NINI)1,2,1
1       NH(I)=NTWO
        IF(IJ-1)21,22,21
21      NUMH(I-1)=NUM
22      NINI=NTWO
        NUM=1
        I=I+1
        GO TO 3
2       NUM=NUM+1
3       IF(NTWO)12,13,12
13      E+0.5*F
12      IF(NK(IJ)-1)14,15,14
15      E=0.5*E
14      IF(NL(IJ)-1)16,17,16
17      E=0.5*E
16      AA=0.0174533*ANGL
        A(IJ)=E*COS(AA)*WEIT
        B(IJ)=E*SIN(AA)*WEIT
        IJ=IJ+1
        IF(IJ-217)18,18,24
305     LAYER=LAYER+950
24      NUMH(I-1)=NUM
```

```
      NHLAS = NH(I-1)+1
      NK(IJ)=0
      NL(IJ)=0
      IJ=IJ-1
      NUMI=I-1
      DO 47 NZ=1,41
      DO 32 I=1,IJ
      NY=NL(I)
      IF(NPAR(I)-2)141,142,150
150   IF(NPAR(I)-3)144,143,144
141   AZ(I) = A(I)*COSRA(NY,NZ)
      BZ(I)=-B(I)*SINRA(NY,NZ)
      GO TO 32
142   AZ(I)=-A(I)*SINRA(NY,NZ)
      BZ(I)=B(I)*COSRA(NY,NZ)
      GO TO 32
143   AZ(I)=B(I)*COSRA(NY,NZ)
      BZ(I)=-A(I)*SINRA(NY,NZ)
      GO TO 32
144   AZ(I)=B(I)*SINRA(NY,NZ)
      BZ(I)=-A(I)*COSRA(NY,NZ)
32    CONTINUE
      DO 33 NY = 1,21
      I = 1
      NHONE = NH(1) + 1
      IJPIJ=1
      DO 35 JK=NHONE,NHLAS
      NS=NUMH(IJPIJ)
      IJPIJ=IJPIJ+1
      DO 38 KL = 1,NS
      NT=NK(I)
      IF(NAR(I))160,160,161
160   PUD(NT)=PUD(NT)+AZ(I)
      SPUD(NT)=SPUD(NT)+BZ(I)
      GO TO 36
161   FUD(NT)=FUD(NT)+AZ(I)
```

```
      SFUD(NT)=SFUD(NT)+BZ(I)
36    I=I+1
38    CONTINUE
      DO 164 IOU = 1,21,2
      TONE=TONE+PUD(IOU)*SINRA(IOU,NY)+FUD(IOU)*COSRA(IOU,NY)
      TTWO=TTWO+SPUD(IOU)*COSRA(IOU,NY)+SFUD(IOU)*SINRA(IOU,NY)
164   CONTINUE
      DO 203 NXX=1,25
      GRID(NXX,NY)=GRID(NXX,NY)+(COSRX(JK,NXX)*TONE)+(SINRX(JK,NXX)+TTWO
     *)
203   CONTINUE
      TONE = 0
      TTWO = 0
      DO 190 IOU=1,21,2
      PUD(IOU)=0
      FUD(IOU)=0
      SPUD(IOU)=0
      SFUD(IOU)=0
190   CONTINUE
35    CONTINUE
33    CONTINUE
      IF(LAYER)300,300,301
301   DO 302 IJK=1,25
      ABC=IJK-1
      IXX=IFIX(41*ABC+NZ+0.1)
      READ(1'IXX)(GRIDR(IJK,JAX),JAX=1,21)
      DO 303 JAX=1,21
      GRIDR(IJK,JAX)=GRIDR(IJK,JAX)+GRID(IJK,JAX)
303   CONTINUE
302   CONTINUE
      GO TO 352
300   DO 350 I=1,25
      DO 351 J=1,21
      GRIDR (I,J)=GRID(I,J)
351   CONTINUE
```

```
350   CONTINUE
352   DO 204 IJK=1,25
      ABC=IJK-1
      IXX=IFIX(41*ABC+NZ+0.1)
      WRITE (1'IXX)(GRIDR(IJK,JAX),JAX=1,21
204   CONTINUE
      DO 206 NI=1,25
      DO 207 NPJ=1,21
      GRID(NI,NPJ)=0
207   CONTINUE
206   CONTINUE
47    CONTINUE
      LAYER=LAYER+1
      IF(LAYER-900)318,318,304
304   DO 210 NX=1,25
      NTH=NX-1
      WRITE (3,55)
55    FORMAT('1',/)
      WRITE (3,107)NTH
107   FORMAT (IX' SECTION X = ',13)
      ABC=NX-1
      DO 216 IJK=1,24
      IXX=IFIX(41*ABC+IJK+0.1)
      READ (1'IXX)(GRIDR(IJK,JAX),JAX=1,21)
216   CONTINUE
      DO 217 IP=1,24
      DO 218 IPT=1,21
      NGRID(IPT)=IFIX(GRIDR(IP,IPT)+0.5)
218   CONTINUE
      WRITE (3,101)NGRID
101   FORMAT ('0',21I4/)
217   CONTINUE
      DO 219 IJK=1,17
      IXX=IFIX(41*ABC+IJK+24.1)
      READ(1'IXX)(GRIDR(IJK,JAX),JAX=1,21)
219   CONTINUE
```

```
      DO 220 IP=1,17
      DO 221 IPT=1,21
      NGRID(IPT)=IFIX(GRIDR(IP,IPT)+0.5)
221   CONTINUE
      WRITE (3,101)NGRID
220   CONTINUE
210   CONTINUE
52    CALL EXIT
      END
```

APPENDIX B

The first six cycles of the phase expansion pathway are shown in detail in this appendix. The sum of angles formula (2.42) was

$$\langle \phi_{\underline{h}} \rangle = \phi_{\underline{h}'} + \phi_{\underline{h}-\underline{h}'} \qquad (2.42)$$

used to estimate the phases of reflections, $\phi_{\underline{h}}$, from 'known' phases $\phi_{\underline{h}'}$ and $\phi_{\underline{h}-\underline{h}'}$. After each cycle phases estimated consistently were added to the 'known' phases and a new set of unknown phases estimated. The initial set of phases was the starting set given in Table VI.

For this space group reflections with one or two indices zero have restricted phase values therefore an estimate which gave such a phase an impossible value was easily detected. Phases estimated by conflicting Σ_2 relationships were not used to develop phases in the next cycle.

The speed of phase propagation and the small number of conflicting phase estimates indicated that the starting set was a good base for phase determination.

Each entry in the phase expansion pathway is of the form:

$$\left.\begin{array}{l} N_1\ (h_1\ k_1\ l_1) = \phi_{\underline{h}'} \\ N_2\ (h_2\ k_2\ l_2) = \phi_{\underline{h}-\underline{h}'} \end{array}\right\}\ N_3\ (h_3\ k_3\ l_3) = \phi_{\underline{h}}$$

where N is the code number of a reflection and (hkl) denotes the phase of the hkl reflection.

List of |E| ≥ 1.80 for NOVOBIOCIN

N	h	k	l	E	N	h	k	l	E	N	h	k	l	E	N	h	k	l	E
1	0	3	5	4.12	34	0	8	15	2.32	67	6	5	12	1.97	100	5	10	0	1.83
2	0	3	6	3.83	35	3	2	9	2.29	68	1	1	13	1.96	101	0	3	23	1.82
3	0	2	5	3.37	36	7	1	8	2.28	69	7	0	15	1.94	102	3	2	10	1.81
4	4	0	25	3.35	37	5	1	17	2.27	70	1	6	18	1.94	103	0	4	24	1.81
5	6	10	1	3.35	38	10	2	0	2.27	71	3	7	19	1.94	104	0	9	13	1.81
6	2	0	30	3.26	39	2	1	29	2.26	72	1	3	8	1.92	105	6	10	10	1.81
7	1	9	13	3.25	40	6	5	0	2.26	73	3	4	25	1.92	106	0	1	10	1.80
8	0	9	16	3.03	41	0	5	11	2.25	74	8	1	1	1.90	107	0	2	15	1.80
9	5	7	7	2.89	42	7	4	18	2.23	75	0	2	22	1.90	108	7	2	6	1.80
10	7	1	13	2.88	43	2	0	29	2.19	76	5	4	13	1.90					
11	1	9	16	2.77	44	4	0	4	2.18	77	0	5	16	1.90					
12	7	1	12	2.74	45	0	7	25	2.17	78	5	9	12	1.90					
13	5	4	12	2.73	46	1	0	29	2.16	79	2	10	9	1.89					
14	6	1	12	2.65	47	6	4	9	2.16	80	4	4	4	1.88					
15	7	1	11	2.61	48	1	7	18	2.15	81	6	4	13	1.88					
16	6	0	17	2.59	49	2	7	17	2.14	82	0	5	20	1.88					
17	4	0	22	2.58	50	0	8	17	2.13	83	3	6	21	1.88					
18	7	2	8	2.58	51	0	4	6	2.08	84	1	7	11	1.88					
19	0	6	10	2.58	52	8	7	8	2.08	85	5	8	6	1.88					
20	6	8	6	2.58	53	6	0	11	2.07	86	5	1	19	1.87					
21	0	4	19	2.56	54	7	4	3	2.07	87	5	7	9	1.87					
22	0	6	11	2.54	55	4	1	21	2.05	88	7	0	5	1.86					
23	6	7	5	2.53	56	5	2	23	2.03	89	7	2	15	1.86					
24	7	2	7	2.49	57	8	2	0	2.03	90	2	8	14	1.86					
25	0	0	22	2.48	58	8	2	1	2.03	91	6	9	1	1.86					
26	0	5	10	2.46	59	1	7	20	2.02	92	6	10	3	1.86					
27	5	2	18	2.44	60	1	6	9	2.01	93	5	0	18	1.84					
28	0	8	18	2.44	61	6	1	17	2.00	94	6	0	12	1.84					
29	7	1	9	2.43	62	2	0	10	1.99	95	5	3	22	1.84					
30	1	9	14	2.43	63	3	9	17	1.99	96	6	4	10	1.84					
31	0	10	9	2.37	64	6	8	1	1.98	97	5	2	21	1.83					
32	6	6	4	2.33	65	3	0	18	1.97	98	4	4	0	1.83					
33	5	10	1	2.33	66	6	1	10	1.97	99	6	7	0	1.83					

Starting set phases underlined

Cycle 1

$$\left.\begin{array}{l} 2\ (0\ 3\ 6) = -\frac{\pi}{2} \\ 19\ (0\ 5\ 10) = \pi \end{array}\right\} 8\ (0\ 9\ 16) = \frac{\pi}{2}$$

$$\left.\begin{array}{l} 2\ (0\ 3\ 6) = -\frac{\pi}{2} \\ 40\ (6\ 5\ 0) = 0 \end{array}\right\} 20\ (6\ 8\ 6) = -\frac{\pi}{2}$$

$$\left.\begin{array}{l} 53\ (6\ 0\ 11) = -\frac{\pi}{2} \\ 53\ (\overline{6}\ 0\ 11) = \frac{\pi}{2} \end{array}\right\} 25\ (0\ 0\ 22) = 0$$

$$\left.\begin{array}{l} 40\ (6\ 5\ 0) = 0 \\ 53\ (\overline{6}\ 0\ 11) = \frac{\pi}{2} \end{array}\right\} 41\ (0\ 5\ 11) = \frac{\pi}{2}$$

$$\left.\begin{array}{l} 19\ (0\ 6\ 10) = \pi \\ 40\ (6\ \overline{5}\ 0) = \pi \end{array}\right\} 66\ (6\ 1\ 10) = 0$$

$$\left.\begin{array}{l} 46\ (\overline{1}\ 0\ 29) = -\frac{\pi}{2} \\ 53\ (6\ 0\ \overline{11}) = -\frac{\pi}{2} \end{array}\right\} 93\ (5\ 0\ 18) = \pi$$

Cycle 2 Code numbers of estimated phases 8, 20, 25, 41, 66,93 from cycle 1

A) checks

$$\left.\begin{array}{l} 8\ (0\ 9\ 16) = \frac{\pi}{2} \\ 20\ (6\ \overline{8}\ \overline{6}) = -\frac{\pi}{2} \end{array}\right\} 66\ (6\ 1\ 10) = 0$$

$$\left.\begin{array}{l} 41\ (0\ 5\ 11) = \frac{\pi}{2} \\ 41\ (0\ \overline{5}\ 11) = -\frac{\pi}{2} \end{array}\right\} 25\ (0\ 0\ 22) = 0$$

B) new estimates

$$\left.\begin{array}{l} 2\ (0\ \overline{3}\ \overline{6}) = \frac{\pi}{2} \\ 41\ (0\ 5\ 11) = \frac{\pi}{2} \end{array}\right\} 3\ (0\ 2\ 5) = \pi$$

$$\left.\begin{array}{l} 8\ (0\ 9\ \overline{16}) = -\frac{\pi}{2} \\ 46\ (1\ 0\ 29) = -\frac{\pi}{2} \end{array}\right\} 7\ (1\ 9\ 13) = \pi$$

$$\left.\begin{array}{l} 25\ (0\ 0\ 22) = 0 \\ 66\ (6\ 1\ \overline{10}) = 0 \end{array}\right\} 14\ (6\ 1\ 12) = 0$$

$$\left.\begin{array}{l} \left.\begin{array}{l} 2\ (0\ 3\ 6) = -\frac{\pi}{2} \\ 41\ (0\ 5\ 11) = \frac{\pi}{2} \end{array}\right\} = 0 \\ \left.\begin{array}{l} 20\ (6\ 8\ 6) = -\frac{\pi}{2} \\ 53\ (\overline{6}\ 0\ 11) = \frac{\pi}{2} \end{array}\right\} = 0 \end{array}\right\} 50\ (0\ 8\ 17) = 0$$

$$\left.\begin{array}{l} 2\ (0\ \overline{3}\ 6) = -\frac{\pi}{2} \\ 20\ (6\ 8\ 6) = -\frac{\pi}{2} \end{array}\right\} 67\ (6\ 5\ 12) = \pi$$

$$\left.\begin{array}{l} 19\ (0\ 6\ 10) = \pi \\ 66\ (6\ 1\ \overline{10}) = 0 \end{array}\right\} 99\ (6\ 7\ 0) = \pi$$

$$\left.\begin{array}{l} 46\ (\overline{1}\ 0\ 29) = -\frac{\pi}{2} \\ 66\ (6\ 1\ \overline{10}) = 0 \end{array}\right\} 86\ (5\ 1\ 19) = -\frac{\pi}{2}$$

Cycle 3 Code numbers of estimated phases 3, 7, 14, 50, 67, 86, 99 from cycle 2

A) Checks

$$\left.\begin{array}{l} 7\ (\overline{1}\ 9\ 13) = \pi \\ 20\ (6\ \overline{8}\ 6) = \frac{\pi}{2} \end{array}\right\} 86\ (5\ 1\ 19) = -\frac{\pi}{2}$$

B) New estimates

$$\left.\begin{array}{l} \left.\begin{array}{l} \left.\begin{array}{l} 3\ (0\ 2\ \overline{5}) = 0 \\ 20\ (6\ 8\ 6) = -\frac{\pi}{2} \end{array}\right\} = -\frac{\pi}{2} \\ 41\ (0\ 5\ \overline{11}) = \frac{\pi}{2} \end{array}\right\} 5\ (6\ 10\ 1) = -\frac{\pi}{2} \\ 67\ (6\ 5\ 12) = \pi \end{array}\right. \qquad \left.\begin{array}{l} \left.\begin{array}{l} 3\ (0\ 2\ 5) = \pi \\ 40\ (6\ 5\ 0) = 0 \end{array}\right\} = \pi \\ 14\ (6\ \overline{1}\ \overline{12}) = \pi \end{array}\right\} 23\ (6\ 7\ 5) = \pi \\ 50\ (0\ 8\ 17) = 0 \end{array}$$

$$\left.\begin{array}{l} 7\ (\overline{1}\ 9\ 13) = \pi \\ 14\ (6\ 1\ \overline{12}) = 0 \end{array}\right\} 33\ (5\ 10\ 1) = \pi \qquad \left.\begin{array}{l} 3\ (0\ 2\ 5) = \pi \\ 19\ (0\ 6\ 10) = \pi \end{array}\right\} 34\ (0\ 8\ 15) = 0$$

$$\left.\begin{array}{l} 14\ (6\ 1\ \overline{12}) = 0 \\ 46\ (\overline{1}\ 0\ 29) = -\frac{\pi}{2} \end{array}\right\} 37\ (5\ 1\ 17) = -\frac{\pi}{2} \qquad \left.\begin{array}{l} 86\ (5\ 1\ 19) = -\frac{\pi}{2} \\ 86\ (5\ 1\ \overline{19}) = \frac{\pi}{2} \end{array}\right\} 38\ (10\ 2\ 0) = 0$$

$$\left.\begin{array}{l} \left.\begin{array}{l} \left.\begin{array}{l} 3\ (0\ \overline{2}\ 5) = 0 \\ 7\ (1\ 9\ 13) = \pi \end{array}\right\} = \pi \\ 93\ (\overline{5}\ 0\ 18) = 0 \end{array}\right\} 48\ (1\ 7\ 18) = \pi \\ 99\ (6\ 7\ 0) = \pi \end{array}\right. \qquad \left.\begin{array}{l} 3\ (0\ 2\ 5) = \pi \\ 93\ (5\ 0\ 18) = \pi \end{array}\right\} 56\ (5\ 2\ 23) = 0$$

$$\left.\begin{array}{l} 14\ (6\ 1\ 12) = 0 \\ 66\ (\overline{6}\ 1\ 10) = \pi \end{array}\right\} 75\ (0\ 2\ 22) = \pi \qquad \left.\begin{array}{l} \left.\begin{array}{l} 3\ (0\ 2\ 5) = \pi \\ 14\ (6\ \overline{1}\ 12) = \pi \end{array}\right\} 0 \\ \left.\begin{array}{l} 50\ (0\ 8\ 17) = 0 \\ 99\ (6\ \overline{7}\ 0) = 0 \end{array}\right\} 0 \end{array}\right\} 61\ (6\ 1\ 17) = 0$$

$2\ (0\ 3\ \overline{6}) = \frac{\pi}{2}$

$86\ (5\ 1\ 19) = -\frac{\pi}{2}$ } $= 0$

$7\ (\overline{1}\ 9\ 13) = \pi$

$40\ (6\ \overline{5}\ 0) = \pi$ } $= 0$

} $76\ (5\ 4\ 13) = 0$

$19\ (0\ 6\ \overline{10}) = \pi$

$86\ (5\ 1\ 19) = -\frac{\pi}{2}$ } $87\ (5\ 7\ 9) = \frac{\pi}{2}$

$14\ (\overline{6}\ \overline{1}\ 12) = 0$

$67\ (6\ 5\ 12) = \pi$ } $103\ (0\ 4\ 24) = \pi$

<u>Cycle 4</u> Code numbers of estimated 5, 23, 33, 34, 37, 38, 48, 56, 61, 75, 76, 87, 103 phases from cycle 3

<u>Checks</u>

$2\ (0\ \overline{3}\ 6) = -\frac{\pi}{2}$

$5\ (6\ 10\ \overline{1}) = -\frac{\pi}{2}$ } $23\ (6\ 7\ 5) = \pi$

$2\ (0\ 3\ 6) = -\frac{\pi}{2}$

$61\ (5\ \overline{1}\ 17) = \frac{\pi}{2}$ } $56\ (5\ 2\ 23) = 0$

$5\ (6\ 10\ 1) = -\frac{\pi}{2}$

$8\ (0\ \overline{9}\ 16) = \frac{\pi}{2}$ } $61\ (6\ 1\ 17) = 0$

$8\ (0\ \overline{9}\ 16) = \frac{\pi}{2}$

$33\ (5\ 10\ 1) = \pi$ } $61\ (5\ 1\ 17) = -\frac{\pi}{2}$

$19\ (0\ 6\ \overline{10}) = \pi$

$56\ (5\ \overline{2}\ 23) = \pi$ } $76\ (5\ 4\ 13) = 0$

$23\ (6\ \overline{7}\ \overline{5}) = 0$

$34\ (0\ 8\ 15) = 0$ } $66\ (6\ 1\ 10) = 0$

B) <u>new estimates</u>

$2\ (0\ \overline{3}\ 6) = -\frac{\pi}{2}$
$33\ (5\ 10\ 1) = \pi$ } $= \pi$

$2\ (0\ 3\ \overline{6}) = \frac{\pi}{2}$
$76\ (5\ 4\ 13) = 0$ } $= \frac{\pi}{2}$

$8\ (0\ 9\ \overline{16}) = -\frac{\pi}{2}$
$56\ (5\ \overline{2}\ 23) = \pi$ } $= \frac{\pi}{2}$

$19\ (0\ 6\ \overline{10}) = \pi$
$37\ (5\ 1\ 17) = -\frac{\pi}{2}$ } $= \frac{\pi}{2}$

$48\ (\overline{1}\ 7\ 18) = \pi$
$53\ (6\ 0\ \overline{11}) = -\frac{\pi}{2}$ } $= \frac{\pi}{2}$

) $9\ (5\ 7\ 7) = \frac{\pi}{2}$

$3\ (0\ \overline{2}\ 5) = 0$
$76\ (5\ 4\ 13) = 0$ } 0

$7\ (\overline{1}\ 9\ 13) = \pi$
$23\ (6\ \overline{7}\ 5) = \pi$ } 0

$33\ (5\ 10\ 1) = \pi$
$50\ (0\ \overline{8}\ 17) = \pi$ } 0

$38\ (10\ 2\ 0) = 0$
$93\ (\overline{5}\ 0\ 18) = 0$ } 0

$40\ (6\ \overline{5}\ 0) = \pi$
$48\ (\overline{1}\ 7\ 18) = \pi$ } 0

) $27\ (5\ 2\ 18) = 0$

$5\ (6\ 10\ \overline{1}) = -\frac{\pi}{2}$
$37\ (\overline{5}\ \overline{1}\ 17) = -\frac{\pi}{2}$ } π

$33\ (\overline{5}\ 10\ 1) = 0$
$61\ (6\ \overline{1}\ 17) = 0$ } 0

) $11\ (1\ 9\ 16) = ?$ (undetermined)

$7\ (1\ 9\ 13) = \pi$
$23\ (6\ \overline{7}\ \overline{5}) = 0$ } $18\ (7\ 2\ 8) = \pi$

$5\ (6\ 10\ \overline{1}) = -\frac{\pi}{2}$
$19\ (0\ \overline{6}\ 10) = \pi$ } $47\ (6\ 4\ 9) = \frac{\pi}{2}$

$53\ (6\ 0\ 11) = -\frac{\pi}{2}$
$87\ (\overline{5}\ 7\ 9) = -\frac{\pi}{2}$ } $59\ (1\ 7\ 20) = \pi$

$5\ (6\ 10\ \overline{1}) = -\frac{\pi}{2}$
$7\ (1\ \overline{9}\ 13) = \pi$ } $= \frac{\pi}{2}$

$20\ (6\ 8\ \overline{6}) = \frac{\pi}{2}$
$48\ (1\ \overline{7}\ 18) = 0$ } $= \frac{\pi}{2}$

$46\ (1\ 0\ 29) = -\frac{\pi}{2}$
$61\ (6\ 1\ \overline{17}) = \pi$ } $\frac{\pi}{2}$

) $12\ (7\ 1\ 12) = \frac{\pi}{2}$

$56\ (\overline{5}\ 2\ 23) = \pi$
$66\ (6\ \overline{1}\ \overline{10}) = \pi$ } 0

$40\ (6\ 5\ 0) = 0$
$76\ (\overline{5}\ \overline{4}\ 13) = 0$ } 0

) $68\ (1\ 1\ 13) = 0$

$61\ (5\ 1\ \overline{17}) = \frac{\pi}{2}$
$48\ (1\ 7\ 18) = \pi$ } $= -\frac{\pi}{2}$

$8\ (0\ 9\ \overline{16}) = -\frac{\pi}{2}$
$61\ (6\ \overline{1}\ 17) = 0$ } $= -\frac{\pi}{2}$

$48\ (1\ 7\ \overline{18}) = 0$
$86\ (5\ 1\ 19) = -\frac{\pi}{2}$ } $= -\frac{\pi}{2}$

) $64\ (6\ 8\ 1) = -\frac{\pi}{2}$

$53\ (6\ 0\ \overline{11}) = -\frac{\pi}{2}$
$103\ (0\ 4\ 24) = \pi$ } $81\ (6\ 4\ 13) = \frac{\pi}{2}$

$3\ (0\ \overline{2}\ 5) = 0$
$33\ (5\ 10\ 1) = \pi$ } $= \pi$

$14\ (6\ 1\ \overline{12}) = 0$
$48\ (\overline{1}\ 7\ 18) = \pi$ } $= \pi$

) $85\ (5\ 8\ 6) = \pi$

$3\ (0\ 2\ 5) = \pi$
$37\ (5\ 1\ 17) = -\frac{\pi}{2}$ } $95\ (5\ 3\ 22) = \frac{\pi}{2}$

$23\ (6\ 7\ \overline{5}) = 0$
$76\ (\overline{5}\ \overline{4}\ 13) = 0$ } $= 0$

$5\ (6\ 10\ \overline{1}) = -\frac{\pi}{2}$
$87\ (\overline{5}\ \overline{7}\ 9) = \frac{\pi}{2}$ } $= 0$

) $72\ (1\ 3\ 8) = 0$

$2\ (0\ 3\ \overline{6}) = \frac{\pi}{2}$
$75\ (0\ 2\ 22) = \pi$ } $77\ (0\ 5\ 16) = -\frac{\pi}{2}$

$20\ (6\ 8\ \overline{6}) = \frac{\pi}{2}$
$37\ (\overline{5}\ \overline{1}\ 17) = -\frac{\pi}{2}$ } $= 0$

$56\ (\overline{5}\ 2\ 23) = \pi$
$67\ (6\ 5\ \overline{12}) = \pi$ } 0

) $84\ (1\ 7\ 11) = 0$

$5\ (6\ 10\ 1) = -\frac{\pi}{2}$
$14\ (\overline{6}\ \overline{1}\ 12) = 0$ } $= \frac{\pi}{2}$

$41\ (0\ 5\ \overline{11}) = \frac{\pi}{2}$
$103\ (0\ 4\ 24) = \pi$ } $-\frac{\pi}{2}$

) $104\ (0\ 9\ 13) = -\frac{\pi}{2}$

<u>Cycle 5</u> Code numbers of estimated phases 9, 12, 18, 27, 47, 59, 64, 68, 72, 77, 81, 84, 85, 95, 104 from cycle 4

<u>Checks</u>

$9\ (5\ 7\ \overline{7}) = -\frac{\pi}{2}$
$27\ (\overline{5}\ \overline{2}\ 18) = \pi$ } $41\ (0\ 5\ 11) = +\frac{\pi}{2}$

$3\ (0\ 2\ \overline{5}) = 0$
$68\ (1\ 1\ 13) = 0$ } $72\ (1\ 3\ 8) = 0$

$5\ (6\ 10\ 1) = -\frac{\pi}{2}$
$9\ (\overline{5}\ \overline{7}\ \overline{7}) = \frac{\pi}{2}$ } $72\ (1\ 3\ 8) = 0$

$7\ (\overline{1}\ 9\ 13) = \pi$
$12\ (7\ \overline{1}\ \overline{12}) = \frac{\pi}{2}$ } $64\ (6\ 8\ 1) = -\frac{\pi}{2}$

$$\left.\begin{array}{l} 9\ (\bar{5}\ \bar{7}\ 7) = \frac{\pi}{2} \\ 20\ (6\ 8\ 6) = -\frac{\pi}{2} \end{array}\right\} 68\ (1\ 1\ 13) = 0$$

$$\left.\begin{array}{l} 12\ (7\ \bar{1}\ 12) = \frac{\pi}{2} \\ 64\ (\bar{6}\ 8\ \bar{1}) = \frac{\pi}{2} \end{array}\right\} 84\ (1\ 7\ 11) = 0 \quad *$$

$$\left.\begin{array}{l} 14\ (\bar{6}\ 1\ 12) = \pi \\ 64\ (6\ 8\ 1) = -\frac{\pi}{2} \end{array}\right\} 104\ (0\ 9\ 13) = -\frac{\pi}{2} \quad *$$

$$\left.\begin{array}{l} 18\ (7\ \bar{2}\ 8) = \pi \\ 40\ (\bar{6}\ 5\ 0) = \pi \end{array}\right\} 72\ (1\ 3\ 8) = 0$$

$$\left.\begin{array}{l} 18\ (7\ \bar{2}\ \bar{8}) = 0 \\ 59\ (\bar{1}\ 7\ 20) = \pi \end{array}\right\} 67\ (6\ 5\ 12) = \pi$$

$$\left.\begin{array}{l} 25\ (0\ 0\ 22) = 0 \\ 47\ (6\ 4\ \bar{9}) = \frac{\pi}{2} \end{array}\right\} 81\ (6\ 4\ 13) = \frac{\pi}{2}$$

$$\left.\begin{array}{l} 9\ (5\ 7\ \bar{7}) = -\frac{\pi}{2} \\ 56\ (\bar{5}\ \bar{2}\ 23) = 0 \end{array}\right\} 77\ (0\ 5\ 16) = -\frac{\pi}{2}$$

$$\left.\begin{array}{l} 27\ (\bar{5}\ 2\ 18) = \pi \\ 66\ (6\ 1\ \overline{10}) = 0 \end{array}\right\} 72\ (1\ 3\ 8) = 0 \quad *$$

$$\left.\begin{array}{l} 37\ (\bar{5}\ \bar{1}\ 17) = -\frac{\pi}{2} \\ 47\ (6\ 4\ \bar{9}) = \frac{\pi}{2} \end{array}\right\} 72\ (1\ 3\ 8) = 0$$

$$\left.\begin{array}{l} 40\ (\bar{6}\ 5\ 0) = \pi \\ 81\ (6\ 4\ 13) = \frac{\pi}{2} \end{array}\right\} 104\ (0\ 9\ 13) = -\frac{\pi}{2}$$

$$\left.\begin{array}{l} 47\ (6\ 4\ 9) = \frac{\pi}{2} \\ 68\ (\bar{1}\ \bar{1}\ 13) = 0 \end{array}\right\} 95\ (5\ 3\ 22) = \frac{\pi}{2}$$

$$\left.\begin{array}{l} 59\ (\bar{1}\ \bar{7}\ 20) = 0 \\ 64\ (6\ 8\ \bar{1}) = -\frac{\pi}{2} \end{array}\right\} 86\ (5\ 1\ 19) = -\frac{\pi}{2}$$

$$\left.\begin{array}{l} 61\ (6\ 1\ 17) = 0 \\ 84\ (\bar{1}\ 7\ \overline{11}) = 0 \end{array}\right\} 85\ (5\ 8\ 6) = \pi \quad *$$

$$\left.\begin{array}{l} 77\ (0\ \bar{5}\ 16) = -\frac{\pi}{2} \\ 85\ (5\ 8\ 6) = \pi \end{array}\right\} 95\ (5\ 3\ 22) = \frac{\pi}{2}$$

$$\left.\begin{array}{l} 84\ (1\ 7\ 11) = 0 \\ 84\ (\bar{1}\ \bar{7}\ 11) = 0 \end{array}\right\} 25\ (0\ 0\ 22) = 0$$

$$\left.\begin{array}{l} 85\ (\bar{5}\ 8\ \bar{6}) = \pi \\ 86\ (5\ 1\ 19) = -\frac{\pi}{2} \end{array}\right\} 104\ (0\ 9\ 13) = -\frac{\pi}{2} \quad *$$

Some inconsistent Σ_2 relationships have occurred at this point. When the weighted tangent formula (equation 3.2) is used to refine phase values, reflections whose phases are not estimated consistently will have small EC values (equation 3.4) and therefore a small weight, w_h, (equation 3.3) in the iterative process. Such phases do not therefore have much influence on the estimation of new phase values.

* this indicates an inconsistent relationship

5 New estimates

$9\ (5\ 7\ 7) = \frac{\pi}{2}$
$48\ (\bar{1}\ \bar{7}\ 18) = 0$ } $4\ (4\ 0\ 25) = \frac{\pi}{2}$

$20\ (6\ 8\ 6) = -\frac{\pi}{2}$
$81\ (\bar{6}\ \bar{4}\ 13) = -\frac{\pi}{2}$ } $= \pi$

$72\ (\bar{1}\ \bar{3}\ 8) = \pi$
$84\ (1\ 7\ 11) = 0$ } $= \pi$

$76\ (\bar{5}\ \bar{4}\ 13) = 0$
$85\ (5\ 8\ 6) = \pi$ } $= \pi$

$47\ (\bar{6}\ 4\ 9) = -\frac{\pi}{2}$
$47\ (6\ 4\ 9) = \frac{\pi}{2}$ } $28\ (0\ 8\ 18) = 0$

$3\ (0\ 2\ \bar{5}) = 0$
$47\ (6\ 4\ 9) = \frac{\pi}{2}$ } $= \frac{\pi}{2}$

$3\ (0\ \bar{2}\ 5) = 0$
$64\ (6\ 8\ \bar{1}) = -\frac{\pi}{2}$ } $= -\frac{\pi}{2}$

$14\ (6\ 1\ \overline{12}) = 0$
$77\ (0\ 5\ 16) = -\frac{\pi}{2}$ } $= -\frac{\pi}{2}$

$68\ (1\ \bar{1}\ 13) = 0$
$87\ (5\ 7\ \bar{9}) = -\frac{\pi}{2}$ } $= -\frac{\pi}{2}$

$21\ (0\ 4\ 19) = \pi$

(Note one indication of $\frac{\pi}{2}$

$32\ (6\ 6\ 4) = -\frac{\pi}{2}$

$5\ (6\ 10\ \bar{1}) = -\frac{\pi}{2}$
$37\ (\bar{5}\ \bar{1}\ 17) = -\frac{\pi}{2}$ } π

$33\ (\bar{5}\ 10\ \bar{1}) = 0$
$61\ (6\ \bar{1}\ 17) = 0$ } 0

$3\ (0\ 2\ 5) = \pi$
$84\ (1\ 7\ 11) = 0$ } π

$37\ (\bar{5}\ 1\ 17) = \frac{\pi}{2}$
$64\ (6\ 8\ \bar{1}) = -\frac{\pi}{2}$ } 0

$66\ (6\ 1\ 10) = 0$
$85\ (\bar{5}\ 8\ 6) = 0$ } 0

$46\ (1\ 0\ 29) = -\frac{\pi}{2}$
$104\ (0\ 9\ \overline{13}) = -\frac{\pi}{2}$ } π

$2\ (0\ 3\ 6) = -\frac{\pi}{2}$
$12\ (7\ 1\ 12) = \frac{\pi}{2}$ } $= 0$

$18\ (7\ \bar{2}\ 8) = \pi$
$19\ (0\ 6\ 10) = \pi$ } $= 0$

$66\ (6\ 1\ 10) = 0$
$72\ (1\ 3\ 8) = 0$ } $= 0$

$42\ (7\ 4\ 18) = 0$

$11\ (1\ 9\ 16) = ?$
still undetermined

$12\ (7\ 1\ 12) = \frac{\pi}{2}$
$37\ (\bar{5}\ \bar{1}\ 17) = -\frac{\pi}{2}$ } $= 0$
$48\ (1\ 7\ 18) = \pi$
$84\ (1\ \bar{7}\ 11) = 0$ } $= \pi$
43 $(2\ 0\ 29) = ?$ undetermined

$37\ (5\ 1\ 17) = -\frac{\pi}{2}$
$68\ (\bar{1}\ \bar{1}\ \overline{13}) = 0$ } $= -\frac{\pi}{2}$
$9\ (5\ \bar{7}\ \bar{7}) = +\frac{\pi}{2}$
$84\ (\bar{1}\ 7\ 11) = 0$ } $= \frac{\pi}{2}$
44 $(4\ 0\ 4) = ?$ undetermined

$9\ (5\ 7\ 7) = \frac{\pi}{2}$
$93\ (\bar{5}\ 0\ 18) = 0$ } $= \frac{\pi}{2}$
$85\ (5\ 8\ 6) = \pi$
$86\ (\bar{5}\ \bar{1}\ 19) = -\frac{\pi}{2}$ } $= \frac{\pi}{2}$
45 $(0\ 7\ 25) = \frac{\pi}{2}$

$3\ (0\ 2\ \bar{5}) = 0$
$18\ (7\ 2\ 8) = \pi$ } $= \pi$
$23\ (6\ 7\ \bar{5}) = 0$
$72\ (1\ \bar{3}\ 8) = \pi$ } $= \pi$
54 $(7\ 4\ 3) = \pi$

$12\ (7\ 1\ \overline{12}) = \frac{\pi}{2}$
$68\ (1\ 1\ 13) = 0$ } 58 $(8\ 2\ 1) = \frac{\pi}{2}$

$18\ (7\ 2\ \bar{8}) = 0$
$27\ (\bar{5}\ \bar{2}\ 18) = \pi$ } 62 $(2\ 0\ 10) = \pi$

$18\ (7\ \bar{2}\ 8) = \pi$
$64\ (\bar{6}\ 8\ 1) = \frac{\pi}{2}$ } $= -\frac{\pi}{2}$
$27\ (\bar{5}\ 2\ 18) = \pi$
$47\ (6\ 4\ \bar{9}) = \frac{\pi}{2}$ } $= -\frac{\pi}{2}$
60 $(1\ 6\ 9) = -\frac{\pi}{2}$

$23\ (6\ \bar{7}\ \bar{5}) = 0$
$59\ (1\ 7\ 20) = \pi$ } 69 $(7\ 0\ 15) = \pi$ undetermined phase must be $\pm\frac{\pi}{2}$

$3\ (0\ 2\ 5) = \pi$
$9\ (5\ 7\ 7) = \frac{\pi}{2}$ } $= -\frac{\pi}{2}$

$19\ (0\ 6\ \overline{10}) = \pi$
$81\ (6\ 4\ 13) = +\frac{\pi}{2}$ } $= -\frac{\pi}{2}$
$66\ (6\ 1\ \overline{10}) = 0$
$104\ (0\ 9\ 13) = -\frac{\pi}{2}$ } $= -\frac{\pi}{2}$
92 $(6\ 10\ 3) = -\frac{\pi}{2}$

$5\ (6\ 10\ \bar{1}) = -\frac{\pi}{2}$
$68\ (\bar{1}\ \bar{1}\ 13) = 0$ } $= -\frac{\pi}{2}$
$19\ (0\ 6\ \overline{10}) = \pi$
$95\ (5\ 3\ 22) = \frac{\pi}{2}$ } $= -\frac{\pi}{2}$
$64\ (6\ 8\ \bar{1}) = -\frac{\pi}{2}$
$68\ (\bar{1}\ 1\ 13) = 0$ } $= -\frac{\pi}{2}$
78 $(5\ 9\ 12) = -\frac{\pi}{2}$

$19\ (0\ \bar{6}\ 10) = \pi$
$104\ (0\ 9\ 13) = -\frac{\pi}{2}$ } $= \frac{\pi}{2}$
$66\ (\bar{6}\ \bar{1}\ 10) = 0$
$81\ (6\ 4\ 13) = \frac{\pi}{2}$ } $= \frac{\pi}{2}$
101 $(0\ 3\ 23) = +\frac{\pi}{2}$

Cycle 6 Code numbers of estimated phases 4, 21, 28, 32, 42, 45, 54, 58, 60, 62, 78, 92, 101 from cycle 5

Checks

$$\left.\begin{array}{l} 4\ (4\ 0\ 25) = \frac{\pi}{2} \\ 7\ (1\ 9\ \overline{13}) = \pi \end{array}\right\} 78\ (5\ 9\ 12) = -\frac{\pi}{2}$$

$$\left.\begin{array}{l} 2\ (0\ 3\ 6) = -\frac{\pi}{2} \\ 21\ (0\ 4\ 19) = \pi \end{array}\right\} 45\ (0\ 7\ 25) = \frac{\pi}{2}$$

$$\left.\begin{array}{l} 32\ (6\ 6\ 4) = \frac{\pi}{2} \\ 38\ (10\ 2\ 0) = 0 \end{array}\right\} 80\ (4\ 4\ 4) = \frac{\pi}{2}$$

$$\left.\begin{array}{l} 32\ (6\ 6\ 4) = -\frac{\pi}{2} \\ 72\ (\overline{1}\ 3\ 8) = 0 \end{array}\right\} 78\ (5\ 9\ 12) = -\frac{\pi}{2}$$

$$\left.\begin{array}{l} 34\ (0\ 8\ \overline{15}) = \pi \\ 42\ (7\ \overline{4}\ 18) = 0 \end{array}\right\} 54\ (7\ 4\ 3) = \pi$$

$$\left.\begin{array}{l} 54\ (7\ 4\ 3) = \pi \\ 62\ (\overline{2}\ 0\ 10) = \pi \end{array}\right\} 76\ (5\ 4\ 13) = 0$$

New estimates

$$\left.\begin{array}{l} 54\ (7\ \overline{4}\ \overline{3}) = 0 \\ 77\ (0\ 5\ 16) = -\frac{\pi}{2} \end{array}\right\} 10\ (7\ 1\ 13) = -\frac{\pi}{2}$$

$$\left.\begin{array}{l} 86\ (\overline{5}\ \overline{1}\ 19) = -\frac{\pi}{2} \\ 92\ (6\ 10\ \overline{3}) = -\frac{\pi}{2} \end{array}\right\} 11\ (1\ 9\ 16) = \pi$$

(plus six estimates in previous cycle of 0, π still undetermined)

$$\left.\begin{array}{l} 60\ (\overline{1}\ \overline{6}\ 9) = \frac{\pi}{2} \\ 92\ (6\ 10\ 13 = -\frac{\pi}{2} \end{array}\right\} 13\ (5\ 4\ 12) = 0$$

$$\left.\begin{array}{l} 28\ (0\ 8\ 18) = 0 \\ 64\ (6\ \overline{8}\ \overline{1}) = -\frac{\pi}{2} \end{array}\right\} 16\ (6\ 0\ 17) = -\frac{\pi}{2}$$

$$\left.\begin{array}{l} 18\ (7\ 2\ 8) = \pi \\ 54\ (\overline{7}\ 4\ 3) = 0 \end{array}\right\} 22\ (0\ 6\ 11) = \pi$$

$$\left.\begin{array}{l} 19\ (0\ 6\ 10) = \pi \\ 54\ (7\ \overline{4}\ \overline{3}) = 0 \end{array}\right\} 24\ (7\ 2\ 7) = \pi$$

$$\left.\begin{array}{l} \left.\begin{array}{l} 2\ (0\ \overline{3}\ 6) = -\frac{\pi}{2} \\ 54\ (7\ 4\ 3) = \pi \end{array}\right\} = \frac{\pi}{2} \\ \left.\begin{array}{l} 40\ (6\ \overline{5}\ 0) = \pi \\ 60\ (1\ 6\ 9) = -\frac{\pi}{2} \end{array}\right\} = \frac{\pi}{2} \\ \left.\begin{array}{l} 58\ (8\ \overline{2}\ 1) = \frac{\pi}{2} \\ 72\ (\overline{1}\ 3\ 8) = 0 \end{array}\right\} = \frac{\pi}{2} \\ \left.\begin{array}{l} 60\ (1\ \overline{6}\ 9) = -\frac{\pi}{2} \\ 99\ (6\ 7\ 0) = \pi \end{array}\right\} = \frac{\pi}{2} \\ \left.\begin{array}{l} 62\ (2\ 0\ \overline{10}) = \pi \\ 86\ (5\ 1\ 19) = -\frac{\pi}{2} \end{array}\right\} = \frac{\pi}{2} \end{array}\right\} 29\ (7\ 1\ 9) = \frac{\pi}{2}$$

$$\left.\begin{array}{l} \left.\begin{array}{l} 92\ (6\ 10\ \overline{3}) = -\frac{\pi}{2} \\ 37\ (\overline{5}\ \overline{1}\ 17) = -\frac{\pi}{2} \end{array}\right\} = \pi \\ \left.\begin{array}{l} 60\ (1\ 6\ \overline{9}) = \frac{\pi}{2} \\ 101\ (0\ 3\ 23) = \frac{\pi}{2} \end{array}\right\} = \pi \end{array}\right\} 30\ (1\ 9\ 14) = \pi$$

$$\left.\begin{array}{l} \left.\begin{array}{l} 19\ (0\ 6\ \overline{10}) = \pi \\ 21\ (0\ 4\ 19) = \pi \end{array}\right\} = 0 \\ \left.\begin{array}{l} 32\ (\overline{6}\ 6\ \overline{4}) = -\frac{\pi}{2} \\ 81\ (6\ 4\ 13) = \frac{\pi}{2} \end{array}\right\} = 0 \end{array}\right\} 31\ (0\ 10\ 9) = 0$$

$$\left.\begin{array}{l} 59\ (1\ 7\ 20) = \pi \\ 60\ (1\ \overline{6}\ 9) = -\frac{\pi}{2} \end{array}\right\} 39\ (2\ 1\ 29) = \frac{\pi}{2}$$

$56\ (\bar{5}\ \bar{2}\ 23) = 0$
$108\ (7\ 2\ 6) = \pi$ } $43\ (2\ 0\ 29) = \pi$ impossible phase value still undetermined

$28\ (0\ 8\ \overline{18}) = 0$
$103\ (0\ \bar{4}\ 24) = \pi$ } $51\ (0\ 4\ 6) = \pi$

$46\ (\bar{1}\ 0\ 29) = -\frac{\pi}{2}$
$80\ (4\ 4\ \bar{4}) = -\frac{\pi}{2}$ } $73\ (3\ 4\ 25) = \pi$

$32\ (6\ \bar{6}\ \bar{4}) = -\frac{\pi}{2}$
$60\ (1\ 6\ 9) = -\frac{\pi}{2}$ } $88\ (7\ 0\ 5) = \pi$ impossible value phase undetermined

$28\ (0\ 8\ 18) = 0$
$61\ (6\ 1\ \overline{17}) = \pi$ } $91\ (6\ 9\ 1) = \pi$

$33\ (5\ 10\ 1) = \pi$
$60\ (1\ \bar{6}\ 9) = -\frac{\pi}{2}$ } $96\ (6\ 4\ 10) = \frac{\pi}{2}$

$80\ (\bar{4}\ 4\ 4) = -\frac{\pi}{2}$
$108\ (7\ \bar{2}\ 6) = \pi$ } $102\ (3\ 2\ 10) = \frac{\pi}{2}$

$34\ (0\ 8\ \overline{15}) = \pi$
$45\ (0\ \bar{7}\ 25) = -\frac{\pi}{2}$ } $106\ (0\ 1\ 10) = \frac{\pi}{2}$

$41\ (0\ 5\ 11) = \frac{\pi}{2}$
$54\ (7\ \bar{4}\ \bar{3}) = 0$ } $36\ (7\ 1\ 8) = \frac{\pi}{2}$

$62\ (\bar{2}\ 0\ 10) = \pi$
$87\ (5\ 7\ 9) = \frac{\pi}{2}$ } $71\ (3\ 7\ 19) = -\frac{\pi}{2}$

$3\ (0\ \bar{2}\ \bar{5}) = \pi$
$45\ (0\ 7\ 25) = \frac{\pi}{2}$ } $82\ (0\ 5\ 20) = -\frac{\pi}{2}$

$20\ (6\ 8\ 6) = -\frac{\pi}{2}$
$60\ (1\ \bar{6}\ 9) = -\frac{\pi}{2}$ } $89\ (7\ 2\ 15) = \pi$

$20\ (6\ \bar{8}\ \bar{6}) = -\frac{\pi}{2}$
$28\ (0\ 8\ 18) = 0$ } $94\ (6\ 0\ 12) = -\frac{\pi}{2}$ impossible value phase undetermined

$27\ (5\ 2\ 18) = 0$
$28\ (0\ 8\ 18) = 0$ } $= 0$

$47\ (6\ 4\ 9) = \frac{\pi}{2}$
$60\ (\bar{1}\ 6\ \bar{9}) = \frac{\pi}{2}$ } $= \pi$

} $100\ (5\ 10\ 0) = ?$ undetermined

Code numbers of estimated phases from cycle 6
10, 13, 16, 22, 24, 29, 30, 31, 36, 39, 51, 71, 73, 82, 89, 91, 96, 102, 106

At this point 80 phases have been estimated and the phase development pathway is well established. A comparison of these phase estimates with the phase values obtained from the refined structure (photographic data) is shown below. The mean error of 29.8° for these 80 phases indicates that the chosen starting set was a good starting point for tangent formula phase determination.

Comparison of estimated phase values with those from the refined structure

N	h	k	l	est.	ref.	ΔØ	N	h	k	l	est.	ref.	ΔØ
2	0	3	6	270	270	0	42	7	4	18	0	3	3
3	0	2	5	180	180	0	45	0	7	25	90	90	0
4	4	0	25	90	90	0	46	1	0	29	270	270	0
5	6	10	1	270	313	43	47	6	4	9	90	31	59
7	1	9	13	180	183	3	48	1	7	18	180	183	3
8	0	9	16	90	90	0	50	0	8	17	0	0	0
9	5	7	7	90	104	14	51	0	4	6	180	180	0
10	7	1	13	270	317	47	53	6	0	11	270	270	0
12	7	1	12	90	65	25	54	7	4	3	180	179	1
13	5	4	12	0	359	1	56	5	2	23	0	3	3
14	6	1	12	0	304	56	58	8	2	1	90	171	81
16	6	0	17	270	270	0	59	1	7	20	180	78	102
18	7	2	8	180	149	31	60	1	6	9	270	328	58
19	0	6	10	180	180	0	61	6	1	17	0	44	44
20	6	8	6	270	288	18	62	2	0	10	180	180	0
21	0	4	19	180	180	0	64	6	8	1	270	260	10
22	0	6	11	180	0	180	66	6	1	10	0	60	60
23	6	7	5	180	144	36	67	6	5	12	180	249	69
24	7	2	7	180	214	34	68	1	1	13	0	97	97
25	0	0	22	0	0	0	71	3	7	19	270	294	24
27	5	2	18	0	26	26	72	1	3	8	0	56	56
28	0	8	18	0	0	0	73	3	4	25	180	216	36
29	7	1	9	90	43	47	75	0	2	22	180	0	180
30	1	9	14	180	109	71	76	5	4	13	0	5	5
31	0	10	9	0	0	0	77	0	5	16	270	270	0
32	6	6	4	270	338	68	78	5	9	12	270	306	36
33	5	10	1	180	240	60	80	4	4	4	90	44	46
34	0	8	15	0	0	0	81	6	4	13	90	102	12
36	7	1	8	90	89	1	82	0	5	20	270	270	0
37	5	1	17	270	247	23	84	1	7	11	0	291	69
38	10	2	0	0	0	0	85	5	8	6	180	242	62
39	2	1	29	90	237	147	86	5	1	19	270	254	16
40	6	5	0	0	0	0	87	5	7	9	90	174	84
41	0	5	11	90	90	0	89	7	2	15	180	178	2

N	h	k	l	est.	ref.	ΔØ
91	6	9	1	180	141	39
92	6	10	3	270	0	90
93	5	0	18	180	180	0
95	5	3	22	90	126	36
96	6	4	10	90	94	4
99	6	7	0	180	180	0
101	0	3	23	90	90	0
102	3	2	10	90	72	18
103	0	4	24	180	180	0
104	0	9	13	270	270	0
106	0	1	10	90	90	0
108	7	2	6	180	131	49

80 ⌊2383
29.79°

OBSERVED AND CALCULATED STRUCTURE FACTORS FOR NOVOBIOCIN

H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC
0	0	4	771	826	0	1	29	8	17	0	3	4	1079	1090	0	4	12	184	181	0	5	23	91	93
0	0	6	466	455	0	1	30	8	23	0	3	5	2462	2502	0	4	13	15	17	0	5	24	70	45
0	0	8	624	601	0	1	31	78	34	0	3	6	2243	2293	0	4	14	246	241	0	5	25	9	5
0	0	10	416	392	0	2	0	880	918	0	3	7	602	645	0	4	15	7	31	0	5	28	92	93
0	0	14	61	42	0	2	1	412	425	0	3	8	79	76	0	4	17	168	172	0	5	29	6	31
0	0	16	199	209	0	2	2	1376	1425	0	3	9	85	120	0	4	18	10	27	0	6	0	657	645
0	0	18	104	104	0	2	3	658	656	0	3	10	132	149	0	4	19	441	435	0	6	1	268	253
0	0	20	113	114	0	2	4	1301	1306	0	3	11	62	97	0	4	21	8	47	0	6	2	285	276
0	0	22	443	432	0	2	5	2370	2441	0	3	12	333	352	0	4	23	29	32	0	6	3	161	155
0	0	24	167	169	0	2	6	77	82	0	3	13	120	129	0	4	24	179	173	0	6	4	118	129
0	0	26	156	170	0	2	7	1011	1015	0	3	14	179	168	0	4	26	93	89	0	6	5	227	227
0	0	28	88	76	0	2	8	7	51	0	3	15	188	197	0	4	28	30	25	0	6	6	86	84
0	1	2	77	141	0	2	9	199	204	0	3	16	112	121	0	4	29	14	48	0	6	7	207	207
0	1	4	1306	1345	0	2	10	92	110	0	3	17	150	148	0	4	30	17	10	0	6	8	95	93
0	1	6	680	671	0	2	11	136	140	0	3	18	121	129	0	5	1	267	256	0	6	9	268	274
0	1	7	35	4	0	2	12	12	41	0	3	21	125	124	0	5	2	542	546	0	6	10	666	657
0	1	8	6	156	0	2	13	213	210	0	3	22	109	109	0	5	3	8	86	0	6	11	579	580
0	1	9	644	668	0	2	14	186	196	0	3	23	236	240	0	5	4	105	127	0	6	12	322	352
0	1	10	879	846	0	2	15	494	486	0	3	24	69	68	0	5	5	199	189	0	6	13	7	55
0	1	12	338	354	0	2	16	114	102	0	3	25	7	28	0	5	6	309	319	0	6	14	273	277
0	1	13	236	249	0	2	17	352	348	0	3	26	116	134	0	5	7	490	524	0	6	16	78	70
0	1	14	510	531	0	2	18	134	135	0	3	27	37	38	0	5	8	649	630	0	6	17	18	30
0	1	15	6	35	0	2	19	6	67	0	3	30	23	17	0	5	9	368	381	0	6	18	160	147
0	1	17	272	269	0	2	20	14	41	0	4	0	223	226	0	5	10	816	803	0	6	19	190	195
0	1	18	234	236	0	2	21	71	58	0	4	1	206	202	0	5	11	644	639	0	6	20	16	26
0	1	19	88	86	0	2	22	239	251	0	4	2	398	393	0	5	12	140	150	0	6	21	137	148
0	1	20	77	67	0	2	23	119	133	0	4	3	166	164	0	5	13	320	324	0	6	22	11	77
0	1	21	153	170	0	2	24	122	150	0	4	4	685	684	0	5	14	277	264	0	6	25	6	50
0	1	22	92	94	0	2	25	22	24	0	4	6	1135	1120	0	5	16	330	344	0	6	26	11	48
0	1	23	11	22	0	2	26	123	104	0	4	7	341	356	0	5	18	135	135	0	6	27	23	26
0	1	25	6	50	0	2	28	27	29	0	4	8	316	312	0	5	19	139	145	0	6	28	30	1
0	1	26	146	149	0	3	1	363	358	0	4	9	221	216	0	5	20	249	246	0	6	29	79	57
0	1	27	16	43	0	3	2	788	753	0	4	10	676	700	0	5	21	77	73	0	7	1	408	394
0	1	28	88	87	0	3	3	463	456	0	4	11	337	330	0	5	22	66	78	0	7	2	7	41

H	K	L	10FO	10FC
0	7	3	194	201
0	7	4	323	331
0	7	5	516	523
0	7	6	132	147
0	7	7	7	67
0	7	8	159	155
0	7	9	63	37
0	7	10	284	292
0	7	11	263	261
0	7	12	133	133
0	7	13	293	277
0	7	14	62	56
0	7	15	216	222
0	7	16	7	50
0	7	18	14	51
0	7	19	81	92
0	7	20	9	68
0	7	22	70	28
0	7	23	12	10
0	7	25	146	159
0	7	27	9	70
0	8	0	118	136
0	8	1	392	392
0	8	2	232	216
0	8	3	71	75
0	8	4	321	338
0	8	5	71	38
0	8	6	37	23
0	8	7	6	56
0	8	8	125	118
0	8	10	250	243
0	8	11	105	103
0	8	12	71	87
	0	13	141	148

H	K	L	10FO	10FC
0	8	14	166	173
0	8	15	294	316
0	8	16	124	137
0	8	17	238	257
0	8	18	263	282
0	8	19	137	142
0	8	21	81	84
0	8	25	78	57
0	8	27	10	32
0	9	2	75	58
0	9	3	61	63
0	9	4	146	154
0	9	5	62	59
0	9	6	9	30
0	9	7	8	28
0	9	8	213	214
0	9	9	215	212
0	9	10	93	100
0	9	11	60	10
0	9	12	149	124
0	9	13	224	234
0	9	14	91	77
0	9	16	334	341
0	9	18	100	79
0	9	19	26	54
0	9	20	128	137
0	9	22	83	33
0	9	23	13	16
0	9	24	69	67
0	9	25	15	11
0	9	26	14	34
0	10	0	164	173
0	10	1	7	54
0	10	2	8	28

H	K	L	10FO	10FC
0	10	3	69	49
0	10	4	172	168
0	10	5	199	199
0	10	6	8	51
0	10	8	63	91
0	10	9	285	282
0	10	11	61	93
0	10	12	135	111
0	10	13	97	103
0	10	14	159	154
0	10	15	109	120
0	10	17	77	91
0	10	18	128	125
0	10	19	133	125
0	10	20	8	82
0	10	21	11	23
0	10	22	14	92
0	10	24	79	58
0	11	1	105	104
0	11	2	8	54
0	11	3	17	3
0	11	4	81	84
0	11	6	68	10
0	11	7	130	135
0	11	8	241	233
0	11	10	8	43
0	11	11	264	269
0	11	12	235	230
0	11	13	7	9
0	11	16	106	88
0	11	17	18	10
0	11	20	133	128
0	11	21	98	81
0	11	22	72	60

H	K	L	10FO	10FC
0	11	23	92	38
0	12	1	9	94
0	12	3	139	148
0	12	4	170	166
0	12	7	10	69
0	12	8	59	44
0	12	9	76	82
0	12	10	173	171
0	12	11	173	196
0	12	12	70	92
0	12	13	15	20
0	12	14	7	97
0	12	15	16	17
0	12	17	80	53
0	12	21	84	104
0	13	1	141	137
0	13	2	6	21
0	13	3	66	9
0	13	4	69	55
0	13	5	31	10
0	13	6	96	75
0	13	7	128	128
0	13	8	8	18
0	13	9	71	81
0	13	10	8	99
0	13	12	13	38
0	13	13	83	71
0	13	14	71	59
0	13	15	14	15
0	13	18	10	10
0	14	0	69	95
0	14	1	6	21
0	14	2	188	173
0	14	3	75	83

H	K	L	10FO	10FC
0	14	4	8	69
0	14	6	122	116
0	14	7	166	150
0	14	8	90	64
0	14	9	43	50
0	14	10	97	86
0	14	12	10	16
0	14	14	26	15
0	14	15	11	4
0	15	1	161	156
0	15	2	8	4
0	15	4	91	96
0	15	5	103	128
0	15	6	99	133
0	15	7	74	39
0	15	8	16	40
0	15	10	26	30
0	15	11	75	87
0	16	0	91	66
0	16	1	6	45
0	16	2	35	13
0	16	4	13	11
1	0	1	101	52
1	0	2	734	784
1	0	3	1202	1213
1	0	4	609	615
1	0	5	30	54
1	0	6	486	497
1	0	7	142	146
1	0	8	361	346
1	0	9	101	116
1	0	10	184	178
1	0	11	88	97
1	0	12	298	311

H	K	L	10FO	10FC
1	0	13	14	7
1	0	14	367	354
1	0	15	115	115
1	0	16	283	275
1	0	17	171	162
1	0	18	156	161
1	0	19	75	74
1	0	20	149	151
1	0	21	8	3
1	0	22	10	43
1	0	23	10	3
1	0	24	9	56
1	0	26	140	139
1	0	27	11	20
1	0	29	107	121
1	1	0	1189	1239
1	1	1	634	665
1	1	2	468	484
1	1	3	949	969
1	1	4	285	291
1	1	5	381	374
1	1	6	843	865
1	1	7	370	387
1	1	8	348	332
1	1	9	393	392
1	1	10	224	205
1	1	11	247	247
1	1	12	275	278
1	1	13	641	657
1	1	14	113	124
1	1	15	285	294
1	1	16	241	237
1	1	17	120	109
1	1	18	171	163
1	1	19	95	77
1	1	20	6	52
1	1	21	82	84
1	1	22	7	96
1	1	23	132	115
1	1	24	113	106
1	1	25	72	86
1	1	26	9	20
1	1	27	98	110
1	1	28	81	91
1	1	30	86	86
1	1	31	106	78
1	2	0	324	330
1	2	1	826	835
1	2	2	603	630
1	2	3	377	384
1	2	4	639	622
1	2	5	753	759
1	2	6	651	656
1	2	7	679	685
1	2	8	362	364
1	2	9	303	320
1	2	10	384	383
1	2	11	522	525
1	2	12	211	221
1	2	13	209	208
1	2	14	357	337
1	2	15	84	89
1	2	16	136	150
1	2	17	61	63
1	2	18	237	235
1	2	19	182	200
1	2	20	156	156
1	2	21	116	110
1	2	22	39	46
1	2	23	104	95
1	2	24	137	129
1	2	25	158	159
1	2	26	126	126
1	2	27	100	98
1	2	28	10	25
1	2	29	15	33
1	2	31	95	44
1	3	0	252	253
1	3	1	1014	1024
1	3	2	450	445
1	3	3	782	781
1	3	4	993	1007
1	3	5	554	540
1	3	6	722	726
1	3	7	241	234
1	3	8	993	1011
1	3	9	514	489
1	3	10	101	92
1	3	11	254	259
1	3	12	338	342
1	3	13	214	212
1	3	14	255	248
1	3	15	96	82
1	3	16	117	109
1	3	18	136	140
1	3	19	181	184
1	3	20	123	137
1	3	21	182	186
1	3	22	162	159
1	3	23	207	191
1	3	24	92	77
1	3	25	102	79
1	3	26	158	162
1	3	27	9	38
1	3	28	8	53
1	4	0	397	385
1	4	1	266	292
1	4	2	247	234
1	4	3	516	516
1	4	4	567	584
1	4	5	474	469
1	4	6	261	249
1	4	7	495	517
1	4	8	382	384
1	4	9	370	357
1	4	10	280	271
1	4	11	260	246
1	4	12	318	305
1	4	13	176	179
1	4	14	136	147
1	4	15	193	183
1	4	16	102	96
1	4	17	169	171
1	4	18	12	44
1	4	19	148	163
1	4	20	153	155
1	4	21	98	77
1	4	22	177	186
1	4	23	89	98
1	4	24	160	165
1	4	25	140	138
1	4	26	79	28
1	4	29	24	82
1	4	30	6	56
1	5	0	270	281
1	5	1	344	329
1	5	2	279	293
1	5	3	163	162
1	5	4	329	330
1	5	5	243	230
1	5	6	603	578
1	5	7	378	403
1	5	8	370	362
1	5	9	161	160
1	5	10	196	207
1	5	11	265	260
1	5	12	282	288
1	5	13	171	173
1	5	14	178	169
1	5	15	67	77
1	5	16	273	267
1	5	17	86	100
1	5	18	83	91
1	5	19	119	114
1	5	20	116	103
1	5	21	162	166
1	5	22	198	189
1	5	23	128	120
1	5	25	97	97
1	5	26	77	73
1	5	28	8	25
1	5	29	7	45
1	6	0	126	148
1	6	1	120	110
1	6	2	75	60
1	6	3	314	310
1	6	4	424	408
1	6	5	331	341
1	6	6	494	484
1	6	7	354	350

H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC
1	6	8	459	474	1	7	16	101	124	1	9	1	212	218	1	10	14	107	129	1	12	10	11	60
1	6	9	621	608	1	7	17	110	98	1	9	2	186	183	1	10	15	166	161	1	12	11	90	86
1	6	10	176	182	1	7	18	281	290	1	9	3	9	61	1	10	17	113	135	1	12	12	25	54
1	6	11	338	346	1	7	19	185	190	1	9	4	110	114	1	10	20	9	76	1	12	13	69	52
1	6	12	288	299	1	7	20	202	210	1	9	5	123	133	1	10	21	6	37	1	12	14	89	72
1	6	13	314	308	1	7	21	7	34	1	9	6	122	118	1	10	22	70	50	1	12	15	13	40
1	6	14	229	236	1	7	22	88	55	1	9	7	68	64	1	11	1	181	184	1	12	16	6	39
1	6	15	176	177	1	7	23	7	54	1	9	8	6	62	1	11	3	6	46	1	12	17	130	125
1	6	16	87	68	1	7	24	74	60	1	9	9	139	141	1	11	4	172	184	1	12	18	79	44
1	6	17	259	256	1	7	25	13	37	1	9	10	8	68	1	11	5	6	69	1	12	19	85	93
1	6	18	299	304	1	7	26	10	16	1	9	11	74	92	1	11	6	22	50	1	12	20	7	97
1	6	19	203	209	1	7	27	21	51	1	9	12	198	193	1	11	7	188	180	1	13	0	91	68
1	6	20	143	147	1	8	0	8	3	1	9	13	474	487	1	11	8	219	216	1	13	1	110	104
1	6	21	164	176	1	8	1	216	218	1	9	14	297	321	1	11	9	151	139	1	13	2	7	27
1	6	22	72	96	1	8	2	233	215	1	9	15	116	116	1	11	10	140	152	1	13	3	200	196
1	6	24	28	36	1	8	3	160	153	1	9	16	340	351	1	11	11	10	91	1	13	4	120	109
1	6	25	80	88	1	8	4	251	248	1	9	17	121	124	1	11	12	125	112	1	13	6	104	88
1	6	27	77	58	1	8	5	142	137	1	9	18	122	143	1	11	13	6	74	1	13	7	9	51
1	6	28	7	33	1	8	6	134	144	1	9	19	85	89	1	11	14	142	138	1	13	8	127	127
1	7	0	109	110	1	8	7	110	135	1	9	20	73	67	1	11	15	79	89	1	13	9	72	40
1	7	1	221	230	1	8	8	63	74	1	9	21	7	34	1	11	16	99	109	1	13	10	6	50
1	7	2	185	169	1	8	9	98	100	1	9	22	107	117	1	11	18	97	99	1	13	11	101	108
1	7	3	425	413	1	8	11	249	258	1	9	24	26	11	1	11	19	103	88	1	13	12	84	68
1	7	4	277	280	1	8	12	200	195	1	10	0	149	129	1	11	20	6	73	1	13	13	92	83
1	7	5	197	201	1	8	13	84	70	1	10	1	153	155	1	11	22	7	54	1	13	14	8	36
1	7	6	117	108	1	8	14	129	156	1	10	2	121	88	1	12	0	162	160	1	13	15	12	46
1	7	7	7	30	1	8	15	177	178	1	10	3	73	36	1	12	1	38	82	1	13	16	21	30
1	7	8	355	355	1	8	16	136	138	1	10	4	103	93	1	12	2	135	149	1	14	0	96	92
1	7	9	168	187	1	8	18	134	146	1	10	5	82	63	1	12	4	136	137	1	14	1	143	147
1	7	10	220	231	1	8	19	146	150	1	10	7	6	80	1	12	5	154	165	1	14	2	126	126
1	7	11	404	400	1	8	21	15	67	1	10	9	73	99	1	12	6	128	126	1	14	3	73	49
1	7	12	229	233	1	8	22	15	51	1	10	10	87	95	1	12	7	194	183	1	14	4	123	118
1	7	13	6	30	1	8	27	18	53	1	10	12	167	154	1	12	8	187	197	1	14	5	102	92
1	7	15	65	70	1	9	0	95	78	1	10	13	90	86	1	12	9	222	230	1	14	7	152	149

H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC
1	14	8	6	61	2	0	18	70	101	2	1	28	97	97	2	3	3	114	107	2	4	9	394	409
1	14	9	13	43	2	0	21	7	12	2	1	29	171	170	2	3	4	635	630	2	4	10	359	363
1	14	10	10	74	2	0	22	140	132	2	1	30	30	57	2	3	5	563	539	2	4	11	243	235
1	14	11	7	37	2	0	23	14	35	2	2	0	299	292	2	3	6	184	184	2	4	12	214	222
1	14	14	9	37	2	0	24	68	64	2	2	1	726	778	2	3	7	511	502	2	4	13	269	268
1	14	15	80	15	2	0	25	22	5	2	2	2	439	437	2	3	8	141	136	2	4	14	195	199
1	15	0	8	28	2	0	27	6	56	2	2	3	597	571	2	3	9	687	671	2	4	15	85	77
1	15	1	16	64	2	0	29	133	145	2	2	4	443	440	2	3	10	437	430	2	4	16	153	152
1	15	2	31	45	2	0	30	231	219	2	2	5	356	338	2	3	11	166	168	2	4	17	88	112
1	15	3	134	129	2	1	0	170	179	2	2	6	247	249	2	3	12	182	196	2	4	18	196	186
1	15	4	73	65	2	1	1	306	321	2	2	7	440	449	2	3	13	174	169	2	4	19	85	38
1	15	5	36	53	2	1	2	321	321	2	2	8	637	594	2	3	14	42	28	2	4	20	73	88
1	15	7	16	22	2	1	3	718	702	2	2	9	432	418	2	3	15	175	182	2	4	21	65	43
1	15	10	83	70	2	1	4	523	525	2	2	10	297	283	2	3	16	136	128	2	4	22	79	73
1	16	1	71	43	2	1	5	469	488	2	2	11	296	303	2	3	17	73	53	2	4	23	101	145
1	16	2	7	54	2	1	6	176	171	2	2	12	323	310	2	3	18	99	113	2	4	24	149	132
1	16	3	77	94	2	1	7	320	334	2	2	13	167	157	2	3	19	63	69	2	4	25	9	56
2	0	0	1582	1587	2	1	8	230	223	2	2	14	75	75	2	3	20	113	120	2	4	26	87	41
2	0	1	665	659	2	1	9	219	222	2	2	15	151	140	2	3	21	71	79	2	4	27	75	72
2	0	2	223	217	2	1	10	136	154	2	2	16	70	95	2	3	22	185	180	2	4	28	85	29
2	0	3	854	841	2	1	11	113	117	2	2	17	162	160	2	3	23	100	103	2	5	0	124	133
2	0	4	997	1002	2	1	12	235	232	2	2	18	140	130	2	3	24	127	133	2	5	1	287	275
2	0	5	943	959	2	1	13	436	410	2	2	19	115	120	2	3	25	195	190	2	5	2	457	437
2	0	6	444	451	2	1	14	187	185	2	2	20	92	74	2	3	26	126	128	2	5	3	363	362
2	0	8	152	162	2	1	15	186	184	2	2	21	74	76	2	3	30	6	14	2	5	4	619	584
2	0	9	249	243	2	1	16	73	62	2	2	22	26	25	2	4	0	8	21	2	5	5	145	126
2	0	10	823	787	2	1	17	276	281	2	2	23	184	171	2	4	1	339	357	2	5	6	184	170
2	0	11	146	151	2	1	18	200	218	2	2	24	107	108	2	4	2	511	515	2	5	7	229	224
2	0	12	358	352	2	1	19	12	59	2	2	25	10	74	2	4	3	102	119	2	5	8	131	123
2	0	13	511	487	2	1	20	19	50	2	2	26	134	122	2	4	4	238	250	2	5	9	229	218
2	0	14	61	64	2	1	22	19	38	2	2	28	9	36	2	4	5	336	355	2	5	10	201	204
2	0	15	265	271	2	1	23	90	91	2	3	0	381	357	2	4	6	550	562	2	5	11	173	175
2	0	16	174	171	2	1	24	71	90	2	3	1	518	494	2	4	7	293	290	2	5	12	66	79
2	0	17	101	107	2	1	25	6	80	2	3	2	679	671	2	4	8	172	178	2	5	13	118	104

H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC
2	5	14	116	117	2	6	18	162	188	2	8	0	62	29	2	9	12	155	160	2	11	5	176	170
2	5	15	151	135	2	6	19	194	192	2	8	1	386	399	2	9	13	175	175	2	11	6	151	177
2	5	16	80	63	2	6	20	151	175	2	8	2	354	340	2	9	14	179	180	2	11	7	254	266
2	5	17	264	272	2	6	21	154	155	2	8	4	98	89	2	9	15	9	46	2	11	8	105	127
2	5	18	102	109	2	6	22	14	34	2	8	5	208	191	2	9	16	78	85	2	11	9	146	131
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2	5	25	95	102	2	7	2	337	346	2	8	12	156	172	2	10	1	6	59	2	11	17	9	46
2	5	26	79	45	2	7	3	82	68	2	8	13	133	128	2	10	2	80	102	2	11	18	6	28
2	5	27	104	56	2	7	4	209	210	2	8	14	248	246	2	10	3	233	240	2	11	19	103	113
2	5	28	85	61	2	7	5	387	371	2	8	15	187	178	2	10	4	86	107	2	11	22	14	66
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2	6	2	391	391	2	7	9	77	83	2	8	20	23	47	2	10	8	91	106	2	12	4	98	87
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2	6	4	603	580	2	7	11	104	113	2	8	24	9	81	2	10	10	135	132	2	12	6	79	68
2	6	5	473	475	2	7	12	63	56	2	8	26	26	28	2	10	12	93	93	2	12	8	125	149
2	6	6	209	196	2	7	13	66	80	2	9	0	96	100	2	10	13	142	144	2	12	9	119	141
2	6	7	270	263	2	7	14	98	102	2	9	1	400	377	2	10	14	45	56	2	12	10	7	74
2	6	8	250	220	2	7	15	171	173	2	9	2	252	250	2	10	15	10	53	2	12	11	8	47
2	6	9	159	179	2	7	16	99	105	2	9	3	97	80	2	10	16	140	139	2	12	14	75	77
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2	6	16	105	112	2	7	25	6	51	2	9	10	100	93	2	11	2	68	76	2	13	1	67	44
2	6	17	209	210	2	7	26	80	63	2	9	11	177	193	2	11	4	148	144	2	13	2	6	69

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2	13	4	18	8	3	0	3	150	148	3	1	9	357	339	3	2	14	100	101	3	3	22	95	84
2	13	5	6	78	3	0	4	159	153	3	1	10	206	209	3	2	15	195	191	3	3	23	73	88
2	13	7	118	100	3	0	5	186	171	3	1	11	501	470	3	2	16	104	118	3	3	24	9	76
2	13	8	119	113	3	0	6	603	617	3	1	12	204	195	3	2	17	92	91	3	3	26	134	138
2	13	9	7	26	3	0	7	568	547	3	1	13	504	475	3	2	19	108	96	3	3	27	7	72
2	13	10	77	33	3	0	8	579	543	3	1	14	248	259	3	2	20	6	79	3	3	29	9	52
2	13	12	114	114	3	0	9	252	247	3	1	15	257	236	3	2	21	6	129	3	4	0	149	145
2	13	13	56	11	3	0	10	428	402	3	1	16	256	260	3	2	22	6	77	3	4	1	663	659
2	13	14	83	105	3	0	11	225	216	3	1	17	197	202	3	2	23	103	88	3	4	2	153	148
2	13	15	8	36	3	0	12	129	136	3	1	18	28	27	3	2	24	83	56	3	4	3	595	600
2	13	16	80	33	3	0	13	202	193	3	1	19	75	80	3	2	25	78	40	3	4	4	294	287
2	14	0	24	20	3	0	14	98	101	3	1	20	74	55	3	2	29	10	98	3	4	5	425	403
2	14	1	11	53	3	0	15	250	239	3	1	21	222	213	3	3	0	617	672	3	4	6	465	437
2	14	2	8	53	3	0	16	138	126	3	1	22	13	44	3	3	1	192	210	3	4	7	237	223
2	14	3	15	21	3	0	17	187	186	3	1	23	67	43	3	3	2	390	376	3	4	8	280	272
2	14	4	97	43	3	0	18	282	289	3	1	24	9	45	3	3	3	447	458	3	4	9	294	303
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2	14	9	8	55	3	0	23	71	91	3	2	0	364	379	3	3	8	9	26	3	4	15	200	217
2	14	10	107	108	3	0	25	68	65	3	2	1	660	652	3	3	9	291	268	3	4	16	108	97
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2	14	14	7	40	3	0	27	84	96	3	2	3	761	718	3	3	11	444	452	3	4	18	132	134
2	15	0	13	24	3	0	29	7	20	3	2	4	158	152	3	3	12	10	50	3	4	19	89	78
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2	15	3	75	56	3	1	1	543	517	3	2	6	280	289	3	3	14	103	109	3	4	21	135	122
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2	15	10	14	46	3	1	6	9	79	3	2	11	214	221	3	3	19	117	101	3	4	27	100	70
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3	5	1	233	224	3	6	8	276	278	3	7	17	109	90	3	9	4	84	96	3	10	18	7	60
3	5	2	174	182	3	6	9	133	137	3	7	19	172	201	3	9	5	206	198	3	10	21	9	64
3	5	3	395	389	3	6	10	234	236	3	7	20	117	120	3	9	6	201	197	3	10	22	8	46
3	5	4	420	410	3	6	11	218	223	3	7	21	72	85	3	9	7	222	225	3	11	0	29	83
3	5	5	99	102	3	6	12	69	93	3	7	22	120	125	3	9	8	217	216	3	11	1	113	136
3	5	6	224	225	3	6	13	234	242	3	7	23	101	73	3	9	9	120	146	3	11	2	182	180
3	5	7	248	249	3	6	14	135	135	3	7	24	79	38	3	9	10	127	138	3	11	3	129	127
3	5	8	436	453	3	6	15	26	55	3	7	25	67	53	3	9	11	99	103	3	11	4	60	30
3	5	9	200	189	3	6	16	87	89	3	8	0	83	75	3	9	13	6	24	3	11	5	32	48
3	5	10	126	115	3	6	17	69	75	3	8	1	278	259	3	9	15	86	98	3	11	6	72	49
3	5	11	362	370	3	6	18	8	64	3	8	2	281	281	3	9	16	167	173	3	11	7	98	101
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3	5	13	121	108	3	6	21	141	180	3	8	4	87	111	3	9	18	107	110	3	11	9	10	51
3	5	14	189	182	3	6	23	6	67	3	8	5	205	212	3	9	19	10	34	3	11	10	6	34
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3	5	18	184	184	3	7	0	154	159	3	8	9	82	83	3	10	0	71	42	3	11	15	86	76
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3	6	5	131	126	3	7	14	110	113	3	9	1	86	63	3	10	15	8	104	3	12	13	17	40
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3	12	18	8	25	4	0	7	259	247	4	1	15	112	117	4	2	22	128	112	4	4	4	599	575
3	13	0	68	38	4	0	8	220	222	4	1	16	113	81	4	2	25	8	50	4	4	5	478	472
3	13	1	11	68	4	0	9	101	121	4	1	17	239	255	4	2	26	94	93	4	4	6	302	291
3	13	3	6	19	4	0	10	75	47	4	1	18	116	124	4	2	28	12	47	4	4	7	199	199
3	13	4	8	17	4	0	11	126	129	4	1	19	174	183	4	3	0	329	322	4	4	8	155	161
3	13	6	14	79	4	0	12	164	170	4	1	20	73	67	4	3	1	461	433	4	4	9	11	53
3	13	8	143	135	4	0	13	283	297	4	1	21	236	235	4	3	2	538	531	4	4	10	162	160
3	13	9	10	72	4	0	14	156	148	4	1	22	128	117	4	3	3	12	18	4	4	11	20	16
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3	13	14	19	24	4	0	19	27	52	4	2	0	187	173	4	3	8	347	320	4	4	16	158	160
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3	14	1	80	58	4	0	21	75	28	4	2	2	240	230	4	3	11	6	59	4	4	18	97	80
3	14	2	6	55	4	0	22	238	224	4	2	3	405	386	4	3	12	158	164	4	4	19	64	44
3	14	3	9	51	4	0	24	11	48	4	2	4	659	657	4	3	13	79	78	4	4	20	90	95
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3	14	5	78	76	4	0	28	22	29	4	2	6	412	380	4	3	15	80	50	4	4	22	10	69
3	14	6	15	50	4	1	0	650	646	4	2	7	507	500	4	3	16	197	206	4	4	23	95	120
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3	14	10	71	58	4	1	2	656	595	4	2	9	337	328	4	3	18	212	206	4	4	25	72	59
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3	15	3	5	40	4	1	7	85	77	4	2	14	66	39	4	3	24	12	20	4	5	3	204	196
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4	0	1	569	571	4	1	10	225	227	4	2	17	107	135	4	3	28	9	14	4	5	6	402	405
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4	0	4	790	745	4	1	12	183	178	4	2	19	138	117	4	4	1	246	261	4	5	8	5	96
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H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC
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4	5	11	282	280	4	6	19	175	156	4	8	1	101	102	4	9	12	95	98	4	11	9	116	103
4	5	12	206	196	4	6	20	25	50	4	8	2	112	98	4	9	13	84	57	4	11	10	105	122
4	5	13	162	160	4	6	21	15	30	4	8	3	299	303	4	9	14	66	42	4	11	11	72	61
4	5	14	136	145	4	6	22	85	113	4	8	4	71	67	4	9	15	13	43	4	11	12	7	60
4	5	15	121	127	4	6	23	12	79	4	8	5	108	99	4	9	16	120	110	4	11	13	95	72
4	5	16	130	118	4	6	24	23	31	4	8	6	154	141	4	9	17	109	117	4	11	14	85	63
4	5	17	24	46	4	6	25	82	96	4	8	7	190	181	4	9	18	91	80	4	11	15	24	11
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4	5	20	120	108	4	7	1	165	155	4	8	10	112	118	4	9	22	73	17	4	12	1	142	118
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4	14	4	111	96	5	1	8	71	58	5	2	17	122	121	5	4	1	235	236	5	5	13	160	168
4	14	5	9	38	5	1	9	205	200	5	2	18	286	284	5	4	2	192	183	5	5	14	120	121
4	14	6	68	43	5	1	10	173	159	5	2	19	14	67	5	4	3	122	117	5	5	15	6	69
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5	1	2	155	155	5	2	11	83	105	5	3	21	85	83	5	5	7	232	224	5	6	22	14	32
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5	7	9	266	274
5	7	10	110	88
5	7	11	197	200
5	7	12	67	46
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5	7	14	20	78
5	7	15	103	91
5	7	16	79	74
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5	7	22	9	30
5	8	0	67	19
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5	8	2	96	109
5	8	3	79	40
5	8	4	215	211
5	8	5	216	213
5	8	6	258	255
5	8	7	171	172
5	8	8	9	27
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5	8	10	195	205
5	8	11	7	87
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5	8	13	139	136
5	8	15	127	135
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6	2	15	161	165
6	2	16	150	160
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6	4	0	103	114
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6	11	2	82	84
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6	11	6	131	126	7	0	20	58	22	7	2	13	129	141	7	4	5	134	135	7	6	1	15	53
6	11	7	89	65	7	0	22	12	41	7	2	14	143	165	7	4	6	11	74	7	6	2	127	131
6	11	8	126	130	7	1	1	128	124	7	2	15	164	162	7	4	7	123	114	7	6	3	94	89
6	11	9	10	88	7	1	2	107	120	7	2	16	17	6	7	4	8	91	81	7	6	4	125	132
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7	0	16	69	70	7	2	9	178	190	7	4	1	95	101	7	5	18	80	85	7	7	18	7	19
7	0	17	7	101	7	2	10	144	167	7	4	2	231	236	7	5	19	76	73	7	8	0	104	101

H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC	H	K	L	10FO	10FC
7	8	1	112	131	7	11	3	6	86	8	1	16	27	29	8	4	0	112	75	8	6	8	79	87
7	8	3	111	129	7	11	4	74	35	8	1	18	88	94	8	4	1	110	108	8	6	10	7	9
7	8	4	93	90	7	11	5	11	44	8	2	0	242	255	8	4	2	15	57	8	6	11	9	43
7	8	5	6	49	7	11	6	8	41	8	2	1	212	236	8	4	3	163	174	8	6	14	9	33
7	8	6	130	137	8	0	0	95	86	8	2	3	169	177	8	4	4	159	154	8	6	15	76	50
7	8	7	6	72	8	0	1	8	12	8	2	4	74	37	8	4	5	89	75	8	7	0	73	25
7	8	8	134	152	8	0	2	118	140	8	2	5	18	55	8	4	6	75	33	8	7	4	6	50
7	8	11	69	37	8	0	3	105	118	8	2	6	96	71	8	4	7	77	79	8	7	5	8	23
7	8	12	6	42	8	0	5	125	114	8	2	7	110	90	8	4	8	12	55	8	7	6	86	44
7	8	13	7	34	8	0	6	148	142	8	2	8	175	176	8	4	9	6	65	8	7	7	6	59
7	8	16	13	53	8	0	7	23	70	8	2	9	138	111	8	4	10	80	90	8	7	8	129	126
7	9	1	7	44	8	0	8	97	107	8	2	10	45	36	8	4	11	11	4	8	7	9	89	44
7	9	2	31	63	8	0	9	6	64	8	2	11	8	93	8	4	12	85	41	8	7	11	71	33
7	9	3	82	72	8	0	10	78	79	8	2	13	9	64	8	4	14	110	106	8	7	14	86	49
7	9	4	6	70	8	0	13	10	85	8	2	14	98	116	8	4	15	98	69	8	8	0	26	45
7	9	5	31	24	8	0	14	19	23	8	2	15	99	82	8	4	16	73	56	8	8	1	79	55
7	9	6	83	28	8	0	16	71	93	8	2	17	93	59	8	5	0	16	41	8	8	2	9	35
7	9	7	10	32	8	0	19	15	31	8	2	18	97	55	8	5	1	7	80	8	8	3	21	75
7	9	8	6	57	8	1	0	74	51	8	2	19	104	78	8	5	2	57	9	8	8	4	6	97
7	9	10	97	86	8	1	1	188	214	8	3	0	66	64	8	5	3	185	171	8	8	6	12	39
7	9	14	76	30	8	1	2	73	90	8	3	1	102	93	8	5	4	156	161	8	8	9	119	96
7	10	0	107	96	8	1	3	143	153	8	3	2	169	164	8	5	5	125	121	8	8	10	12	22
7	10	1	85	91	8	1	4	125	124	8	3	3	75	90	8	5	6	81	113	8	8	11	9	55
7	10	2	154	148	8	1	5	105	92	8	3	4	123	131	8	5	7	102	102	8	9	0	6	48
7	10	3	112	104	8	1	6	73	91	8	3	5	8	63	8	5	9	112	95	8	9	1	10	14
7	10	4	86	84	8	1	7	82	77	8	3	6	83	81	8	5	11	20	28	8	9	2	22	15
7	10	5	68	62	8	1	8	77	120	8	3	8	6	69	8	5	13	111	88	8	9	3	80	61
7	10	6	11	35	8	1	9	93	116	8	3	10	12	66	8	5	15	22	61	8	9	4	6	33
7	10	8	97	84	8	1	10	146	141	8	3	11	96	110	8	6	0	112	133	8	9	7	7	52
7	10	10	23	45	8	1	11	76	72	8	3	13	122	121	8	6	1	6	42	8	10	0	8	22
7	10	11	9	4	8	1	12	126	128	8	3	14	135	115	8	6	2	86	88	8	10	1	84	82
7	10	12	14	41	8	1	13	163	152	8	3	15	27	35	8	6	3	27	37	9	0	2	12	28
7	11	0	13	30	8	1	14	140	124	8	3	16	6	81	8	6	6	7	39	9	0	4	115	120
7	11	1	110	117	8	1	15	89	53	8	3	18	116	120	8	6	7	122	130	9	0	6	12	37

H	K	L	10FO	10FC
9	0	7	10	28
9	0	10	18	44
9	0	11	28	51
9	0	12	85	107
9	0	14	85	18
9	1	1	80	64
9	1	2	146	150
9	1	3	14	17
9	1	4	9	75
9	1	5	26	49
9	1	7	71	57
9	1	8	160	132
9	1	10	6	24
9	1	12	11	25
9	1	13	16	45
9	1	14	11	16
9	2	0	98	100
9	2	1	139	135
9	2	3	106	119
9	2	4	6	36
9	2	5	72	103
9	2	7	7	63
9	2	8	9	27
9	2	10	10	71
9	2	11	71	23
9	2	12	6	45
9	2	13	8	57
9	2	14	97	58
9	3	0	125	127
9	3	1	8	35
9	3	3	72	75
9	3	4	71	82
9	3	5	88	67
9	3	6	92	89
9	3	7	83	93
9	3	10	12	58
9	3	11	11	36
9	3	13	98	73
9	4	0	9	83
9	4	2	9	64
9	4	3	104	68
9	4	4	22	25
9	4	6	9	72
9	4	7	92	65
9	4	8	69	50
9	4	9	6	63
9	4	10	98	67
9	4	11	6	68
9	4	12	73	63
9	5	0	26	13
9	5	1	19	44
9	5	2	86	101
9	5	4	8	69
9	5	5	100	111
9	5	6	6	87
9	5	7	10	23
9	5	8	5	28
9	5	9	78	85
9	5	10	12	81
9	5	11	7	98
9	6	0	23	5
9	6	1	6	12
9	6	2	6	61
9	6	3	7	53
9	6	4	7	77
9	6	5	94	85
9	6	6	10	45
9	6	8	98	66
9	6	9	8	71
9	7	0	8	51
9	7	1	6	51
9	7	2	8	51
9	7	3	16	57
9	7	4	36	51
9	7	5	81	74
10	0	2	87	62
10	0	4	85	91
10	0	6	6	68
10	1	1	31	33
10	1	2	8	21
10	1	4	98	69
10	1	5	10	53
10	1	6	20	9
10	2	0	154	148
10	2	1	7	30
10	2	4	79	38
10	2	5	98	49
10	3	1	7	22
10	3	2	7	24

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The Crystal Structure of Novobiocin

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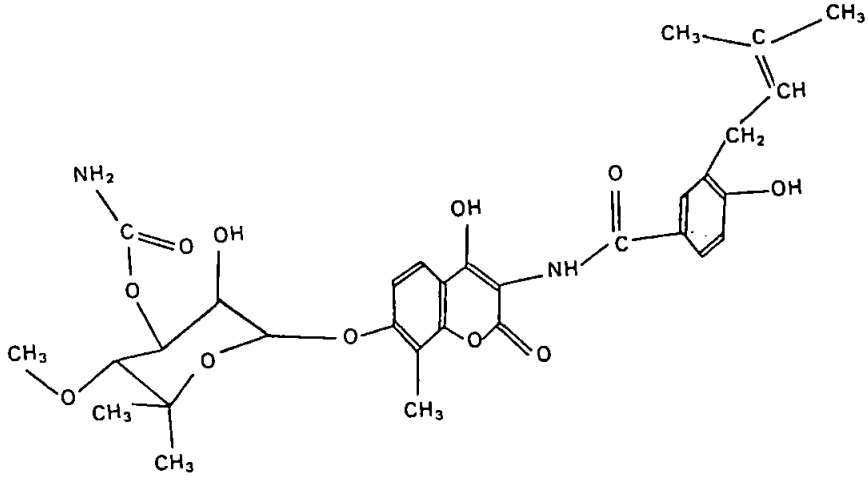
The structure of the antibiotic novobiocin with formula $C_{31}H_{36}N_2O_{11}.H_2O$ has been determined.



It crystallizes in the orthorhombic system with space group $P2_12_12_1$ and four molecules in a unit cell of dimensions $a=8{\cdot}577$, $b=13{\cdot}610$, $c=26{\cdot}357$ Å (standard deviation 0·20%). The structure was solved by the weighted tangent formula from intensity measurements obtained by visual comparison and the equi-inclination Weissenberg method. Full-matrix least-squares refinement yielded an R value of 0·14 for observed data using isotropic thermal parameters and excluding hydrogen atoms from the refinement. The coumarin and substituted benzene ring joined by the peptide bond all lie in one plane, with the isobutenyl and sugar groups on the same side of this plane. The structure contains one water molecule per asymmetric unit hydrogen-bonded to three novobiocin molecules; this plays a major role in stabilizing the structure. In addition there is likely to be some intermolecular interaction between the nitrogen of the carbamyl group and the –O– of an adjacent sugar ring, and intramolecular hydrogen bonding between the peptide oxygen and the –OH of the coumarin system. The structure viewed normally to the main plane of the molecule forms a series of almost overlapping rings (the coumarin with the substituted benzene). The crystal studied contained approximately one Ca^{2+} ion per 35 novobiocin molecules.

Introduction

Novobiocin, 7-[3-*O*-carbamyl-5,5-dimethyl-4-*O*-methyl-α-L-lyxosyl]-4-hydroxy-3-[4-hydroxy-3-(3-methylbut-2-enyl)benzamido]-8-methylcoumarin (Golding & Rickards, 1963), exhibits a fairly broad spectrum of antibacterial activity and is active mainly against Gram-positive bacteria, particularly *Staphylococcus aureus*. It is used clinically for treatment of penicillin-resistant staphylococcal infections.

Strong evidence exists to suggest that novobiocin inhibits growth by combining with Mg^{2+} ions (Brock, 1967), further evidence suggests however that this does not provide the complete explanation of the mode of action of the antibiotic (Morris & Russell, 1971). The presence and positioning of the carbamyl group are of major importance to the activity of novobiocin (Hoeksema & Smith, 1961). Brock (1967) suggests that this group may interact with the enolic group or some other electron-donating group of the molecule. The antibacterial activity of novobiocin cannot be attributed to any particular part of its unique chemical structure; the intact molecule appears to be necessary for full activity.

Novobiocin crystallizes in two forms (Hoeksema & Smith, 1961). Form 2 was used throughout this study. The three-dimensional crystal and molecular structure described in this paper has been determined in the anticipation that this will lead, with evidence already

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obtained, to a plausible explanation of the activity of the antibiotic. A recent review describes the discovery and characteristics of novobiocin and its antibacterial action (Morris & Russell, 1971).

Experimental

Novobiocin was obtained from the Boots Pure Drug Company as the calcium salt. This was converted to the free acid by hydrolysis in 0·1 *M* HCl, the free acid was then extracted from the aqueous solution using ethyl acetate and obtained as pale-yellow crystals by slow evaporation of the solvent. The crystals obtained were of form 2.

The crystal used for X-ray intensity measurements was an irregular flat plate approximately 0·7 × 0·5 × 0·1 mm. The unit-cell dimensions are $a = 8{\cdot}577$, $b = 13{\cdot}610$, $c = 26{\cdot}357$ Å with standard deviation 0·20%. The systematic absences $h00$, $h = 2n+1$, $0k0$, $k = 2n+1$, $00l$, $l = 2n+1$ indicated space group $P2_12_12_1$. The density measured by flotation in a mixture of dichloromethane and chloroform was 1330 kg m^{-3}. The cal-

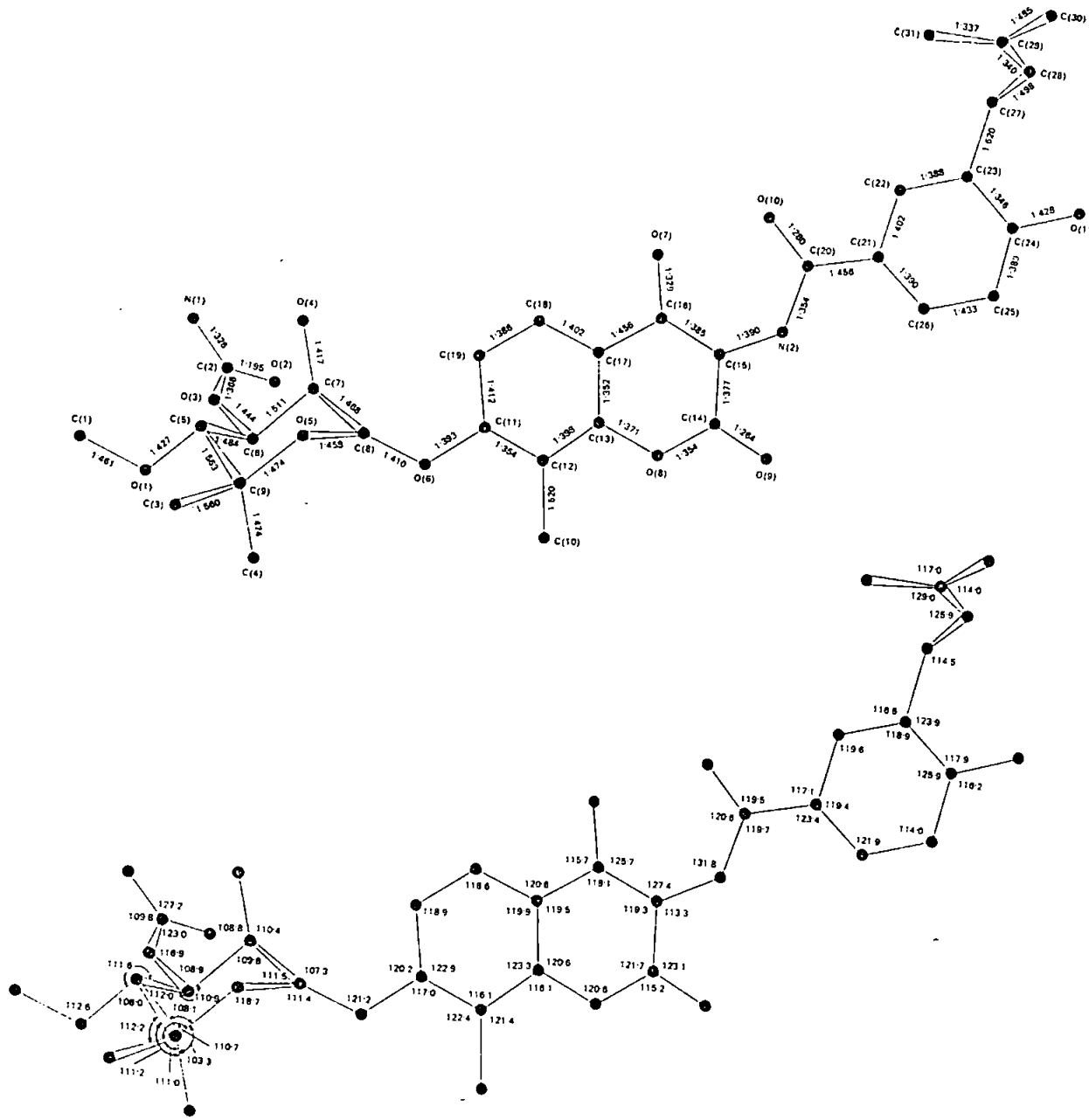

Fig. 1. Interatomic distances and interbond angles in the novobiocin molecule [the molecule is shown in perspective except for the sugar ring which has been rotated through approximately 80° about C(8)].

culated density was 1360 kg m^{-3} for four molecules per unit cell (including one water molecule per asymmetric unit).

Data for intensity measurement were obtained by the equi-inclination method on a Stoe–Weissenberg camera using Ni-filtered Cu $K\alpha$ radiation ($\lambda = 1{\cdot}5418$ Å) and the multiple-film technique. The crystal for these measurements was rotated about the b axis. The intensities of the X-ray reflexions were obtained by visual comparison with a precalibrated density scale obtained from the 205 reflexion. Constant exposure conditions were used and the interlayer scale factor set at unity. The intensities of 3030 unique reflexions were examined of a possible 3987 in the Cu $K\alpha$ sphere of reflexion. A total of 2487 reflexions were of measurable intensity. The absorption coefficient $\mu = 876$ m^{-1}; no absorption correction was applied to the intensity measurements.

Structure determination

Some initial calculations were carried out on an IBM 1130 computer with programs written by the authors. The major computations were carried out using the X-RAY 70 system on the ICL 1906 A at the Atlas Computer Laboratory, Chilton. In all the calculations the scattering factor tables given in *International Tables for Crystallography* (1962) were used.

The structure was solved using the weighted tangent formula (Germain, Main & Woolfson, 1971).

$$\tan \phi_{\mathbf{h}} = \frac{\sum_{\mathbf{h}'} \omega_{\mathbf{h}'} |E_{\mathbf{h}'} E_{\mathbf{h}-\mathbf{h}'}| \sin(\phi_{\mathbf{h}'} + \phi_{\mathbf{h}-\mathbf{h}'})}{\sum_{\mathbf{h}'} \omega_{\mathbf{h}'} |E_{\mathbf{h}'} E_{\mathbf{h}-\mathbf{h}'}| \cos(\phi_{\mathbf{h}'} + \phi_{\mathbf{h}-\mathbf{h}'})} = \frac{T_{\mathbf{h}}}{B_{\mathbf{h}}},$$

where

$$\omega_{\mathbf{h}'} = 0{\cdot}5 + 0{\cdot}5 \tanh [0{\cdot}5 \,.\, EC(\mathbf{h}) \,.\, EC(\mathbf{h}') \,.\, EC(\mathbf{h}-\mathbf{h}') \times \sigma_3/\sigma_2^{1{\cdot}5}],$$

where

$$EC(\mathbf{h}) = (T_{\mathbf{h}}^2 + B_{\mathbf{h}}^2)^{1/2},$$

$$\sigma_r = \sum_{j=1}^{N} Z_j^r,$$

Z_j is the atomic number of the jth atom, N is the total number of atoms per unit cell.

The starting set consisted of five reflexions with high $|E_{\mathbf{h}}|$. Three origin-determining reflexions and one enantiomorph-defining reflexion were chosen to conform to procedures described by Hauptman & Karle (1956) and Karle & Hauptman (1956). One reflexion was phased from the $\sum_1$ relationship (Hauptman & Karle, 1953). The starting set was chosen from a preliminary list of reflexions with $E \geq 1{\cdot}80$ (112 reflexions); due regard was paid to their ability to interact to form $\sum_2$ relationships with other reflexions within the $E \geq 1{\cdot}80$ list. The starting set was expanded within this E list by the sum of angles formula

$$\langle \alpha(\mathbf{h}) \rangle = \alpha(\mathbf{h}') + \alpha(\mathbf{h}-\mathbf{h}')$$

to check on the rate of phase determination and to test for inconsistencies at an early stage in the refinement process. Details of the starting set are given in Table 1.

Tangent refinement was carried out in a series of seven cycles, the E limit being lowered after each cycle. Phase estimates for all $E \geq 1{\cdot}40$ (355 reflexions) were obtained. An E map using these reflexions showed

Table 1. *The starting set for phase determination*

	Reflexion h	k	l	E	Phase	Number of $\sum_2$ interactions within $E \geq 1{\cdot}80$ list
Origin-fixing reflexions	6	5	0	2·14	0	11
	6	0	11	2·05	$-\pi/2$	11
	1	0	29	2·19	$-\pi/2$	6
Enantiomorph-defining reflexion	0	3	6	3·61	$-\pi/2$	24
$\sum_1$ reflexion	0	6	10	2·21	π	15

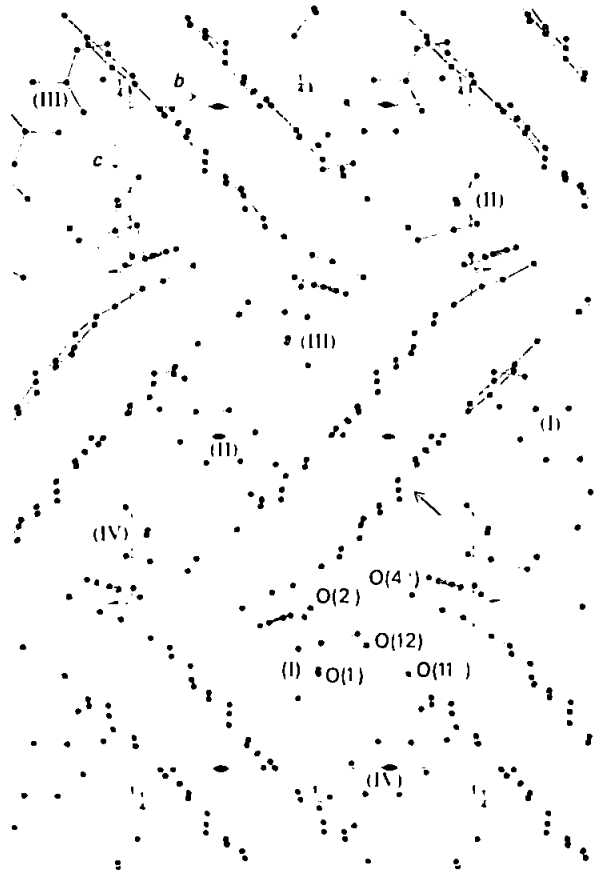

Fig. 2. The novobiocin crystal structure viewed along the x axis (hydrogen bonds to the water molecule are shown as dashed lines).

clearly the 24 atoms of the coumarin and benzene ring system joined by the peptide bond. Most of the remaining non-hydrogen atoms could have been located from this map but some atoms in the isobutenyl side chain and the sugar system were not clearly defined. The usual Fourier technique using the known atomic position to phase the observed F_o was used to determine the position of the remaining non-hydrogen atoms plus one additional atom assumed to be associated with a water molecule.

Refinement of the structure

The 45 atoms located on the electron-density map resulted in an R value of 0·26, where $R=\sum||F_o|-|F_c||/\sum|F_o|$. Five cycles of full-matrix least-squares refinement minimizing the function $\sum\omega[|F_o|-|F_c|]^2$ with individual isotropic temperature factors, unit weights and including refinement of interlayer scale factors resulted in $R=0{\cdot}15$. An F_o-F_c synthesis showed an excess of electron density in the region of the double bond C(28)–C(29) corresponding to approximately 2·0 e Å^{-3}. The possibility of this electron density being due to the presence of dihydronovobiocin was eliminated by a study of the n.m.r. spectrum of the crystalline material. Assumed disorder within the crystal structure with the isobutenyl chain in some other configuration could not account for the observed excess electron density. Atomic absorption spectrophotometric analysis showed that the crystalline free acid contained some calcium, and therefore it is assumed that Ca^{2+} ions are held in this region. The refinement process was continued with the inclusion of this further atomic position. The temperature factor was held constant at 5·0 Å^2. Site occupancy refinement for the assumed Ca^{2+} position indicated an occupancy of approximately 1 Ca^{2+} ion to 35 novobiocin molecules. A weighting function $\omega=1/[\Delta F]^2$ was used in the final refinement process, where $\Delta F=0{\cdot}055F_o+1{\cdot}17$ this equation obtained from a graph of $|\Delta F|$ *vs* $|F_o|$. The final R value was 0·14 using only the observed reflexions, individual isotropic temperature factors and omitting the 38 hydrogen atoms; the largest shift/error was 0·1009. An F_o-F_c Fourier synthesis indicated significant anisotropic thermal vibrations in some atoms. No further refinement was carried out pending the determination of an accurate data set.

Discussion of the structure

The final parameters are given in Table 2. A list of the observed and calculated structure factors and phase angles for all reflexions examined is given in Table 3. The bond distances and angles are listed with their standard deviations in Tables 4 and 5 and are shown in context in Fig. 1.

The coumarin and substituted benzene, with the peptide bond, all lie approximately in one plane, with the isobutenyl side chain on the same side of the main plane as the sugar ring. The temperature factors of the carbon atoms of the isobutenyl side chain are significantly higher than average, consistent with the lack of rigid bonding of this group and probable distortion due to the proximity of the assumed Ca^{2+} ions in some molecules. The bond distances C(23)–C(27) at 1·620 Å and C(29)–C(31) at 1·337 Å are respectively considerably higher and lower than the expected value of approximately 1·54 Å; this is assumed to result from the presence of the Ca^{2+} ions in this region. The distance of 2·446 Å between O(7) and O(10) suggests the presence of intramolecular hydrogen bonding between these atoms.

Table 2. *Final positional and temperature parameters*

Positional parameters are given as fractions of cell edges × 10^4. Temperature factors are of the form $\exp(-B\sin^2\theta/\lambda^2)$ and are given in Å^2. Standard deviations in parentheses are with respect to the last figures given.

	x	y	z	B
C(1)	6006 (17)	4830 (12)	8932 (5)	4·93 (27)
C(2)	7865 (14)	4397 (11)	7749 (4)	3·68 (20)
C(3)	3235 (16)	6578 (12)	7976 (5)	4·83 (26)
C(4)	1831 (16)	5467 (12)	8578 (5)	4·46 (25)
C(5)	4333 (13)	4858 (10)	8201 (4)	3·40 (19)
C(6)	5095 (12)	4615 (10)	7711 (4)	3·17 (18)
C(7)	3977 (12)	4085 (10)	7363 (4)	3·16 (18)
C(8)	2616 (13)	4709 (10)	7262 (4)	3·76 (21)
C(9)	2837 (13)	5497 (10)	8125 (4)	3·39 (19)
C(10)	4435 (13)	6765 (10)	6241 (4)	3·20 (18)
C(11)	2091 (13)	6110 (10)	6708 (4)	3·31 (18)
C(12)	2695 (12)	6707 (9)	6347 (3)	2·87 (17)
C(13)	1619 (11)	7237 (9)	6058 (3)	2·49 (15)
C(14)	1275 (12)	8328 (9)	5368 (4)	3·11 (18)
C(15)	9691 (12)	8374 (9)	5448 (3)	2·92 (17)
C(16)	9034 (12)	7813 (10)	5831 (4)	3·11 (18)
C(17)	68 (12)	7233 (9)	6150 (3)	2·87 (17)
C(18)	9470 (12)	6670 (9)	6551 (4)	3·08 (17)
C(19)	486 (13)	6083 (10)	6826 (4)	3·46 (20)
C(20)	7400 (12)	9156 (9)	5021 (4)	3·04 (18)
C(21)	6936 (13)	9876 (10)	4641 (4)	3·45 (20)
C(22)	5343 (12)	9941 (9)	4526 (3)	2·84 (17)
C(23)	4824 (14)	653 (11)	4190 (4)	4·01 (22)
C(24)	5879 (13)	1221 (10)	3951 (4)	3·45 (20)
C(25)	7474 (16)	1229 (12)	4042 (5)	4·68 (26)
C(26)	7977 (14)	509 (11)	4404 (4)	4·03 (22)
C(27)	2989 (18)	624 (12)	4045 (5)	5·01 (28)
C(28)	2122 (28)	1554 (19)	4157 (8)	8·68 (53)
C(29)	1610 (22)	1840 (15)	4614 (6)	6·58 (38)
C(30)	842 (23)	2818 (17)	4615 (7)	7·57 (45)
C(31)	1725 (30)	1377 (20)	5060 (9)	9·44 (60)
N(1)	8954 (14)	3736 (10)	7853 (4)	5·01 (23)
N(2)	8931 (11)	9013 (8)	5120 (3)	3·42 (17)
Ca	2192 (45)	867 (33)	4580 (13)	5·00*
O(1)	5338 (9)	5416 (7)	8522 (3)	3·55 (14)
O(2)	8059 (10)	5220 (8)	7603 (3)	4·57 (18)
O(3)	6420 (9)	3987 (7)	7807 (3)	4·00 (16)
O(4)	3511 (11)	3195 (8)	7597 (3)	4·66 (18)
O(5)	1871 (8)	5028 (6)	7731 (3)	3·36 (14)
O(6)	3136 (8)	5517 (6)	6973 (2)	3·16 (13)
O(7)	7548 (9)	7835 (7)	5973 (3)	3·91 (15)
O(8)	2226 (9)	7804 (6)	5676 (3)	3·34 (13)
O(9)	1956 (11)	8799 (7)	5019 (3)	4·37 (17)
O(10)	6360 (11)	8624 (8)	5234 (3)	5·01 (19)
O(11)	5320 (13)	1914 (9)	3587 (4)	5·68 (22)
O(12)	7468 (12)	6861 (8)	8154 (4)	5·67 (22)

* Held constant.

Table 3. *Observed and calculated structure factors*

Each entry lists in order, l, $10F_o$, $10F_c$ and $\varphi \times 10^3/2\pi$, where φ is the phase angle in radians. Unobserved reflexions were given $0{\cdot}5 \times$ threshold value and are denoted by *.

Table 4. *Bond distances and their standard deviations* (Å) *after weighted least-squares refinement*

C(19)–C(18)	1·386 (16)	C(23)–C(27)	1·620 (19)
C(18)–C(17)	1·402 (15)	C(27)–C(28)	1·498 (30)
C(17)–C(13)	1·352 (13)	C(28)–C(29)	1·340 (28)
C(13)–C(12)	1·398 (14)	C(29)–C(30)	1·485 (30)
C(12)–C(11)	1·354 (15)	C(29)–C(31)	1·337 (30)
C(11)–C(19)	1·412 (16)	C(11)–O(6)	1·393 (14)
C(17)–C(16)	1·456 (15)	O(6)—C(8)	1·410 (15)
C(16)–C(15)	1·385 (15)	C(8)—C(7)	1·468 (17)
C(15)–C(14)	1·377 (15)	C(7)—C(6)	1·511 (15)
C(14)–O(8)	1·354 (13)	C(6)—C(5)	1·484 (14)
O(8)—C(13)	1·371 (12)	C(5)—C(9)	1·563 (17)
C(16)–O(7)	1·329 (13)	C(9)—O(5)	1·474 (13)
C(12)–C(10)	1·520 (15)	O(5)—C(8)	1·458 (13)
C(14)–O(9)	1·264 (14)	C(7)—O(4)	1·417 (16)
C(15)–N(2)	1·390 (15)	C(6)—O(3)	1·444 (14)
N(2)—C(20)	1·345 (14)	O(3)—C(2)	1·368 (15)
C(20)–O(10)	1·280 (15)	C(2)—O(2)	1·195 (17)
C(20)–C(21)	1·456 (16)	C(2)—N(1)	1·326 (18)
C(21)–C(26)	1·390 (18)	C(5)—O(1)	1·427 (14)
C(26)–C(25)	1·433 (20)	O(1)—C(1)	1·461 (16)
C(25)–C(24)	1·389 (18)	C(9)—C(3)	1·560 (21)
C(24)–C(23)	1·346 (18)	C(9)—C(4)	1·474 (16)
C(23)–C(22)	1·388 (17)	Ca^{2+}–C(27)	1·602 (38)
C(22)–C(21)	1·402 (15)	Ca^{2+}–C(28)	1·455 (45)
C(24)–O(11)	1·428 (16)	Ca^{2+}–C(29)	1·417 (49)
		Ca^{2+}–C(31)	1·497 (44)

Fig. 2 shows the structure viewed along the a axis, showing the back-to-back arrangement of molecules related by the twofold screw axis along this direction. Fig. 3 shows part of the structure viewed normal to the plane containing the coumarin and substituted benzene system (in the direction of the arrow in Fig. 2). From this it can be seen that the molecule extends $2a$ in the x direction and the molecules related by the twofold screw axis (represented by full and dashed lines in Fig. 3) almost overlap in certain regions. In particular one half of the coumarin system of molecule (I) nearly overlaps with the substituted benzene ring of molecule (II) and the same half of the coumarin system of molecule (II) almost overlaps the substituted benzene ring of molecule (I) in the next unit cell. The separation of these rings is approximately 3·4 Å and hence they are attracted by van der Waals forces. Only molecules related by the twofold screw axis along the x direction are affected by this attraction of the overlapping rings.

There is one water molecule per asymmetric unit shown as O(12) in Fig. 3. The water molecule is in a position where hydrogen bonding can be assumed to

Table 5. *Bond angles and their standard deviations* (°)

C(19)–C(18)–C(17)	118·57 (0·95)	C(23)–C(24)–O(11)	117·85 (1·05)
C(18)–C(17)–C(13)	119·89 (0·96)	C(25)–C(24)–O(11)	116·21 (1·13)
C(17)–C(13)–C(12)	123·29 (0·93)	C(22)–C(23)–C(27)	116·45 (1·11)
C(13)–C(12)–C(11)	116·13 (0·92)	C(24)–C(23)–C(27)	123·87 (1·12)
C(12)–C(11)–C(19)	122·88 (1·06)	C(23)–C(27)–C(28)	114·50 (1·44)
C(11)–C(19)–C(18)	118·91 (1·03)	C(27)–C(28)–C(29)	125·90 (1·94)
C(18)–C(17)–C(16)	120·59 (0·90)	C(28)–C(29)–C(30)	114·00 (1·76)
C(12)–C(13)–O(8)	116·12 (0·82)	C(28)–C(29)–C(31)	128·97 (2·17)
C(17)–C(16)–C(15)	118·13 (0·91)	C(30)–C(29)–C(31)	117·01 (1·81)
C(16)–C(15)–C(14)	119·34 (1·00)	C(19)–C(11)–O(6)	120·17 (0·97)
C(15)–C(14)–O(8)	121·67 (0·95)	C(12)–C(11)–O(6)	116·95 (0·94)
C(14)–O(8)—C(13)	120·56 (0·80)	C(11)–O(6)—C(8)	121·23 (0·83)
O(8)—C(13)–C(17)	120·56 (0·90)	O(6)—C(8)—O(5)	111·43 (1·00)
C(13)–C(17)–C(16)	119·52 (0·93)	O(6)—C(8)—C(7)	107·29 (0·90)
C(17)–C(16)–O(7)	115·72 (0·90)	C(8)—C(7)—C(6)	109·80 (1·03)
O(7)—C(16)–C(15)	125·71 (1·02)	C(7)—C(6)—C(5)	110·86 (0·88)
C(11)–C(12)–C(10)	122·42 (0·97)	C(6)—C(5)—C(9)	112·00 (0·85)
C(13)–C(12)–C(10)	121·38 (0·91)	C(5)—C(9)—O(5)	108·07 (0·93)
C(15)–C(14)–O(9)	123·06 (1·04)	C(9)—O(5)—C(8)	118·68 (0·78)
O(8)—C(14)–O(9)	115·19 (0·94)	O(5)—C(8)—C(7)	111·54 (0·85)
C(16)–C(15)–N(2)	127·39 (0·94)	C(8)—C(7)—O(4)	110·43 (0·93)
C(14)–C(15)–N(2)	113·27 (0·94)	C(6)—C(7)—O(4)	108·82 (0·85)
C(15)–N(2)—C(20)	131·77 (0·98)	C(7)—C(6)—O(3)	108·90 (0·97)
N(2)—C(20)–O(10)	120·63 (1·05)	C(5)—C(6)—O(3)	109·06 (0·83)
N(2)—C(20)–C(21)	119·69 (0·98)	C(6)—O(3)—C(2)	116·91 (1·00)
O(10)–C(20)–C(21)	119·54 (0·99)	O(3)—C(2)—O(2)	112·98 (1·14)
C(20)–C(21)–C(26)	123·42 (1·03)	O(2)—C(2)—N(1)	127·19 (1·19)
C(20)–C(21)–C(22)	117·15 (1·01)	N(1)—C(2)—O(3)	109·76 (1·20)
C(21)–C(22)–C(23)	119·61 (1·04)	C(6)—C(5)—O(1)	111·63 (0·89)
C(22)–C(23)–C(24)	118·94 (1·09)	C(9)—C(5)—O(1)	105·99 (0·96)
C(23)–C(24)–C(25)	125·87 (1·20)	C(5)—O(1)—C(1)	112·64 (0·99)
C(24)–C(25)–C(26)	113·99 (1·23)	C(5)—C(9)—C(3)	112·18 (0·97)
C(25)–C(26)–C(21)	121·94 (1·12)	C(5)—C(9)—C(4)	111·19 (0·96)
C(26)–C(21)–C(22)	119·40 (1·07)	O(5)—C(9)—C(3)	110·72 (0·89)
		O(5)—C(9)—C(4)	103·26 (0·91)
		C(3)—C(9)—C(4)	111·04 (1·11)

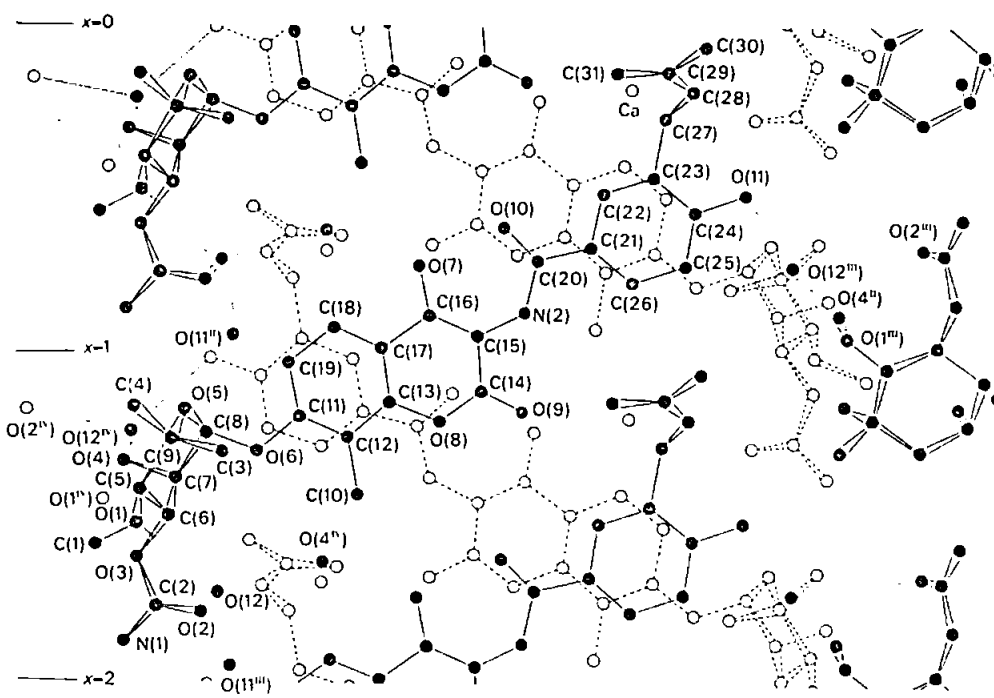

Fig. 3. Part of the novobiocin structure viewed normal to the main plane of the molecule (in the direction of the arrow in Fig. 2) showing the near overlapping of some rings in symmetry-related molecules and the hydrogen bonds to the water molecule (dotted lines).

take place between itself and three novobiocin molecules. Fig. 2 shows that two of these are related by the twofold screw axis along x (effectively holding opposite ends of the molecules together), the remaining hydrogen bonds are to O(1) and O(2) on a symmetry related sugar ring, resulting in a continuous linkage of the sugar rings in the y direction by hydrogen bonds.

There is possibly further intermolecular hydrogen bonding between O(5) and the carbamyl nitrogen N(1) on adjacent molecules (Fig. 3), the separation of these atoms being 3·083 Å. Thus the sugar rings of adjacent molecules are linked in the x direction. The hydrogen-bond distances involved between the donor and acceptor atoms are shown in Table 6. The distance of 4·481 Å between N(1) and the enolic oxygen O(7) on the coumarin system eliminates the possibility of interaction between these groups (Brock, 1967).

Table 6. *Distances and their standard deviations* (Å) *between donor and acceptor atoms involved in hydrogen bonding*

Donor	Acceptor	Type of bond	
O(4)	O(12)	OH ···H_2O	2·814 (14)
O(12)	O(2)	H_2O···C=O	2·712 (15)
O(12)	O(1)	H_2O···–O–	2·854 (14)
O(11)	O(12)	OH ···H_2O	2·771 (14)
O(7)	O(10)	OH ···C=O	2·446 (12)
N(1)	O(5)	NH ···–O–	3·075 (15)

At this stage it would seem highly desirable to recrystallize the novobiocin again in an attempt to produce crystals suitable for more accurate data collection and possibly to eliminate the presence of Ca^{2+} ions. This work is in progress and further discussion of the structure will be published later.

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