1979

THE STRUCTURAL AND PHYSICAL PROPERTIES OF CRYSTALLINE ANTIBIOTIC MATERIALS

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http://hdl.handle.net/10026.1/2046

http://dx.doi.org/10.24382/3828
University of Plymouth

THE STRUCTURAL AND PHYSICAL PROPERTIES OF CRYSTALLINE ANTIBIOTIC MATERIALS

BY

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A THESIS SUBMITTED FOR THE
DEGREE OF
DOCTOR OF PHILOSOPHY
OF THE
COUNCIL FOR NATIONAL ACADEMIC AWARDS

PLYMOUTH POLYTECHNIC
SCHOOL OF
MATHEMATICAL SCIENCES

NOVEMBER 1979

TABLE OF CONTENTS

		Page
Abstract		xii
Chapter 1:	The Determination of Structure by the Scattering of X-rays from a Single Crystal	1
	Introduction	1
1.1	The basis for crystal structure analysis	4
1.2	The scattering of a wave packet	4
1.3	The scattering of X-rays by atoms	10
1.4	Diffraction from a crystal	11
1.5	The reciprocal lattice	12
1.6	Intensity data collection and the Weissenberg method	13
1.7	Determination of cell dimensions	14
1.8	Determination of space group	16
1.9	Factors affecting observed intensities:	18
	Absorption	18
	Primary and secondary extinction	19
	Atomic vibration - the temperature factor	20
	Polarisation	20
	The Lorentz factor	21
1.10	The structure factor	21
1.11	The Wilson Plot	23
1.12	Structure determination methods:	24
	Heavy atom technique	25
	Direct methods	26
	Application of direct methods	29
1.13	Structure completion:	31
	Fourier F_0 synthesis	31
	Difference ΔF synthesis	32

		Page
1.14	Least-squares refinement and residual index, R	33
1.15	Accuracy of bond lengths and angles	34
Chapter 2:	The Crystal and Molecular Structure of 7-chloro-2-methyl-5-phenyl-3-propyl[2,3-b]-imidazolyl quinoline	37
2.1	Introduction	37
2.2	Crystal preparation	38
2.3	Space group and unit cell determination	38
2.4	Intensity data collection and preliminary treatment	39
2.5	Structure determination	39
2.6	Refinement of the structure	44
2.7	Discussion	46
Chapter 3:	The Crystal and Molecular Structure of the methyl ester of 5,5-dimethyl-2-(2-phenoxymethyl-5-oxo-1,3-oxazolin-4-ylidene)-1,3-thiazolidine-4-carboxylic acid	58
3.1	Introduction	58
3.2	Experimental	59
3.3	Structure determination	60
3.4	Structure refinement	61
3.5	Discussion	69
Chapter 4:	The Crystal and Molecular Structure of the Phenyl Ester of Carbenicillin (Carfecillin)	83
4.1	Introduction	83
4.2	Crystal preparation and preliminary X-ray studies	83
4.3	Intensity data collection and preliminary treatment	86
4.4	Structure determination	88
4.5	Structure refinement	94
4.6	Discussion	95
4.7	A comparison of the conformation of the penicillin nucleus with known penicillins and cephalosporins	101

		Page
Chapter 5:	The Configuration and Conformation of the side-chain substituents in penicillin derivatives	115
5.1	Introduction	115
5.2	Side chain configuration and associated nuclear magnetic resonance	115
5.3	Relative orientations of the C(17) side-chain substituent in the phenyl ester of carbenicillin	121
	(i) Relative orientation in fresh solution	127
	(ii) Relative orientation after equilibration	128
5.4	Side-chain configuration and associated circular dichroism	148
5.5	Further possibilities for conformational change outside the penicillin nucleus	163
Appendices		
A	Computer programs for the calculation of orthorhombic Patterson function, (1), and electron density Fourier synthesis, (2), in space group $P2_12_12$	165
B	List of structure factors for	181
	(i) SH62 P2(1)2(1)2: $C_{20}H_{18}N_3C\ell$	182
	(ii) SNP P2(1)/C: $C_{17}H_{18}N_2O_5S$	194
	(iii) CARF P2(1): Phenyl ester of carbenicillin	204
References		211

LIST OF TABLES

Table		Page
2.1	Co-ordinates of non-hydrogen atoms in $C_{20}H_{18}N_3C\ell$	53
2.2	Bond lengths in $C_{20}H_{18}N_3C\ell$	54
2.3	Bond angles in $C_{20}H_{18}N_3C\ell$	55
2.4	Co-ordinates of hydrogen atoms in $C_{20}H_{18}N_3C\ell$	56
2.5	Thermal parameters for $C_{20}H_{18}N_3C\ell$	57
3.1	Starting set of reflexions with associated phases and equivalent sign relations	62
3.2	Co-ordinates of non-hydrogen atoms in $C_{17}H_{18}N_2O_5S$	76
3.3	Bond lengths in $C_{17}H_{18}N_2O_5S$	77
3.4	Bond angles in $C_{17}H_{18}N_2O_5S$	78
3.5	Co-ordinates of hydrogen atoms in $C_{17}H_{18}N_2O_5S$	80
3.6(a)	Thermal parameters for non-hydrogen atoms in $C_{17}H_{18}N_2O_5S$	81
(b)	Thermal parameters for hydrogen atoms in $C_{17}H_{18}N_2O_5S$	82
4.1	Co-ordinates of non-hydrogen atoms in carfecillin	108
4.2	Bond lengths in carfecillin	109
4.3	Bond lengths in carfecillin	110
4.4	Co-ordinates of hydrogen atoms in carfecillin	111
4.5	Thermal parameters for carfecillin	112/113
4.6	Planarity of the penicillin nucleus in carfecillin	114
5.1	β lactam proton magnetic resonance spin-spin splitting coupling constants, J, and chemical shifts, δ	147
5.2	Circular dichroism data for the principal Cotton Effects	162

LIST OF ILLUSTRATIONS

Figure		Page
1.1	Path of integration in the complex k' plane	8
1.2	Oscillation geometry	14
1.3	Intensity distribution curves	18
2.1	Progress of the structure determination during electron density Fourier synthesis of $C_{20}H_{18}N_3C\ell$	42/43
2.2	Sectioning of SHELX 'BLOC' refinement	45
2.3	Bond lengths in $C_{20}H_{18}N_3C\ell$	47
2.4	Bond angles in $C_{20}H_{18}N_3C\ell$	48
2.5	The crystal structure of $C_{20}H_{18}N_3C\ell$ viewed along a	49
2.6	The crystal structure of $C_{20}H_{18}N_3C\ell$ viewed along c	50
3.1	Sectioning for SHELX 'BLOC' refinement	63
3.2	Schematic labelling of the non-hydrogen atoms in $C_{17}H_{18}N_2O_5S$	64
3.3	Bond lengths in $C_{17}H_{18}N_2O_5S$	65
3.4	Bond angles in $C_{17}H_{18}N_2O_5S$	66
3.5	The crystal structure of $C_{17}H_{18}N_2O_5S$ viewed along b	67
3.6	The crystal structure of $C_{17}H_{18}N_2O_5S$ viewed along a	68
4.1	Perspective view of the twinning of carfecillin crystals	84
4.2	Twinning of crystals of carfecillin	85
4.3	Wilson Plot for carfecillin	87
4.4	Progress of electron density Fourier synthesis during the structure determination of carfecillin	92/93
4.5	SHELX 'BLOC' sectioning of carfecillin	94
4.6	Schematic labelling and inter-atomic distances in carfecillin	97
4.7	Bond angles in carfecillin	98
4.8	The crystal structure of carfecillin viewed along a	99
4.9	The crystal structure of carfecillin viewed along b	100

		Page
4.10	Conformation of thiazolidine rings of known penicillin structures	104
4.11	Bonding geometry at β lactam nitrogen in some penicillins and cephalosporins	105
4.12	The comparative structural arrangements of the nuclei of penicillins, Δ^3 and Δ^2 - cephalosporins	106
5.1	Geometrical parameters used in describing the relative side-chain orientation in the phenyl ester of carbenicillin	123
5.2	Transformation of co-ordinate axes	124
5.3	Contour plot of n.m.r. shielding values for protons in the vicinity of a benzene ring	126
5.4	Distances of C(17) and its substituents from H(C(6))	129
5.5	Theoretical geometry about C(17)	132
5.6	Conformation of the C(15) = O(16) chromophore with respect to C(17) substituents in amoxycillin and ampicillin	151

PROTON MAGNETIC RESONANCE SPECTRA:

Trace			Page
1	(a)	Methyl ester of carbenicillin in fresh solution	135
	(b)	Methyl ester of carbenicillin after equilibration	
2	(a)	Ethyl ester of carbenicillin in fresh solution	136
	(b)	Ethyl ester of carbenicillin after equilibration	
3	(a)	Methyl ester of carbenicillin, β lactam H^1 resonance in solution after time 't'	137
	(b)	Ethyl ester of carbenicillin, β lactam H^1 resonance in solution after time 't'	
4	(a)	Phenyl ester of carbenicillin after equilibration	138
	(b)	Phenyl ester of carbenicillin, β lactam H^1 resonance in solution after time 't'	
5	(a)	D-Amino-phenylacetamido penicillanic acid (ampicillin)	139
	(b)	D-Amino-phenylacetamido penicillanic acid (ampicillin), β lactam H^1 resonance at varying sample concentration 'c'	
6	(a)	D- Amino-hydroxybenzyl penicillin (amoxycillin)	140
	(b)	D- Amino-hydroxybenzyl penicillin (amoxycillin), β lactam H^1 resonance at varying sample concentration 'c'	
7	(a)	D-Tyrosyl penicillin	141
	(b)	D-Tyrosyl penicillin, β lactam H^1 resonance at varying sample concentration 'c'	
8	(a)	L-Amino-phenylacetamido penicillanic acid	142
	(b)	L-Amino-hydroxybenzyl penicillin	
9	(a)	L-Amino-hydroxybenzyl penicillin and L-amino-phenylacetamido penicillanic acid, β lactam H^1 resonances	143
	(b)	L-Tyrosyl penicillin and D-tyrosyl penicillin, β lactam H^1 resonances	
10	(a)	L-Tyrosyl penicillin	144
	(b)	L-Tyrosyl penicillin, β lactam H^1 resonance at varying sample concentration 'c'	

CIRCULAR DICHROISM SPECTRA

CD		Page
(1)	Phenethicillin Potassium	154
(2)	Predicted CD spectrum of 6-APA	155
(3)	Amino-phenylacetamido penicillanic acid	156
(4)	Amino-hydroxybenzyl penicillin	157
(5)	Tyrosyl penicillin	158
(6)	Methyl ester of carbenicillin	159
(7)	Ethyl ester of carbenicillin	160
(8)	Phenyl ester of carbenicillin	161

ACKNOWLEDGEMENTS

I wish to convey my thanks to the Governors of the Polytechnic and to Dr. C.M. Gillett for providing the facilities and ancillary services for this work, and to express my deep gratitude to Dr. M.O. Boles for his patience, humour and helpful supervision and to Dr. R.J. Girven for his sage advice in the early stages of this work.

The support and advice, provided by Beecham Pharmaceuticals, are gratefully acknowledged and I am indebted to Mr. A.E. Bird and his colleagues at the Chemotherapeutic Research Centre, Brockham Park, for supplies of purified compounds.

My thanks are also extended to Dr. G. Kirk of the Chelsea School of Pharmacy, for supplies of novel heterocyclic compounds and to Dr. L. Re and co-workers at Snamprogetti Società per Azioni for supply of their suspected novel class of unsaturated penicillins.

The structure determinations bear witness to the excellent service and useful advice of Drs. M. Elder and M. Pickering, and Miss P. Machin of the Science Research Council Microdensitometer Service at Daresbury.

I wish to thank also Dr. K. Bancroft of the School of Environmental Sciences, Plymouth Polytechnic, for assistance and guidance in the collection of n.m.r. spectra and Westfield College, University of London, for chiroptical facilities, particularly Dr. P.M. Scopes of the Department of Chemistry.

I have appreciated the assistance of the departmental technicians and workshop staff and the services provided by the Learning Resources Centre and Reprographic Department.

I wish to extend my thanks to Mr. R. Srodzinski for his unstinting determination to provide photographic excellence during the preparation of this thesis and to Mrs. S. Tolan and Miss G. Stock for undertaking to demonstrate their 'code-breaking' talents in typing.

My greatest appreciation must be for those forebearing friends who remained such throughout, and for the devoted support given by my Mother during this work.

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. P.A.C. Gane under my supervision during the years 1976-1979.

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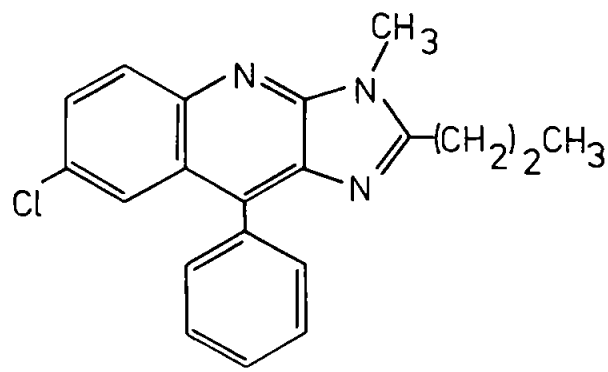
ABSTRACT

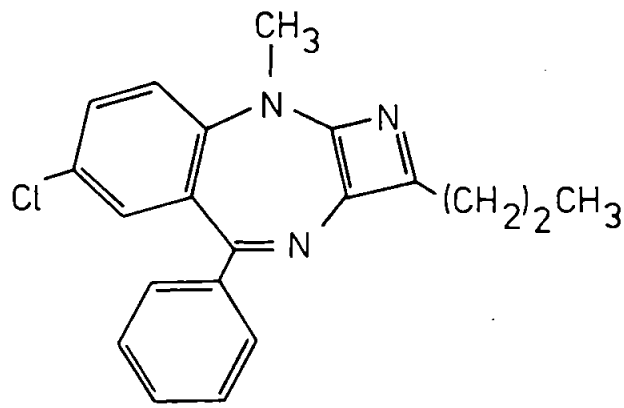
Effectiveness in biological chemical environments virtually defines the term 'drug' when applied to any attempt to modify that environment by the introduction of an influence in terms of a specific compound or group of compounds. Interest in the configuration of the molecules involved in such modifications led to the X-ray structure determinations, discussed in the thesis, of the following three compounds;

(i) 7-chloro-2-methyl-5-phenyl-3-propyl[2,3-b]-imidazolyl quinoline.



(1)

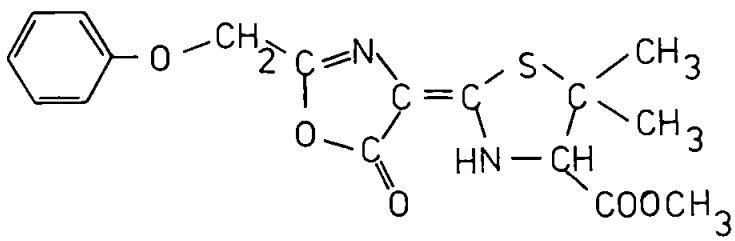
Derived from the psychoactive drug Librium, it was thought to conform to the structure,



(2)

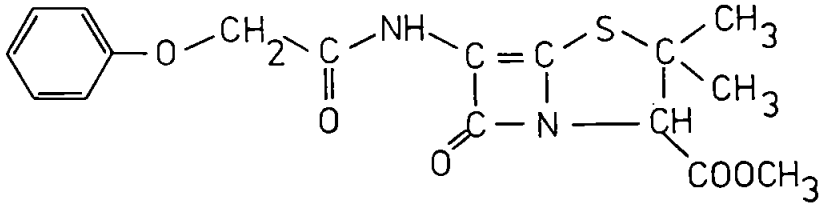
containing the highly strained 4-membered monocyclic azete system (Shenoy, a thesis submitted for the degree of Doctor of Philosophy, University of London, 1975), and suggested as one of the first examples of possible stable 4-membered azacyclobutadiene rings.

(ii) The methyl ester of 5,5-dimethyl-2-(2-phenoxymethyl-5-oxo-1,3-oxazolin-4-ylidene)-1,3-thiazolidine-4-carboxylic acid.



(3)

$C_{17}H_{18}N_2O_5S$ was first reported by Brandt, Bassignani and Re, (1976, Tetrahedron Letters No. 44, pp 3979-3982), to have configuration (4),



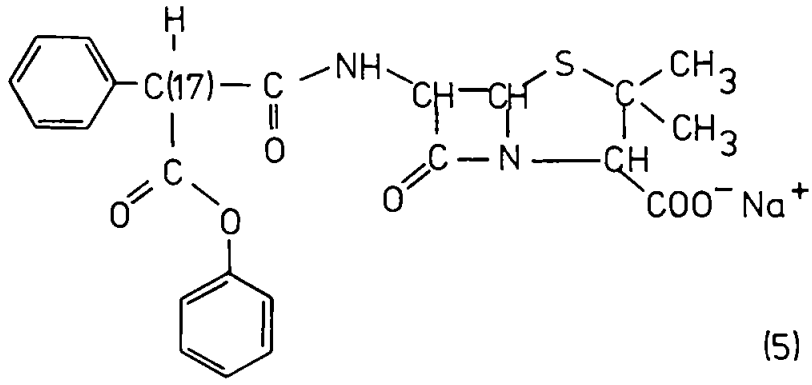
(4)

i.e. that of a novel class of DL-5,6-didehydropenicillins. Its reported weak antibacterial activity, thought to be associated with the unsaturated nature of the penicillin nucleus promoted its X-ray structure analysis.

Subsequently, Bachi and Vaya, (1977, Tetrahedron Letters No. 25, pp 2209-2212), suggested the configuration (3) which has been confirmed by the structure determination.

A comparison of the proposed derivations of (3) and (4) is made, and the conformation of the unconstrained thiazolidine ring is discussed in comparison with the constraining effect of adjacent β lactams in the nuclei of known penicillin structures.

(iii) The phenyl ester of carbenicillin (carfecillin).



(5)

The crystal structure is used to facilitate a comparison of the configurations of both the penicillin nucleus and the side-chain substituents of C(17) with other penicillin derivatives of known crystal structure.

The conformation in aqueous solution about C(17) is reflected in the modification of H^1 n.m.r. signals from the β lactam protons for the methyl and ethyl esters of carbenicillin between the two epimers. A

similar effect is noted for the two diastereoisomers of amino-hydroxybenzyl penicillin, amino-phenylacetamido penicillanic acid and a tyrosyl penicillin.

To evaluate a correlation between the absolute configuration and n.m.r. studies, circular dichroism spectra from penicillin compounds have been characterised and the mutarotation exhibited by the esters of carbenicillin used to describe configurational equilibria about C(17) in terms of their characteristic n.m.r. spectra.

CHAPTER 1

The Determination of Structure by the Scattering of X-Rays from a Single Crystal

INTRODUCTION

The bioactivity of organic compounds may depend upon numerous factors such as the reactivity and relative orientation of constituent functional groups. Overall molecular size and shape often results in preferential receptor site occupation in certain 'lock-and-key' mechanisms. The crystalline solid state consists of a regular array of molecules with relative orientations which minimise the potential energy associated with both inter-molecular and inter-atomic interactions. Crystal structure analysis reveals the molecular geometry associated with the crystalline state, and a study of structures within a group of similar compounds, provides the possibility of distinguishing these geometrical features which play an important part in biological activity.

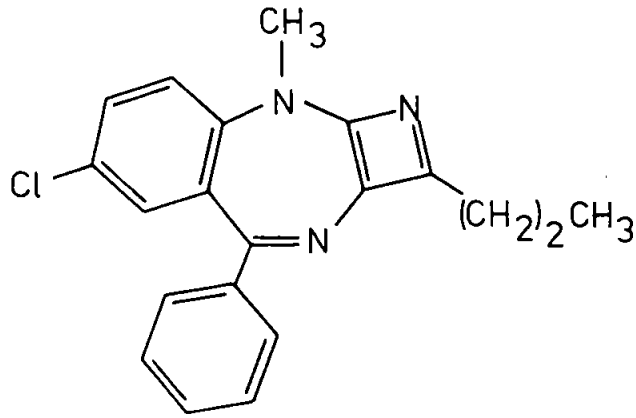
Assignment of molecular structure can be achieved without the analysis of the crystalline state by techniques which provide limited information by their use alone, but in conjunction, provide a means whereby the most probable structure may be derived. Constituent atoms are analysed by mass spectrometry, and molecular weight analysis results in determination of the number of each elemental atoms in the molecule. Nuclear magnetic resonance methods, provide a description of the chemical environment about some constituent atomic sites, enabling a possible ordering of particular groups to be achieved. Further analysis by optical methods can be made to study the relative configuration of certain optically active groups. However, the structure solution satisfying the results obtained by these

methods is often not unique and only limited information regarding the geometrical features of the molecule is obtained.

The techniques of single crystal analysis discussed in this chapter, bear a particular emphasis upon those used in the determination of the crystal structures described in the following chapters, and are progressively developed from the more general aspects of the scattering of radiation and regarded as a particular solution to the scattering process. The various analyses of X-ray diffraction data centre upon the determination of diffracted intensity, and associated phase angle, and the Fourier synthesis of the model structure with its subsequent least-squares refinement.

This thesis describes the use of the methods of X-ray crystallography to assign the structures of three compounds in the crystalline state, which were considered of interest either by virtue of their proposed novel structures or to further the comparison of known groups of biologically active compounds.

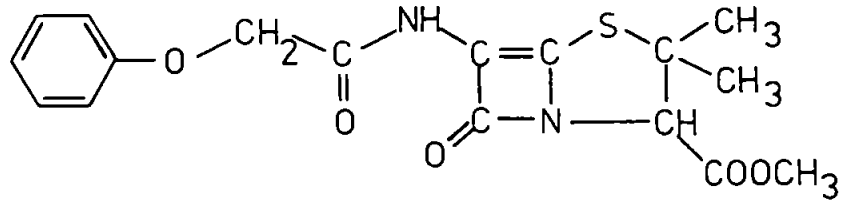
The novel pharmacologically active azete, I, is of interest because it is derived from the psychoactive drug Librium, and contains the highly strained azete system. Only a few azete compounds have been reported, and the stability of monocyclic azetes is very low.



I

Structure determination of the dehydropenicillin, II, was carried out as a preliminary to the investigation of the reported weak

antibacterial activity, suggested to result from the "unsaturated"

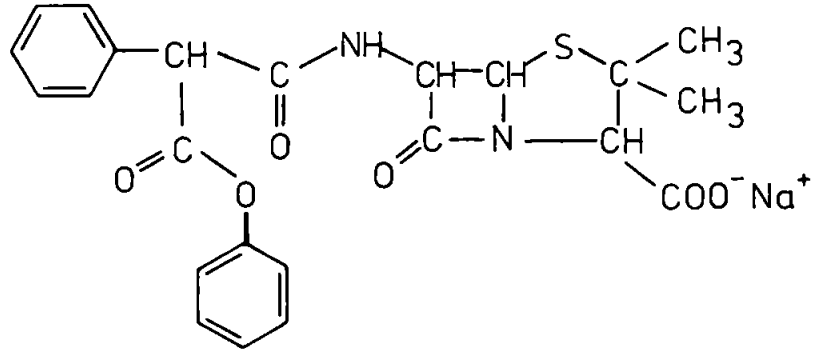


II

nature of the penicillin nucleus.

Chapters 2 and 3 describe the eventual reassignment of structure to both compounds I and II.

The esters of carbenicillin are shown to undergo change of the side-chain substituents in solution, (ref Ch. 5); investigation of the nature of the processes involved was facilitated by the X-ray structural investigations of the phenyl ester of carbenicillin, III, described in Chapter 4.



III

The configuration and conformation of the side-chain substituents in some penicillin derivatives of known structure is discussed in Chapter 5 and compared with that of three esters of carbenicillin, including III, using distinguishing features arising from H^1 nuclear magnetic resonance spectra and circular dichroism measurements.

1.1 The basis for crystal structure analysis

The laws of diffraction alone can provide the basis for crystal structure analysis; however, such laws result from the observations made from a particular state of matter ie. the crystalline state, whereas the techniques of analysis stem from the combination of fundamental aspects of matter, relating more to the scattering process itself. Diffraction can therefore be considered to emerge as a direct result of constraints applied to the scattering process by the spatial geometry within the crystal. Thus, a complete description of the basic scattering process, and its particular solution under certain constraining conditions for X-ray diffraction, such as elastic wave/matter interactions and subsequent interference phenomena, is used to form a basis for the development of the mathematical models used in the structure analyses discussed later.

The field of X-ray crystallography is well served by many standard texts; particular use has been made of the works of M.J. Buerger[1], E.W. Nuffield[2], M.F.C. Ladd and R.A. Palmer[3], M.M. Woolfson[4], G.H. Stout and L.H. Jensen[5], and International Tables for X-ray Crystallography[6].

1.2 The scattering of a wave packet

The wave-particle description of matter, developed largely from an understanding of energy/matter interactions and their eventual equivalency, has resulted in considerable information concerning the nature of energy scattering processes, in particular, those associated with the atom and its constituents.

The crystalline state provides a unique interaction with incident radiation which is described by the modification of a wave packet under the influence of a potential[7,8].

Motion under the influence of a potential V, which is appreciably

different from zero only within a sphere surrounding the origin of radius 'a', may be described by the Schrödinger equation

$$\left(-\frac{\hbar^2}{2\mu}\nabla^2 + V\right)\psi = E\psi \qquad \text{... Eqn 1.1}$$

where ψ describes a wave packet, E the energy eigenvalue associated with it and μ the reduced mass.

Simplification of Eqn. 1.1 is achieved using the substitutions $k^2 = \frac{2\mu E}{\hbar^2}$ and $U = \frac{2\mu V}{\hbar^2}$ to become

$$(\nabla^2 + k^2)\ \psi = U\psi \qquad \text{... Eqn 1.2}$$

Considering $U\psi$ as a temporary inhomogeneity , Eqn. 1.2 can be solved by the superposition of a particular solution and complementary function. Formally, a particular solution can be constructed in terms of the Green's function $G(\underset{\sim}{r}, \underset{\sim}{r}')$ which is a solution of the equation

$$(\nabla^2 + k^2)\ G\ (\underset{\sim}{r}, \underset{\sim}{r}') = -4\pi\ \delta(\underset{\sim}{r} - \underset{\sim}{r}') \qquad \text{... Eqn 1.3}$$

where the Dirac delta function has the property

$$\int \delta(\underset{\sim}{r} - \underset{\sim}{r}')\ d^3r' = 1 \qquad \text{... Eqn 1.4}$$

provided the region of integration includes $\underset{\sim}{r}' = \underset{\sim}{r}$.

Hence, by Eqn. 1.4

$$\int U\ (\underset{\sim}{r}')\ \psi\ (\underset{\sim}{r}')\ \ \delta(\underset{\sim}{r} - \underset{\sim}{r}')\ d^3r' = U(\underset{\sim}{r})\psi\ (\underset{\sim}{r}) \qquad \text{... Eqn 1.5}$$

and provides the inhomogeneity $U\psi$ as required.

Thus, the expression

$$\frac{-1}{4\pi}\int G(\underset{\sim}{r}, \underset{\sim}{r}')\ \ U(\underset{\sim}{r}')\ \psi\ (\underset{\sim}{r}')\ d^3r' \qquad \text{... Eqn 1.6}$$

solves Eqn. 1.2 adequately.

The complementary function is found as an arbitrary solution of the homogeneous equation

$$(\nabla^2 + k^2)\ \psi = 0 \qquad \text{... Eqn 1.7}$$

which is the Schrödinger equation for a free particle (no scattering) and has the solution corresponding to a plane wave. Choosing a suitable normalisation factor establishes the integral equation.

$$\psi_{\underset{\sim}{k}}(\underset{\sim}{r}) = \frac{1}{(2\pi)^{3/2}}\ e^{j\underset{\sim}{k}.\underset{\sim}{r}} - \frac{1}{4\pi}\int G(\underset{\sim}{r},\ \underset{\sim}{r}')\ U(\underset{\sim}{r}')\psi_{\underset{\sim}{k}}(\underset{\sim}{r}')\ d^3r'$$

$$\text{... Eqn 1.8}$$

as a particular set of solutions of the Schrödinger equation.

The magnitude of the wave vector $\underset{\sim}{k}$ has a definite value, fixed by the energy eigenvalue, but its direction is determined physically by the direction of incidence. A complete knowledge of $\underset{\sim}{k}$, however, does not completely define $\psi_{\underset{\sim}{k}}(\underset{\sim}{r})$ in Eqn. 1.8, for there remains an infinite choice of Green's function $G(\underset{\sim}{r},\ \underset{\sim}{r}')$. To determine suitable Green's functions, the solution to Eqn. 1.3 must be found.

$$(\nabla^2 + k^2)\ G(\underset{\sim}{r}) = -\ 4\pi\ \delta(\underset{\sim}{r}) \qquad \text{... Eqn 1.9}$$

describes a simplified expression of Eqn. 1.3

Defining $\delta(\underset{\sim}{r})$ by

$$\delta(\underset{\sim}{r}) = \frac{1}{(2\pi)^3}\int e^{j\underset{\sim}{k}'.\underset{\sim}{r}}\ d^3k' \qquad \text{... Eqn 1.10}$$

suggests the application of the Fourier transform description of $G(\underset{\sim}{r})$, well known in diffraction processes, giving

$$G(\underset{\sim}{r}) = \int g(\underset{\sim}{k}')\ e^{j\underset{\sim}{k}'.\underset{\sim}{r}}\ d^3k' \qquad \text{... Eqn 1.11}$$

Substitution in Eqn 1.9 gives

$$(\nabla^2 + k^2)\int g(\underset{\sim}{k}')\ e^{j\underset{\sim}{k}'.\underset{\sim}{r}}\ d^3k' = -\ \frac{1}{2\pi^2}\int e^{j\underset{\sim}{k}'.\underset{\sim}{r}}\ d^3k'$$

$$\text{... Eqn 1.12}$$

and hence,

$$\int g(\underset{\sim}{k}')\ (-k'^2 + k^2)\ e^{j\underset{\sim}{k}'\cdot\underset{\sim}{r}}\ d^3k'$$

$$= -\frac{1}{2\pi^2}\int e^{j\underset{\sim}{k}'\cdot\underset{\sim}{r}}\ d^3k' \qquad \text{... Eqn 1.13}$$

which in turn gives

$$g(\underset{\sim}{k}') = \frac{1}{2\pi^2}\left(\frac{1}{k'^2 - k^2}\right) \qquad \text{... Eqn 1.14}$$

furnishing the Fourier representation in reciprocal k-space, (ref § 1.5),

$$G(\underset{\sim}{r}) = \frac{1}{2\pi^2}\int \frac{e^{j\underset{\sim}{k}'\cdot\underset{\sim}{r}}}{k'^2 - k^2}\ d^3k' \qquad \text{... Eqn 1.15}$$

Evaluation of this integral over all space is conveniently carried out by expressing in terms of polar co-ordinates, letting the colatitude θ coincide with the angle between the vectors $\underset{\sim}{k}'$ and $\underset{\sim}{r}$. Thus, Eqn. 1.15 becomes

$$G(\underset{\sim}{r}) = \frac{1}{2\pi^2}\int_{-\infty}^{+\infty}\int_0^{2\pi}\int_0^{\pi} \frac{e^{jk'r\cos\theta}}{k'^2 - k^2}\ k'^2 \sin\theta\ d\theta\ d\phi\ dk'$$

$$\text{... Eqn 1.16}$$

which may more conveniently be rewritten to give

$$G(r) = -\frac{1}{\pi r}\frac{d}{dr}\int_{-\infty}^{+\infty} \frac{e^{jk'r}}{k'^2 - k^2}\ dk' \qquad \text{... Eqn 1.17}$$

following the integration over θ and ϕ.

The resulting integrand has simple poles on the real axis in the complex k' plane at $k' = \pm k$ and a solution may be found by using an integration path such as in Fig. 1.1, avoiding the poles.

Since r is taken to be positive, a closed contour produced by a semicircle, in the upper half plane, joining the path of integration along the real axis from $-\infty$ to $+\infty$ will suffice.

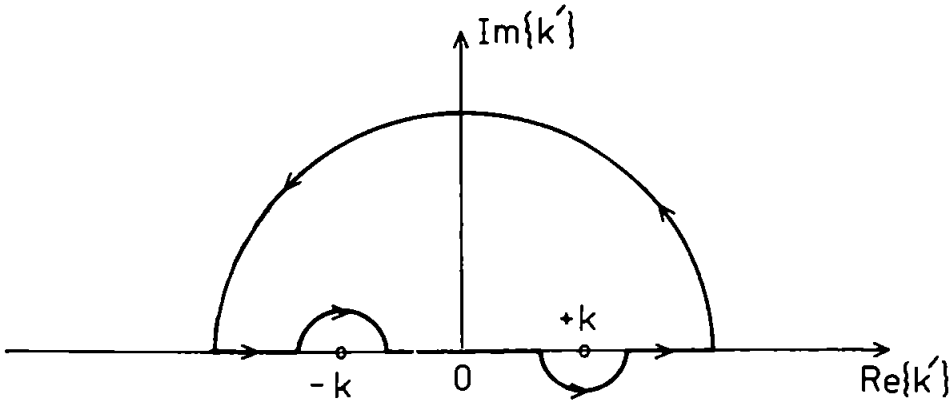


Fig. 1.1. Path of integration in the complex k' plane

Thus, Eqn. 1.17 becomes

$$G(r) = -\frac{1}{\pi r}\frac{d}{dr}\oint \frac{e^{jk'r}}{k'^2 - k^2}\,dk' \qquad \text{... Eqn 1.18}$$

in which, by the use of the residue theorem

$$\oint \frac{e^{jk'r}}{k'^2 - k^2}\,dk' = 2\pi j\left(\frac{e^{jkr}}{2k} - \frac{e^{-jkr}}{2k}\right)$$

... Eqn 1.19

Hence,

$$G(r) = \frac{e^{jkr}}{r} + \frac{e^{-jkr}}{r} \qquad \text{... Eqn 1.20a}$$

which can be expressed as

$$G(r) = G_+(r) + G_-(r) \qquad \text{... Eqn 1.20b}$$

where $G_+(r)$ and $G_-(r)$ are both Green's functions, each satisfying Eqn. 1. 9.

Thus, the Green's functions required as solutions to Eqn. 1.3 are

$$G_{\pm}(\underset{\sim}{r}, \underset{\sim}{r}') = \frac{\exp(\pm jk|\underset{\sim}{r} - \underset{\sim}{r}'|)}{|\underset{\sim}{r} - \underset{\sim}{r}'|} \qquad \text{... Eqn 1.21}$$

and substitution in the integral equation, Eqn. 1. 8, results in two distinct eigensolutions, denoted by $\psi^{(+)}$ and $\psi^{(-)}$ given by

$$\psi^{(\pm)}_{\underset{\sim}{k}}(\underset{\sim}{r}) = \frac{1}{(2\pi)^{3/2}} e^{j\underset{\sim}{k}.\underset{\sim}{r}} - \frac{1}{4\pi} \int \frac{\exp(\pm jk \left|\underset{\sim}{r} - \underset{\sim}{r}'\right|)}{\left|\underset{\sim}{r} - \underset{\sim}{r}'\right|} U(\underset{\sim}{r}')\psi^{(\pm)}_{\underset{\sim}{k}}(\underset{\sim}{r}')\, d^3r'$$

... Eqn. 1.22

In view of the fact that U exists only for values of r' <a, the integrand can be closely approximated. If r is chosen so large that the quadratic term can be neglected, and if, further, $\underset{\sim}{r}'$ in the denominator of the integrand is neglected, as is the case in all macroscopic measurement techniques, then

$$\psi^{(\pm)}_{\underset{\sim}{k}}(\underset{\sim}{r}) \sim \frac{1}{(2\pi)^{3/2}} e^{j\underset{\sim}{k}.\underset{\sim}{r}} - \frac{e^{\pm jkr}}{4\pi r} \int e^{\mp j\underset{\sim}{k}'.\underset{\sim}{r}'} U(\underset{\sim}{r}')\, \psi^{(\pm)}_{\underset{\sim}{k}}(\underset{\sim}{r}')\, d^3r'$$

... Eqn 1.23

where $\underset{\sim}{k}' = k\hat{\underset{\sim}{r}}$.

This asymptotic expression can be written as

$$\psi^{(\pm)}_{\underset{\sim}{k}}(\underset{\sim}{r}) \sim \frac{1}{(2\pi)^{3/2}} \left(e^{j\underset{\sim}{k}.\underset{\sim}{r}} + \frac{e^{\pm jkr}}{r} f^{(\pm)}_{\underset{\sim}{k}}(\hat{\underset{\sim}{r}}) \right)$$

(r large) ... Eqn. 1.24

where

$$f^{(\pm)}_{\underset{\sim}{k}}(\hat{\underset{\sim}{r}}) = -\frac{(2\pi)^{3/2}}{4\pi} \int e^{\mp j\underset{\sim}{k}'.\underset{\sim}{r}'} U(\underset{\sim}{r}')\, \psi^{(\pm)}_{\underset{\sim}{k}}(\underset{\sim}{r}')\, d^3r'$$

... Eqn. 1.25

Physically, $\psi^{(+)}$, when modified by $\exp\left(-j\frac{Et}{\hbar}\right)$, represents the outgoing solution of the Schrödinger equation. Thus, Eqn. 1.24, describes the wave function $\psi^{(+)}$ in terms of an incident planewave, of energy and direction governed by the wave vector $\underset{\sim}{k}$, and a scattered outgoing radial wave falling in amplitude

as the inverse of the distance from the scatterer and with a modified amplitude, defined as the scattering amplitude, $f_{\underset{\sim}{k}}(\hat{\underset{\sim}{r}})$. The term $f_{\underset{\sim}{k}}(\hat{\underset{\sim}{r}})$, in turn, is dependent upon the scattering potential and whether the process is elastic, (Thomson scattering), or inelastic, (Compton scattering), determines the energy of the radial wave and thus, the wave vector $\underset{\sim}{k}'$. It is therefore, the scattering amplitude and its associated phase which furnishes the means by which the properties of the process can be determined. The effective measurement of $f_{\underset{\sim}{k}}(\hat{\underset{\sim}{r}})$ forms the basis for the methods of structure analysis considered in the following discussion.

1.3 The scattering of X-rays by atoms

Section 1.2 adequately described the interaction of a wave packet, for example on X-ray photon, with a central potential such as that of a free electron. However, the electrons surrounding atoms are not free but bound into definite energy states. Thus, Thomson scattering corresponds to the electron remaining in the same energy state after scattering, whereas Compton scattering will involve the transition of the electron between energy states with the absorption or emission of discrete energy quanta.

The coherently scattered component will suffer from ordered interference and provides a systematic means of analysing the scattering centres involved. Hence, the observationally valuable part of the scattering amplitude is related to an outgoing wave of energy unchanged from that of the incident wave ie. $k' = k$. The ratio of the amplitude of the coherently scattered component from an atomic electron, contributing an idealised spherically symmetric charge distribution to that from an electron situated at the origin is defined as the scattering factor for that electron, f_e. If an atom contains Z electrons then the total ratio of amplitudes will be the sum of the individual ratios, $(fe)_i$,

for each electron and is defined as the atomic scattering factor, fa, given by

$$fa = \sum_{i=1}^{Z} (fe)_i \qquad \text{... Eqn. 1.26}$$

Scattering factors used in this work were taken from International Tables for X-ray Crystallography, Vol III, or Acta. Cryst., A24 (1968) 321.

The scattering factor describes a reduction in amplitude of the scattered wave with increase of scattering angle: at zero scattering angle $fa = Z$.

1.4 Diffraction from a crystal

Section 1.2 discussed the scattering of a photon of wave vector $\underset{\sim}{k} = \frac{2\pi}{\lambda} \hat{\underset{\sim}{k}}$ from a single scattering centre. The condition that all atoms in a three dimensional array (crystal lattice) should scatter in phase in some direction can be fulfilled by three conditions. Defining a scattering vector $\underset{\sim}{s}$, such that

$$\underset{\sim}{s} = \frac{n}{2\pi}\underset{\sim}{k} = \frac{n\hat{\underset{\sim}{k}}}{\lambda}, \quad \text{(n integer)} \qquad \text{... Eqn. 1.27}$$

the three conditions that atoms separated by $\underset{\sim}{a}$, $\underset{\sim}{b}$ or $\underset{\sim}{c}$ should scatter in phase, where the vectors $\underset{\sim}{a}$, $\underset{\sim}{b}$ and $\underset{\sim}{c}$ are the three vectors which define the array, are

$$\begin{aligned} \underset{\sim}{a} \cdot \underset{\sim}{s} &= h \\ \underset{\sim}{b} \cdot \underset{\sim}{s} &= k \\ \underset{\sim}{c} \cdot \underset{\sim}{s} &= \ell \end{aligned} \qquad \text{.... Eqns 1.28}$$

where h, k and ℓ are integers. Eqns. 1.28 are the Laue equations, so called, after the first demonstration of diffraction of X-rays from a regular crystal lattice by von Laue in 1912.

The three integers h, k and ℓ can be chosen to uniquely define

a given interference maximum, (known as the X-ray reflexion), and are the Miller indices of that reflexion. Eqns. 1.28 also describe a family of planes in the crystal space and so, therefore, do the Miller indices.

1.5 The Reciprocal Lattice

To examine the scattering vector $\underset{\sim}{s}$, and hence the crystal structure, it is necessary to describe $\underset{\sim}{s}$ in terms of three basis vectors, which themselves relate to the vectors $\underset{\sim}{a}$, $\underset{\sim}{b}$ and $\underset{\sim}{c}$ which in turn define the unit cell of the crystal ie, that parallelepiped which, reproduced by close packing in three dimensions, gives the whole crystal.

The three vectors $\underset{\sim}{a}^*$, $\underset{\sim}{b}^*$ and $\underset{\sim}{c}^*$ are used to define the reciprocal lattice where $\underset{\sim}{a}^*$, $\underset{\sim}{b}^*$ and $\underset{\sim}{c}^*$ satisfy the complete set of relationships.

$$\begin{array}{lll} \underset{\sim}{a} \cdot \underset{\sim}{a}^* = 1 & \underset{\sim}{a} \cdot \underset{\sim}{b}^* = 0 & \underset{\sim}{a} \cdot \underset{\sim}{c}^* = 0 \\ \underset{\sim}{b} \cdot \underset{\sim}{a}^* = 0 & \underset{\sim}{b} \cdot \underset{\sim}{b}^* = 1 & \underset{\sim}{b} \cdot \underset{\sim}{c}^* = 0 \\ \underset{\sim}{c} \cdot \underset{\sim}{a}^* = 0 & \underset{\sim}{c} \cdot \underset{\sim}{b}^* = 0 & \underset{\sim}{c} \cdot \underset{\sim}{c}^* = 1 \end{array} \qquad \text{... Eqns. 1.29}$$

which uniquely define $\underset{\sim}{a}^*$, $\underset{\sim}{b}^*$ and $\underset{\sim}{c}^*$ in terms of the real space vectors $\underset{\sim}{a}$, $\underset{\sim}{b}$ and $\underset{\sim}{c}$ such that

$$\underset{\sim}{a}^* = \frac{\underset{\sim}{b} \wedge \underset{\sim}{c}}{\underset{\sim}{a} \cdot (\underset{\sim}{b} \wedge \underset{\sim}{c})}$$

$$\underset{\sim}{b}^* = \frac{\underset{\sim}{c} \wedge \underset{\sim}{a}}{\underset{\sim}{a} \cdot (\underset{\sim}{b} \wedge \underset{\sim}{c})} \qquad \text{...Eqns. 1.30}$$

$$\underset{\sim}{c}^* = \frac{\underset{\sim}{a} \wedge \underset{\sim}{b}}{\underset{\sim}{a} \cdot (\underset{\sim}{b} \wedge \underset{\sim}{c})}$$

Thus, using Eqns. 1.29, the scattering vector, $\underset{\sim}{s}$, can be

defined, such that it satisfies Eqns. 1.28, by

$$\underset{\sim}{s} = h\underset{\sim}{a}^* + k\,\underset{\sim}{b}^* + \ell\,\underset{\sim}{c}^* \qquad \text{... Eqn. 1.31}$$

Thus, each set of Miller indices is related to a particular scattering vector $\underset{\sim}{s}_{hk\ell}$. The spacing between the real space planes defined by $(hk\ell)$ is given by

$$d_{hk\ell} = \frac{1}{\left|\underset{\sim}{s}_{hk\ell}\right|} \quad . \qquad \text{... Eqn. 1.32}$$

Bragg[9] showed that an X-ray beam incident at an angle $\theta_{hk\ell}$ to the family of planes $(hk\ell)$ was diffracted such that the diffraction maximum occurred along a path also at angle θ to the same planes, given that $\theta_{hk\ell}$ satisfied the relation

$$2d_{hk\ell} \sin \theta_{hk\ell} = \lambda \qquad \text{... Eqn. 1.32}$$

Hence, the scattering vector $\underset{\sim}{s}_{hk\ell}$ determines the unique Bragg angle $\theta_{hk\ell}$.

1.6 Intensity data collection and the Weissenberg method

The photographic density produced by the impingement of an X-ray photon on a film is related to the square of the amplitude of the oscillation associated with the wave packet, and thus, provides the means by which the intensity of each reflexion can be measured. The range of intensity that can be measured by a single film is limited by the saturation response of the emulsion. An increase in the available measurement range was achieved using the multiple film technique, so that each reflexion intensity was measured within the linear optical density response, given by the relation $\log_{10}\left(\frac{I}{I_o}\right)$ where the logarithmic argument recorded the fraction of total possible measurable intensity. Photographic densities were measured by the Science Research Council microdensitometer service at Daresbury, consisting of an Optronics International System P-1000 Photoscan interfaced to a Computer Automation ALPHA -16 mini-computer with 16K

of 16-bit words of core store.

A Philips PW 10/10 X-ray generator operating at 34kV, 20mA using a Cu tube and Ni filter was used to generate nearly monochromatic CuKα X-radiation (λ = 1.5418Å). All X-ray measurements were obtained using either Stoe or Nonius Weissenberg cameras. Application of the Weissenberg camera to data collection is described in refs. 3, 4 and 5. Zero layer Weissenberg photographs were obtained by the normal beam method and upper layers by the equi-inclination method. Similar exposure times were used for each layer and the inter-layer scale factors were set initially at 1; they were subsequently refined during the least-squares refinement, (§ 1.14).

1.7 Determination of Cell Dimensions

Fig 1.2 shows a greatly magnified crystal, mounted to rotate about an axis corresponding to a unit cell edge. The family of planes perpendicular to this axis will diffract to the nth order maximum at an angle Ω_n to the zero order maximum if

$$r\sin\Omega_n = n\lambda \qquad \text{..... Eqn.1.34}$$

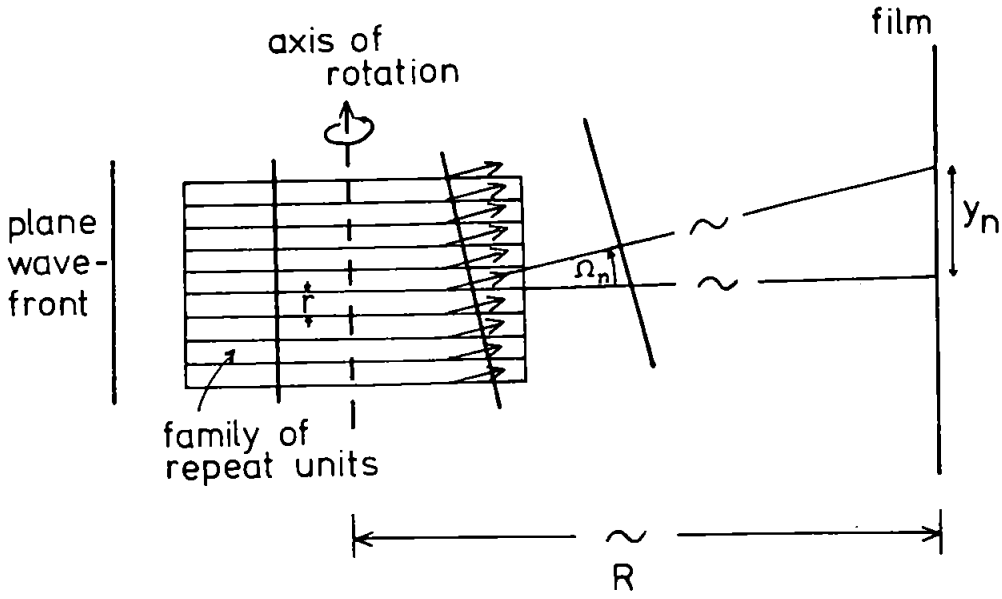


Fig.1.2 Oscillation geometry.

is satisfied, where r is the repeat distance along the rotation axis. Thus, a measurement of the separation of the zero layer and n^{th} lines, y_n, from the resulting oscillation photograph, is related to Ω_n by

$$\tan \Omega_n = \frac{y_n}{R} \qquad \text{... Eqn. 1.35}$$

Hence, r is obtained from Eqn. 1.34 as

$$r = \frac{n\lambda}{\sin\left(\tan^{-1} \frac{y_n}{R}\right)} \qquad \text{Eqn. 1.36}$$

where R is the true film radius.

The lengths of the two remaining unit cell axes were obtained from zero layer Weissenberg photographs, by measuring the perpendicular distance between equivalent axial reflexions on opposite halves of the film.

The angles between the unit cell axes, defined by

$$\alpha = \cos^{-1} (\hat{\underset{\sim}{b}} \cdot \hat{\underset{\sim}{c}})$$

$$\beta = \cos^{-1} (\hat{\underset{\sim}{a}} \cdot \hat{\underset{\sim}{c}}) \qquad \text{... Eqns. 1.37}$$

$$\gamma = \cos^{-1} (\hat{\underset{\sim}{a}} \cdot \hat{\underset{\sim}{b}})$$

were determined from the separation of the axial lines on zero level Weissenberg photographs.

Accurate cell dimensions were obtained from zero level Weissenberg photographs, calibrated using an annealed gold wire, taken about two axial directions. The separation between pairs of equivalent high angle reflexions on either half of the film was measured and the corresponding value of θ subsequently calculated gave a small statistical spread for d by Eqn. 1.32, of standard deviation ~0.03Å.

1.8 Determination of Space Group

The fundamental repetition characteristics within a crystal can be expressed in terms of one of the 14 Bravais lattices. All the possible unique combinations of symmetry elements, or point groups, places the unit cell within one of 32 crystal classes. Consideration of both the Bravais lattice and crystal class together with any translation operators describes the unit cell in terms of one of the 230 space groups.

Since X-ray diffraction is dependent upon the spatial array of atoms within a crystal, then any operation relating equivalent parts of that array will be displayed in the resulting symmetry of oscillation and Weissenberg photographs. Thus, space group determination requires knowledge of the number of molecules within the unit cell and the identification of any symmetry elements by which they are related.

The number of molecules, N, per unit cell was obtained from the measured density of the crystal using the relation

$$N = \frac{V \rho N_o}{M} \qquad \text{... Eqn. 1.38}$$

where

V = volume of unit cell, in m^3

ρ = measured density, kgm^{-3}

N_o (Avogadro's number) = 6.023×10^{26} molecules/kilo-gramme molecular weight

M = molecular weight.

Symmetry elements involving translation operators result in extinction of certain families of reflexions by destructive interference. Such symmetry elements encountered in the non-centrosymmetric space groups $P2_12_12$ and $P2_1$, and the centrosymmetric space group $P2_1/c$ discussed later include 2-fold screw axes

and glide planes.

A 2-fold screw axis (2_1) effectively halves the separation between planes normal to the axis and the reciprocal nature of the diffraction array results in doubling of the separation of interference maxima, or equivalently, causing phase cancellation of all odd-order reflexions from these planes. The 2_1 axis, however, has no simple ordered effect on the spacing of other planes and thus, only axial reflexions of order 2n + 1, (n integer), suffer regular extinction.

The effect upon the diffraction array of a glide plane is very similar to that of a 2_1 axis. Upper level Weissenberg photographs were used to distinguish between axial absences caused by a 2_1 axis, and those which resulted from rotation of the crystal about an axis in the glide plane normal to the translation vector of the glide (ref. 3 - Chapter 2).

A combination of symmetry elements within a space group results in a linear superposition of the systematic absences derived from the individual elements.

Consideration of the systematic absences alone does not uniquely define all space groups for they provide no information regarding the presence or otherwise of a centre of symmetry. A test which may be used to detect a centre of symmetry is the N(z) test, suggested by Howells, Phillips and Rogers (1950)[10], and considers a cumulative distribution curve for intensities. N(z) is the fraction of reflexions with intensities less than or equal to z times the mean intensity. Fig. 1.3 shows a theoretical plot of N(z) against z for centric and acentric distributions. The consistently lower values of N(z) in the case of the acentric, compared with those for the centric distributions, modelled the tendency for the intensity of reflexions from the noncentrosymmetric space group crystals, ($P2_1 2_1 2$ (ref Chapter 2)

and $P2_1$ (ref. Chapter 4)), to be more closely distributed about their mean than were those from the centrosymmetic crystals, ($P2_1/c$ (ref. Chapter 3)), discussed in this thesis.

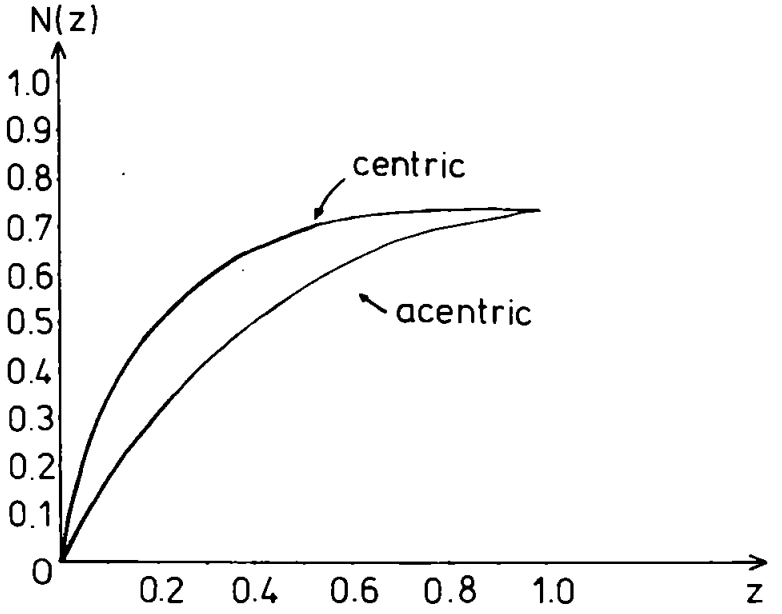


Fig. 1.3 Intensity distribution curves.

1.9 Factors affecting observed intensities

Absorption

Attenuation of the X-ray beam whilst passing through a crystal of thickness t is given by

$$I = I_o e^{-\mu_\lambda t} \qquad \text{... Eqn. 1.39}$$

where I_o is the intensity of the incident beam and I that of the emergent beam. μ_λ, the linear absorption coefficient, is expressed in terms of the mass absorption coefficient $\left(\frac{\mu}{\rho}\right)_{\lambda,\ E_i}$ for a given wavelength λ and element E_i by

$$\mu_\lambda = \rho \sum_i \frac{P_i}{100} \left(\frac{\mu}{\rho}\right)_{\lambda,\ E_i} \qquad \text{... Eqn. 1.40}$$

where ρ is the density of the compound composed of P_i% of each element E_i.

Correction factors are integral parts of many crystallographic programs provided the crystal shape is known. Numerical approximations are used to provide a value for the average length of the X-ray beam in the crystal for given reflexions. Interpolation of intra-crystal path lengths between fixed 'grid' points, separated by known path lengths within the crystal, was used to provide an absorption correction from the SHELX[21] 'ABSC' routine (ref. Chapter 4).

Primary and Secondary Extinction

Whilst the orientation of the crystal is such as to meet the Bragg condition, every point in the crystal is exposed to both the incident beam and part of the diffracted beam. Any systematic effect by crystal interaction with a diffracted beam, results in primary extinction, and assumes a perfectly regular geometry within the crystal, which in practice is confined only to microscopic regions of the crystal known as mosaic blocks. The mosaic structure of such ideally imperfect crystals, introduced by kinetic/thermal energy during crystallisation, is such that effects due to primary extinction can be considered random and therefore, neglected.

Observations relating the intensities of reflexions relies upon the assumption that all planes contributing to a reflexion receive the same incident intensity. However, in the case of the most intense reflexions, transmission of the incident intensity through the crystal is attenuated by reflexion from those planes first encountered by the beam, thus, planes deeper in the crystal receive less radiation and contribute a reduced intensity to the diffracted beam, such that the reflexion suffers from secondary extinction. A compromise was therefore made between a reduction in crystal size and the resulting lowering of diffraction intensity such that only a few very strong reflexions were affected, and these were omitted at the final stage of refinement.

Atomic Vibration - the temperature factor

Atoms, bound within a crystal structure, are consequently at a potential energy minimum related to the bonding processes within the molecule. Thus, any additional random energy, for example, a rise in temperature, will result in vibrational motion of the atoms. The electron cloud is, therefore, spread over a larger region than if the atom were totally at rest, resulting in a faster fall off of scattering amplitude with increasing Bragg angle. The effective atomic scattering factor, $(f_a)_T$, for an atom undergoing isotropic vibration may be expressed in terms of that for the stationary atom, f_a, by

$$(f_a)_T = f_a \, e^{\frac{-B(\sin^2 \theta)}{\lambda^2}} \qquad \text{... Eqn. 1.41}$$

where the exponential term is known as the Debye-Waller factor[11,12]. The isotropic temperature factor, B, is related to the mean square displacement, $\overline{U^2}$, of the atom, normal to the reflecting plane by

$$B = 8\pi^2 \, \overline{U^2} \qquad \text{... Eqn. 1.42}$$

Anisotropic vibration is described in terms of a symmetric 3 x 3 matrix of the mean-square amplitudes of vibration U_{ij}, such that Eqn. 1.41 becomes

$$(f_a)_T = f_a \exp\left[-2\pi^2 \left(U_{11} h^2 a^{*2} + U_{22} k^2 b^{*2} + U_{33} \ell^2 c^{*2} + 2U_{12} hka^*b^* + 2U_{13} h\ell a^* c^* + 2U_{23} k\ell b^* c^*\right)\right] \qquad \text{... Eqn. 1.43}$$

Polarisation

X-ray reflexion by a crystal plane is more efficient for that component of the incident beam which is parallel to the plane than that which is perpendicular to it. Thus, an unpolarised incident X-ray beam becomes partially polarised upon diffraction. For such an unpolarised incident beam, the measured intensity must be corrected by a factor $\frac{1}{p}$, where

$$p = \frac{1 + \cos^2 2\theta}{2} \qquad \text{... Eqn. 1.44}$$

defines the polarisation, p, in terms of the Bragg angle.

The Lorentz-factor

Methods of collecting intensity data, in general, involve the rotation of the crystal in the X-ray beam such that each reciprocal lattice point passes through the sphere of reflexion, satisfying a given Bragg condition. Thus, the time taken for the reciprocal lattice point to pass through the sphere varies with its position in reciprocal space and its direction of approach. For a Weissenberg equi-inclination setting angle of μ, the measured intensity is corrected by the application of a factor $\frac{1}{L}$, where

$$L = \frac{\sin\theta}{\sin 2\theta \sqrt{(\sin^2\theta - \sin^2\mu)}} \qquad \text{... Eqn. 1.45}$$

is the Lorentz factor.

Thus, the corrected intensity of a given reflexion $I(hk\ell)$ is obtained from the measured intensity by

$$I(hk\ell) = \frac{I(hk\ell)}{Lp} \text{ (measured)} \qquad \text{... Eqn. 1.46}$$

1.10 The Structure Factor

In section 1.2, the solution of the scattering process, expressed in terms of the Green's function given by the Fourier transform in Eqn. 1.11, describes the diffraction pattern in terms of the scattering object. In the case of X-ray diffraction, the scattering object is the electron density $\rho(\underset{\sim}{r})$ and the diffraction pattern $F(\underset{\sim}{k})$ of the unit cell is therefore the Fourier transform of the electron density within that cell of Volume V.

Hence,

$$F(\underset{\sim}{k}) = \int_V \rho(\underset{\sim}{r})\, e^{j\underset{\sim}{k}\cdot\underset{\sim}{r}}\, d^3r \qquad \text{... Eqn. 1.47}$$

and

$$\rho(\underset{\sim}{r}) = \int_{V^*} F(\underset{\sim}{k})\, e^{-j\underset{\sim}{k}\cdot\underset{\sim}{r}}\, d^3k \qquad \text{... Eqn. 1.48}$$

where $V^* = \frac{1}{V}$ is the reciprocal lattice unit cell volume.

A crystal is defined by the convolution of the unit cell with the crystal lattice, thus the Fourier transform (F T) of the crystal ie. the complete X-ray diffraction pattern, by the convolution theorem can be expressed as $\sum_{hk\ell} F(\underset{\sim}{s}_{hk\ell}) = FT\{$ unit cell * reciprocal lattice $\}$

$$= F(\underset{\sim}{k}) \times \text{reciprocal lattice} \qquad \text{... Eqn. 1.49}$$

Thus, $F(\underset{\sim}{s}_{hk\ell})$, where $\underset{\sim}{s}_{hk\ell}$ are the scattering vectors given by Eqn. 1.31, exists only at the discrete reciprocal lattice points. Hence, Eqns. 1.47 and 1.48 reduce to

$$F(\underset{\sim}{s}_{hk\ell}) = \int_V \rho(\underset{\sim}{r})\, e^{j2\pi \underset{\sim}{s}_{hk\ell}\cdot\underset{\sim}{r}}\, d^3r \qquad \text{... Eqn. 1.50}$$

$$\rho(\underset{\sim}{r}) = \frac{1}{V}\sum_{(h\,k\,\ell)} F(\underset{\sim}{s}_{hk\ell})\, e^{-j2\pi \underset{\sim}{s}_{hk\ell}\cdot\underset{\sim}{r}} \qquad \text{... Eqn. 1.51}$$

The vectors $\underset{\sim}{s}_{hk\ell}$ are described by Miller indices h, k and ℓ, and so, therefore, $F(\underset{\sim}{s}_{hk\ell})$ can be written as the familiar structure factor $F(hk\ell)$. Splitting the electron density in Eqn. 1.50 into the individual electron densities, $\rho_n(\underset{\sim}{r}')$ for each of the N atoms making up the unit cell, $F(hk\ell)$ becomes

$$F(hk\ell) = \sum_{n=1}^{N} \int_V \rho_n(\underset{\sim}{r}')\, e^{j2\pi \underset{\sim}{s}_{hk\ell}\cdot(\underset{\sim}{r}_n + \underset{\sim}{r}')}\, d^3r'$$

$$= \sum_{n=1}^{N} (f_a)_n\, e^{j2\pi \underset{\sim}{s}_{hk\ell}\cdot\underset{\sim}{r}_n} \qquad \text{... Eqn. 1.52}$$

where $\underset{\sim}{r}_n$ is the vector position of the nth atom and $(f_a)_n$ is the atomic scattering factor given in Eqn. 1.26.

The structure factor is therefore complex and may be expressed as

$$F\ (hk\ell) = \left|F\ (hk\ell)\right| \quad e^{j\phi_{hk\ell}} \qquad \text{... Eqn. 1.53}$$

where $\left|F\ (hk\ell)\right|$ is the ratio of the scattering amplitude from the unit cell to that from a point electron and $\phi_{hk\ell}$ gives the phase relative to that of a point electron situated at the unit cell origin. Calculation of the structure factor is facilitated by symmetry reduction of Eqn. 1.52 specific to each space group (the programs in Appendix A use the symmetry reduced expressions for $F\ (hk\ell)$ given in International Tables for X-ray Crystallography , Vol. 1)[8].

1.11 The Wilson Plot

Assuming that the temperature factor, B, is the same for each atom and is isotropic, the structure factor modulus $\left|F\ (hk\ell)_R\right|$, for atoms at rest, is related to the measured intensity by

$$I\ (hk\ell) = K \left|F\ (hk\ell)_R\right|^2 \exp\left(-2B\ \left(\frac{\sin^2\theta}{\lambda^2}\right)\right) \qquad \text{... Eqn. 1.54}$$

where K is a scale factor. To place the relative intensities $I\ (hk\ell)$, and thus $\left|F\ (hk\ell)\right|^2$, on an approximately absolute basis, K and B must be determined.

The theoretical average absolute intensity, $\langle I_{abs}\rangle$, is given by

$$\langle I_{abs}\rangle = \sum_{i=1}^{N} (f_{a_i})_T^2 \qquad \text{... Eqn. 1.55}$$

for N atoms in the unit cell.

Thus, since B is assumed constant, by Eqn. 1.41 and 1.55

$$\langle I_{abs}\rangle = \exp\left(-2B\ \left(\frac{\sin^2\theta}{\lambda^2}\right)\right) \sum_{i=1}^{N} f_{a_i}^2$$

$$= \exp\left(-2B\ \left(\frac{\sin^2\theta}{\lambda^2}\right)\right) \left\langle \left|F\ (hk\ell)_R\right|^2 \right\rangle \qquad \text{... Eqn. 1.56}$$

Hence, by Eqn. 1.54

$$\frac{\langle I(hk\ell)\rangle}{\sum_{i=1}^{N} f_{a_i}^2} = K \exp\left(-2B\,\frac{(\sin^2\theta)}{\lambda^2}\right) \quad \text{... Eqn. 1.57}$$

To form a linear relation, Eqn. 1.57 becomes

$$\ln\left(\frac{\langle I\,(hk\ell)\rangle}{\sum_{i=1}^{N} f_{a_i}^2}\right) = \ln K - 2B\,\frac{(\sin^2\theta)}{\lambda^2} \quad \text{... Eqn. 1.58}$$

However, the f_a's are not constant, but vary with scattering angle as $\frac{\sin\theta}{\lambda}$. Division of reciprocal space into thin concentric shells minimises the effect of this variation. Thus, $\langle I\,(hk\ell)\rangle_\theta$, is taken as the average value of the relative intensities within each shell and the f_{a_θ}'s are chosen suitably for that shell.

From Eqn. 1.58, a plot of $\ln\left(\frac{\langle I\,(hk\ell)\rangle}{\sum_{i=1}^{N} f_{a_i}^2}\right)_\theta$ against $\frac{\sin^2\theta}{\lambda^2}$ is a straight line of slope $-2B$ and co-ordinate intercept $\ln K$. The graph is termed a Wilson plot (A.J.C.Wilson[13]). The theory, Eqn. 1.54, assumes a random distribution of identical atoms throughout the unit cell, in practice it is found that the Wilson plot can be used to determined values of K and B adequate for preliminary data analysis without this restriction (ref. Ch. 4).

1.12 Structure determination methods

The intensity of an X-ray reflexion from a crystal is proportional to $|F(hk\ell)|^2$ and thus only the moduli of the structure factors are directly obtainable from the diffraction data. The solution of a structure rests with the computation of the electron density from Eqn. 1.51 which requires the determination of the phases of a large number of reflexions.

There are two major techniques which can lead to a full structure determination; the heavy atom method and direct methods. These techniques are particularly relevant to the present work and their application is discussed in detail.

Heavy Atom Technique

A. L. Patterson[14] defined the centrosymmetric function $P(\underset{\sim}{r})$ in terms of the autocorrelation of the electron density $\rho(\underset{\sim}{r})$ as

$$P(\underset{\sim}{r}) = P(-\underset{\sim}{r}) = V(\rho(\underset{\sim}{r}) * \rho(\underset{\sim}{r}))$$

$$= V\int_V \rho(\underset{\sim}{r}')\, \rho(\underset{\sim}{r}' + \underset{\sim}{r})\, d^3r' \qquad \text{... Eqn. 1.59}$$

and by Eqn. 1.51 this becomes

$$P(\underset{\sim}{r}) = \frac{1}{V} \sum_{(hk\ell)} \left|F(hk\ell)\right|^2 e^{-j2\pi \underset{\sim}{s}_{hk\ell}\cdot\underset{\sim}{r}} \qquad \text{... Eqn. 1.60}$$

Since $\rho(\underset{\sim}{r})$ depends upon the number of electrons in the atom, Z, $P(\underset{\sim}{r})$ has peaks of weight $Z_i\, Z_j$ at $\underset{\sim}{r}_i - \underset{\sim}{r}_j$ where i, j take all values from 1 to N, the number of atoms in the unit cell. Computation of $P(\underset{\sim}{r})$ requires no knowledge of the phases, $\phi_{hk\ell}$, of the structure factors, $F(hk\ell)$. Thus, provided the atomic number of an atom is sufficiently large, ($\sim$ twice that of the average of the remaining atoms for structures of the size discussed later) the vector between it and its symmetry related atoms may be found, which, with a suitable choice of origin, gives the position of that 'heavy' atom within the unit cell.

If the electron density $\rho(\underset{\sim}{r}')$ for each atom were concentrated at the atom origin ($\underset{\sim}{r}' = 0$), then Eqn. 1.59 would result in more discernable peaks. Such a process is equivalent to considering a point atom at rest. By a rearrangement of Eqn. 1.41, the scattering factor for an atom at rest is given by

$$f_a = (f_a)_T \exp\left(B\frac{(\sin^2\theta)}{\lambda^2} \right) \qquad \text{... Eqn. 1.61}$$

A point atom at rest has a scattering factor equal to its

atomic number, thus, by simple identity

$$Z = \frac{Z}{f_a} \quad (f_a)_T \exp\left(B \frac{(\sin^2\theta)}{\lambda^2}\right) \qquad \text{... Eqn. 1.62}$$

and since

$$\left|F(hk\ell)\right| = \sum_{i=1}^{N} (f_{a_i})_T \; e^{j2\pi \underline{s}_{hk\ell}\cdot \underline{r}_i} \qquad \text{... Eqn. 1.63}$$

then the modulus of the structure factor for point atoms at rest is

$$\left|F(hk\ell)\right|_{point} = \sum_{i=1}^{N} Z_i \; e^{j2\pi \underline{s}_{hk\ell}\cdot \underline{r}_i}$$

$$= \frac{\sum_{i=1}^{N} Z_i}{\sum_{i=1}^{n} f_{a_i}} \left|F(hk\ell)\right| \exp\left(B\frac{(\sin^2\theta)}{\lambda^2}\right) \qquad \text{... Eqn. 1.64}$$

Use of $\left|F(hk\ell)\right|^2_{point}$ in Eqn. 1.60 results in a sharpened Patterson function with the advantage of more localised peaks with consequently less overlap.

Oversharpening can introduce peaks due to diffraction ripples, which, together with series termination effects caused by the finite range of h, k and ℓ used in the summation can lead to spurious results. Use of both the Patterson and sharpened Patterson functions together gave the most reliable heavy atom positions. The particular crystal system and its space group symmetry enables Eqn. 1.60 to be reduced to its simplest form, as expressed, for example, in the computation for space group $P2_12_12$ in Appendix A.

Direct Methods

The determination of relationships between the structure factors can lead to sufficient information about their phases to obtain the electron density directly from Eqn. 1.51.

For any complex numbers a_i, b_i, the Cauchy inequality is given by

$$\left|\sum_{i=1}^{N} a_i b_i\right|^2 \leq \sum_{i=1}^{N} |a_i|^2 \sum_{i=1}^{N} |b_i|^2$$

... Eqn. 1.65

Harker and Kasper (1948)[15] applied this relation to structure factors. In order to consider relating structure factors for any h, k and ℓ, it is necessary to normalise all $F(hk\ell)$ to remove that affect due to sin θ fall off of the scattering factors. Such normalised structure factors are often defined by

$$|E(hk\ell)| = \frac{|F(hk\ell)|}{\sqrt{\left(\sum_{i=1}^{N} (f_{a_i})_T^2\right)}\,\varepsilon} \qquad \text{... Eqn. 1.66}$$

and can be written

$$E(hk\ell) = \sum_{i=1}^{N} n_i \, e^{j2\pi \underset{\sim}{s}_{hk\ell}\cdot \underset{\sim}{r}_i} \qquad \text{... Eqn. 1.67}$$

where $n_i = \dfrac{(f_{a_i})_T}{\sqrt{\left(\sum_{i=1}^{N} (f_{a_i})_T^2\right)}}$ ... Eqn. 1.68

In the case of a centrosymmetric structure, the phase $\phi_{hk\ell}$ is given by 0 or π and so only the real part of $E(hk\ell)$ need be considered ie, from Eqn. 1.67

$$E(hk\ell) = \sum_{i=1}^{n} n_i \cos 2\pi \underset{\sim}{s}_{hk\ell}\cdot \underset{\sim}{r}_i \qquad \text{... Eqn. 1.69}$$

Letting $a_i = \sqrt{n_i}$ and $b_i = \sqrt{(n_i)} \cos 2\pi \underset{\sim}{s}_{hk\ell}\cdot \underset{\sim}{r}_i$ the Cauchy inequality, Eqn. 1.65, becomes

$$E(hk\ell)^2 \leq \sum_{i=1}^{N} n_i \sum_{i=1}^{N} n_i \cos^2 2\pi \underset{\sim}{s}_{hk\ell}\cdot \underset{\sim}{r}_i \qquad \text{... Eqn. 1.70}$$

which reduces to

$$E(hk\ell)^2 \leq \sum_{i=1}^{N} n_i \left(\frac{1}{2}\sum_{i=1}^{N} n_i + \frac{1}{2}\sum_{i=1}^{N} n_i \cos 2\pi 2\underset{\sim}{s}_{hk\ell}\cdot \underset{\sim}{r}_i\right)$$

... Eqn. 1.71

by a trigonometrical identity.

Defining a unitary structure factor

$$|U(hk\ell)|^2 = \frac{|E(hk\ell)|^2}{\left(\sum_{i=1}^{N} n_i\right)^2} \qquad \text{... Eqn. 1.72}$$

Eqn. 1.71 becomes

$$U(hk\ell)^2 \leq \frac{1}{2} + \frac{1}{2} U(2h\ 2k\ 2\ell) \qquad \text{... Eqn. 1.73}$$

which written as

$$U(2h\ 2k\ 2\ell) \geq 2U(hk\ell)^2 - 1 \qquad \text{... Eqn. 1.74}$$

enables the sign of $U(2h\ 2k\ 2\ell)$, and hence $E(2h\ 2k\ 2\ell)$, to be determined from the magnitude of $E(hk\ell)$, provided the magnitude of $U(hk\ell)$ is sufficiently large.

Many such inequalities may be found by suitable partitioning of $E(hk\ell)$ taking into account any possible symmetry reduction within the space group concerned.

If inequalities are produced by taking the sum and difference of $E(hk\ell)$ and $E(h'\ k'\ \ell')$, then, assuming that $|E(hk\ell)|$ and $|E(h'k'\ell')|$ are both large enough,

$$s(hk\ell)\ s(h'\ k'\ \ell')\ s(h-h',\ k-k',\ \ell-\ell') = 1 \qquad \text{... Eqn. 1.75}$$

where $s(hk\ell)$ refers to the sign of $E(hk\ell)$. Sayre[16], Cochran[17] and Zachariasen[18] (1952) separately showed that Eqn. 1.75 is approximately true even for $|E|$'s smaller than was necessary to satisfy the inequality relations. Eqn. 1.75 provides the means of computing a sign - relation expansion pathway for all E's considered large enough to satisfy or nearly satisfy the inequality relations.

For non-centrosymmetric structures, $\phi_{hk\ell}$ must be determined by the use of general phase relations. From Sayre[16] it was suggested that if the phases of a few reflexions are known, then

an expectation value for others may be estimated by

$$\langle\phi_{hk\ell}\rangle = \phi_{h'k'\ell'} + \phi_{h-h',\ k-k',\ \ell-\ell'} \qquad \text{... Eqn. 1.76}$$

Further expectation values may be found by the tangent expression

$$\langle\tan\phi_{hk\ell}\rangle = \frac{\sum_{(h'k'\ell')} |E(h'k'\ell')||E(h-h',\ k-k',\ \ell-\ell')|\sin(\phi_{h'k'\ell'}+\phi_{h-h',\ k-k',\ \ell-\ell'})}{\sum_{(h'k'\ell')} |E(h'k'\ell')||E(h-h',\ k-k',\ \ell-\ell')|\cos(\phi_{h'k'\ell'}+\phi_{h-h',\ k-k',\ \ell-\ell'})}$$

... Eqn. 1.77

derived by Karle and Hauptman (1956)[23].

Cochran[19] (1955) investigated the general form of the probability distribution of $\langle\phi_{hk\ell}\rangle$ and showed that the probability of $\phi_{hk\ell}$ being within 20° of $\langle\phi_{hk\ell}\rangle$ is 0.31 and the probability that it is within 40° is 0.57.

Application of Direct Methods

The sign relations and the tangent formula predict structure factor phases from a starting set of predetermined phases. In the structures under consideration, three of the starting set phases must be chosen so as to fix the origin of the unit cell on each of its three axes. Since, three linearly independent vectors are necessary to define any Euclidean space uniquely, then the three starting set phases must be those of reflexions whose associated scattering vectors $\underset{\sim}{s}_{hk\ell}$ are linearly independent. Using the three reciprocal axis vectors $\underset{\sim}{a}^*$, $\underset{\sim}{b}^*$ and $\underset{\sim}{c}^*$ as a set of basis vectors then by the definition of $\underset{\sim}{s}_{hk\ell}$ (Eqn. 1.31),

$$\underset{\sim}{s}_{hk\ell} = \begin{pmatrix} h \\ k \\ \ell \end{pmatrix} = \underset{\sim}{h} \qquad \text{... Eqn. 1.78}$$

A set of n vectors are said to be linearly dependent if there exists a set of n integers a, ... a_n, not all zero such that

$$\sum_{i=1}^{n} a_i \underset{\sim}{h}_i = 0 \qquad \text{... Eqn. 1.79}$$

Since the starting set must be those phases of some of the most intense reflexions, Eqn. 1.79, alone, would restrict the possibility of even finding a starting set. However, symmetry within each space group allows one of a number of permissible origins to be chosen so that certain linear combinations of phases whose values are independent of the choice of permissible origin remain unchanged. Such linear combinations are structure seminvariants. Eqn. 1.79 can thus be modified to become

$$\sum_{i=1}^{n} a_i \underset{\sim}{h}_i = 0 \pmod{\underset{\sim}{\omega}_s} \qquad \text{... Eqn. 1.80}$$

ie, for the vectors $\underset{\sim}{h}_i$ to be linearly independent modulo $\underset{\sim}{\omega}_s$, there must not exist any set of n integers $a_i \ldots a_n$, at least one of which being incongruent to zero modulo ω_i, for some i, such that Eqn. 1.80 holds, where $\underset{\sim}{\omega}_s$ is defined as the seminvariant modulus, having components ω_i, and is defined by the space group.

Thus the three origin determining reflexions must be chosen such that the triple $\underset{\sim}{h}_1$, $\underset{\sim}{h}_2$ and $\underset{\sim}{h}_3$ is linearly independent modulo $\underset{\sim}{\omega}_s$ ie. such that the vectors $\underset{\sim}{h}_1$, $\underset{\sim}{h}_2$, $\underset{\sim}{h}_3$, $\underset{\sim}{h}_1+\underset{\sim}{h}_2$, $\underset{\sim}{h}_2+\underset{\sim}{h}_3$, $\underset{\sim}{h}_3+\underset{\sim}{h}_1$ and $\underset{\sim}{h}_1+\underset{\sim}{h}_2+\underset{\sim}{h}_3$ are each linearly independent modulo $\underset{\sim}{\omega}_s$.[22,23]

In non-centrosymmetric space groups, the phase of a further reflexion must be fixed in order to define the enantiomorph. The enantiomer of a structure gives rise to a change of sign of $\phi_{hk\ell}$. The enantiomorph determining reflexion must be chosen with phase other than 0 or π (since these are not affected by change of $+\phi$ to $-\phi$) and must be a structure seminvariant. The phase of the enantiomorph reflexion is thus chosen to lie within the range 0 to π or π to 2π. Certain space groups restrict the choice to $\pm\frac{\pi}{2}$ by their inherent symmetry. Otherwise, a choice of $\frac{\pi}{4}$ or $\frac{3\pi}{4}$ will give a maximum

error of $\pm\frac{\pi}{4}$.

The use of a starting set, found by the above rules, may be sufficient to determine the phases of a large number of reflexions of high E. If the number of reliably estimated phases is not large enough, then many trials using less reliable estimation of phase may be carried out using different starting sets, known as the multisolution method, (MULTAN[20], SHELX[21]).

Further relations between $E(hk\ell)$'s have been developed by Karle and Karle (1964, 1966)[24,25], using a symbolic addition method in which a limited number of reflexions with high $|E(hk\ell)|$ and many interactions are given symbols to represent their phases and solutions evaluating these symbols are subsequently related to the auxiliary phase determining relations, already described, to form a reliability check and the possibility of a multisolution approach within the expansion pathway.

1.13 Structure Completion

The previous sections described methods of obtaining the phase of those reflexions either associated with the position of a heavy atom, or which take part in more general phase relationships. In the first case, the structure has to be extended to determine the remaining atomic positions, whereas, in the second case, often only a partial structure can be determined and the structure remains to be completed.

Fourier F_o-Synthesis

The electron density synthesis Eqn. 1.51 is calculated using as Fourier coefficients the observed structure factor amplitudes combined with calculated phases, based on atomic positions included in the model. Atoms included in the model appear with increased peak height together with the appearance of smaller peaks suggesting

positions of further atoms in the structure.

If phases used in the synthesis are computed by direct methods then their associated $|E(hk\ell)|$'s can be used to produce an 'E-map'. Atomic positions are indicated by the higher peaks in the map. Further atomic positions may be found by the Fourier method using phase angles calculated from the accepted atomic positions and measured $|F(hk\ell)|$'s.

Syntheses used in the early stages of structure determination often suffer from series termination effects that cause spurious rippling in the map especially if a restricted set of amplitudes is used; (this caused particular problems in the structure determination described in Ch. 4).

Difference ΔF-Synthesis

The difference, $\Delta\rho(\underset{\sim}{r})$, between the observed electron density, as given by F_o synthesis, and the calculated electron density from the structure model is defined as

$$\Delta\rho(\underset{\sim}{r}) = \frac{1}{V} \sum_{(hk\ell)} (|F_o(hk\ell)| - |F_c(hk\ell)|)\, e^{j\alpha_c}\, e^{-j2\pi \underset{\sim}{s}_{hk\ell}\cdot\underset{\sim}{r}} \qquad \text{... Eqn. 1.81}$$

where α_c is the phase of $F_c(hk\ell)$.

Atoms which are correctly placed do not give rise to peaks on this map, but missing atoms are shown by distinct positive peaks and incorrectly placed atoms by negative 'holes'. Careful use of difference syntheses can result in information concerning assignment of atom type due to the difference in electron density between alternatives. Anisotropic thermal vibration may also be distinguished from the appearance of asymmetrically placed peaks and 'holes' surrounding given atom positions.

Optimisation of the information obtained from the ΔF synthesis

was used to check the placing of hydrogen atoms within the structure model following geometrical calculation of their positions.

1.14 Least-Squares Refinement and Residual Index, R

The structure factor F(hkℓ), as described by

$$F(hk\ell) = \sum_{i=1}^{n} f_{a_i} \, e^{-B_i \frac{(\sin^2\theta)}{\lambda^2}} \, . e^{j2\pi \underset{\sim}{s}_{hk\ell} \cdot \underset{\sim}{r}_i} \qquad \text{... Eqn. 1.82}$$

is a function of the position vector $\underset{\sim}{r}_i$ of each atom and of the temperature factor B_i (or U_{ij}).

For small errors in these parameters, an extension of the total differential gives

$$\Delta F(hk\ell) - \sum_{i=1}^{N} \left(\frac{\partial F(hk\ell)}{\partial B_i} . \Delta B_i + \frac{\partial F(hk\ell)}{\partial \underset{\sim}{r}_i} . \Delta \underset{\sim}{r}_i \right) = 0$$

... Eqn. 1.83

If, however, ΔF(hkℓ) is given by the difference between the observed and calculated structure factor, $(F_o(hk\ell) - F_c(hk\ell))$ and F(hkℓ) is substituted by F_c (hkℓ) within the summation, Eqn. 1.83 is usually not identically zero. A revised calculated structure factor $F_c'(hk\ell)$, may therefore be obtained by

$$F_c'(hk\ell) = F_c(hk\ell) + \sum_{i=1}^{N} \left(\frac{\partial F_c(hk\ell)}{\partial B_i} \cdot \Delta B_i + \frac{\partial F_c(hk\ell)}{\partial \underset{\sim}{r}_i} . \Delta \underset{\sim}{r}_i \right)$$

... Eqn. 1.84

and thus,

$$F_o(hk\ell) - F_c'(hk\ell) = \Delta F(hk\ell) - \sum_{i=1}^{N} \left(\frac{\partial F_c(hk\ell)}{\partial B_i} \cdot \Delta B_i + \frac{\partial F_c(hk\ell)}{\partial \underset{\sim}{r}} . \Delta \underset{\sim}{r}_i \right)$$

... Eqn. 1.85

The range of (hkℓ) provides a set of equations of

the form 1.85 and a least-squares solution for the error in the parameters is calculated which minimises

$$D = \sum_{(h\ k\ \ell)} (F_o(hk\ell) - F_c'(hk\ell))^2 \qquad \text{... Eqn. 1.86}$$

Convergence to the best set of values is indicated by the minimised static value of the residual index, R, after successive cycles of refinement where

$$R = \frac{\sum_{(h\ k\ \ell)} \left| |F_o(hk\ell)| - |F_c(hk\ell)| \right|}{\sum_{(h\ k\ \ell)} |F_o(hk\ell)|} \qquad \text{... Eqn. 1.87}$$

1.15 Accuracy of Bond Lengths and Angles

Bond lengths and angles are functions of the atomic position vectors $\underset{\sim}{r}_i$ which are parameters of the least-squares refinement. The error, in terms of standard deviation, of a function f related to n independent variables x_i (i = 1 to n) is given by

$$\sigma_f = \sqrt{\left(\sum_{i=1}^{n} \left[\frac{\partial f}{\partial x_i}\right]^2 \sigma^2_i \right)} \qquad \text{... Eqn. 1.88}$$

where σ_i^2 is the variance of variable x_i.

The bond length, as a function of the position vectors $\underset{\sim}{r}_1$ and $\underset{\sim}{r}_2$ between atoms 1 and 2 is given by

$$\ell_{12} = |\underset{\sim}{r}_2 - \underset{\sim}{r}_1| = |\underset{\sim}{r}_{12}| \qquad \text{... Eqn. 1.89}$$

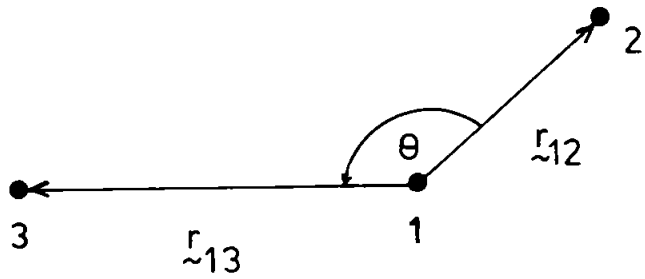
which reduces to

$$\ell_{12} = \sqrt{(x_{12}^2 a^2 + y_{12}^2 b^2 + z_{12}^2 c^2)} \qquad \text{... Eqn. 1.90}$$

where x , y and z are the components of $\underset{\sim}{r}$ related to orthogonal axes of units a,b and c respectively.

Thus, if the atoms are uncorrelated, the standard deviation in ℓ is given by Eqn. 1.88 as

$$\sigma_\ell = \sqrt{\left((\sigma^2_{x_1} + \sigma^2_{x_2}) \left(\frac{x_{12}\, a}{\ell}\right)^2 + (\sigma^2_{y_1} + \sigma^2_{y_2}) \left(\frac{y_{12} b}{\ell}\right)^2 + (\sigma^2_{z_1} + \sigma^2_{z_2}) \left(\frac{z_{12} c}{\ell}\right)^2 \right)} \qquad \text{... Eqn. 1.91}$$



Similarly, the bond angle, θ, between bonds 1-2 and 1-3 may be obtained by the scalar product of the interatomic vectors ie.

$$\underset{\sim}{r}_{12} \cdot \underset{\sim}{r}_{13} = \ell_{12}\,\ell_{13} \cos\theta \qquad \text{... Eqn. 1.92}$$

which when related to orthogonal axes gives

$$\theta = \cos^{-1} \frac{(x_{12}\,x_{13} + y_{12}\,y_{13} + z_{12}\,z_{13})}{\ell_{12}\,\ell_{13}} \qquad \text{... Eqn. 1.93}$$

and therefore by equation 1.88

$$\sigma_\theta = \left(\frac{\sigma^2_{\underset{\sim}{r}_1}\,\ell^2_{23}}{\ell^2_{12}\,\ell^2_{13}} + \frac{\sigma^2_{\underset{\sim}{r}_2}}{\ell^2_{12}} + \frac{\sigma^2_{\underset{\sim}{r}_3}}{\ell^2_{13}} \right)^{\frac{1}{2}} \qquad \text{... Eqn. 1.94}$$

where $\sigma^2_{\underset{\sim}{r}_1}$, $\sigma^2_{\underset{\sim}{r}_2}$ and $\sigma^2_{\underset{\sim}{r}_3}$ are the variance in positions of the atoms 1,2 and 3 respectively, (ref. 7, Ch 17).

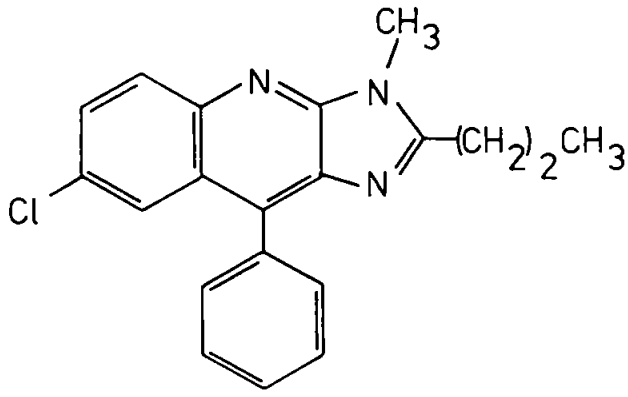
The deviations calculated using the relations described take no account of errors such as errors in data collection, unit cell parameters etc. Thus, such a treatment of errors should be viewed as a measure of accuracy with respect to self-consistent parametrical errors.

Sections 1.6 to 1.15 have discussed the major crystallographic

measurements and computations required to carry out a single crystal structure analysis. These are developed further in the following chapters and applied to the structures referred to in the Introduction.

CHAPTER TWO

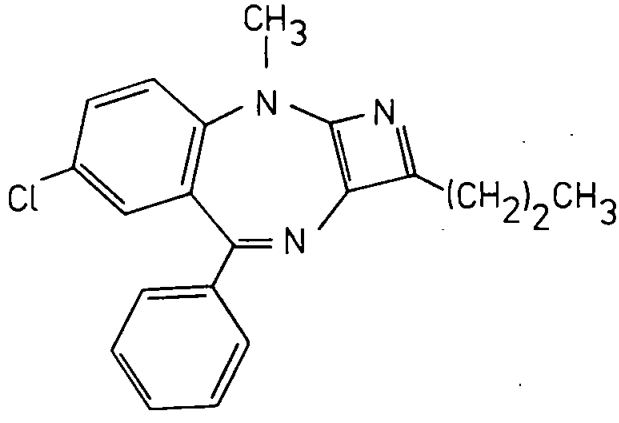
The Crystal and Molecular Structure of 7-Chloro-2-Methyl-5-Phenyl-3-Propyl [2,3 -b] - Imidazolyl Quinoline.



(1)

2.1 Introduction

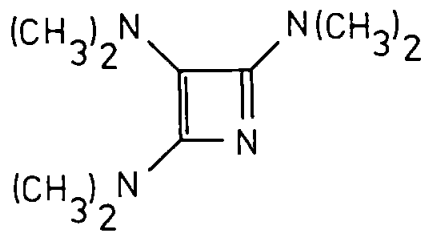
The title compound (1) $C_{20}H_{18}N_3Cl$, provided by Dr G. Kirk, Department of Pharmacy of Chelsea College, University of London, was considered of interest because it is derived from the psychoactive drug Librium, and was thought to conform to the structure,



(2)

i.e., that of a 7-chloro-1-methyl-5-phenyl-3-propyl-azeto - [2,3 -b] [1,4] benzodiazepine containing the highly strained 4-membered azete system.

Shenoy, (1975)[26], assigned the structure (2) on the basis of C^{13}, H^{1} n.m.r., U.V. and mass spectroscopy data. No examples of stable 4-membered azacyclobutadiene rings had previously been reported, though Seybold et al, (1973)[27], inferred that they had prepared tris (dimethylamino) azacyclobutadiene (3),



(3)

though the stability of the monocyclic azete is very low.

2.2 Crystal Preparation

R. J. Girven, (1977)[28], prepared crystals of the compound in order that configuration (2) could be confirmed by structure determination. The best crystallisation solvent was found to be acetone; using, as solution, 104 mg of starting material in 5 cm^3 acetone and heating to 45°C followed by cooling to room temperature over two hours, colourless rod shaped crystals were obtained, up to 1 cm in length.

2.3 Space Group and Unit Cell Determination

Preliminary X-ray investigations showed the crystals to belong to the orthorhombic system, and to have unit cell dimensions, a = 7.43 (3), b = 21.56 (3) and c = 10.69 (3) Å, V = 1712.44 $Å^3$, with

elongation of the crystal along the $\underset{\sim}{a}^*$ reciprocal crystallographic axis.

Weissenberg photographs revealed systematic absences 0k0, k = 2n + 1 and h00, h = 2n + 1, (n integer). The density was determined by flotation in potassium iodide solution to be 1293 kgm^{-3}. For a structure having Z = 4 molecules per unit cell, the calculated density was found to be 1303 kgm^{-3} using Eqn. 1.38. It was thus concluded that the crystal symmetry must be that of the space group $P2_12_12$.

2.4 Intensity Data Collection and Preliminary Treatment

Equi-inclination Weissenberg techniques were employed using the multifilm method of data collection. The Stoe camera was used throughout with CuKα radiation. Crystals were rotated about the $\underset{\sim}{a}^*$ reciprocal axis and intensity data collected for the zero layer and five upper layers, using exposure periods of 5 days. Additional data was collected for each level using 12 hours exposure, in order to bring the most intense reflexions within the measurable range.

Intensity measurements were carried out at the SRC Microdensitometer Service, Daresbury. 2222 reflexions were of measurable intensity.* Lp corrections and conversion to F_o values were carried out by the HKLF and MERG routines of the SHELX program, resulting in an overall isotropic temperature factor, of estimated value U = 0.074$Å^2$ from a modified K-curve[60] and linear absorption coefficient μ (CuKα) = 1898 m^{-1}.

2.5 Structure Determination

The presence of a chlorine atom in the molecule enabled the structure to be solved by the heavy atom method.

The space group $P2_12_12$ has equivalent positions in the unit cell given by

*A possible 3869 reflexions are contained within the experimental Cu sphere.

$$\left.\begin{array}{l} x,\ y,\ z \\ \bar{x},\ \bar{y},\ z \\ \frac{1}{2} + x,\ \frac{1}{2} - y,\ \bar{z} \\ \frac{1}{2} - x,\ \frac{1}{2} + y,\ \bar{z} \end{array}\right\}$$

for the corresponding atoms in each of the four molecules within the unit cell.

The Patterson function, given in Eqn. 1.60, reduces to the form

$$P(u,v,w) = \frac{8}{V} \sum_{hk\ell} |F(hk\ell)|^2 \cos 2\pi hu \cos 2\pi kv \cos 2\pi \ell w \qquad \text{... Eqn. 2.1}$$

in the orthorhombic system, where the multiplying constant $\frac{8}{V}$, normalises spatially (V) and symmetrically (8).

A peak at the point (u,v,w) in the Patterson map indicates that there exist, within the unit cell, atoms at (x_1, y_1, z_1) and (x_2, y_2, z_2), such that

$$\left.\begin{array}{l} u = x_1 - x_2 \\ v = y_1 - y_2 \\ w = z_1 - z_2 \end{array}\right\} \qquad \text{... Eqns. 2.2}$$

Thus, the equivalent positions in $P2_12_12$ correspond to Patterson peaks at

$$\left.\begin{array}{l} (u_1, v_1, w_1) = (2x,\ 2y,\ 0) \\ (u_2, v_2, w_2) = (\frac{1}{2},\ \frac{1}{2} - 2y,\ 2z) \\ (u_3, v_3, w_3) = (\frac{1}{2} - 2x,\ \frac{1}{2},\ 2z) \end{array}\right\} \qquad \text{... Eqn. 2.3}$$

ie.

	$(2x,\ 2y)$	on the w = 0 section,
	$(\frac{1}{2}-2y,\ 2z)$	" " u = ½ " ,
and	$(\frac{1}{2}-2x,\ 2z)$	" " v = ½ " .

Computation of P(u,v,w) was performed in both the unsharpened form (Eqn. 2.1) and the sharpened form using the sharpening function

given in Eqn. 1.64, by the use of program (1) given in Appendix A. Peaks consistent with Eqns. 2.3 gave a solution for the chlorine atomic position at

$$\left.\begin{aligned} x &= 0.091 \\ y &= 0.198 \\ z &= 0.225 \end{aligned}\right\} \qquad \text{... Eqns. 2.4}$$

Further synthesis of the electron density (Eqn. 1.51) can be expressed in terms of the spatial co-ordinates (X, Y, Z) within the $P2_12_12$ unit cell by

$$\begin{aligned} \rho(X,Y,Z) = \frac{8}{V} \Bigg\{ & \sum_{\substack{hk\ell \\ (h+k\,=\,2n)}} |F(hk\ell)| \, [\cos 2\pi hX \cos 2\pi kY \cos 2\pi \ell Z \cos\alpha(hk\ell) \\ & - \sin 2\pi hX \sin 2\pi kY \sin 2\pi \ell Z \sin\alpha(hk\ell)] \\ & - \sum_{\substack{hk\ell \\ (h+k\,=\,2n+1)}} |F(hk\ell)| \, [\sin 2\pi hX \sin 2\pi kY \cos 2\pi \ell Z \cos\alpha(hk\ell) \\ & - \cos 2\pi hX \cos 2\pi kY \sin 2\pi \ell Z \sin\alpha(hk\ell)] \Bigg\} \qquad \text{... Eqn. 2.5} \end{aligned}$$

where

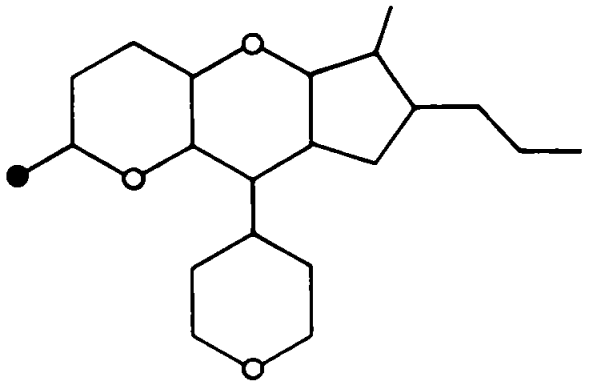
$$\alpha(hk\ell) = \tan^{-1} \left\{ \frac{-\sin 2\pi(hx + \frac{h+k}{4}) \sin 2\pi(ky - \frac{h+k}{4}) \sin 2\pi \ell z}{\cos 2\pi(hx + \frac{h+k}{4}) \cos 2\pi(ky - \frac{h+k}{4}) \cos 2\pi \ell z} \right\}$$

... Eqn. 2.6

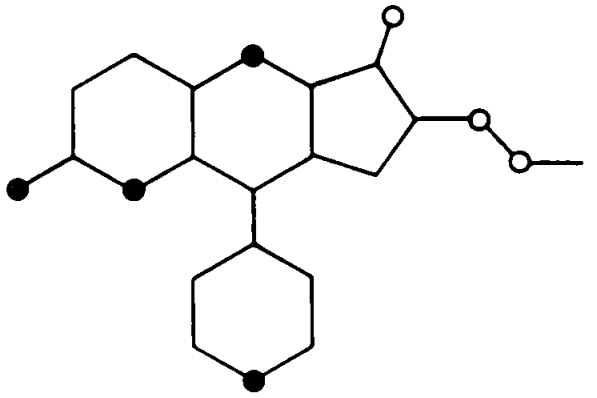
is the phase of reflexion (hkℓ) contributed by an atom at (x,y,z). The program written to compute this function is given in Appendix A (2).

Fig 2.1 shows the progress of the Fourier syntheses in the structure determination process; where the symbols • and o describe respectively those atoms used in the synthesis and those derived from it.

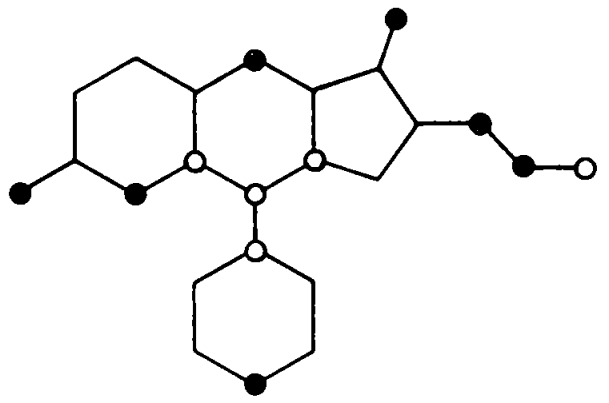
Initial phasing of Eqn. 2.5 was carried out using those observed reflexions $F_o(hk\ell)$ thought to have a significant scattering contribution from the chlorine atom already found by the Patterson



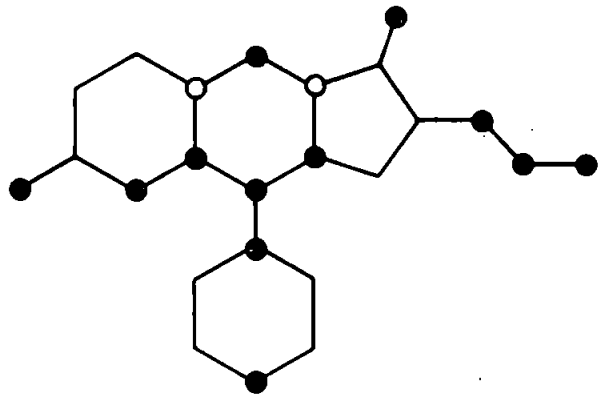
A.



B.

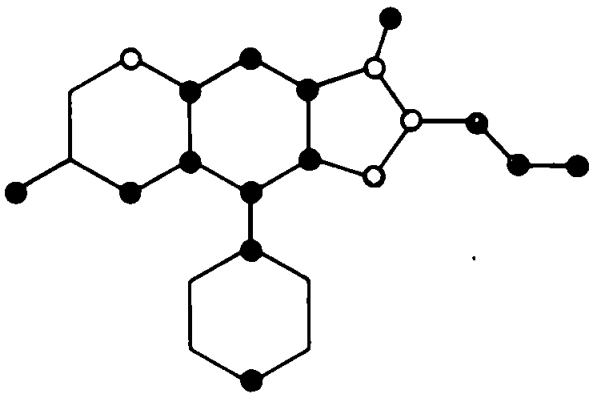


C.

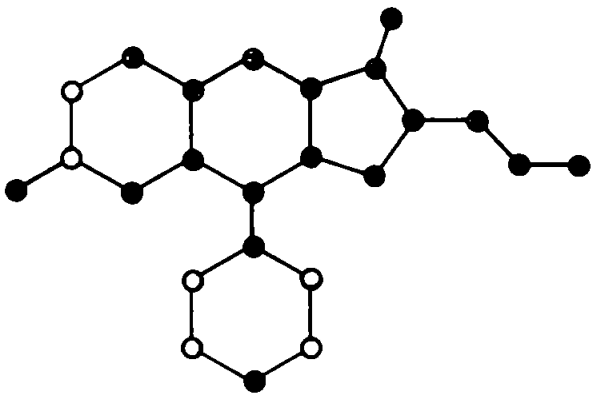


D.

Fig 2.1. Progress of the structure determination during electron density Fourier Synthesis.



E.



F.

Fig 2.1 (contd.)

method. The criterion used for selection of reflexions in this initial phasing was

$$|F_{\bar{c}}(hk\ell)|_{c\ell} > 0.3|F_o(hk\ell)| \qquad \text{... Eqn. 2.7}$$

where $|F_c(hk\ell)|_{c\ell}$ is the calculated structure factor modulus for the chlorine atom alone. The resultant synthesis, using 668 reflexions produced three further peaks thought to be consistent with new atomic positions. The newly found interatomic vectors were calculated, and corresponding Patterson peaks used to verify the new positions as C(3), N(11) and C(18), Fig. 2.1 (A).

Further use of Eqn. 2.5 was made to produce subsequent syntheses using those observed reflexions satisfying the inequality

$$|F_c(hk\ell)| > 0.3\ |F_o(hk\ell)| \qquad \text{... Eqn. 2.8}$$

where $|F_c(hk\ell)|$ is the calculated structure factor modulus for all derived atoms, and corresponding values of $\alpha(hk\ell)$, calculated by Eqn. 2.6, for all derived atomic positions.

The six-membered ring containing N(11) was quickly recognised to show a departure from the expected structure (2), as early as synthesis (D) and the spatial relation with the derived positions of C(21) confirmed the attachment of C(21) to an alternative conjugation. The side chain C(22), C(23), and C(24) had also therefore to branch from the same ring system as C(21) which proved inconsistent with the existence of a four-membered azete ring. Subsequent synthesis revealed the existence of a five-membered ring which when used in the phase calculation allowed the determination of the two remaining six-membered rings in the final synthesis.

2.6 Refinement of the Structure

Six cycles of unweighted full matrix least-squares isotropic refinement resulted in an unweighted R factor of 0.1498 excluding

H atoms from the refinement.

Anisotropic refinement was carried out using SHELX 'BLOC' sectioning, partitioning the structure into 6 sections, Fig. 2.2, to maintain the number of parameters refined in any one cycle within the maximum of 112, compatible with the limited storage capacity of the ICL 1903A machine used.

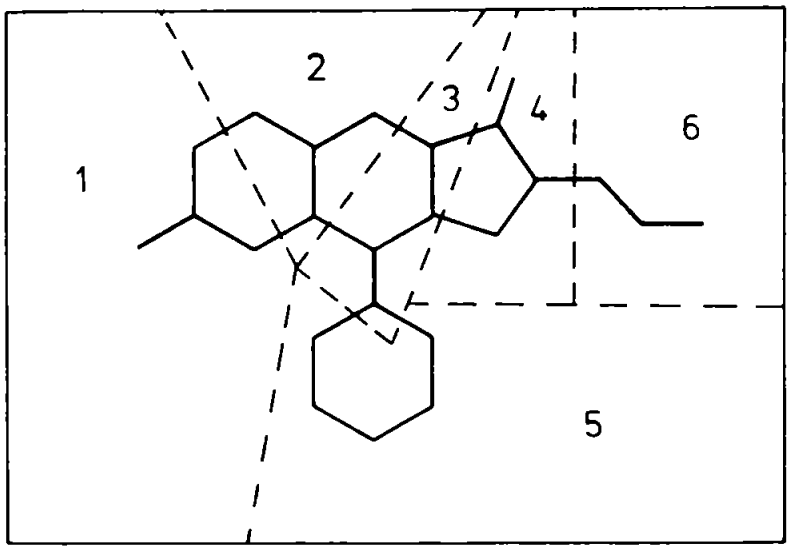


Fig. 2.2 Sectioning for SHELX 'BLOC' refinement. These sections were refined in pairs, to provide the following overlap.

Cycle	Sections	Refined
1	1	2
2	2	3
3	3	4
4	4	5
5	5	6
6	6	1

H atoms were included in the anisotropic refinement with temperature factors fixed at the values of the isotropic temperature factor of the carrier atom, obtained at the isotropic refinement stage, the bond length being fixed at 1.08Å, resulting initially in R = 0.1121. Final anisotropic refinement was carried out with the omission of nine low order reflexions showing severe intensity reduction by secondary extinction.

The final value of the unweighted R factor for the refined structure was 0.0821. Structure factors are listed in Appendix B (ref. SH62 $P2_12_12$).

2.7 Discussion

The final co-ordinates of the non-hydrogen atoms are given in Table 2.1. The bond distances and angles are listed with their standard deviations in Tables 2.2 and 2.3, and are illustrated in Figs. 2.3 and 2.4. The H atom co-ordinates are given in Table 2.4. Thermal parameters are listed with their standard deviations in Table 2.5. Fig 2.5 gives a view of the complete unit cell contents along $\underset{\sim}{a}$ and Fig 2.6 shows the complete unit cell contents viewed along $\underset{\sim}{c}$.

The structure exists with the whole of the conjugated ring system and linear side chains virtually coplanar; the separation between the planes of adjacent parallel molecules ~3.6Å. However, the side chain phenyl group is constrained such that the plane of the ring makes an angle of ~65° to the plane of the remaining molecule effected by rotation about C(8) - C(15); a feature which maintains a parallel relationship with the plane of the same group in the adjacent molecule with a perpendicular interplanar separation ~1.8Å as shown in Fig 2.6.

Synthesis of the compound, (Shenoy, 1975)[26], was an indirect

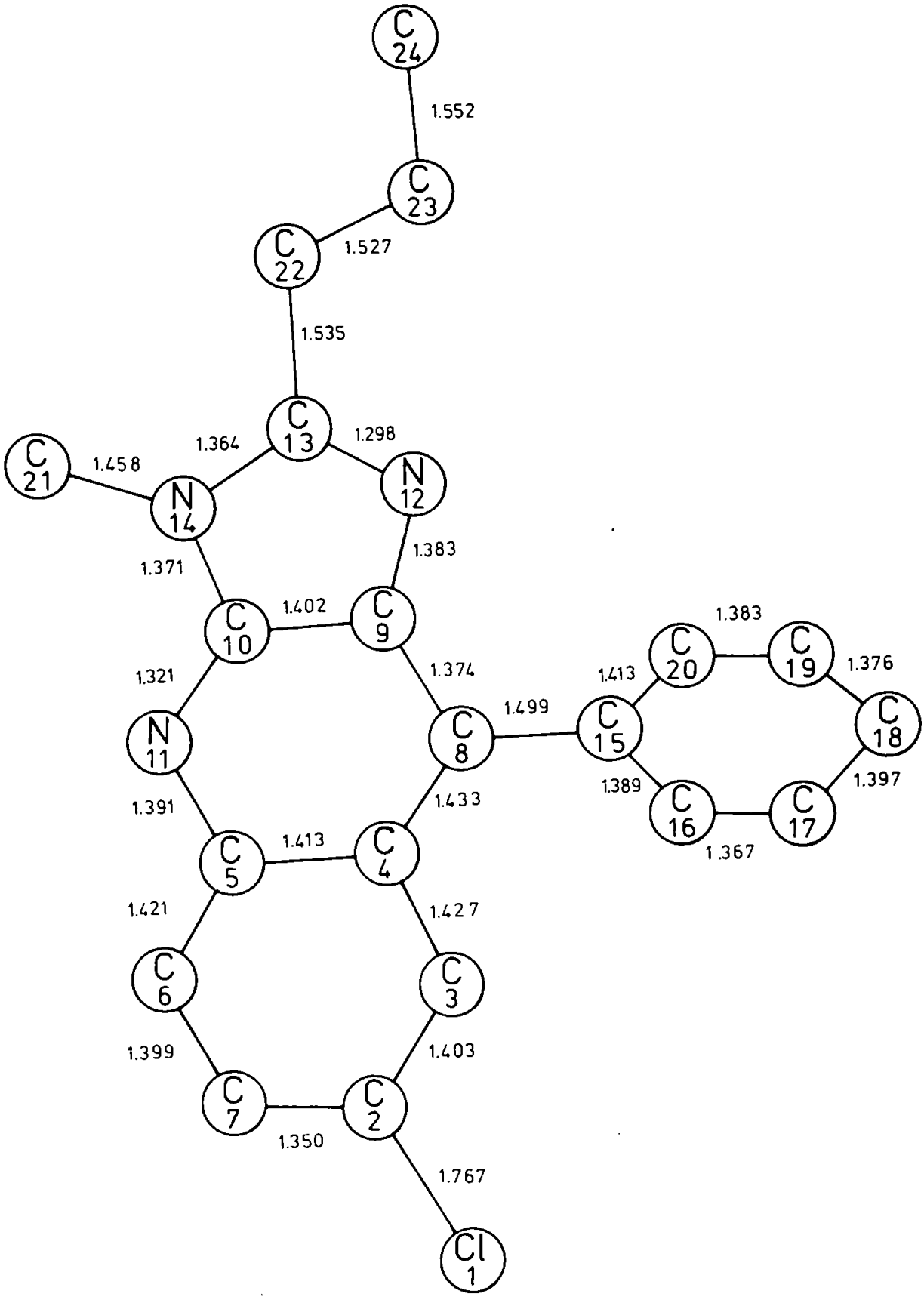


Figure 2.3 Bond lengths in $C_{20}H_{18}N_3C\ell$

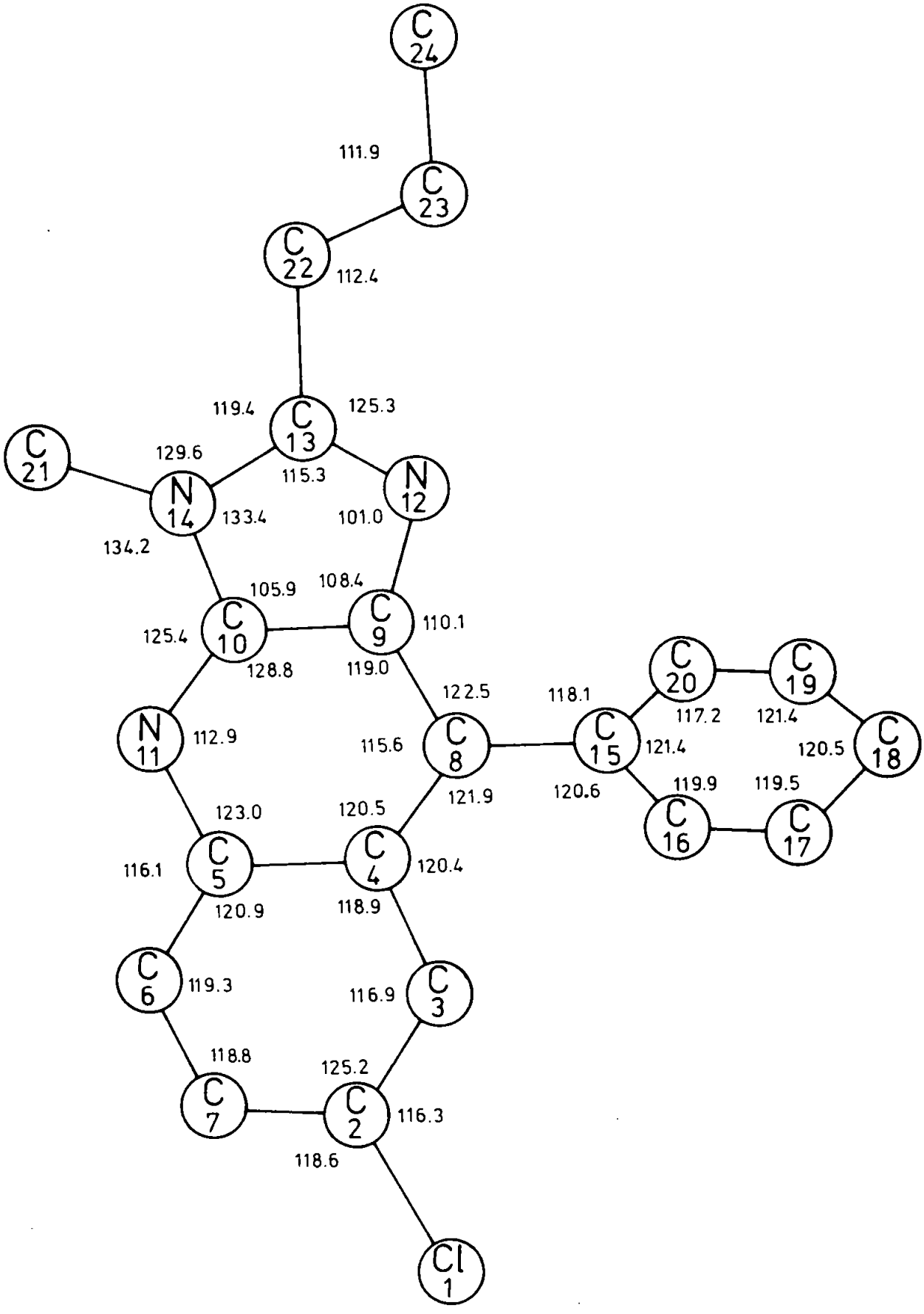


Figure 2.4 Bond angles in $C_{20}H_{18}N_3C\ell$

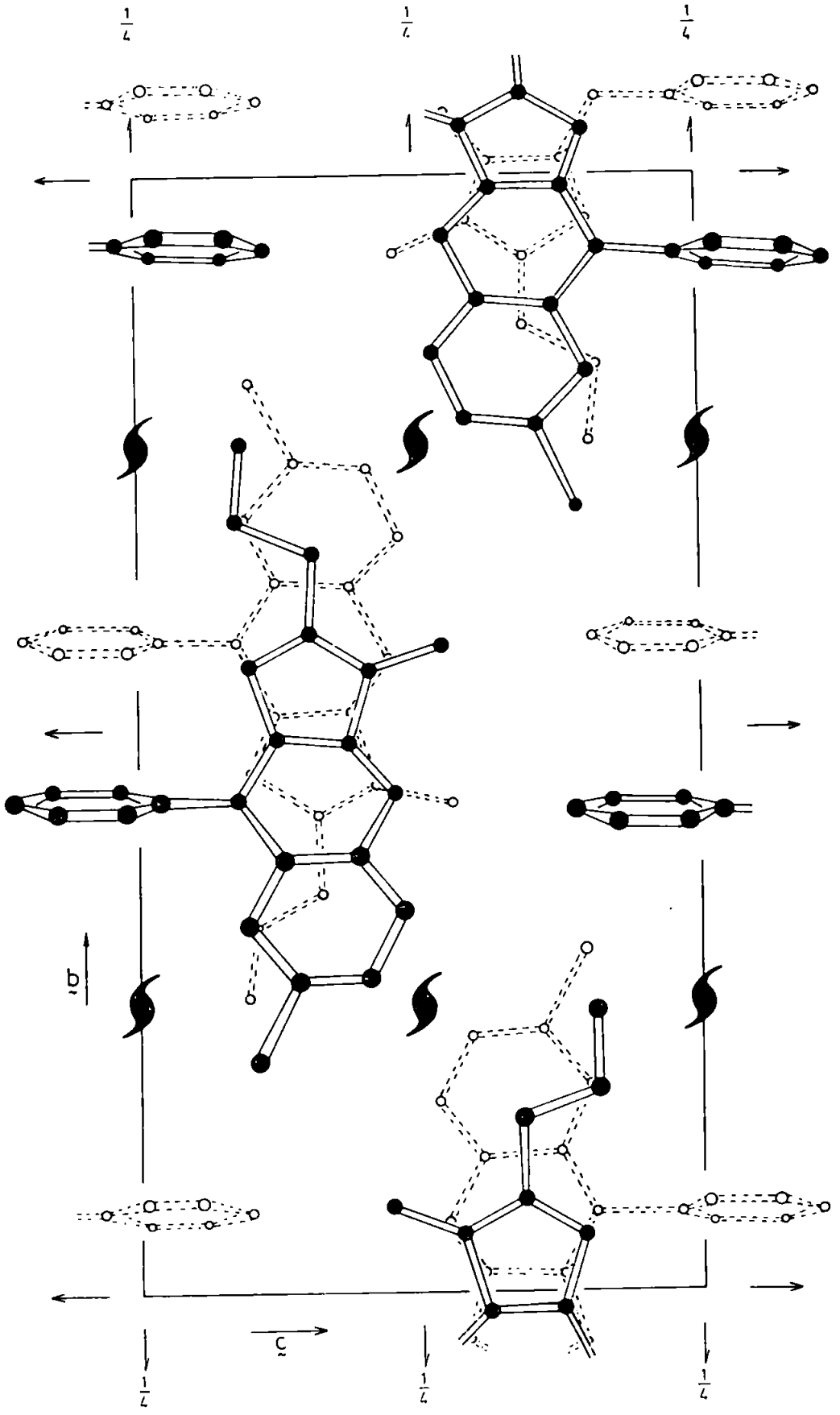


Figure 2.5 The Crystal structure of $C_{20}H_{18}N_3C\ell$ viewed along **a**.

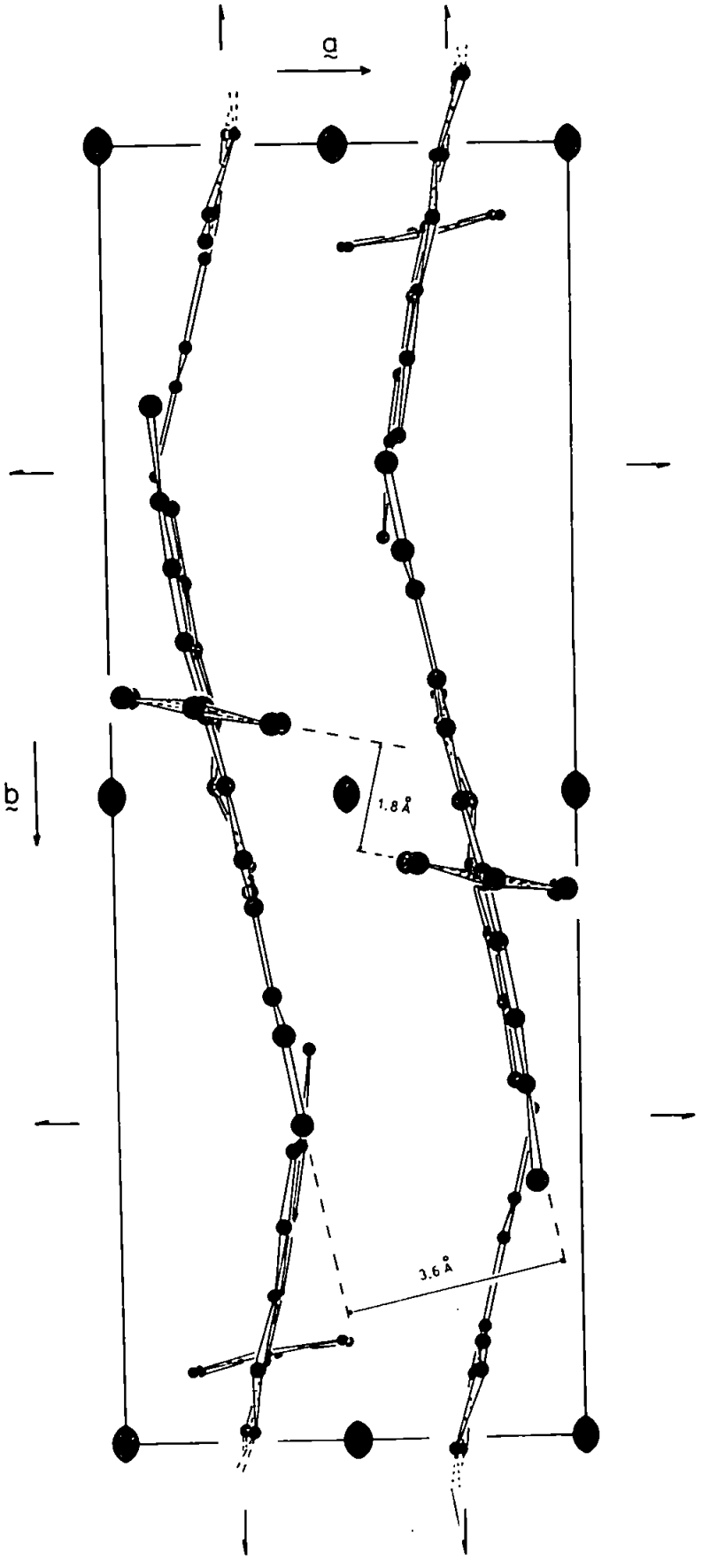
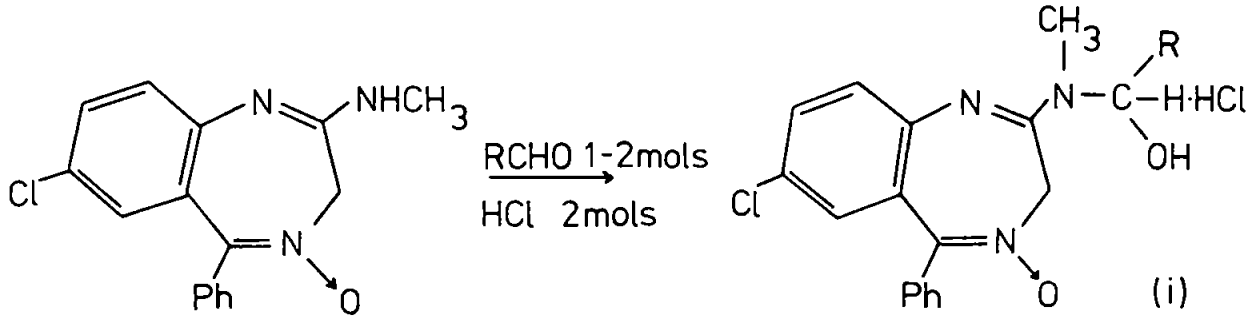
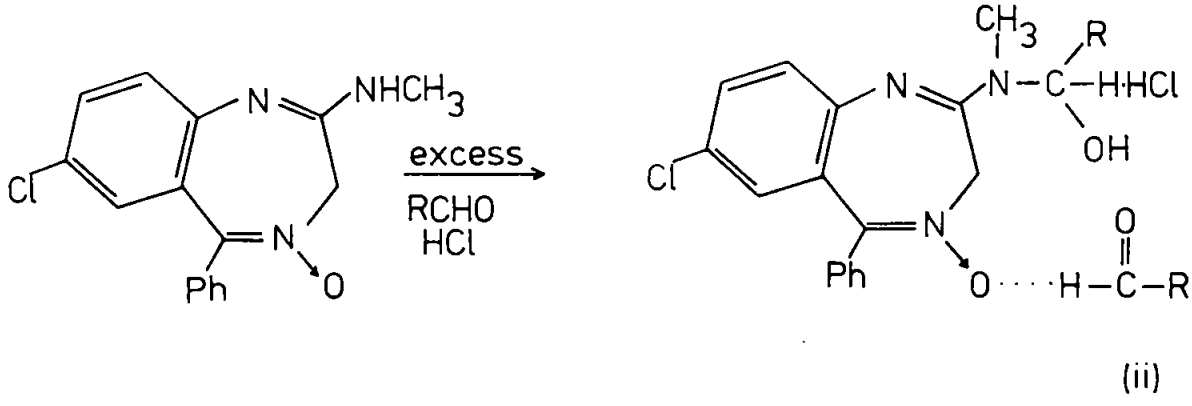


Figure 2.6 The Crystal structure of $C_{20}H_{18}N_3C\ell$ viewed along c.

result of an attempt to react aldehydes with chlordiazepoxide under acidic conditions.

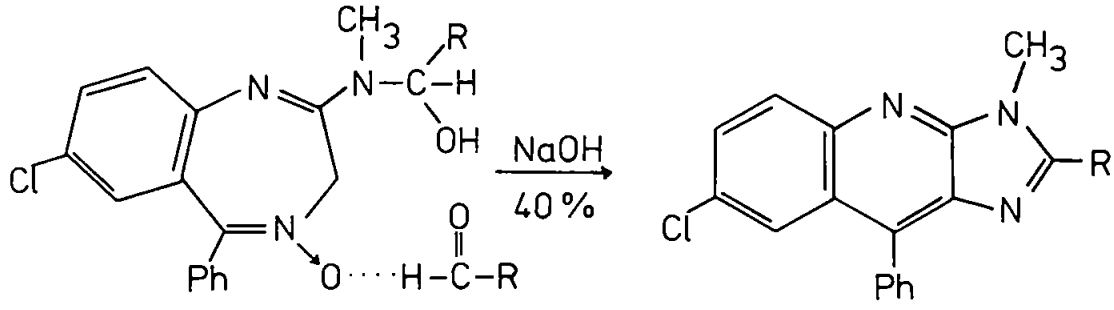


The reaction was repeated using forced conditions (excess aldehyde) resulting in an adduct



Treatment of methanolic solutions of the adduct with 40% aqueous NaOH, gave products which analysed for C, H, N and Cℓ only. Carboxylic acid was shown to be present in the mother liquor after acidification, indicating the removal of the O from the N-oxide function. In the case of butyraldehyde used in reaction (ii), the H^1 n.m.r. spectrum of the product after treatment with 40% NaOH showed peaks for: a n-propyl side chain, a deshielded N-methyl group and only eight aromatic protons; features fully consistent with both the proposed azete structure, (2), and that found by the X-ray structure analysis described here, to correspond to structure (1).

It is therefore concluded that the reaction formalism,



(iii)

involving the cleavage of the seven-membered ring, becomes the only interpretation consistent with the evidence provided by structure determination.

The activity of the parent chlordiazepoxide as an antianxiety agent is greatly dependent upon the existence of the (C = N→O) system, though removal of the O from the N-oxide function is not critical for retention of activity (F. J. Patracek, Chemistry of Psychopharmacological Agents)[29]. It might be expected therefore, that breaking of the C = N bond would result in a diminishing of the typical anticonvulsant activity. However, the structure of the resultant compound is more akin to that of a tricyclic antidepressant though retaining the unsaturated nature within the central ring system similar to that of the parent compound.

TABLE 2.1. Final co-ordinates obtained from least-squares refinement. Co-ordinates are given as fractions of cell edges x 10^4. Standard deviations in parentheses are with respect to the last figures given .

	x	y	z
Cℓ(1)	913(4)	1966(1)	2172(3)
C(2)	1217(13)	2702(3)	2869(9)
C(3)	1441(10)	3203(3)	2046(8)
C(4)	1734(10)	3797(3)	2594(6)
C(5)	1802(10)	3847(4)	3912(7)
C(6)	1547(13)	3319(4)	4685(8)
C(7)	1257(14)	2740(4)	4129(10)
C(8)	2062(9)	4328(3)	1820(6)
C(9)	2449(9)	4869(3)	2444(6)
C(10)	2433(10)	4870(4)	3756(6)
N(11)	2142(9)	4403(3)	4529(5)
N(12)	2189(7)	459(2)	7992(5)
C(13)	2002(9)	787(3)	6987(6)
N(14)	2196(8)	468(3)	5893(5)
C(15)	1961(10)	4294(3)	421(6)
C(16)	325(10)	4195(3)	9826(6)
C(17)	246(14)	4180(4)	8549(7)
C(18)	1818(17)	4266(3)	7853(7)
C(19)	3439(13)	4366(4)	8443(8)
C(20)	3568(10)	4376(3)	9734(7)
C(21)	2088(14)	689(4)	4607(7)
C(22)	1603(9)	1485(3)	6947(7)
C(23)	1413(11)	1766(3)	8252(8)
C(24)	1025(14)	2473(3)	8200(10)

TABLE 2.2.. Bond lengths and their standard deviations (Å) after final least-squares refinement

Cℓ(1) - C(2)	1.767(8)
C(2) - C(3)	1.403(11)
C(2) - C(7)	1.350(14)
C(3) - C(4)	1.427(10)
C(4) - C(5)	1.413(10)
C(4) - C(8)	1.433(9)
C(5) - C(6)	1.421(12)
C(5) - N(11)	1.391(10)
C(6) - C(7)	1.399(13)
C(8) - C(9)	1.374(9)
C(8) - C(15)	1.499(9)
C(9) - C(10)	1.402(9)
C(9) - N(12)	1.383(10)
C(10) - N(14)	1.371(10)
C(10) - N(11)	1.321(10)
N(12) - C(13)	1.298(9)
C(13) - N(14)	1.364(9)
C(13) - C(22)	1.535(9)
N(14) - C(21)	1.458(9)
C(15) - C(16)	1.389(10)
C(15) - C(20)	1.413(10)
C(16) - C(17)	1.367(10)
C(17) - C(18)	1.397(15)
C(18) - C(19)	1.376(15)
C(19) - C(20)	1.383(11)
C(22) - C(23)	1.527(11)
C(23) - C(24)	1.552(10)

TABLE 2.3. Bond angles (°) and their standard deviations

C(3) - C(2) - Cℓ(1)	116.3 (0.7)
C(7) - C(2) - Cℓ(1)	118.6 (0.6)
C(7) - C(2) - C(3)	125.2 (0.7)
C(4) - C(3) - C(2)	116.9 (0.7)
C(5) - C(4) - C(3)	118.9 (0.7)
C(8) - C(4) - C(3)	120.4 (0.6)
C(8) - C(4) - C(5)	120.5 (0.6)
C(6) - C(5) - C(4)	120.9 (0.7)
N(11) - C(5) - C(4)	123.0 (0.7)
N(11) - C(5) - C(6)	116.1 (0.7)
C(5) - C(6) - C(7)	119.3 (0.8)
C(6) - C(7) - C(2)	118.8 (0.8)
C(15) - C(8) - C(4)	121.9 (0.6)
C(15) - C(8) - C(9)	122.5 (0.6)
C(9) - C(8) - C(4)	115.6 (0.6)
C(10) - C(9) - C(8)	119.0 (0.6)
N(12) - C(9) - C(8)	110.1 (0.3)
C(10) - C(9) - N(12)	108.4 (0.3)
C(9) - C(10) - N(11)	128.8 (0.7)
N(14) - C(10) - N(11)	125.4 (0.6)
N(14) - C(10) - C(9)	105.9 (0.3)
C(10) - N(11) - C(5)	112.9 (0.6)
C(9) - N(12) - C(13)	101.0 (0.2)
N(14) - C(13) - N(12)	115.3 (0.6)
C(22) - C(13) - N(12)	125.3 (0.6)
C(22) - C(13) - N(14)	119.4 (0.6)
C(21) - N(14) - C(13)	129.6 (0.6)
C(21) - N(14) - C(10)	134.2 (0.7)
C(13) - N(14) - C(10)	133.4 (0.7)
C(16) - C(15) - C(8)	120.6 (0.6)
C(20) - C(15) - C(8)	118.1 (0.6)
C(20) - C(15) - C(16)	121.4 (0.6)
C(17) - C(16) - C(15)	119.9 (0.7)
C(18) - C(17) - C(16)	119.5 (0.9)
C(19) - C(18) - C(17)	120.5 (0.8)
C(20) - C(19) - C(18)	121.4 (0.8)
C(19) - C(20) - C(15)	117.2 (0.7)
C(23) - C(22) - C(13)	112.4 (0.6)
C(24) - C(23) - C(22)	111.9 (0.7)

TABLE 2.4. Co-ordinates of hydrogen atoms.

Co-ordinates are given as fractions of cell edges $x10^4$. The heavy atom associated with each hydrogen atom is also given. Standard deviations in parentheses are with respect to the last figures given. (e.s.d.'s quoted refer to those of the parent atom).

	x	y	z
H(C3)	1385(10)	3147(3)	1042(8)
H(C6)	1554(13)	3370(4)	5690(8)
H(C7)	1091(14)	2330(4)	4698(10)
H(C16)	14(10)	4186(3)	812(6)
H(C17)	- 1024(14)	4095(4)	8088(7)
H(C18)	1749(17)	4273(3)	6844(7)
H(C19)	4648(13)	4420(4)	7893(8)
H(C20)	4020(10)	4400(3)	693(7)
H(C21)(1)	2322(14)	305(4)	3980(7)
H(C21)(2)	3092(14)	1043(4)	4448(7)
H(C21)(3)	766(14)	880(4)	4435(7)
H(C22)(1)	2682(9)	1716(3)	6455(7)
H(C22)(2)	355(9)	1555(3)	6448(7)
H(C23)(1)	2655(11)	1690(3)	8754(8)
H(C23)(2)	323(11)	1539(3)	8738(8)
H(C24)(1)	912(14)	2655(3)	9138(10)
H(C24)(2)	- 219(14)	2552(3)	7702(10)
H(C24)(3)	2114(14)	2703(3)	7718(10)

TABLE 2.5. Anisotropic temperature factors are expressed as $\exp[-2\pi^2(U_{11}h^2a^{*2} + U_{22}k^2b^{*2} + U_{33}\ell^2c^{*2} + 2U_{12}hka^*b^* + 2U_{13}h\ell\, a^*\, c^* + 2U_{23}k\ell b^*c^*)]$. Isotropic temperature factors are expressed as $\exp[-2\pi^2U(h^2a^{*2}+k^2b^{*2}+\ell^2\, c^{*2})]$. The unit of Uij is $Å^2 \times 10^4$. Standard deviations in parentheses are with respect to the last figures given.

	U or U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
Cℓ(1)	586(22)	344(10)	921(21)	64(13)	-75(14)	-54(12)
C(2)	281(37)	281(37)	584(61)	102(39)	-83(44)	23(36)
C(3)	53(52)	422(41)	361(44)	40(35)	-22(33)	8(30)
H(C3)	239(32)					
C(4)	1(46)	407(38)	259(37)	32(30)	15(29)	19(30)
C(5)	1(47)	506(46)	315(41)	53(35)	-23(31)	50(33)
C(6)	443(68)	620(55)	353(48)	165(42)	33(43)	-11(44)
H(C6)	459(62)					
C(7)	269(66)	492(52)	642(65)	218(47)	41(48)	-34(44)
H(C7)	417(41)					
C(8)	1(38)	351(36)	222(33)	51(28)	60(28)	27(26)
C(9)	1(43)	351(37)	213(33)	8(26)	16(26)	19(27)
C(10)	1(47)	520(47)	273(37)	-14(31)	17(28)	29(33)
N(11)	218(46)	491(41)	257(32)	- 3(28)	4(28)	37(33)
N(12)	6(33)	321(28)	278(27)	22(25)	-16(24)	43(22)
C(13)	1(39)	460(39)	296(35)	46(33)	-54(30)	-61(29)
N(14)	124(37)	417(34)	248(29)	103(26)	-16(25)	4(27)
C(15)	88(43)	256(34)	251(36)	-17(27)	-26(28)	2(28)
C(16)	301(53)	354(37)	227(37)	31(30)	-67(30)	-36(33)
H(C16)	278(32)					
C(17)	669(75)	439(45)	347(48)	- 9(36)	-109(42)	-138(45)
H(C17)	386(38)					
C(18)	1127(99)	336(39)	263(41)	-24(34)	27(50)	-12(47)
H(C18)	464(40)					
C(19)	508(77)	562(51)	438(51)	-16(38)	301(43)	-5(44)
H(C19)	422(41)					
C(20)	215(51)	375(39)	372(40)	- 3(31)	163(31)	22(32)
H(C20)	256(32)					
C(21)	555(66)	591(56)	321(42)	110(38)	15(41)	-26(49)
H(C21)(1)	472(41)					
H(C21)(2)	472(41)					
H(C21)(3)	472(41)					
C(22)	128(44)	361(33)	446(41)	165(33)	-56(32)	37(27)
H(C22)(1)	296(32)					
H(C22)(2)	296(32)					
C(23)	273(55)	324(35)	703(58)	45(36)	-19(42)	34(30)
H(C23)(1)	383(40)					
H(C23)(2)	383(40)					
C(24)	615(64)	336(37)	969(76)	-19(45)	38(62)	67(41)
H(C24)(1)	573(47)					
H(C24)(2)	573(47)					
H(C24)(3)	573(47)					

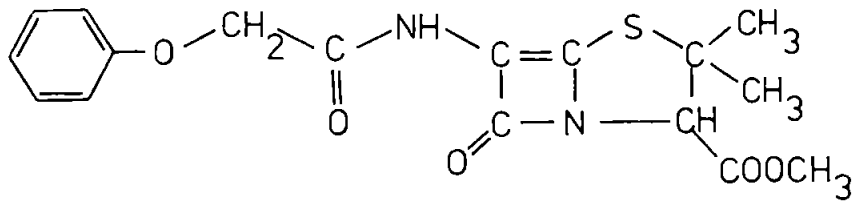
CHAPTER 3

The Crystal and Molecular Structure of the Methyl Ester of 5, 5-Dimethyl-2-(2-Phenoxymethyl-5-Oxo-1, 3-Oxazolin-4-Ylidene) -1, 3-Thiazolidine-4-Carboxylic Acid

3.1 Introduction

$C_{17}H_{18}N_2O_5S$ was first reported by Brandt, Bassignani and Re (1976)[30,31] to have the configuration I,

I



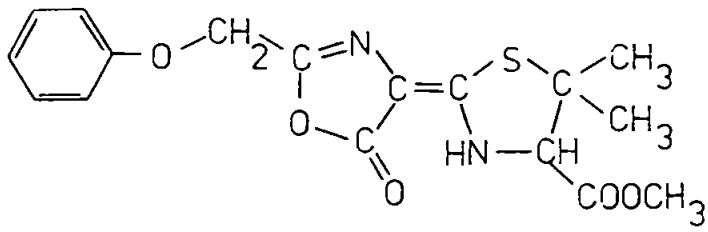
i.e. that of a DL-5, 6-didehydropenicillin, afforded on the basis of spectroscopic data (I.R., H^1 and C^{13} n.m.r., mass spectrometry).

X-ray structure analysis was undertaken as a preliminary to investigation of the reported weak antibacterial activity of the dehydropenicillin and to establish a possible correlation with the "unsaturated" nature of the penicillin nucleus.

Subsequently, Bachi and Vaya (1977)[32] suggested the configuration II on the basis of a comparative study of U.V. and I.R.

spectra.

II



3.2 Experimental

The compound was obtained from Snamprogetti Società per Azioni, Monterotondo, Rome. Single crystals were prepared in the following manner: 30 mg starting material was dissolved in 2 cm^3 ethyl acetate and to this solution 1 cm^3 cyclohexane was added slowly to prevent clouding. The solution was placed in a water bath warmed to not more than 40^oC and protected from light and rapid evapor ation. The compound crystallised as clear needle-shaped single crystals on slowly cooling the solution.

The unit cell dimensions were determined from zero level equi-inclination Weissenberg photographs, the camera radius was determined from high-angle reflexions from an annealed gold wire. Systematic absences $h0\ell$, $\ell = 2n + 1$ and $0k0$, $k = 2n + 1$ (n integer), indicated space group $P2_1/c$ in the monoclinic system, with a = 10.60 (3) b = 15.53 (3), c = 12.63 (3) Å, $\beta = 61.97^o$, V = 1835.25 $Å^3$, and linear absorption coefficient μ (CuKα) = $1714m^{-1}$.

Data for intensity measurement were obtained by the equi-inclination method on Stoe and Nonius Weissenberg cameras using Ni-filtered CuKα radiation (λ = 1.5418 Å) and the multiple film technique. The crystals used for these measurements were rotated about the $\underset{\sim}{c}$ crystallographic axis with the long edge of the crystal parallel to the rotation axis. The X-ray films showed severe reduction in intensity of reflexion at high sin θ after crystals had prolonged exposure to X-rays; 4 different crystals were used for collection of intensity data. The intensities of the X-ray reflexions were measured by the Science Research Council microdensitometer at Daresbury. A total of 1738 reflexions were of measur able intensity. Lp corrections and conversion to F_o values were carried out by the HKLF and MERG routines of the SHELX program, resulting in an overall isotropic temperature factor, of estimated value U = 0.066 $Å^2$.

3.3 Structure Determination

The structure was solved by direct methods, (Germain, Main and Woolfson (1970, 1971), and Germain and Woolfson (1968)[20], Hauptman and Karle (1956)[22], Karle and Hauptman (1956))[23], using the starting set given in Table 3.1 with the three origin determining reflexions marked ORIG and the multisolution phase assignments marked MULT. Expansion was carried out with reflexions, having $|E(hk\ell)| > 1.2$, to 290 signs using 2395 relations with 4096 permutations and 116 quartets. The choice of the three origin determining reflexions was governed by Eqn. 1.80 such that the triple $\underset{\sim}{h}_1$, $\underset{\sim}{h}_2$, $\underset{\sim}{h}_3$ was linearly independent modulo $\underset{\sim}{\omega}_s$. The seminvariant modulus $\underset{\sim}{\omega}_s$ in the case of space group $P2_1/c$ is given by Hauptman (1974)[33] as

$$\underset{\sim}{\omega}_s = \begin{pmatrix} 2 \\ 2 \\ 2 \end{pmatrix} \qquad \text{... Eqn. 3.1}$$

Thus, for the three origin determining reflexions,

$$\underset{\sim}{h}_1 = \begin{pmatrix} 5 \\ 2 \\ 1 \end{pmatrix} = \begin{pmatrix} 1 \\ 0 \\ 1 \end{pmatrix} ,$$

$$\underset{\sim}{h}_2 = \begin{pmatrix} -5 \\ 7 \\ 1 \end{pmatrix} = \begin{pmatrix} -1 \\ 1 \\ 1 \end{pmatrix}$$

and $$\underset{\sim}{h}_3 = \begin{pmatrix} 4 \\ 5 \\ 1 \end{pmatrix} = \begin{pmatrix} 0 \\ 1 \\ 1 \end{pmatrix} \quad \text{modulo} \begin{pmatrix} 2 \\ 2 \\ 2 \end{pmatrix} \qquad \text{... Eqns. 3.2}$$

combination gives

$$\underset{\sim}{h}_1 + \underset{\sim}{h}_2 = \begin{pmatrix} 0 \\ 9 \\ 2 \end{pmatrix} = \begin{pmatrix} 0 \\ 1 \\ 0 \end{pmatrix} ,$$

$$\underset{\sim}{h}_2 + \underset{\sim}{h}_3 = \begin{pmatrix} -1 \\ 12 \\ 2 \end{pmatrix} = \begin{pmatrix} -1 \\ 0 \\ 0 \end{pmatrix}$$

and $$\underset{\sim}{h}_3 + \underset{\sim}{h}_1 = \begin{pmatrix} 9 \\ 7 \\ 2 \end{pmatrix} = \begin{pmatrix} 1 \\ 1 \\ 0 \end{pmatrix} \quad \text{modulo} \begin{pmatrix} 2 \\ 2 \\ 2 \end{pmatrix}$$

... Eqns. 3.3

together with

$$\underset{\sim}{h}_1 + \underset{\sim}{h}_2 + \underset{\sim}{h}_3 = \begin{pmatrix} 4 \\ 14 \\ 3 \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix} \quad \text{modulo} \begin{pmatrix} 2 \\ 2 \\ 2 \end{pmatrix}$$

... Eqn. 3.4

such that the triple $\underset{\sim}{h}_1$, $\underset{\sim}{h}_2$, $\underset{\sim}{h}_3$ is linearly independent modulo $\underset{\sim}{\omega}_s$ as required.

All the 25 non hydrogen atoms were obtained from the E map produced from an expansion pathway using the permutation of sign relations ++++--+++++++-- (Table 3.1) such that the configuration II, (§ 3.1), suggested by Bachi and Vaya (1977)[32], was subsequently recognised and confirmed.

3.4 Structure Refinement

Four cycles of unweighted full matrix least-squares isotropic

TABLE 3.1 Starting set of reflexions with associated phases and equivalent sign relations.

h k l	E	Phase °	Sign Relations
5 2 1	3.540	0	ORIG +
-5 7 1	2.615	0	ORIG +
4 5 1	1.945	0	ORIG +
-1 4 1	3.396	0, 180	MULT + -
0 8 2	2.274	0, 180	MULT + -
0 3 2	2.896	0, 180	MULT + -
-2 7 1	2.297	0, 180	MULT + -
4 7 9	1.975	0, 180	MULT + -
-1 1 5	1.800	0, 180	MULT + -
3 6 4	1.305	0, 180	MULT + -
6 3 6	2.113	0, 180	MULT + -
-2 8 5	2.468	0, 180	MULT + -
4 7 1	1.869	0, 180	MULT + -
-2 5 1	1.950	0, 180	MULT + -
-6 3 2	2.557	0, 180	MULT + -

refinement including interlayer and overall scale factor refinement produced R = 0.175. Anisotropic refinement initially resulted in R = 0.106 with hydrogen atoms included in the refinement. The hydrogen atoms were given isotropic temperature factors fixed at the values of the isotropic temperature factor of the atom to which they are bonded, obtained at the isotropic refinement stage, the bond length being fixed at 1.08 Å.

An $F_o - F_c$ synthesis revealed a significant positive peak adjacent to O(19) which could not be removed during refinement. Further refinement was carried out with O(19) replaced by O(19(2)) in the position indicated by the $F_o - F_c$ peak. A further $F_o - F_c$ synthesis showed a positive peak at the previous O(19) position, (O(19(1))). The ratio of the peak heights in both syntheses (~2:1) was taken as an indication of alternative site occupation, in part confirmed by the departure from the expected benzene C-C bond length (1.39 Å) in the phenyl group attached to O(19) illustrated in particular by the bond C(22) - C(23), (1.36 Å). Anisotropic refinement of the O(19(1)), O(19(2)) positions, performed using site occupation factors of 0.7 and 0.3 respectively, finally resulted in R = 0.096.

Refinement at the anisotropic stage was carried out using SHELX 'BLOC' sectioning, with the structure partitioned into 6 sections, Fig. 3.1., to keep the number of independent variables refined in any one cycle within the maximum of 112.

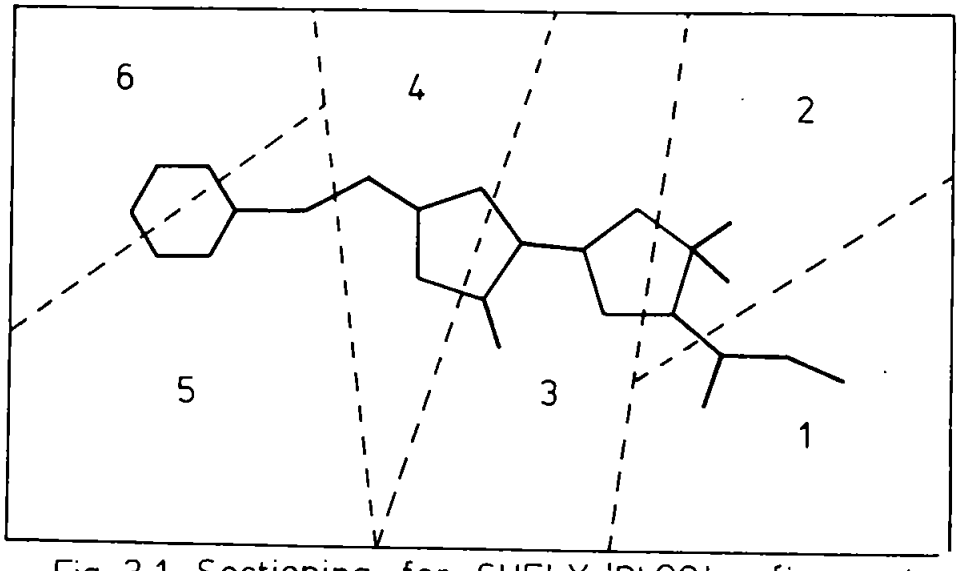


Fig. 3.1 Sectioning for SHELX 'BLOC' refinement

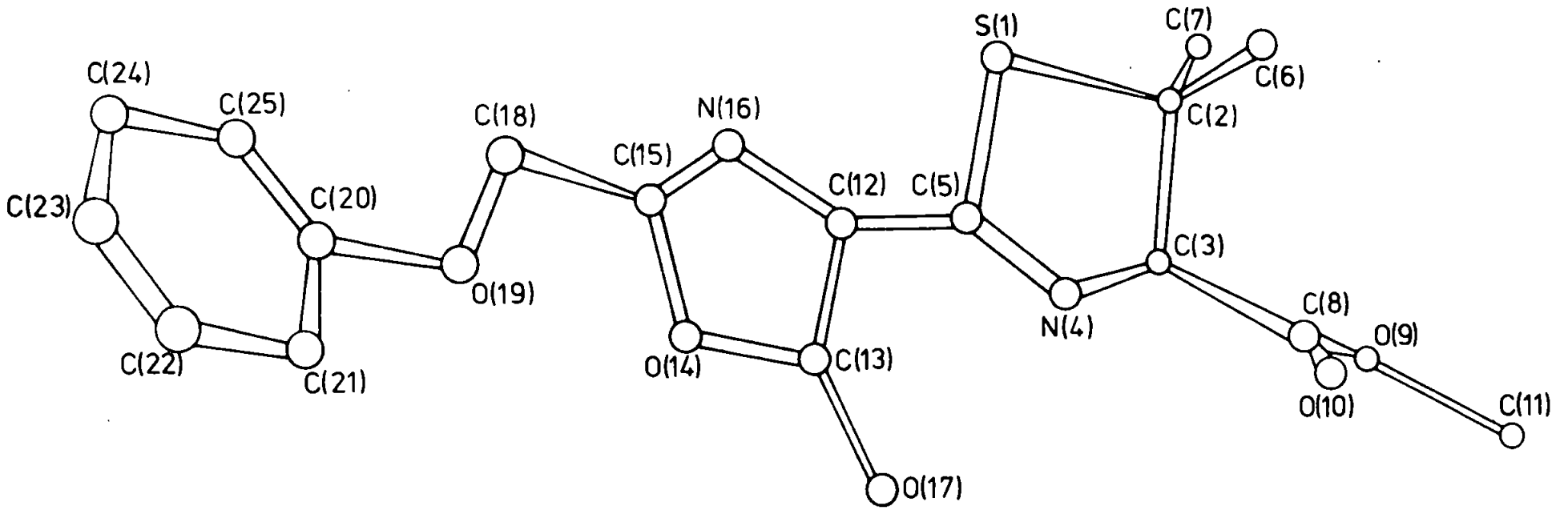


Fig.3.2 Schematic labelling of the non hydrogen atoms in the title compound, ($C_{17}H_{18}N_2O_5S$)

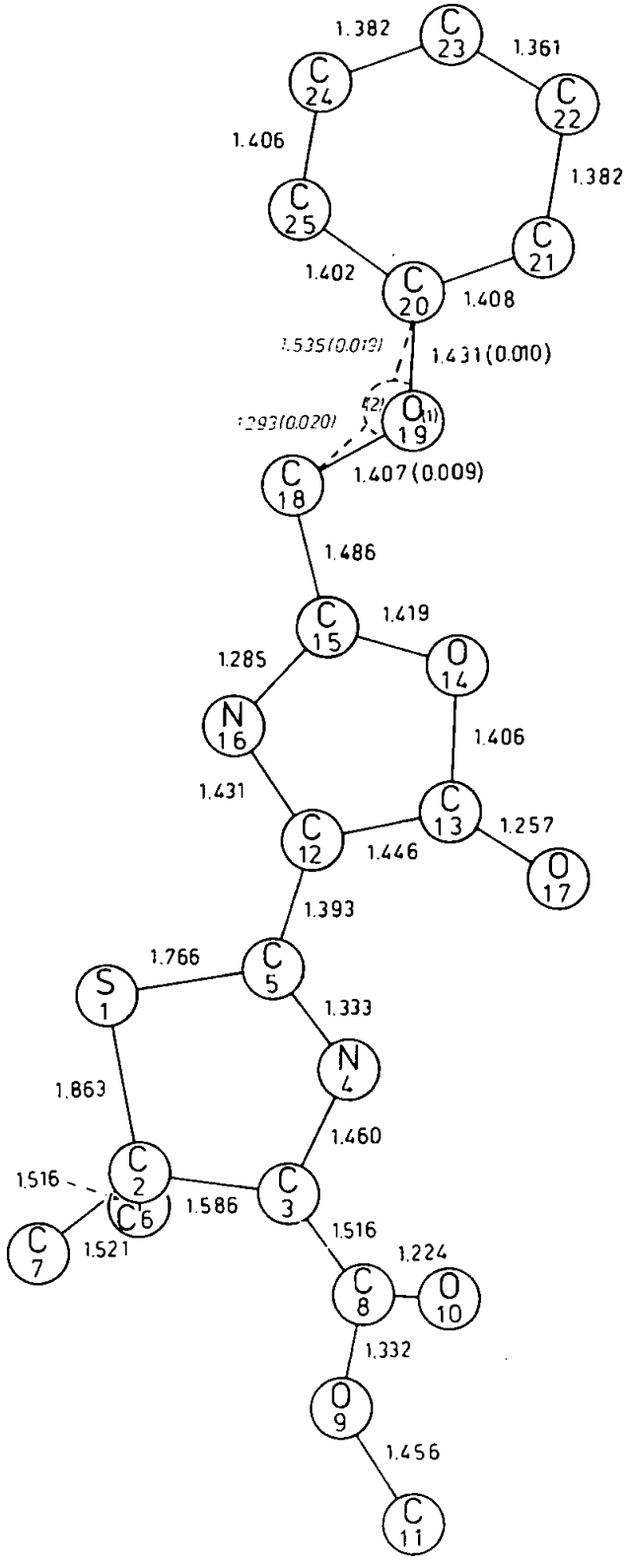


Figure 3.3 Bond lengths in $C_{17}H_{18}N_2O_5S$

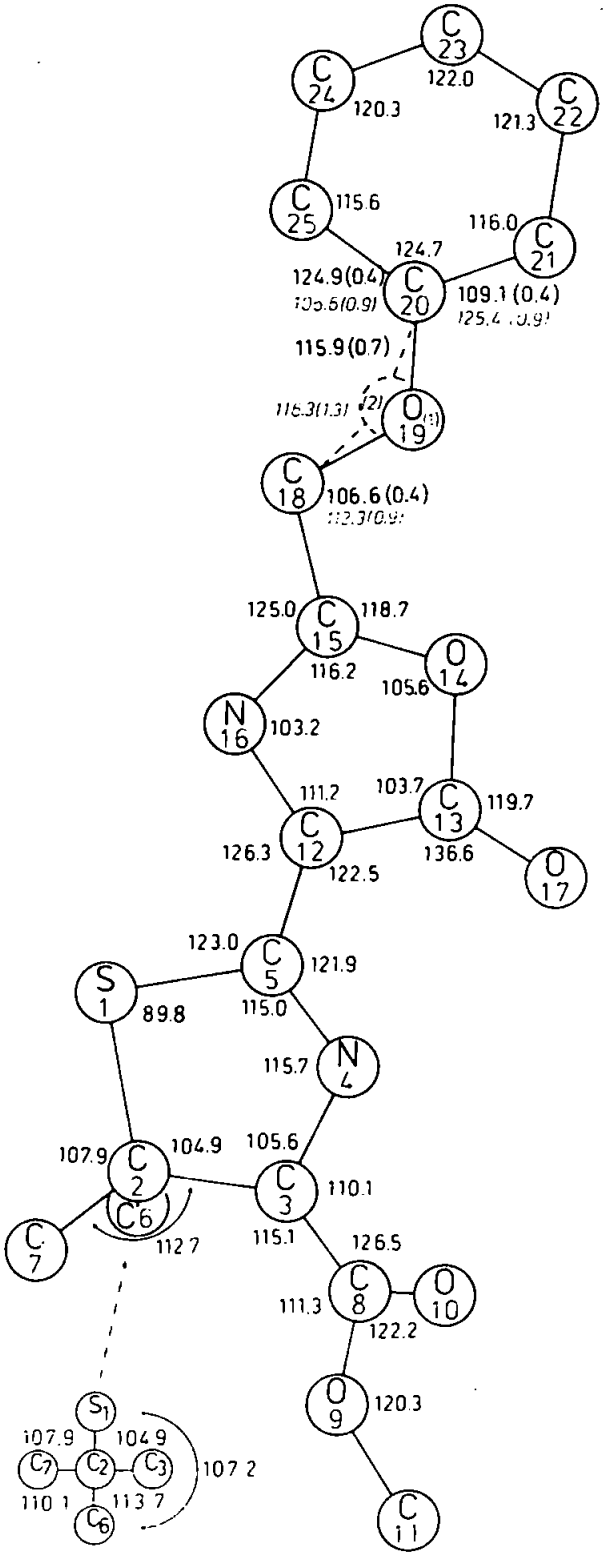


Figure 3.4 Bond angles in $C_{17}H_{18}N_2O_5S$

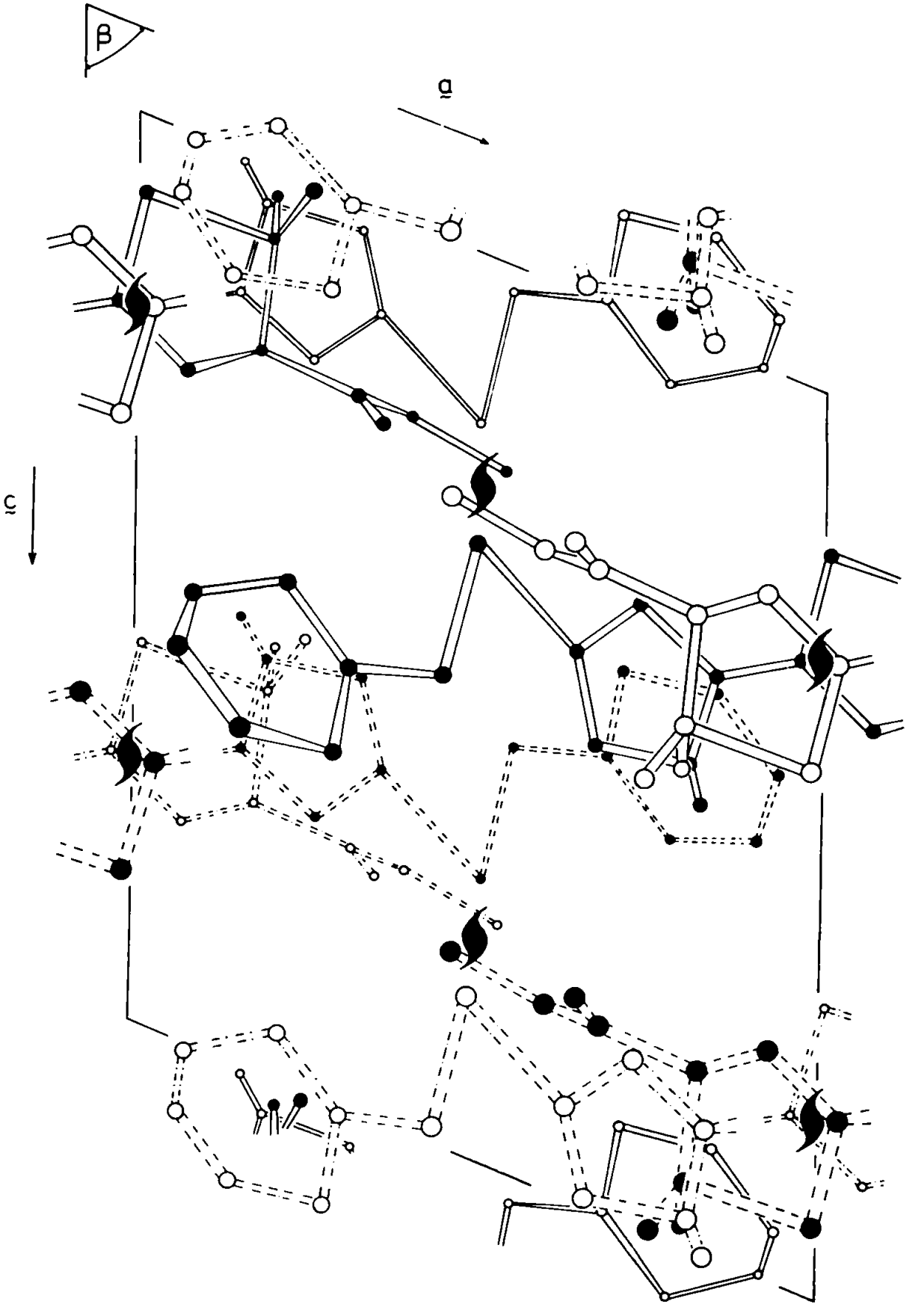


Fig.3.5 The crystal structure viewed along $\underset{\sim}{b}$, $(C_{17}H_{18}N_2O_5S)$

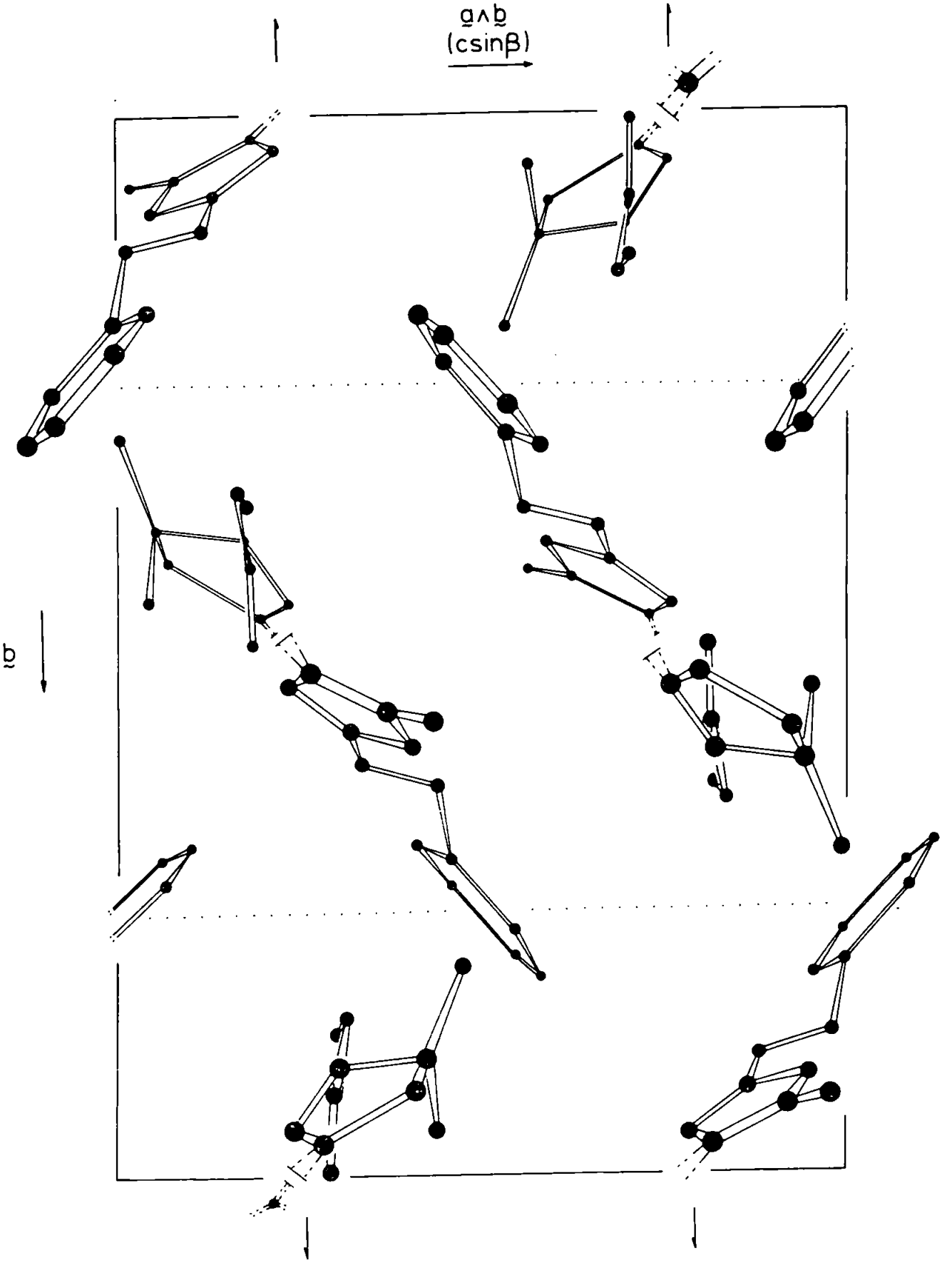


Fig. 3.6 The crystal structure viewed along a, ($C_{17}H_{18}N_2O_5S$)

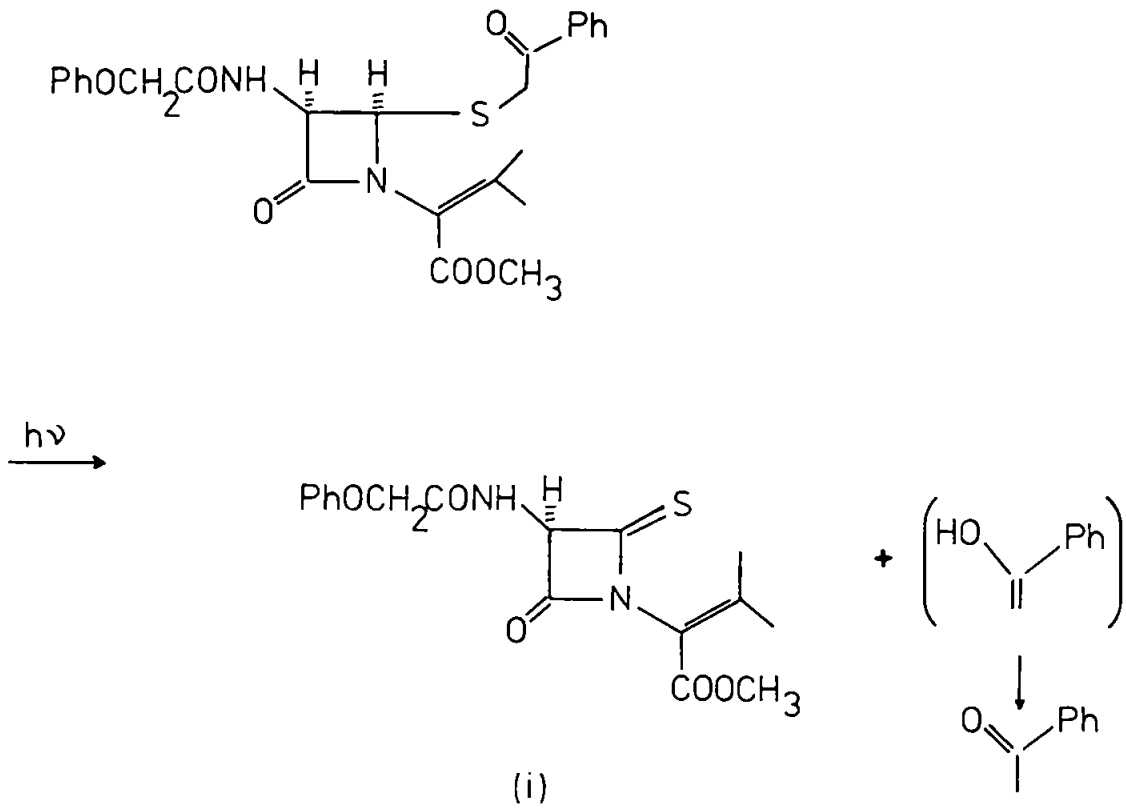
Sections were paired during refinement to provide overlap and overall refinement in 6 cycles.

The residual electron density in the final difference map was within -0.39 and 0.42 $e\mathring{A}^{-3}$. No further improvement could be made in F_o/F_c correlation probably due to the deterioration of the crystals during prolonged X-ray exposure. Structure factors are listed in Appendix B (ref. SNP P2(1)/C).

3.5 Discussion

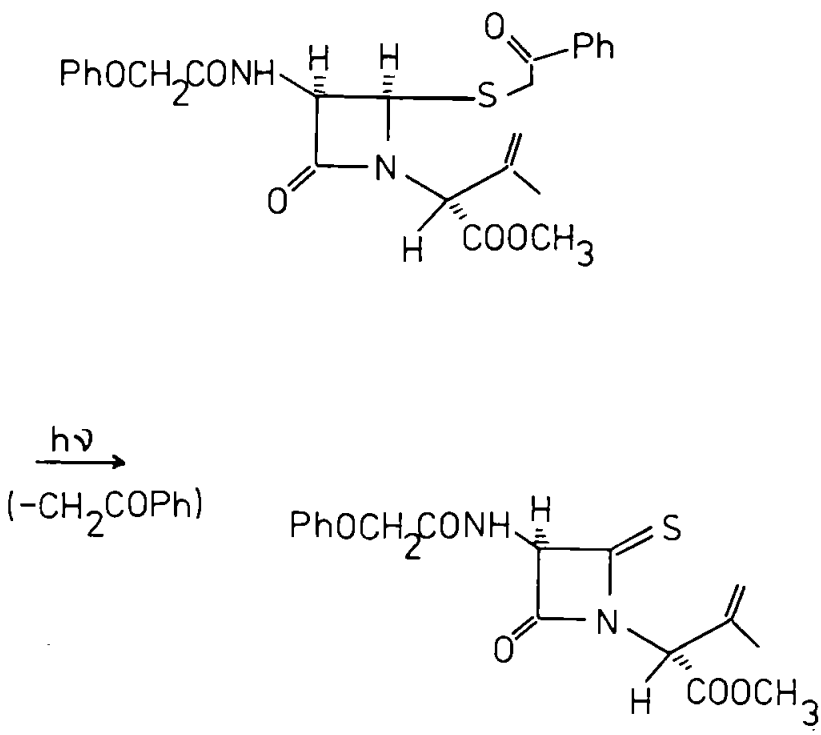
The final co-ordinates of the non-hydrogen atoms are given in Table 3.2 with their schematic labelling illustrated in Fig. 3.2. The bond distances and angles are listed with their standard deviations in Tables 3.3 and 3.4 and illustrated in Figs. 3.3 and 3.4. The H atom co-ordinates are given in Table 3.5. Thermal parameters are listed with their standard deviations in Table 3.6(a) and (b). Fig. 3.5 gives a view of the complete unit cell contents along $\underset{\sim}{b}$ and Fig. 3.6 shows the complete unit cell contents viewed along $\underset{\sim}{a}$.

Synthesis of the DL-5, 6-dehydropenicillins was reported by Brandt, Bassignani and Re (1976b)[31] to result from an intramolecular alkylation of a novel class of 2-azetidinones when treated with Et_3N alone. The 4-thioxo-2-azetidinones (i) and (ii); amongst the first examples of 2-azetidinones carrying a 4-thioxo group; formed the direct synthetic precursor of the 'dehydropenicillins', and were obtained, by a Norrish type II photoelimination reaction, on irradiation of the corresponding 4-acylmethylthio -2-azetidinones with U.V. light[30].


PhOCH2CONH
H
H
O
Ph
S
N
O
COOCH3
hν
PhOCH2CONH
H
S
N
O
COOCH3
HO
Ph
O
Ph

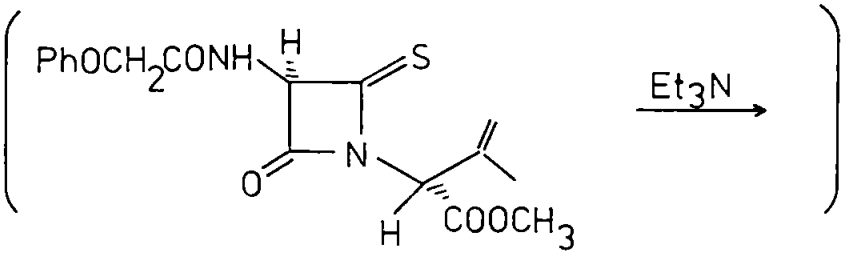
(i)

and

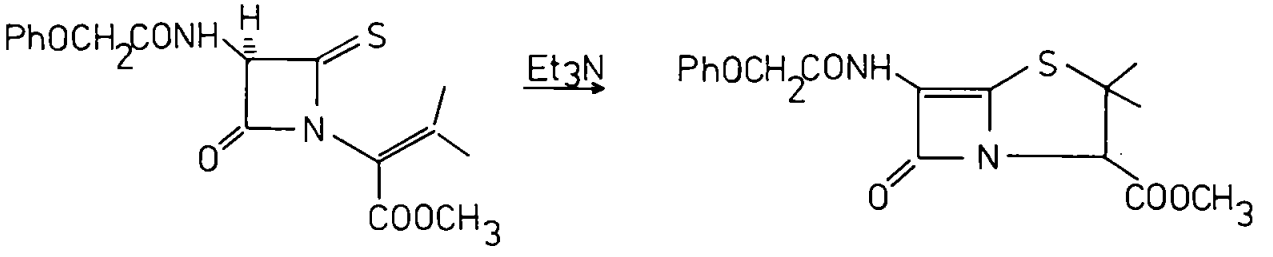

PhOCH2CONH
H
H
O
Ph
S
N
O
H
COOCH3
hν
(-CH2COPh)
PhOCH2CONH
H
S
N
O
H
COOCH3

(ii)

The suggested intramolecular alkylation, proceeding to the 'dehydropenicillin', I, was then represented by

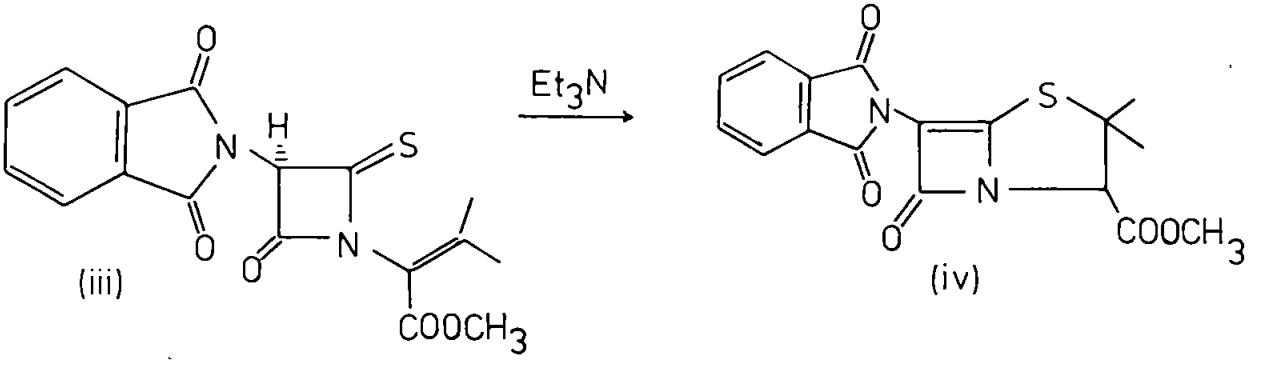


(ii)



(i) I

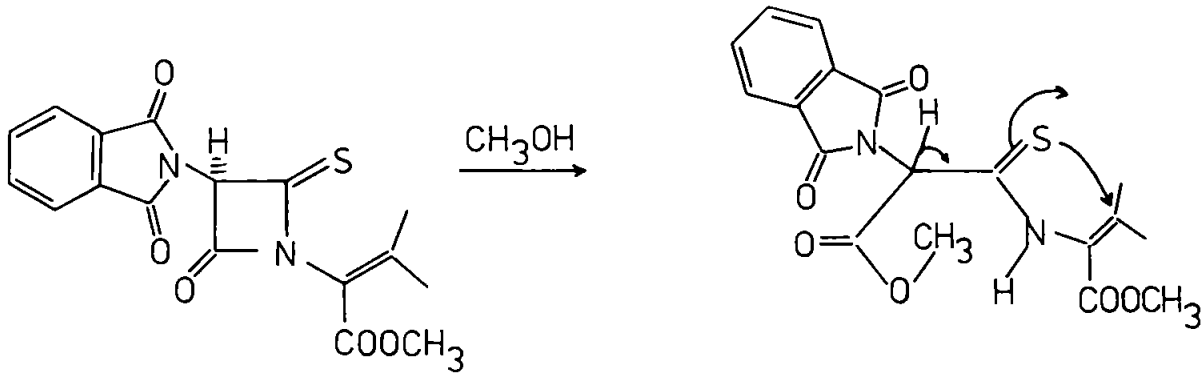
By analogy, Bachi and Vaya (1977)[32] expected the conversion of the thiomalonimide, (iii), by treatment with silica gel or Et_3N, into the corresponding 'dehydropenicillin ', (iv), thus,



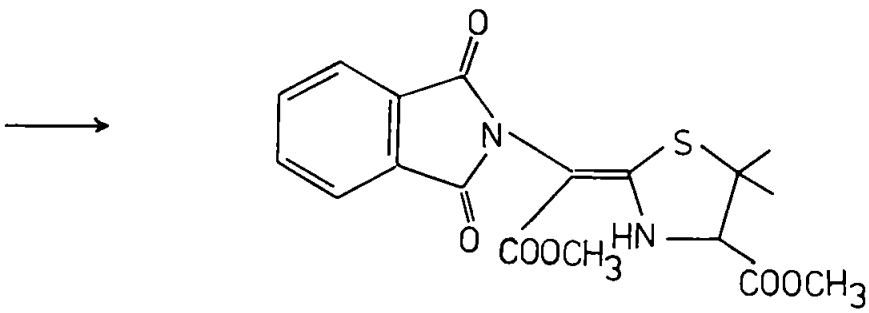
(iii) (iv)

- in their hands, however, this transformation did not occur.

Treatment of the thiomalonimide, (iii), with Et_3N in absolute methanol for 5 min., afforded, after preparative t.l.c., two compounds of the same molecular weight. Structure (v) was assigned to the less polar compound and structure (vi) to the more polar compound. The major product, (v), was obtained by a nucleophilic attack of methanol at C-2 followed by a spontaneous ring closure to the thiazolidine.

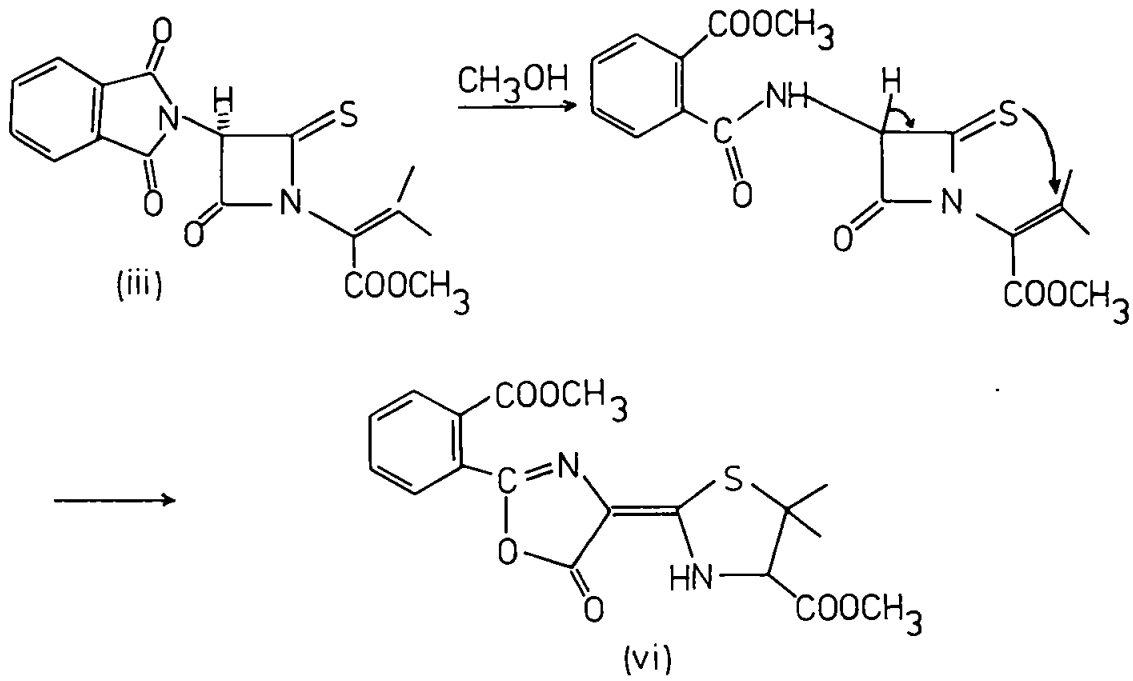


(iii)



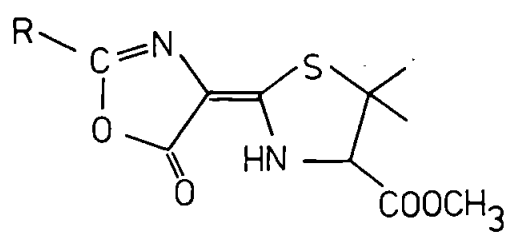
(v)

The formation of (vi) required a competitive methanolysis of the phthalamido group in (iii) to the phthaleamic ester which rearranged to the thiazolidylideneoxazolone.



A similar intramolecular rearrangement in which the azetidinone ring is cleaved by a neighbouring acylamino group occurs in many penicillins[58].

The assignment of structure (vi) to the more polar compound was corroborated by comparison of its U.V. spectrum with the spectra of other thiazolid ylideneoxazolones of structure (vii) prepared by a different route,



(vii)

and with those of 4-(1-thioalkylidene) - and 4 -(1-aminoalkylidene) - oxazolones[59].

The similarity between the spectral data of (vi), and that of the 'dehydropenicillin' suggested by Brandt et al[31], together with the apparent inability to produce compound (iv), led Bachi and Vaya[32] to propose the alternative structure, II.

The compound was claimed by Brandt, Bassignani and Re (1976b)[31] to show only a very weak antibacterial activity when tested against B. subtilis and Staph. aureus by the agar diffusion disc assay. It is therefore of interest to compare the conformation of the part of the molecule in common with that of penicillins, the thiazolidine ring. Boles and Girven (1976a and b)[34,35] compare the conformation of thiazolidine rings of known penicillin structures. The ring exists with four of its five atoms nearly coplanar and with the remaining atom out of this plane.

In this structure, the plane containing the atoms S(1), C(2) and C(5) is defined by

$$-0.2073x - 2.4491y + z = -0.8991 \qquad \text{... Eqn. 3.5}$$

where x,y and z are measured in fractions of cell edges $\underset{\sim}{a}$, $\underset{\sim}{b}$ and $\underset{\sim}{c}$ respectively. N(4) is 0.19 Å out of this plane, and may be considered, as in comparable structures, to be the fourth atom in the plane, though showing rather more distortion about the plane than is the case in penicillins. C(3) is 0.6 Å out of the plane defined above. The structure of the thiazolidine ring is therefore similar to that in phenoxymethylpenicillin[36], p-bromopenicillin[37] and potassium benzyl penicillin[38,39], (ref Chap. 4, $4.7).

The N(4) - C(5) bond length of 1.333 Å in the present compound is significantly less than the equivalent bond length in penicillin nuclei where the thiazolidine ring is constrained by the adjacent β lactam eg. in amoxycillin trihydrate[40],

ampicillin anhydrate[34] and ampicillin trihydrate[41], the N(4) - C(5) bond lengths are 1.49, 1.45, and 1.47 Å respectively. This feature results in a marked difference in the bond angle S(1) - C(5) - N(4) between the thiazolidine ring of the title compound, (115.0°) and that of constrained penicillin nuclei of typical value ~105.5°.

O(19(1)) and O(19(2)) have positions almost symmetrical about the expected O(19) - C(20) bond with bond angles O(19(1)) - C(20) - C(21) and O(19(2)) - C(20) - C(25) being 109.1° and 106.6°, and bond angles O(19(2)) - C(20) - C(21) and O(19(1)) - C(20) - C(25) being 125.4° and 124.9° respectively, compared with the expected values of 120°. This suggests that the co-ordinates of the atoms in the benzene ring attached to O(19) have refined to the values of the weighted mean of the alternative configurations.

Table 3.2. Final coordinates obtained from least-squares refinement. Coordinates are given as fractions of cell edges x 10^4. Standard deviations in parentheses are with respect to the last figures given.

	x	y	z
S(1)	95(3)	4164(2)	1227(3)
C(2)	1928(13)	3912(8)	990(12)
C(3)	1831(12)	3999(7)	2277(11)
N(4)	738(9)	4650(6)	2906(7)
C(5)	- 255(11)	4733(6)	2548(9)
C(6)	2938(17)	4553(10)	83(13)
C(7)	2297(17)	3003(9)	489(14)
C(8)	3215(13)	4248(8)	2275(11)
O(9)	4011(10)	3558(6)	2172(9)
O(10)	3581(10)	4975(6)	2388(10)
C(11)	5365(13)	3650(11)	2194(14)
C(12)	-1502(10)	5204(6)	3204(8)
C(13)	-1837(10)	5604(6)	4340(9)
O(14)	-3196(6)	5966(4)	4710(6)
C(15)	-3549(9)	5775(6)	3782(8)
N(16)	-2609(8)	5353(5)	2881(7)
O(17)	-1238(8)	5685(5)	4989(7)
C(18)	-4939(11)	6082(7)	3904(11)
O(19(1))	-5397(11)	6750(6)	4755(11)
O(19(2))	-5858(20)	6238(14)	5007(18)
C(20)	-6858(11)	7007(7)	5220(9)
C(21)	-7166(13)	7653(8)	6090(10)
C(22)	-8453(14)	8077(7)	6469(11)
C(23)	-9336(12)	7895(7)	5991(10)
C(24)	-9031(10)	7251(7)	5150(11)
C(25)	-7744(11)	6787(6)	4716(10)

Table 3.3 Bond lengths and their standard deviations (Å) after final least-squares refinement

S(1)	- C(2)	1.863(15)
S(1)	- C(5)	1.766(11)
C(2)	- C(3)	1.586(22)
C(2)	- C(6)	1.515(18)
C(2)	- C(7)	1.521(19)
C(3)	- C(8)	1.516(21)
C(3)	- N(4)	1.459(13)
N(4)	- C(5)	1.333(17)
C(5)	- C(12)	1.392(13)
C(8)	- O(9)	1.331(17)
C(8)	- O(10)	1.224(17)
O(9)	- C(11)	1.456(20)
C(12)	- C(13)	1.446(15)
C(12)	- N(16)	1.432(16)
C(13)	- O(17)	1.256(17)
C(13)	- O(14)	1.407(12)
O(14)	- C(15)	1.419(15)
C(15)	- N(16)	1.285(11)
C(15)	- C(18)	1.486(17)
C(18)	- O(19(1))	1.407(9)
C(18)	- O(19(2))	1.293(20)
O(19(1))	- C(20)	1.431(10)
O(19(2))	- C(20)	1.535(19)
C(20)	- C(21)	1.408(16)
C(20)	- C(25)	1.403(19)
C(21)	- C(22)	1.381(19)
C(22)	- C(23)	1.361(23)
C(23)	- C(24)	1.383(16)
C(24)	- C(25)	1.407(14)

Table 3.4 Bond angles (°) and their standard deviations

C(5)	- S(1)	- C(2)	89.8 (0.6)
C(3)	- C(2)	- S(1)	104.9 (0.7)
C(6)	- C(2)	- S(1)	107.2 (1.1)
C(6)	- C(2)	- C(3)	113.7 (1.3)
C(7)	- C(2)	- S(1)	107.9 (1.1)
C(7)	- C(2)	- C(3)	112.7 (1.2)
C(7)	- C(2)	- C(6)	110.1 (1.0)
N(4)	- C(3)	- C(2)	105.6 (1.1)
C(8)	- C(3)	- C(2)	115.1 (0.9)
C(8)	- C(3)	- N(4)	110.1 (1.0)
C(5)	- N(4)	- C(3)	115.7 (1.0)
N(4)	- C(5)	- S(1)	115.0 (0.7)
C(12)	- C(5)	- N(4)	121.9 (1.0)
C(12)	- C(5)	- S(1)	123.0 (1.1)
O(9)	- C(8)	- C(3)	111.3 (1.1)
O(10)	- C(8)	- C(3)	126.5 (1.2)
O(10)	- C(8)	- O(9)	122.2 (1.4)
C(11)	- O(9)	- C(8)	120.3 (1.1)
C(13)	- C(12)	- C(5)	122.5 (1.2)
N(16)	- C(12)	- C(5)	126.3 (1.0)
N(16)	- C(12)	- C(13)	111.2 (0.8)
O(14)	- C(13)	- C(12)	103.7 (1.0)
O(17)	- C(13)	- C(12)	136.6 (0.9)
O(17)	- C(13)	- O(14)	119.7 (0.8)
C(15)	- O(14)	- C(13)	105.6 (0.7)
N(16)	- C(15)	- O(14)	116.2 (0.9)
C(18)	- C(15)	- O(14)	118.7 (0.8)
C(18)	- C(15)	- N(16)	125.0 (1.1)
C(15)	- N(16)	- C(12)	103.2 (0.9)
O(19(1))	- C(18)	- C(15)	106.6 (0.4)
O(19(2))	- C(18)	- C(15)	112.3 (0.9)
C(20)	- O(19(1))	- C(18)	115.9 (0.7)
C(20)	- O(19(2))	- C(18)	116.3 (1.3)
C(21)	- C(20)	- O(19(1))	109.1 (0.4)

Table 3.4 (continued)

C(21)	- C(20)	- O(19(2))	125.4 (0.9)
C(25)	- C(20)	- O(19(1))	124.9 (0.4)
C(25)	- C(20)	- O(19(2))	106.6 (0.9)
C(25)	- C(20)	- C(21)	124.7 (1.1)
C(22)	- C(21)	- C(20)	116.0 (1.4)
C(23)	- C(22)	- C(21)	121.3 (1.1)
C(24)	- C(23)	- C(22)	122.0 (1.1)
C(25)	- C(24)	- C(23)	120.3 (1.3)
C(24)	- C(25)	- C(20)	115.6 (1.0)

Table 3.5 Coordinates of hydrogen atoms.

Coordinates are given as fractions of cell edges x 10^4. The heavy atom associated with each hydrogen atom is also given. Standard deviations in parentheses are with respect to the last figures given

	x	y	z
H(C3)	1542(12)	3378(7)	2711(11)
H(N4)	962(9)	4869(6)	3228(7)
H(C6)(1)	2689(17)	5197(10)	442(13)
H(C6)(2)	2823(17)	4514(10)	-722(13)
H(C6)(3)	4024(17)	4397(10)	-128(13)
H(C7)(1)	1566(17)	2561(9)	1155(14)
H(C7)(2)	3380(17)	2841(9)	280(14)
H(C7)(3)	2180(17)	2959(9)	-313(14)
H(C11)(1)	5851(13)	3024(11)	2100(14)
H(C11)(2)	5164(13)	3930(11)	3042(14)
H(C11)(3)	6077(13)	4063(11)	1471(14)
H(C18)(1)	-5673(11)	5548(7)	4137(11)
H(C18)(2)	-4799(11)	6369(7)	3077(11)
H(C21)	-6405(13)	7797(8)	6407(10)
H(C22)	-8777(14)	8553(7)	7173(11)
H(C23)	-292(12)	8273(7)	6248(10)
H(C24)	-9806(10)	7092(7)	4853(11)
H(C25)	-7436(11)	6305(6)	4022(10)

Table 3.6(a) Thermal parameters for non hydrogen atoms

Anisotropic temperature factors are expressed as

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

The units of U_{ij} are $Å^2 \times 10^4$. Standard deviations in parentheses are with respect to the last figures given.

	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
S(1)	612(19)	658(20)	543(25)	-326(15)	-379(15)	243(16)
C(2)	457(76)	367(73)	524(93)	-249(61)	-246(65)	110(56)
C(3)	407(70)	327(65)	392(81)	-35(51)	-168(57)	80(50)
N(4)	441(51)	435(52)	264(53)	-65(38)	-216(38)	45(41)
C(5)	491(65)	290(54)	242(59)	-29(42)	-222(49)	-33(47)
C(6)	806(112)	741(107)	396(96)	-48(79)	-232(82)	-96(88)
C(7)	717(104)	500(90)	759(110)	-368(80)	-351(84)	159(76)
C(8)	368(71)	402(76)	461(81)	-54(60)	-172(57)	87(60)
O(9)	568(60)	436(55)	759(71)	-143(48)	-344(52)	170(47)
O(10)	503(60)	341(49)	866(78)	-124(48)	-267(54)	27(42)
C(11)	341(79)	773(109)	750(114)	-190(85)	-227(74)	120(71)
C(12)	423(57)	280(54)	272(59)	-18(41)	-155(44)	-12(43)
C(13)	429(58)	333(55)	351(65)	-145(43)	-218(48)	97(41)
O(14)	374(36)	471(41)	442(43)	-169(33)	-205(31)	125(32)
C(15)	339(51)	354(54)	377(63)	-23(44)	-250(44)	7(42)
N(16)	421(48)	426(47)	394(53)	-67(40)	-230(39)	83(39)
O(17)	559(50)	817(61)	474(53)	-250(42)	-293(41)	183(42)
C(18)	608(72)	458(60)	717(80)	-266(56)	-398(61)	234(53)
O(19(1))	590(67)	566(65)	1036(88)	-365(63)	-454(62)	169(51)
O(19(2))	267(107)	633(144)	559(123)	194(114)	-83(86)	176(97)
C(20)	612(67)	673(68)	497(64)	-142(53)	-287(52)	293(54)
C(21)	807(85)	884(86)	678(78)	-244(67)	-409(64)	256(68)
C(22)	944(88)	633(75)	574(77)	-166(59)	-335(68)	285(66)
C(23)	628(68)	556(65)	638(77)	34(55)	-149(59)	200(52)
C(24)	452(55)	518(69)	944(89)	51(62)	-336(55)	12(50)
C(25)	570(62)	367(55)	584(70)	77(49)	-259(54)	18(46)

Table 3.6(b) Thermal parameters for H atoms. Isotropic temperature factors are expressed as exp $[-2\pi^2 U(h^2 a^{*2} + k^2 b^{*2} + \ell^2 c^{*2})]$. The units of U are $Å^2 \times 10^4$. Standard deviations in parentheses are with respect to the last figures given.

	U
H(C3)	390(46)
H(N4)	322(34)
H(C6)(1)	681(66)
H(C6)(2)	681(66)
H(C6)(3)	681(66)
H(C7)(1)	619(63)
H(C7)(2)	619(63)
H(C7)(3)	619(63)
H(C11)(1)	616(62)
H(C11)(2)	616(62)
H(C11)(3)	616(62)
H(C18)(1)	512(41)
H(C18)(2)	512(41)
H(C21)	717(49)
H(C22)	661(46)
H(C23)	660(47)
H(C24)	609(45)
H(C25)	494(37)

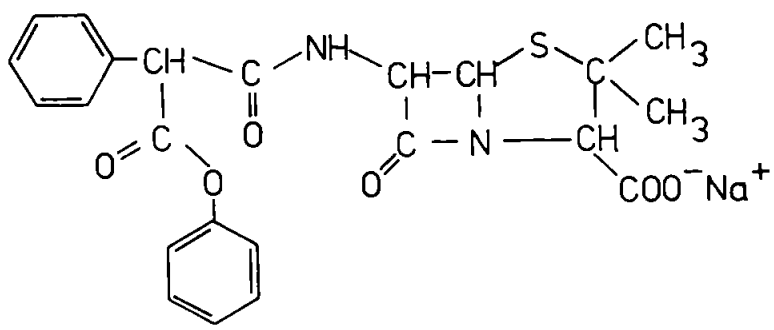
CHAPTER 4

The Crystal and Molecular Structure of the Phenyl Ester of Carbenicillin (Carfecillin).

4.1 Introduction

Esters of carbenicillin have been shown by optical rotatory measurements to undergo side-chain configurational modification in solution (ref. Ch. 5). To facilitate a study of the mechanism by which such modification takes place within the side-chain of penicillin compounds, the crystal structure of the phenyl ester of carbenicillin, I, has been determined.

I



Experimental

4.2 Crystal Preparation and Preliminary X-ray Studies

The compound was obtained from Beecham Research Laboratories. Preparation of suitable crystalline material was performed in the following manner: 2 g starting material was dissolved in 7 cm^3 distilled water at 48°C and to this solution 18 cm^3 of ethanol and 18 cm^3 isopropanol was added slowly to prevent clouding. The solution was maintained at 4°C and protected from light for 7 days. After filtering and drying, flat plate-like crystals were obtained.

Preliminary X-ray investigations of these crystals revealed a doubling of the reflexions indicating that the crystals were themselves twinned. All reflexions other than the Okℓ's were doublets, showing that the ab and ac planes of the twin forms are parallel. Investigation under the microscope of each crystal used, revealed a system of twinning, common to them all, where the coincidence of the ab planes occurred between the flat plate-like faces of the constituent crystals as shown, in perspective, in Fig. 4.1. Fig. 4.2, I and II shows

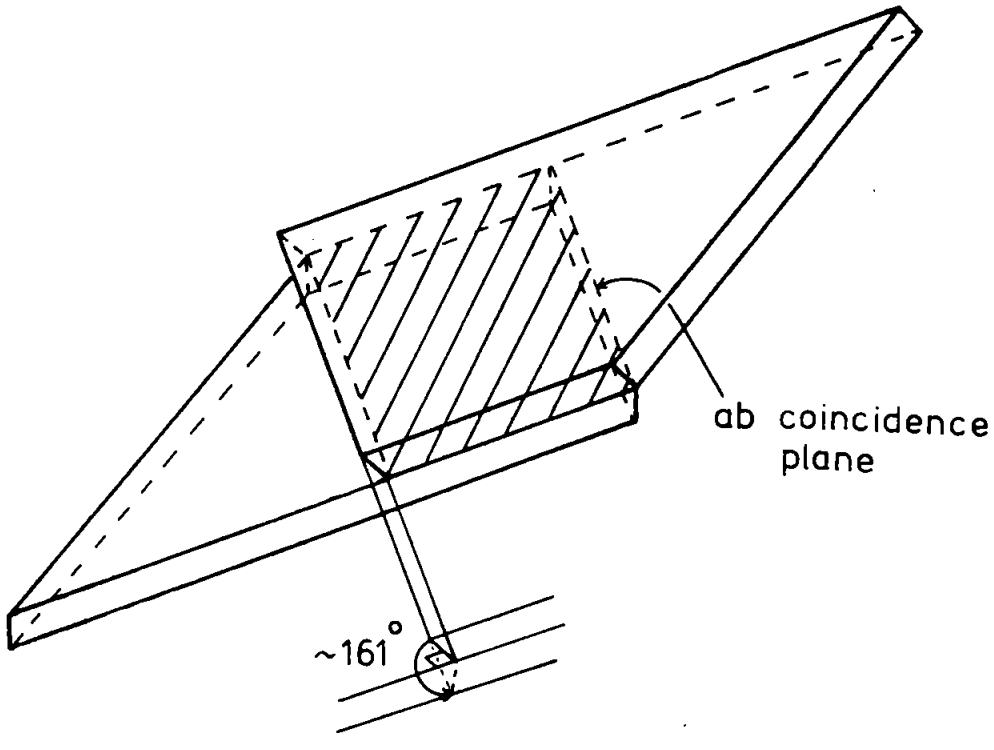
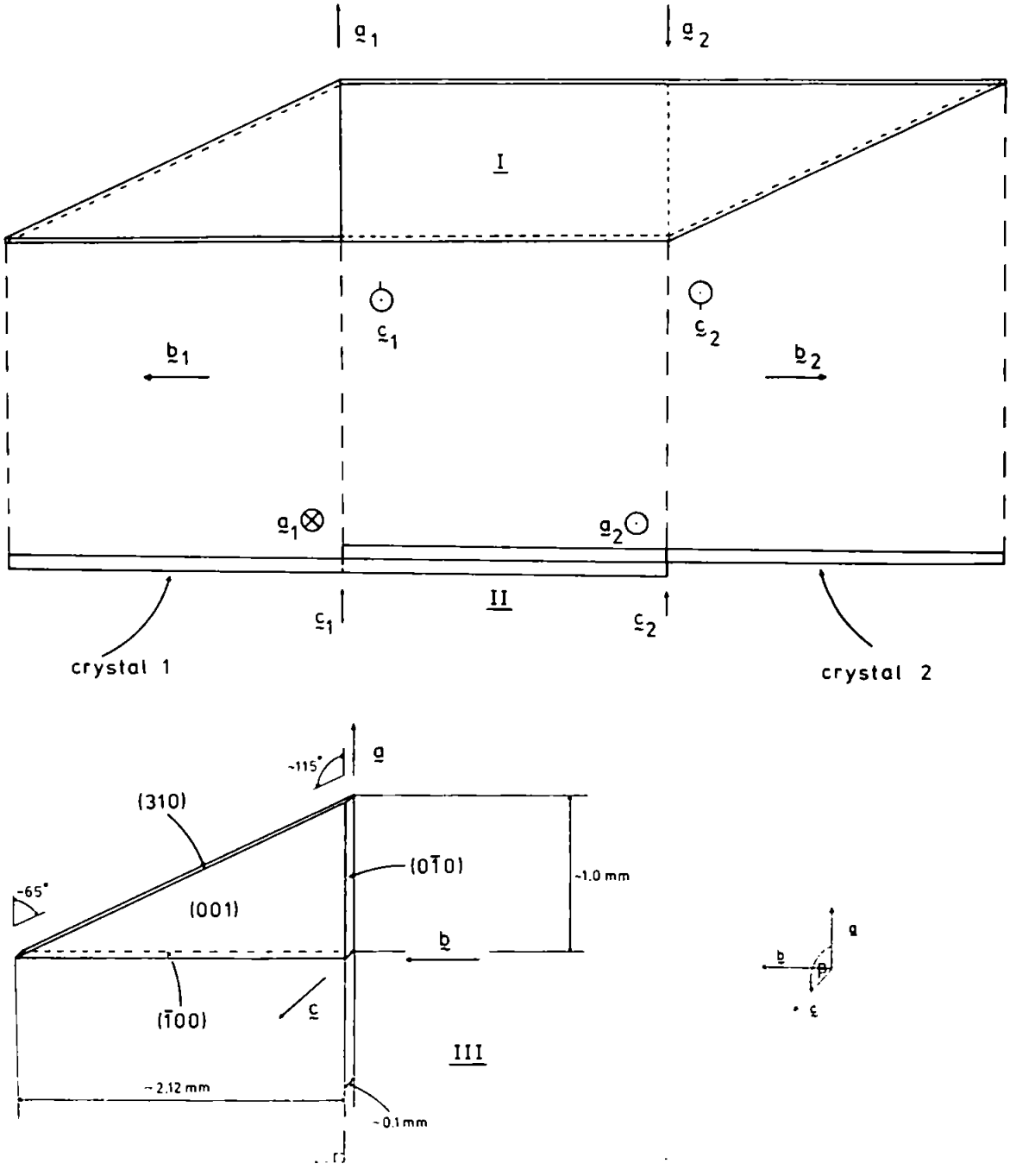


Fig.4.1 Perspective view of the twinning of Carfecillin crystals.



Twinning of crystals of Carfecillin

Fig. 4.2

two aspects of one of the twin crystals. The overlapping coincidence plane proved impossible to cleave and it was found necessary to remove the triangular 'tail' of one twin for single crystal X-ray analysis, Fig. 4.2, III.

The unit-cell dimensions were determined from zero level equi-inclination Weissenberg photographs, the camera radius was determined from high-angle reflexions from an annealed gold wire. Systematic absences 0k0, k = 2n + 1, (n integer), together with there being two molecules per unit cell, indicated space group $P2_1$ in the monoclinic system, with a = 8.77(3), b = 6.20(3), c = 21.40(3) Å, $\beta = 99.5^{o}$, V = 1147.65 $Å^3$, μ(CuKα) = 1476 m^{-1}.

4.3 Intensity Data Collection and Preliminary Treatment

Data for intensity measurement were obtained by the equi-inclination method on Stoe and Nonius Weissenberg cameras using Ni-filtered CuKα radiation (λ = 1.5418 Å) and the multiple film technique. The crystals for these measurements were rotated about the $\underset{\sim}{b}^*$ reciprocal crystallographic axis with the $(\bar{1}\ 0\ 0)$ face of the crystal parallel to the rotation axis. The x-ray films showed some reduction in intensity of reflexion at high sin θ after crystals had prolonged exposure to X-rays, 5 different crystals of very similar dimensions were used for collection of intensity data. The intensities of the X-ray reflexions were measured by the Science Research Council microdensitometer at Daresbury. A total of 1208 reflexions were of measurable intensity.

A Wilson Plot (ref Ch. 1, §1.11) was used to estimate an overall scale factor and isotropic temperature factor to place the $|F_o(hk\ell)|$ data on an absolute scale. Groups of reflexions, within a shell of the reciprocal reflexion sphere containing ~100 reflexions were chosen omitting those with Miller indices containing only 0's or 1's, since they are close to the reciprocal origin, and thus

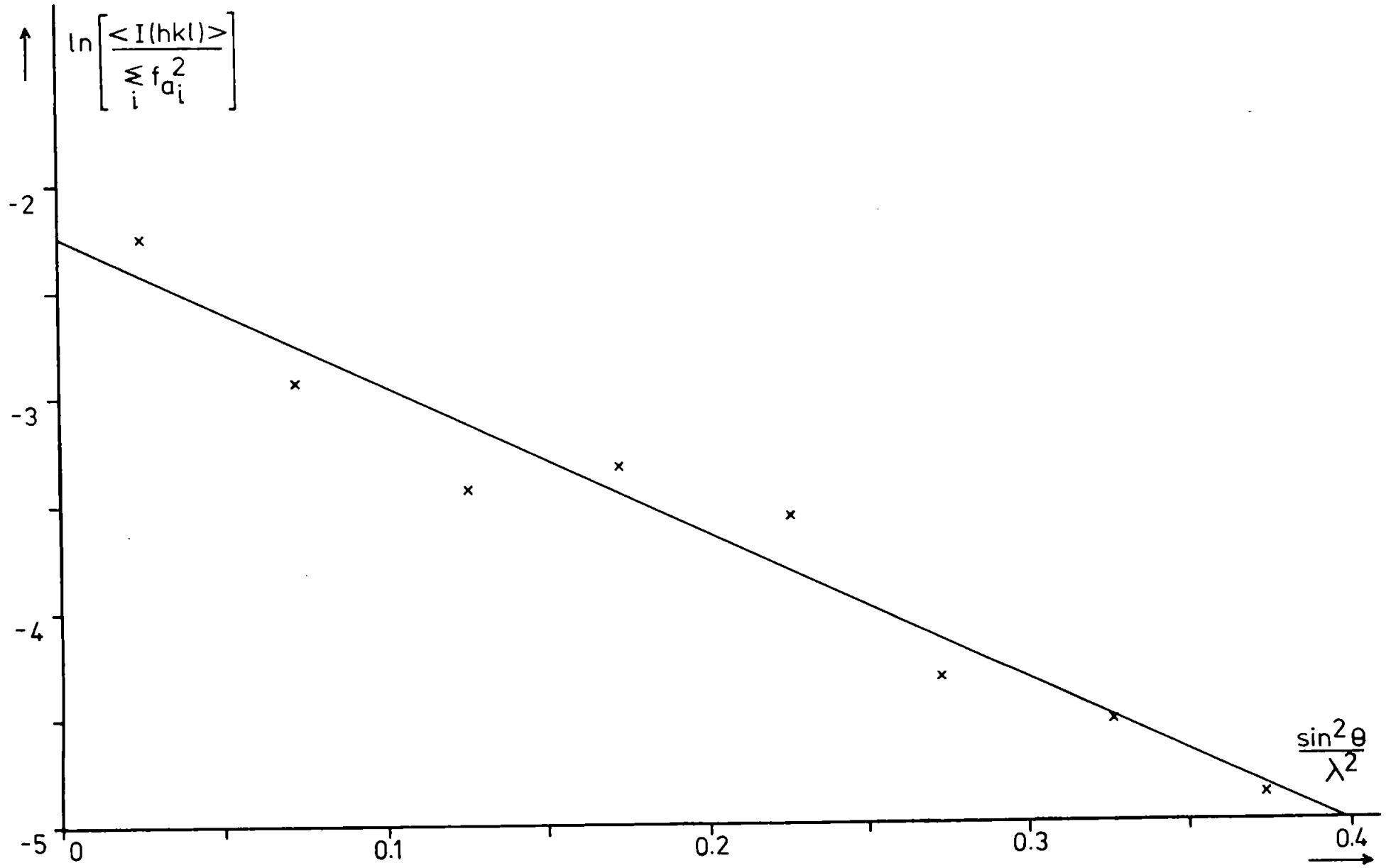


Fig.4.3 Wilson Plot for Carfecillin

affected by the physical constraints of intensity measurement related to the proximity of the reciprocal origin, extinction and camera back stop interference. Such shells were described by radii lying between ha* values. Computation of $(\sin^2\theta)/\lambda^2$ for each reflexion resulted in the following distribution of reflexions within each shell;

$(\sin^2\theta)/\lambda^2$	No. of reflexions with h, k or $\ell \neq 0$ or 1	$\langle I(hk\ell)\rangle$
0 - 0.05	111	357.2
0.05 - 0.10	198	179.2
0.10 - 0.15	237	108.7
0.15 - 0.20	251	121.1
0.20 - 0.25	177	96.5
0.25 - 0.30	110	44.8
0.30 - 0.35	68	36.3
0.35 - 0.40	26	25.8

A plot of $\ln\left(\frac{\langle I(hk\ell)\rangle}{\sum_{i=1}^{N} f_{a_i}^2}\right)$ against $(\sin^2\theta)/\lambda^2$ is given in Fig. 4.3, where f_{a_i} is the atomic scattering factor for the ith atom at rest in the unit cell containing N atoms. The measured gradient (-6.875 = -2B) resulted in an estimated isotropic temperature factor B = 3.5 $\mathring{A}^2$ and an overall scale factor of 0.325, given by $\sqrt{(e^{-2.25})}$, where the exponent is the intercept when $(\sin^2\theta)/\lambda^2 = 0$.

4.4 Structure Determination

The major computations were carried out with the SHELX program. Initial determination of the sulphur atom position was by means of a Patterson function of the form given in Eqn. 1.60, which, upon symmetry reduction in the monoclinic system, becomes

$$P(u,v,w) = \frac{4}{V} \sum_{(hk\ell)} \left[|F(hk\ell)|^2 \cos 2\pi (hu + \ell w) + |F(\bar{h}k\ell)|^2 \cos 2\pi (hu - \ell w) \right] \cos 2\pi kv \quad \text{... Eqn. 4.1}$$

The equivalent positions in the space group $P2_1$ are (x,y,z) and (-x, ½+y, -z), resulting in expected Patterson peaks at

$$\left.\begin{aligned} (u_1, v_1, w_1) &= (2x, \tfrac{1}{2}, 2z) \\ \text{and} \quad (u_2, v_2, w_2) &= (-2x, \tfrac{1}{2}, -2z) \end{aligned}\right\} \quad \text{... Eqns. 4.2}$$

ie. peaks at (2x, 2z) and (1-2x, 1-2z) on the v = ½ section, corresponding to the centrosymmetric nature of the Patterson function.

A sharpened Patterson function; in this instance performed by replacing the coefficient $|F(hk\ell)|^2$ in Eqn. 4.1 by $|E(hk\ell)|.|F(hk\ell)|$, where $E(hk\ell)$ is the normalised structure factor given by Eqn. 1.66; resulted in values for x and z, consistent with Eqns. 4.2, of,

$$\left.\begin{aligned} x &= 0.2648 \\ z &= 0.1501 \end{aligned}\right\} \quad \text{... Eqns. 4.3}$$

with the y value chosen as zero for convenience.

Electron density Fourier synthesis was carried out using reflexions initially phased upon the sulphur atom alone using the expression

$$\rho(X,Y,Z) = \frac{4}{V} \left\{ \sum_{\substack{hk\ell \\ (k=2n)}} \left[|F(hk\ell)| \cos 2\pi (hX + \ell Z) \cos (2\pi kY - \alpha(hk\ell)) + |F(\bar{h}k\ell)| \cos 2\pi(-hX + \ell Z) \cos(2\pi kY - \alpha(\bar{h}k\ell)) \right] \right.$$

$$- \sum_{\substack{hk\ell \\ (k=2n+1)}} \left[|F(hk\ell)| \sin 2\pi(hX + \ell Z) \sin (2\pi kY - \alpha(hk\ell)) \right.$$

$$\left. \left. + |F(\bar{h}k\ell)| \sin 2\pi(-hX + \ell Z) \sin (2\pi kY - \alpha(\bar{h}k\ell)) \right] \right\} \quad \text{... Eqn. 4.4}$$

where $\alpha(hk\ell)$ is the phase of $F(hk\ell)$ given by

$$\alpha(hk\ell) = \tan^{-1}\left(\frac{B}{A}\right) \quad \text{... Eqn. 4.5}$$

where

$$\left.\begin{aligned} A &= 2\cos 2\pi(hx + \ell z + \tfrac{k}{4})\cos 2\pi(ky - \tfrac{k}{4}) \\ \text{and} \qquad & \\ B &= 2\cos 2\pi(hx + \ell z + \tfrac{k}{4})\sin 2\pi(ky - \tfrac{k}{4}) \end{aligned}\right\} \quad \ldots \text{ Eqns. 4.6}$$

and x, y and z are the phasing atom co-ordinates. However, interpretation of the resultant map proved difficult on two accounts.

Firstly, by the nature of Eqns. 4.6, phasing on an atom placed on y = 0, results in the calculated phase, $\alpha(hk\ell)$, having a value of $-\frac{k\pi}{2}$. Any arbitrary value given to y, results in the same effective value of $\alpha(hk\ell)$, since a change in y represents a shift of origin along the y axis; the origin itself being arbitrary and fixed only by choice. Thus, Eqn. 4.4 reduces in the case of one atom on y = 0 to the form

$$\rho\,(XYZ) = \frac{4}{v}\left\{\sum_{\substack{hk\ell \\ (k=2n)}} \left[\,|F(hk\ell)|\,\cos 2\pi(hX + \ell Z) + |F(\bar{h}k\ell)|\cos 2\pi(-hX + \ell Z)\right]\cos 2\pi kY \cdot \cos n\pi \right.$$

$$\left. - \sum_{\substack{hk\ell \\ (k=2n+1)}} \left[\,|F(hk\ell)|\,\sin 2\pi(hX + \ell Z) + |F(\bar{h}k\ell)|\sin 2\pi(-hX + \ell Z)\right] \cos 2\pi kY\,\cos n\pi \right\} \quad \ldots \text{ Eqn. 4.7}$$

from which it can be seen that the Y Fourier component follows a cosine variation and is therefore symmetrical about Y = 0, resulting in a mirror plane on Y = 0, and thus increasing the symmetry such that it belongs to space group $P2_1/m$. Hence, an atom derived from the first Fourier synthesis appears at x, y, z and x, -y, z; choosing one of these positions only, breaks the mirror symmetry and weights any subsequent synthèses toward the reduced $P2_1$ symmetry.

Secondly, due to the plate-like shape of the crystal, early attempts at Fourier synthesis using phases calculated from deduced atomic positions (x,y,z) yielded satellite positions (-x, ½-y, -z) caused initially by the above centrosymmetric effect. This was enhanced by absorption of diffracted intensity in passing

through extended intra-crystal path lengths for reflexions (hkℓ) with ℓ large compared with h, in contrast with relatively shorter intra-crystal path lengths for h large compared with ℓ: similarly, layers with k large, for all h and ℓ, suffer relatively large absorption, which confined early Fourier syntheses to a dominance of low order k reflexions with the result that the pseudo mirror symmetry was not easily removed. The anomalous behaviour was overcome by the application of the numerical absorption correction, based on the SHELX 'ABSC' routine, to the observed data.

Fig. 4.4 shows the progress of the electron density synthesis using absorption corrected data; where the symbols • and o describe respectively those atoms used in the synthesis and those derived from it. C(2) derived from synthesis A appeared as two identical strong peaks directly above and below S(1) when viewed along the $\underset{\sim}{b}$ axis at $y \approx \pm \frac{1}{4}$, however, the nature of the Y component in Eqn. 4.7 together with a predominance of low order k values at this early stage led to doubt concerning the validity of this peak. Indeed a trial synthesis using C(2) on $y = -\frac{1}{4}$ proved intractable when compared with inter-atomic vector peaks in the Patterson map. The Patterson map, however, revealed a peak at (0, 0.3, 0) ie. directly above S(1), as in the case of synthesis A, but indicating a y co-ordinate of $\pm$ 0.3. Choosing y = −0.3, successfully broke the mirror symmetry in A and produced synthesis B, from which, part of the β lactam could be derived. Completion of the penicillin nucleus was achieved by synthesis E and the side-chain determination proceeded rapidly, though the benzene rings appeared as unresolved electron density distributions defining the planes of the rings only until the final synthesis using the completed remaining molecule, defined the individual atoms satisfactorily.

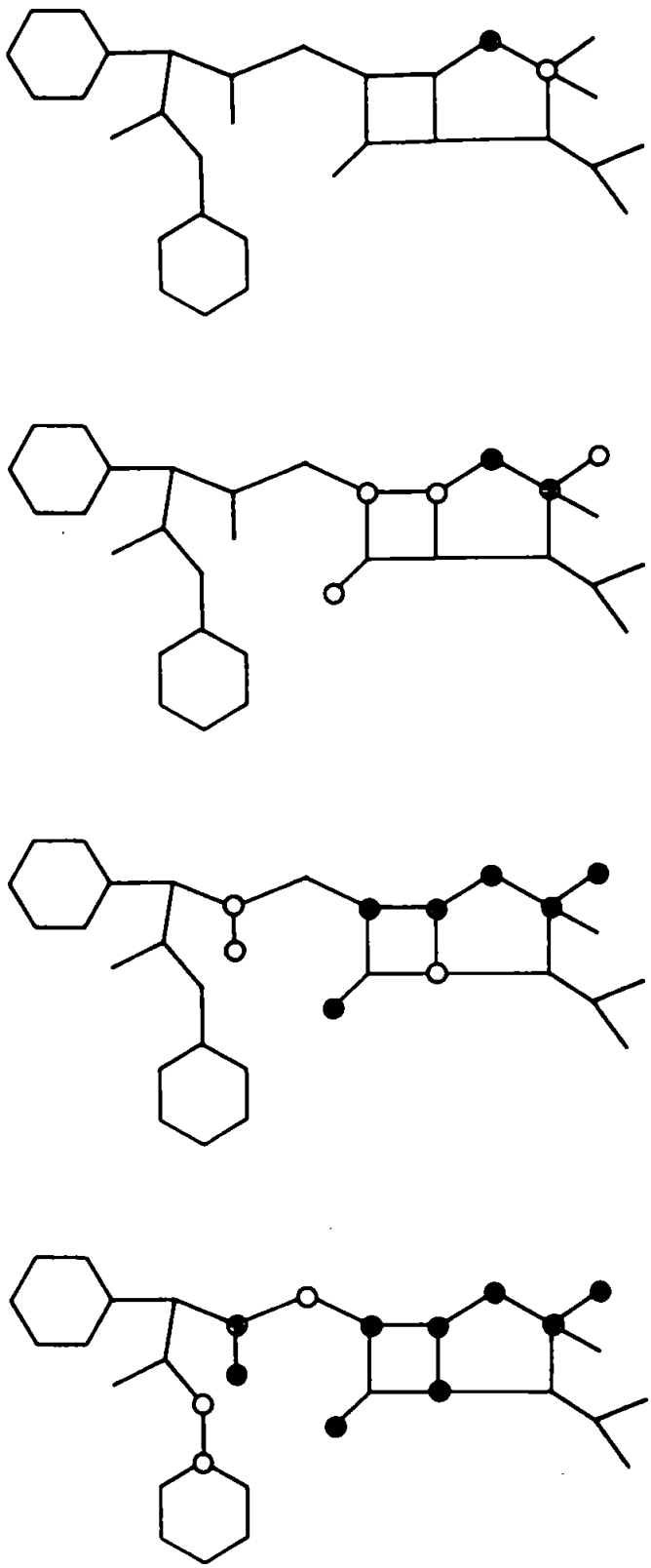
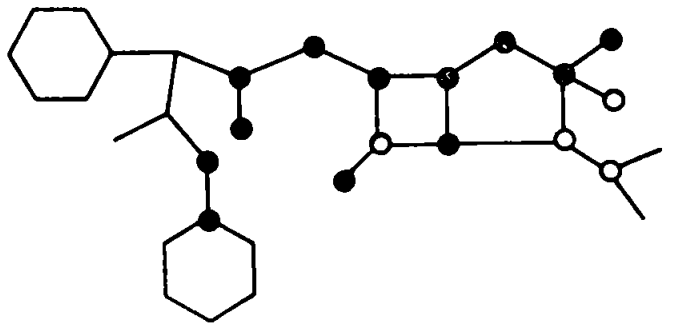
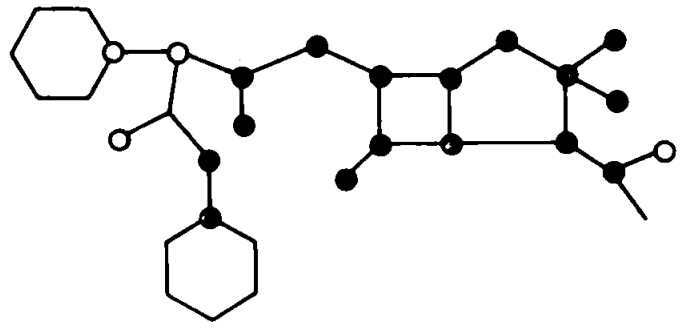


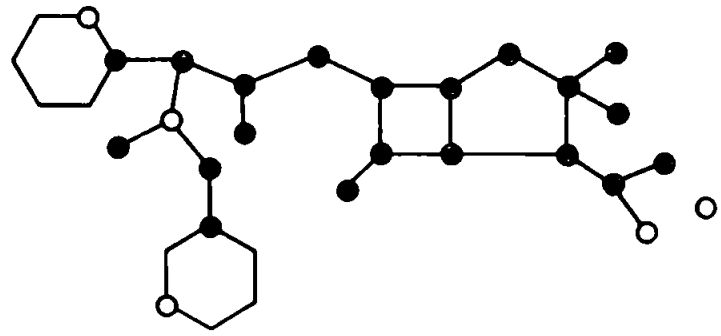
Fig. 4.4. Progress of Electron Density Fourier Synthesis during the Structure determination of Carfecillin



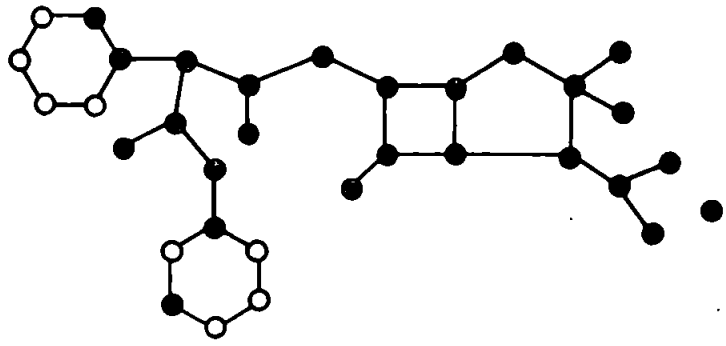
E.



F.



G.



H.

Fig. 4.4 (Continued)

4.5 Structure Refinement

Three cycles of unweighted full matrix least-squares refinement with individual isotropic temperature factors and refinement of scale factors resulted in R = 0.120. An F_o-F_c Fourier synthesis showed considerable anisotropic thermal vibrations, associated particularly with the sulphur atom. Anisotropic refinement using the SHELX 'BLOC' to section the structure, (Fig. 4.5), with hydrogen atoms included in the refinement, resulted in R = 0.095 after omitting ten low order reflexions suffering from severe extinction. The hydrogen atoms were given isotropic temperature factors fixed at the values of the isotropic temperature factor of the atom to which they are bonded, obtained at the isotropic refinement stage, the bond length being fixed at 1.08 Å. The benzene rings were refined as rigid hexagons with bond lengths fixed at 1.395 Å. No further improvement could be made in F_o/F_c correlation probably due to the deterioration of the crystals during prolonged X-ray exposure and the possible inaccuracy inherent in the use of an absorption correction applied to the intensity data based on the assumption that all the crystals used during data collection were of the same dimensions. The observed and calculated structure factors are given in Appendix B (ref. CARF P2(1)).

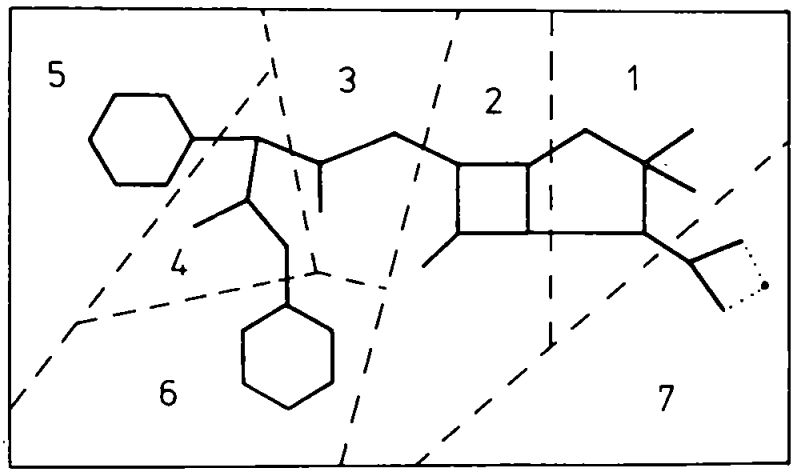
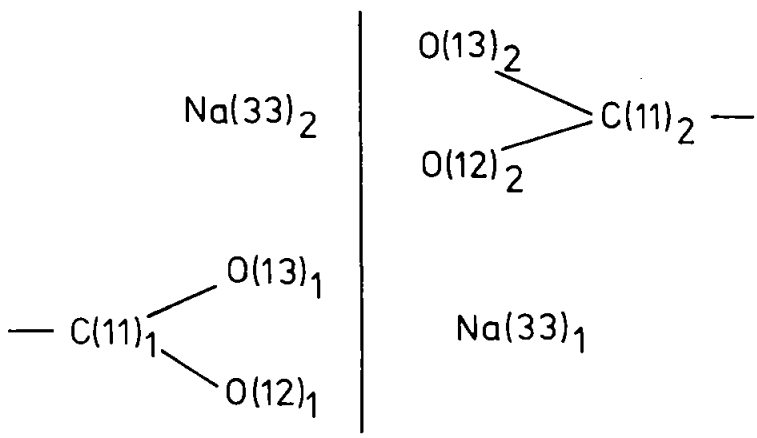


Fig. 4.5 SHELX 'BLOC' sectioning of Carfecillin.

4.6 Discussion

The final co-ordinates of the non-hydrogen atoms are given in Table 4.1. The bond distances and angles are listed with their standard deviations in Tables 4.2 and 4.3. The H atom co-ordinates are given in Table 4.4. Thermal parameters for all atoms are listed in Table 4.5. Fig. 4.6 shows the schematic labelling of the non hydrogen atoms together with inter-atomic distances: Fig. 4.7 shows bond angles. Fig. 4.8 gives a view of the complete unit cell contents along $\underset{\sim}{a}$ and Fig. 4.9 shows the structure viewed along $\underset{\sim}{b}$.

With reference to Fig. 4.8, it can be seen that the coincidence ab plane (in the twinned crystal) passes through the ionic $O^{-}Na^{+}$ system where Na(33) lies almost equidistant from $O^{-}(12)$ and O(13). Upon crystallisation from solution, two molecules could combine in one of two ways, depending on the charge distribution within the carboxylic radical. Each single crystal in the twin has an atomic arrangement thus

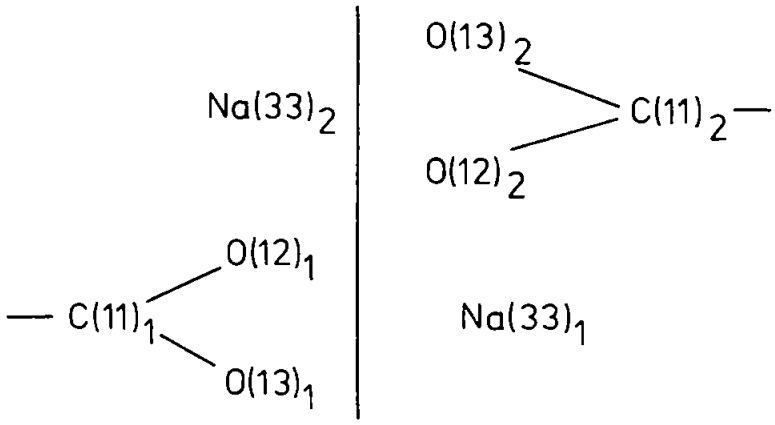


(i)

ab plane

whereas at the crystal interface, the following atom arrangement

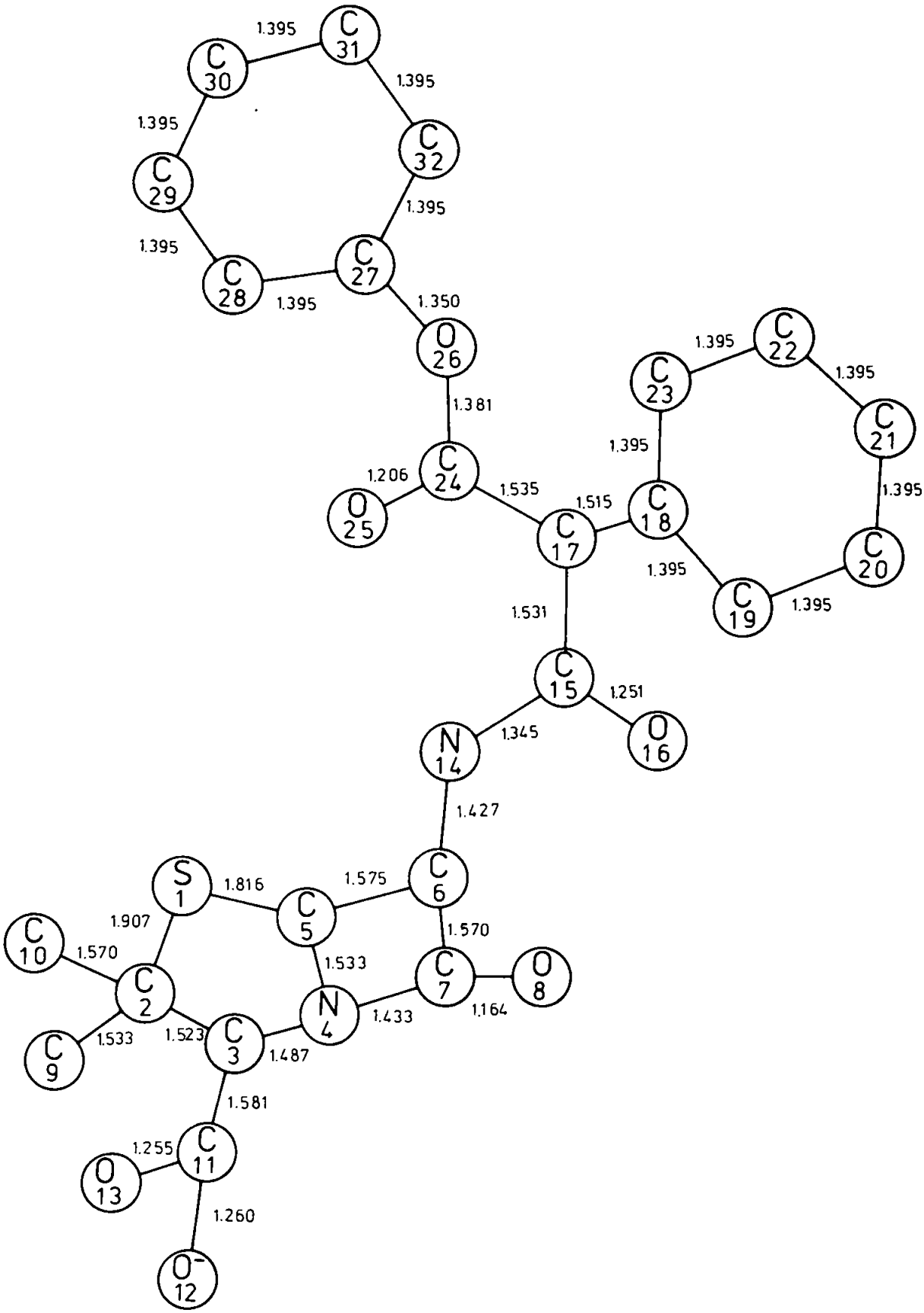
provides another orientation for similar crystal growth of the twin.



(ii)

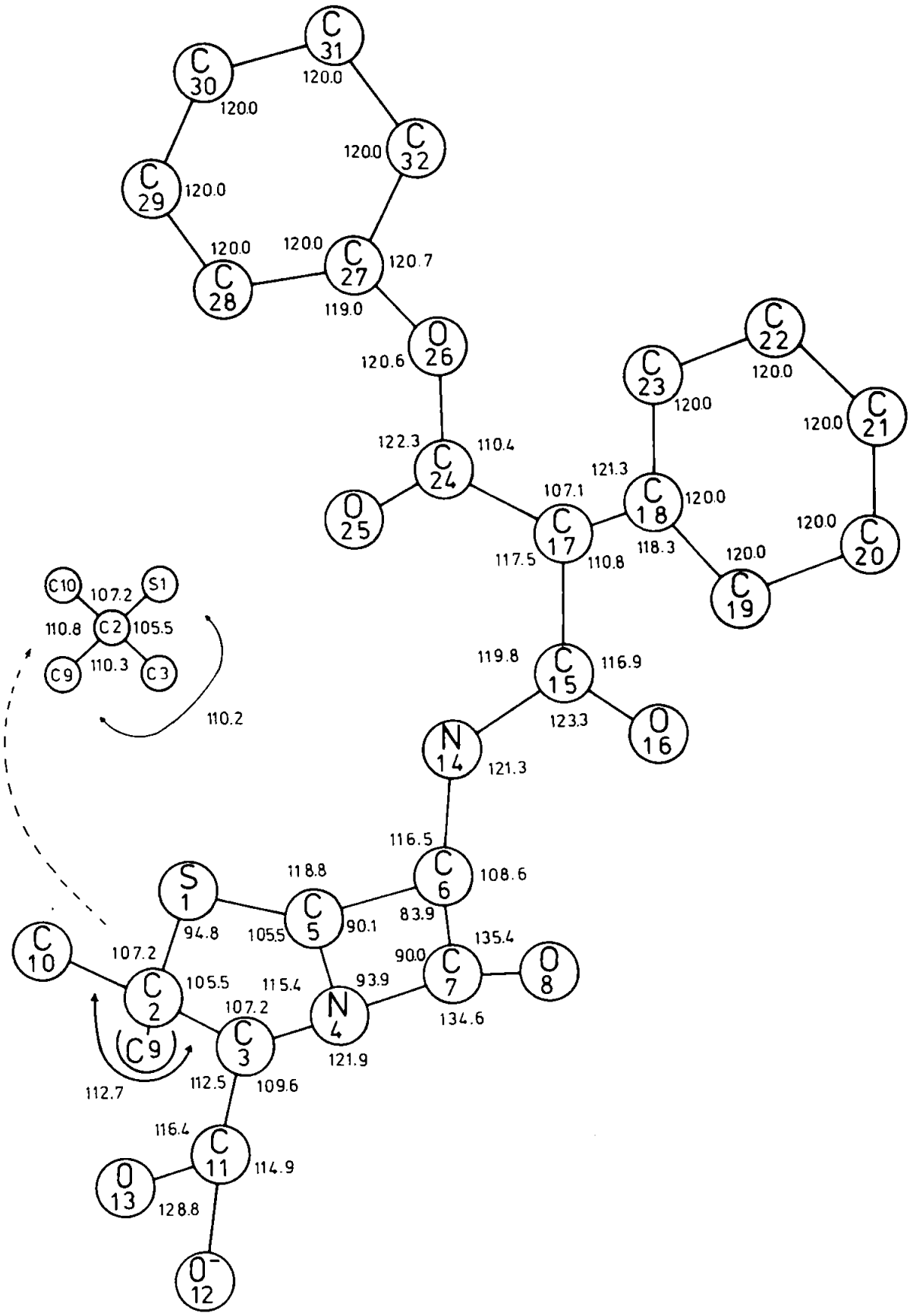
ab interface plane

Given the interface arrangement (ii) in the twinned crystal, growth from solution can take place as in Fig. 4.8. Thus, a macro-crystal is seen to develop containing two single crystals oriented 180° with respect to each other about an axis perpendicular to the flat ab face. The nature of this twinning could explain the difficulty encountered when attempting cleavage of the twin along the coincident plane.



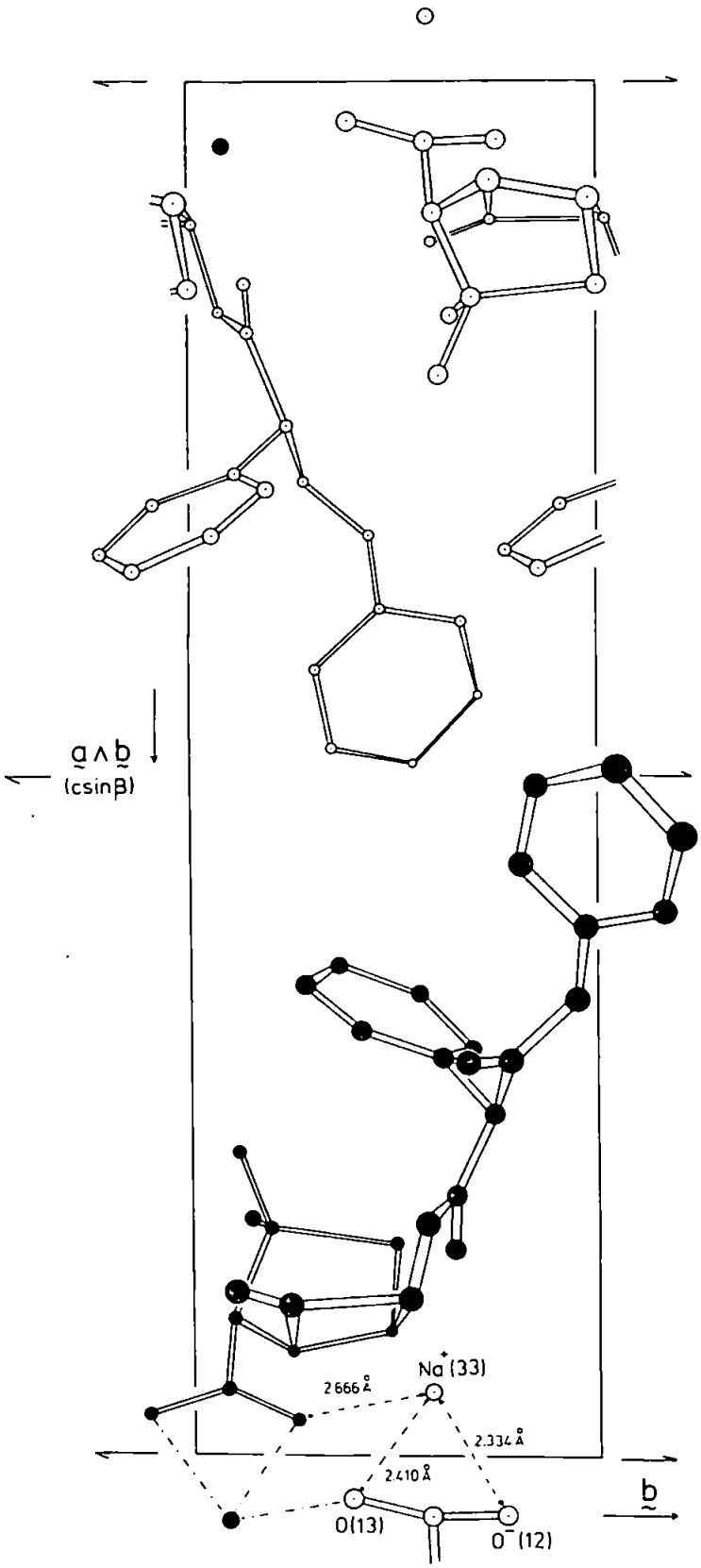
Schematic labelling and inter-atomic distances in Carfecillin

Fig. 4.6



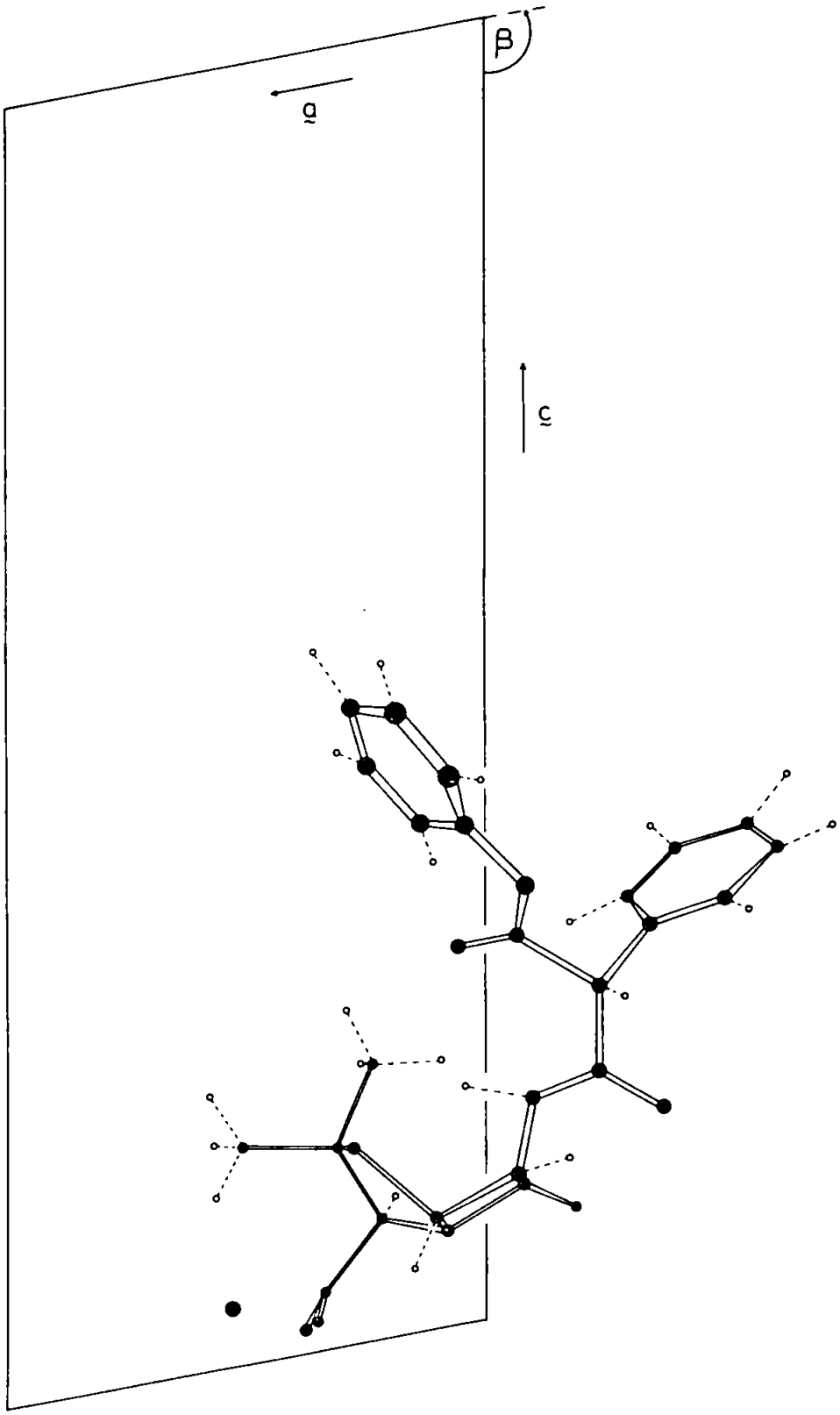
Bond angles in Carfecillin

Fig. 4.7



The crystal structure of Carfecillin viewed along a

Fig. 4.8



The crystal structure of Carfecillin viewed along b

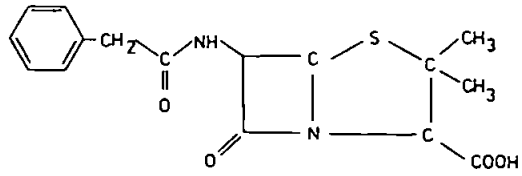
Fig. 4.9

4.7 A comparison of the conformation of the penicillin nucleus with known penicillins and cephalosporins

Some penicillins and cephalosporins, which have been the subject of detailed and accurate X-ray crystal structural studies, are listed together with their structural formulae.

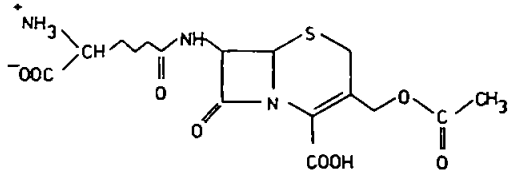
Potassium benzyl penicillin (pen. G)
(Crowfoot et al 1949, Pitt 1952)

(ref. no.) 38 , 39



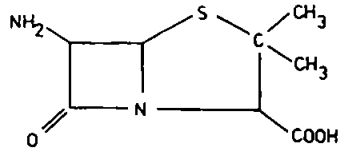
Cephalosporin C
(Hodgkin, Maslen 1961)

42



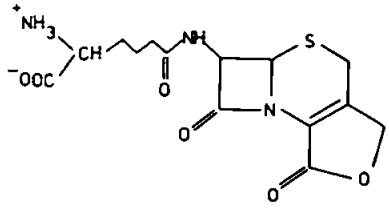
6 - amino - penicillanic acid (6-APA)
(Diamond 1963)

43



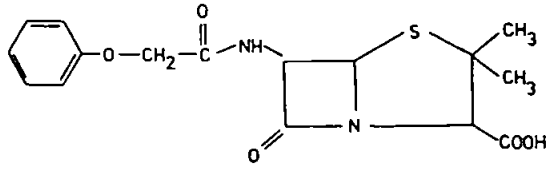
Cephalosporin C_c (ceph C_c)
(Diamond 1963)

43



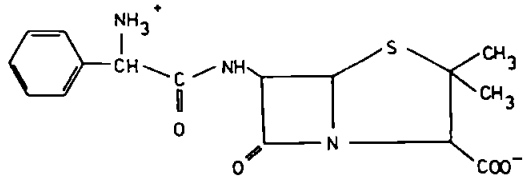
Phenoxymethylpenicillin (pen-V)
(Abrahamsson et al 1963)

36



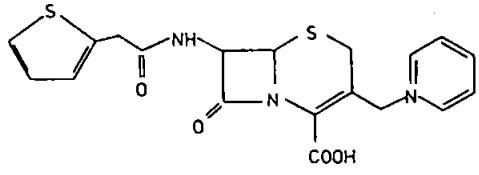
Ampicillin
(trihydrate James et al 1968)
(anhydrate Boles, Girven 1976)

41 , 34



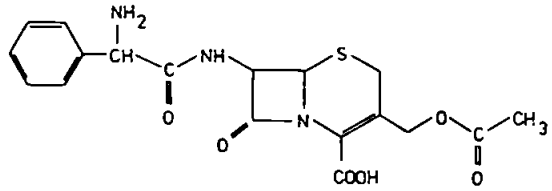
Cephaloridine hydrochloride monohydrate
(Sweet and Dahl, 1970)

44



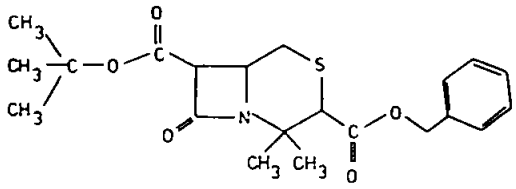
Cephaloglycin
(Sweet and Dahl 1970)

44



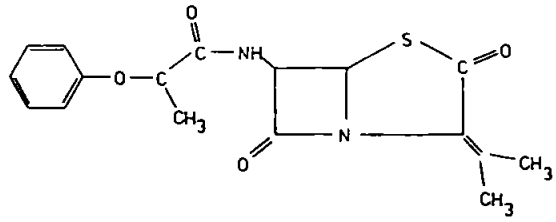
Synthetic β- lactam syn-ceph
(Kalyani and Hodgkin 1970)

45



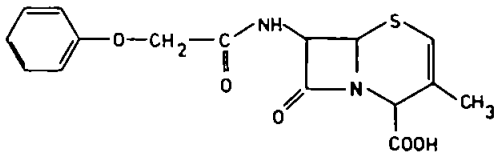
Anhydro - α - phenoxyethyl penicillin (an - pen)
(Simon and Dahl 1970)

46



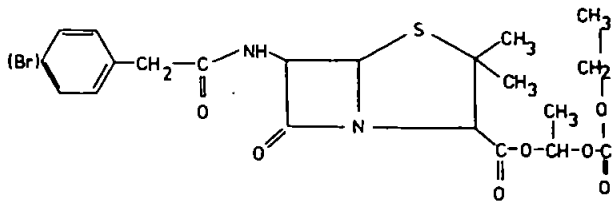
Phenoxymethyl - Δ^2 - deacetoxylcephalosporin (2-cephem)
(Sweet and Dahl 1970)

44



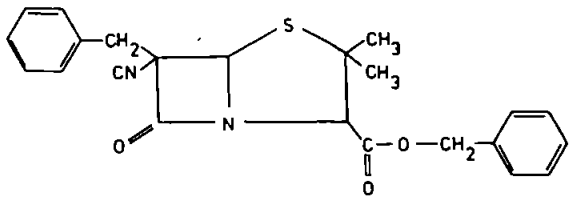
(pBr) benzyl pen. 1'
diethyl carbonate ester
(Csoregh, Palm 1976)

47



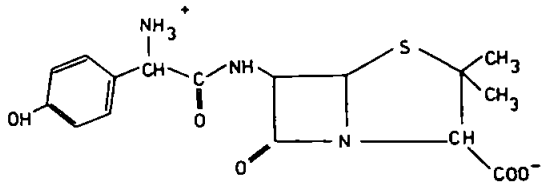
Benzyl 6α - benzyl 6_β - isocyano - penicillanate
(Girven 1977)

28



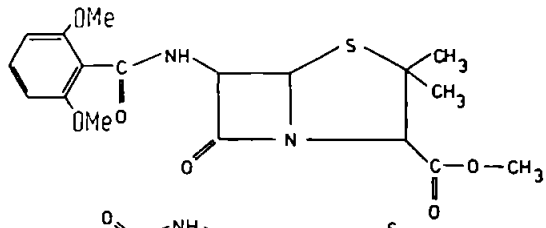
Amoxycillin trihydrate
(Boles, Girven, Gane 1977)

48



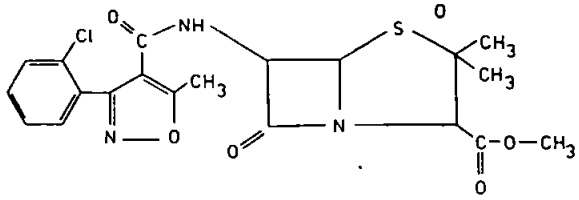
Methicillin methyl ester
(Blanpain, Melebeck, Durant 1977)

49



Cloxacillin Methyl Ester
(Blanpain, Durant 1976)

50



Contrasts between the very different molecular forms of molecules known to inhibit activity amongst bacterial enzymes, indicates that requirements for activity may not be very conformationally restrictive. Penicillin and cephalosporin antibiotics are similar stereo-chemically, not in their detailed dimensions or conformation, but because each, in its β lactam ring, contains a N-CO bond with characteristics differing from the usual amide or unstrained β lactam.

The decrease in amide character and the peculiarly distinctive behaviour of the fused β lactam would appear to be necessary for any bactericidal action.

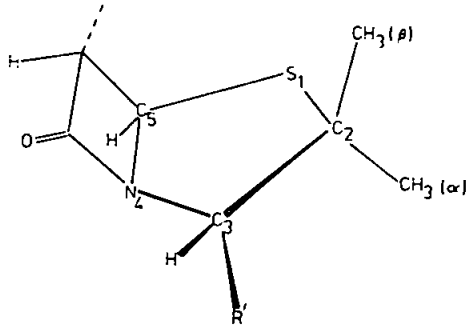
It is possible to facilitate a comparison amongst the reported structures by considering the molecular conformation within the penicillin nucleus. Two distinctive features are illustrated in Figs. 4.10 and 4.11.

The effect of the constraining power enforced by the β lactam upon the adjoining thiazolidine ring, consisting of five atoms, maintains four of them almost coplanar, with the remaining atom removed from this plane. The non-coplanar atom differs in both Type A and B (Fig 4.10). Particular thiazolidine ring conformations appropriate to the known penicillin structures are shown. In carfecillin, C(3) is 0.42 Å out of the plane defined by S(1), C(5), N(4) and C(2) (Table 4.6). The thiazolidine ring in carfecillin is therefore very similar to that of the thiazolidine ring in phenoxymethyl penicillin, p-bromopenicillinV and potassium benzyl penicillin, characterised by C(3) out of the common plane, and thus belongs to type A.

The significance of the chemical and biological activity of β lactam compounds is perhaps centered on the relative geometry of the β lactam nitrogen atom with its three substituents. The perpendicular distances of N(4) from the plane containing its neighbouring atoms appropriate to the known structures are shown in Fig. 4.11. Inclusion

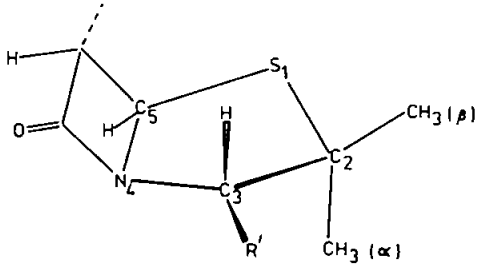
CONFORMATION OF THIAZOLIDINE RINGS OF KNOWN PENICILLIN STRUCTURES

TYPE A



PHENOXYMETHYL – PENICILLIN penicillin V	C_3 down	0·51 Å
6-AMINO-PENICILLANIC ACID	N_4 up	0·4 Å
p-BROMOPENICILLIN V	C_3 down	0·4 Å
POTASSIUM BENZYL PENICILLIN penicillin G	C_3 down	0·5 Å
(pBr) benzyl pen. 1' diethyl carbonate ester	C_3 down	0·51 Å

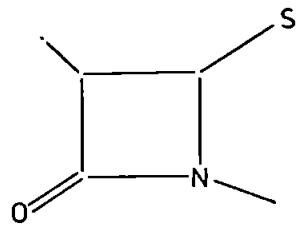
TYPE B



AMPICILLIN ANHYDRATE	C_2 down	0·71 Å
AMPICILLIN TRIHYDRATE	S_1 up	0·84 Å
PENICILLIN V SULPHOXIDE	S_1 up	?
AMOXYCILLIN TRIHYDRATE	S_1 up	0·83 Å
METHICILLIN METHYL ESTER	S_1 up	?
CLOXACILLIN METHYL ESTER	S_1 up	?

Fig. 4.10

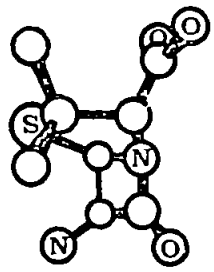
BONDING GEOMETRY AT β-LACTAM NITROGEN IN SOME PENICILLINS AND CEPHALOSPORINS



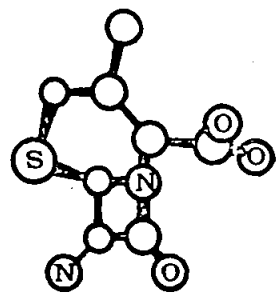
COMPOUND	DISTANCE OF N ATOM FROM PLANE OF 3 SUBSTITUENTS (Å)	
METHICILLIN METHYL ESTER	0·44	
PEN G	0·40	
PEN V	0·40	
CLOXACILLIN METHYL ESTER	0·39	
(pBr) benzyl pen. 1' diethyl carbonate ester	0·38	
AMOXYCILLIN TRIHYDRATE	0·38	
AMPICILLIN TRIHYDRATE	0·38	
AMPICILLIN ANHYDRATE	0·35	
6-APA	0·32	
CEPH C_c	0·32	
CEPHALORIDINE	0·24	
CEPHALOGLYCINE	0·22	
AN-PEN	0·42	Inactive
2-CEPHEM	0·06	Inactive
SYN-CEPH	0·10	Inactive

Fig. 4.11

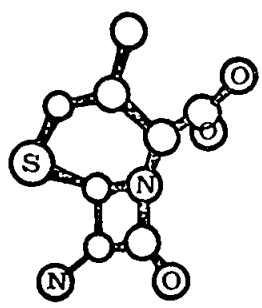
of the 'inactive' compounds must be borne with reference to significant differences from the antibiotics. The comparative structural arrangements of the nuclei of penicillins, Δ^3 -cephalosporins and Δ^2 - cephalosporins are given in Fig. 4.12 (a), (b) and (c) respectively.



(a) penicillins



(b) Δ^3 – cephalosporins



(c) Δ^2 – cephalosporins

Fig. 4.12 The comparative structural arrangements of the nuclei of penicillins, Δ^3 & Δ^2- cephalosporins.

The pyramidal nature of N(4) is described by the deviation of N(4) from the plane defined by C(3), C(5) and C(7). In carfecillin, N(4) is 0.47 Å out of this plane; a similar value to that for methicillin methyl ester (0.44 Å), but somewhat greater than a typical distance of 0.35 Å for reported bactericidally active β lactam antibiotics. N(4) is also significantly out of the plane of the remaining β lactam constituents C(5), C(6) and C(7) being 0.28 Å distant , (Table 4.6).

The following chapter discusses the side-chain configuration in carfecillin, both in the crystalline state and in solution, forming a correlation between side-chain configurations of penicillin derivatives of known structure and observed H^1 n.m.r. and circular dichroism characteristics associated with those configurations.

Table4.1 Final co-ordinates obtained from least-squares refinement . Co-ordinates are given as fractions of cell edges x 10^4. Standard deviations in parentheses are with respect to the last figures given.

	x	y	z
S(1)	2614(6)	0(0)	1492(3)
C(2)	2842(23)	-3049(42)	1580(10)
C(3)	2004(22)	-3992(37)	960(9)
N(4)	670(17)	-2555(26)	733(7)
C(5)	958(22)	- 143(47)	860(9)
C(6)	- 712(23)	100(40)	1032(10)
C(7)	- 712(28)	-2592(43)	1009(11)
O(8)	-1514(18)	-3948(33)	1146(9)
C(9)	2097(23)	-3847(38)	2137(8)
C(10)	4622(21)	-3547(33)	1695(9)
C(11)	3078(22)	-4157(40)	437(8)
O(12)	3325(18)	-6048(24)	263(6)
O(13)	3440(15)	-2410(22)	204(6)
N(14)	- 799(17)	771(27)	1663(7)
C(15)	-2133(20)	1481(37)	1822(8)
O(16)	-3398(14)	1419(22)	1454(6)
C(17)	-2141(21)	2461(35)	2478(8)
C(18)	-3141(13)	1142(24)	2848(7)
C(19)	-2605(13)	- 878(24)	3073(7)
C(20)	-3491(13)	-2141(24)	3416(7)
C(21)	-4913(13)	-1383(24)	3536(7)
C(22)	-5448(13)	638(24)	3311(7)
C(23)	-4562(13)	1900(24)	2968(7)
C(24)	- 575(25)	2783(45)	2908(9)
O(25)	565(15)	1680(26)	2930(6)
O(26)	- 654(16)	4472(25)	3322(7)
C(27)	441(17)	4721(25)	3840(6)
C(28)	1219(17)	6686(25)	3936(6)
C(29)	2253(17)	7048(25)	4495(6)
C(30)	2509(17)	5445(25)	4958(6)
C(31)	1730(17)	3480(25)	4862(6)
C(32)	696(17)	3118(25)	4303(6)
Na(33)	4871(8)	881(13)	457(3)

Table 4.2 Bond lengths and their standard deviations (Å) after final least-squares refinement.

S(1)	-	C(2)	1.907(26)
S(1)	-	C(5)	1.816(19)
C(2)	-	C(3)	1.523(28)
C(2)	-	C(9)	1.533(31)
C(2)	-	C(10)	1.570(27)
C(3)	-	N(4)	1.487(25)
C(3)	-	C(11)	1.580(29)
N(4)	-	C(5)	1.533(33)
N(4)	-	C(7)	1.434(30)
C(5)	-	C(6)	1.576(30)
C(6)	-	C(7)	1.570(36)
C(6)	-	N(14)	1.427(26)
C(7)	-	O(8)	1.164(32)
C(11)	-	O(12)	1.260(29)
C(11)	-	O(13)	1.255(28)
N(14)	-	C(15)	1.345(24)
C(15)	-	O(16)	1.251(19)
C(15)	-	C(17)	1.530(26)
C(17)	-	C(24)	1.535(26)
C(17)	-	C(18)	1.515(24)
C(18)	-	C(19)	1.395(20)
C(18)	-	C(23)	1.395(18)
C(19)	-	C(20)	1.395(20)
C(20)	-	C(21)	1.395(18)
C(21)	-	C(22)	1.395(20)
C(22)	-	C(23)	1.395(20)
C(24)	-	O(25)	1.206(28)
C(24)	-	O(26)	1.381(29)
O(26)	-	C(27)	1.350(18)
C(27)	-	C(28)	1.395(21)
C(27)	-	C(32)	1.395(19)
C(28)	-	C(29)	1.395(17)
C(29)	-	C(30)	1.395(19)
C(30)	-	C(31)	1.395(21)
C(31)	-	C(32)	1.395(17)
Na(33)....		O(12)	2.334
Na(33)....		O(13)	2.410

Table 4.3 Bond angles (°) and their standard deviations.

C(5)	-	S(1)	-	C(2)	94.8(1.1)
C(3)	-	C(2)	-	S(1)	105.4(1.5)
C(9)	-	C(2)	-	S(1)	110.3(1.6)
C(9)	-	C(2)	-	C(3)	110.3(1.8)
C(10)	-	C(2)	-	S(1)	107.2(1.5)
C(10)	-	C(2)	-	C(3)	112.7(1.8)
C(10)	-	C(2)	-	C(9)	110.8(1.6)
N(4)	-	C(3)	-	C(2)	107.2(1.7)
C(11)	-	C(3)	-	C(2)	112.5(1.6)
C(11)	-	C(3)	-	N(4)	109.6(1.5)
C(5)	-	N(4)	-	C(3)	115.4(1.4)
C(7)	-	N(4)	-	C(3)	121.9(1.7)
C(7)	-	N(4)	-	C(5)	94.0(1.6)
N(4)	-	C(5)	-	S(1)	105.5(1.4)
C(6)	-	C(5)	-	S(1)	118.7(1.4)
C(6)	-	C(5)	-	N(4)	90.1(1.6)
C(7)	-	C(6)	-	C(5)	83.9(1.8)
N(14)	-	C(6)	-	C(5)	116.6(1.6)
N(14)	-	C(6)	-	C(7)	108.6(1.8)
C(6)	-	C(7)	-	N(4)	90.0(1.7)
O(8)	-	C(7)	-	N(4)	134.6(2.5)
O(8)	-	C(7)	-	C(6)	135.4(2.3)
O(12)	-	C(11)	-	C(3)	115.0(1.9)
O(13)	-	C(11)	-	C(3)	116.5(2.0)
O(13)	-	C(11)	-	O(12)	128.1(1.9)
C(15)	-	N(14)	-	C(6)	121.3(1.4)
O(16)	-	C(15)	-	N(14)	123.3(1.6)
C(17)	-	C(15)	-	N(14)	119.8(1.4)
C(17)	-	C(15)	-	O(16)	116.9(1.6)
C(24)	-	C(17)	-	C(15)	117.6(1.6)
C(18)	-	C(17)	-	C(15)	110.9(1.6)
C(18)	-	C(17)	-	C(24)	107.1(1.4)
C(19)	-	C(18)	-	C(17)	118.3(1.3)
C(23)	-	C(18)	-	C(17)	121.7(1.4)
C(23)	-	C(18)	-	C(19)	120.0(1.3)
C(18)	-	C(19)	-	C(20)	120.0(1.2)
C(21)	-	C(20)	-	C(19)	120.0(1.3)
C(22)	-	C(21)	-	C(20)	120.0(1.3)
C(23)	-	C(22)	-	C(21)	120.0(1.2)
C(18)	-	C(23)	-	C(22)	120.0(1.3)
O(25)	-	C(24)	-	C(17)	127.1(2.1)
O(26)	-	C(24)	-	C(17)	110.4(1.8)
O(26)	-	C(24)	-	O(25)	122.3(1.8)
C(27)	-	O(26)	-	C(24)	120.6(1.5)
C(28)	-	C(27)	-	O(26)	119.0(1.3)
C(32)	-	C(27)	-	O(26)	120.7(1.4)
C(32)	-	C(27)	-	C(28)	120.0(1.1)
C(27)	-	C(28)	-	C(29)	120.0(1.3)
C(30)	-	C(29)	-	C(28)	120.0(1.4)
C(31)	-	C(30)	-	C(29)	120.0(1.1)
C(32)	-	C(31)	-	C(30)	120.0(1.3)
C(27)	-	C(32)	-	C(31)	120.0(1.4)

Table4.4Co-ordinates of hydrogen atoms. Co-ordinates are given as fractions of cell edges x 10^4. The heavy atom associated with each hydrogen atom is also given. Standard deviations in parentheses are with respect to the last figures given.

	x	y	z
H(C3)	1664(22)	-5618(37)	1055(9)
H(C5)	1248(22)	1067(47)	536(9)
H(C6)	-1616(23)	1097(40)	777(10)
H(C9)(1)	2666(23)	-3143(38)	2576(8)
H(C9)(2)	2251(23)	-5576(38)	2155(8)
H(C9)(3)	878(23)	-3474(38)	2071(8)
H(C10)(1)	5164(21)	-2925(33)	1315(9)
H(C10)(2)	4733(21)	-5282(33)	1713(9)
H(C10)(3)	5178(21)	-2866(33)	2139(9)
H(N14)	386(17)	623(27)	1896(7)
H(C17)	-2552(21)	4065(35)	2345(8)
H(C19)	-1504(13)	-1465(24)	2980(7)
H(C20)	-3076(13)	-3705(24)	3590(7)
H(C21)	-5599(13)	-2360(24)	3802(7)
H(C22)	-6549(13)	1224(24)	3404(7)
H(C23)	-4977(13)	3464(24)	2794(7)
H(C28)	1021(17)	7927(25)	3578(6)
H(C29)	2855(17)	8569(25)	4570(6)
H(C30)	3309(17)	5725(25)	5391(6)
H(C31)	1928(17)	2239(25)	5220(6)
H(C32)	94(17)	1597(25)	4228(6)

Table 4.5 Anisotropic temperature factors are expressed as exp $[-2\pi^2(U_{11} h^2a^{*2} + U_{22}k^2b^{*2} + U_{33}\ell^2c^{*2} + 2U_{12}hka^*b^* + 2U_{13}h\ell a^*c^* + 2U_{23}k\ell b^*c^*)]$. Isotropic temperature factors are expressed as exp $[-2\pi^2U(h^2a^{*2} + k^2b^{*2} + \ell^2c^{*2})]$. The units of Uij are $Å^2 \times 10^4$. Standard deviations in parentheses are with respect to the last figures given.

	U or U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
S(1)	201(24)	137(43)	268(30)	-39(28)	4(20)	37(27)
C(2)	182(109)	55(169)	268(127)	-78(102)	-28(90)	-68(98)
C(3)	361(114)	1(116)	293(117)	16(102)	-62(95)	-39(102)
H(C3)	164(99)					
N(4)	135(82)	10(103)	171(82)	-51(69)	-65(65)	31(68)
C(5)	203(102)	506(167)	187(116)	216(126)	-51(85)	69(126)
H(C5)	287(118)					
C(6)	260(107)	260(142)	326(136)	-62(117)	120(92)	202(113)
H(C6)	249(111)					
C(7)	308(131)	376(177)	357(141)	-148(119)	-44(106)	-149(125)
O(8)	467(102)	431(124)	904(134)	0(112)	252(95)	-194(103)
C(9)	443(128)	140(127)	181(104)	93(102)	187(94)	-112(114)
H(C9)(1)	270(109)					
H(C9)(2)	270(109)					
H(C9)(3)	270(109)					
C(10)	301(112)	1(133)	339(117)	-50(97)	-6(90)	54(97)
H(C10)(1)	253(126)					
H(C10)(2)	253(126)					
H(C10)(3)	253(126)					
C(11)	387(111)	182(150)	221(99)	20(107)	81(86)	-137(106)
O(12)	624(105)	119(104)	298(85)	21(70)	188(73)	120(76)
O(13)	355(83)	117(92)	212(83)	26(64)	134(66)	-71(65)
N(14)	278(82)	130(108)	211(81)	-45(73)	34(66)	141(73)
H(N14)	215(48)					
C(15)	135(91)	450(155)	131(95)	112(99)	68(74)	89(99)
O(16)	223(64)	78(87)	344(71)	-118(66)	-13(55)	107(63)
C(17)	226(103)	201(139)	180(99)	-152(93)	-28(80)	121(95)
H(C17)	203(57)					
C(18)	142(91)	450(157)	257(106)	-109(117)	63(80)	-42(110)
C(19)	312(117)	318(165)	327(126)	144(109)	102(100)	-36(106)
H(C19)	393(88)					
C(20)	552(161)	484(184)	386(138)	85(128)	-101(117)	-129(137)
H(C20)	393(88)					
C(21)	449(159)	963(245)	390(133)	-151(164)	168(121)	-197(177)
H(C21)	393(88)					
C(22)	292(110)	1073(265)	582(159)	-24(169)	165(112)	49(145)
H(C22)	393(88)					
C(23)	264(116)	436(158)	497(144)	12(125)	94(104)	145(115)
H(C23)	393(88)					
C(24)	294(122)	628(197)	265(116)	-196(126)	38(96)	-43(128)
O(25)	258(75)	510(114)	231(75)	-157(73)	-51(59)	-4(77)
O(26)	370(83)	316(119)	433(93)	-118(79)	-144(70)	13(72)
C(27)	422(131)	697(187)	281(118)	-77(136)	-4(96)	-231(141)
C(28)	467(143)	942(227)	236(117)	-128(127)	-78(100)	-20(148)
H(C28)	452(65)					

(continued)

Table 4.5. (continued)

	U or U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C(29)	572(155)	798(214)	400(135)	-408(145)	4(116)	-175(152)
H(C29)	452(65)					
C(30)	898(202)	1080(339)	565(192)	120(207)	-207(153)	-425(226)
H(C30)	452(65)					
C(31)	1213(245)	1324(315)	58(116)	294(159)	-126(127)	-382(250)
H(C31)	452(65)					
C(32)	815(204)	464(189)	509(151)	-90(141)	101(133)	-43(154)
H(C32)	452(65)					
Na(33)	336(38)	65(48)	364(43)	-79(36)	133(31)	-9(34)

Table 4.6. Planarity of the penicillin nucleus in carfecillin.

Equations expressed as Px + Qy + Rz = S in direct space; with x, y and z given as fractions of cell edges a, b and c respectively.

	P	Q	R	S	Deviations (Å) of atoms from planes	
(a)	Planarity of the thiazolidine ring					
	-0.3810	-0.0072	1	0.0496	S(1)	0.00
					C*(2)	0.06
					C*(3)	0.42
					N(4)	0.00
					C(5)	0.00
(b)	Pyramidal nature of N(4)					
	0.0365	0.0359	1	0.0890	C(3)	0.00
					N*(4)	0.47
					C(5)	0.00
					C(7)	0.00
(c)	Deviation of N(4) and O(8) from the plane of the remaining β lactam constituents					
	0.1018	-0.0085	1	0.0958	N*(4)	0.28
					C(5)	0.00
					C(6)	0.00
					C(7)	0.00
					O*(8)	0.14

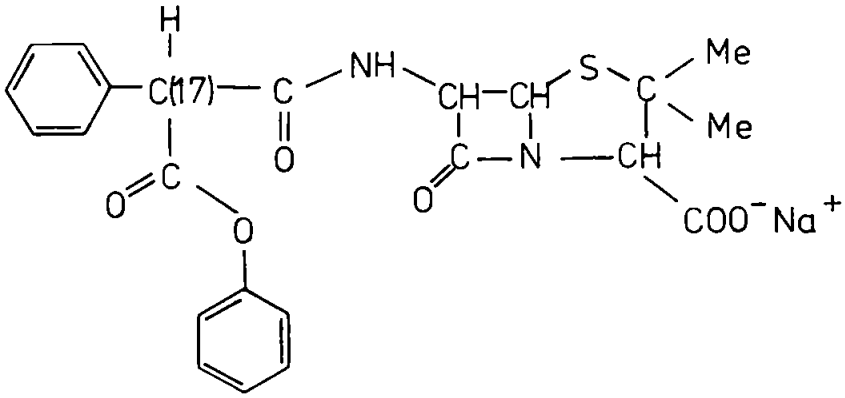
* Atoms not used to define the planes.

CHAPTER 5

The Configuration and Conformation of the side-chain substituents in Penicillin Derivatives

5.1 Introduction

The crystal structure of the phenyl ester of carbenicillin, discussed in Chapter 4, is used to facilitate a comparison of the configuration about C(17) with other penicillin derivatives of known crystal structure to form a characterisation and evaluation of the spectroscopic differences evident in the H^1 nuclear magnetic resonance and circular dichroism spectra of the respective side-chain diastereoisome ric configurations.



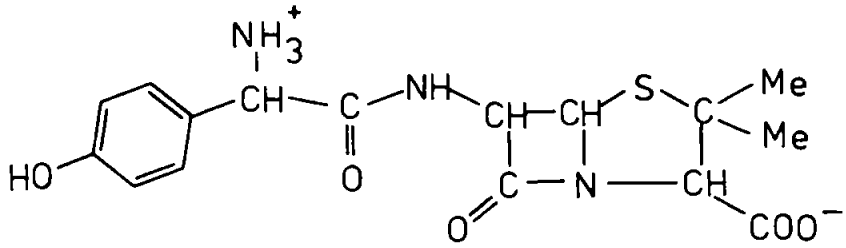
5.2 Side Chain Configuration and Associated Nuclear Magnetic Resonance

Configuration of the side chain about C(17) is shown to notably affect the H^1 n.m.r. resonance from the β lactam protons H(C(5)) and H(C(6)). In the crystalline solid state, the configuration of the

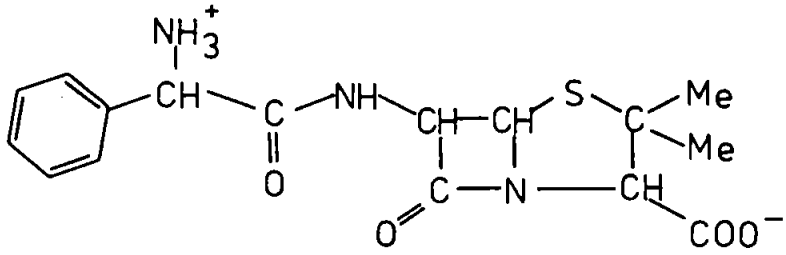
methyl and ethyl esters of carbenicillin, as in the structure of the phenyl ester of carbenicillin (ref. Ch. 4), when examined by n.m.r. in fresh solution, (t = 0), is characterised by a singlet β lactam proton peak (Traces 1(a) and 2(a)). However, after about 30 minutes a steady state is reached (at 35°C) in D_2O solution, characterised by four β lactam proton peaks superimposed upon the singlet which in turn suffers a reduction in intensity, for the methyl and ethyl esters, (Traces 1(b), 2(b), 3(a) and 3(b)), but this effect is not apparent in the case of the phenyl ester (Traces 4(a) and 4(b)). To investigate the cause of this effect; its association with configuration about C(17): and the apparently anomalous behaviour of the phenyl ester, H^1 n.m.r. studies of the two diastereoisomers of amino-hydroxybenzyl penicillin[40], amino-phenylacetamido penicillanic acid[34] and a tyrosyl penicillin have been made.

The three penicillin derivatives amino-hydroxybenzyl penicillin (i), amino-phenylacetamido penicillanic acid (ii), and the tyrosyl penicillin (iii) are known to crystallise, under different conditions,

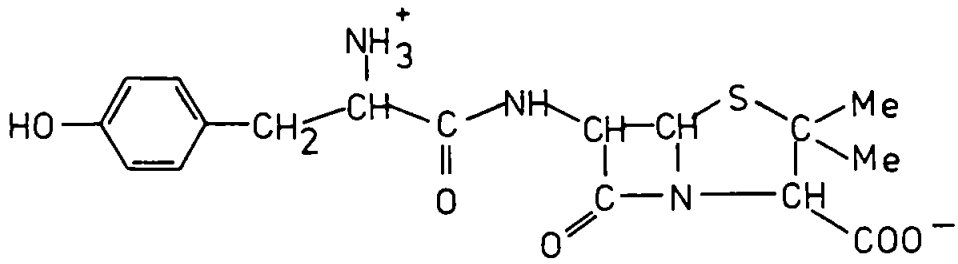
(i) Amino-hydroxybenzyl penicillin



(ii) Amino-phenylacetamido penicillanic acid



(iii) the tyrosyl penicillin



to accommodate the side chain in either the D or L forms, (N.B. D and L being assigned by optical measurements from the two C(17) configurational epimers, the penicillin nuclei remaining unchanged). These configurations are stable in solution at room temperature and no epimerisation of the two diastereoisomers takes place. Thus, the H^1 n.m.r. spectra of these compounds in the side chain D and L forms have been used to assert that the feature distinguishing the diastereoisomers of each compound is the association of a single β lactam proton peak with the side chain D form and a system of four peaks with the side chain L form (Traces 5(a), 6(a), 7(a), 8(a), 8(b), 9(a), 9(b), 10(a)). This characteristic phenomenon was demonstrated to be purely intra-molecular by determining that there was no dependence of the β lactam proton resonance upon concentration (Traces 5(b), 6(b), 7(b), 10(b), (1), (2), (3) & (4)[each Trace is shown with a scale expansion illustrating no observed change in the resonance feature at very low concentration]).

The spectra were obtained by using a Perkin-Elmer R12b nuclear magnetic resonance spectrometer with D_2O as solvent, and $NaOD/D_2O$ to form the sodium salt of the amino compounds. The spectrum produced by n.m.r. due to the β lactam protons, is conventionally described as an AB system:[51, 52] one in which two protons are coupled together, having a coupling constant J comparable to the chemical shift δ which could be introduced by the comparative shielding of one proton by environmental effects imposed by chemical groups and their position relative to the proton within the molecular configuration.

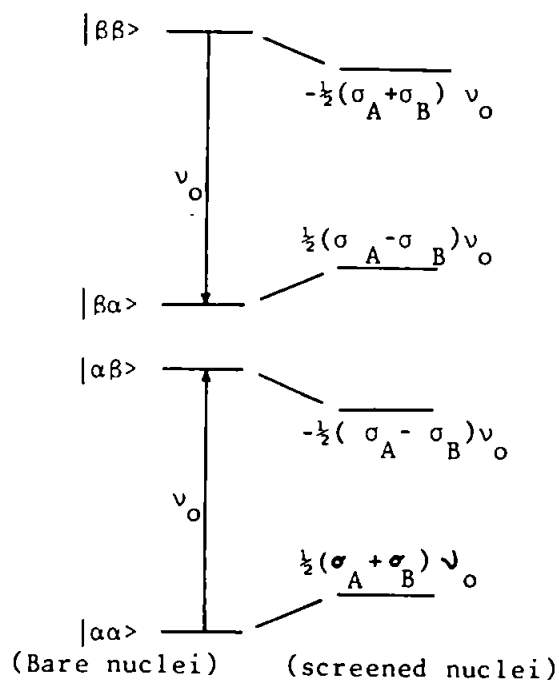
The spin Hamiltonian for the AB spectrum is given by:-

$$H = -\nu_o (1 - \sigma_A) I_{ZA} - \nu_o (1 - \sigma_B) I_{ZB} + J \underset{\sim}{I}_A \cdot \underset{\sim}{I}_B \qquad \text{..... Eqn. 5.a}$$

where ν_o is the frequency at which resonance takes place for the bare proton above; σ is the screening constant which is isotropic due to the exclusive use of liquids for the purpose of n.m.r. measurements; $\underset{\sim}{I}$ is the nuclear spin with I_Z as its Z component. The Hamiltonian describes the two Zeeman energies for the protons A and B in the molecular environment, combined with the spin-spin coupling. Each proton can have two spin states $|\alpha>$ and $|\beta>$; the combined system can be described using the set of basis functions,

$$\phi_1 = |\alpha\alpha>,\ \phi_2 = |\alpha\beta>,\ \phi_3 = |\beta\alpha> \text{ and } \phi_4 = |\beta\beta>.$$

The term $\nu_o (1 - \sigma) I_Z$ describes the chemical shift δI_Z, where $\delta_A I_{ZA}$, splits ϕ_1 from ϕ_3 and ϕ_4, and $\delta_B I_{ZB}$ splits ϕ_1 from ϕ_2 and ϕ_4 in terms of frequency units.



The operator $\underset{\sim}{I}_A \cdot \underset{\sim}{I}_B$ can be split into two parts

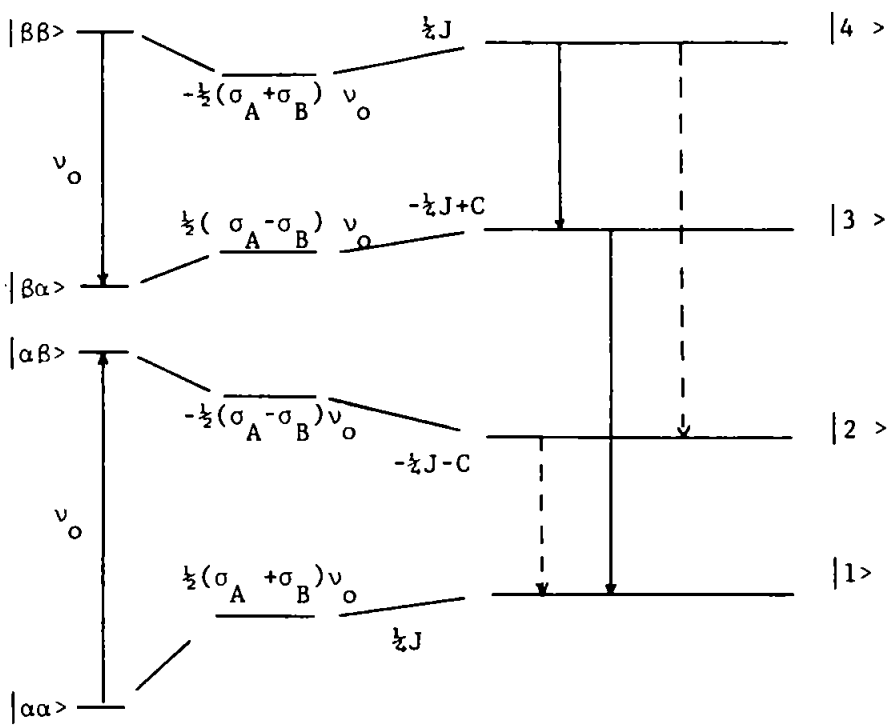
$$\underset{\sim}{I}_A \cdot \underset{\sim}{I}_B = I_{ZA} I_{ZB} + \tfrac{1}{2} (I_A^+ I_B^- + I_A^- I_B^+) \qquad \cdots \text{ Eqn. 5.b}$$

where

$$I^+ |\alpha\rangle = 0, \; I^+ |\beta\rangle = |\alpha\rangle$$

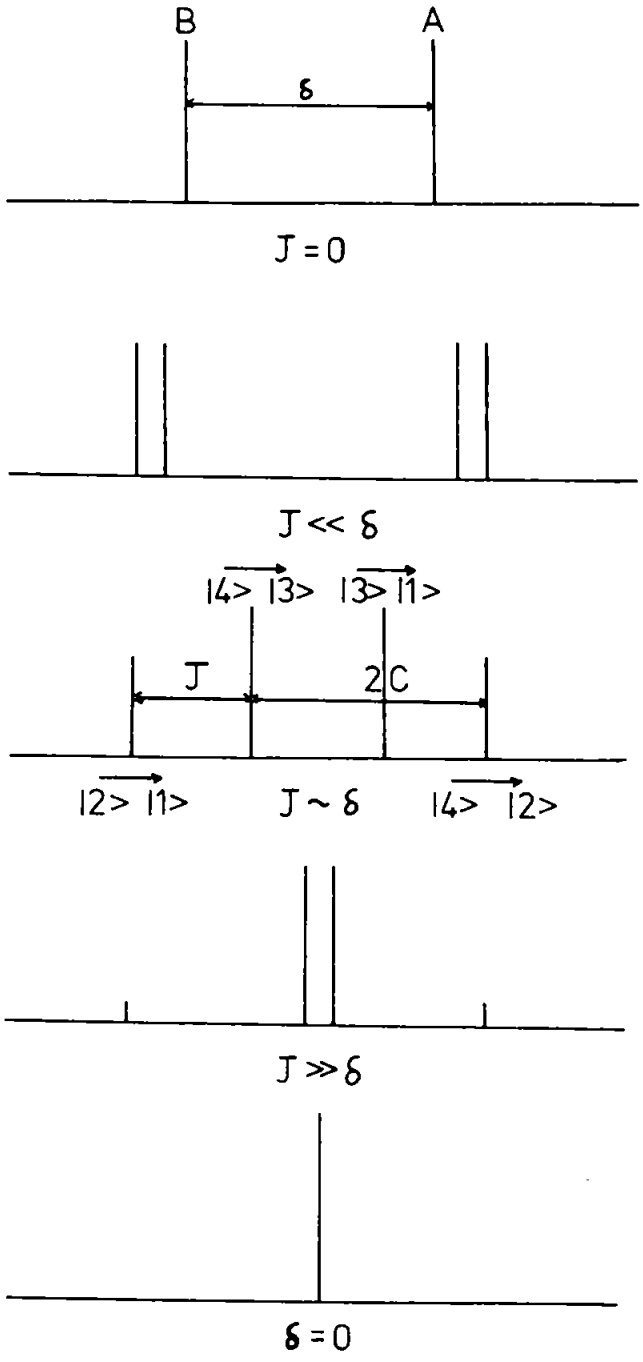
$$I^- |\alpha\rangle = |\beta\rangle \text{ and } I^- |\beta\rangle = 0. \qquad \cdots \text{ Eqns. 5.c}$$

The effect of $\frac{1}{2} (I_A^+ I_B^- + I_A^- I_B^+)$ is to mix ϕ_2 with ϕ_3 and produces a further change of frequency of these states by, $\mp C$ respectively where $C = \frac{1}{2}\sqrt{(J^2+\delta^2)}$. The term $I_{ZA} I_{ZB}$ shifts the levels of all states (in frequency units) by $\pm\frac{1}{4}J$ depending on each $I_Z = \pm\frac{1}{2}$ where $|\alpha\rangle$ has $I_Z = \frac{1}{2}$ and $|\beta\rangle$ has $I_Z = -\frac{1}{2}$.



(Spin-spin coupling)

For varying coupling J, relative to the difference in chemical environment for each proton δ, the spectra appear thus, in which the intensity of each resonance is related to the transition probability.



From this analysis, it can be seen that the spectra of the D forms of the penicillin derivatives correspond to $\delta = 0$, i.e. no difference in the screening tensor $\underset{\sim}{\sigma}$ between the β lactam protons. However, the L forms have spectra corresponding to a chemical shift between the proton resonances of the same order of magnitude as the coupling, or less. The single β lactam proton peak of the fresh solution of methyl and ethyl carbenicillin esters indicates that the compounds crystallise in the configuration corresponding to the D diastereoisomer of other penicillin derivatives. Equilibrium between the two configurations is reached in solution and the mixture of the two gives rise to a superposition of the singlet upon the spin-spin split spectrum.

The phenyl ester of carbenicillin, however, is known from optical measurements, (§. 5.4), to reach equilibrium more rapidly than either the methyl or ethyl esters, yet its n.m.r. spectrum shows no shielding of the β lactam protons with time i.e. the resonance remains a singlet. An explanation of the anomaly is afforded by the consideration of the relative orientation of the phenyl group C(27) C(28) C(29) C(30) C(31) C(32), with respect to the β lactam protons, (H(C(5)) and H(C(6)), Fig. 4.6. Coupling constants and chemical shifts for those compounds showing spin-spin splitting of the β lactam proton resonance are given in Table 5.1

5.3 Relative orientations of the C(17) side-chain substituent in the phenyl ester of carbenicillin

Benzene rings can affect H^1 n.m.r. signals by introducing ring current shifts. When an aromatic ring is within a magnetic field, a current is induced which arises from the circulation of the delocalised π - electrons. This ring current produces a local magnetic field which opposes the externally applied field in the area above and below the plane of the aromatic ring, but reinforces it otherwise. The ring current effect, therefore, can produce magnetic shielding, or

deshielding, of a proton depending upon the aspect of the aromatic ring that is presented to the proton. The geometric form of the region of influence of ring current shifts corresponds to cones whose axes coincide with the direction of the principal symmetry axis. The special case of axial symmetry, in benzene, results in a spherical cone with half-angle $54^{o}44'$. The model described is useful for semi-quantitative estimations of the shielding effect due to the phenyl group (C(27), C(28), C(29), C(30), C(31), C(32)) of the β lactam protons (H(C(5)), H(C(6))) in carfecillin, during equilibration.

Relative orientations of the benzene ring with respect to H(C(6)), (the β lactam proton nearest the ring and hence, most likely to suffer shielding), have been calculated for all the substituent positions possible at C(17).

Inter-atomic vectors may be described by a basis set of three orthogonal unit vectors $\hat{\underset{\sim}{\rho}}$, $\hat{\underset{\sim}{\mu}}$ and $\hat{\underset{\sim}{n}}$ as shown in Fig. 5.1, where $\hat{\underset{\sim}{\rho}}$, $\hat{\underset{\sim}{\mu}}$ lie in the plane of the benzene ring and $\hat{\underset{\sim}{n}}$, $(= \hat{\underset{\sim}{\mu}} \wedge \hat{\underset{\sim}{\rho}})$, perpendicular to the plane. Determination of the basis set relied on the definition of the plane in terms of the scalar product.

$$\underset{\sim}{r} \cdot \underset{\sim}{n} = 0 \qquad \text{... Eqn. 5.1}$$

where $\underset{\sim}{r}$ describes any vector in the plane and $\underset{\sim}{n}$, $(= n\hat{\underset{\sim}{n}})$ a vector normal to the plane.

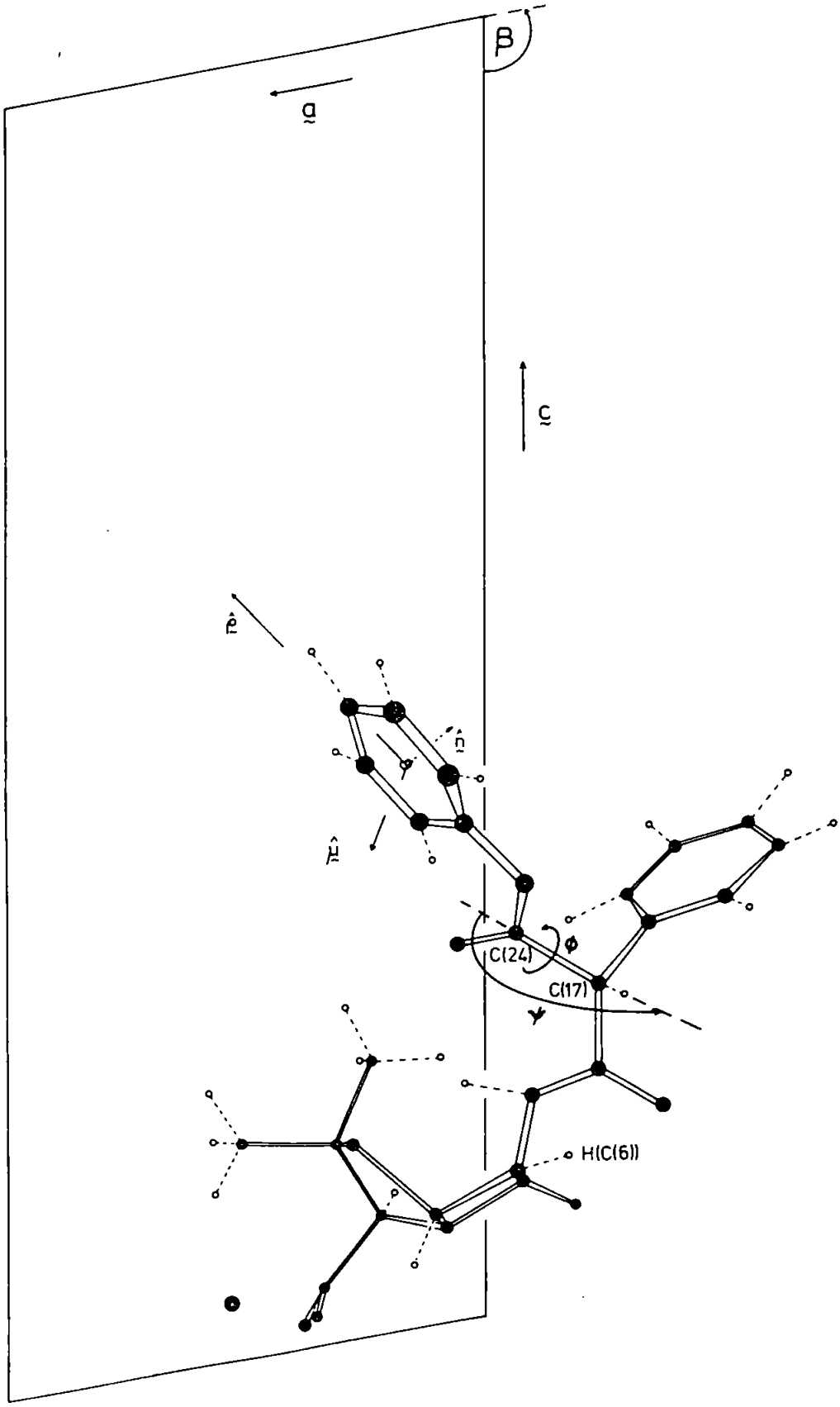
Two vectors in the plane of the benzene ring are the inter-atomic vectors

$$\underset{\sim}{C(27)\,C(28)} = \begin{pmatrix} 0.0778 \\ 0.1965 \\ 0.0096 \end{pmatrix} \qquad \text{... Eqn. 5.2}$$

and

$$\underset{\sim}{C(27)\,C(32)} = \begin{pmatrix} 0.0255 \\ -0.1603 \\ 0.0463 \end{pmatrix} \qquad \text{... Eqn. 5.3}$$

with components expressed as fractions of the unit cell vectors $\underset{\sim}{a}$, $\underset{\sim}{b}$ and $\underset{\sim}{c}$ respectively. These vectors can be used in Eqn. 5.1 to determine the



Geometrical parameters used in describing the relative side-chain orientation of the phenyl ester of carbenicillin

Fig. 5.1

direction of $\underset{\sim}{n}$ as,

$$\underset{\sim}{n} = \begin{pmatrix} -0.6082 \\ 0.1919 \\ 1 \end{pmatrix} \qquad \text{... Eqn. 5.4}$$

(there being no necessity to uniquely define its magnitude).

$\underset{\sim}{\rho}$ is conveniently defined with respect to the unit cell vectors $\underset{\sim}{a}$, $\underset{\sim}{b}$ and $\underset{\sim}{c}$ as the interatomic vector $\underset{\sim}{C(27)\ C(30)}$, such that

$$\underset{\sim}{\rho} = \begin{pmatrix} 0.2068 \\ 0.0724 \\ 0.1118 \end{pmatrix} \qquad \text{... Eqn. 5.5}$$

Thus, satisfying the definition of the plane,

$$\underset{\sim}{\rho} \cdot \underset{\sim}{n} \simeq 0 \qquad \text{... Eqn. 5.6}$$

is found to be true.

The two vectors $\underset{\sim}{\rho}$ and $\underset{\sim}{n}$ are referred to the unit cell vectors $\underset{\sim}{a}$, $\underset{\sim}{b}$ and $\underset{\sim}{c}$ which are not orthogonal ($\beta = 99.5^{\circ}$). To produce an orthogonal basis, it is necessary to transform $\underset{\sim}{\rho}$ and $\underset{\sim}{n}$ to refer to orthogonalised unit basis vectors $\delta\hat{\underset{\sim}{a}}$, $\hat{\underset{\sim}{b}}$ and $\hat{\underset{\sim}{c}}$, as shown in Fig. 5.2, where

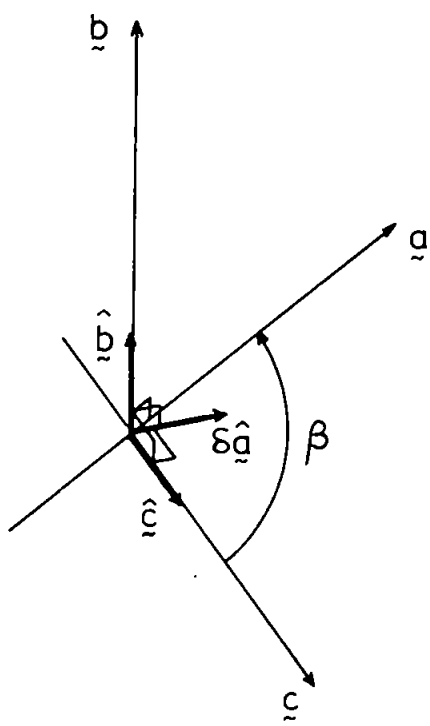


Fig. 5.2 Transformation of co-ordinate axes.

the operation δ satisfies the reorientation of $\underset{\sim}{a}$ such that

$$\left.\begin{aligned} \delta\underset{\sim}{a}.\underset{\sim}{c} &= 0 \\ \text{and} \quad \delta\underset{\sim}{a}.\underset{\sim}{b} &\doteq 0 \end{aligned}\right\} \qquad \text{... Eqns. 5.7}$$

and premultiplies the magnitude of $\underset{\sim}{a}$ by sin β.

Thus, expressed in terms of the basis $\delta\hat{\underset{\sim}{a}}$, $\hat{\underset{\sim}{b}}$ and $\hat{\underset{\sim}{c}}$,

$$\underset{\sim}{\rho} = \begin{pmatrix} \rho_1 & \delta a \\ \rho_2 & b \\ \rho_3 & c \end{pmatrix} = \begin{pmatrix} 1.7888 \\ 0.4489 \\ 2.3925 \end{pmatrix} \qquad \text{... Eqn. 5.8}$$

and

$$\underset{\sim}{n} = \begin{pmatrix} n_{1/\delta a} \\ n_{2/b} \\ n_{3/c} \end{pmatrix} = \begin{pmatrix} -0.0703 \\ 0.0310 \\ 0.0467 \end{pmatrix} \qquad \text{... Eqn. 5.9}$$

where $\underset{\sim}{\rho}$ and $\underset{\sim}{n}$ still satisfy Eqn. 5.6.

Conversion to unit vectors results in

$$\hat{\underset{\sim}{\rho}} = \begin{pmatrix} 0.5922 \\ 0.1486 \\ 0.7920 \end{pmatrix} \qquad \text{... Eqn. 5.10}$$

and

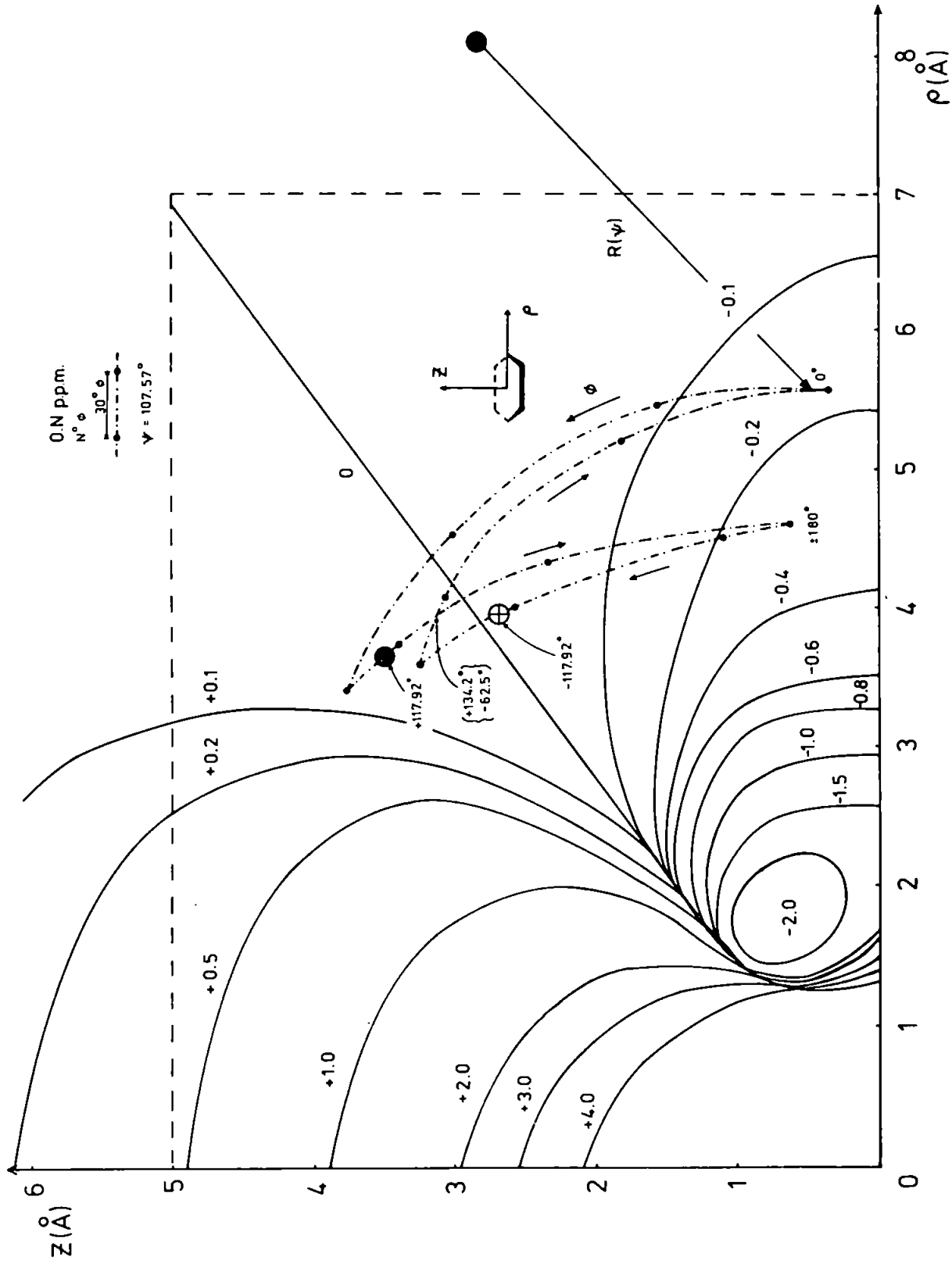
$$\hat{\underset{\sim}{n}} = \begin{pmatrix} -0.7819 \\ 0.3448 \\ 0.5194 \end{pmatrix} \qquad \text{... Eqn. 5.11}$$

Definition of the remaining vector, $\hat{\underset{\sim}{\mu}}$, in the plane of the ring is given by the vector product

$$\hat{\underset{\sim}{\mu}} = \hat{\underset{\sim}{\rho}} \wedge \hat{\underset{\sim}{n}} \qquad \text{... Eqn. 5.12}$$

such that

$$\hat{\underset{\sim}{\mu}} = \begin{pmatrix} -0.1959 \\ -0.9269 \\ 0.3204 \end{pmatrix} \qquad \text{... Eqn. 5.13}$$



Contour plot of n.m.r. shielding values for protons in the vicinity of a benzene ring

Fig.5.3

Fig. 5.3 is a contour plot of shift in n.m.r. shielding values in p.p.m., which will be experienced by hydrogen atoms as a result of the ring currents associated with the benzene ring, (from Johnson and Bovey, 1958)[53]. The z direction is along $\hat{\underset{\sim}{n}}$ ie, the hexagonal axis of the benzene ring while ρ is the direction in the plane of the carbon atoms, ie, the $\hat{\underset{\sim}{\rho}}$, $\hat{\underset{\sim}{\mu}}$ plane.

(i) Relative orientation in fresh solution

In the crystalline solid state the orientation of the ester substituent benzene ring with respect to H(C(6)) is described by the vector between H(C(6)) and the centre of the benzene ring, $\underset{\sim}{v}_1$, expressed in terms of the newly found basis set $\hat{\underset{\sim}{\rho}}$, $\hat{\underset{\sim}{\mu}}$ and $\hat{\underset{\sim}{n}}$. The vector $\underset{\sim}{v}_1$ may be conveniently obtained by considering the vector sum

$$\underset{\sim}{v}_1 = \underset{\sim}{H(C(6))\,C(17)} + \underset{\sim}{C(17)\,C(27)} + \tfrac{1}{2}\underset{\sim}{\varrho} \qquad \text{... Eqn. 5.14}$$

which gives

$$\underset{\sim}{v}_1 = \begin{pmatrix} 2.6737 \\ 2.4722 \\ 7.7512 \end{pmatrix} \qquad \text{... Eqn. 5.15}$$

expressed in terms of $\delta\hat{\underset{\sim}{a}}$, $\hat{\underset{\sim}{b}}$ and $\hat{\underset{\sim}{c}}$.

By letting

$$\underset{\sim}{v}_1 = \ell\hat{\underset{\sim}{\rho}} + m\hat{\underset{\sim}{\mu}} + p\hat{\underset{\sim}{n}} \qquad \text{... Eqn. 5.16}$$

ℓ, m and n are given by

$$\left.\begin{aligned} \ell &= \underset{\sim}{v}_1 \cdot \hat{\underset{\sim}{\rho}} \\ m &= \underset{\sim}{v}_1 \cdot \hat{\underset{\sim}{\mu}} \\ p &= \underset{\sim}{v}_1 \cdot \hat{\underset{\sim}{n}} \end{aligned}\right\} \qquad \text{... Eqns. 5.17}$$

and describe the components of $\underset{\sim}{v}_1$ in terms of the basis set $\hat{\underset{\sim}{\rho}}$, $\hat{\underset{\sim}{\mu}}$ and $\hat{\underset{\sim}{n}}$ such that

$$\underset{\sim}{v}_1 = \begin{pmatrix} 8.0897 \\ -0.3318 \\ 2.7878 \end{pmatrix} \qquad \text{... Eqns. 5.18}$$

The distance from H(C(6)) to the centre of the ring is thus given by the two components

$$\rho = \sqrt{(\ell'^2 + m^2)}$$

$$\approx 8.1 \text{ Å parallel to the plane of ring}$$

... Eqn. 5.19

and $z = p \approx 2.8$ Å perpendicular to the plane of ring

... Eqn. 5.20

It can be seen from Fig. 5.3 that these co-ordinates lie outside the region of influence, (indicated by the 'dashed' rectangle), considered sufficient to effect any shielding of H(C(6)). Thus, the configuration of the phenyl ester of carbenicillin in fresh solution, assumed to be the configuration described above, i.e. that of the crystalline state, is such that no shielding of the β lactam protons would be expected; as evidenced by the singlet H^1 n.m.r. peak in Trace 4(b). Comparable distances between H(C(6)) and the ester substituents in the cases of the methyl and ethyl esters of carbenicillin could also be expected to result in the β lactam singlet in fresh solution.

(ii) Relative orientation after equilibration

During equilibration, a change of the ester substituent position at C(17) is described either by the replacement of the benzene substituent C(18) C(19) C(20) C(21) C(22) C(23) or of H(C(17)) by the ester. Hence, if the phenyl ester is to effect shielding of the β lactam proton H(C(6)) it must result in a closer approach between the ester and H(C(6)) than that described in the crystalline state.

To study the closeness of approach of any C(17) substituent position to H(C(6)) the unit interatomic vectors $C(17)\widehat{\sim}C(24)$, $C(17)\widehat{\sim}H(C(17))$ and $C(17)\widehat{\sim}C(18)$ were determined. The distance from H(C(6)) to the end of each unit vector was given by 4.32, 3.89 and 4.33 Å respectively as shown in Fig. 5.4.

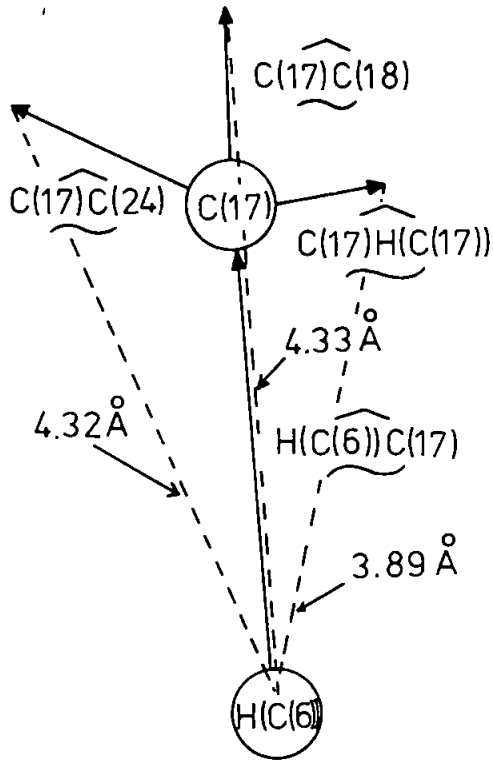


Fig. 5.4 Distances of C(17) & its substituents from H(C(6)).

Hence, substitution of the ester at H(C(17)) provides the closest approach to H(C(6)). This change of orientation can be effected by a rotation of the bond C(17) - C(24) to C(17) - H(C(17)) through the bond angle C(24) - C(17) - H(C(17)) given by

$$\cos \psi = C(17)\widehat{C}(24) \,.\, C(17)\widehat{H}(C(17)) \qquad \text{... Eqn. 5.21}$$

which upon substitution gives

$$\psi = 107.57^{\circ} \qquad \text{... Eqn. 5.22}$$

A general rotation of a co-ordinate system through an angle ψ about an axis inclined to the $(\hat{\underset{\sim}{\rho}}, \hat{\underset{\sim}{\mu}}, \hat{\underset{\sim}{n}})$ axes with direction cosines ℓ, m and n respectively is represented by the matrix

$$R(\psi) = \begin{pmatrix} \cos\psi + \ell^2(1-\cos\psi) & \ell m(1-\cos\psi) + n\sin\psi & \ell n(1-\cos\psi) - m\sin\psi \\ \ell m(1-\cos\psi) - n\sin\psi & \cos\psi + m^2(1-\cos\psi) & mn(1-\cos\psi) + \ell\sin\psi \\ \ell n(1-\cos\psi) + m\sin\psi & mn(1-\cos\psi) - \ell\sin\psi & \cos\psi + n^2(1-\cos\psi) \end{pmatrix}$$

... Eqn. 5.23

The axis of rotation is the vector perpendicular to $C(17)\hat{\underset{\sim}{C}}(24)$ and $C(17)\hat{\underset{\sim}{H}}(C(17))$, ie. given by their vector product. The direction cosines are therefore found to be

$$\left.\begin{aligned} \ell &= 0.5651 \\ m &= -0.0341 \\ n &= -0.8244 \end{aligned}\right\} \qquad \text{... Eqns. 5.24}$$

Hence, the matrix operator for the rotation of the ester substituent is given by

$$R(107.57^{\circ}) = \begin{pmatrix} 0.1139 & -0.8110 & -0.5740 \\ 0.7608 & -0.3004 & 0.6053 \\ -0.6390 & -0.5021 & 0.5829 \end{pmatrix}$$

... Eqn. 5.25

Since this operates upon $\hat{\underset{\sim}{\rho}}$, $\hat{\underset{\sim}{\mu}}$ and $\hat{\underset{\sim}{n}}$, the vector from C(17) to the centre of the benzene ring $(= C(17)\underset{\sim}{C}(27) + \frac{1}{2}\underset{\sim}{\rho})$ remains unchanged when substituted at H(C(17)), but $H(C(6))\underset{\sim}{C}(17)$ is operated on by R such that

$$R(107.57^{\circ})\,[H(C(6))\underset{\sim}{C}(17)] = \begin{pmatrix} -1.5267 \\ 3.4786 \\ -0.5084 \end{pmatrix} \qquad \text{... Eqn. 5.26}$$

expressed in terms of the basis $\hat{\underset{\sim}{\rho}}$, $\hat{\underset{\sim}{\mu}}$ and $\hat{\underset{\sim}{n}}$.

Rotation about the C(17) -C(24) bond must now be considered to allow for conformational change about this bond.

As an approximation it is assumed that $\hat{\underset{\sim}{\rho}}$ is parallel to $C(17)\hat{\underset{\sim}{C}}(24)$; this is verified by taking the scalar product between $\hat{\underset{\sim}{\rho}}$

and $\widehat{C(17)C(24)}$ resulting in

$$\hat{\underset{\sim}{\rho}} \cdot \widehat{C(17)C(24)} = 0.95$$

$$\simeq 1 \text{ as required} \qquad \ldots \text{ Eqn. 5.27}$$

Hence, rotation about C(17) - C(24) is in the $\hat{\underset{\sim}{\mu}}$, $\hat{\underset{\sim}{n}}$ plane and the rotation matrix R, (Eqn. 5.23), reduces to

$$R'(\phi) = \begin{pmatrix} 1 & 0 & 0 \\ 0 & \cos\phi & -\sin\phi \\ 0 & \sin\phi & \cos\phi \end{pmatrix} \qquad \ldots \text{ Eqn. 5.28}$$

where ϕ is the angle of rotation.

Thus, a rotation ψ associated with a change in configuration about C(17) is accompanied by a rotation ϕ about C(17) - C(24) associated with a change in conformation.

Severe constraint upon the rotation ϕ is expected due to the vicinity of the two carbonyl groups C(15) = O(16) and C(24) = O(25). The relative orientation of the carbonyl groups in the crystalline state was assumed in some measure to represent the most probable and energetically favourable conformation, and indeed, in solution, conformation of such groups is strongly localised such that the mutually repellant nature of the carbonyls is accommodated within the constraints of the surrounding groups[54,55]. Thus, to maintain the same relative orientation between the carbonyls, ϕ is given by

$$\phi = \pm \frac{\psi}{109.47} \times \frac{(360)}{3}$$

$$= \pm 117.92^{\circ} \qquad \ldots \text{ Eqn. 5.29}$$

where 109.47° is the theoretical tetrahedral bond angle as illustrated in Fig. 5.5. However, orientation with respect to the benzene ring

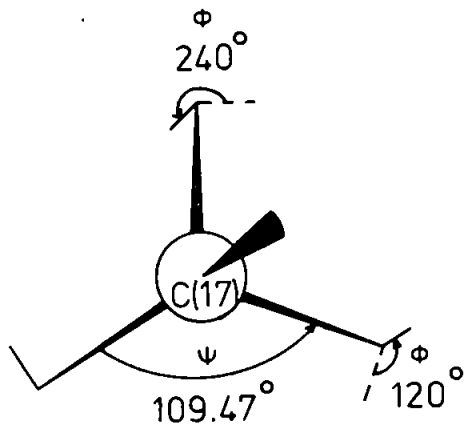


Fig. 5.5 Theoretical geometry about C(17).

C(18) C(19) C(20) C(21) C(22) and C(23) constrains ϕ to take the value - 117.92°.

The transformed vector H(C(6)) $\underset{\sim}{C}$(17) therefore becomes

$$R'\ (-117.92^{\circ}).\left(R\ (107.57^{\circ})\ [H(C(6))\ \underset{\sim}{C}(17)]\right) = \begin{pmatrix} -1.5267 \\ -2.0779 \\ -2.8357 \end{pmatrix}$$

... Eqn. 5.30

and the vector from H(C(6)) to the centre of the ring, $\underset{\sim}{v}_2$, is given by

$$\underset{\sim}{v}_2 = \begin{pmatrix} -1.5267 \\ -2.0779 \\ -2.8357 \end{pmatrix} + \underset{\sim}{C(17)\,C(27)} + \tfrac{1}{2}\underset{\sim}{\rho}$$

$$= \begin{pmatrix} 3.8232 \\ -1.2748 \\ -2.5852 \end{pmatrix} \qquad \text{... Eqn. 5.31}$$

expressed in terms of the basis set $\hat{\underset{\sim}{\rho}}$, $\hat{\underset{\sim}{\mu}}$ and $\hat{\underset{\sim}{n}}$.

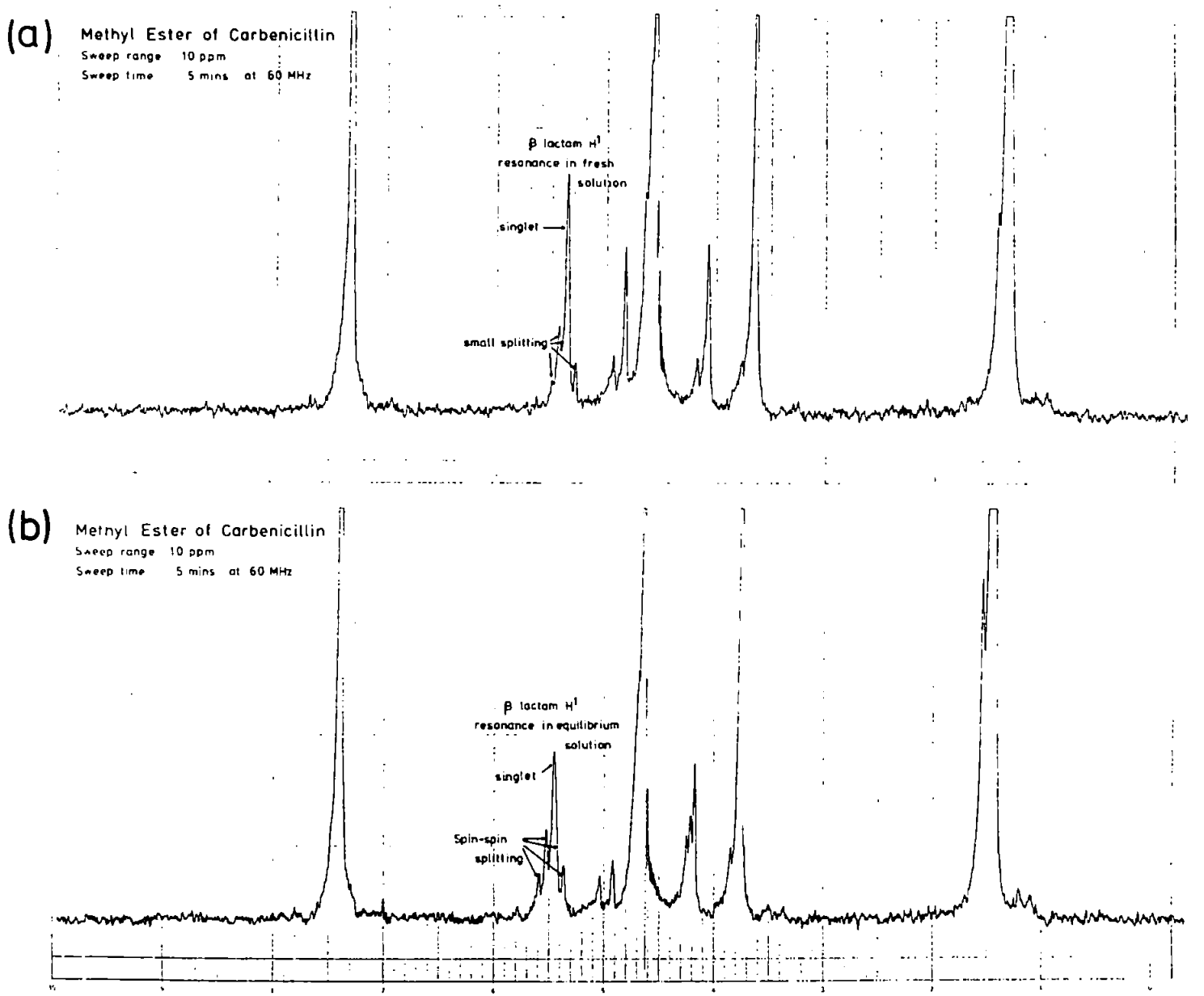
Since the operators $R(\psi)$ and $R'(\phi)$ rotate the co-ordinate axes ($\hat{\underset{\sim}{\rho}}$, $\hat{\underset{\sim}{\mu}}$, $\hat{\underset{\sim}{n}}$) by the same transformation as that experienced by the ester side chain, the vectors $\underset{\sim}{C(17)\,C(27)}$ and $\tfrac{1}{2}\underset{\sim}{\rho}$ remain rotationally invariant under $R(\psi)$ and $R'(\phi)$, then it is only the vector $\underset{\sim}{H(C(6))\,C(17)}$ that undergoes transformation.

The vector $\underset{\sim}{v}_2$ can be reduced to components parallel and perpendicular to the plane of the ring by Eqns. 5.19 and 5.20, such that

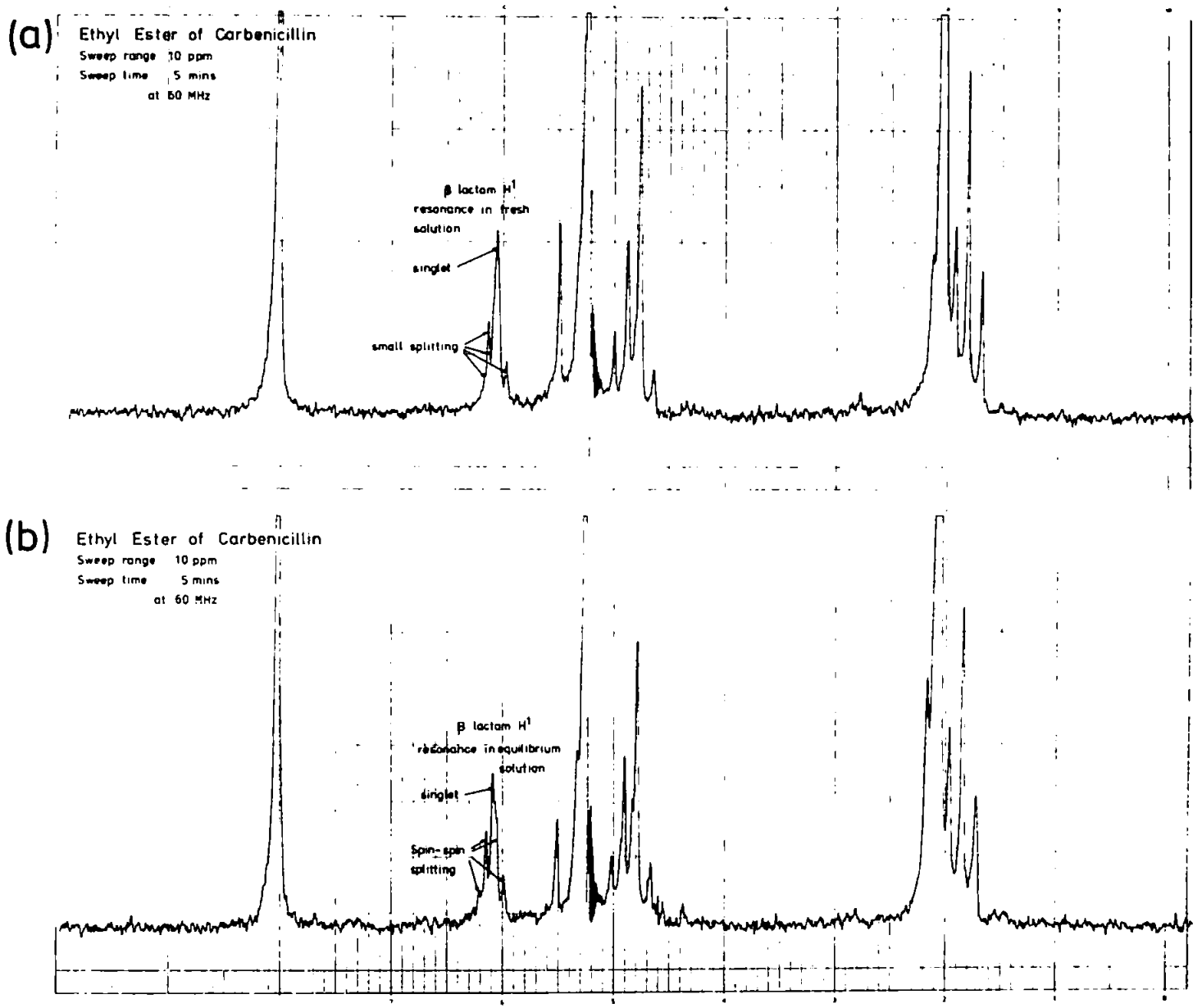
$$\left.\begin{aligned} \rho &\approx 4.03\ \text{Å} \\ \text{and} \qquad & \\ z &\approx 2.59\ \text{Å} \end{aligned}\right\} \qquad \text{... Eqns. 5.32}$$

Fig. 5.3 illustrates that these co-ordinates result in an aspect of the ester substituent benzene ring, at position ⊗, lying close to the zero shielding contour, thus, no change in the β lactam proton n.m.r. signal during equilibration is to be expected for the phenyl ester of carbenicillin. The trajectory followed by the ring under arbitrary rotation ϕ, ie. conformational change, is also plotted at 30° intervals from which it can be seen that for approximately 75% of the period time, the ring orientation is such that the magnitude of the n.m.r. shielding is between $\pm$ 0.1 p.p.m. The n.m.r. spectra of the β lactam protons in the methyl and ethyl esters of carbenicillin show a splitting during equilibration, with the coupling J (in frequency units) of the order of the chemical shift δ. J was found to be 4.0Hz and 2C to be 9.0Hz; using a 60MHz oscillator, δ was estimated to be $\pm$ 0.13 p.p.m. ie. of comparable

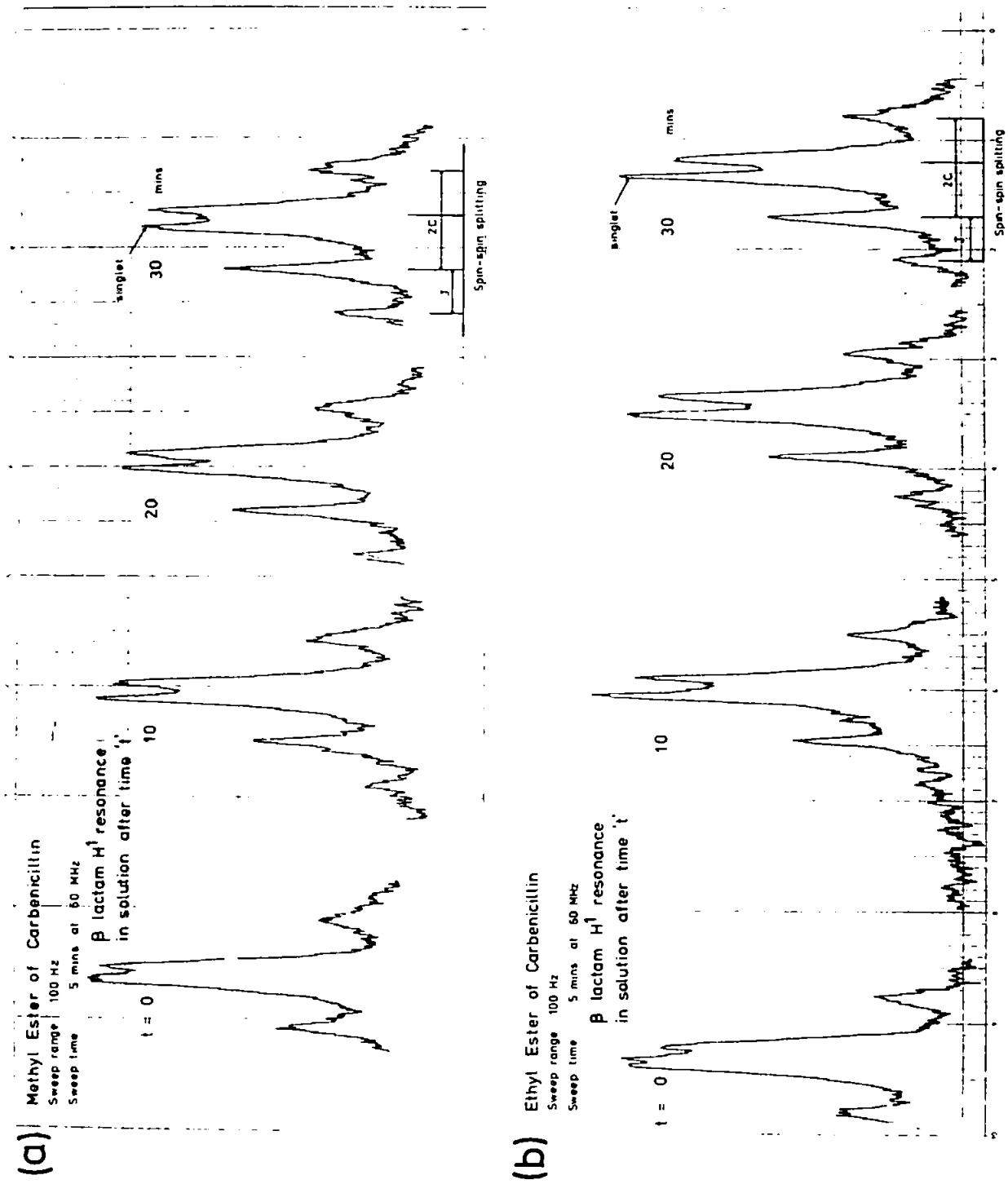
magnitude to that expected above.


(a)
Methyl Ester of Carbenicillin
Sweep range 10 ppm
Sweep time 5 mins at 60 MHz
β lactam H1
resonance in fresh
solution
singlet
small splitting
(b)
Metnyl Ester of Carbenicillin
Sweep range 10 ppm
Sweep time 5 mins at 60 MHz
β lactam H1
resonance in equilibrium
solution
singlet
Spin-spin
splitting

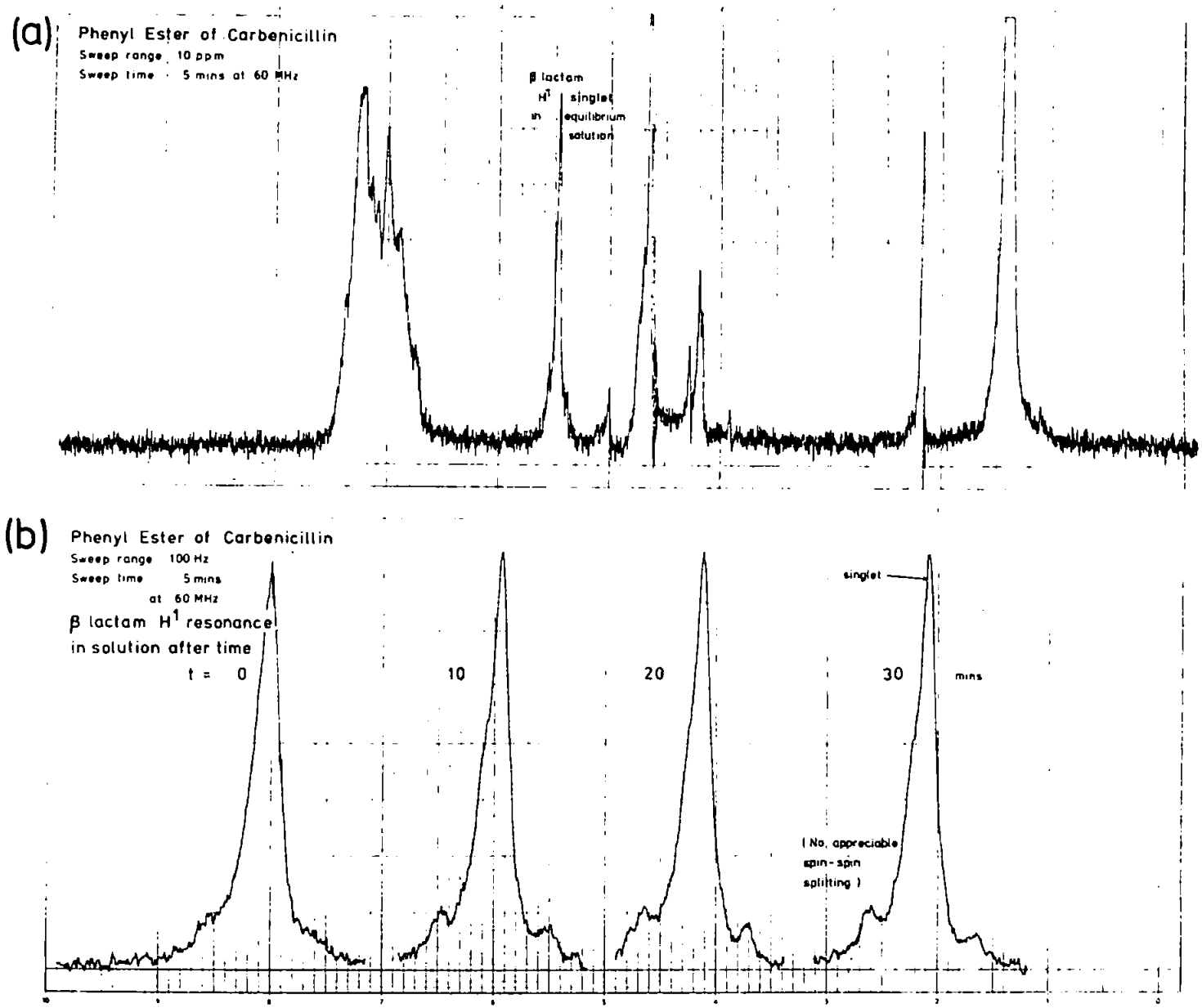
Trace 1


(a)
Ethyl Ester of Carbenicillin
Sweep range 10 ppm
Sweep time 5 mins
at 60 MHz
β lactam H1
resonance in fresh
solution
singlet
small splitting
(b)
Ethyl Ester of Carbenicillin
Sweep range 10 ppm
Sweep time 5 mins
at 60 MHz
β lactam H1
resonance in equilibrium
solution
singlet
Spin-spin
splitting

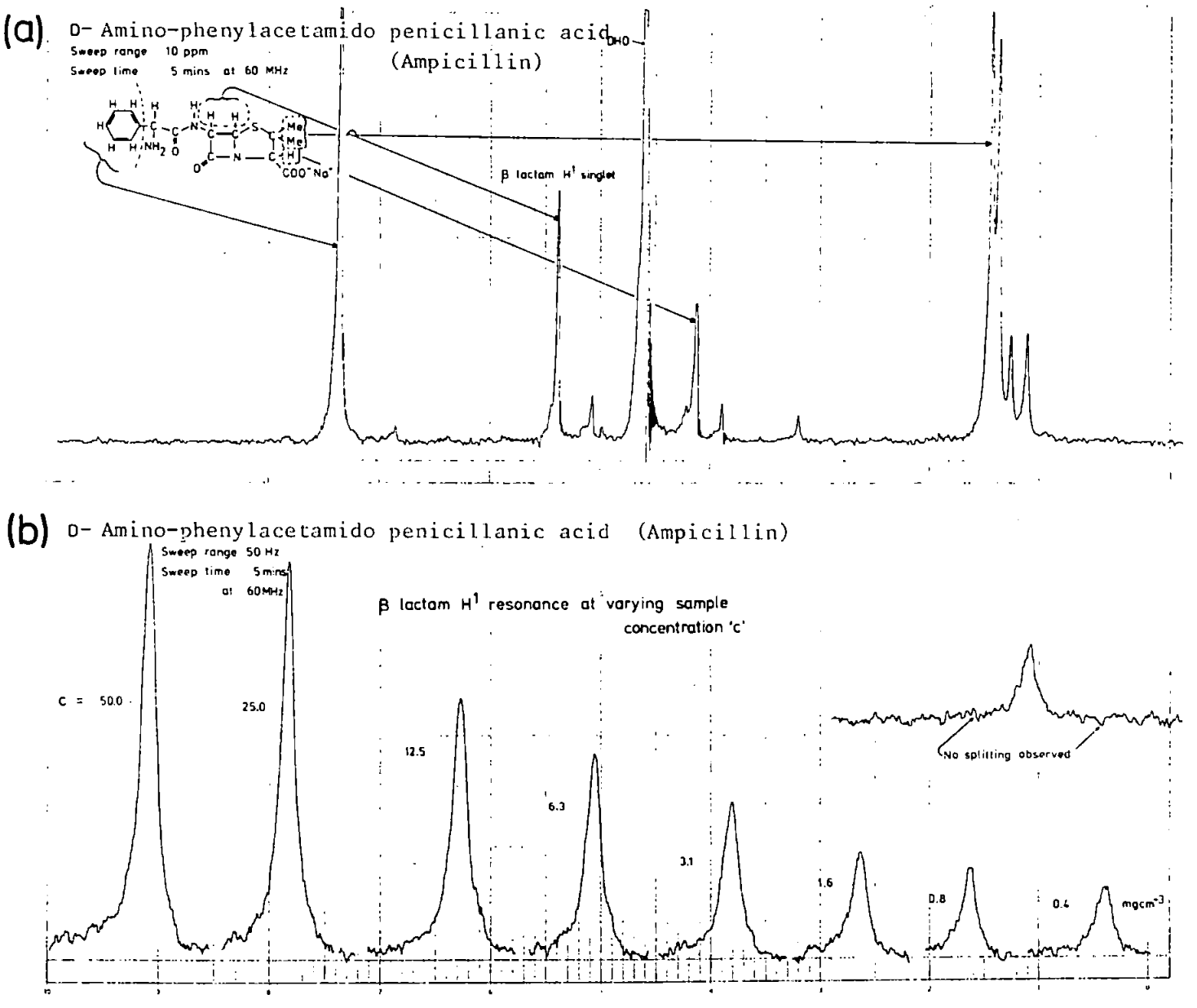
Trace 2


(a)
Methyl Ester of Carbenicillin
Sweep range 100 Hz
Sweep time 5 mins at 60 MHz
β lactam H¹ resonance
in solution after time 't'
t = 0
10
20
30
mins
singlet
J
2C
Spin-spin splitting
(b)
Ethyl Ester of Carbenicillin
Sweep range 100 Hz
Sweep time 5 mins at 60 MHz
β lactam H¹ resonance
in solution after time 't'
t = 0
10
20
30
mins
singlet
J
2C
Spin-spin splitting

Trace 3


(a)
Phenyl Ester of Carbenicillin
Sweep range 10 ppm
Sweep time 5 mins at 60 MHz
β lactam
H¹ singlet
in equilibrium
solution
(b)
Phenyl Ester of Carbenicillin
Sweep range 100 Hz
Sweep time 5 mins
at 60 MHz
β lactam H¹ resonance
in solution after time
t = 0
10
20
30
mins
singlet
(No. appreciable
spin-spin
splitting)

Trace 4


(a)
D- Amino-phenylacetamido penicillanic acid
(Ampicillin)
Sweep range 10 ppm
Sweep time 5 mins at 60 MHz
β lactam H¹ singlet
DHO
(b)
D- Amino-phenylacetamido penicillanic acid (Ampicillin)
Sweep range 50 Hz
Sweep time 5 mins
at 60MHz
β lactam H¹ resonance at varying sample concentration 'c'
C = 50.0
25.0
12.5
6.3
3.1
1.6
0.8
0.4
mgcm⁻³
No splitting observed

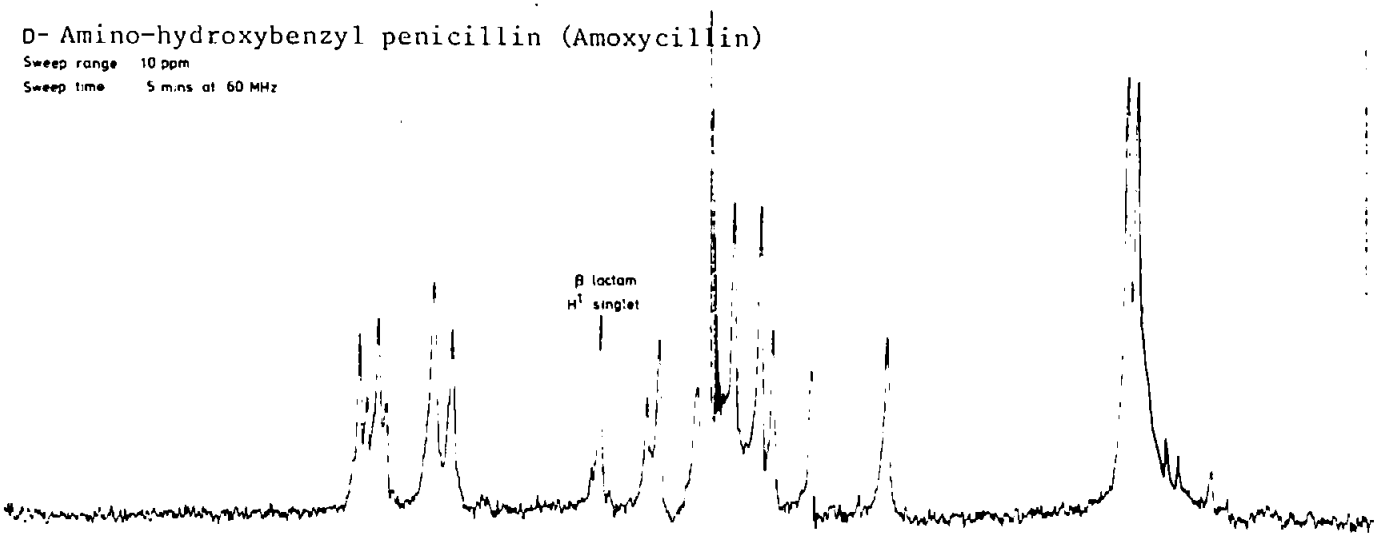
Trace 5

Trace 6

(a) D- Amino-hydroxybenzyl penicillin (Amoxycillin)

Sweep range 10 ppm

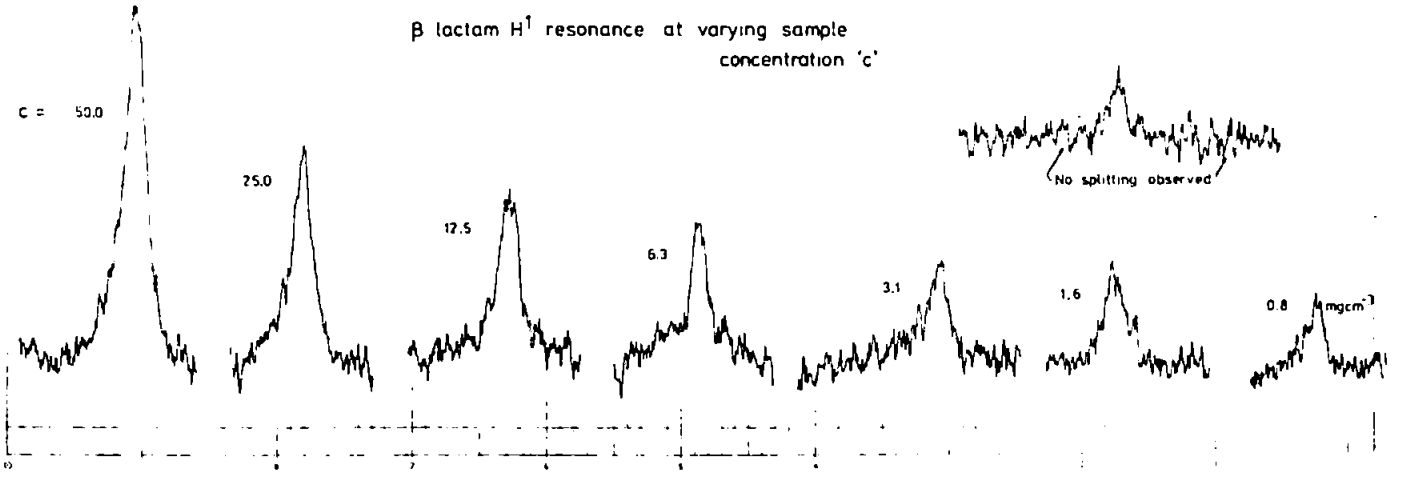
Sweep time 5 mins at 60 MHz

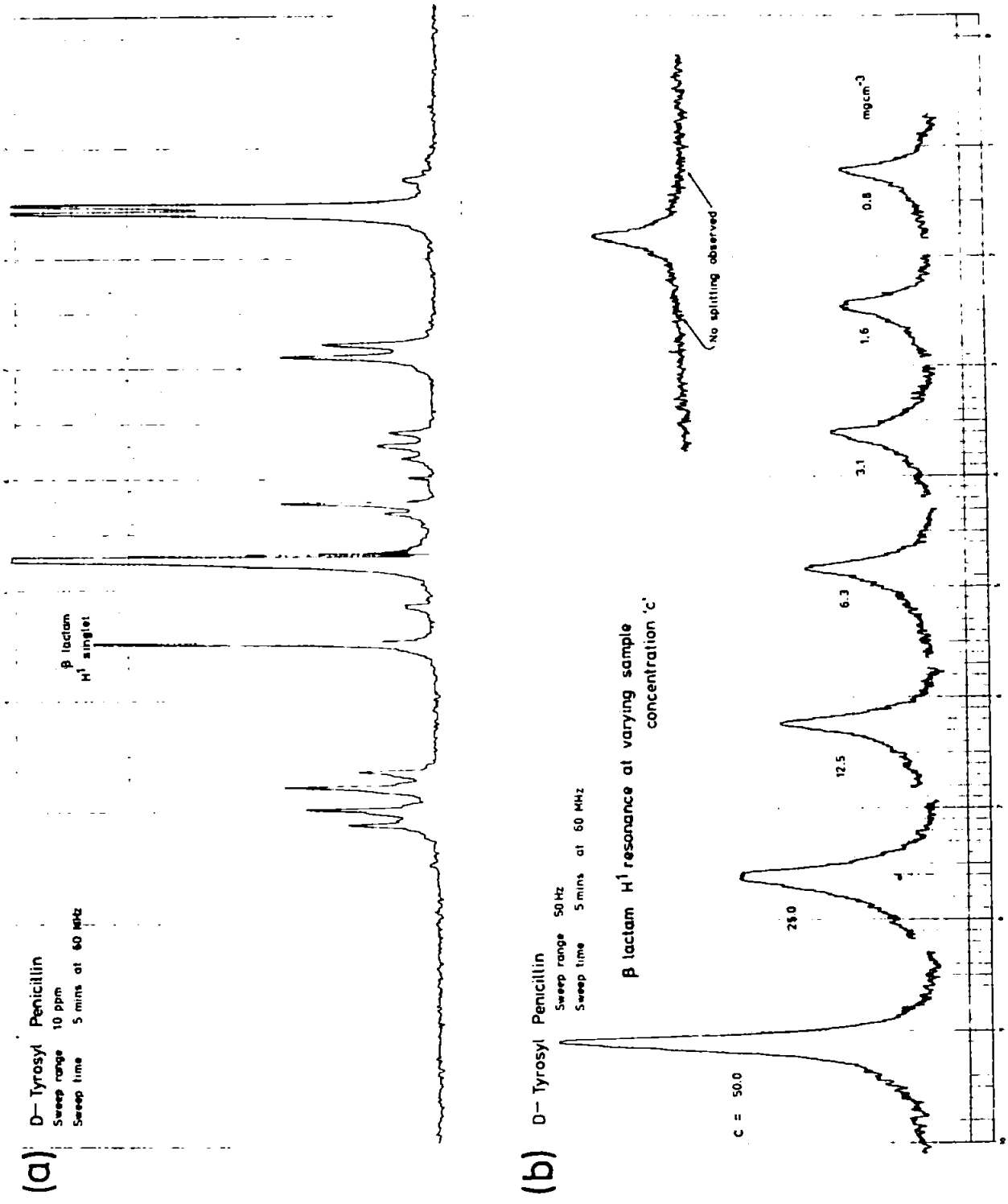


(b) D- Amino-hydroxybenzyl penicillin (Amoxycillin)

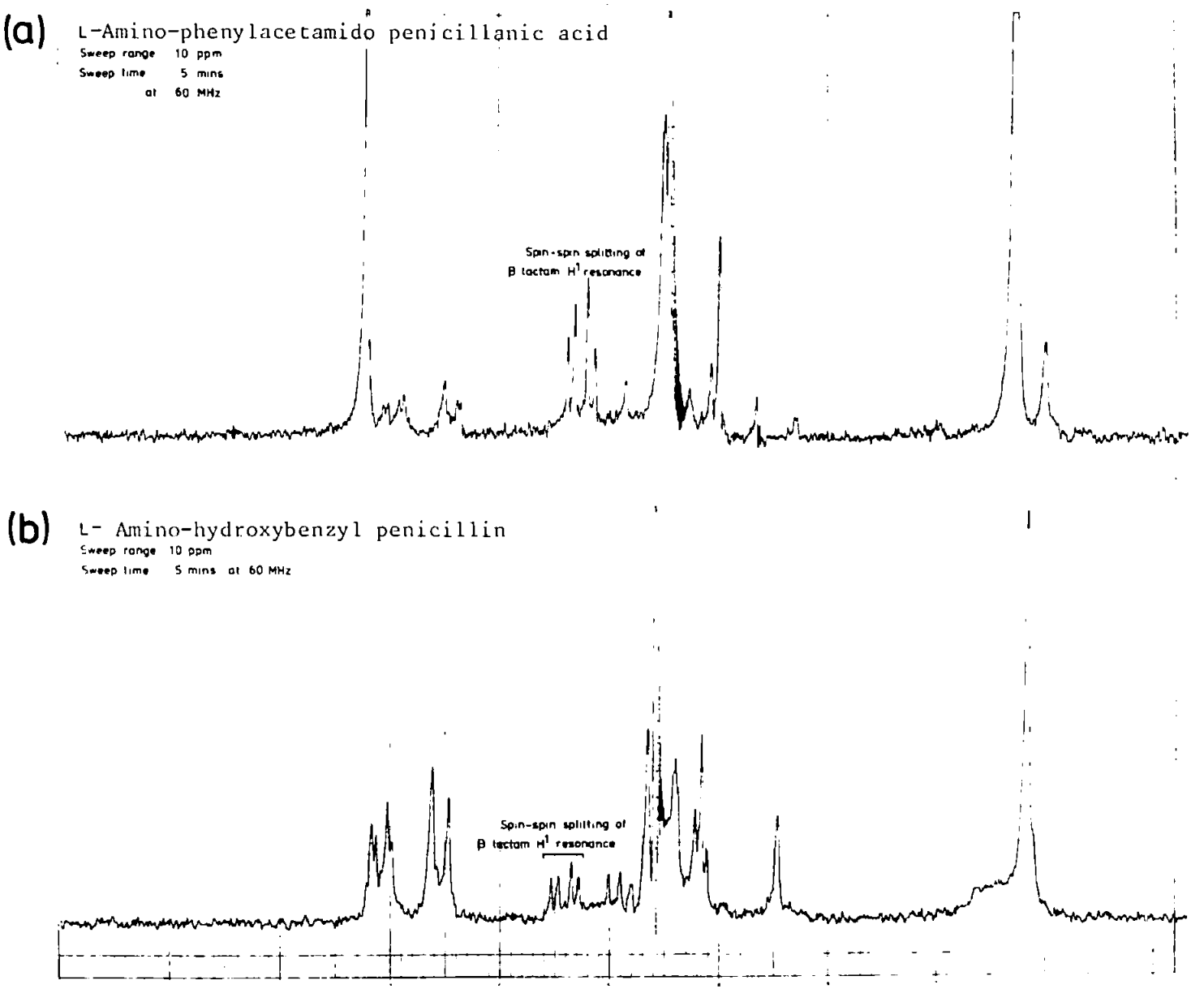
Sweep range 50 Hz

Sweep time 5 mins at 60 MHz

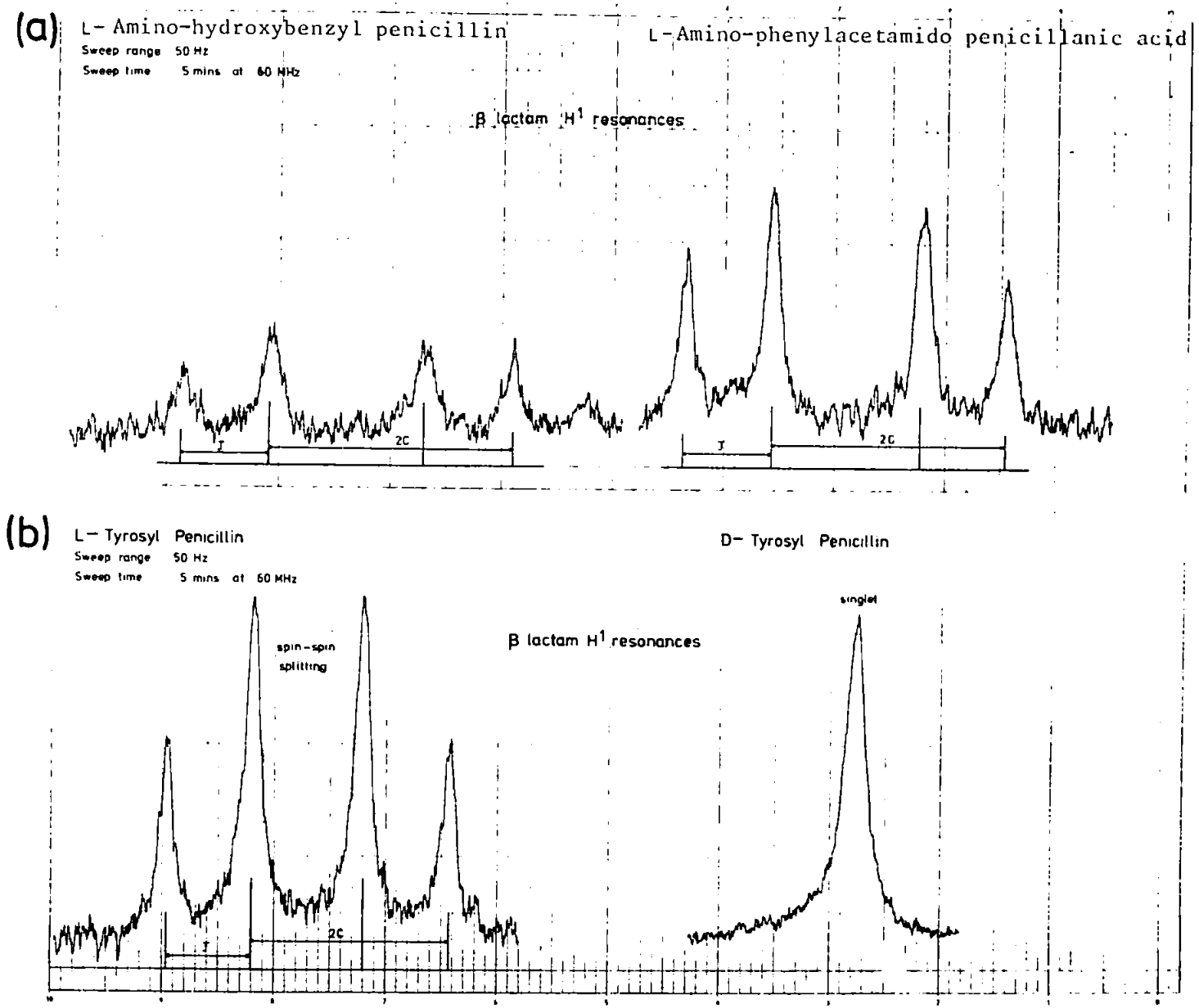



(a)
D– Tyrosyl Penicillin
Sweep range 10 ppm
Sweep time 5 mins at 60 MHz
β lactam
H1 singlet
(b)
D– Tyrosyl Penicillin
Sweep range 50 Hz
Sweep time 5 mins at 60 MHz
β lactam H1 resonance at varying sample concentration 'c'
No splitting observed
c = 50.0
25.0
12.5
6.3
3.1
1.6
0.8
mgcm-3

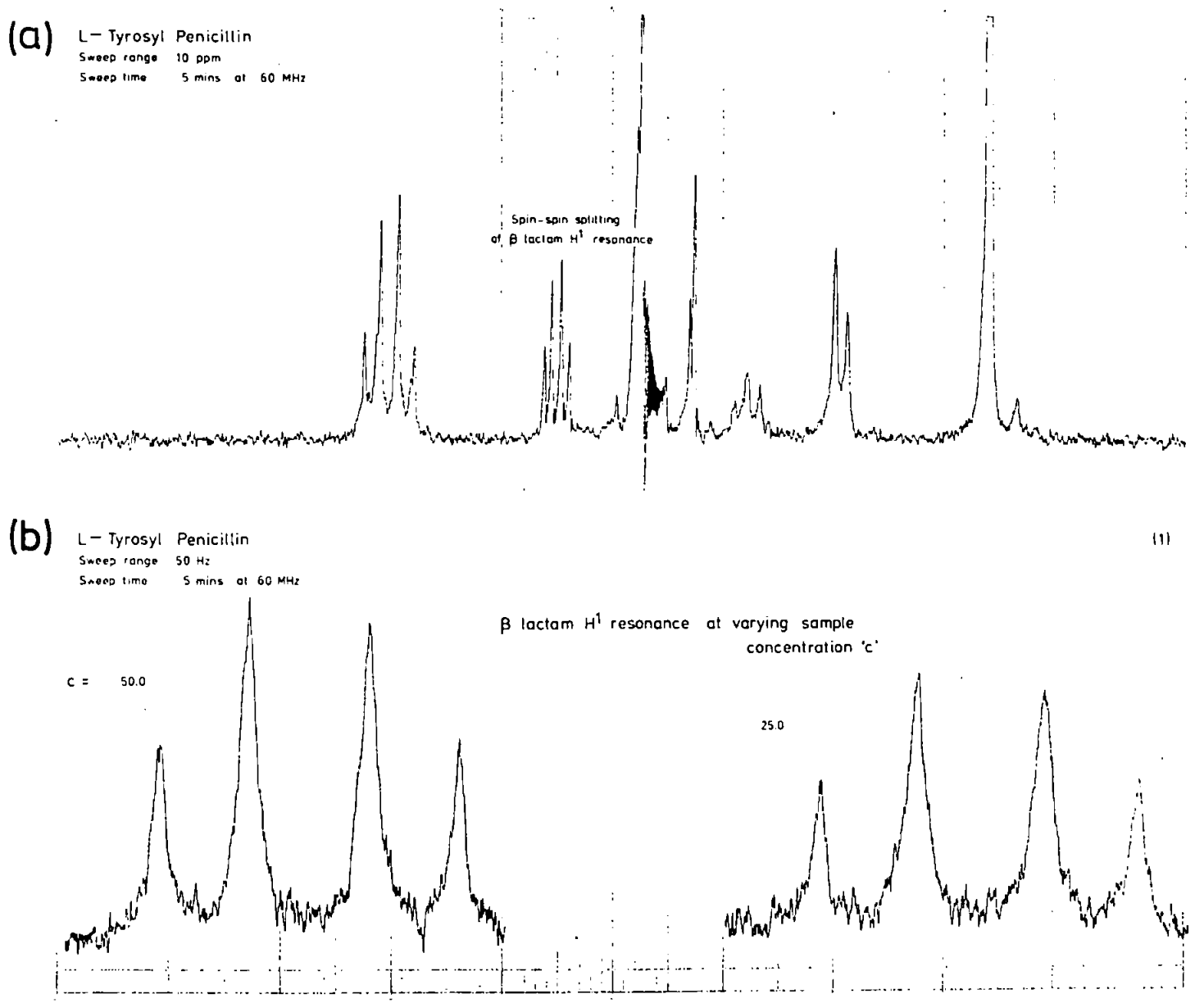
Trace 7


(a)
L-Amino-phenylacetamido penicillanic acid
Sweep range 10 ppm
Sweep time 5 mins
at 60 MHz
Spin-spin splitting of
β lactam H1 resonance
(b)
L- Amino-hydroxybenzyl penicillin
Sweep range 10 ppm
Sweep time 5 mins at 60 MHz
Spin-spin splitting of
β lactam H1 resonance

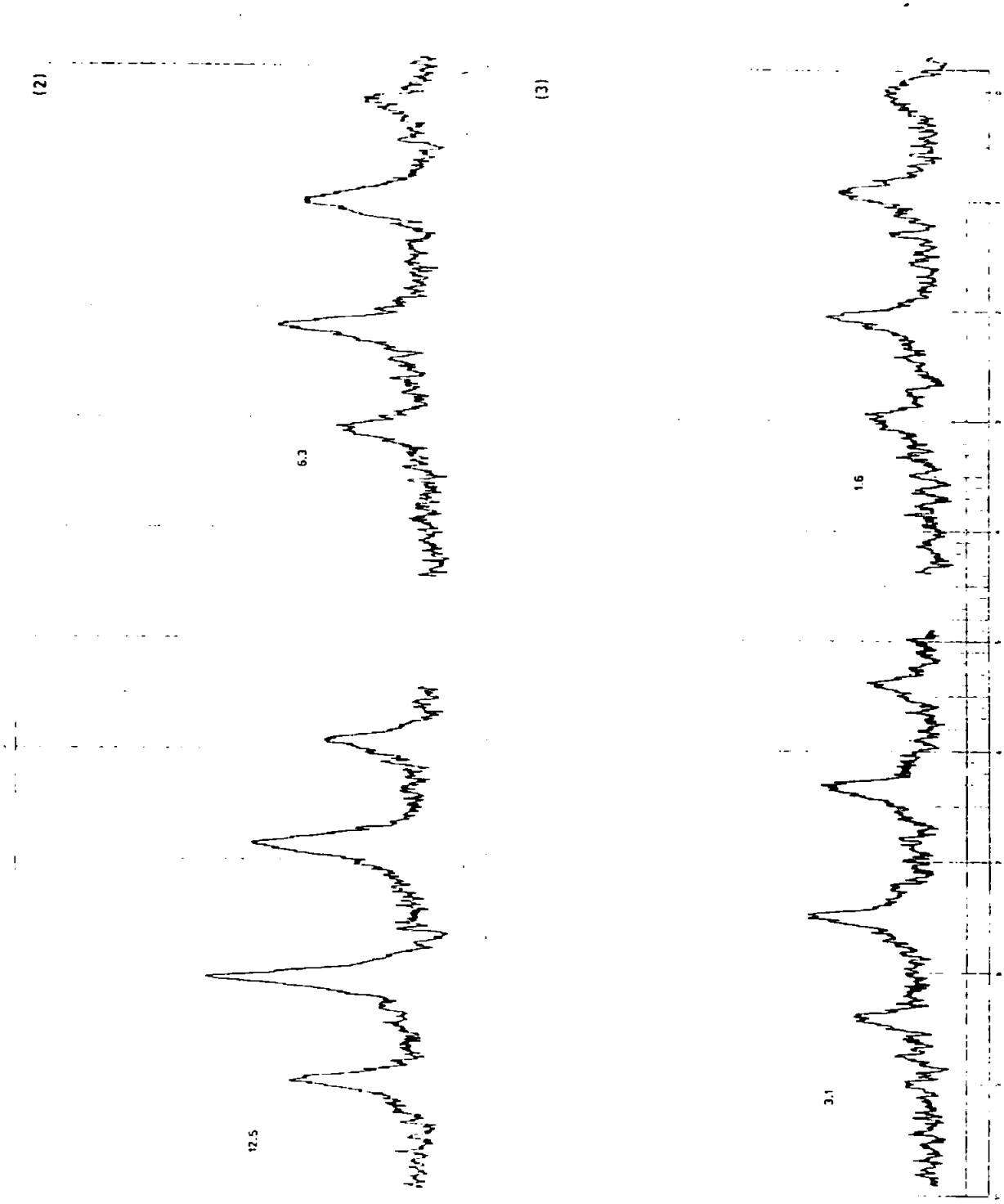
Trace 8


(a)
L- Amino-hydroxybenzyl penicillin
Sweep range 50 Hz
Sweep time 5 mins at 60 MHz
L-Amino-phenylacetamido penicillanic acid
β lactam H¹ resonances
J
2C
J
2C
(b)
L- Tyrosyl Penicillin
Sweep range 50 Hz
Sweep time 5 mins at 60 MHz
D- Tyrosyl Penicillin
spin-spin splitting
β lactam H¹ resonances
singlet
J
2C

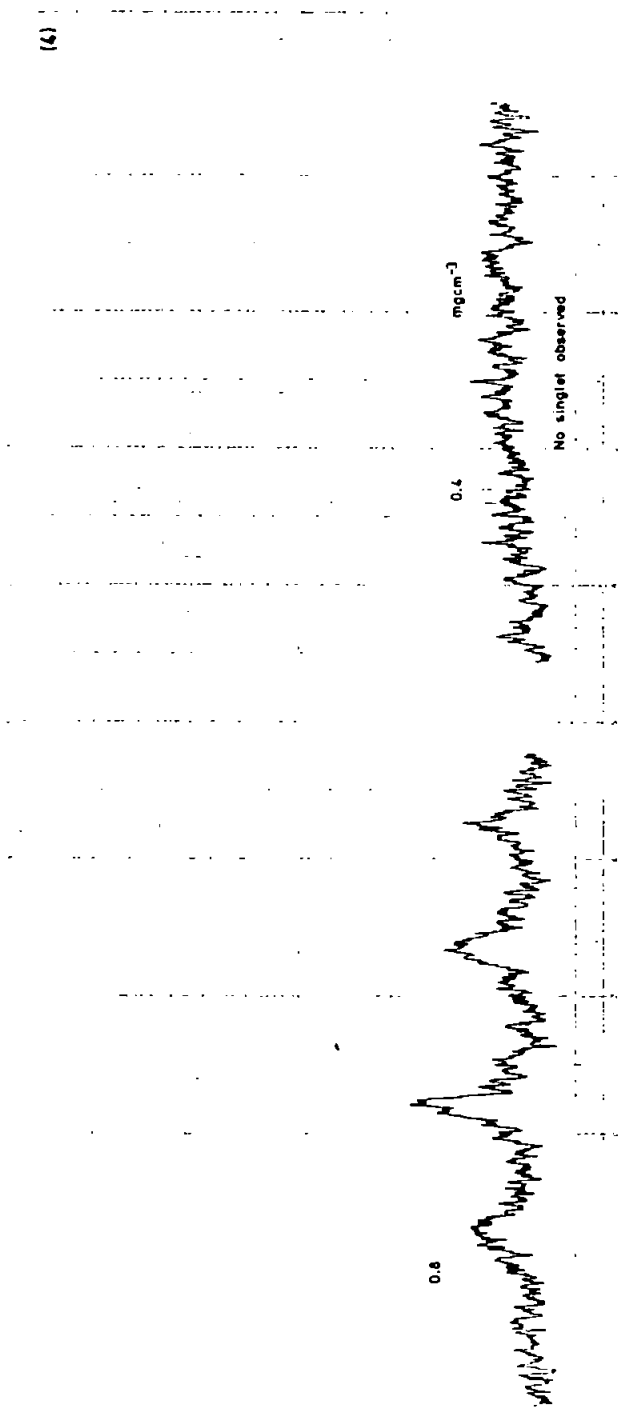
Trace 9


(a)
L– Tyrosyl Penicillin
Sweep range 10 ppm
Sweep time 5 mins at 60 MHz
Spin-spin splitting
of β lactam H1 resonance
(b)
L– Tyrosyl Penicillin
Sweep range 50 Hz
Sweep time 5 mins at 60 MHz
(1)
β lactam H1 resonance at varying sample
concentration 'c'
c = 50.0
25.0

Trace 10



Trace 10 (b) contd.



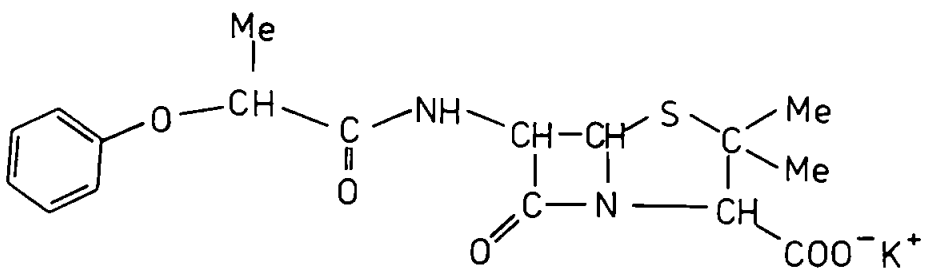
Trace 10 (b) contd.

Table 5.1 β lactam proton magnetic resonance spin-spin splitting coupling constants, J, and chemical shifts, δ, in solution at 35°C.

	J(Hz)	δ (p.p.m.)	Solvent
L-Tyrosyl penicillin,	3.80	0.13	D_2O/NaOD
L-Amino-phenylacetamido penicillanic acid,	3.90	0.16	"
L-Amino-hydroxybenzyl penicillin,	3.95	0.17	"
Methyl ester of carbenicillin,	4.00	0.13	D_2O } after equilibration
Ethyl ester of carbenicillin.	4.00	0.13	" } after equilibration

5.4 Side Chain Configuration and Associated Circular Dichroism

Stenlake, et al, (1972)[56], used the chiroptical technique of circular dichroism, (CD), as a method of assay to determine the proportions of D and L diastereoisomers in samples of phenethicillin potassium.



The spectrum obtained for the D and L epimers alone is shown in CD(1) where $\Delta\varepsilon$ is the difference ($\varepsilon_L - \varepsilon_R$) between left and right circularly polarised light. The reversal in Cotton Effect, (CE), between the (+)ve $\Delta\varepsilon$ value for the D and the (-)ve value for the L compounds both at 269nm and 276nm was shown to follow a linear relationship.

Boyd, et al, (1975)[57], computed a theoretical CD spectrum for penicillin nuclei using a molecular orbital model based on 6-aminopenicillanic acid, (6-APA). The predicted CD spectrum based on Extended Huckel Molecular Orbital theory is shown in CD(2), in which the exponential half-

width parameter for the transitions has a value chosen to most closely agree with experimental spectra of 6-APA. Two main features are evident: (i) the (+)ve CE at about 236nm is predicted to involve molecular orbitals designated S-N $\pi \rightarrow$ amide π^* with some mixing from S n $\rightarrow$ amide π^* ie. the sulphide chromophoric behaviour shows a dependence upon the relative vicinity of the β lactam amide chromophore; (ii) the shorter wavelength (-)ve CE at about 199nm is assigned to the β lactam n $\rightarrow \pi^*$ transition, however, a reversal to a (+)ve CE has been noted experimentally under conditions of varying pH. The (-)ve CE seems consistent with experimentally low pH values (~3.0), and the reversal of CE for higher pH values could be explained by a small mixing of the amino group orbitals into the amide orbitals.

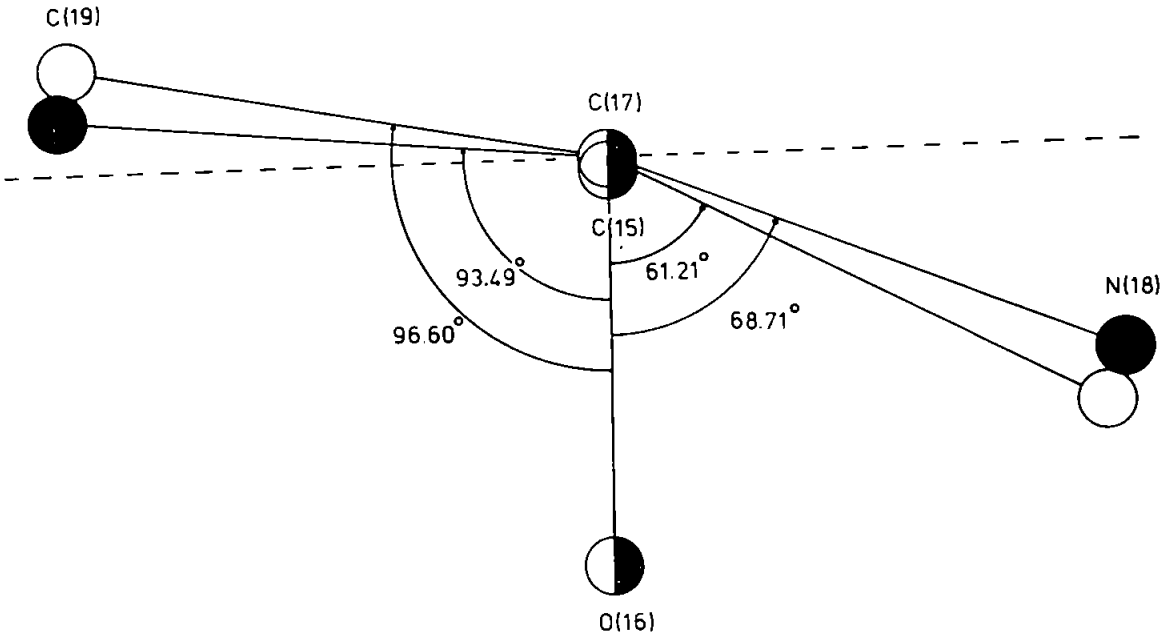
An empirical discussion of the contribution made by the side chain chromophores to the CD spectra of penicillin derivatives is facilitated by the observance of features modifying the spectrum of the penicillin nucleus discussed above.

The CD spectrum of amino-phenylacetamido penicillanic acid is shown in CD(3). The spectrum for the D epimer resembles closely that of the penicillin nucleus alone CD(2), however, the short wavelength (-)ve CE occurs at 206nm compared with the unperturbed β lactam amide transition at 199nm. As in the case of phenethicillin potassium CD(1), a (+)ve CE is noted in the long wavelength region at 259nm and 267nm. The spectrum of the L epimer, however, differs by a reversal of sign of $\Delta\varepsilon$ at 206nm and at the long wavelength CE at 261 nm and 266 nm. The reversal in CE at the short wavelength must be associated with the difference in side chain configuration since no pH change occurred to effect any change in the amide n $\rightarrow \pi^*$ transition. Similarly, the longer wavelength CE reversal must also be associated with the side chain configurational change since little or no contribution to the spectrum by the penicillin nucleus is apparent at such wavelengths. The side chain chromophores sensitive to configurational change about

C(17) are those that are either substituents of C(17) or suffer no insulation from chromophores in such a site. Thus, the study of the CD spectra of selected penicillin derivatives enables an assignment to be made of which chromophore(s) contributes the variation in spectra both between each derivative and their respective D and L diastereoisomers.

In the case of amino-phenylacetamido penicillanic acid, the differing spectral features, ie, reversal of CE's between the D and L forms already described, could be assigned to the possible chromophoric transitions in either the benzene, amine or carbonyl C(17) substituents.

Amino-hydroxybenzyl penicillin differs from amino-phenylacetamido penicillanic acid in respect to the para-substituted hydroxyl of the C(17) benzene substituent only. The absorption wavelength for a disubstituted benzene is not greatly affected by para-substitution of an electron donating group and as shown in CD(4), the spectrum of amino-hydroxybenzyl penicillin shows CE's associated with the diastereoisomeric configuration, at only slightly longer wavelengths than those of amino-phenylacetamido penicillanic acid. However, change of sign of the CE at 270-278 nm between the D and L forms of amino-hydroxybenzyl penicillin is opposite to that of amino-phenylacetamido penicillanic acid suggesting that this band is due either to a weak benzene $\pi \rightarrow \pi^*$ transition or pH sensitive amine transitions. Fig. 5.6 illustrates the C(17) substituent orientations relative to the C(17) carbonyl substituent C(15) = O(16) in amoxycillin and ampicillin (the D epimers of amino-hydroxybenzyl penicillin and phenylacetamido penicillanic acid respectively), (James, Hall and Hodgkin, (1968)[41] Boles and Girven, (1976a)[34] and Boles, Girven and Gane, (1978))[40], in which it can be seen that any CE due to the carbonyl chromophore should exhibit very little difference between like isomeric configurations of the two compounds, thus, again confining the 270-278 nm absorption band as representative of weak benzene or amine transitions as described above.



Conformation of the C(15)=O(16) chromophore with respect to C(17) substituents in amoxycillin and ampicillin.

Fig.5.6

* CD is sensitive to relative configuration of those chromophores in the immediate vicinity of C(17). Since two of the chromophores are carbonyl groups and, therefore, identical, C(17) can be regarded as not constituting an asymmetric centre for the purpose of CD measurements.

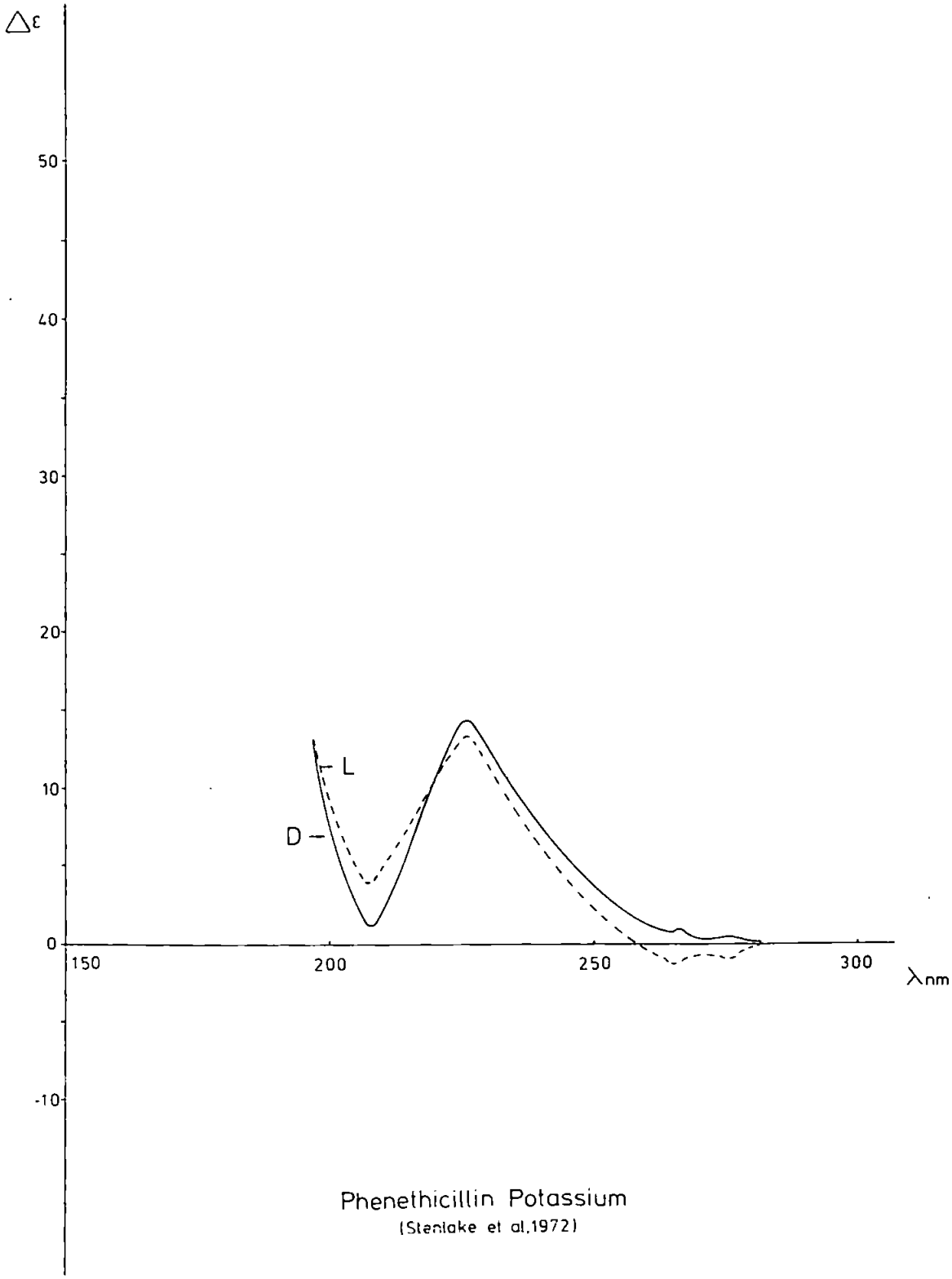
The tyrosyl penicillin provides the opportunity to study the CD spectrum of a derivative very similar to amino-hydroxybenzyl penicillin and amino-phenylacetamido penicillanic acid but with chromophoric insulation of the benzene ring by the intervening C(17) substituent methylene group. The spectrum shown in CD(5) exhibits no reversal of CE between the D and L forms ie. the insulation of the benzene has removed any CE sensitivity to side chain configuration within the recorded wavelength range, and thus, it can be concluded that both the long and short wavelength absorption bands are associated with π electron transitions of the benzene chromophore. Some reduction in the (+)ve $\Delta\varepsilon$ at 190-195 nm for the L form could be attributed to the carbonyl or amine chromophores.

The absence of CE reversal between the spectra for D and L phenethicillin potassium in the short wavelength band would indicate that either some mixing of the absorption by the amine substituent, present in the above compounds, but replaced by a methyl group in phenethicillin, or the remoteness of the benzene due to the intervening oxygen, have a marked effect on the benzene absorption at this wavelength.

The methyl, ethyl and phenyl esters of carbenicillin show distinctive (-)ve CE's at 206-208 nm, in fresh solution, similar to the D forms, amoxycillin and ampicillin. Although C(17) does not constitute an asymmetric centre in the case of carbenicillin,* the short wavelength CE is sensitive to mutarotation. CD(6), CD(7) and CD(8) show the CD spectra of the methyl, ethyl and phenyl esters respectively, in fresh solution and subsequently at 10, 20 and 30 minutes and after 24 hours. All three esters exhibit mutarotation and the(-)ve CE at 206-208 nm in fresh solution virtually disappears when equilibrium is reached between the two side chain substituent configurations in good agreement with the H^1 n.m.r. spectra previously discussed. The phenyl ester reaches equilibrium more rapidly than eitherthe methyl or ethyl esters

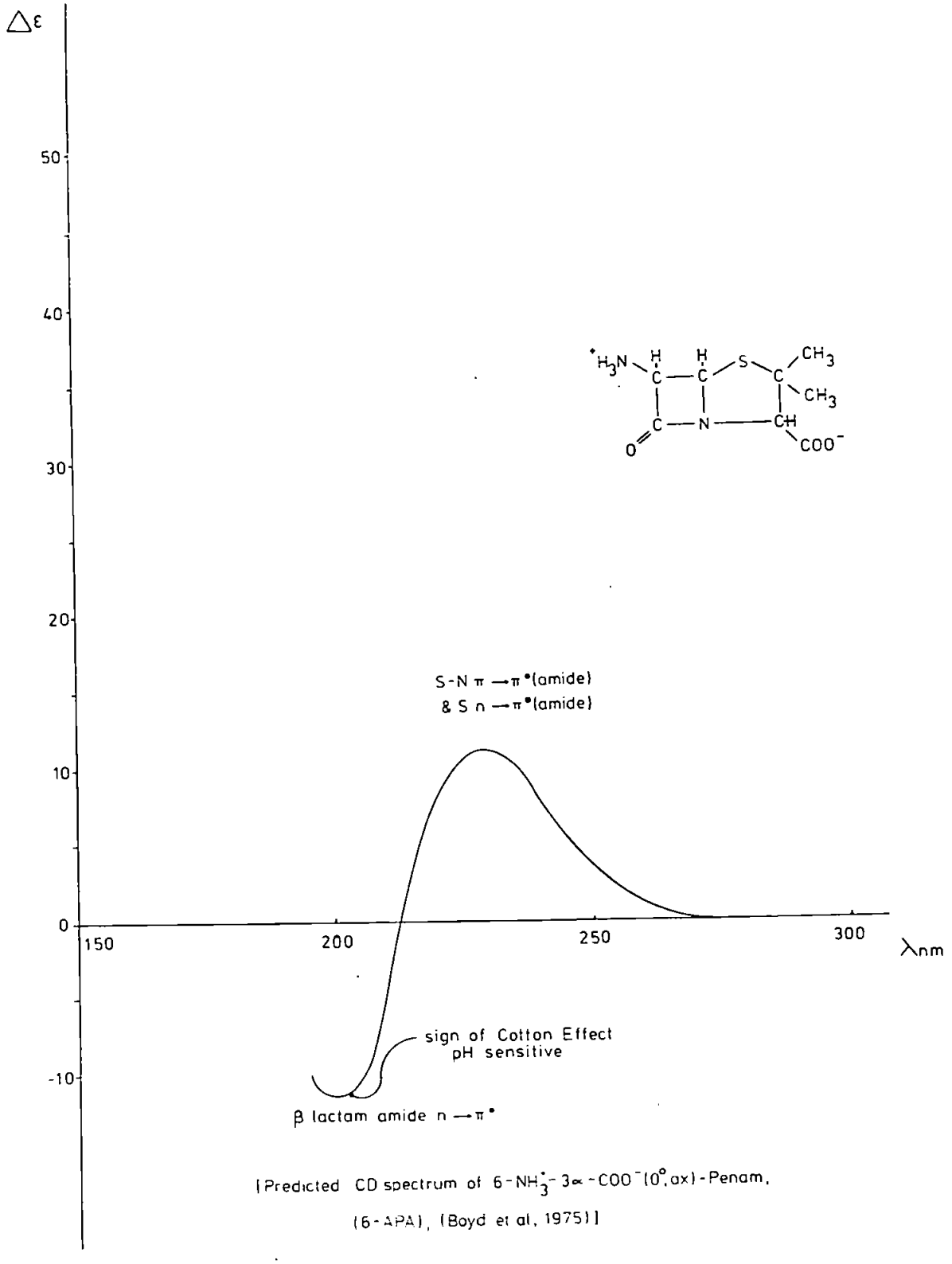
suggesting that the mechanism is susceptible to the molecular environment. The CD spectra, recorded at 27°C show slower rates of equilibration than those recorded at 35°C for the methyl and ethyl esters during H^1 n.m.r. studies. Hence, the mechanism would appear to involve the temporary separation of the mildly acidic hydrogen H(C(17)), as suggested in the model previously used to describe the anomalous behaviour of the H^1 n.m.r. spectrum of the phenyl ester after equilibration, (§. 5.3).

The $\Delta\varepsilon$ values for the salient CE's for the D and L epimers of amino-phenylacetamido penicillanic acid, amino-hydroxybenzyl penicillin, the tyrosyl penicillin and the methyl, ethyl and phenyl esters of carbenicillin are given in Table 5.2; the spectra being recorded in aqueous solution at 27°C.



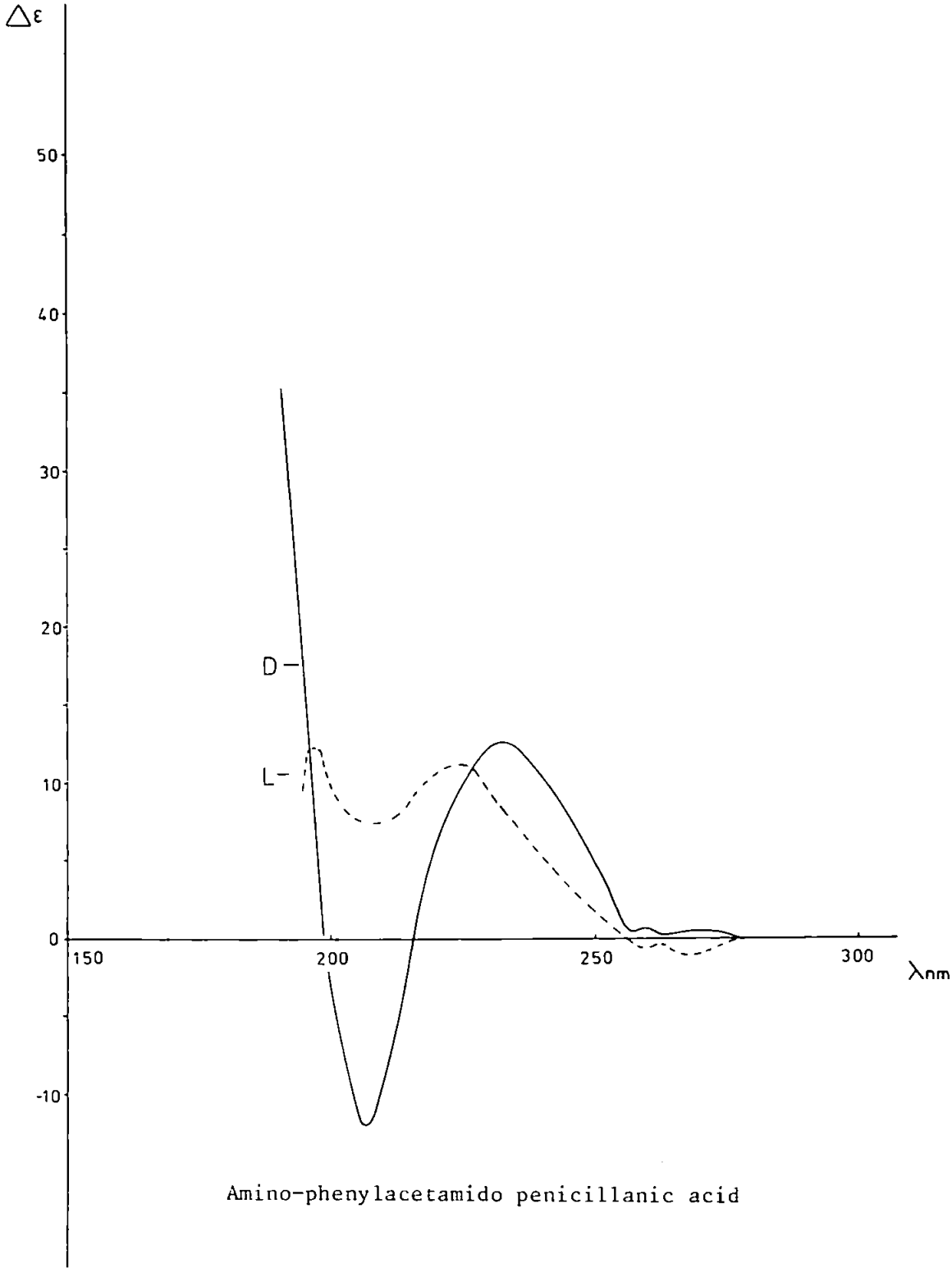
Phenethicillin Potassium
(Stenlake et al.,1972)

CD(1)



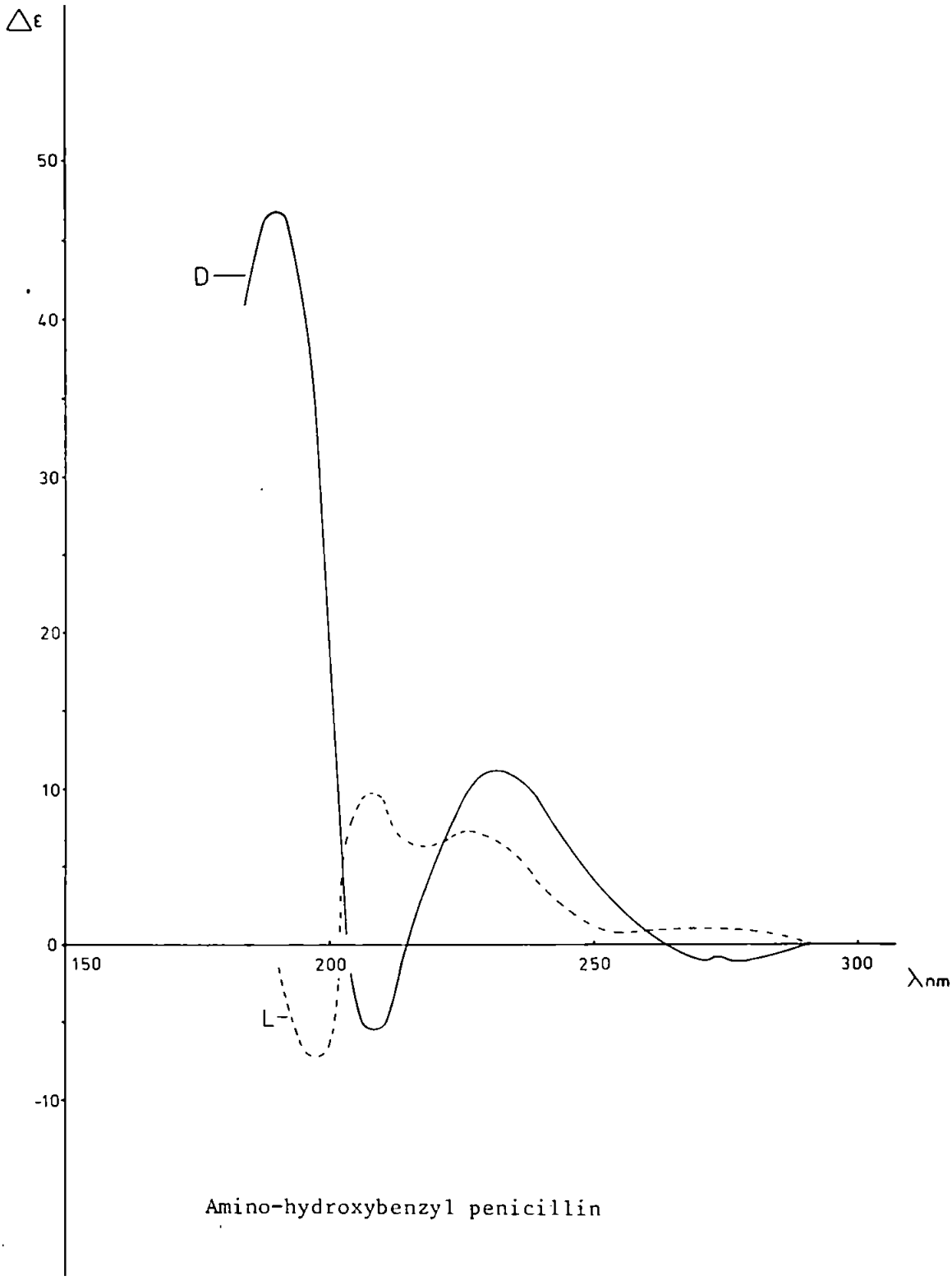
[Predicted CD spectrum of 6-NH_3^+-3α-COO^-(0°,ax)-Penam, (6-APA), (Boyd et al, 1975)]

CD (2)



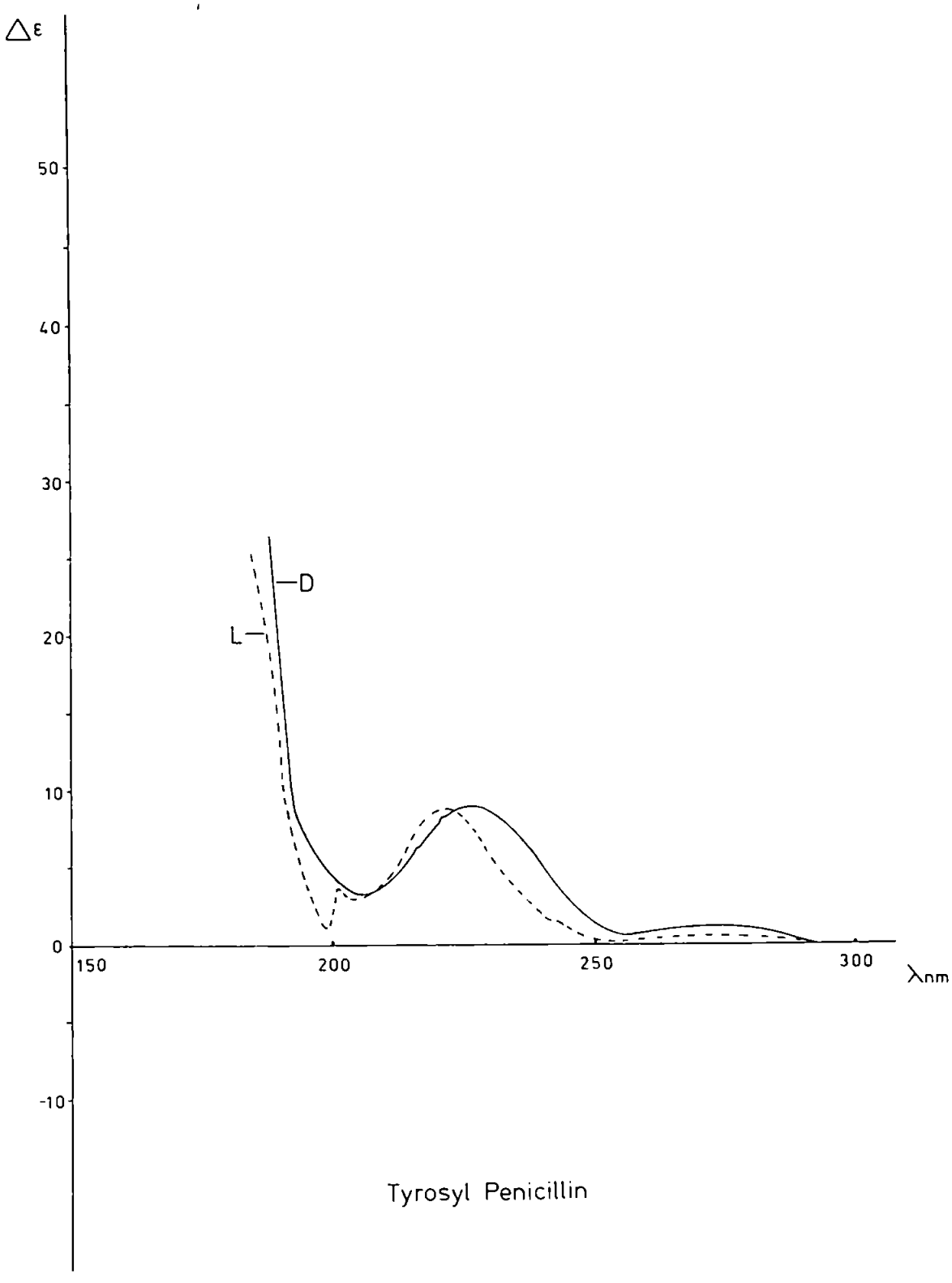
Amino-phenylacetamido penicillanic acid

CD (3)

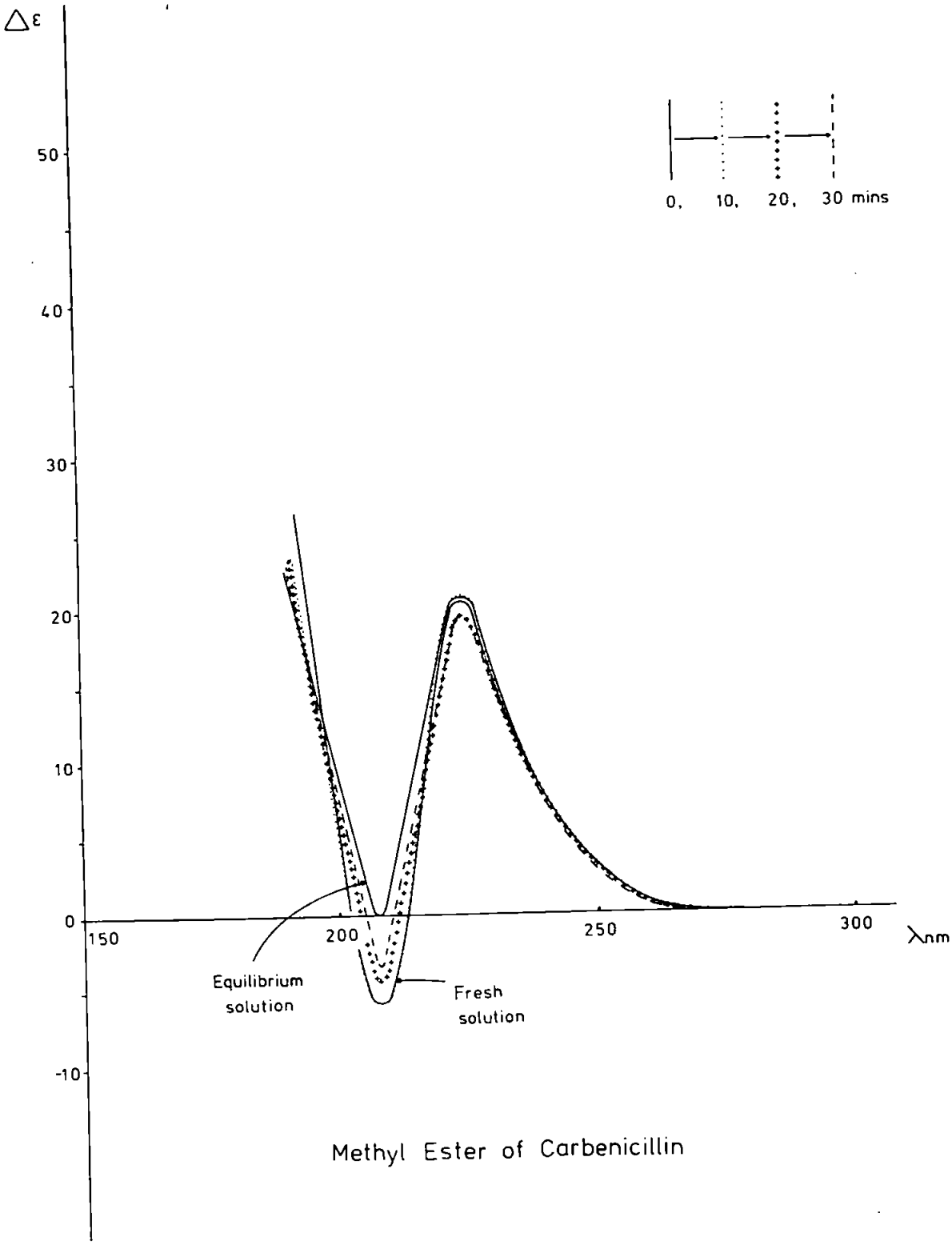


Amino-hydroxybenzyl penicillin

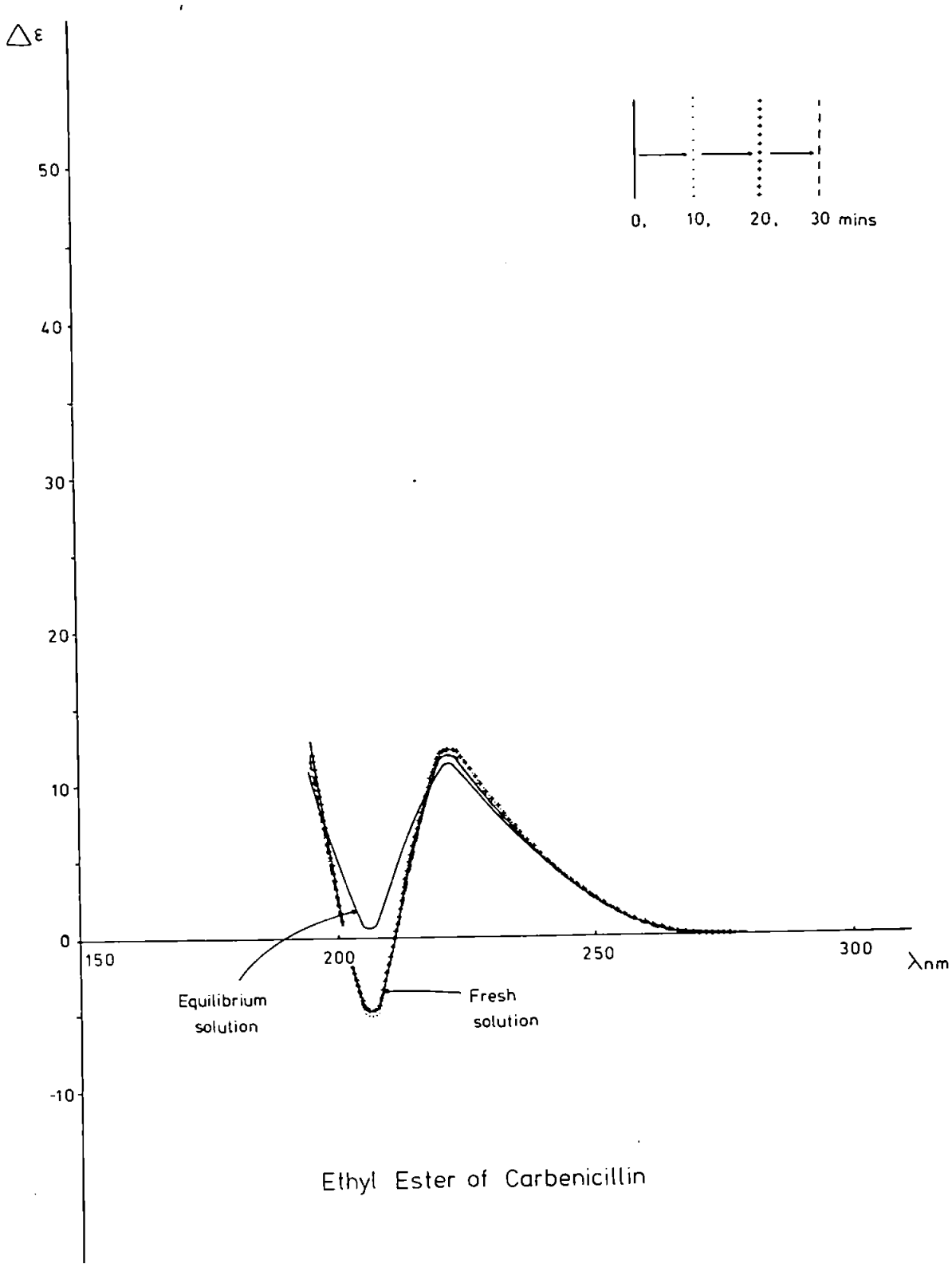
CD (4)



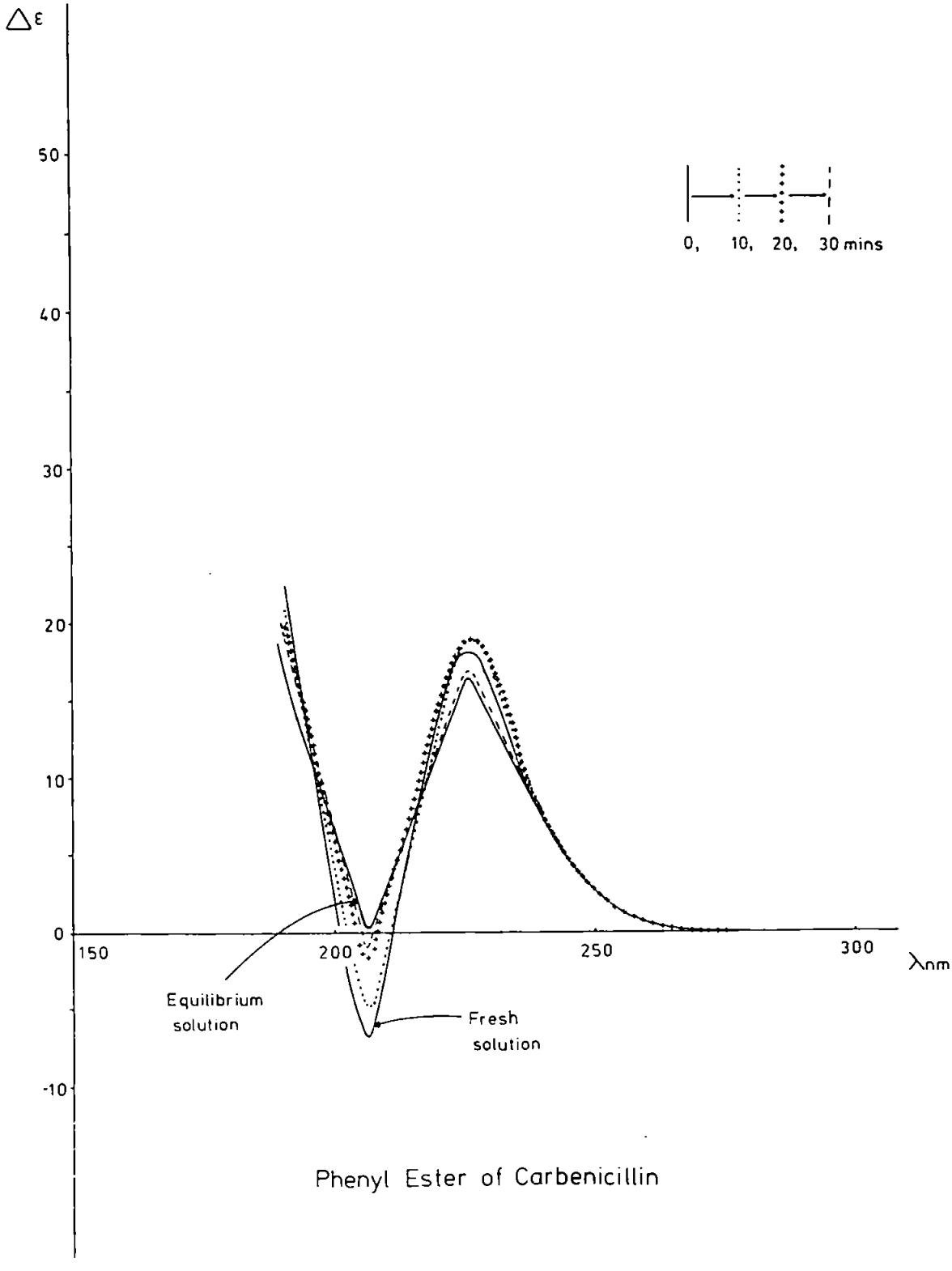
CD (5)


Δε
50
40
30
20
10
0
-10
150
200
250
300
λnm
0, 10, 20, 30 mins
Equilibrium solution
Fresh solution
Methyl Ester of Carbenicillin

CD (6)


Δε
50
40
30
20
10
0
-10
150
200
250
300
λnm
0, 10, 20, 30 mins
Equilibrium solution
Fresh solution
Ethyl Ester of Carbenicillin

CD (7)


Δε
50
40
30
20
10
0
-10
150
200
250
300
λnm
0, 10, 20, 30 mins
Equilibrium solution
Fresh solution
Phenyl Ester of Carbenicillin

CD(8)

Table 5.2. Circular dichroism data for the principal Cotton Effects of the following penicillin derivatives in aqueous solution at 27°C. The units of the molar extinction coefficient, ε , are given in $dm^3 mol^{-1} cm^{-1}$, where $\Delta\varepsilon$ $[=(\varepsilon_L - \varepsilon_R)]$ expresses the difference in absorption between left and right circularly polarised light.

	$\Delta\varepsilon$	λ(nm)
D-Tyrosyl penicillin monohydrate	+ 0.29	275
	+ 8.75	235
	+ 7.10	221
	+ 5.74	217
	+ 7.38	195*
L-Tyrosyl penicillin trihydrate	+ 0.15	275
	+ 7.46	228
	+ 3.37	202
	+10.10	190*
D-Amino-phenylacetamido penicillanic acid anhydrate (Ampicillin anhydrate)	+ 0.64	268
	+ 0.96	260
	+12.48	232
	-10.24	206
	+ 4.16	195*
D-Amino-phenylacetamido penicillanic acid trihydrate (Ampicillin trihydrate)	+ 0.34	267
	+ 0.67	259
	+12.73	232
	-11.39	206
	+29.82	192*
L-Amino-phenylacetamido penicillanic acid trihydrate	- 0.31	266
	- 0.13	261
	+10.59	224
	+11.65	198*
D-Amino-hydroxybenzyl penicillin trihydrate (Amoxycillin trihydrate)	- 0.49	278
	- 0.74	270
	+10.83	234
	- 6.40	207
	+46.25	190*
L-Amino-hydroxybenzyl penicillin trihydrate	+ 0.96	275
	+ 2.89	243
	+ 7.45	226
	+ 9.62	208
	- 7.45	198*

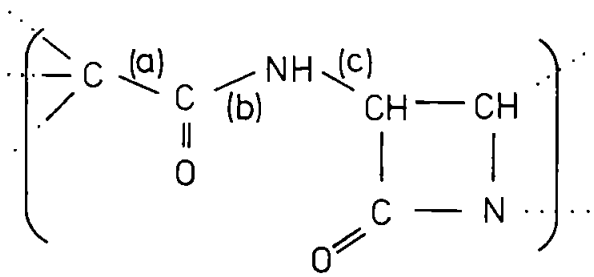
* denotes the shortest recorded wavelength

5.5 Further possibilities for conformational change outside the penicillin nucleus

The discussion related to the spectroscopic evidence above has been confined to a description of the relative orientation of side-chain substituents of C(17) only. It has been suggested, (§. 5.3), that this description is satisfactory in explaining the characteristic behaviour of the β lactam proton resonance and precludes the possibility of changes in conformation elsewhere within the molecule.

It is of interest to speculate what other possible conformational changes might take place in solution and how, if at all, such changes would be reflected in the relevant proton resonance and circular dichroism spectra.

The penicillin derivatives used in the previous discussion, (§. 5.2, 5.3, and 5.4), all have the common side-chain and β lactam constituents, with bonds labelled (a), (b) and (c) for convenience.



The constraining nature of the interstitial carbonyl group in the side-chain provides conformational stability of the bonds (a) and (b), where (b) takes on the nature of an amide-carbonyl bond common to rigid chain protein structures. Thus, possible conformational change of the side-

chain is limited to bond (c) where linkage is made with the β lactam of the penicillin nucleus. Rotation about (c) is, however, a remote possibility due to the constraints applied by possible repelling effects of the carbonyl groups in the side-chain and β lactam respectively.

Any progressive change of conformation of the side-chain in solution would be evidenced in both the proton resonance and circular dichroism spectra by either a superposition of shielding and unshielding effects or a progressive change in Cotton Effect for all the penicillin derivatives.

Free rotation of the side-chain in solution would result in the characteristic β lactam proton resonance being time averaged between the shielded and unshielded extrema.

It may therefore be concluded that the discrete nature of the differences in the respective H^1 n.m.r. and CD spectra of the D and L side-chain C(17) epimers precludes interpretation which does not include the constraints of rigidity within the side-chain substituents of the stable diastereoisomeric configurations.

APPENDIX A

COMPUTER PROGRAMS FOR THE CALCULATION OF ORTHORHOMBIC PATTERSON FUNCTION, (1), AND ELECTRON DENSITY FOURIER SYNTHESIS, (2), IN SPACE GROUP $P2_12_12$.

FORTRAN COMPILATION BY 4XFAT MX 5A DATE

```
0001                    LIST
0002                    WORK(ED,ICLF-DEFAULT)
0003                    PROGRAM(GANE)
0004                    INPUT 1,2=CRD
0005                    OUTPUT 3=LPT
0006                    OUTPUT 4=TPR
0007                    COMPLEX, INTEGER AND LOGICAL
0008                    TRACE 2
0009                    END
```

Program (1)

```
      MASTER PATT
C
C     PATTERSON FUNCTION FOR ORTHORHOMBIC SYSTEM
C
      DIMENSION NH(2000),NK(2000),NL(2000),FOBSS(2000)
      DIMENSION COSRU(11,11),COSRV(31,31),COSRW(31,31)
      DIMENSION UPATT(31), FPATT(31,31,10)
      DIMENSION FSCAT(101),F(5),NPATT(31)
      DIMENSION LINE(51)
      DIMENSION NUMH(2000)
      READ (2,303) NAST, IBLK
303   FORMAT(2A3)
C     READ IN ATOM TYPE AND TEMPERATURE FACTOR
      READ(2,5) NTYPE,BETA
5     FORMAT(I5,F10.5)
      WRITE(3,600) NTYPE,BETA
600   FORMAT(24H SHARPENING ATOM TYPE = ,I5,4X,5H B = ,F10.5)
C     READ IN UNIT CELL DIMENSIONS
      READ(2,1) A,B,C
1     FORMAT(3F10.5)
      ASQ = A*A
      BSQ = B*B
      CSQ = C*C
C     READ IN SCATTERING FACTORS
      DO 200 N = 1,5
      READ(2,2) Y
2     FORMAT(F3)
      KK = 14*N-14
      JMT = IFIX(KK+1.5)
      DO 200 I = 1,14
      MI = JMT + I
      READ(2,3) FSCAT(MI)
3     FORMAT(F10.5)
200   CONTINUE
100   CONTINUE
      NUMR = 0
      NOUT = 0
      NA = 1
      NUM(1) = 0
C     READ IN H,K,L AND OBSERVED F(HKL)
11    READ(2,4) J,K,L,FOBS
4     FORMAT (3I5,F9.5)
      IF(J-999) 7,8,7
8     SCALE = FOBS
      GO TO 11
7     IF(J-999) 9,10,9
C     CALCULATE SIN(THETA)/LAMBDA FOR HKL REFLEXION
9     DJKL = SQRT(J*J/ASQ + K*K/BSQ + L*L/CSQ)
      IF(NUMR.EQ.0) GO TO 301
      IF(J.GT.NH(NUMR)) GO TO 300
      GO TO 301
300   NA = NA + 1
      NUM(NA) = NUMR
301   SINLAM = 0.5 * DJKL
      NUMR = NUMR + 1
      NH(NUMR) = J
      NK(NUMR) = K
      NL(NUMR) = L
C     INTERPOLATE SCATTERING FACTOR FOR INCREASING THETA
      RANGE = 0.05
      N = 1
17    IF(SINLAM-RANGE) 14,14,16
16    RANGE = RANGE + 0.05
```

```
0073                N = N + 1
0074                GO TO 17
0075          14    DO 15 MN = 1,5
0076                FU = 14*MN - 14
0077                NUT = IFIX(FU+0.5)
0078                NQ = NUT + 1
0079                F(MN) = 20 + (FSCAT(NQ)-FSCAT(NQ+1))*(RANGE-SINLAM)+FSCAT(NQ+1)
0080          15    CONTINUE
0081          C     SHARPEN THE OBSERVED F(HKL)
0082                FOBS = FOBS/SCALE
0083                FOBS = FOBS/F(NTYPE)*EXP(BETA*SINLAM*SINLAM)
0084                FOBSQ = FOBS*FOBS
0085          C     REDUCE THE SYMMETRY FACTOR FOR H,K OR L = 0
0086                IF(J) 52,53,52
0087          53    FOBSQ = 0.5*FOBSQ
0088          52    IF(K) 54,55,54
0089          55    FOBSQ = 0.5*FOBSQ
0090          54    IF(L) 56,57,56
0091          57    FOBSQ = 0.5* FOBSQ
0092          56    FORIG = FORIG + FOBSQ
0093                FOBSS(NTHR) = FOBSQ
0094                GO TO 11
0095          C     SET UP TRIGONOMETRIC ARRAYS
0096          10    NA = NA + 1
0097                WRITE (3,400) NA
0098          400   FORMAT (1H4 NA = MAX H + 2 = ,I5)
0099                NUM(NA) = NUMR
0100                DO 61 I = 1,31
0101                DO 61 IJ = 1,31
0102                FI = I-1
0103                FJ = IJ-1
0104                COSRV(I,IJ) = COS(0.104719*FI*FJ)
0105                COSRU(I,IJ) = COS(0.209439*FI*FJ)
0106          61    CONTINUE
0107          60    CONTINUE
0108                DO 202 I = 1,11
0109                DO 201 IJ = 1,11
0110                FI = I-1
0111                FJ = IJ-1
0112                COSRW(I,IJ) = COS(0.314159*FI*FJ)
0113          201   CONTINUE
0114          202   CONTINUE
0115          C     CALCULATE PATTERSON FUNCTION IN VW PLANE FOR BLOCKS OF H
0116                NUI = NUM(NA) + 1
0117                WRITE (3,401) NUI
0118          401   FORMAT (1H4 NUI = MAX I + 1 = ,I5)
0119                DO 902 NU = 1,31
0120                DO 901 NV = 1,31
0121                DO 903 NB = 1,NUI
0122                NC = NUM(NB) + 1
0123                ND = NUM(NB+1)
0124                IF (NC.GT.ND) GO TO 903
0125                DO 904 I = NC,ND
0126                NKI = NK(I) + 1
0127                NLI = NL(I) + 1
0128                FPATT(NV,NW,NB) = FPATT(NV,NW,NB) + FOBSS(I)*COSRV(NKI,NV)
0129              C *COSRW(NLI,NW)
0130          904   CONTINUE
0131          903   CONTINUE
0132          901   CONTINUE
0133          902   CONTINUE
0134                DO 300 NU = 1,11
0135                WRITE(3,307)
0136          307   FORMAT('1',/)
0137                NTH = NU + 1
0138                WRITE(3,306) NTH
```

```
0139          306   FORMAT(1X,13H SECTION J = ,I3)
0140                DO 302 NW = 1,31
0141                DO 700 I = 1,31
0142                IPATT(I) = 0
0143          700   CONTINUE
0144                DO 301 NV = 1,31
0145                DO 302 MB = 1,MJ
0146          C     CALCULATE THE PATTERSON FUNCTION
0147                IPATT(NV) = IPATT(NV) + COSRU(MA,MB)*FPATT(NV,NW,MB)
0148          302   CONTINUE
0149          C     PRINT OUT A GRID REPRESENTING THE AMPLITUDE (*REFERS TO >99)
0150                IPATT(NV) = IFIX((IPATT(NV)*99./FORIG*10.) + 0.5)
0151                LINE(NV) = IBLK
0152                IF(IPATT(NV).GT.99) GO TO 500
0153                IF(IPATT(NV).LT.-99) GO TO 501
0154                GO TO 501
0155          500   IPATT(NV) = 99
0156                LINE(NV) = MAST
0157                GO TO 501
0158          501   IPATT(NV) = -99
0159                LINE(NV) = MAST
0160          301   CONTINUE
0161                WRITE(3,309) (IPATT(I),I = 1,31)
0162          309   FORMAT('0',31I3)
0163                WRITE(3,702) (LINE(I),I = 1,31)
0164          702   FORMAT('+',31A3)
0165          302   CONTINUE
0166          300   CONTINUE
0167          1000  STOP
0168                END

END OF SEGMENT, LENGTH   992, NAME  PATT
```

Program (1) performs the computation of the function

$$P(u,v,w) = \frac{8}{V} \sum_{(hk\ell)} |F(hk\ell)|^2 \cos 2\pi hu \cos 2\pi kv \cos 2\pi \ell w \qquad \text{... Eqn A.1}$$

for all observed reflexions (hkℓ) at discrete chosen values of (u,v,w). The output format is that of a discrete plot of P(u,v,w) over a 31 x 31 array of points (v,w) for 11 fixed values of u, ranging one quarter of the unit cell.

FORTRAN ARRAY VARIABLE	DEFINITION
NH(I) NK(I) NL(I)	Reflexion indices h k ℓ
COSRU(I,J) COSRV(I,J) COSRW(I,J)	Discrete values of the cosine components in Eqn A.1.
UPATT(I) FPATT(I,J,K)	Values of the Patterson function during synthesis.
FSCAT(I)	Scattering factors used in the Sharpening function.
F(I)	Value of scattering factor for given Bragg angle.
NPATT(I)	Integer value of scaled Patterson function.
LINE(I)	Overprint variable of NPATT(I).
NUMH(I)	Number of reflexions of given h index.

LINES	
14 : 19	Set array variable dimensions.
20 21	Input NAST as ** and IBLK as 'blank'.
22 : 26	Input the element (NTYPE), used as the average scattering atom, whose scattering factors are used in the sharpening process, and an average isotropic temperature factor (BETA).
27 : 32	Input unit cell dimensions. (D = a, E = b, C = cA)

33 : 44 Input scattering factors (Int. Tables for X-ray Crystallography Vol. 3.) in order of atom type 1...5.

45 : 48 Set variables FORIG (value of Patterson function at the origin) and counters NUMR (number of input reflexions), MA and NUMH(I).

49 : 51 (hkℓ) and observed $|F_0(hk\ell)|$ read in as variables J,K,L, and FOBS.

52 53 54 If J is given the value of 100, FOBS is the layer scale factor for given data.

55 Test for J = 999 as end of reflexion data flag.

56 : 67 Calculates $d_{hk\ell}$ as DJKL and places hkℓ data in layers of given h provided input is also in layers of given ascending h.

68 : 80 Interpolates the scattering factor data for that value of $(\sin\theta)/\lambda$, for reflexion hkℓ, using a difference formula.

81 : 84 Applies the Sharpening factor (if required) as expressed in Eqn. 1.64.

85 : 91 Applies symmetry reduction factor to $|F_0(hk\ell)|^2$ for h, k or ℓ = 0.

92 Sums contribution by $|F_0(hk\ell)|^2$ at the origin.

93 94 Places Patterson coefficients in array for each reflexion.

95 : 114 Calculates discrete values of cos2πkv and cos2πℓw for arrays of $\left.\begin{matrix}k\\ \ell\end{matrix}\right\}$ 0.....30

and $\left.\begin{matrix}v\\ w\end{matrix}\right\}$ 0.....30

115 : 133 Calculates the Patterson function at discrete values of (v,w) for blocks of fixed h index, excluding u variation.

134 : 139 Print section U = section number heading.

140 : 145 Sets up nested DO LOOPS for output map.

146
:
:
148

Calculates the Patterson function for varying discrete u levels.

149
:
:
:
:
:
:
168

The value of the Patterson function is scaled such that $P'(u,v,w) = \frac{P(u,v,w) \times 99}{P(0,0,0) \times 10}$ and then output as an integer. If $|P'(u,v,w)| > 99$, then $P'(u,v,w)$ is set to 99 and overprinted by **. This scaling was found the most convenient for the problem in hand.

Program (2)

```
0010                MASTER SFED
0011          C
0012          C     P2(1)2(1)2
0013          C
0014                DIMENSION X(100),Y(100),Z(100),FSCAT(100)
0015                DIMENSION AFUD(31,11),BFUD(31,11),APUD(31,11),BPUD(31,11)
0016                DIMENSION A(2000),B(2000)
0017                DIMENSION AZ(2000),BZ(2000),NK(2000)
0018                DIMENSION NL(2000),NPAR(2000),NH(12),NUMH(12)
0019                DIMENSION NTYPE(100),F(5),COSRA(31,61),COSRX(11,21),SINRX(11,21)
0020                DIMENSION SINRA(31,61),NGRID(10600),GRID(11,31)
0021                NUT = 0
0022                DO 110 I = 1,31
0023                DO 115 J = 1,11
0024                AFUD(I,J) = 0
0025                BFUD(I,J) = 0
0026                APUD(I,J) = 0
0027                BPUD(I,J) = 0
0028          115   CONTINUE
0029          110   CONTINUE
0030                SCALE = 1.0
0031                DO 111 I=1,2000
0032                AZ(I)=1
0033                BZ(I)=1
0034                NK(I)=0
0035                NL(I)=0
0036                NPAR(I)=0
0037                A(I)=0
0038                B(I)=0
0039          111   CONTINUE
0040                DO 230 I=1,11
0041                DO 231 J=1,31
0042                GRID(I,J)=0
0043          231   CONTINUE
0044          230   CONTINUE
0045                DO 112 I=1,12
0046                NH(I)=0
0047                NUMH(I)=0
0048          112   CONTINUE
0049                NTYPX=1
0050                I=1
0051                IJ=1
0052                NTOT=200
0053                TOTE=0
0054                TTOD=0
0055                N=1
0056              5 READ(2,101) X(N),Y(N),Z(N),NTYPE(N)
0057                WRITE(3,101) X(N),Y(N),Z(N),NTYPE(N)
0058          101   FORMAT(3F7.4,I5)
0059                L=N
0060                N=N+1
0061                IF(X(L)-2)5,2,2
0062              2 NA=N-2
0063                READ(2,102) D,E,C
0064          102   FORMAT(3F10.5)
0065                ASQ=D*D
0066                BSQ=E*E
0067                CSQ=C*C
0068                DO 3 NH=1,5
0069                READ(2,104)N
0070          104   FORMAT(I3)
0071                FUN=16*N-14
0072                NUT= IFIX(FUN+0.5)
```

```
0073          DO 6 M=1,14
0074          MM = NJT+M
0075          READ(2,103) FSCAT(MM)
0076        6 CONTINUE
0077        3 CONTINUE
0078      103 FORMAT(F10.5)
0079       11 READ(2,105) J,K,L,FOBS
0080      105 FORMAT(3I3,F6.2)
0081          IF(J-100) 7,6,7
0082        6 SCALE=FOBS
0083          GO TO 11
0084        7 IF(J-999) 9,10,9
0085        9 DJKL=SQRT(J*J/ASQ+K*K/BSQ+L*L/CSQ)
0086          STHLAM=0.5*DJKL
0087          RANGE = 0.05
0088          N=1
0089       17 IF(STHLAM-RANGE) 14,14,16
0090       16 RANGE=RANGE+0.05
0091          N=N+1
0092          GO TO 17
0093       14 DO 15 II=1,5
0094          FJ=14*II-14
0095          NJT=IFIX(FJ+0.5)
0096          NN=NJT+N
0097          F(MM)=20*(FSCAT(NN)-FSCAT(NN+1))*(RANGE-STHLAM)+FSCAT(NN+1)
0098       15 CONTINUE
0099          SUMFA=0
0100          SUMFB=0
0101          DO 13 I=1,14
0102          CJKL=(J*J)/A+(J+K)/4.0
0103          CTHKEE=(K*K)/B-(J+K)/4.0
0104          CTHKEE=L*2/C
0105          G=4*COS(6.283186*CTHKE)*COS(6.283186*CTHD)*COS(6.283186*CTHKEE)
0106          H=-4*SIN(6.283186*CTHKE)*SIN(6.283186*CTHD)*SIN(6.283186*CTHKEE)
0107          MM=NTYPE(I)
0108          SUMFA=SUMFA+F(MM)*G)
0109          SUMFB=SUMFB+F(MM)*H)
0110       13 CONTINUE
0111          FINT=SQRT((SUMFA*SUMFA)+(SUMFB*SUMFB))
0112          FINT = FINT/EXP(5.*STHLAM*STHLAM)
0113          FPAR=0.3 * FOBS
0114          IF(FINT-FPAR)11,11,21
0115    C     ACCEPT FOBS IF FINT.GT.0.3*FOBS
0116    C
0117    21    SUMFA = FOBS*SUMFA/FINT
0118          SUMFB=FOBS*SUMFB/FINT
0119          NK(IJ)=K
0120          NL(IJ)=L
0121          JKSUM=J+K
0122          IF(K-NYMX) 28,197,197
0123    197   NYMX = K + 1
0124       28 IF(JKSUM)24,25,26
0125       25 NPAR(IJ)=1
0126          GO TO 27
0127       24 NPAR(IJ)=2
0128          GO TO 27
0129       26 JKSUM=JKSUM-2
0130          GO TO 28
0131       27 NK(IJ)=NK(IJ)+1
0132          NL(IJ)=NL(IJ)+1
0133          IF(J-NINI)31,32,31
0134    31    NM(I) = J + 1
0135          IF(IJ-1)41,42,41
0136       41 NUMM(I-1)=NUM
0137       42 NINI=J
0138          NUM=1
```

```
0139                I=I+1
0140                GO TO 43
0141             32 NUM=NUM+1
0142             43 IF(J) 52,53,52
0143             53 SUMFA=0.5 * SUMFA
0144                SUMFB=0.5 * SUMFB
0145             52 IF(NK(IJ)-1) 54,55,54
0146             55 SUMFA=0.5 * SUMFA
0147                SUMFB=0.5 * SUMFB
0148             54 IF(L)191,192,191
0149            192 SUMFA=0.5*SUMFA
0150                SUMFB=0.5*SUMFB
0151            191 A(IJ)=SUMFA
0152                B(IJ)= SUMFB
0153                IJ=IJ+1
0154                GO TO 11
0155             10 NUMH(I-1) = NUM
0156                IJ = IJ - 1
0157                WRITE (3,301) IJ
0158            301 FORMAT(I6)
0159                NUMI=I-1
0160                DO 66 I=1,51
0161                DO 61 J=1,61
0162                FI=I-1
0163                FJ=J-1
0164                COSRA(I,J)=COS(0.10472 * FI * FJ)
0165                SINRA(I,J) = SIN(0.10472 * FI * FJ)
0166             61 CONTINUE
0167             66 CONTINUE
0168                DO 201 II=1,11
0169                DO 201 JJ=1,21
0170                FI=II-1
0171                FJ=JJ-1
0172                COSRX(II,JJ)=COS(.314159 * FI * FJ)
0173                SINRX(II,JJ) = SIN(.314159 * FI * FJ)
0174            201 CONTINUE
0175            200 CONTINUE
0176                DO 79 NZT=1,51
0177                NZTLF=2*NZT-1
0178                NZZ=NZT-1
0179                WRITE(3,199) NZZ
0180            199 FORMAT(I5)
0181                DO 79 I=1,IJ
0182                NC = NL(I)
0183                AZ(I)=A(I)*COSRA(NC,NZTLF)
0184                BZ(I)=-B(I)*SINRA(NC,NZTLF)
0185         79     CONTINUE
0186                I = 1
0187                DO 31 JK = 1,NUMI
0188                JN = NH(JK)
0189                IS = NUMH(JN)
0190                DO 88 KL = 1,IS
0191                NT = NX(I)
0192                IF(NPAR(I) - 1) 92,92,91
0193         92     APUD(NT,JN) = APUD(NT,JN) + AZ(I)
0194                BPUD(NT,JN) = BPUD(NT,JN) + BZ(I)
0195                GO TO 93
0196         91     AFUD(NT,JN) = AFUD(NT,JN) + AZ(I)
0197                BFUD(NT,JN) = BFUD(NT,JN) + BZ(I)
0198         93     I =I + 1
0199         88     CONTINUE
0200         31     CONTINUE
0201                DO 80 NY = 1,51
0202                NYY = NY - 1
0203                DO 300 JK = 1,NUMI
0204                DO 164 IDD = 1,NYMX
```

```
0205                JN = NI(JF)
0206                TONE=TONE+APUD(IOU,JN)*COSRA(IOU,NY)
0207              C  =BPUD(IOU,JN)*COSRA(IOU,NY)
0208                TTWO=TTWO+BPUD(IOU,JN)*SINRA(IOU,NY)
0209              C =APUD(IOU,JN)*SINRA(IOU,NY)
0210        164   CONTINUE
0211                DO 203 NXX = 1,11
0212                GRID(NXX,NY) = GRID(NXX,NY) + (COSRX(JN,NXX)*TONE)
0213              C + (SINRX(JN,NXX)*TTWO)
0214        203   CONTINUE
0215                TONE = 0
0216                TTWO = 0
0217        300   CONTINUE
0218        80    CONTINUE
0219                DO 500 IOU = 1,NYMX
0220                DO 501 JN = 1,NDIM
0221                JN = NI(JF)
0222                APUD(IOU,JN) = 0
0223                BPUD(IOU,JN) = 0
0224                APUD(IOU,JN) = 0
0225                BPUD(IOU,JN) = 0
0226        501   CONTINUE
0227        500   CONTINUE
0228                DO 83 I=1,11
0229                DO 84 IP=1,31
0230                GRID=(31*NZZ)+YS1*(I-1)+IP
0231                NGRID(IPN)=IFIX(GRID(I,IP)+0.5)
0232           84 CONTINUE
0233           83 CONTINUE
0234                DO 430 I=1,11
0235                DO 431 J=1,31
0236                GRID(I,J) = 0
0237        431   CONTINUE
0238        430   CONTINUE
0239           70 CONTINUE
0240                PX=0
0241                DO 151 I=1,10571
0242                IF(NGRID(I)-PX)152,152,153
0243          153 PX=NGRID(I)
0244          152 CONTINUE
0245          151 CONTINUE
0246                T = 997/PX
0247                DO 154 I=1,10571
0248                NGRID(I)=IFIX(NGRID(I)*T)
0249          154 CONTINUE
0250                DO 211 NI=1,11
0251                NI4=4I-1
0252                WRITE(3,58)
0253           58 FORMAT('1',/)
0254                WRITE(3,114) NTH
0255        114   FORMAT(1X,13H SECTION X = ,I3)
0256                I=961*(NI-1)+1
0257          211 WRITE(3,400) (NGRID(J),J=I,961+I)
0258          400 FORMAT('0',5I13)
0259                STOP
0260                END                                                    END1J500

END OF SEGMENT, LENGTH  1865, NAME  SEED
```

Program (2) performs the computation of the function

$$\rho(X,Y,Z) = \frac{8}{V}\left\{\sum_{\substack{(hk\ell)\\ h+k=2n}} |F(hk\ell)|[\cos 2\pi hX \cos 2\pi kY \cos 2\pi \ell Z \cos\alpha(hk\ell) - \sin 2\pi hX \sin 2\pi kY \sin 2\pi \ell Z \sin\alpha(hk\ell)] - \sum_{\substack{(hk\ell)\\ h+k=2n+1}} |F(hk\ell)|[\sin 2\pi hX \sin 2\pi kY \cos 2\pi \ell Z \cos\alpha(hk\ell) - \cos 2\pi hX \cos 2\pi kY \sin 2\pi \ell Z \sin\alpha(hk\ell)]\right\} \quad \text{... Eqn. A.2}$$

where $\alpha(hk\ell) = \tan^{-1}\left(\frac{B}{A}\right)$ ... Eqn. A.3

where A, B depend upon atom co-ordinates (x,y,z) such that

$$\left.\begin{aligned} A &= 4\cos 2\pi\left(hx + \frac{h+k}{4}\right) \cos 2\pi\left(ky - \frac{h+k}{4}\right) \cos 2\pi \ell z \\ B &= -4\sin 2\pi\left(hx + \frac{h+k}{4}\right) \sin 2\pi\left(ky - \frac{h+k}{4}\right) \sin 2\pi \ell z \end{aligned}\right\} \text{... Eqns. A.4}$$

Intensity symmetry in space group $P2_12_12$ results in

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

and

$$\alpha(hk\ell) = -\alpha(\bar{h}\bar{k}\bar{\ell}) = -\alpha(\bar{h}k\ell) = -\alpha(h\bar{k}\ell) = -\alpha(hk\bar{\ell})$$

$$\forall\ h + k = 2n$$

and

$$\alpha(hk\ell) = -\alpha(\bar{h}\bar{k}\bar{\ell}) = \pi-\alpha(\bar{h}k\ell) = \pi-\alpha(h\bar{k}\ell) = -\alpha(hk\bar{\ell})$$

$$\forall\ h + k = 2n + 1$$

where n is integer.

The output format is that of a discrete plot of $\rho(X,Y,Z)$ over an array of points (Y,Z) for 11 fixed values of X, ranging one quarter of the unit cell.

FORTRAN ARRAY VARIABLE	DEFINITION
X(I)	Input atomic co-ordinates x
Y(I)	y
Z(I)	z
AFUD(I,J)	$= \sum_{hk\ell} \lvert F(hk\ell)\rvert \cos\alpha(hk\ell) \cos 2\pi\ell Z$
BFUD(I,J)	$= \sum_{hk\ell} \lvert F(hk\ell)\rvert \sin\alpha(hk\ell) \sin 2\pi\ell Z$

$h + k = 2n$

APUD(I,J)	$= \sum_{hk\ell} \lvert F(hk\ell)\rvert \cos\alpha(hk\ell) \cos 2\pi\ell Z$
BPUD(I,J)	$= \sum_{hk\ell} \lvert F(hk\ell)\rvert \sin\alpha(hk\ell) \sin 2\pi\ell Z$

$h + k = 2n + 1$

A(I) B(I)	Calculated A for each reflexion. B
AZ(I) BZ(I)	A(I) $\cos 2\pi\ell Z$ B(I) $\sin 2\pi\ell Z$
NH(I) NK(I) NL(I)	Reflexion indices h k ℓ
NPAR(I)	Parity store distinguishing h + k = 2n and h + k = 2n + 1.
NUMH(I)	Number of reflexions of given h.
NTYPE(I)	Atom type number to distinguish scattering factors.
F(I)	Scattering factor store.
COSRX(I,J) SINRX(I,J)	Arrays of discrete values for $\cos 2\pi hX$. $\sin 2\pi hX$
COSRA(I,J) SINRA(I,J)	Arrays of discrete values for $\cos 2\pi kY$, $\cos 2\pi\ell Z$ $\sin 2\pi kY$, $\sin 2\pi\ell Z$
GRID(I,J)	Calculated value of $\rho(X,Y,Z)$ for fixed X
NGRID(I)	Calculated integer value of $\rho(X,Y,Z)$ for complete map.

LINES

14 : 20	Set array variable dimensions.
21 : 48	Set arrays to zero (only needed for previously occupied array storage).
49 : 55	Set values for counters and end of data flag NINI = 999.
56 : 62	Input atom co-ordinates x,y,z and atom type for selection of scattering factors.
63 : 67	Input unit cell dimensions D = a E = b C = cÅ

69 : 78 Input scattering factors in order of atom type N.

79 : 98 Input reflexion data in terms of indices (hkℓ) and observed $|F_0(hk\ell)|$. Layer scale factors are input as F_0 data when h = 100 preceding each layer data batch. Scattering factors are difference interpolated for the calculated $(\sin\theta)/\lambda$ of each reflexion.

99 : 110 Calculation of $\sum_{hk\ell} |F_c(hk\ell)|A$

and $\sum_{hk\ell} |F_c(hk\ell)|B$

111 : 116 Calculated $|F_c(hk\ell)|$ produced and tested for significant contribution to observed $|F_0(hk\ell)|$ using inequality Eqn. 2.8.

117 118 If $|F_0(hk\ell)|$ accepted as Fourier coefficient, it becomes phased on the input atoms.

119 : 141 Parity and counters set for each reflexion.

142 : 150 Symmetry factor reduction of A and B in the case of h, k or ℓ = 0.

151 : 159 A and B stored in arrays A(I), B(I) and the number of accepted reflexions counted and output.

160 : 175 Discrete arrays for spatial cosine and sine variations calculated.

176 : 185 A and B are computed in conjunction with (ℓ,Z) variation within the synthesis i.e. $\cos 2\pi\ell Z \cos\alpha(hk\ell)$ and $\sin 2\pi\ell Z \sin\alpha(hk\ell)$.

186 : 200 Calculation of APUD(I,J), BPUD(I,J);
AFUD(I,J), BFUD(I,J);
as defined in array variable, for given Z.

201 : 210 Calculation of terms including all Y variation in batches of given Z.

211 : 214 GRID(I,J) contains values of the synthesis for X and Y variation for fixed Z.

```
215
 :                    Disused arrays reset to zero for recycling.
227

228                   Values for the completed synthesis are placed
 :                    in NGRID(I) for  Y = 0........30
 :                          for each  X = 0........10
 :                    in turn for each Z = 0........30
233

234                   Scales the maximum value of |ρ(X,Y,Z)|
 :                    to 99.
249

250                   Outputs discrete values of integer ρ(X,Y,Z)
 :                    on 11 levels of X for a 31 x 31 array of
260                   (Y,Z) points.
```

APPENDIX B

LIST OF STRUCTURE FACTORS FOR

(i) SH62 P2(1)2(1)2: 7-chloro-2-methyl-5-phenyl-3-propyl[2,3-b]-imidazolyl quinoline.

(ii) SNP P2(1)/C: Methyl ester of 5,5-dimethyl-2-(2-phenoxymethyl-5-oxo-1,3-oxazolin-4-yli dene)-1,3-thiazolidine-4-carboxylic acid.

(iii) CARF P2(1): Phenyl ester of carbenicillin.

(i)

OBSERVED AND CALCULATED STRUCTURE FACTORS FOR SH62 P2(1)2(1)2

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	3	0	126	120	0	10	0	315	253	6	17	0	107	81	0	2	1	96	49	-6	6	1	120	114
2	3	0	84	77	2	10	0	453	427	0	18	0	64	56	1	2	1	485	520	-5	6	1	81	93
3	3	0	371	356	4	10	0	75	76	1	18	0	475	474	3	2	1	133	118	-4	6	1	266	250
5	3	0	229	217	6	10	0	169	148	2	18	0	255	281	5	2	1	186	168	-3	6	1	186	170
6	3	0	709	783	4	11	0	257	201	3	18	0	118	104	6	2	1	221	222	-2	6	1	198	226
0	4	0	175	66	5	11	0	250	250	6	18	0	93	85	-6	3	1	491	489	-1	6	1	308	320
1	4	0	563	651	6	11	0	194	204	1	19	0	136	135	-4	3	1	479	483	0	6	1	453	414
2	4	0	55	79	0	12	0	324	312	4	19	0	218	251	-3	3	1	324	336	1	6	1	322	304
3	4	0	437	440	1	12	0	235	252	5	19	0	61	57	-2	3	1	359	318	2	6	1	242	197
4	4	0	121	114	3	12	0	380	371	6	19	0	69	60	-1	3	1	487	492	3	6	1	203	162
6	4	0	490	494	5	12	0	136	127	0	20	0	76	75	0	3	1	58	40	4	6	1	258	246
2	5	0	121	97	6	12	0	99	102	1	20	0	317	323	1	3	1	479	493	5	6	1	97	86
3	5	0	102	80	1	13	0	389	393	2	20	0	109	92	2	3	1	361	335	6	6	1	118	116
4	5	0	177	186	2	13	0	86	81	3	20	0	124	109	3	3	1	380	326	-6	7	1	91	92
5	5	0	44	57	4	13	0	87	101	1	21	0	174	177	4	3	1	548	491	-5	7	1	307	303
6	5	0	459	479	5	13	0	97	96	3	21	0	156	157	6	3	1	442	488	-4	7	1	94	108
0	6	0	831	985	6	13	0	230	228	4	21	0	57	54	-6	4	1	384	344	-3	7	1	294	282
2	6	0	113	56	0	14	0	309	273	5	21	0	111	108	-5	4	1	170	171	-2	7	1	165	138
3	6	0	83	53	1	14	0	220	206	0	22	0	140	132	-4	4	1	182	149	-1	7	1	120	119
4	6	0	163	147	2	14	0	112	89	2	22	0	52	45	-3	4	1	197	192	0	7	1	695	683
5	6	0	175	165	3	14	0	145	155	3	22	0	168	192	-2	4	1	285	246	1	7	1	99	131
6	6	0	181	166	4	14	0	164	158	5	22	0	44	37	0	4	1	130	111	2	7	1	165	145
1	7	0	65	70	5	14	0	265	254	1	23	0	141	155	2	4	1	273	255	3	7	1	308	291
2	7	0	247	235	1	15	0	270	260	2	23	0	50	51	3	4	1	215	195	4	7	1	86	108
3	7	0	166	173	2	15	0	121	105	3	23	0	126	50	4	4	1	165	171	5	7	1	309	305
6	7	0	90	96	3	15	0	74	74	0	24	0	65	59	5	4	1	180	166	-6	8	1	152	131
5	7	0	145	143	4	15	0	78	84	2	24	0	58	64	6	4	1	357	345	-5	8	1	305	286
1	8	0	326	308	5	15	0	291	301	3	24	0	97	114	-6	5	1	86	76	-4	8	1	162	179
2	8	0	357	290	0	16	0	534	507	4	24	0	66	61	-4	5	1	164	171	-3	8	1	80	65
4	8	0	35	45	2	16	0	211	205	2	25	0	76	94	-2	5	1	275	274	-2	8	1	249	233
5	8	0	121	101	3	16	0	325	338	6	0	1	71	72	-1	5	1	522	498	-1	8	1	334	328
1	9	0	300	289	4	16	0	95	82	-6	1	1	55	42	0	5	1	166	125	0	8	1	538	491
2	9	0	359	316	5	16	0	253	241	-6	2	1	240	224	1	5	1	505	499	1	8	1	346	313
3	9	0	339	343	1	17	0	129	122	-5	2	1	147	178	2	5	1	296	261	2	8	1	251	237
4	9	0	131	136	2	17	0	198	201	-3	2	1	107	122	4	5	1	175	157	3	8	1	91	59
6	9	0	137	126	3	17	0	220	231	-1	2	1	441	500	6	5	1	60	54	4	8	1	168	168

OBSERVED AND CALCULATED STRUCTURE FACTORS FOR SHO2 P2(1)2(1)2 PAGE 2

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
5	8	1	301	294	-4	12	1	89	87	-4	15	1	243	250	-1	18	1	61	28	1	22	1	57	45
6	8	1	141	131	-3	12	1	91	81	-3	15	1	155	158	0	18	1	216	222	2	22	1	94	90
-5	9	1	91	112	-2	12	1	330	316	-2	15	1	138	135	2	18	1	193	180	5	22	1	118	143
-4	9	1	107	80	-1	12	1	479	472	0	15	1	429	385	3	18	1	81	88	-4	23	1	79	88
-3	9	1	361	357	0	12	1	253	227	2	15	1	144	137	5	18	1	135	133	-2	23	1	48	48
-2	9	1	181	174	1	12	1	491	462	3	15	1	168	153	-5	19	1	88	89	-1	23	1	70	75
-1	9	1	125	116	2	12	1	334	322	4	15	1	244	250	-4	19	1	94	109	0	23	1	155	152
1	9	1	120	116	3	12	1	101	75	5	15	1	202	198	-3	19	1	227	221	1	23	1	62	79
2	9	1	188	169	4	12	1	74	96	6	15	1	128	125	-2	19	1	131	138	4	23	1	76	88
3	9	1	373	359	5	12	1	101	102	-6	16	1	89	77	-1	19	1	184	185	-3	24	1	70	83
4	9	1	101	85	-6	13	1	186	191	-5	16	1	107	111	0	19	1	107	9	-2	24	1	105	124
5	9	1	101	104	-5	13	1	168	157	-4	16	1	165	192	1	19	1	190	178	-1	24	1	97	115
6	9	1	86	69	-4	13	1	154	148	-3	16	1	163	158	2	19	1	136	138	0	24	1	134	17
-5	10	1	101	96	-3	13	1	227	215	-2	16	1	329	336	3	19	1	225	224	1	24	1	101	111
-4	10	1	116	114	-2	13	1	182	212	-1	16	1	224	236	4	19	1	97	105	2	24	1	110	117
-3	10	1	195	171	-1	13	1	181	160	0	16	1	177	168	5	19	1	89	84	3	24	1	67	86
-2	10	1	87	89	0	13	1	234	206	1	16	1	244	222	-5	20	1	109	114	4	24	1	59	72
-1	10	1	563	524	1	13	1	161	169	2	16	1	337	335	-4	20	1	71	78	-3	25	1	39	77
0	10	1	154	126	2	13	1	199	199	3	16	1	168	163	-3	20	1	127	121	-2	25	1	50	56
1	10	1	532	549	3	13	1	227	217	4	16	1	176	179	-2	20	1	63	60	-1	25	1	72	79
2	10	1	92	91	4	13	1	149	145	5	16	1	108	102	-1	20	1	58	48	0	25	1	100	112
3	10	1	201	169	5	13	1	164	160	6	16	1	94	75	1	20	1	67	52	1	25	1	69	85
4	10	1	110	100	6	13	1	189	187	-5	17	1	148	145	2	20	1	65	58	2	25	1	49	60
5	10	1	100	96	-6	14	1	172	176	-4	17	1	79	84	3	20	1	127	124	3	25	1	40	76
-6	11	1	115	107	-5	14	1	93	89	-3	17	1	107	123	4	20	1	65	83	-2	26	1	70	82
-4	11	1	84	74	-4	14	1	217	239	-2	17	1	139	150	5	20	1	115	106	1	0	2	247	266
-3	11	1	206	196	-2	14	1	207	211	-1	17	1	256	236	-5	21	1	150	153	2	0	2	166	148
-2	11	1	140	142	-1	14	1	88	73	0	17	1	390	363	-3	21	1	69	58	3	0	2	118	103
-1	11	1	622	624	0	14	1	117	102	1	17	1	248	248	-1	21	1	104	99	4	0	2	243	255
0	11	1	376	356	1	14	1	87	75	2	17	1	168	162	0	21	1	74	59	5	0	2	45	41
1	11	1	592	615	2	14	1	213	209	3	17	1	124	104	1	21	1	104	98	6	0	2	99	118
2	11	1	138	145	4	14	1	219	235	4	17	1	86	80	3	21	1	142	137	-6	1	2	151	144
3	11	1	208	192	5	14	1	88	88	5	17	1	147	143	-5	22	1	114	139	-5	1	2	97	129
4	11	1	73	82	6	14	1	171	179	-5	18	1	145	130	-2	22	1	93	86	-4	1	2	79	67
6	11	1	128	114	-6	15	1	147	140	-3	18	1	84	89	-1	22	1	59	48	-3	1	2	103	114
-5	12	1	98	108	-5	15	1	201	195	-2	18	1	194	181	0	22	1	58	62	-1	1	2	336	387

OBSERVED AND CALCULATED STRUCTURE FACTORS FOR SHO2 P2(1)2(1)2

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	1	2	353	370	-1	4	2	299	262	-1	7	2	248	262	-2	10	2	313	297	-1	13	2	155	149
2	1	2	639	757	0	4	2	310	283	0	7	2	312	294	-1	10	2	324	298	0	13	2	104	87
3	1	2	117	110	1	4	2	298	274	1	7	2	271	248	0	10	2	390	361	1	13	2	136	162
4	1	2	61	83	2	4	2	248	223	2	7	2	336	287	1	10	2	309	310	2	13	2	67	49
5	1	2	104	121	3	4	2	343	295	3	7	2	264	272	2	10	2	315	311	4	13	2	57	72
6	1	2	126	141	4	4	2	81	112	4	7	2	200	218	3	10	2	73	66	5	13	2	208	204
-6	2	2	93	82	5	4	2	97	87	5	7	2	61	58	4	10	2	214	221	-5	14	2	152	146
-5	2	2	37	33	6	4	2	220	214	-6	8	2	104	99	5	10	2	164	160	-4	14	2	92	76
-4	2	2	324	332	-6	5	2	249	236	-5	8	2	191	165	6	10	2	132	145	-3	14	2	224	201
-3	2	2	338	379	-5	5	2	195	223	-4	8	2	191	171	-6	11	2	158	142	-1	14	2	175	177
-2	2	2	384	361	-4	5	2	58	46	-3	8	2	335	321	-5	11	2	94	80	0	14	2	140	130
-1	2	2	349	373	-3	5	2	282	266	-2	8	2	313	297	-4	11	2	208	185	1	14	2	180	169
0	2	2	516	723	-2	5	2	65	39	-1	8	2	309	303	-3	11	2	279	265	2	14	2	124	36
1	2	2	352	391	-1	5	2	437	483	0	8	2	123	87	-2	11	2	197	178	3	14	2	204	225
2	2	2	383	365	0	5	2	85	55	1	8	2	313	316	-1	11	2	213	205	4	14	2	83	79
3	2	2	375	361	1	5	2	479	463	2	8	2	315	302	0	11	2	131	127	5	14	2	141	143
4	2	2	326	326	2	5	2	65	46	3	8	2	335	306	1	11	2	222	195	-5	15	2	173	151
5	2	2	37	23	3	5	2	299	268	4	8	2	177	169	2	11	2	189	191	-4	15	2	80	79
6	2	2	95	98	4	5	2	53	53	5	8	2	176	175	3	11	2	300	262	-3	15	2	64	65
-6	3	2	71	48	5	5	2	205	217	-6	8	2	109	105	4	11	2	187	204	-2	15	2	154	151
-5	3	2	173	192	6	5	2	221	233	-6	9	2	97	93	5	11	2	83	84	-1	15	2	167	151
-4	3	2	321	299	-6	6	2	74	62	-5	9	2	135	139	6	11	2	157	147	0	15	2	132	112
-3	3	2	129	133	-5	6	2	146	153	-4	9	2	142	136	-6	12	2	100	101	1	15	2	151	163
-2	3	2	254	230	-4	6	2	229	244	-3	9	2	173	144	-5	12	2	128	123	2	15	2	158	153
-1	3	2	417	445	-3	6	2	255	244	-2	9	2	233	103	-3	12	2	252	246	4	15	2	77	76
0	3	2	28	29	-2	6	2	91	65	-1	9	2	225	234	-2	12	2	142	127	5	15	2	150	164
1	3	2	418	443	-1	6	2	440	432	0	9	2	308	250	-1	12	2	166	182	-4	16	2	131	115
2	3	2	220	239	0	6	2	464	444	1	9	2	236	226	0	12	2	143	130	-3	16	2	211	233
3	3	2	136	143	1	6	2	441	445	2	9	2	227	209	1	12	2	189	165	-2	16	2	86	97
4	3	2	294	301	2	6	2	101	84	3	9	2	164	158	2	12	2	141	137	-1	16	2	102	82
5	3	2	182	139	3	6	2	251	257	4	9	2	130	145	3	12	2	267	235	0	16	2	171	156
-6	4	2	255	212	4	6	2	243	234	5	9	2	133	138	5	12	2	121	126	1	16	2	95	92
-5	4	2	93	94	5	6	2	158	147	6	9	2	105	94	6	12	2	111	112	2	16	2	93	89
-4	4	2	97	101	-4	7	2	228	212	-6	10	2	129	135	-5	13	2	204	210	3	16	2	219	232
-3	4	2	292	310	-3	7	2	261	263	-5	10	2	167	159	-4	13	2	56	62	4	16	2	131	112
-2	4	2	234	227	-2	7	2	319	304	-4	10	2	234	206	-2	13	2	53	58	-3	17	2	100	109

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	17	2	209	210	-2	21	2	55	63	-5	1	3	97	83	-6	4	3	148	57	6	6	3	97	97
-1	17	2	192	186	-1	21	2	78	77	-4	1	3	61	78	-5	4	3	51	50	-5	7	3	155	134
0	17	2	61	52	1	21	2	80	73	-3	1	3	313	329	-4	4	3	344	330	-4	7	3	188	184
1	17	2	189	188	2	21	2	61	59	-2	1	3	444	426	-3	4	3	161	165	-3	7	3	109	115
2	17	2	219	206	3	21	2	97	107	-1	1	3	86	58	-2	4	3	68	84	-2	7	3	454	450
3	17	2	110	102	4	21	2	106	119	0	1	3	72	90	-1	4	3	103	93	-1	7	3	277	266
4	17	2	111	46	-4	22	2	89	12	1	1	3	84	69	0	4	3	194	166	0	7	3	345	376
5	17	2	73	62	-3	22	2	96	102	2	1	3	451	433	1	4	3	105	92	1	7	3	269	265
-4	18	2	135	134	-2	22	2	73	68	3	1	3	328	339	2	4	3	70	85	3	7	3	115	116
-3	18	2	115	121	-1	22	2	72	75	4	1	3	67	69	3	4	3	188	153	4	7	3	190	171
-2	18	2	106	83	1	22	2	73	71	5	1	3	101	85	4	4	3	314	329	5	7	3	135	137
-1	18	2	186	177	2	22	2	69	72	6	1	3	129	124	5	4	3	56	55	-5	8	3	239	242
0	18	2	269	239	3	22	2	85	106	-5	2	3	100	83	-6	5	3	169	155	-4	8	3	64	72
1	18	2	191	168	-4	23	2	61	77	-4	2	3	231	221	-5	5	3	85	108	-3	8	3	293	318
2	18	2	91	94	-1	23	2	84	67	-3	2	3	254	275	-4	5	3	165	159	-2	8	3	328	327
3	18	2	127	115	0	23	2	70	62	-2	2	3	262	267	-3	5	3	134	119	-1	8	3	173	170
4	18	2	118	140	1	23	2	71	72	-1	2	3	290	266	-2	5	3	209	203	0	8	3	652	717
-5	19	2	54	71	4	23	2	68	74	0	2	3	278	290	-1	5	3	555	544	1	8	3	161	181
-4	19	2	74	71	-3	24	2	66	81	1	2	3	268	281	0	5	3	389	386	2	8	3	360	312
-3	19	2	87	91	-2	24	2	62	73	2	2	3	264	257	1	5	3	338	359	3	8	3	324	304
-2	19	2	147	131	-1	24	2	75	74	3	2	3	275	267	2	5	3	225	208	4	8	3	81	61
-1	19	2	171	167	1	24	2	65	80	4	2	3	215	232	3	5	3	133	124	5	8	3	267	251
1	19	2	168	168	2	24	2	65	67	5	2	3	82	98	4	5	3	141	169	-6	9	3	131	125
2	19	2	142	142	3	24	2	64	77	-6	3	3	171	151	5	5	3	98	103	-4	9	3	126	126
3	19	2	87	63	-2	25	2	56	66	-5	3	3	137	148	6	5	3	141	142	-3	9	3	382	363
4	19	2	78	78	-1	25	2	91	107	-4	3	3	96	102	-6	6	3	120	101	-2	9	3	292	274
5	19	2	64	73	0	25	2	57	64	-3	3	3	142	101	-4	6	3	236	251	-1	9	3	163	164
-5	20	2	91	102	1	25	2	94	104	-2	3	3	238	238	-3	6	3	319	306	0	9	3	56	70
-2	20	2	105	111	2	25	2	62	67	-1	3	3	409	453	-2	6	3	395	385	1	9	3	187	147
-1	20	2	114	116	1	0	3	111	120	0	3	3	217	229	-1	6	3	288	278	2	9	3	307	276
0	20	2	124	122	2	0	3	129	80	1	3	3	412	437	0	6	3	61	35	3	9	3	398	376
1	20	2	111	114	3	0	3	391	408	2	3	3	248	262	1	6	3	296	275	4	9	3	140	114
2	20	2	108	111	4	0	3	141	141	3	3	3	144	110	2	6	3	416	389	6	9	3	120	122
3	20	2	99	97	5	0	3	248	290	4	3	3	103	92	3	6	3	345	319	-5	10	3	85	88
-4	21	2	109	116	6	0	3	103	93	5	3	3	142	148	4	6	3	243	233	-4	10	3	236	217
-3	21	2	100	107	-6	1	3	130	122	6	3	3	132	132	5	6	3	69	34	-3	10	3	166	161

OBSERVED AND CALCULATED STRUCTURE FACTORS FOR SH62 P2(1)2(1)?

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	10	3	254	242	3	13	3	189	179	-4	17	3	119	123	-3	22	3	77	87	-5	2	4	86	86
-1	10	3	396	355	5	13	3	87	78	-3	17	3	152	159	-2	22	3	84	86	-4	2	4	138	137
0	10	3	64	50	0	13	3	154	154	-2	17	3	130	130	-1	22	3	48	42	-3	2	4	101	111
1	10	3	379	376	-5	14	3	82	75	0	17	3	71	58	1	22	3	53	33	-2	2	4	126	125
2	10	3	266	240	-4	14	3	139	100	2	17	3	132	131	2	22	3	87	83	-1	2	4	203	207
3	10	3	174	160	-3	14	3	202	187	3	17	3	160	152	3	22	3	67	95	0	2	4	87	95
4	10	3	206	221	-2	14	3	210	219	4	17	3	110	121	-1	23	3	76	67	1	2	4	223	187
5	10	3	87	93	-1	14	3	105	104	-5	18	3	83	91	0	23	3	108	99	2	2	4	118	130
-5	11	3	77	69	0	14	3	225	218	-4	18	3	100	110	1	23	3	67	75	3	2	4	102	115
-4	11	3	195	182	1	14	3	113	97	-2	18	3	112	109	2	23	3	99	63	4	2	4	136	137
-3	11	3	71	40	2	14	3	229	218	-1	18	3	148	137	4	23	3	41	35	5	2	4	89	80
-2	11	3	60	82	3	14	3	194	202	0	18	3	52	52	-3	24	3	58	75	-6	3	4	183	178
-1	11	3	332	329	4	14	3	175	147	1	18	3	146	134	-2	24	3	72	78	-5	3	4	60	66
0	11	3	33	20	-6	15	3	91	79	2	18	3	103	112	2	24	3	73	76	-4	3	4	226	181
1	11	3	347	319	-5	15	3	131	114	4	18	3	113	109	3	24	3	58	72	-3	3	4	74	95
2	11	3	84	66	-4	15	3	86	95	5	18	3	85	85	-2	25	3	40	40	-2	3	4	104	106
4	11	3	188	182	-2	15	3	214	250	-5	19	3	66	70	-1	25	3	55	74	-1	3	4	142	147
5	11	3	75	67	-1	15	3	146	119	-4	19	3	96	102	1	25	3	61	66	0	3	4	260	288
-6	12	3	119	103	0	15	3	197	182	-3	19	3	70	83	0	0	4	527	604	1	3	4	163	125
-4	12	3	189	205	1	15	3	126	131	-1	19	3	106	107	1	0	4	288	287	2	3	4	120	96
-3	12	3	158	167	2	15	3	240	241	0	19	3	86	70	2	0	4	76	73	3	3	4	86	89
-2	12	3	61	64	4	15	3	100	86	1	19	3	109	111	3	0	4	186	216	4	3	4	202	196
-1	12	3	255	253	5	15	3	116	123	3	19	3	78	81	4	0	4	160	151	5	3	4	71	63
1	12	3	261	247	-6	15	3	89	80	4	19	3	99	101	-6	1	4	78	73	6	3	4	187	176
2	12	3	53	75	-5	16	3	99	114	5	19	3	75	68	-5	1	4	78	93	-6	4	4	192	168
3	12	3	180	153	-4	16	3	108	117	-2	20	3	60	59	-4	1	4	140	154	-5	4	4	126	131
4	12	3	203	192	-3	16	3	68	62	-1	20	3	91	101	-3	1	4	131	159	-4	4	4	114	96
6	12	3	113	100	-2	16	3	117	121	0	20	3	82	80	-2	1	4	400	400	-3	4	4	204	217
-6	13	3	176	164	-1	16	3	88	97	1	20	3	96	98	-1	1	4	317	309	-2	4	4	116	105
-5	13	3	91	80	0	16	3	262	239	2	20	3	68	51	0	1	4	65	28	-1	4	4	180	154
-3	13	3	174	184	1	16	3	97	83	4	20	3	64	48	1	1	4	304	317	0	4	4	54	24
-2	13	3	134	136	2	16	3	151	112	-2	21	3	56	55	2	1	4	385	410	1	4	4	172	167
-1	13	3	139	134	3	16	3	74	61	-1	21	3	62	61	3	1	4	152	145	2	4	4	98	125
0	13	3	256	240	4	16	3	130	115	1	21	3	56	63	4	1	4	138	139	3	4	4	231	200
1	13	3	130	143	5	16	3	97	110	2	21	3	60	55	5	1	4	74	99	4	4	4	107	103
2	13	3	151	125	-6	17	3	73	100	4	21	3	68	56	6	1	4	80	71	5	4	4	131	131

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
6	4	4	177	173	-6	8	4	102	105	-4	11	4	244	242	-4	14	4	191	193	1	17	4	157	163
-6	5	4	170	176	-5	8	4	88	76	-3	11	4	221	234	-3	14	4	164	161	2	17	4	139	141
-5	5	4	107	118	-4	8	4	139	115	-2	11	4	124	140	-2	14	4	81	102	3	17	4	81	69
-3	5	4	62	75	-3	8	4	88	104	0	11	4	225	213	-1	14	4	78	96	4	17	4	70	71
-2	5	4	227	223	-2	8	4	198	196	1	11	4	47	49	0	14	4	140	146	5	17	4	78	88
-1	5	4	264	277	-1	8	4	292	305	2	11	4	143	123	1	14	4	84	87	-2	18	4	63	48
0	5	4	237	204	0	8	4	364	346	3	11	4	217	233	2	14	4	97	85	-1	18	4	121	124
1	5	4	269	260	1	8	4	316	298	4	11	4	235	256	3	14	4	153	173	0	18	4	125	99
2	5	4	236	222	2	8	4	202	205	6	11	4	162	159	4	14	4	191	195	1	18	4	131	113
3	5	4	69	77	3	8	4	98	97	-6	12	4	98	98	5	14	4	108	98	2	18	4	39	52
5	5	4	121	109	4	8	4	128	127	-5	12	4	210	200	6	14	4	138	143	-5	19	4	61	53
6	5	4	160	132	5	8	4	86	74	-4	12	4	134	134	-5	15	4	120	113	-4	19	4	121	116
-6	6	4	138	127	6	8	4	91	95	-3	12	4	92	113	-4	15	4	96	97	-3	19	4	129	155
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-1	6	4	221	198	-1	9	4	317	313	3	12	4	106	102	3	15	4	76	67	4	19	4	108	123
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1	6	4	202	212	1	9	4	329	317	5	12	4	205	201	5	15	4	111	111	-2	20	4	92	88
2	6	4	131	112	2	9	4	267	280	6	12	4	106	106	-4	16	4	143	146	-1	20	4	76	76
3	6	4	198	203	3	9	4	125	112	-6	13	4	116	119	-3	16	4	83	82	1	20	4	67	81
4	6	4	272	233	4	9	4	57	55	-5	13	4	78	59	-2	16	4	254	284	2	20	4	91	91
5	6	4	198	168	5	9	4	102	100	-4	13	4	100	95	-1	16	4	105	101	3	20	4	65	73
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3	22	4	55	78	3	2	5	132	129	1	5	5	335	324	-6	9	5	107	106	1	12	5	130	134
4	22	4	53	62	4	2	5	327	331	2	5	5	142	144	-5	9	5	77	69	2	12	5	146	147
-4	23	4	52	66	5	2	5	188	206	3	5	5	181	165	-4	9	5	129	137	3	12	5	135	130
-2	23	4	60	62	-6	3	5	226	230	4	5	5	127	135	-3	9	5	82	90	4	12	5	55	63
-1	23	4	68	73	-5	3	5	171	169	5	5	5	86	88	-2	9	5	208	211	5	12	5	82	71
0	23	4	145	175	-4	3	5	133	142	6	5	5	175	176	-1	9	5	129	113	-6	13	5	131	127
1	23	4	76	69	-3	3	5	260	256	-6	6	5	104	101	1	9	5	135	116	-5	13	5	118	102
2	23	4	57	57	-2	3	5	117	91	-5	6	5	89	103	2	9	5	219	208	-4	13	5	66	57
-2	24	4	116	142	-1	3	5	176	170	-4	6	5	69	74	3	9	5	88	78	-3	13	5	129	140
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1	24	4	66	70	1	3	5	157	191	-1	6	5	254	232	5	9	5	72	72	-1	13	5	208	208
2	24	4	127	142	2	3	5	118	99	0	6	5	122	94	6	9	5	101	106	0	13	5	85	65
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0	2	5	252	213	-2	5	5	141	136	3	8	5	190	194	-2	12	5	148	136	2	15	5	189	196
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5	15	5	142	159	3	19	5	100	123	0	1	6	104	72	-1	4	6	221	196	0	7	6	174	174
-5	16	5	72	76	4	19	5	103	98	2	1	6	218	208	0	4	6	233	194	1	7	6	76	95
-4	16	5	108	111	5	19	5	103	101	3	1	6	262	242	1	4	6	220	193	2	7	6	47	45
-3	16	5	206	209	-5	20	5	56	63	4	1	6	201	200	2	4	6	241	251	3	7	6	211	221
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0	16	5	288	280	0	20	5	71	65	-5	2	6	124	135	5	4	6	92	89	6	7	6	132	119
1	16	5	218	212	1	20	5	109	117	-4	2	6	153	151	6	4	6	127	110	-6	8	6	121	129
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3	16	5	194	214	5	20	5	54	66	-2	2	6	226	198	-4	5	6	142	152	-2	8	6	145	164
4	16	5	114	104	-3	21	5	56	69	-1	2	6	106	108	-3	5	6	120	126	-1	8	6	151	145
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-4	17	5	101	107	0	21	5	59	57	1	2	6	120	96	-1	5	6	275	277	1	8	6	150	150
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1	17	5	88	103	-2	22	5	64	63	5	2	6	130	124	3	5	6	129	124	-6	9	6	109	104
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1	18	5	181	203	-2	24	5	34	37	0	3	6	370	383	-1	6	6	220	225	4	9	6	72	76
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-1	19	5	109	105	-3	1	6	243	261	-4	4	6	94	81	-4	7	6	104	118	3	10	6	93	86
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-3	11	6	80	62	6	14	6	98	89	4	18	6	45	53	4	1	7	68	77	6	4	7	110	105
-2	11	6	132	150	-6	15	6	93	105	-5	19	6	58	71	5	1	7	75	72	-6	5	7	168	169
-1	11	6	234	234	-5	15	6	76	76	-4	19	6	52	49	-5	2	7	79	82	-5	5	7	103	109
1	11	6	244	230	-4	15	6	95	88	-3	19	6	134	160	-4	2	7	145	137	-4	5	7	183	187
2	11	6	132	146	-3	15	6	158	166	-2	19	6	59	59	-3	2	7	236	236	-3	5	7	194	194
5	11	6	75	78	-2	15	6	79	80	2	19	6	62	58	-2	2	7	179	192	-2	5	7	130	129
6	11	6	133	116	-1	15	6	139	131	3	19	6	138	154	-1	2	7	125	92	-1	5	7	158	140
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1	7	7	205	196	3	10	7	106	96	-5	14	7	96	96	-1	18	7	73	83	-1	2	8	141	148
2	7	7	287	288	4	10	7	284	292	-4	14	7	74	70	0	18	7	108	90	0	2	8	48	43
3	7	7	93	99	5	10	7	175	181	-3	14	7	151	138	1	18	7	74	83	1	2	8	156	138
4	7	7	166	181	6	10	7	82	80	-2	14	7	57	43	4	18	7	117	121	2	2	8	225	229
5	7	7	121	114	-6	11	7	93	119	-1	14	7	139	140	5	18	7	47	55	3	2	8	135	129
6	7	7	104	103	-5	11	7	85	74	0	14	7	107	97	-4	19	7	47	56	4	2	8	180	185
-5	8	7	202	199	-4	11	7	169	155	1	14	7	144	136	-2	19	7	66	57	5	2	8	72	70
-4	8	7	151	161	-3	11	7	228	244	3	14	7	135	153	2	19	7	55	65	-6	3	8	136	123
-3	8	7	346	390	-1	11	7	243	244	6	14	7	79	69	4	19	7	52	58	-4	3	8	187	163
-2	8	7	99	108	1	11	7	247	236	5	14	7	94	93	-2	20	7	71	81	-3	3	8	80	83
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0	8	7	69	77	4	11	7	161	163	-3	15	7	79	100	2	20	7	74	80	-1	3	8	89	82
1	8	7	179	186	5	11	7	85	80	-2	15	7	116	110	-2	21	7	74	78	0	3	8	154	132
2	8	7	100	116	6	11	7	131	121	2	15	7	115	108	-1	21	7	50	50	1	3	8	87	85
3	8	7	387	383	-6	12	7	114	116	3	15	7	84	99	1	21	7	51	68	2	3	8	50	38
4	8	7	159	161	-5	12	7	85	72	5	15	7	128	134	2	21	7	71	77	3	3	8	87	75
5	8	7	198	201	-4	12	7	182	178	-5	16	7	78	86	0	0	8	212	197	4	3	8	143	136
-6	9	7	118	136	-3	12	7	102	120	-4	16	7	92	90	1	0	8	133	138	6	3	8	139	129
-3	9	7	202	223	-2	12	7	156	166	-3	16	7	136	149	2	0	8	190	176	-6	4	8	106	106
-2	9	7	279	278	0	12	7	67	76	-1	16	7	81	74	3	0	8	129	121	-5	4	8	78	82
-1	9	7	229	246	2	12	7	160	163	0	16	7	134	122	4	0	8	89	104	-4	4	8	304	305
0	9	7	325	308	3	12	7	115	108	1	16	7	77	77	-5	1	8	107	107	-3	4	8	131	30
1	9	7	233	243	4	12	7	183	177	2	16	7	58	41	-4	1	8	86	71	-2	4	8	118	138
2	9	7	282	292	5	12	7	85	74	3	16	7	136	144	-3	1	8	106	121	-1	4	8	70	66
3	9	7	214	223	6	12	7	139	122	4	16	7	108	84	-2	1	8	202	204	1	4	8	62	67
4	9	7	39	31	-6	13	7	98	110	5	16	7	77	89	-1	1	8	146	143	2	4	8	136	126
6	9	7	131	136	-5	13	7	164	153	-2	17	7	132	136	0	1	8	76	46	4	4	8	275	308
-6	10	7	87	86	-3	13	7	190	194	-1	17	7	79	74	1	1	8	144	148	5	4	8	88	79
-5	10	7	178	180	-2	13	7	104	112	0	17	7	57	43	2	1	8	193	207	6	4	8	116	109
-4	10	7	283	283	-1	13	7	136	120	1	17	7	74	77	3	1	8	112	117	-6	5	8	167	174
-3	10	7	103	100	0	13	7	50	49	2	17	7	136	131	4	1	8	74	74	-5	5	8	100	86
-2	10	7	232	238	1	13	7	120	134	4	17	7	63	43	5	1	8	109	111	-4	5	8	151	155
-1	10	7	116	118	2	13	7	111	108	5	17	7	55	51	-6	2	8	166	62	-3	5	8	67	77
0	10	7	201	182	3	13	7	186	194	-5	18	7	47	56	-4	2	8	185	181	-2	5	8	245	260
1	10	7	114	122	5	13	7	130	143	-4	18	7	118	120	-3	2	8	131	130	-1	5	8	72	83

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	5	8	52	51	0	8	8	368	358	4	11	8	174	185	0	16	8	45	37	3	2	9	179	198
1	5	8	75	84	1	8	8	200	193	3	11	8	78	91	2	16	8	58	49	5	2	9	62	60
2	5	8	247	263	2	8	8	355	378	-5	12	8	106	95	4	16	8	60	58	6	2	9	109	104
3	5	8	194	78	3	8	8	82	83	-4	12	8	177	173	5	16	8	72	78	-6	3	9	40	58
4	5	8	157	162	4	8	8	155	147	-3	12	8	112	111	-4	17	8	52	52	-4	3	9	55	26
5	5	8	100	87	-6	9	8	77	79	-2	12	8	110	119	-3	17	8	82	86	-3	3	9	212	193
6	5	8	169	168	-4	9	8	187	189	-1	12	8	138	143	3	17	8	78	87	-1	3	9	226	228
-6	6	8	132	151	-3	9	8	168	165	1	12	8	133	135	4	17	8	46	50	1	3	9	218	237
-5	6	8	138	154	-2	9	8	86	76	2	12	8	111	117	-2	18	8	87	86	3	3	9	211	194
-4	6	8	189	183	-1	9	8	119	118	3	12	8	118	110	-1	18	8	113	120	6	3	9	30	38
-3	6	8	259	284	0	9	8	190	174	4	12	8	171	170	1	18	8	119	121	-6	4	9	181	212
-2	6	8	303	298	1	9	8	130	113	5	12	8	106	100	2	18	8	84	85	-5	4	9	75	86
-1	6	8	134	129	2	9	8	88	75	-4	13	8	70	54	-3	19	8	49	67	-3	4	9	152	136
0	6	8	136	134	3	9	8	173	171	-3	13	8	97	102	-2	19	8	72	70	-1	4	9	51	40
1	6	8	131	125	4	9	8	180	189	-2	13	8	117	122	-1	19	8	59	55	3	4	9	148	145
2	6	8	286	309	5	9	8	68	58	0	13	8	59	36	0	19	8	60	48	5	4	9	88	88
3	6	8	273	235	-5	10	8	88	86	2	13	8	114	124	1	19	8	60	57	6	4	9	203	213
4	6	8	185	176	-4	10	8	189	165	3	13	8	101	98	2	19	8	68	71	-5	5	9	164	186
5	6	8	144	149	-3	10	8	164	164	4	13	8	48	61	-1	20	8	48	49	-4	5	9	134	149
6	6	8	162	169	-2	10	8	159	178	-3	14	8	102	97	1	20	8	63	69	-3	5	9	209	225
-5	7	8	158	137	-1	10	8	231	212	-3	14	8	130	137	1	0	9	52	28	-1	5	9	91	106
-4	7	8	116	135	0	10	8	237	235	-2	14	8	128	124	2	0	9	87	98	0	5	9	146	143
-3	7	8	167	198	1	10	8	227	223	-1	14	8	62	60	3	0	9	326	337	1	5	9	100	96
-2	7	8	173	187	2	10	8	161	173	1	14	8	64	60	5	0	9	177	190	3	5	9	226	224
-1	7	8	248	259	3	10	8	162	166	2	14	8	119	132	-4	1	9	65	70	4	5	9	149	147
0	7	8	147	149	4	10	8	171	167	3	14	8	126	138	-3	1	9	117	127	5	5	9	193	193
1	7	8	255	259	5	10	8	89	84	5	14	8	103	97	-1	1	9	94	91	6	5	9	77	82
2	7	8	175	182	-5	11	8	85	91	-5	15	8	57	62	0	1	9	193	188	-6	6	9	84	99
3	7	8	192	188	-4	11	8	190	189	-4	15	8	71	66	1	1	9	103	87	-4	6	9	115	111
4	7	8	123	129	-3	11	8	87	77	0	15	8	67	65	3	1	9	123	127	-3	6	9	128	141
5	7	8	154	138	-2	11	8	144	170	1	15	8	54	55	4	1	9	71	72	0	6	9	102	77
6	7	8	83	83	-1	11	8	92	90	4	15	8	65	72	6	1	9	82	79	3	6	9	145	132
-4	8	8	155	144	0	11	8	43	59	5	15	8	60	63	-3	2	9	184	191	4	6	9	118	110
-3	8	8	86	85	1	11	8	88	94	-3	16	8	72	81	-1	2	9	91	100	5	6	9	61	58
-2	8	8	369	384	2	11	8	153	168	-4	16	8	73	56	0	2	9	59	62	6	6	9	93	102
-1	8	8	188	200	3	11	8	76	88	-2	16	8	38	31	1	2	9	90	99	-5	7	9	146	143

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
-4	7	9	114	100	1	10	9	157	147	-2	10	9	58	81	1	6	10	140	135	0	12	10	55	52
-3	7	9	105	112	2	10	9	78	76	2	10	9	50	76	3	6	10	97	109	1	12	10	79	78
-2	7	9	170	176	3	10	9	108	117	3	0	10	63	69	4	6	10	74	84	2	12	10	59	64
-1	7	9	104	93	4	10	9	85	83	-5	1	10	118	118	5	6	10	104	99	4	12	10	60	59
0	7	9	319	316	5	10	9	80	70	-3	1	10	99	101	-5	7	10	74	73	-4	13	10	40	43
1	7	9	91	101	-5	11	9	62	47	-2	1	10	110	114	-4	7	10	100	42	4	13	10	44	42
2	7	9	180	172	-4	11	9	101	88	-1	1	10	57	76	-3	7	10	82	74	-2	14	10	63	58
3	7	9	103	113	-2	11	9	120	144	0	1	10	167	174	-2	7	10	82	92	-1	14	10	63	62
4	7	9	96	105	0	11	9	102	110	1	1	10	74	80	0	7	10	74	87	0	14	10	101	76
5	7	9	144	139	2	11	9	122	135	2	1	10	108	115	2	7	10	78	94	1	14	10	58	60
-5	8	9	63	71	4	11	9	108	90	3	1	10	104	97	3	7	10	87	71	2	14	10	66	52
-4	8	9	86	76	5	11	9	48	51	5	1	10	106	119	5	7	10	75	72	0	15	10	66	62
-3	8	9	123	123	-5	12	9	72	87	-5	2	10	63	52	-5	8	10	99	85	-2	16	10	67	64
-2	8	9	52	31	-3	12	9	64	63	-4	2	10	75	69	-3	8	10	64	66	2	16	10	65	69
-1	8	9	68	70	0	12	9	48	38	-3	2	10	65	65	-1	8	10	110	108	5	0	11	98	108
0	8	9	226	197	3	12	9	61	70	-2	2	10	97	96	0	8	10	132	40	-3	1	11	55	71
1	8	9	70	68	5	12	9	89	87	0	2	10	105	103	1	8	10	116	102	-2	1	11	62	61
2	8	9	56	28	-4	13	9	57	71	1	2	10	172	156	3	8	10	99	85	0	1	11	46	21
3	8	9	121	125	-2	13	9	100	102	2	2	10	96	97	-5	9	10	66	59	2	1	11	58	66
4	8	9	70	81	-1	13	9	58	54	3	2	10	75	63	-1	9	10	55	36	3	1	11	66	73
5	8	9	69	68	0	13	9	79	81	5	2	10	50	52	1	9	10	54	39	-4	2	11	70	74
-4	9	9	83	94	1	13	9	59	56	-4	3	10	84	88	4	9	10	56	66	-3	2	11	60	69
-3	9	9	131	130	2	13	9	96	105	-1	3	10	177	204	5	9	10	66	61	-2	2	11	43	46
-2	9	9	184	191	-3	14	9	28	100	0	3	10	96	79	-4	10	10	52	63	-1	2	11	66	84
-1	9	9	150	150	-2	14	9	86	85	1	3	10	196	200	-1	10	10	56	33	3	2	11	57	72
0	9	9	179	188	2	14	9	85	86	4	3	10	75	85	0	10	10	145	37	4	2	11	64	77
1	9	9	149	151	3	14	9	83	92	-3	4	10	105	113	1	10	10	56	31	-3	3	11	195	21
2	9	9	176	199	-1	16	9	45	54	-2	4	10	76	78	4	10	10	59	63	-1	3	11	102	100
3	9	9	135	127	1	16	9	52	50	2	4	10	82	74	-1	11	10	75	73	-3	4	11	79	83
4	9	9	92	88	-2	17	9	49	49	3	4	10	106	112	0	11	10	86	98	3	4	11	71	84
-5	10	9	83	72	2	17	9	50	50	5	4	10	53	37	1	11	10	79	71	5	4	11	51	62
-4	10	9	90	82	-2	18	9	46	51	1	5	10	102	93	3	11	10	55	67	-1	5	11	94	85
-3	10	9	112	115	-1	18	9	64	56	-5	6	10	105	101	-4	12	10	52	62	1	5	11	83	81
-2	10	9	81	75	0	18	9	87	81	-4	6	10	72	87	-3	12	10	50	57	-1	6	11	81	66
-1	10	9	152	150	1	18	9	53	59	-3	6	10	109	109	-2	12	10	61	62	1	6	11	70	68
0	10	9	53	50	2	18	9	48	48	-1	6	10	143	134	-1	12	10	81	74	3	6	11	58	56

OBSERVED AND CALCULATED STRUCTURE FACTORS FOR SH62 P2(1)2(1)2

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
-4	7	11	42	55	-2	11	11	48	40	0	2	12	37	29	-3	6	12	51	51	0	8	12	166	157
4	7	11	47	56	-1	13	11	60	64	2	2	12	74	58	-2	6	12	103	92	2	8	12	90	92
-4	8	11	41	30	1	13	11	68	61	4	2	12	37	43	-1	6	12	63	48	-2	9	12	82	81
-2	8	11	71	60	-2	14	11	44	41	-2	3	12	55	48	1	6	12	62	47	-1	9	12	49	46
-1	8	11	62	57	0	14	11	100	42	-1	3	12	53	62	2	6	12	95	93	1	9	12	54	45
1	8	11	67	60	2	14	11	41	41	1	3	12	57	59	3	6	12	40	54	2	9	12	76	83
2	8	11	70	61	0	0	12	180	170	2	3	12	51	46	-2	7	12	91	81	2	1	13	36	40
-2	9	11	53	56	-3	1	12	45	50	-2	5	12	50	39	0	7	12	117	78	-2	2	13	35	47
0	9	11	59	59	2	1	12	83	76	-1	5	12	56	41	2	7	12	86	79	2	2	13	41	46
2	9	11	49	60	4	1	12	46	43	1	5	12	57	44	-2	8	12	92	94	-1	4	13	104	25
0	10	11	55	31	-2	2	12	78	60	2	5	12	51	40										

(ii)

OBSERVED AND CALCULATED STRUCTURE FACTORS FOR SNP P2(1)/C

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	0	0	238	-256	7	4	0	111	-95	6	9	0	100	94	0	18	0	70	-82	-7	3	1	61	-71
3	0	0	361	401	9	4	0	102	-81	7	9	0	172	171	1	18	0	70	-69	-5	3	1	182	162
4	0	0	151	113	10	4	0	144	-128	9	9	0	70	56	3	18	0	69	-112	-4	3	1	475	-455
6	0	0	391	309	1	5	0	768	-786	10	9	0	119	106	-11	1	1	39	-50	-3	3	1	409	-415
7	0	0	66	79	2	5	0	249	-231	0	10	0	353	376	-9	1	1	136	126	-2	3	1	302	307
9	0	0	114	97	6	5	0	115	112	1	10	0	87	82	-7	1	1	136	-110	-1	3	1	1043	1018
1	1	0	183	-216	7	5	0	178	-157	2	10	0	69	-45	-6	1	1	230	-217	0	3	1	98	-54
2	1	0	135	107	8	5	0	85	61	3	10	0	133	115	-5	1	1	190	-174	1	3	1	112	110
3	1	0	257	271	9	5	0	95	-84	4	10	0	95	49	-4	1	1	456	-521	2	3	1	516	-508
4	1	0	99	103	0	6	0	392	-462	6	10	0	279	275	-3	1	1	648	-732	4	3	1	111	79
5	1	0	304	324	1	6	0	453	-466	9	10	0	99	80	-2	1	1	117	-110	5	3	1	227	207
6	1	0	261	-245	2	6	0	76	-46	2	11	0	85	-74	3	1	1	148	-145	7	3	1	55	62
8	1	0	91	-77	3	6	0	316	-348	3	11	0	262	258	4	1	1	287	-299	8	3	1	188	-208
2	2	0	101	66	4	6	0	150	-137	4	11	0	183	196	5	1	1	541	-562	-10	4	1	78	80
3	2	0	454	516	6	6	0	350	-358	6	11	0	108	96	5	1	1	276	254	-7	4	1	67	63
4	2	0	125	-100	7	6	0	99	-90	7	11	0	84	61	7	1	1	115	-95	-8	4	1	274	-235
5	2	0	139	115	9	6	0	134	-116	0	12	0	105	84	8	1	1	126	-102	-6	4	1	52	62
6	2	0	336	331	10	6	0	151	-133	3	12	0	211	230	9	1	1	134	-149	-4	4	1	46	55
7	2	0	71	73	2	7	0	82	-83	5	12	0	142	128	-10	2	1	56	50	-3	4	1	247	254
8	2	0	103	85	3	7	0	101	-48	6	12	0	222	223	-9	2	1	90	79	-2	4	1	485	460
11	2	0	63	45	4	7	0	209	-175	8	12	0	112	98	-3	2	1	79	-92	-1	4	1	1434	1492
1	3	0	2068	-2035	5	7	0	131	-117	9	12	0	74	97	-7	2	1	113	116	0	4	1	500	471
2	3	0	299	-279	6	7	0	87	64	1	13	0	87	-49	-6	2	1	65	-44	2	4	1	442	403
3	3	0	167	-145	7	7	0	271	255	4	13	0	127	-103	-4	2	1	333	332	3	4	1	339	335
4	3	0	479	-512	8	7	0	81	-72	5	13	0	110	114	-3	2	1	415	415	4	4	1	284	298
5	3	0	265	257	10	7	0	90	62	2	14	0	89	106	-2	2	1	606	573	5	4	1	224	197
7	3	0	230	-212	0	8	0	361	-335	3	14	0	180	191	1	2	1	333	302	6	4	1	290	286
9	3	0	101	78	1	8	0	372	-392	4	14	0	191	-221	2	2	1	266	286	7	4	1	92	87
11	3	0	46	-9	2	8	0	170	169	6	14	0	69	32	3	2	1	61	56	8	4	1	105	118
0	4	0	169	-132	3	8	0	79	-71	1	15	0	101	-87	4	2	1	219	-201	9	4	1	70	75
1	4	0	712	713	4	8	0	138	-138	2	15	0	85	99	5	2	1	805	835	-10	5	1	99	95
2	4	0	453	-469	6	8	0	69	-67	0	16	0	122	-91	7	2	1	115	105	-9	5	1	55	16
3	4	0	334	324	9	8	0	101	-87	1	16	0	127	-133	10	2	1	132	142	-8	5	1	177	183
4	4	0	217	-239	2	9	0	114	-75	3	16	0	110	-103	11	2	1	56	79	-7	5	1	186	176
5	4	0	337	-319	3	9	0	85	-76	6	16	0	63	-8	-9	3	1	59	-58	-5	5	1	294	300
6	4	0	256	-239	4	9	0	132	109	4	17	0	74	-97	-8	3	1	250	217	-4	5	1	93	97

OBSERVED AND CALCULATED STRUCTURE FACTORS FOR SNP P2(1)/C

H	K	L	10FO	10FC
-3	5	1	252	-276
-2	5	1	552	543
-1	5	1	319	285
0	5	1	126	-102
1	5	1	429	423
3	5	1	311	300
4	5	1	449	427
5	5	1	220	197
6	5	1	56	54
8	5	1	149	-155
9	5	1	122	131
-8	6	1	93	72
-7	6	1	179	-160
-6	6	1	75	77
-5	6	1	265	-264
-4	6	1	196	-131
-3	6	1	462	445
-2	6	1	607	533
-1	6	1	115	120
0	6	1	445	-417
1	6	1	246	242
2	6	1	54	-58
3	6	1	116	94
4	6	1	352	313
5	6	1	65	-43
6	6	1	88	-79
7	6	1	83	-76
8	6	1	106	-111
9	6	1	79	77
10	6	1	65	90
-10	7	1	37	46
-8	7	1	75	61
-7	7	1	271	273
-6	7	1	149	-151
-5	7	1	352	368
-4	7	1	419	414
-3	7	1	37	48
-2	7	1	512	517
-1	7	1	163	115
1	7	1	233	220
2	7	1	215	-194
3	7	1	278	265
4	7	1	347	313
6	7	1	115	118
7	7	1	89	-107
9	7	1	72	-54
11	7	1	44	-52
-9	8	1	60	-52
-7	8	1	186	-169
-6	8	1	67	-51
-5	8	1	144	-135
-4	8	1	342	-330
-3	8	1	87	63
-2	8	1	590	553
-1	8	1	276	-258
1	8	1	156	-123
2	8	1	42	-11
3	8	1	177	133
4	8	1	99	-91
5	8	1	175	-172
6	8	1	249	-246
7	8	1	153	-133
9	8	1	68	-62
-3	9	1	48	43
-6	9	1	62	-62
-5	9	1	106	-100
-3	9	1	318	321
-2	9	1	117	117
-1	9	1	294	-286
0	9	1	180	-153
1	9	1	65	61
2	9	1	97	83
3	9	1	127	115
4	9	1	110	-88
5	9	1	294	-301
6	9	1	85	97
-9	10	1	70	62
-6	10	1	73	-61
-5	10	1	267	278
-4	10	1	110	-96
-3	10	1	168	-166
-2	10	1	82	54
-1	10	1	124	-106
2	10	1	166	159
4	10	1	162	-149
5	10	1	194	-195
6	10	1	81	-81
7	10	1	196	-202
8	10	1	115	-115
9	10	1	75	-75
-7	11	1	123	-116
-5	11	1	132	-151
-4	11	1	78	56
-3	11	1	135	145
-2	11	1	133	-141
-1	11	1	388	-410
0	11	1	58	47
1	11	1	152	-166
2	11	1	128	-137
3	11	1	91	-81
4	11	1	86	-83
5	11	1	192	-197
8	11	1	103	-106
-5	12	1	83	72
-4	12	1	176	206
-3	12	1	173	179
-1	12	1	118	104
1	12	1	256	260
2	12	1	25	50
4	12	1	95	-88
5	12	1	112	108
6	12	1	64	78
8	12	1	91	-83
-7	13	1	56	-60
-5	13	1	57	-62
-4	13	1	215	-246
-3	13	1	35	-115
-2	13	1	62	63
-1	13	1	60	-71
1	13	1	109	105
2	13	1	272	-322
3	13	1	117	-129
4	13	1	90	-76
-5	14	1	71	57
-4	14	1	120	145
-1	14	1	171	192
0	14	1	112	109
2	14	1	148	212
3	14	1	136	157
5	14	1	106	110
6	14	1	58	70
-4	15	1	104	-115
1	15	1	52	74
2	15	1	97	-130
5	15	1	59	61
-4	16	1	55	60
-3	16	1	110	143
-2	16	1	96	102
0	16	1	51	74
1	16	1	96	-109
3	16	1	54	71
-2	17	1	65	73
0	17	1	66	86
1	17	1	61	86
3	17	1	75	-92
4	17	1	76	90
3	18	1	45	68
4	18	1	44	74
-10	0	2	90	88
-9	0	2	160	132
-7	0	2	158	179
-6	0	2	76	78
-5	0	2	408	-418
-4	0	2	162	187
-3	0	2	203	-203
-2	0	2	328	-355
4	0	2	87	117
5	0	2	143	-119
6	0	2	208	-199
7	0	2	155	-152
8	0	2	193	-223
9	0	2	91	141
-8	1	2	55	-74
-7	1	2	39	54
-6	1	2	195	-200
-5	1	2	339	-336
-4	1	2	223	-221
-3	1	2	176	-171
0	1	2	605	-555
3	1	2	363	351
4	1	2	263	-291
5	1	2	253	-243
6	1	2	184	-195
8	1	2	85	-91
9	1	2	126	-199
-10	2	2	81	69
-7	2	2	200	212
-6	2	2	135	120
-5	2	2	74	-59
-4	2	2	261	-227

OBSERVED AND CALCULATED STRUCTURE FACTORS FOR SNP P2(1)/C

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
-3	2	2	324	-365	-10	5	2	65	68	0	7	2	396	389	-6	10	2	87	-89	1	14	2	205	242
-2	2	2	75	-96	-6	5	2	110	-111	1	7	2	403	391	-5	10	2	118	-130	2	14	2	77	83
-1	2	2	236	-256	-5	5	2	196	-210	2	7	2	134	159	-4	10	2	105	121	3	14	2	163	-195
0	2	2	1194	-1123	-4	5	2	221	-227	3	7	2	56	70	-3	10	2	87	104	4	14	2	86	-73
2	2	2	190	133	-3	5	2	43	6	4	7	2	157	160	-2	10	2	91	97	6	14	2	103	-114
3	2	2	215	-220	-2	5	2	92	-98	5	7	2	181	179	0	10	2	115	119	-3	15	2	104	-139
5	2	2	217	194	-1	5	2	100	-95	6	7	2	103	116	1	10	2	227	-224	-1	15	2	121	-144
7	2	2	78	37	0	5	2	133	-109	8	7	2	93	-131	2	10	2	89	-95	0	15	2	92	-85
9	2	2	105	-160	1	5	2	484	-458	-9	8	2	67	-55	3	10	2	94	79	5	15	2	128	-151
-8	3	2	114	-97	2	5	2	233	221	-6	8	2	127	-131	4	10	2	86	100	6	15	2	79	-95
-6	3	2	428	-447	3	5	2	214	-226	-4	8	2	77	-77	6	10	2	119	-122	-1	16	2	79	113
-5	3	2	212	-234	4	5	2	293	306	0	8	2	630	-669	7	10	2	86	-95	0	16	2	92	97
-3	3	2	141	-123	5	5	2	131	-128	1	8	2	114	122	-5	11	2	96	102	1	16	2	100	113
0	3	2	1822	-1731	6	5	2	321	-338	2	8	2	115	110	-3	11	2	124	140	3	16	2	82	-94
1	3	2	553	-515	7	5	2	95	-85	3	8	2	150	143	0	11	2	77	67	5	16	2	94	88
2	3	2	386	-362	8	5	2	159	-209	4	8	2	149	160	1	11	2	212	206	2	17	2	87	-141
3	3	2	568	-564	-7	6	2	102	-115	5	8	2	109	-105	2	11	2	306	332	3	17	2	82	-105
4	3	2	611	-606	-6	6	2	143	-144	7	8	2	79	92	3	11	2	251	270	0	18	2	81	103
5	3	2	115	-122	-4	6	2	193	207	11	8	2	62	-28	4	11	2	79	-76	-8	1	3	127	-114
6	3	2	204	-203	-3	6	2	135	-141	-9	9	2	86	88	7	11	2	87	-60	-7	1	3	105	98
7	3	2	205	-234	-2	6	2	77	-94	-7	9	2	144	136	-4	12	2	140	169	-6	1	3	203	196
10	3	2	99	-137	-1	6	2	407	-371	-6	9	2	115	116	-1	12	2	78	55	-5	1	3	220	-189
-6	4	2	126	-126	0	6	2	611	-606	-5	9	2	144	147	1	12	2	100	77	-4	1	3	72	55
-5	4	2	75	34	1	6	2	187	188	-4	9	2	198	214	2	12	2	177	201	-3	1	3	271	291
-4	4	2	81	104	2	6	2	179	-164	-3	9	2	160	176	3	12	2	170	-162	-2	1	3	159	-113
-3	4	2	335	-359	3	6	2	270	-247	-2	9	2	105	109	6	12	2	129	-138	-1	1	3	400	449
-2	4	2	138	131	4	6	2	621	625	0	9	2	206	283	-4	13	2	84	109	1	1	3	259	-256
0	4	2	169	-150	5	6	2	168	140	1	9	2	352	378	-2	13	2	135	-203	3	1	3	152	-153
1	4	2	269	-266	6	6	2	67	56	2	9	2	240	228	-1	13	2	170	-194	4	1	3	263	-296
3	4	2	210	-202	7	6	2	150	173	3	9	2	459	490	0	13	2	138	-142	6	1	3	185	164
4	4	2	75	-53	8	6	2	82	-153	4	9	2	210	203	3	13	2	94	-110	7	1	3	191	171
5	4	2	54	-42	-8	7	2	97	73	5	9	2	78	99	4	13	2	212	-223	8	1	3	173	-161
6	4	2	80	53	-6	7	2	228	240	6	9	2	167	177	6	13	2	103	-121	9	1	3	314	387
7	4	2	99	119	-3	7	2	72	75	7	9	2	180	-229	8	13	2	68	-70	10	1	3	147	210
8	4	2	76	-104	-2	7	2	400	382	8	9	2	104	126	-4	14	2	86	105	-10	2	3	71	65
9	4	2	82	-36	-1	7	2	52	-19	9	9	2	79	101	-1	14	2	260	295	-8	2	3	167	157

OBSERVED AND CALCULATED STRUCTURE FACTORS FOR SNP P2(1)/C

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	2	3	162	148	-2	5	3	84	-59	8	7	3	127	-140	-7	11	3	90	82	-3	0	4	78	46
-4	2	3	320	303	-1	5	3	590	-662	9	7	3	117	-143	-4	11	3	275	265	-2	0	4	288	-265
-3	2	3	180	159	0	5	3	381	-355	10	7	3	89	-89	-2	11	3	140	-149	-1	0	4	421	-417
-2	2	3	104	97	1	5	3	71	35	-9	8	3	62	-59	-1	11	3	261	219	5	0	4	575	-625
-1	2	3	401	411	2	5	3	155	103	-8	8	3	91	-70	0	11	3	272	249	6	0	4	394	-420
2	2	3	169	-145	3	5	3	378	-363	-7	8	3	101	-94	2	11	3	140	135	7	0	4	247	-278
3	2	3	212	236	4	5	3	110	-92	-6	8	3	112	85	3	11	3	234	241	9	0	4	176	259
4	2	3	163	153	5	5	3	118	-120	-5	8	3	290	-272	5	11	3	137	-123	-8	1	4	75	51
5	2	3	493	545	6	5	3	181	-168	-4	8	3	114	-114	6	11	3	195	162	-6	1	4	81	-69
6	2	3	167	-115	7	5	3	193	-169	-2	8	3	368	-362	-2	12	3	230	-232	-5	1	4	158	-133
7	2	3	179	144	9	5	3	101	-100	-1	8	3	425	-439	-1	12	3	226	-213	-3	1	4	92	61
8	2	3	98	64	-8	6	3	85	65	0	8	3	131	-126	4	12	3	183	-167	-2	1	4	199	182
9	2	3	144	163	-7	6	3	71	79	2	8	3	259	-252	5	12	3	99	89	-1	1	4	98	119
-8	3	3	121	-104	-5	6	3	47	-47	3	8	3	246	-231	-5	13	3	113	-123	0	1	4	97	-77
-6	3	3	79	34	-4	6	3	96	-79	4	8	3	91	-79	-1	13	3	262	253	1	1	4	398	-398
-2	3	3	115	74	-2	6	3	156	-138	5	8	3	297	-278	0	13	3	185	173	2	1	4	211	198
1	3	3	273	-299	1	6	3	264	240	6	8	3	136	-103	1	13	3	134	-107	3	1	4	434	440
4	3	3	151	35	2	6	3	107	-100	7	8	3	85	86	2	13	3	203	195	4	1	4	328	-350
5	3	3	108	105	3	6	3	251	-212	9	8	3	89	-84	3	13	3	93	95	5	1	4	154	138
7	3	3	224	-213	4	6	3	364	334	10	8	3	86	-69	4	13	3	115	111	6	1	4	90	-83
8	3	3	366	374	5	6	3	96	-53	-3	9	3	253	248	6	13	3	100	-101	7	1	4	232	221
-6	4	3	175	-136	6	6	3	117	-80	-2	9	3	131	-143	8	13	3	135	116	8	1	4	155	-172
-5	4	3	123	103	7	6	3	83	59	0	9	3	130	121	-5	14	3	126	74	-9	2	4	106	-93
-4	4	3	194	159	8	6	3	94	103	1	9	3	102	63	-1	14	3	74	86	-8	2	4	97	-68
-3	4	3	77	68	9	6	3	92	-87	2	9	3	103	85	2	14	3	174	185	-5	2	4	128	-125
-2	4	3	485	516	-7	7	3	185	-171	3	9	3	305	290	-5	15	3	85	-90	-4	2	4	186	-162
-1	4	3	606	643	-5	7	3	239	214	-7	10	3	92	-74	-2	15	3	89	-91	-3	2	4	249	216
1	4	3	324	-304	-4	7	3	72	66	-6	10	3	89	79	2	15	3	100	114	-2	2	4	198	-178
4	4	3	410	393	-3	7	3	138	125	-4	10	3	296	-288	-4	16	3	94	120	-1	2	4	137	-106
5	4	3	239	263	-2	7	3	109	-104	-3	10	3	306	-310	3	16	3	74	-61	0	2	4	425	409
8	4	3	273	287	-1	7	3	432	-439	-2	10	3	484	-490	5	16	3	43	-86	1	2	4	521	328
10	4	3	119	-132	0	7	3	398	-403	-1	10	3	339	-323	-3	17	3	78	-87	2	2	4	473	-516
-7	5	3	235	-189	1	7	3	536	-568	1	10	3	89	-60	3	17	3	70	-94	4	2	4	121	-117
-5	5	3	169	-140	3	7	3	257	-242	2	10	3	240	-236	-6	0	4	133	-117	5	2	4	308	-328
-4	5	3	127	-89	5	7	3	92	54	5	10	3	97	-78	-5	0	4	174	-166	6	2	4	175	-165
-3	5	3	58	-40	6	7	3	372	-373	7	10	3	126	-96	-4	0	4	89	-69	7	2	4	76	-66

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
-9	3	4	76	-76	-1	5	4	276	280	-4	8	4	85	-81	-5	11	4	107	88	-2	1	5	242	271
-6	3	4	230	-207	0	5	4	99	-99	-3	8	4	132	133	-1	11	4	92	78	-1	1	5	499	524
-5	3	4	104	-66	1	5	4	362	-321	-2	8	4	192	195	0	11	4	74	50	0	1	5	466	501
-4	3	4	152	126	2	5	4	304	-291	1	8	4	147	131	1	11	4	115	115	2	1	5	63	-51
-3	3	4	148	-127	3	5	4	159	143	2	8	4	346	357	-7	12	4	92	-116	3	1	5	148	162
-1	3	4	144	139	4	5	4	149	104	3	8	4	314	315	-5	12	4	111	-108	4	1	5	58	73
0	3	4	153	-174	5	5	4	293	-304	4	8	4	167	-169	-4	12	4	136	-144	5	1	5	339	-289
1	3	4	594	-579	6	5	4	344	361	5	8	4	219	186	-3	12	4	169	-167	7	1	5	87	90
2	3	4	162	-139	7	5	4	74	66	6	8	4	234	191	-2	12	4	135	-133	8	1	5	53	-48
3	3	4	110	-90	-8	6	4	100	96	8	8	4	145	132	-1	12	4	210	-222	9	1	5	86	120
4	3	4	157	-135	-5	6	4	276	280	9	8	4	96	-40	0	12	4	95	-103	-5	2	5	48	-22
5	3	4	441	410	-4	6	4	320	328	-8	9	4	69	80	1	12	4	98	-107	-4	2	5	112	-100
6	3	4	262	267	-3	6	4	263	254	-6	9	4	82	85	2	12	4	122	-118	-3	2	5	171	-148
7	3	4	242	244	-2	6	4	299	308	-5	9	4	125	111	3	12	4	147	-152	-2	2	5	228	191
8	3	4	217	251	-1	6	4	314	-294	-4	9	4	92	96	4	12	4	207	-175	-1	2	5	282	-258
9	3	4	131	217	0	6	4	542	576	-3	9	4	178	-182	5	12	4	88	-85	0	2	5	115	64
-8	4	4	92	91	1	6	4	275	262	-2	9	4	242	-219	7	12	4	64	-88	1	2	5	179	-128
-7	4	4	109	-104	2	6	4	415	423	-1	9	4	118	-100	8	12	4	136	-172	2	2	5	591	-596
-6	4	4	192	134	3	6	4	488	462	0	9	4	283	287	9	12	4	70	-68	3	2	5	185	-137
-5	4	4	152	150	4	6	4	348	347	1	9	4	159	-148	-2	13	4	99	-99	4	2	5	437	-445
-4	4	4	184	165	5	6	4	362	374	2	9	4	101	93	-1	13	4	151	151	5	2	5	140	-149
-3	4	4	313	325	6	6	4	180	184	3	9	4	124	-98	1	13	4	89	117	6	2	5	68	48
-2	4	4	209	209	0	6	4	65	63	7	9	4	210	179	5	13	4	154	140	7	2	5	163	-195
-1	4	4	252	249	-5	7	4	102	-93	8	9	4	87	50	6	13	4	102	94	9	2	5	174	-248
1	4	4	412	397	-4	7	4	93	-77	-7	10	4	102	-89	-4	14	4	140	-152	10	2	5	53	-120
2	4	4	251	222	-3	7	4	108	105	-2	10	4	327	-349	-3	14	4	113	-113	-8	3	5	70	71
3	4	4	190	197	-2	7	4	133	-79	-1	10	4	69	-55	-1	14	4	113	-109	-6	3	5	60	50
4	4	4	174	176	-1	7	4	90	-79	0	10	4	170	-155	1	14	4	171	-168	-5	3	5	182	169
5	4	4	76	-41	0	7	4	92	-75	1	10	4	213	-221	2	14	4	80	-92	-4	3	5	132	-114
6	4	4	98	94	1	7	4	226	-201	2	10	4	124	-124	5	14	4	112	-110	-3	3	5	177	-176
8	4	4	188	242	2	7	4	314	-322	3	10	4	168	155	0	15	4	139	124	-2	3	5	197	179
9	4	4	138	191	5	7	4	64	-85	4	10	4	215	-189	4	16	4	106	88	-1	3	5	71	37
-6	5	4	196	-183	6	7	4	78	75	5	10	4	155	-122	-1	17	4	64	-75	1	3	5	177	164
-4	5	4	69	50	7	7	4	245	256	8	10	4	128	-109	-8	1	5	93	85	2	3	5	125	69
-3	5	4	167	-127	8	7	4	70	86	10	10	4	75	-113	-7	1	5	128	137	3	3	5	58	-43
-2	5	4	280	-276	-5	8	4	203	192	-6	11	4	112	87	-6	1	5	56	-53	4	3	5	169	161

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
5	3	5	393	-375	-3	6	5	300	310	4	8	5	134	-96	9	11	5	121	135	0	0	6	82	-64
6	3	5	308	-304	-2	6	5	111	69	5	8	5	110	108	-3	12	5	109	-105	1	0	6	296	-300
7	3	5	127	120	-1	6	5	157	146	6	8	5	215	195	-2	12	5	111	-105	4	0	6	371	-404
8	3	5	75	93	0	6	5	209	208	7	8	5	221	220	-1	12	5	208	227	7	0	6	341	410
9	3	5	89	-122	1	6	5	305	303	8	8	5	123	130	3	12	5	167	-181	-8	1	6	54	47
-7	4	5	89	-93	2	6	5	128	-116	10	8	5	112	165	4	12	5	72	-58	-5	1	6	56	55
-6	4	5	50	-45	3	6	5	107	85	11	8	5	51	89	6	12	5	85	57	-4	1	6	149	124
-5	4	5	71	70	4	6	5	74	74	-6	9	5	44	-51	8	12	5	39	-45	-3	1	6	149	130
-4	4	5	53	56	5	6	5	172	-150	-3	9	5	123	103	-5	13	5	55	67	-2	1	6	218	208
-3	4	5	140	116	6	6	5	85	79	-2	9	5	224	-203	-2	13	5	201	240	0	1	6	264	-284
-2	4	5	235	245	7	6	5	220	257	-1	9	5	49	-16	1	13	5	164	108	1	1	6	469	477
-1	4	5	191	102	8	6	5	96	-144	1	9	5	57	40	4	13	5	199	184	3	1	6	182	194
0	4	5	231	-246	-3	7	5	69	-80	2	9	5	107	-104	5	13	5	114	-143	4	1	6	258	-255
1	4	5	555	-542	-5	7	5	71	69	3	9	5	94	101	6	13	5	96	86	5	1	6	80	-55
2	4	5	331	-306	-4	7	5	54	-57	4	9	5	241	-247	8	13	5	54	70	6	1	6	267	313
3	4	5	414	-341	-3	7	5	220	-242	5	9	5	68	-63	9	13	5	37	61	7	1	6	52	61
4	4	5	321	-274	-2	7	5	229	-234	6	9	5	144	129	-3	14	5	70	-71	-7	2	6	80	71
5	4	5	402	-396	-1	7	5	303	-313	-3	10	5	131	-130	1	14	5	53	-57	-6	2	6	118	-115
7	4	5	83	-100	0	7	5	397	-372	-2	10	5	167	162	2	14	5	69	-56	-5	2	6	121	-119
8	4	5	39	-34	1	7	5	121	-103	-1	10	5	246	243	4	14	5	108	-115	-4	2	6	123	-122
10	4	5	95	-169	2	7	5	272	-253	0	10	5	210	221	8	14	5	62	-72	-3	2	6	260	-255
-6	5	5	56	-59	3	7	5	110	-97	2	10	5	208	214	-4	15	5	62	-65	-2	2	6	104	-100
-5	5	5	183	-170	4	7	5	73	-62	4	10	5	261	252	-2	15	5	126	155	-1	2	6	217	234
-3	5	5	151	-135	5	7	5	170	-144	5	10	5	163	147	0	15	5	105	-123	0	2	6	194	-165
-1	5	5	221	-198	6	7	5	99	71	6	10	5	60	49	4	15	5	129	140	1	2	6	294	-264
0	5	5	296	257	10	7	5	65	-89	8	10	5	145	144	-1	16	5	53	-56	2	2	6	74	-34
2	5	5	230	-237	-7	8	5	77	75	10	10	5	92	126	0	17	5	43	-58	3	2	6	180	140
3	5	5	285	-291	-5	8	5	55	53	-5	11	5	57	66	1	17	5	68	-72	5	2	6	197	-183
5	5	5	242	-250	-4	8	5	152	149	-4	11	5	109	122	2	17	5	54	-77	7	2	6	85	109
6	5	5	117	-111	-3	8	5	216	231	-2	11	5	84	68	-7	0	6	103	86	0	2	6	98	-118
7	5	5	165	-179	-2	8	5	378	397	-1	11	5	156	-129	-6	0	6	135	-114	-8	3	6	77	80
8	5	5	180	-207	-1	8	5	274	270	1	11	5	95	101	-5	0	6	302	-289	-7	3	6	55	-61
-8	6	5	63	81	0	8	5	178	175	5	11	5	158	139	-4	0	6	145	-124	-6	3	6	86	-81
-7	6	5	60	-47	1	8	5	69	46	6	11	5	174	169	-3	0	6	286	-286	-5	3	6	104	97
-6	6	5	80	75	2	8	5	160	175	7	11	5	152	144	-2	0	6	281	-256	-4	3	6	237	245
-4	6	5	111	-108	3	8	5	256	233	8	11	5	61	-89	-1	0	6	277	268	-3	3	6	212	207

OBSERVED AND CALCULATED STRUCTURE FACTORS FOR SNP P2(1)/C

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	3	6	395	405	4	6	6	150	137	2	9	6	245	-255	6	10	6	57	-71	5	3	7	75	67
-1	3	6	334	319	5	6	6	83	69	3	9	6	166	-164	2	11	6	68	-93	6	3	7	255	290
0	3	6	182	196	6	6	6	94	-81	4	9	6	134	-127	-5	1	7	116	-125	7	3	7	72	61
1	3	6	370	326	7	6	6	147	140	5	9	6	200	-202	-4	1	7	173	-182	8	3	7	230	208
2	3	6	472	460	-5	7	6	144	-133	6	9	6	151	-141	-3	1	7	86	-85	9	3	7	148	-137
3	3	6	112	110	-4	7	6	145	-170	7	9	6	380	-379	-1	1	7	57	-68	-5	4	7	112	-119
4	3	6	159	156	-3	7	6	83	111	8	9	6	264	-299	1	1	7	565	-613	-5	4	7	105	-113
5	3	6	340	331	-2	7	6	104	76	-4	10	6	84	96	2	1	7	106	-94	-3	4	7	132	-122
6	3	6	413	413	0	7	6	190	-194	-1	10	6	96	105	3	1	7	155	-137	-2	4	7	92	-33
7	3	6	140	125	1	7	6	116	-101	3	10	6	123	122	4	1	7	513	-485	-1	4	7	235	-268
-4	4	6	132	-124	2	7	6	220	-132	4	10	6	104	-103	5	1	7	190	176	0	4	7	543	-525
-3	4	6	276	-269	3	7	6	107	85	5	10	6	78	74	7	1	7	269	-281	1	4	7	331	-337
-2	4	6	100	-37	4	7	6	242	-239	6	10	6	72	59	8	1	7	144	-126	3	4	7	477	-496
1	4	6	60	59	5	7	6	234	-215	-1	11	6	67	-84	9	1	7	157	160	4	4	7	242	-206
2	4	6	238	-135	6	7	6	118	-127	3	11	6	120	-117	10	1	7	111	-89	5	4	7	131	33
3	4	6	345	-259	7	7	6	76	-70	5	11	6	118	-121	-6	2	7	65	-75	6	4	7	84	-74
5	4	6	142	-111	1	7	6	244	-340	7	11	6	169	-168	-5	2	7	216	-232	7	4	7	267	-259
6	4	6	65	49	-4	8	6	58	61	8	11	6	135	-145	-4	2	7	85	-86	8	4	7	236	223
-5	5	6	71	70	-3	8	6	160	164	9	11	6	18	-71	-1	2	7	93	-92	9	4	7	106	-96
-3	5	6	147	137	-2	8	6	83	86	-4	12	6	58	52	0	2	7	307	-309	10	4	7	226	-223
-2	5	6	296	323	-1	8	6	98	77	1	12	6	89	113	1	2	7	286	-320	-4	5	7	142	141
-1	5	6	206	178	0	8	6	135	-111	3	12	6	169	-186	2	2	7	36	18	-3	5	7	186	204
0	5	6	191	202	1	8	6	54	63	5	12	6	65	76	3	2	7	155	-147	-2	5	7	71	72
1	5	6	78	-61	2	8	6	139	115	9	12	6	68	101	4	2	7	97	-91	0	5	7	86	-76
2	5	6	464	433	3	8	6	135	-194	-4	13	6	53	42	5	2	7	86	50	1	5	7	217	193
4	5	6	79	-59	5	8	6	110	109	-2	13	6	109	140	6	2	7	330	-327	2	5	7	292	290
5	5	6	216	191	6	8	6	167	-144	2	13	6	134	142	7	2	7	101	-117	3	5	7	72	78
8	5	6	92	-72	7	8	6	91	-95	5	13	6	108	115	9	2	7	152	-117	4	5	7	172	155
-6	6	6	106	136	8	8	6	66	-94	-4	14	6	56	-63	10	2	7	152	-151	5	5	7	386	358
-5	6	6	82	77	-5	9	6	101	-97	0	14	6	67	-78	-6	3	7	58	76	6	5	7	154	135
-4	6	6	134	137	-4	9	6	118	-124	3	14	6	145	-151	-4	3	7	164	-170	7	5	7	197	200
-3	6	6	102	-108	-3	9	6	215	-216	8	14	6	69	74	-1	3	7	122	103	8	5	7	160	147
-2	6	6	222	-136	-2	9	6	81	-67	-2	15	6	74	94	0	3	7	330	-332	11	5	7	152	130
0	6	6	84	49	-1	9	6	132	123	-1	15	6	83	97	1	3	7	82	64	-5	5	7	88	-109
1	6	6	39	46	0	9	6	97	-95	4	15	6	119	140	3	3	7	55	42	-4	5	7	103	-112
3	6	6	383	-345	1	9	6	114	-115	5	15	6	51	78	4	3	7	138	137	-3	5	7	70	75

OBSERVED AND CALCULATED STRUCTURE FACTORS FOR SNP P2(1)/C

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	6	7	160	-153	-2	10	7	97	111	1	16	7	96	-127	10	2	8	88	67	5	5	8	111	98
-1	6	7	179	-134	-1	10	7	93	-94	1	17	7	47	62	-4	3	8	165	165	6	5	8	139	135
1	6	7	184	174	0	10	7	109	96	2	17	7	61	98	-3	3	8	76	-69	8	5	8	130	114
2	6	7	197	-200	1	10	7	181	184	-4	0	8	154	135	-2	3	8	74	-60	9	5	8	88	45
4	6	7	35	30	2	10	7	110	122	-3	0	8	280	317	-1	3	8	119	113	10	5	8	172	-140
7	6	7	109	-104	3	10	7	171	157	-2	0	8	188	196	0	3	8	154	141	-3	6	8	137	-127
8	6	7	142	-117	4	10	7	319	318	1	0	8	190	262	1	3	8	224	-261	-1	6	8	128	-115
9	6	7	132	-113	5	10	7	73	-72	2	0	8	177	226	3	3	8	186	-182	0	6	8	222	-265
-2	7	7	92	102	6	10	7	128	-118	4	0	8	136	-146	5	3	8	262	273	1	6	8	211	-222
-1	7	7	73	-64	8	10	7	84	-77	5	0	8	185	216	6	3	8	47	66	2	6	8	231	-260
1	7	7	72	-37	9	10	7	80	73	7	0	8	139	136	7	3	8	83	-53	3	6	8	107	-119
2	7	7	261	276	-5	11	7	72	-89	8	0	8	329	352	8	3	8	101	89	6	6	8	196	-198
4	7	7	337	253	-2	11	7	173	-211	9	0	8	304	295	9	3	8	228	-186	7	6	8	297	-286
5	7	7	496	470	1	11	7	89	-80	10	0	8	107	86	10	3	8	154	-100	8	6	8	284	-270
7	7	7	296	275	2	11	7	202	-206	11	0	8	179	138	12	3	8	110	-75	9	6	8	103	-94
8	7	7	100	99	4	11	7	156	-141	-1	1	8	114	-115	-5	4	8	84	-82	12	6	8	141	-89
9	7	7	154	-140	7	11	7	210	-225	0	1	8	113	64	-4	4	8	92	-96	-3	7	8	126	125
-3	8	7	112	131	8	11	7	125	-142	2	1	8	46	32	-3	4	8	112	107	-2	7	8	141	-135
-2	8	7	67	-65	10	11	7	75	-78	3	1	8	159	-169	0	4	8	147	-150	-1	7	8	109	-113
-1	8	7	135	-125	-2	12	7	115	153	4	1	8	178	-170	1	4	8	122	-136	0	7	8	86	-80
1	8	7	286	307	0	12	7	123	-137	5	1	8	199	210	2	4	8	210	-219	1	7	8	89	58
4	8	7	67	47	4	12	7	157	165	6	1	8	85	58	4	4	8	121	109	2	7	8	99	101
5	8	7	120	113	6	12	7	82	-76	9	1	8	178	-144	5	4	8	58	-53	3	7	8	275	309
7	8	7	154	158	7	12	7	181	-162	-5	2	8	73	81	6	4	8	246	-241	4	7	8	62	-70
8	8	7	149	-152	0	13	7	141	146	-4	2	8	92	74	7	4	8	51	-47	6	7	8	147	127
9	8	7	90	84	1	13	7	67	-54	-3	2	8	219	235	8	4	8	88	-69	7	7	8	312	340
-3	9	7	78	-35	4	13	7	154	-171	-2	2	8	134	145	9	4	8	138	93	8	7	8	118	88
-2	9	7	105	-114	5	13	7	176	-197	0	2	8	124	101	12	4	8	94	-65	-5	8	8	74	66
-1	9	7	156	133	6	13	7	74	-84	1	2	8	108	91	-4	5	8	102	97	-1	3	9	83	-90
0	9	7	111	124	9	13	7	63	-81	2	2	8	116	103	-3	5	8	92	55	0	3	9	89	-66
1	9	7	207	-225	0	14	7	134	-159	3	2	8	231	232	-2	5	8	170	-167	1	3	9	68	-56
2	9	7	165	-139	5	14	7	109	-123	4	2	8	63	63	-1	5	8	53	32	3	3	9	159	-132
3	9	7	71	-54	6	14	7	91	-111	5	2	8	54	39	1	5	8	49	53	5	3	9	39	-56
5	9	7	99	30	0	15	7	92	109	6	2	8	225	217	2	5	8	87	64	7	3	9	185	-173
7	9	7	153	-138	5	15	7	78	-98	8	2	8	201	207	3	5	8	67	-66	9	3	9	174	-159
11	9	7	72	69	6	15	7	73	-94	9	2	8	256	238	4	5	8	90	75	-3	9	9	88	-87

OBSERVED AND CALCULATED STRUCTURE FACTORS FOR SNP P2(1)/C

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	9	8	176	-176	0	1	9	140	-147	-5	4	9	57	61	-3	7	9	66	53	7	12	9	100	105
0	9	8	93	-15	2	1	9	44	49	-4	4	9	71	89	1	7	9	211	203	8	12	9	111	115
3	9	8	142	144	3	1	9	241	-243	-1	4	9	42	39	2	7	9	73	52	0	13	9	97	-109
4	9	8	193	168	4	1	9	274	-293	0	4	9	101	100	3	7	9	136	184	1	13	9	69	-75
8	9	8	182	158	5	1	9	317	-350	1	4	9	120	147	4	7	9	362	366	2	13	9	71	-90
-1	10	8	111	103	6	1	9	333	-379	2	4	9	88	90	6	7	9	122	87	3	13	9	69	-75
0	10	8	128	112	7	1	9	184	-205	3	4	9	229	-219	7	7	9	96	97	4	13	9	59	-74
2	10	8	173	170	8	1	9	191	172	4	4	9	204	159	8	7	9	229	-202	5	13	9	57	-45
3	10	8	126	114	9	1	9	95	62	5	4	9	312	320	10	7	9	75	71	0	14	9	62	67
5	10	8	165	146	10	1	9	183	-189	6	4	9	246	225	-2	8	9	47	-66	1	14	9	70	78
6	10	8	214	236	11	1	9	117	-70	7	4	9	429	449	-1	8	9	62	-59	2	14	9	117	158
11	10	8	79	69	-5	2	9	60	64	8	4	9	250	245	1	8	9	99	-104	5	14	9	92	100
-4	11	8	79	-74	-4	2	9	110	107	11	4	9	134	129	2	8	9	261	-239	7	14	9	78	68
-3	11	8	143	-139	-3	2	9	89	74	-5	5	9	94	113	4	8	9	153	-128	-4	0	10	117	106
-2	11	8	92	-109	-2	2	9	35	89	-4	5	9	117	127	5	8	9	117	-105	-2	0	10	146	-141
-1	11	8	136	-158	1	2	9	240	253	-3	5	9	61	66	10	8	9	67	-63	-1	0	10	155	161
0	11	8	92	-64	2	2	9	86	77	0	5	9	143	126	11	8	9	52	-52	0	0	10	169	191
2	11	8	89	-50	3	2	9	106	93	1	5	9	273	305	-2	9	9	47	59	1	0	10	169	-186
3	11	8	96	-75	4	2	9	91	83	2	5	9	98	-99	-1	9	9	58	62	2	0	10	269	299
4	11	8	185	156	5	2	9	51	69	4	5	9	116	96	1	9	9	110	-131	3	0	10	30	-5
9	11	8	100	76	6	2	9	276	253	5	5	9	74	52	2	9	9	65	43	6	0	10	227	-205
0	12	8	161	124	7	2	9	351	349	6	5	9	60	41	3	9	9	137	123	8	0	10	76	-61
1	12	8	89	114	8	2	9	346	324	7	5	9	69	51	4	9	9	125	99	9	0	10	97	-86
3	12	8	179	168	9	2	9	178	-154	9	5	9	191	149	7	9	9	75	-53	10	0	10	57	-72
6	12	8	123	133	10	2	9	165	141	10	5	9	123	99	0	10	9	70	82	-4	1	10	52	-58
9	12	8	87	68	11	2	9	150	131	-2	6	9	73	-61	4	10	9	99	-108	0	1	10	165	-162
-2	13	8	57	-66	-5	3	9	47	42	0	6	9	90	121	5	10	9	172	-183	1	1	10	39	22
0	13	8	99	-116	-2	3	9	105	99	1	6	9	119	-132	7	10	9	119	-99	2	1	10	131	-139
6	13	8	79	-70	-1	3	9	63	-47	2	6	9	131	-119	-3	11	9	64	-70	3	1	10	122	-129
3	14	8	167	193	1	3	9	115	86	3	6	9	34	47	-1	11	9	102	-106	5	1	10	64	-61
5	14	8	100	-86	2	3	9	55	-49	5	6	9	172	-160	0	11	9	74	-52	6	1	10	139	-131
8	14	8	71	64	4	3	9	102	97	7	6	9	220	216	1	11	9	80	-91	8	1	10	138	-127
6	15	8	69	-38	5	3	9	178	-170	8	6	9	39	-46	4	11	9	92	-85	9	1	10	148	-147
5	16	8	69	-37	6	3	9	238	-258	-4	7	9	57	64	1	12	9	81	86	-3	2	10	74	-80
-5	1	9	55	-57	7	3	9	72	-62	-3	7	9	75	76	2	12	9	138	149	-2	2	10	121	-133
-1	1	9	86	-91	0	3	9	106	-98	-2	7	9	96	112	3	12	9	75	73	-1	2	10	58	64

OBSERVED AND CALCULATED STRUCTURE FACTORS FOR SNP P2(1)/C

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	2	10	79	84	9	3	10	224	-215	0	6	10	84	-120	1	8	10	85	-87	5	10	10	120	-118
2	2	10	112	86	10	3	10	150	-126	1	6	10	74	80	2	8	10	131	-142	4	10	10	55	-48
3	2	10	23	-41	-2	4	10	69	-82	4	6	10	104	97	3	8	10	169	-167	5	10	10	85	81
4	2	10	76	-75	1	4	10	152	149	6	6	10	119	-103	4	8	10	129	137	7	10	10	193	-202
5	2	10	164	-160	3	4	10	159	151	7	6	10	186	167	6	8	10	85	-75	0	11	10	79	80
6	2	10	144	-141	5	4	10	277	-252	8	6	10	156	-132	8	8	10	42	-43	3	11	10	134	140
8	2	10	137	103	7	4	10	138	127	-3	7	10	52	45	10	8	10	96	87	9	11	10	94	71
9	2	10	144	-134	9	4	10	87	-69	-2	7	10	69	73	-3	9	10	78	78	-1	12	10	56	51
10	2	10	101	-104	-3	5	10	71	-77	-1	7	10	157	167	-2	9	10	56	61	0	12	10	46	-34
-3	3	10	127	-132	1	5	10	80	-82	0	7	10	145	166	-1	9	10	155	160	2	12	10	48	37
-1	3	10	89	102	3	5	10	326	-305	2	7	10	86	88	0	9	10	175	194	3	12	10	75	-85
0	3	10	275	-301	4	5	10	209	-208	4	7	10	193	-203	1	9	10	95	111	5	12	10	103	104
1	3	10	138	-152	5	5	10	90	-65	6	7	10	342	316	2	9	10	68	75	0	13	10	56	-65
2	3	10	138	-131	6	5	10	46	-55	7	7	10	63	59	3	9	10	180	177	1	13	10	63	-65
3	3	10	396	-394	7	5	10	67	-64	8	7	10	157	-136	6	9	10	142	128	4	13	10	51	-53
4	3	10	287	-288	8	5	10	121	-133	10	7	10	78	65	7	9	10	54	51	5	13	10	101	-101
5	3	10	136	-113	9	5	10	184	-173	-3	8	10	94	-105	9	9	10	167	152	6	13	10	91	-106
6	3	10	252	-262	-4	6	10	48	-53	-1	8	10	80	-79	0	10	10	93	-99	2	14	10	95	129
7	3	10	75	-73	-1	6	10	115	-138	0	8	10	42	-35	1	10	10	76	-86	5	14	10	62	74
8	3	10	145	-135																				

(iii)

OBSERVED AND CALCULATED STRUCTURE FACTORS FOR CARE P2(1)

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	0	0	375	395	5	5	0	124	65	3	2	1	247	236	1	5	1	226	200	-7	2	2	128	161
2	0	0	202	134	-6	5	1	102	112	4	2	1	276	273	2	5	1	208	196	-5	2	2	122	120
4	0	0	364	369	-7	5	1	209	205	5	2	1	335	326	3	5	1	190	183	-3	2	2	446	516
5	0	0	503	529	-5	5	1	76	49	0	2	1	145	136	4	5	1	108	108	-2	2	2	156	192
6	0	0	137	115	-4	5	1	160	132	-6	3	1	120	122	-8	0	2	121	109	0	2	2	297	221
7	0	0	355	352	-2	5	1	152	147	-5	3	1	177	169	-7	0	2	105	99	1	2	2	581	583
8	0	0	135	117	-1	5	1	255	226	-4	3	1	130	139	-5	0	2	143	130	2	2	2	209	209
2	1	0	161	160	1	5	1	224	210	-3	3	1	134	132	-4	0	2	240	193	3	2	2	144	133
3	1	0	231	193	2	5	1	157	166	-2	3	1	317	291	-3	0	2	443	425	4	2	2	265	249
4	1	0	90	82	3	5	1	315	259	-1	3	1	164	150	-2	0	2	569	570	5	2	2	187	163
5	1	0	189	185	5	5	1	438	461	1	3	1	219	211	-1	0	2	308	305	6	2	2	147	136
6	1	0	137	139	6	5	1	193	192	2	3	1	325	317	0	0	2	159	135	7	2	2	119	127
7	1	0	160	141	8	5	1	121	109	3	3	1	402	373	1	0	2	366	383	-6	3	2	214	232
8	1	0	146	161	9	5	1	127	125	4	3	1	207	198	2	0	2	462	403	-5	3	2	69	85
9	1	0	150	144	-9	1	1	123	134	5	3	1	246	236	3	0	2	485	458	-4	3	2	134	147
1	2	0	129	119	-8	1	1	119	120	6	3	1	151	156	4	0	2	274	261	-3	3	2	135	129
2	2	0	111	113	-7	1	1	116	118	8	3	1	112	124	5	0	2	320	303	-2	3	2	319	292
3	2	0	253	244	-5	1	1	300	265	-8	4	1	91	101	6	0	2	348	346	-1	3	2	246	243
4	2	0	244	251	-4	1	1	370	347	-7	4	1	90	90	10	0	2	153	152	0	3	2	484	471
5	2	0	160	156	-3	1	1	345	319	-6	4	1	233	262	-8	1	2	240	251	1	3	2	88	70
6	2	0	155	124	-2	1	1	300	297	-4	4	1	114	102	-7	1	2	150	150	2	3	2	168	156
7	2	0	163	135	-1	1	1	452	510	-3	4	1	207	183	-6	1	2	246	238	3	3	2	295	257
8	2	0	171	164	1	1	1	157	165	-2	4	1	110	104	-5	1	2	154	143	4	3	2	316	306
1	3	0	556	510	2	1	1	336	311	-1	4	1	272	291	-4	1	2	262	265	5	3	2	128	101
2	3	0	167	172	3	1	1	124	93	1	4	1	33	10	-3	1	2	203	192	8	3	2	59	68
3	3	0	219	213	4	1	1	158	131	2	4	1	166	157	-2	1	2	415	402	9	3	2	149	163
4	3	0	114	95	5	1	1	105	90	3	4	1	268	246	-1	1	2	770	851	-7	4	2	182	167
5	3	0	94	97	6	1	1	234	252	4	4	1	210	192	1	1	2	394	384	-6	4	2	99	89
6	3	0	157	159	7	1	1	200	207	5	4	1	142	112	2	1	2	176	158	-5	4	2	124	149
1	4	0	262	249	8	1	1	149	156	6	4	1	97	107	3	1	2	244	203	-4	4	2	100	103
2	4	0	72	71	-5	2	1	129	116	-5	5	1	76	37	4	1	2	193	189	-3	4	2	145	153
3	4	0	225	239	-4	2	1	120	101	-4	5	1	71	131	5	1	2	236	225	-2	4	2	49	41
1	5	0	218	228	-3	2	1	425	461	-3	5	1	134	103	6	1	2	315	304	-1	4	2	242	283
2	5	0	144	130	-2	2	1	276	329	-2	5	1	160	95	7	1	2	107	97	0	4	2	235	223
3	5	0	196	198	-1	2	1	184	220	-1	5	1	164	171	-9	2	2	116	113	1	4	2	169	146
4	5	0	102	159	1	2	1	217	205	0	5	1	156	104	-8	2	2	143	146	2	4	2	104	129

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
3	4	2	170	153	2	1	3	459	440	-3	4	3	42	26	-7	1	4	164	164	2	3	4	210	193
4	4	2	277	238	3	1	3	328	301	-2	4	3	210	196	-6	1	4	109	93	3	3	4	151	129
8	4	2	88	69	5	1	3	105	98	-1	4	3	261	307	-5	1	4	98	95	4	3	4	245	237
-5	5	2	85	129	6	1	3	131	117	0	4	3	77	54	-4	1	4	182	178	6	3	4	243	233
-4	5	2	170	113	-4	2	3	111	111	1	4	3	274	262	-3	1	4	665	710	4	3	4	121	143
-2	5	2	176	165	-3	2	3	241	312	3	4	3	46	45	-2	1	4	347	323	-4	4	4	140	158
-1	5	2	126	133	-2	2	3	104	119	4	4	3	233	223	-1	1	4	210	203	-5	4	4	54	57
0	5	2	183	196	-1	2	3	100	184	5	4	3	124	135	0	1	4	241	206	-4	4	4	207	210
1	5	2	188	164	0	2	3	645	555	6	4	3	104	130	1	1	4	617	717	-2	4	4	64	66
2	5	2	359	330	2	2	3	482	452	7	4	3	62	72	2	1	4	512	493	-1	4	4	180	191
3	5	2	152	65	4	2	3	163	146	8	4	3	104	113	3	1	4	114	101	0	4	4	199	138
4	5	2	76	36	5	2	3	222	237	-5	5	3	112	115	4	1	4	251	232	1	4	4	106	116
5	5	2	72	124	6	2	3	145	141	-3	5	3	102	62	5	1	4	144	152	3	4	4	193	145
-8	0	3	166	172	-5	3	3	93	129	-2	5	3	176	157	6	1	4	206	192	6	4	4	100	111
-7	0	3	87	100	-4	3	3	189	203	-1	5	3	122	124	7	1	4	111	114	5	4	4	289	252
-6	0	3	224	132	-3	3	3	104	134	0	5	3	126	102	-5	2	4	149	151	6	4	4	215	223
-4	0	3	81	74	-5	3	3	145	146	1	5	3	192	190	-4	2	4	312	379	-3	5	4	150	72
-3	0	3	226	210	-4	3	3	123	122	2	5	3	242	208	-3	2	4	136	156	-1	5	4	129	142
-2	0	3	776	829	-3	3	3	266	259	3	5	3	105	137	-2	2	4	246	343	1	5	4	129	95
-1	0	3	312	315	-2	3	3	289	274	5	5	3	75	145	-1	2	4	179	236	1	5	4	187	183
1	0	3	951	1032	-1	3	3	510	500	-10	0	4	99	114	0	2	4	509	430	2	5	4	159	71
2	0	3	696	764	0	3	3	487	459	-8	0	4	192	202	1	2	4	304	265	3	5	4	132	193
3	0	3	355	350	1	3	3	216	204	-6	0	4	271	256	2	2	4	160	141	-10	0	5	94	97
4	0	3	235	237	2	3	3	92	64	-4	0	4	144	135	3	2	4	202	285	-7	0	5	188	167
5	0	3	340	337	3	3	3	220	107	-3	0	4	318	293	4	2	4	83	70	-6	0	5	343	324
6	0	3	281	258	4	3	3	117	99	-2	0	4	426	442	5	2	4	171	143	-5	0	5	127	122
7	0	3	131	110	5	3	3	222	216	-1	0	4	648	674	6	2	4	266	261	-4	0	5	349	307
-8	1	3	299	314	6	3	3	145	127	0	0	4	427	497	-8	3	4	121	116	-3	0	5	530	528
-7	1	3	239	231	7	3	3	134	121	2	0	4	209	160	-7	3	4	179	195	-1	0	5	57	56
-6	1	3	219	264	3	3	3	116	141	3	0	4	163	166	-5	3	4	188	197	0	0	5	498	585
-5	1	3	234	208	0	3	3	70	73	4	0	4	66	51	-4	3	4	87	73	1	0	5	691	756
-4	1	3	202	194	-3	4	3	86	105	5	0	4	363	362	-3	3	4	146	144	2	0	5	237	210
-3	1	3	563	563	-7	4	3	127	150	6	0	4	102	91	-2	3	4	340	344	3	0	5	353	332
-2	1	3	269	240	-6	4	3	177	232	7	0	4	128	134	-1	3	4	382	338	4	0	5	293	266
0	1	3	554	645	-5	4	3	206	196	8	0	4	86	83	0	3	4	360	351	5	0	5	307	288
1	1	3	183	132	-4	4	3	260	254	0	0	4	160	164	1	3	4	204	179	7	0	5	119	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
8	0	5	290	336	5	3	5	192	170	3	0	6	120	109	-2	3	6	366	392	-4	1	7	247	223
9	0	5	166	178	6	3	5	245	243	6	0	6	104	92	-1	3	6	303	291	-5	1	7	162	158
-6	1	5	139	211	7	3	5	146	155	7	0	6	128	120	0	3	6	315	274	-3	1	7	394	382
-5	1	5	162	167	8	3	5	81	70	8	0	6	161	169	1	3	6	230	168	-2	1	7	581	584
-4	1	5	81	56	0	3	5	62	70	-10	1	6	97	108	2	3	6	55	40	-1	1	7	218	211
-3	1	5	256	231	-7	4	5	123	140	-8	1	6	73	95	3	3	6	230	159	0	1	7	303	264
-2	1	5	254	258	-6	4	5	190	203	-6	1	6	144	134	4	3	6	137	95	1	1	7	361	353
-1	1	5	203	173	-5	4	5	66	63	-5	1	6	161	149	5	3	6	130	155	3	1	7	502	493
0	1	5	430	411	-3	4	5	223	236	-4	1	6	290	258	6	3	6	90	75	4	1	7	96	107
1	1	5	455	433	-2	4	5	135	108	-3	1	6	423	431	8	3	6	86	97	5	1	7	127	118
2	1	5	339	264	-1	4	5	322	355	-2	1	6	374	375	9	3	6	111	125	-7	1	7	112	119
3	1	5	521	510	0	4	5	104	68	-1	1	6	147	142	-8	4	6	87	100	-6	1	7	139	114
4	1	5	247	240	1	4	5	202	170	0	1	6	506	497	-7	4	6	130	164	-5	1	7	243	228
6	1	5	127	120	2	4	5	221	193	1	1	6	114	112	-6	4	6	141	139	-4	1	7	276	277
-9	2	5	137	145	3	4	5	225	211	2	1	6	371	367	-5	4	6	64	60	-3	1	7	237	239
-8	2	5	146	134	5	4	5	202	176	3	1	6	551	554	-4	4	6	136	117	-2	1	7	192	161
-3	2	5	237	243	6	4	5	100	100	5	1	6	236	230	-3	4	6	232	227	-1	1	7	449	401
-2	2	5	270	308	7	4	5	66	74	6	1	6	159	158	-2	4	6	109	100	0	1	7	338	348
-1	2	5	156	146	-4	5	5	212	138	8	1	6	145	145	-1	4	6	239	266	1	1	7	163	135
0	2	5	130	118	-3	5	5	206	120	-6	2	6	233	292	0	4	6	94	100	2	1	7	611	613
1	2	5	276	274	-2	5	5	290	224	-5	2	6	131	145	1	4	6	97	116	3	1	7	525	522
2	2	5	150	126	-1	5	5	111	93	-4	2	6	193	202	2	4	6	346	303	5	1	7	173	168
3	2	5	130	122	0	5	5	122	141	-3	2	6	105	143	3	4	6	191	157	6	1	7	109	110
4	2	5	137	133	1	5	5	112	90	-2	2	6	66	46	4	4	6	104	89	7	1	7	155	154
6	2	5	149	151	3	5	5	111	135	-1	2	6	223	182	6	4	6	129	120	8	1	7	164	181
-8	3	5	135	157	4	5	5	159	135	0	2	6	264	259	7	4	6	93	110	-10	2	7	110	102
-7	3	5	140	175	-10	3	6	109	87	1	2	6	334	305	8	4	6	93	115	-6	2	7	201	245
-6	3	5	91	112	-7	0	6	285	285	2	2	6	187	145	-5	5	6	115	93	-4	2	7	96	107
-4	3	5	213	235	-6	0	6	104	73	4	2	6	255	231	-4	5	6	199	199	-3	2	7	147	207
-3	3	5	112	115	-5	0	6	303	302	6	2	6	252	276	-3	5	6	234	203	-2	2	7	270	351
-2	3	5	293	254	-4	0	6	417	395	-8	3	6	79	95	-1	5	6	160	98	-1	2	7	121	115
-1	3	5	292	270	-3	0	6	642	652	-7	3	6	83	89	0	5	6	93	89	0	2	7	221	204
1	3	5	246	213	-2	0	6	431	413	-6	3	6	161	177	1	5	6	114	125	1	2	7	83	73
2	3	5	234	245	-1	0	6	322	269	-5	3	6	152	139	2	5	6	107	116	2	2	7	162	118
3	3	5	148	160	0	0	6	561	576	-4	3	6	175	162	3	5	6	118	153	4	2	7	212	171
4	3	5	217	203	2	0	6	123	134	-3	3	6	184	173	-7	0	7	261	260	5	2	7	248	219

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
6	2	7	162	166	-[illegible]	[illegible]	3	136	134	2	2	8	307	283	-4	3	8	188	162	-2	2	9	219	221
8	2	7	95	93	-[illegible]	[illegible]	3	161	152	3	2	8	220	194	-3	3	8	226	236	-1	2	9	283	292
-9	3	7	89	63	-[illegible]	[illegible]	8	195	193	5	2	8	191	156	-2	3	8	144	76	0	2	9	359	344
-7	3	7	165	193	-5	[illegible]	3	211	209	0	2	8	82	131	-1	3	8	117	66	1	2	9	283	264
-6	3	7	132	158	-4	[illegible]	3	141	121	-9	3	8	93	131	0	3	8	89	78	2	2	9	250	183
-5	3	7	95	74	-3	[illegible]	8	119	100	-8	3	8	95	126	1	3	8	134	49	3	2	9	129	191
-3	3	7	290	279	-2	[illegible]	8	564	574	-7	3	8	157	230	2	3	8	168	221	4	2	9	196	165
-2	3	7	129	131	-1	[illegible]	8	250	252	-6	3	8	122	156	-9	0	9	171	162	5	2	9	205	188
-1	3	7	427	425	0	[illegible]	3	220	190	-5	3	8	71	57	-7	0	9	102	84	-7	3	9	46	74
0	3	7	414	374	1	[illegible]	3	280	262	-4	3	8	100	90	-6	0	9	124	130	-6	3	9	265	268
1	3	7	140	112	2	[illegible]	3	226	206	-3	3	8	114	120	-5	0	9	366	342	-5	3	9	75	88
2	3	7	286	279	4	[illegible]	8	333	372	-2	3	8	176	164	-4	0	9	190	169	-4	3	9	153	145
3	3	7	307	276	6	[illegible]	3	136	176	-1	3	8	307	296	-3	0	9	345	346	-3	3	9	131	196
4	3	7	169	169	2	[illegible]	3	117	135	0	3	8	264	261	-2	0	9	371	362	-2	3	9	257	265
5	3	7	95	92	-7	1	3	233	225	1	3	8	196	144	-1	0	9	435	411	-1	3	9	246	263
6	3	7	178	130	-5	1	3	154	135	2	3	8	222	219	0	0	9	340	324	0	3	9	219	215
7	3	7	146	142	-4	1	3	326	323	3	3	8	75	66	1	0	9	180	161	1	3	9	189	174
8	3	7	85	92	-3	1	8	138	120	4	3	8	183	170	2	0	9	263	212	2	3	9	180	166
-8	4	7	69	73	-2	1	3	270	242	5	3	8	193	136	3	0	9	220	234	3	3	9	145	122
-5	4	7	99	108	-1	1	3	132	76	6	3	8	180	175	4	0	9	265	236	4	3	9	213	204
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1	4	7	213	237	5	1	3	127	132	-4	4	8	235	231	-3	1	9	425	387	-2	4	9	112	100
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6	4	7	123	132	-8	2	3	169	16[illegible]	-1	4	8	201	179	1	1	9	251	207	2	4	9	166	185
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1	5	9	89	107	-5	3	10	209	245	1	0	11	230	196	0	3	11	162	163	-5	2	12	145	110
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PLYMOUTH POLYTECHNIC

THE STRUCTURAL AND PHYSICAL PROPERTIES OF CRYSTALLINE ANTIBIOTIC MATERIALS

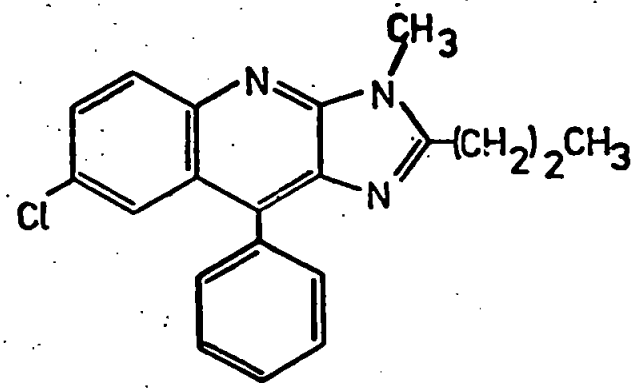
by

P.A.C. GANE

ABSTRACT OF THESIS

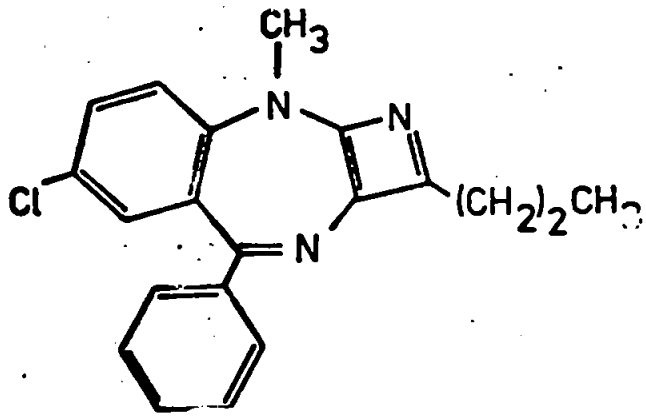
Effectiveness in biological chemical environments virtually defines the term 'drug' when applied to any attempt to modify that environment by the introduction of an influence in terms of a specific compound or group of compounds. Interest in the configuration of the molecules involved in such modifications led to the X-ray structure determinations, discussed in the thesis, of the following three compounds:

(i) 7-chloro-2-methyl-5-phenyl-3-propyl[2,3-b]-imidazolyl quinoline.



(1)

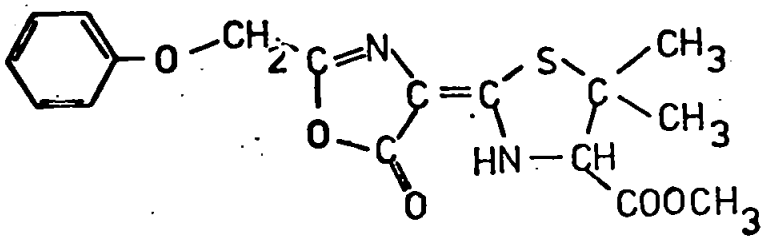
Derived from the psychoactive drug Librium, it was thought to conform to the structure:



(2)

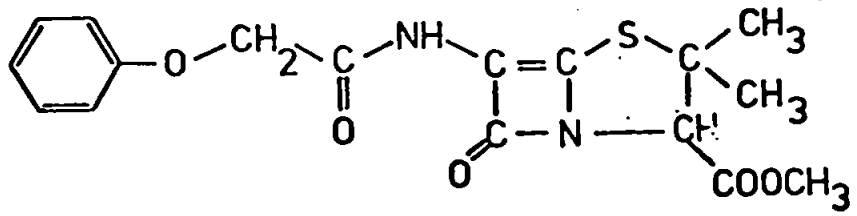
containing the highly strained 4-membered monocyclic azete system (Shenoy, a thesis submitted for the degree of Doctor of Philosophy, University of London, 1975), and suggested as one of the first examples of possible stable 4-membered azacyclobutadiene rings.

(ii) The methyl ester of 5,5-dimethyl-2-(2-phenoxymethyl-5-oxo-1,3-oxazolin-4-ylidene)-1,3-thiazolidine-4-carboxylic acid.



(3)

$C_{17}H_{18}N_2O_5S$ was first reported by Brandt, Bassignani and Re, (1976, Tetrahedron Letters No. 44, pp 3979-3982), to have configuration (4),



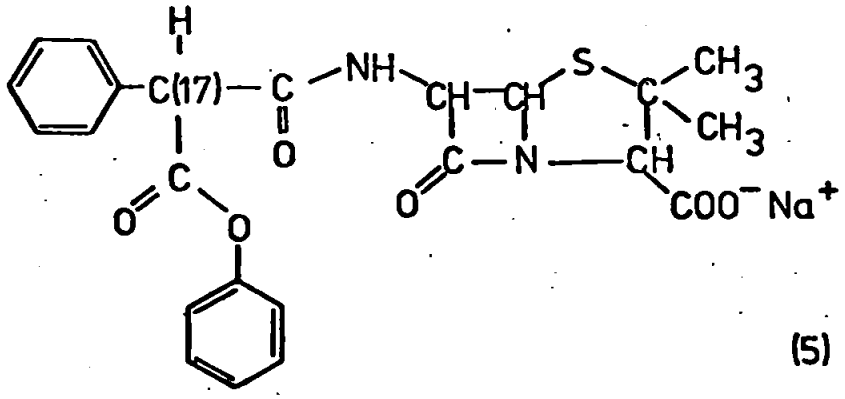
(4)

i.e. that of a novel class of DL-5,6-didehydropenicillins. Its reported weak antibacterial activity, thought to be associated with the unsaturated nature of the penicillin nucleus promoted its X-ray structure analysis.

Subsequently, Bachi and Vaya, (1977, Tetrahedron Letters No. 25, pp 2209-2212), suggested the configuration (3) which has been confirmed by the structure determination.

A comparison of the proposed derivations of (3) and (4) is made, and the conformation of the unconstrained thiazolidine ring is discussed in comparison with the constraining effect of adjacent β lactams in the nuclei of known penicillin structures.

(iii) The phenyl ester of carbenicillin (carfecillin).



(5)

The crystal structure is used to facilitate a comparison of the configurations of both the penicillin nucleus and the side-chain substituents of C(17) with other penicillin derivatives of known crystal structure.

The conformation in aqueous solution about C(17) is reflected in the modification of H^1 n.m.r. signals from the β lactam protons for the methyl and ethyl esters of carbenicillin between the two epimers. A similar effect is noted for the two diastereoisomers of amino-hydroxybenzyl penicillin, amino-phenylacetamido penicillanic acid and a tyrosyl penicillin.

To evaluate a correlation between the absolute configuration and n.m.r. studies, circular dichroism spectra from penicillin compounds have been characterised and the mutarotation exhibited by the esters of carbenicillin used to describe configurational equilibria about C(17) in terms of their characteristic n.m.r. spectra.