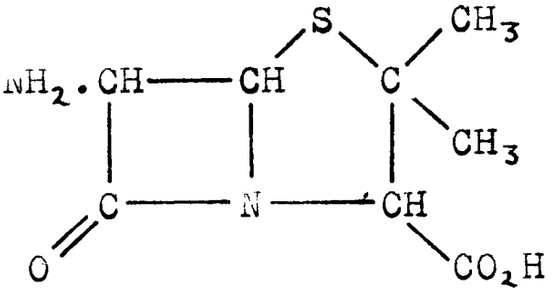
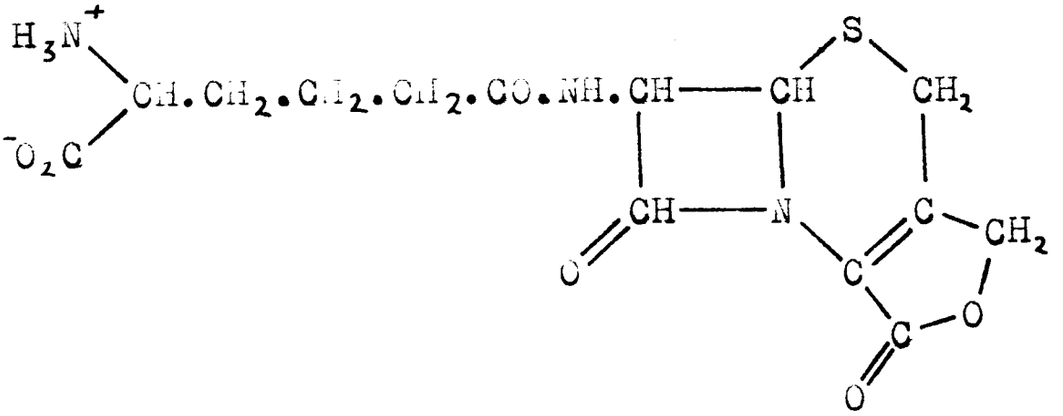
ABSTRACT

The crystal structure of 6-amino penicillanic acid (C_8, H_{12}, N_2, O_3, S) (I) and cephalosporin Cc ($C_{14}, H_{17}, O_6, N_3, S; CH_3CO_2H$), (II) have been determined by X-ray crystallographic analysis.



(I)



(II)

6-aminopenicillanic acid crystallises in flat orthorhombic plates,

Crystal data.

$a = 6.24 \pm .02$ Å, $b = 14.83 \pm .04$ Å, $c = 10.49 \pm .03$ Å

space group $P2_12_12_1$ $Z = 4$.

Using copper K_α radiation layers okl - 4kl and hk0 to hk8 were recorded on equi-inclination Wissenberg photographs. A total of 988 reflections were measured visually out of a possible 1170 in the copper sphere. A Patterson function was calculated using data sharpened by the finction $\frac{1}{f^2} \exp.(3 \sin^2\theta)$, where f was the scattering factor for carbon; from this a superposition function was calculated. It was possible to obtain all atomic positions from this. These were refined using anistropic and isotropic least squares procedures, the final positions have calculated deviations of about .01Å. The final agreement factor was 11.6% using isotropic and 9.7% anistropic temperature factors.

The structure is similar to that reported by Crowfoot, Bunn, Rogers-Low and Turner-Jones for potassium benzyl penicillin except that in the thiazolidine ring the atom out of the plane of the

other four is the nitrogen instead of the carbon to which the carboxyl group is attached, as in the other penicillins. The bond between the sulphur and the gem-dimethyl group is longer in all the penicillins than the generally accepted value for a C - S single bond (1.86 Å compared with 1.81 - 1.82 Å). The length of the C - N bond in the β-lactam group falls between that of a C - N single bond and that of an ordinary C - N peptide bond; this is probably caused by the lack of resonance in the fused β-lactam ring.

Cephalosporin Cc crystalises in flat orthorhombic needles (a = 5.04 ± .0 Å, b = 7.00 ± .02 Å, c = 54.6 ± .2 Å), space group $P2_12_12_1$. 1521 Reflections out of a possible 2580 in the copper sphere were measured visually on equi-inclination Wissenberg films (layers okl - 3kl and hol - h5l). The structure was determined and refined in a similar fashion to 6-aminopenicillanic acid, though because of the larger size of the molecule three rounds of Fourier refinement were needed before it was possible to use the least square procedure. The final agreement after eight rounds of least squares refinement was 10.6%, with calculated standard deviations of about .01 Å.

OTHER

The structure was identical to that proposed by Abraham and Newton. The bond distances were all within the expected range of values. The α-amino group of the side chain together with the acetic acid of crystallisation formed a hydrogen bond system around the screw axis parallel to b. There was no significant difference between the lengths bonds of the β-lactam group in cephalosporin Cc and in 6-aminopenicillanic acid.

V

A CRYSTALLOGRAPHIC STUDY

of the
STRUCTURE OF SOME ANTIBIOTICS

A Thesis Submitted
for the
Degree of Doctor of Philosophy
of the

University of Oxford

Trinity Term, 1963.

R. D. Diamand
New College.

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CHAPTER I

Introduction

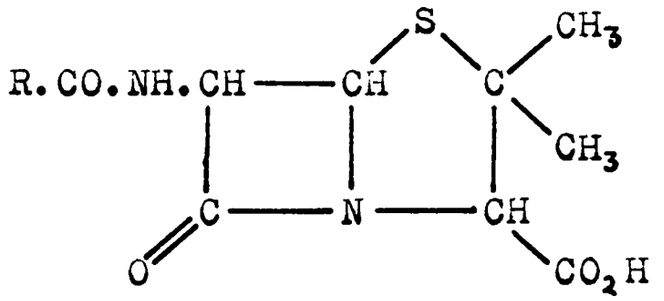
CHAPTER I

INTRODUCTION

This thesis is concerned with the crystal structure of two of the penicillin family of antibiotics, 6-amino-penicillanic acid (referred to as 6-APA), and cephalosporin Cc, the γ-lactone of the antibiotic cephalosporin C (referred to as ceph. Cc and ceph. C respectively; see end paper for numbered diagrams). The original penicillins were introduced into medicine as a result of much war-time work in Great Britain and the U.S.A. Later, bacterial strains emerged that were capable of destroying penicillin by means of an enzyme, penicillinase. These have since increased in number, especially in hospitals, posing a very serious medical problem. This has been attacked by measures to control the spread of resistant strains, and by searching for new antibiotics and penicillins that are resistant to penicillinase.

The early workers had found that the nature of the side chain R of the penicillin molecule (I) could be varied to some extent by putting appropriate side chain precursors into the fermentation medium. The resulting

penicillins differed both in chemical and antibiotic properties.

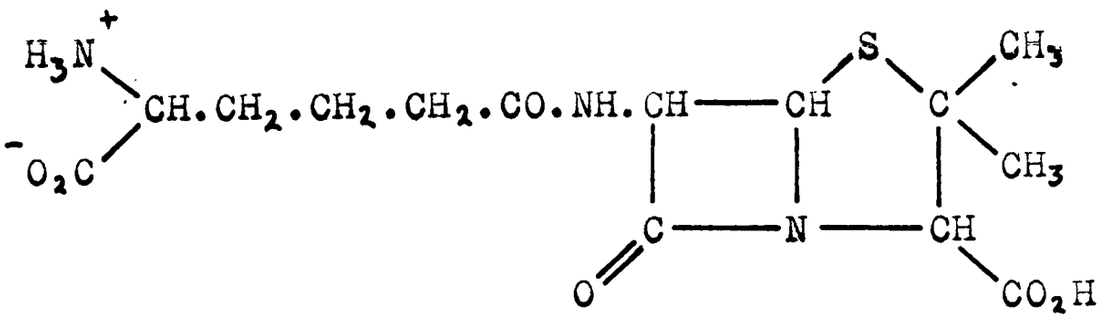


I

Nevertheless the range of side chains that could be introduced in this way was limited. Batchelor, Doyle, Naylor and Rollinson[17] found that if they left out the side chain precursor entirely they could obtain the penicillin nucleus, 6-aminopenicillanic acid, with a free amino group at N14, instead of the peptide linkage R-CO-NH-. A great many side chains can be added to the nucleus by condensation with the appropriate acid chloride. This facility for making a large number of different penicillins has led to the discovery of several medically useful modifications with increased acid stability or resistance to penicillinase. It has also enabled a systematic study to be made of the chemical and biological effects of different side chains.

Another series of antibiotics, some of whose members show resistance to penicillinase, are the cephalosporins. Their activity was first discovered by G. Brotzu who isolated the fungus from a sewage outfall in Sardinia, and noted its antibiotic activity. These results were published in an obscure journal (21), but it was not till they were brought to the attention of Sir Howard Florey that any further development took place. The problem was then taken up by E.P. Abraham and G.G.F. Newton and their co-workers.

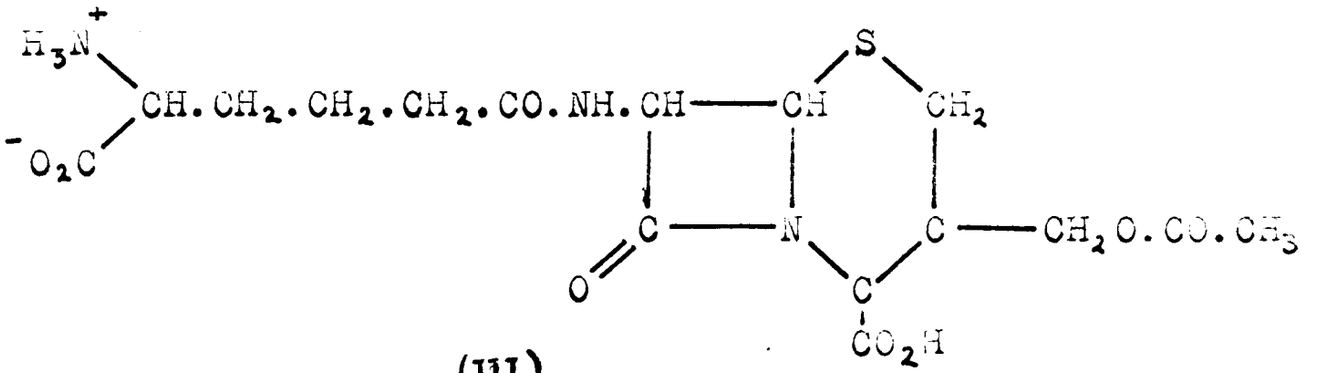
They isolated from the culture a group of active steroids, the cephalosporin P family (23) and cephalosporin N (9) which proved to be D-(4-amino-4-carboxybutyl) penicillin. (II)



II

They found that this was contaminated with a related but acid-stable product cephalosporin C. The

elucidation of its structure proved much more difficult than that of cephalosporin N, but it was eventually shown by chemical and X-ray studies (Abraham and Newton (7) Crowfoot-Hodgkin and Maslen (36)) to have the structure, (III)



(III)

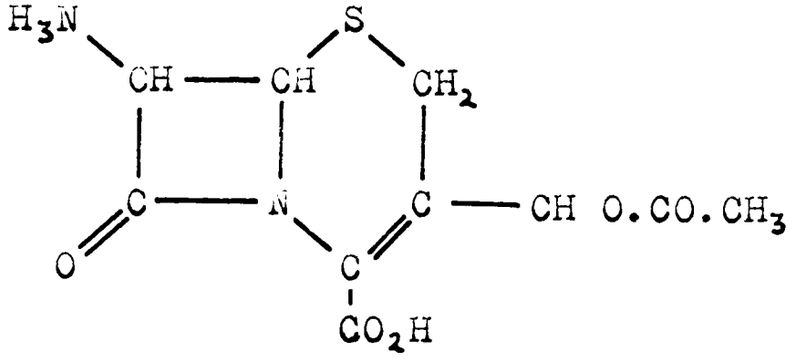
Only cephalosporin C showed any real promise as a practical antibiotic. Cephalosporin P was not very active in vivo, and resistant strains developed very easily. Cephalosporin N was more successful, but did not show sufficient advantages over the penicillins already available.

The antibiotic activity of cephalosporin C was similar in its mode of action, which involved the lysis of growing cell walls, to that of the ordinary penicillins. It is, however, only about ·1% as active as penicillin against many bacteria, but as its rate of hydrolysis by penicillinase is in the order of

10^{-4} of that of benzyl penicillin, it looks very promising as a practical antibiotic.

As it acts as a competitive inhibitor of the action of penicillinase from Bacillus cereus, (though not of that from Staphyloccus aureus), it would act synergistically with penicillin.

In the penicillins the side chain has a very great effect on the biological activity of the antibiotic. In order to try to increase the effectiveness of the cephalosporins Loder, Newton and Abraham (45) produced 7-amino-cephalosporanic acid (IV) by mild acid hydrolysis



IV

of the parent cephalosporin C. They were able to produce a number of different derivatives of 7-amino-cephalosporanic acid; the phenylacetyl derivative had

an activity of the same order of magnitude as benzyl-penicillin against Staph. aureus, without any loss of resistance to penicillinase. Their process only produced the cephalosporin nucleus in very low yield, so that the practical usefulness of the resulting antibiotics was very limited. Since then, Morin, Jackson, Flynn and Roeske (49) have found a method of producing 7-aminocephalosporanic acid at up to 70% yield, by the action of nitrosyl chloride on cephalosporin C in formic acid. This promises to be a method for producing a wide range of useful antibiotics.

Crystallographic Studies

The work on Na, K, and Rb benzyl penicillin by Crowfoot, Bunn, Rogers-Low and Turner-Jones (26), was a major advance in the use of X-ray crystallographic techniques. It was also responsible for the general acceptance of the β-lactam structure for penicillin. However, as at the time all calculations had to be done either by hand or on punched-card machinery, it was not possible to carry the refinement of the structures very far. The accuracy of the structures was also limited through lack of data, for the original crystals

were poor and gave very few reflections with spacings of $d<1{\cdot}2$Å . This last defect was overcome by Pitt (55), who was able to collect data for the whole of the copper sphere; however the degree to which he could refine the structure was also limited through lack of computing facilities.

It is difficult to estimate the size of the errors in Pitt's published co-ordinates as these are taken from the final Fourier map, and the co-ordinates used to calculate the phases had been adjusted to give reasonable molecular dimensions. The published co-ordinates are, therefore, biased towards the theoretical structure so that the differences between theoretical and observed bond lengths are bound to be less than the true errors in the analysis; substantially less, as the structure is non-centrosymmetric. In this case the mean shift, in the refinement described by Pitt was 0·04Å , and the mean agreement between final bond distances and theoretical ones 0·02Å , so it is unlikely that any difference less than about 0·04Å is significant.

The chemistry of the penicillins has proved unusual, particularly in relation to the β-lactam ring system. The lack of stability of this has been attributed by

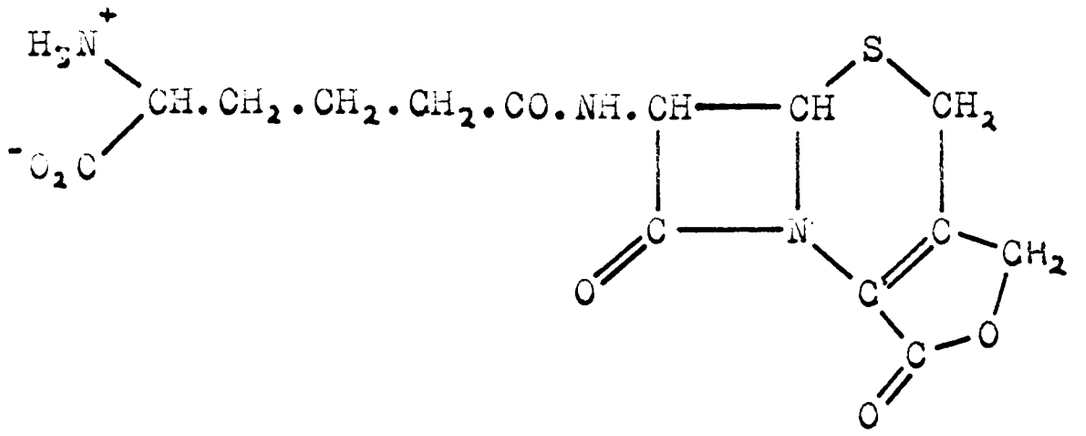
Woodward (69) to the limited resonance that is possible in the fused β-lactam ring in comparison with the ordinary peptide linkages, because of the non-planarity of the former system. In order to try and elucidate this, and to obtain a better physical description of the whole penicillin molecule, than had been possible in the earlier investigations which had been limited in their accuracy by lack of computing facilities, it was decided at Oxford to undertake investigations into a number of different penicillins. These were; phenoxymethylpenicillin by Abrahamsson, Hodgkin and Maslen (10); p-bromophenoxymethylpenicillin by K. Watson (personal communication) and J. Rollett and A. Vaciago's further refinement of Pitt's (55) data for potassium benzylpenicillin.

It was felt that 6-aminopenicillanic acid would be a useful member of this group as, being smaller, the errors should be less; and, since it does not contain the side chain, it provides a means of finding out if this has any noticeable effect on the molecular structure. 6-APA is in fact one of the most accurate of these determinations, with a calculated standard deviation for

the bond distances of about 0·01Å , the true standard deviation is somewhat larger, about 0·02-0·03Å (P. 53.) p-Bromophenoxy-methylpenicillin has slightly higher calculated standard deviations, and the errors in the other two penicillins are about twice that in 6-APA.

The structure of cephalosporin C was determined by Crowfoot-Hodgkin and Maslen (**36**). The crystals were very small and only gave reflections with spacing greater than 1·04Å . The structure was crystallographically very difficult, as there were two molecules to the asymmetric unit, and these deviate only slightly from positions related by a displacement of c/2. Because of this difficulty and the limited nature of the data, very little attempt was made to refine the structure beyond the rather unsatisfactory state published, with a R value of 0·26 and bond distances differing from accepted values by an average of 0·07 Å. It was expected that cephalosporin Cc, (V), the γ-lactone of cephalosporin C (**III**), would be more suitable for refinement because it was much simpler crystallographically with only one molecule per asymmetric unit, and also because it gave reflections out to the limit of the

copper sphere. The second part of this thesis is concerned with the solution and refinement of the structure of cephalosporin Cc.



Cephalosporin Cc. (V)

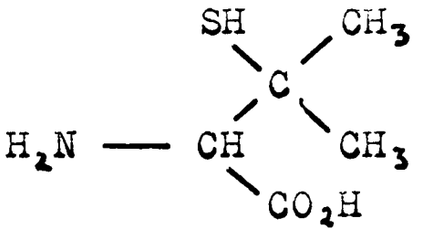
The final positions determined for this structure have proved quite accurate, the calculated standard deviation is about 0·01Å, with a true standard deviation of about twice this. The structure determination has fully confirmed the chemical formula proposed by Abraham and Newton (7).

CHAPTER 2

CHEMISTRY of PENICILLIN

The medical potential of penicillin became clear following its isolation in crude form by various workers at Oxford. They had used the original mould discovered by Fleming (32), and by 1942 they were able to obtain samples of the barium salt up to 80% pure. At first all the work was done at Oxford, but it was soon taken up by other groups in Britain and America. Because of the military importance of penicillin the publication of work was restricted, and results were communicated to other groups either through the Committee for Penicillin studies in Great Britain or the Committee on Medical Research in the U.S.A. After the war all the work that had been carried out was published together in the "Chemistry of Penicillin" (24). The determination of the chemical structure of the penicillins proved extremely difficult. At that time there were no model compounds containing a fused β-lactam ring and it was not realised how different this would be from other lactams and peptides. Woodward later suggested (69) that this was probably due to the absence of resonance stabilisation in the β-lactam ring (p.8).

Degradative work was started at Oxford as soon as possible on the minute quantities of partially purified penicillin that were available. The first fragments to be characterised were penicillamine, (I),



I

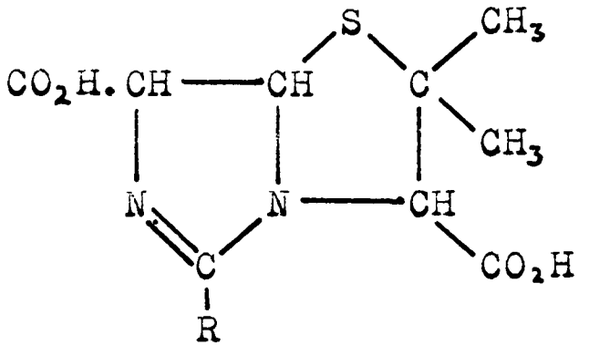
which was obtained on hydrolysis with hot dilute mineral acid (5), and the penilloaldehydes. The exact formulae for aldehydes varied, depending on the culture producing the penicillin. They all, however, had the following general formula

$$R \cdot CO \cdot NH \cdot CH_2 \cdot CHO$$

where R was $CH_3 \cdot CH_2CH{=}CH \cdot CH_2-$ in the Oxford material, $CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2-$ in that from Imperial College, London, and the benzyl group in the American material. It was this variability which showed that penicillin was not a single chemical substance, but

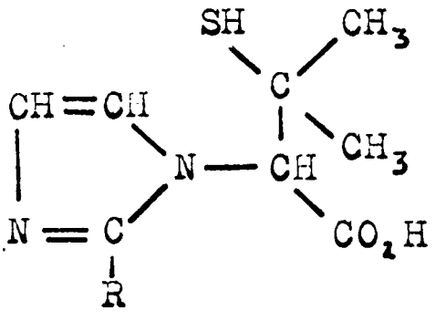
a group of related compounds.

Another early degradation product to be isolated was penillic acid (2), (18), now known to be (II).

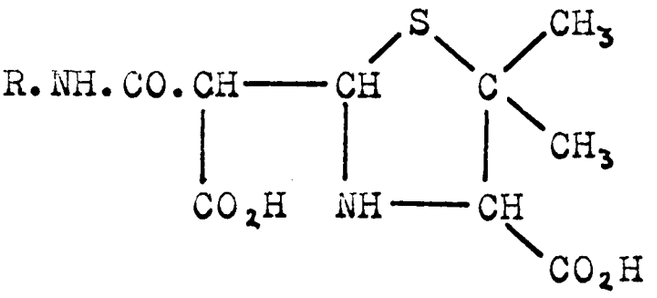


II

On adding mercuric chloride to the penillic acid, CO_2 was liberated, to give penillamine. This had a free SH group and three ionizable centres. It gave penicillaminic acid (the sulphonic acid of penicillamine). From these and other facts the formula of penillamine was deduced to be (III), (6)



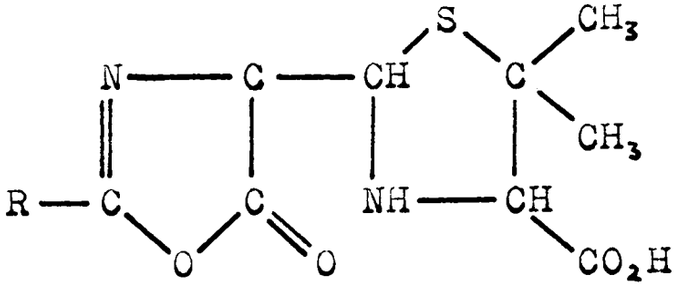
From the mode of formation of penillamine from penillic acid, and the knowledge that the latter contained no free thiol group, Abraham, Chain, Baker and Robinson (3) proposed (II) for the structure of the penillic acid. When penicillin was kept at pH 10 for 15 minutes it was transformed into penicilloic acid, containing one basic and two acidic groups. This contained no free SH group or α-amino acid. On treatment with mercuric chloride it gave penicillamine, and liberated one molecule of CO_2 per molecule of penicillin. Abraham, Baker, Chain and Robinson (6) put forward the following formula to represent penicilloic acid (IV).



The same authors later discovered that penicillin was inactivated to penicilloic acid by a bacterial enzyme,

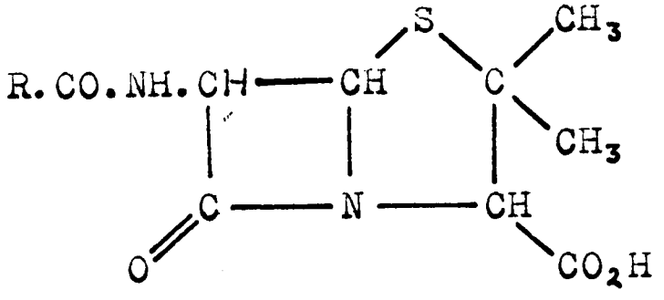
penicillinase.

By the end of 1943, there was sufficient evidence to justify putting forward possible structional formulae for the penicillins. Both the Oxford group (3) and the American groups (74) independently suggested the thiazolidine-oxazolone, (V),

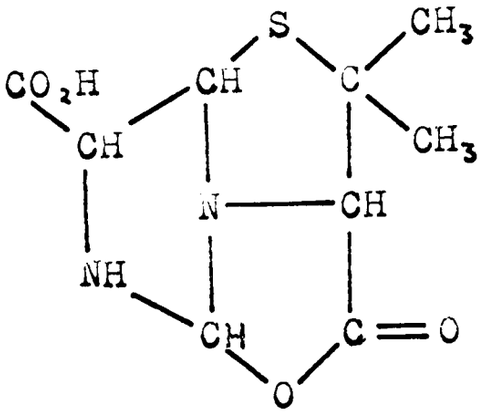


V

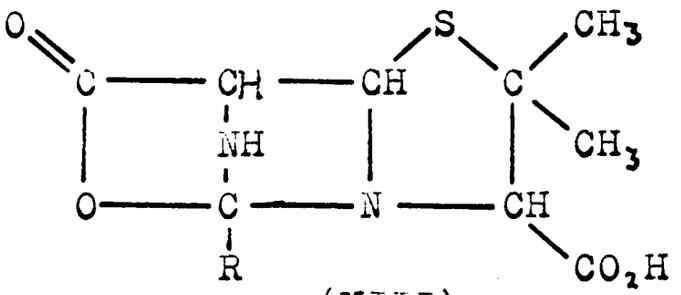
and, as a possible though less likely alternative, the β-lactam formula, (VI). At the same time Bently, Catch, Cook, Elvidge, Hall and Heilbron (18) proposed the tricyclic formula, (VII). This had to be altered, as it was possible to isolate the methyl ester of penicillamine from penicillin in which the free carboxyl group had been esterified (72). (This showed that the carboxyl group of penicillamine was free in penicillin). The tricylic formula was therefore revised to (VIII).



VI .



(VII)



(VIII)

The salient features of penicillin chemistry which had to be accounted for were;

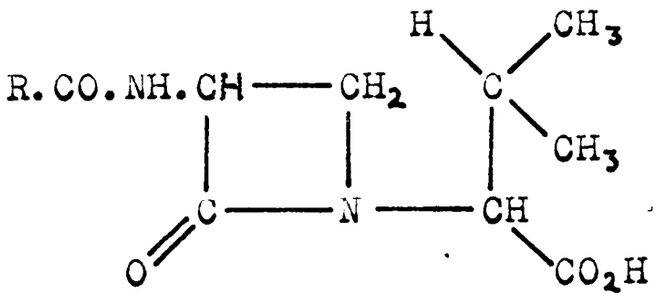
1) The absence of any basic group in the penicillin. This was most easily explained on the basis of the β-lactam formula. In the oxazolone formula (V), the nitrogen of the thiazolidine ring might reasonably be expected to be involved in electron donation towards the carboxyl group of the oxazolone, and so lose some of its basic

properties. There was no very plausible explanation for the non-basicity of the tricyclic formula.

2) The isomerisation of penicillin to penillic acid.

3) Conversion of penicillin to penicilloic-acid under mild conditions.

4) The formation of desthiopenicillin (IX)



IX

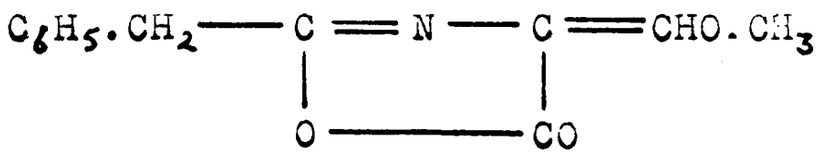
when penicillin is boiled with Raney nickel (47). This strongly supported the β-lactam formula, though the β-lactam ring in desthiopenicillin is much more stable than in penicillin itself.

There was no general acceptance of any of the possible formulae till the β-lactam structure was proved to be correct by the X-ray work of Crowfoot, Bunn, Rogers-Low and Turner-Jones (26). At the same

time there was a growing body of other evidence in favour of the β-lactam ring formulation. Woodward (68,69) and Abraham, Baker and Chain (4) supported the β-lactam formula on chemical grounds.

Penicillin Synthesis.

There were numerous attempts to synthesise all three postulated structures. Thus in the "Chemistry of Penicillin" (24, p. 861) there is a list of about 150 different attempts at ring closure from benzylpenicilloic acid and its derivatives (IV). None of these was successful, though some produced slight traces of antibiotic activity. Other synthetic routes were also tried, and benzyl penicillin was eventually synthesised in yields of about 0·1% by the condensation of penicillamine hydrochloride and 2-benzyl-4-methoxymethylene-5(4)oxazolone. (X)

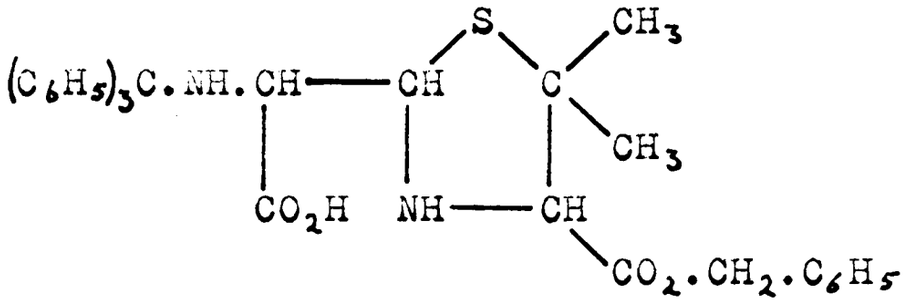


X

This was carried out in pyridine containing triethylamine and produced an inactive intermediate which was then isolated and heated in pyridine and hydrogen chloride. That one of the products was indeed benzylpenicillin was proved by radioactive tracer techniques and also by comparing the biological action of the crude product with that of benzylpenicillin on a number of different bacteria. However, this synthesis was of no practical use as the yield could not be improved, nor was it possible to elucidate the course of the reaction.

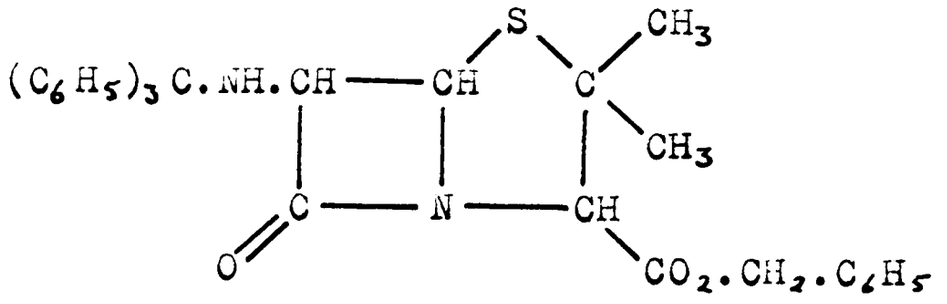
The first rational synthesis of penicillin was achieved by Sheehan and his co-workers (61). This depended on finding a ring-closing agent for the penicilloates (IV) that would not destroy any penicillin formed, and would also suppress the alternative azlactonization; using N,N'-dicyclohexylcarbodiimide in dioxane-water solution they obtained penicillin in 10% yields.

Later they described a different route involving the synthesis of 6-amino-penicillanic acid (62,63). They formed the trityl "desacylpenicilloates" (XI).



XI

On treating this with N,N'-diisopropylcarbodiimide, in cold dioxane-water solution, ring closure occurred giving the benzyl 6-tritylaminopenicillanate. (XII).



XII

The ester was saponified to the 6-tritylamino-penicillanic acid. Detritylation was then carried out using 2 equivalents of N hydrochloric acid in

isopropyl alcohol, to give 6-aminopenicillanic acid. This synthesis gave a racemic mixture; so to obtain the natural D penicillin, Sheehan ran a parallel series of reactions starting from the D- desacylpenicilloates (XI), obtained by degrading natural penicillin. The final synthetic 6-amino penicillanic acid was shown to be in every way identical to the natural product. Finally the 6-aminopenicillanic acid could be converted to any desired penicillin by reacting it with the appropriate acid chloride.

Natural 6-aminopenicillanic acid

Sometime before the synthesis of 6-APA by Sheehan (p.20) there were reports by Murao and Sakaguchi (1950,60) and Murao (1955,50) that if benzylpenicillin was added to a submerged fermentation of Penicillium chrysogonum Q 176, hydrolysis of the penicillin to 6-APA and phenyl acetic acid took place. However, this result has not been confirmed by other workers (16).

Kato (40) was the first to suggest that 6-APA is formed if no suitable side-chain precursor be added to the fermentation mixture. Later, during some work on

p-aminobenzyl penicillin, Batchelor, Doyle, Nayler and Rolinson (17) found discrepancies between biological and chemical assays for penicillin in the control fermentations to which no side chain precursor had been supplied. The assays agreed when p-aminophenylacetic acid was added as a precursor. They showed that the descrepancy was due to 6-APA which was detected by the chemical but not by the biological assay. They proved that 6-APA was present by reaction with phenylacetyl-chloride to give benzylpenicillin. The full investigation of the fermentation, purification and properties of 6-APA is described in a series of papers in the Proc. Roy. Soc. (1961) from which the following account is derived. (13, 14, 15, 16, 25 and 28).

They obtained the highest yield of 6-APA by fermenting Penicillium chrysogonum Wis 51. 20 with a maximum aeration rate at the beginning of the fermentation, and reducing this to 60% of the maximum after 64 hours. The 6-APA could not be extracted from the fermentation liquor by organic solvents, so use was made of this to remove the penicillins by solvent extraction after the culture filtrate had been reduced under vacuum to one tenth of its original volume. After

adjusting the pH of the residual liquor to 3·0, the resultant precipitate was removed, and the clarified solution extracted with n-butyl acetate. 3 vols. of acetone were then added, many of the impurities forming a semi-solid precipitate. The supernatent solution was removed and concentrated to one twentieth of the original volume of the culture filtrate. The resulting solution which had a concentration of about 1·75 mg./ml. was then fractionated on a Dowex-1 resin conditioned with hydrochloric acid. By concentrating the resultant solution under vacuum and then adjusting the pH to the isoelectric point of 4·3, crystalline 6-APA of 95% purity was obtained. 6-APA was recrystallised by dissolving it in water to give a concentration of 100 mg/ml. and adding 10% hydrochloric acid slowly with stirring till the pH was 4·3. The crystalline 6-APA was washed with cold water and cold acetone and then dried.

The crystals used in this X-ray analysis were prepared in the above way, though the crystallisation was slowed down as much as possible in order to produce the larger single crystals.

CHAPTER 3

SOLUTION AND REFINEMENT OF 6-AMINOPENICILLANIC ACID

6-Aminopenicillanic acid crystallised in flat orthorhombic plates elongated along a, which gave parallel extinction.

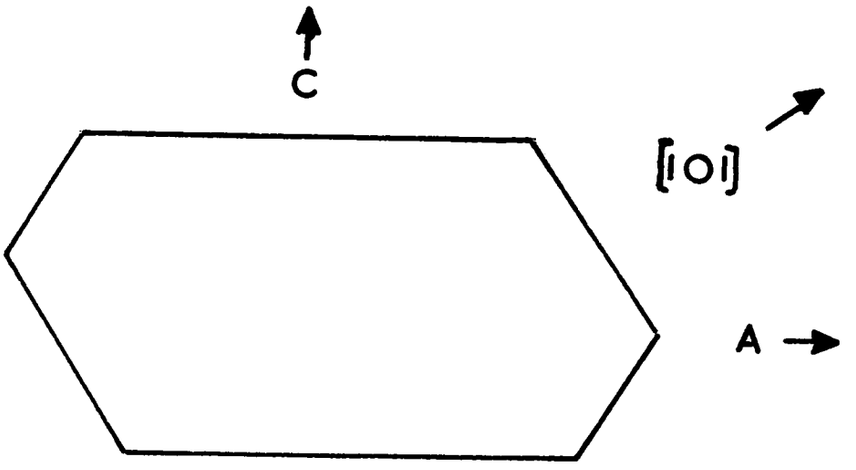


Fig. 31

Typical dimensions of the larger crystals were:

0·8	mm.	parallel	to	a
0·02	mm.	"	"	b
0·4	mm.	"	"	c

Crystal Data

Formula: $C_8 H_{12} N_2 O_3 S$

Molecular weight 216·2

$a = 6{\cdot}24 \pm {\cdot}02$ Å

$b = 14{\cdot}83 \pm {\cdot}04$ Å

$c = 10{\cdot}49 \pm {\cdot}03$ Å

$V = 970{\cdot}5$ Å^3

Dobs $1{\cdot}482 \pm {\cdot}003$ gm/cm^3

Dcalc $1{\cdot}484 \pm {\cdot}008$ gm/cm^3

$Z = 4$

Fooo = 456 electrons

$\mu = 25{\cdot}6$ cm^{-1}

Systematic absences:

h k l,	none.
h o o,	$h \neq 2n$ only
o k o,	$k \neq 2n$ only
o o l,	$l \neq 2n$ only

so the space group must be P $2_1 2_1 2_1$

CELL DIMENSIONS

6-APA gave strong reflections right out to the limit of the copper sphere. The high-angle reflections were used to measure the cell dimensions using the Straumanis method of loading the film.

In this method the $\theta=0^{\circ}$ position is found by measuring symmetrically related low-angle spots, and finding the position half-way between them . The $\theta=90^{\circ}$ position is located in a similar way. The diameter of the film is found directly from the difference between these two positions, so that shrinkage is automatically allowed for. For high-angle reflections any uncertainty in the exact value for the diameter of the films becomes much less important, as the value actually measured is $(90^{\circ}-\theta)$. Also any inaccuracy of measurement becomes less important as at high angles $\sin\theta$ changes much more slowly with θ.

In order to allow for systematic errors which normally include eccentricity of the specimen, absorption, and the film not forming an exact circle,

the value of d is plotted against some function of θ, and extrapolated to $\theta = 90^{\circ}$ (51).

Collection of Intensity Data

Most of the crystals were extremely thin, so the thickest were chosen for X-ray analysis. Even so, considerable spot-shape trouble was likely to be encountered. This problem would be worst when photographing the subsidiary axis, since this involved rotation about the medium length axis, as opposed to the main axis where rotation would be around the needle direction.

Absorption

The linear absorption coefficient for 6-APA is 25.6 cm^{-1}, so that percentage absorptions along the three axes are as follows:-

a = 87% b = 0% c = 63%

However, as the plate is in general about 0·2mm. thick, the path length along a for an angle of 5° would be ·02 mm., with an absorption of 40% ,

for 10° of 27%,

for 20° of 15%.

Thus except for a few reflections, for which either the incident or the reflected beams are along the crystal axis, absorption is not important. It was felt that any absorption corrections would not be worth the effort involved; most of the reflections having a large absorption, also have a very small area on the film. For these reflections any spot-shape correction would decrease the intensity, whereas any absorption correction would increase it. So the result of omitting both corrections is to leave an error usually less than that due to either spot shape or absorption alone.

The intensities of the reflections were measured using the multiple-film equi-inclination Weissenberg

technique, with three films in a pack. The following layers were collected:-

main axis, 0kl - 4kl; subsidiary axis, hko - hk8; a total of 988 independent reflections were measured out of a possible 1170 in the copper sphere.

For each layer a long exposure of about 4 - 6 days was taken, together with a short exposure of about 12 hours. Two intensity strips were prepared for each axis, one for the lower layers and one for the higher ones. The intensities were measured visually, and film-to-film ratios calculated from reflections measured on two films. On scaling up reflections common to more than one film, greatest weight was given to measurements made nearest the middle of the intensity scale.

Lorentz, polarisation, and inclination corrections were calculated on the Mercury computer, using a programme written by Dr. C. K. Prout. This applied the

function,

$$Lp^{-1} = \frac{\sqrt{\sin^2\theta - \sin^4\theta - (\xi^2/4)}}{1 - 2(\sin^2\theta - \sin^4\theta)}$$

which allows for all the above corrections at the same time.

Layer scaling

As a result of differing exposure times, and also of using different intensity scales for the various layers, each layer was on a different and unknown scale. In order to correlate these, the ratios between reflections common to two different layers were used.

If the errors have a normal distribution the best estimate of the scale factors is obtained using a least-squares procedure. In this the sum $R = \Sigma \omega_i \Delta_i^2$ is minimised, where Δ_i is the error in any particular equation and ω_i is the weight attached to it.

Dr. John Rollett has written a programme for solving

these equations given all the values of the type $\sqrt{\omega}\, I_{az}$ and $\sqrt{\omega}\, I_{za}$, where I_{az} is the sum of the intensities (or some function of the intensities) on layer a that are common to layers a and z. As there will be the same number of unknowns as equations in the least square procedure he has added the additional condition that the sum of the square of the scales should be a constant (59).

The simplest way of determining the observational equations consists of taking all the reflections common to layers a and b, forming $\sum I_{ab}$, and $\sum I_{ba}$. This is equivalent to putting ω for each individual reflection equal to I.

This gives the greatest weight to the most intense reflections which are those most liable to suffer from extinction and absorption effects. Also they are on the bottom film, so that any error in film-to-film ratio will have a large effect on them.

Therefore if least-squares techniques are used, where each reflection is given its own weight, the scale factors should preferably be determined by giving the greatest weight to the films having the largest number

of reflection on them.

Thus a more satisfactory scheme is to give each reflection equal weight. In order to do this it is necessary to calculate the ratios between the intensities observed on the two different axes for each common reflection .

In this case it was decided to write a programme to do this (Appendix), and to print out the results in a form suitable for input into Dr. Rollett's "scale two-axis data" programme. This latter was modified so that any individual reflection can easily be removed from the equations if desired.

The programme also forms the common I, subject to limitations of maximum and minimum I and ξ : and prints these in suitable form for "scale two-axis data".

Each reflection was then multiplied by the appropriate layer scale factor. When a reflection occurred on two different layers the mean of the scaled intensities was taken. This was done using a programme written by Dr. T. F. Lai.

Some time later Dr. C. K. Prout wrote an experimental programme to carry out all these operations from the

observed intensities to the final set of correlated and sorted F's in one operation. This programme also contained a facility for applying individual weights to each reflection. As there is no convenient method for determining the real errors in visually measured data, the scheme used was one that was considered reasonable, giving the highest weights to those reflections observed in the middle range of the intensity strip on the most densely populated film, and giving lowest weights to the two extreme ends of the final intensity range. The faintest spots could not be measured accurately, and the most intense were liable to extinction errors, and were also liable to be affected by any cumulative errors in film-to-film ratios. The scale factors obtained in this way were used in the last three isotropic rounds of least-squares refinement in which individual weights were given to each reflection.

During the refinement it was also decided to use scale factors derived from the calculated structure factors. For this purpose a programme was written to carry out an agreement analysis layer by layer using the uncorrelated F^2 from each axis (Appendix).

Table 3.1 shows the different scale factors obtained by the various methods. In general the different methods agreed to within 10% of each other, apart from the layers HK7 and HK8, which have comparatively few common reflections.

Table 3.1

Layer scales derived by the different scaling methods

Layer	Method of scaling a	b	c	d	e	f
0 K L	1.78	2.51	2.52	2.39	2.45	(g)
1 K L	1.32	1.19	1.64	1.70	1.63	1.83
2 K L	0.63	0.61	0,69	0.71	0.73	0.73
3 K L	0.40	0.36	0.41	0.44	0.47	0.48
4 K L	0.53	0.49	0.58	0.61	0.65	0.63
H K 0	0.53	0.37	0.53	0.62	0.62	0.59
H K 1	0.89	0.79	1.05	1.06	1.08	0.99
H K 2	0.30	0.28	0.31	0.33	0.36	0.26
H K 3	0.57	0.38	0.71	0.81	0.78	0.73
H K 4	0.48	0.43	0.54	0.50	0.54	0.49
H K 5	0.50	0.47	0.49	0.52	0.59	0.51
H K 6	0.38	0.31	0.34	0.35	0.38	0.40
H K 7	0.90	1.05	1.11	1.10	1.15	1.43
H K 8	1.04		1.07	1.22	1.19	1.81

a) derived from F^2 using the 'scale two axis data' programme.

b) derived from individual ratios using the 'scale two axis data' programme.

c) as b, but bad reflections removed.

d) from ALS 7 using 'agreement analysis on original data'.

e) as d but using ILS 4.

f) scales derived from DR o6 (p.34)

g) A remeasured set of intensities for OKL were used for this set.

Structure Determination

The absolute scale and the temperature factor were determined from two-dimensional Wilson plots of the HKO and OKL zones.

Patterson sythesis

In order to increase the resolution obtainable from the Patterson synthesis it was decided to sharpen the data. The danger of doing this to excess is that the Fourier series is no longer convergent, and so it is possible to get spurious effects from the diffraction ripples introduced.

S. Abrahamsson (10, 36) has done some experimental work on this with the closely related crystal structures of cephalosporin C and Penicillin V. He found that using a sharpening function of the form

$$Fm^2 = \frac{F^2}{\hat{f}^2} \exp.(K \operatorname{Sin}^2 \theta) \qquad 3.1$$

where $\hat{f}$ is a scattering factor either for one of the constituent atoms, or for a weighted mean, no serious diffraction effects occurred with $K = 2B/\lambda^2$, i.e. sharpening to point atoms at rest. He then calculated a series of syntheses with increasing values of K. Even when the outer terms were increased to 50 times their

value when $K = 2B/\lambda^2$ the Patterson synthesis still retained the same basic features. The heavy atom peaks showed up more clearly, and not till very heavy sharpening was used did diffraction ripples become a serious problem.

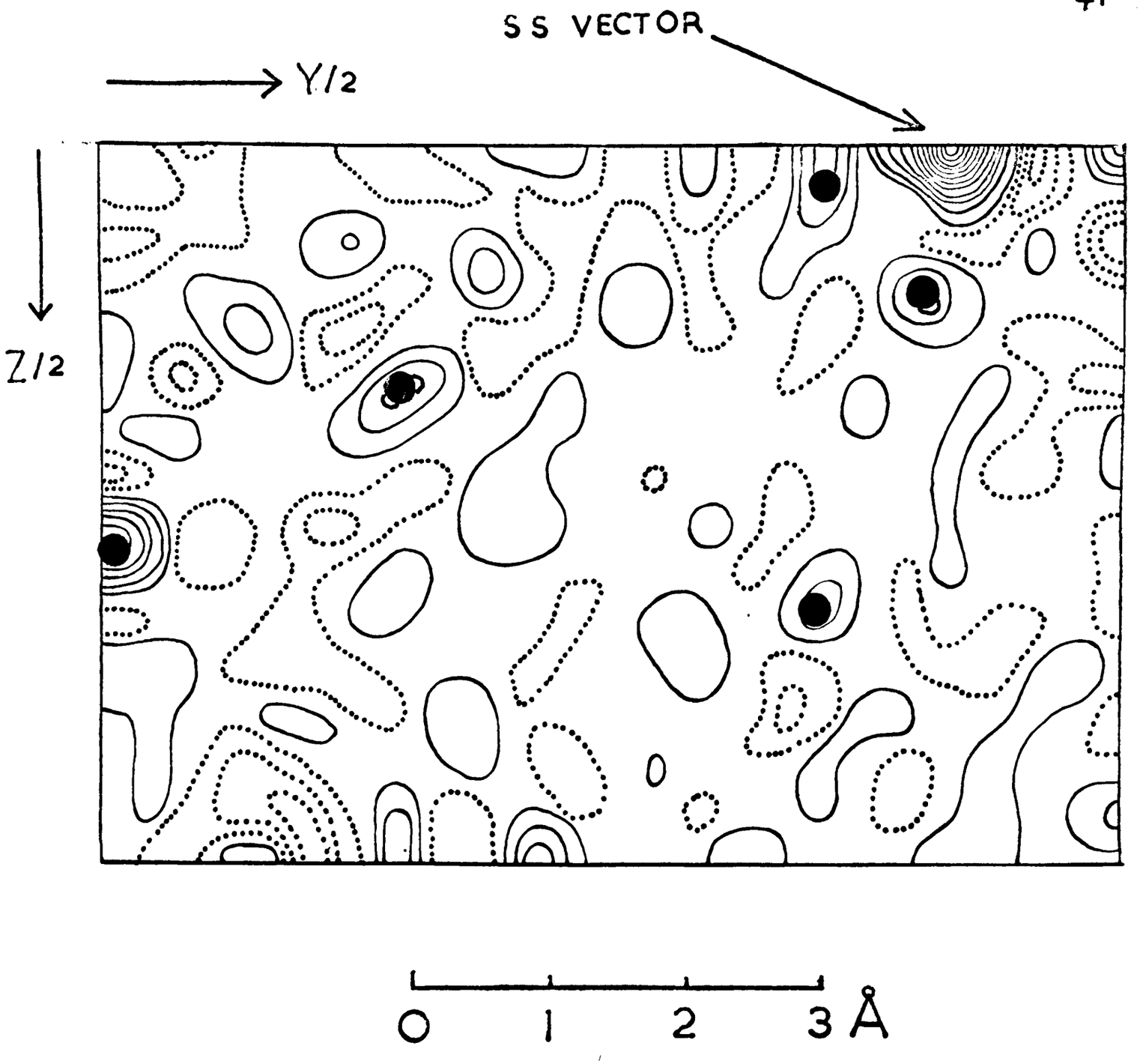
The form factor for sulphur drops off more slowly with increasing θ than that of the light atoms, so by giving greater weight to the outer terms the S-S vectors would be enhanced in relation to light atom - light atom vectors, and S-light atom vectors, making them much easier to pick out.

A Patterson series was calculated using terms modified by the expression 3·1, with K = 3, and the $\hat{f}$ = f carbon, which corresponds to sharpening to point atoms at rest.

The vectors between atoms in the space-group $P\ 2_1\ 2_1\ 2_1$ form the group P mmm, so the Patterson synthesis was calculated from 0 - ½ in x,y, and z.

Vectors between symmetry-related atoms will be:-

½ + 2x	2y	½
½	½ + 2y	2z
2x	½	½ + 2z



● Sulphur to light atom vectors in the section.

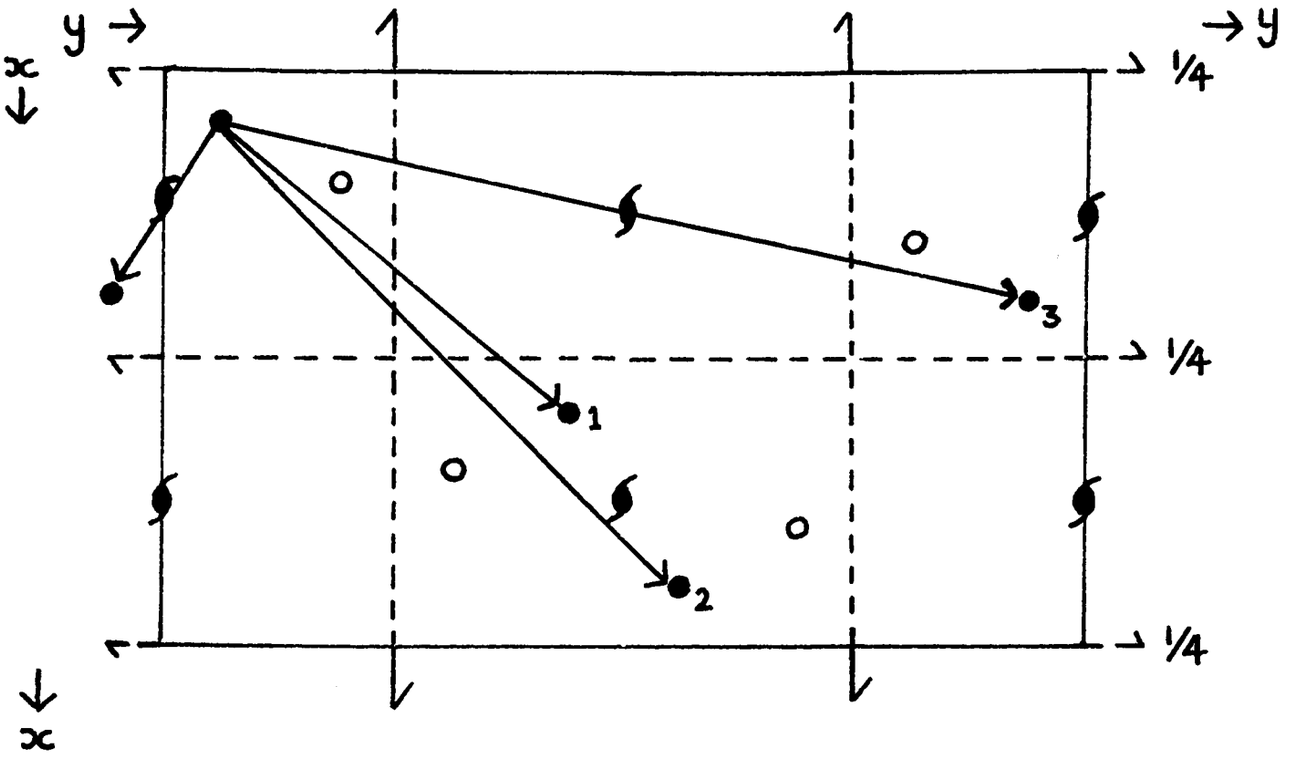
Figure 3.2 6-APA. Harker section at x = ½.

These fall on the three sections at

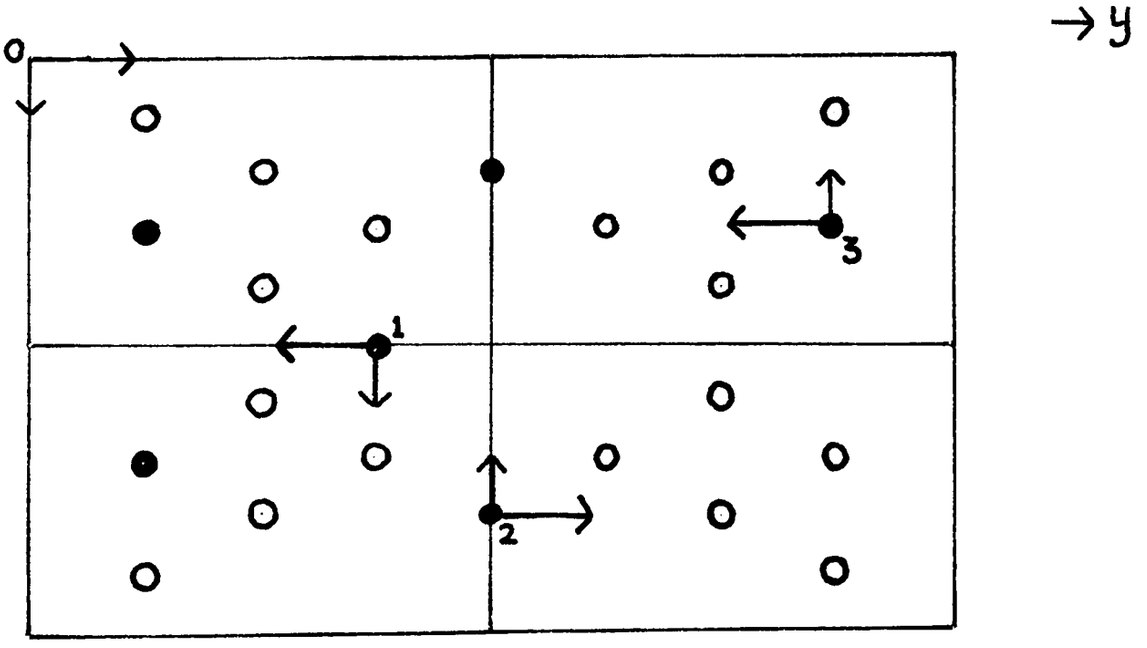
$$x = \tfrac{1}{2}, \text{ (fig. 3 2)}, \quad y = \tfrac{1}{2}, \quad z = \tfrac{1}{2}.$$

The S - S peaks were easily picked out, they correspond to a sulphur position at x = .045, y = .0396, z = 0 cell translations. As the peaks at $x = \tfrac{1}{2}$. $y = \tfrac{1}{2}$ are on special positions these have double the weight of the other single S - S vector.

Knowing the position of the heavy atom it was possible to construct a superposition function on the symmetry-related sulphur peaks (Lipson and Cochran (73) Chap. 6, Buerger (22)). If the origin of the Patterson is placed successively on the S - S vectors which correspond to the view of the other three symmetry related S peaks from the first one, then a sulphur-light atom peak related to sulphur 1, will superimpose on the corresponding S_2 - light atom peak, and similarly on S_3 and S_4 (Fig. 3.3). If the Pattersons are oriented as indicated in the diagram then the peaks will correspond in position to the real space diagram. If they are all kept in the same orientation the positions will have opposite signs. The reason for this can be seen from the diagram. The superpositions at 1 and 3 are



Real space



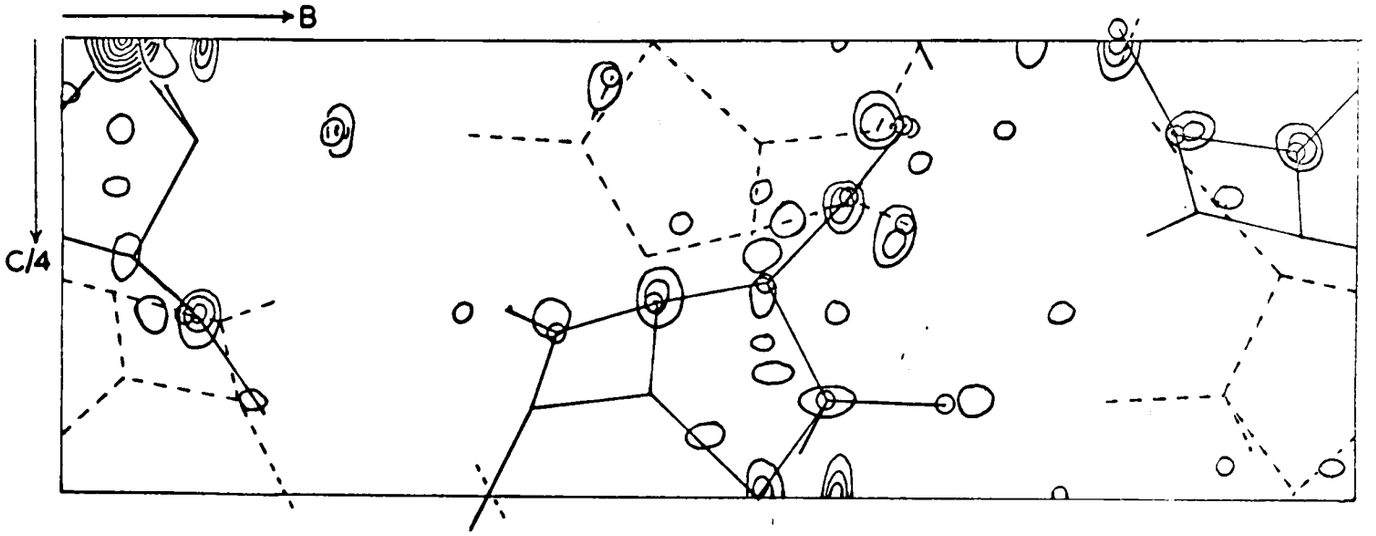
Vector space

● heavy atom, heavy-heavy atom vector.

○ light atom, heavy-light atom vector.

Figure 3.3

reversed in the y direction; at positions 0 and 2 the diagram is symmetrical with respect to y, so that the coincidences will occur with an opposite y co-ordinate from that which would occur if the indicated rotations had been made. Similar arguments apply in the other two directions. As there is no crystallographic way of telling the absolute configuration of the molecule apart from anomalous dispersion it is of no crystallographic importance which convention is finally adapted. The configuration given here is the correct absolute configuration (10). If $\rho_0, \rho_1 \cdots \rho_n$ are the values of the constituent Patterson densities at any point where they all superimpose, then various functions can be formed. These are usually the product function $\rho_0 \rho_1 \rho_2 \cdots \rho_n$, the sum function $\sum \rho_0 + \rho_1 + \rho_2 + \cdots \rho_n$ and the minimum function which is the minimum value of any of $\rho_0, \rho_1 \cdots \rho_n$. This latter can be drawn very easily by hand as it simply involves contouring in all those areas which are surrounded by the same number of contours from each superposition. This is most easily done by forming the superposition 0 - 1 in one colour, then the superposition between this and 2 in another and



o Positions of atoms in the sections.

Fig. 3.4.

6-aminopenicillanic acid sections from the minumum function between x = 0 and x = ½. The mirror related molecules are shown dotted.

so on. The mimumum function is then surrounded by contours of all possible colours.

A minimum function was plotted in the way described; however, the z co-ordinate of the sulphur atom was effectively zero. This meant that the result of the superposition map was to give two images of the molecule one on top of the other related by a false mirror plane at z = 0. At the same time a minimum function was calculated on the Mercury computer using a program written by Dr. S. Abrahamsson. (Fig. 3·4)

A later analysis of the superposition peak heights when the structure had been solved gave the following results (Table 3·2)

Table 3·2

Analysis of peak heights in the minimum function

Value of Patterson function (arbitrary scale)	True peaks,	Spurious	Diffraction ripple
>200	3	-	1
150-200	7	-	2
100-150	4	19	4

From this minimum function the positions of the various atoms were found. As the general form of the

penicillin nucleus had already been determined, use was made of this knowledge in breaking down the false mirror symmetry. First of all the two atoms next to the sulphur were found, and from these the general orientation of the thiazolidine ring was fixed. It was then a simple matter to find the remaining atoms of the ring. The positions of all the other atoms were determined roughly in the same way.

They were all then recalculated from the original Patterson peaks. Where there was no overlap between adjacent peaks this gave four independent estimates of each co-ordinate of any one atom. However, there was usually a certain amount of overlap, so that often only two accurate co-ordinates could be obtained from one peak. This meant that as a rule two or three estimates were made of any one co-ordinate. Compared with the final positions these co-ordinates showed the following differences:-

co-ordinate:-	x	y	z
average deviation:-	0·09Å	0·05Å	0·08Å
maximum deviation:-	0·3Å	0·12Å	0·3Å
atom for which max. deviation occurred:-	C_{11}	C_5	N_{14}

The Patterson peak due to N_{14} had its centre on the mirror plane at z = 0. The peak was, however, elongated in the z direction, indicating that it was in fact due to the superposition of two peaks with small and opposite z co-ordinates. Thus though it was possible to tell that N_{14} had a small z co-ordinate, it was not possible to apply a sign to it, or to measure it properly. Rather than guess its co-ordinate on chemical grounds it was decided to leave it at zero, even though this would inevitably involve some error.

The above discussion shows that in this case, had there not been a false mirror plane, an automatic refinement process which merely picked the highest peaks would have solved this structure.

Fourier refinement

The resulting structure was chemically reasonable, and there were no short non-bonded contacts apart from the N - H.....O distances in the hydrogen bond system. This system was associated with the two-fold screw axis parallel to a(p. 86). The bond distances were compared with those to be expected from general experience (Sutton, 65), and with those found by Pitt (55) for

potassium benzyl penicillin.

The average deviation from the expected value was ·12Å . There will of course, be some uncertainty in the choice of "standard" distance. However, this will usually be much smaller than the errors in the proposed structure in the early stages of the refinement. These differences were considered acceptable at this stage.

A set of three-dimensional structure factors were calculated using the positions obtained from the Patterson. These gave a residual or R factor of 25%, the formfactors used were those given by Vaciago (66) for sulphur, by Freeman (33) for oxygen and nitrogen, by Berghius et al. (19) for carbon, and McWeeny (46) for hydrogen. Two Fourier synthises were then calculated ($\rho_{p.obs.}$, $\rho_{p.diff.}$), one using the observed terms and the other the difference between the observed and calculated terms.

When the calculated structure factor was less than a quarter of the observed value, the term was excluded from the summation, as the phase was unlikely to be correct.

In the resulting observed Fourier map all the atoms

that had been put in came up at reasonable heights of between 9 and 15 $e/Å^3$ for C N, and O. In the difference Fourier there were several peaks of about 1 $e/Å^3$. Any atom that had been left out would be expected to give a peak of about half its final value. if the rest of the structure were correct. (Luzzati (44), Hodgkin and Trueblood (37). So these small peaks could not correspond to possible atomic sites, but were most probably due to random errors in the phases.

The position of the centre of the peaks on the observed Fourier synthesis were found using a programme written by Dr. Sparks. This derives the best fit of a Gaussian ellipsoid to the 19 points surrounding the peak. The centre of the ellipsoid is then taken as the centre of the peak.

Later on these results were used to find out what value of n (n - shift, rule Schoemaker et al. (64)) would have given the best convergence. The average value of the best n - shift for each of the 14 atoms came to 2·1, in reasonable agreement with theory.

The overall temperature factor was adjusted by means of a plot of $\ln \left(\Sigma |F_{obs.}| \,/\, \Sigma |F_{calc}| \right) / \sin^2\theta$ for

a number of ranges of $Sin^2\theta$, in a similar fashion to a Wilson plot.

Least-squares technique

The least squares programme used in the refinement of both the structures described in this thesis was one written by Dr. J. S. Rollett (48) for MERCURY (ALS). This uses the block diagonal least squares approximation to the normal matrix. Down the main diagonal alternate 3 x 3 blocks for position parameters and 6 x 6 for thermal vibration parameters are calculated, together with a 2 x 2 matrix for overall scale and temperature factors. In this system the interactions between different atoms are ignored, as are the interactions between position and temperature factors for any one atom. The temperature factor applied is

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

Later an isotropic version of this programme (ILS) became available in which the temperature factor was represented by the single variable B in the expression

$$e^{-B\ Sin^2\theta/\lambda^2}$$

Ideally each reflection should be given a weight inversely proportional to its standard deviation. With visually estimated data there is no convenient means of measuring this for any individual reflection. So it is common to assume that the errors are some function of either Fo or θ, and to calculate a weight for each reflection according to some simple scheme. As in the case of individual reflections the weight should be inversely proportional to the standard deviation, for any group of reflections. The true standard deviations are unknown; however, if the value of the difference between observed and calculated structure factors is taken as Δ, and the average value of the standard deviation for any range of Fobs. is $\bar{\sigma}$, then $\bar{\sigma} \propto \sum \left(\frac{\Delta^2}{n}\right)^{\frac{1}{2}}$

This value of $\bar{\sigma}$ will allow for any errors in $F_{calc.}$ as well as $F_{fobs.}$

John Rollett's programme has three different weighting schemes available.

$\sqrt{w}$ =

1) $|F_o| / F^*$ if $|F_o| < F^*$

$F^* / |F_o|$ if $|F_o| \geq F^*$

2) 1 if $|F_o| < F^*$

$F^*/|F_o|$ if $|F_o| \geq F^*$

3) $$\sqrt{\frac{1}{1 + \{(|F_o| - b)/a^2\}}}$$

where a, b and F^* are pre-set parameters.

As the only programme available for agreement analysis totalled only $|\Delta|$, not Δ^2 ; $\Sigma|\Delta|$ was taken instead of $(\Sigma\Delta^2)^{\frac{1}{2}}$, and the weighting scheme chosen so that as far as possible $\omega|\Delta|$ for any range of $F_{obs.}$ is constant. The weighting scheme initially chosen was 2 with a value of F^* of 22 electrons on the absolute scale.

At a later stage in the refinement a convenient method of applying individual weights became available (p.34). These individual weights were used in the last three cycles of least squares refinement.

Standard Deviations

The standard deviations were calculated from the variances of the normal equations obtained from the least squares programme. If it is assumed that

the values of Δ are a measure of σ, then

$$\sigma_i^2 = \frac{V_{ii}\Sigma\omega\Delta^2}{(m-n)} \qquad \text{(Cruickshank(27)}$$

where V_{ii} is the variance of parameter i, m the number of observations and n the number of parameters refined. This is only true if ω has been chosen so that for any systematic group of $F_{obs.}$, $\overline{\omega\Delta^2}$ is constant,

As only the terms in the diagonal blocks are taken into account in calculating the standard deviation, the value derived must be lower than the true value, the magnitude of the difference depending on the size of the off diagonal terms that have been ignored and of any systematic errors that are present. The figures quoted in this thesis have been calculated using the block diagonal approximation. Though the relationship of these figures to the true standard deviations is unknown, it is unlikely that the true positional standard deviations are more than twice the calculated figures, even allowing for possible systematic errors. The true standard deviations of the thermal parameters are likely to

be much larger than the calculated ones, as spot shape, absorption, and extinction errors are all compensated for by false thermal parameters, so that the calculated standard deviations are of no real significance.

Least-squares refinement

A total of 16 rounds of least squares refinement were carried out on 6-APA. (table 3·3) These were divided into 9 anisotropic rounds and 7 isotropic rounds, individual weights being used for the last three rounds. Hydrogen positions were introduced at the end of the fourth anisotropic round (ALS4).

Table 3.3 Progress of Refinement

Stage	Fourier	R Value %	Shift (Å) Av.	Max.
Positions from Patterson	ρ_{obs} ρ_{diff}	25		
Positions from 19 pt. Routine		22.2		
1st (Anisotropic) least squares refinement weight 2, $F^* = 22e$		15.6	.039	.21
ALS 2		11.6	.011	.053
ALS 3	$\rho_{3\ diff}$	11.5	.0054	.025
ALS 4, (hydrogen atoms added after this)		10.6	.0031	.009
ALS 5		10.4	.0061	.020
ALS 6		10.0	.0034	.015
ALS 7	$\rho_{7\ diff.}$ phased on heavy atoms only. $\sin^2\theta \leq .75$	9.8	.002	.009
2nd set of correlated F^2 used				
ALS 8		9.7	.005	.015
ALS 9			.001	.0043
Positions from ALS 9 with isotropic temperature factors		11.5		
ILS 1		11.6	.005	.015
ILS 2		11.1	.0015	.006
ILS 3 weight 3, a = 10, b = 4		11.1	.0045	.017

Table 3.3 continued.

Stage		Fourier	R Value %	Shift (Å) Av.	Shift (Å) Max.
ILS 4			11.9	.002	.007
ILS 5	individual weights		11.8	.0067	.025
ILS 6			11.8	.0027	.01
ILS 7		Final obs and diff. Fouriers.	11.7	.0015	.005

Using the positions derived from the first Fourier maps (ρ_{obs} and ρ_{diff},) four consecutive cycles of anisotropic least squares refinement were carried out, during which the R value fell from 15·6% to 10·6% (hydrogen atoms were introduced in the last of these rounds). The temperature factors were all reasonable, varying between equivalent isotropic temperature factors of around 2Å^2 for the ring atoms and up to 4 2 Å^2 for some of the side groups. There were no very high or low values indicating either a wrongly placed atom, or a wrongly assigned atom type.

All the bond lengths were within ·05Å of the expected values; the following bonds had the greatest diviation from these values (table 3·4)

Table 3·4

Bond	Length (Å)	Expected Value(Å)
$S_1 - C_5$	1·87	1·81
$C_{11} = O_{13}$	1·25	1.26 a or 1.23 b
$C_{11} - O_{12}$	1.29	1.26 a or 1·36 b
$C_2 - C_{10}$	1·50	1.54
$C_2 - C_9$	1.57	1.54

a) if carboxyl group is ionized

b) of carboxyl group is unionized

At this stage it was considered worth while to introduce all the hydrogen atoms, although it was not possible to find well defined positive peaks in all the positions. (The location of the hydrogen atoms is described more fully on page 64).

A further three cycles of least squares refinement were carried out (rounds ALS 5,6,7) with the hydrogen atoms included in the structures factor calculation, but not in the least squares refinement, during which the R value fell to 10%. The temperature factor of the hydrogen atoms was fixed at 4Å^2, the value of the largest temperature factor in the rest of the molecule; the exact value is not significant as at Sin θ = ·5 the difference between the structure factor contribution of a hydrogen atom with a B of 4Å^2 and one of 5Å^2 is 0·006 electrons and at Sin θ = 1, 0·001 electrons.

The average shift was now 0·002Å, compared with 0·003Å in round ALS4. However, the maximum shift was still 009Å ; of the same order of magnitude as the standard deviation. There was no very significant shifts in bond length, the largest was an decrease of ·02Å in C_2 - C_{10} from 1·506Å to 1·485Å . Since the shifts were still the same size as the standard deviations it was considered worth while to continue refinement.

As the agreement in structure factors was now good, it was decided to redetermine the layer scales by comparison with the calculated structure factors. This was done using the method described on page 34 . This second set of intensities was used in round ALS 8 onwards. A difference Fourier was calculated using these intensities in which only those terms for which $Sin^2\theta < .75$ were included; it was hoped that this would increase the resolution of the hydrogen atoms. (p. 64)

Using the second set of correlated F^2 two rounds of least squares refinement were carried out (ALS 8 and 9) By this time the maximum shift had fallen to a third of the standard deviation, so that the anisotropic refinement was finished at this point. The final positions and temperature factors are given in tables 3.5 and 3.6 on page 69, and the magnitudes and directions of the vibrational ellipsoid in table 3.7.

Isotropic Refinement

By this stage an isotropic least squares programme was available. As experience of other workers at Oxford (Armstrong and Prout, 12) had been that this

produced slightly better bond lengths and angles, it was decided to carry out a number of rounds of isotropic least squares. This procedure is probably safer if there is only a small amount of anisotropy, as in the anisotropic case many systematic errors such as absorption and spot shape errors can be compensated for by erroneous anisotropic temperature factors.

Two rounds of least squares were carried out with the original weighting scheme; for rounds ALS 3 and 4 the scheme was changed to 3 with a = 10e b = 4e as this gave more nearly constant values of $\overline{\omega\Delta^2}$ (p.52). The initial value of the temperature factor for each atom was obtained by averaging the equivalent isotropic temperature factors in the three axial directions. The R value rose from 9.7% to 11.5% on applying isotropic temperature factors and fell to 11.1% at the end of ILS 4.

Individual weights

As mentioned on page 34 , Dr. C. K. Prout has written a programme to perform the whole scaling and sorting process in one operation; it also incorporates a facility for giving each reflection an individual weight. In order to see if this procedure made any significant difference, at set of F^2 were prepared using individually weighted data. Three rounds of least squares were carried out using this data (ILS 5,6,7). The final positions are shown in tables 3.5 and 3.6 on page 69. Table 3.8 shows the differences between the positions from ALS 9, ILS4, and ILS7 together with the average standard deviations.

The differences are consistent with the standard deviations, and it cannot be said that any of these three sets of positions are significantly different. The change of weighting scheme in ALS 5-6 seems to have had more effect than the change from anisotropic to isotropic

temperature factors. The small change in R value between the anisotropic and isotropic refinements, shows that for most atoms there is not sufficient improvement in agreement to warrant the five additional parameters per atom required for anisotropic refinement. This may not be the case for the methyl and carboxyl side groups where the anisotropic temperature factors vary in a chemically sensible fashion, the direction of the minimum of the vibration ellipsoid being along the bond direction.

The bond distances and angles derived from the various different refinement procedures are not sufficiently different for any one set to be picked out as being better than the others, especially as there is no group like a benzene ring whose bond distances and angles are well known, that can be used as a check on accuracy. The differences in the standard deviation between the different series of refinements were less than 10%

The results from the DR 06 data using individual weights have been taken as the final set of positions as it was felt that this weighting scheme is better than the others, and the additional parameters required

for anisotropic refinement were not justified by the improvement in agreement.

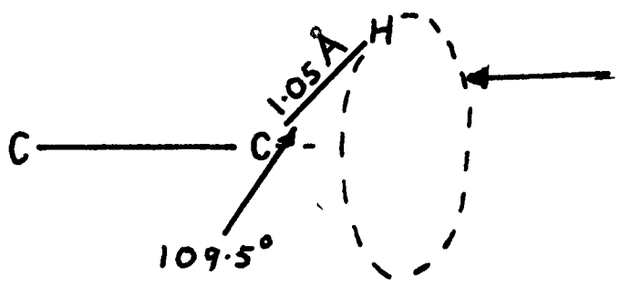
Determination of Hydrogen Positions

As was mentioned on page 59 the positions of the hydrogen atoms was introduced at the end of ALS 4. These positions were derived from a difference Fourier ρ_3. The three tertiary hydrogen atoms could be placed fairly accurately from chemical knowledge; the corresponding peaks in the difference Fourier were easily picked out. The two methyl groups were more difficult to place as their peaks were not as well defined as the other hydrogen atoms. In particular there was only a very small peak in a position corresponding to one of the hydrogens attached to C_{10}. The difficulty in picking up these hydrogen atoms may be partly due to the higher thermal motion of the side goup atoms to which they are attached, and partly to the possibility of a large rotational vibration.

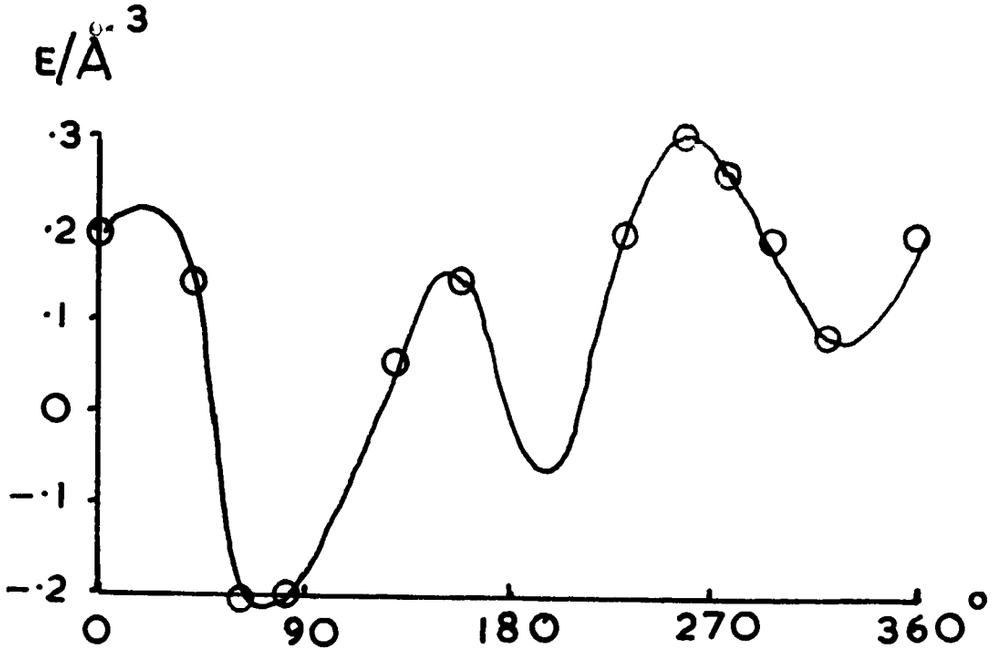
Later another difference map was calculated using the positions from ALS 6; for which $Sin^2\theta < .75$. As the limited Fourier excludes all those terms for which

the hydrogen contribution is negligible, the hydrogen peaks should show up on this slightly more clearly than on the full Fourier.

The hydrogen position which had not appeared in ρ_3, did not appear either in this Fourier map as a peak. So the electron density around the circumference of the circle, which would be described by the hydrogen atoms attached to C_{10} rotating around the bond C_2 - C_{10}, was plotted out.



This should go through three peaks, each separated by 120°, if the hydrogen atoms do in fact give peaks on the Fourier. Figure 3.5 shows that for C_{10} there can be no doubt of the correct orientation of the methyl group and that in fact all three hydrogen atoms



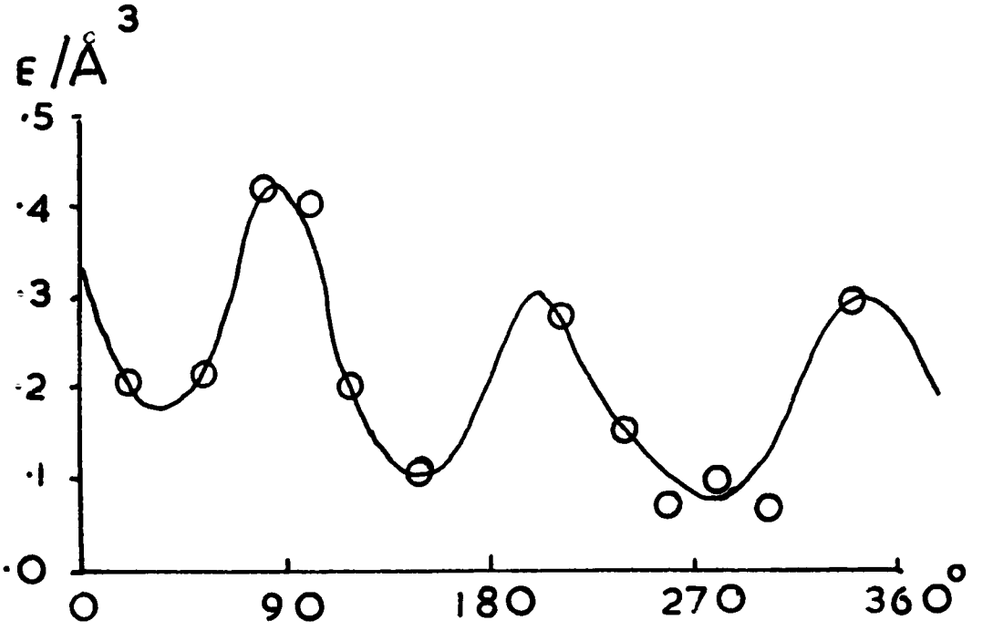


Fig. 3.5 Graph of electron density around C_{10}.

Fig. 3.6 Graph of electron density around N_{14}.

do show up and that the absence of a positive peak for one of them is due to the lower background at this point. As would be expected both methyl groups are in the staggered configuration.

As one of the peaks that had been assumed to be part of a NH_3^+ group (N_{14}) had fallen in height, the same procedure was adopted as for C_{10}; the results for this are shown in fig. 3.6. This shows that there is a peak in the correct position, however as there are a number of spurious peaks at about this height it cannot be taken as conclusive proof of the presence of hydrogen atoms.

Table 3.9 gives the electron density at the positions finally chosen for the hydrogens. As these were largely fixed by geometric considerations these positions do not in general coincide with the peak maximum; this is usually displaced by up to 0.3Å from the quoted positions. As the general background level is about the same order of magnitude as the hydrogen peak heights this is not very surprising. The hydrogen atoms are numbered by multiplying the number of the atom to which they are bonded by ten, and if there is

more than one hydrogen bonded to one atom then they are distinguished by adding 0, 1, or 2. For example, one of the hydrogens in the methyl group of C_9 would be H_{91}.

Table 3.5 6-APA Final Co-ordinates from ALS 9 and ILS 7

	x/a		y/b		3/c	
ATOM	ALS	ILS	ALS	ILS	ALS	ILS
S1	1.0420	1.0412	0.0414	0.0414	-0.0011	-0.0014
C2	0.8373	0.8371	0.0974	0.0975	0.1016	0.1005
C3	0.8556	0.8564	0.0517	0.0515	0.2353	0.2354
N4	0.9553	0.9561	-0.0350	-0.0351	0.2133	0.2135
C5	1.1263	1.1241	-0.0413	-0.0406	0.1179	0.1183
C6	1.0676	1.0675	-0.1418	-0.1419	0.1006	0.1017
C7	0.8714	0.8732	-0.1176	-0.1195	0.1829	0.1831
O8	0.7019	0.7007	-0.1557	-0.1553	0.2096	0.2082
C9	0.6140	0.6141	0.0765	0.0757	0.0448	0.0437
C10	0.8750	0.8779	0.1972	0.1973	0.1019	0.1022
C11	0.9942	0.9935	0.1065	0.1073	0.3271	0.3266
O12	0.8920	0.8919	0.1548	0.1547	0.4080	0.4078
O13	1.1900	1.1896	0.1017	0.1020	0.3169	0.3173
N14	1.0250	1.0273	-0.1764	-0.1763	-0.0275	-0.0280

Table 3.5 6-APA Final Co-ordinates from ALS 9 and ILS 7

	x/a/a	y/b	3/c
H30	0.7020	0.0430	0.2735
H50	1.1731	-.1844	0.1506
H60	1.2832	-.0313	0.1536
H90	0.4955	0.1046	0.1025
H91	0.6048	0.1013	-.0488
H92	0.5936	0.0058	0.0404
H100	1.0275	0.2114	0.1397
H101	0.8649	0.2229	0.0088
H102	0.7608	0.2286	0.1613
H140	1.0400	-.2430	0.0500
H141	1.1500	0.1750	0.0930
H142	0.8800	-.1520	-.0600

Table 3.6 6 APA Temperature Factors

Anisotropic

$$\exp- 10^{-5}(B_{11}h^2 + B_{22}k^2 + B_{33}l^2 + B_{23}kl + B_{13}kl + B_{12}hk)$$

Isotropic $\exp-(B \sin^2\theta \quad ^2)$

Atom	B_{11}	B_{22}	B_{33}	B_{23}	B_{13}	B_{12}	Isotropic
S1	1631	259	556	131	326	-19	2.66
C2	1379	294	384	118	-658	-143	2.56
C3	1217	141	518	-255	-385	-149	1.74
N4	2025	147	600	-137	195	76	2.44
C5	1513	217	656	-337	130	225	2.46
C6	2064	207	599	-40	72	392	2.46
C7	2431	252	380	65	639	102	2.95
O8	2064	207	599	-40	72	392	3.92
C9	1892	412	395	-252	-657	235	3.04
C10	2886	187	880	129	204	43	3.36
C11	2713	211	488	75	-344	-215	2.17
O12	2002	198	607	-277	-11	-69	2.66
O13	1166	394	821	-346	-444	-57	3.30
N14	1589	242	290	20	97	37	2.38

Table 3.7. Root mean square displacement in the three principle directions of the vibrational ellipsoid.

Atom	$\left(\overline{U^2}\right)^{\frac{1}{2}}$ (Å)	Direction cosines with respect to x	y	z
S1	0.195	0.60	0.36	0.71
	0.176	0.68	-0.70	-0.22
	0.151	0.42	0.61	-0.67
C2	0.200	-0.60	0.62	0.51
	0.169	0.54	0.78	-0.31
	0.113	-0.599	0.09	-0.80
C3	0.189	-0.33	-0.37	0.86
	0.158	-0.86	0.49	-0.15
	0.089	-0.49	-0.79	-0.36
N4	0.201	-0.98	-0.03	-0.20
	0.186	-0.19	-0.29	0.94
	0.121	0.08	-0.96	-0.28
C5	0.212	-0.07	-0.55	0.83
	0.178	-0.94	-0.24	-0.24
	0.117	0.33	-0.80	-0.50
C6	0.211	-0.92	-0.39	-0.04
	0.183	0.01	0.14	0.99
	0.137	0.39	-0.91	-0.13
C7	0.228	0.93	0.13	0.33
	0.167	0.17	-0.98	-0.10
	0.131	-0.31	-0.15	0.94
O8	0.291	0.71	-0.40	0.58
	0.198	-0.22	0.66	0.72
	0.150	-0.67	-0.64	0.38

Table 3.7 continued

		x	y	z
C9	0.234	-0.50	-0.77	0.40
	0.188	-0.78	0.60	0.18
	0.122	0.38	0.22	0.90
C10	0.24	0.93	0.02	0.36
	0.22	-0.36	0.18	0.92
	0.14	0.04	0.98	-0.18
C11	0.198	-0.81	0.35	0.47
	0.155	0.50	0.01	0.86
	0.146	0.29	0.94	-0.18
O12	0.202	0.49	-0.47	0.73
	0.198	-0.86	-0.20	0.45
	0.124	-0.06	-0.86	-0.51
O13	0.243	-0.13	-0.66	0.74
	0.185	-0.45	0.71	0.55
	0.139	-0.88	-0.26	-0.38
N14	0.178	0.97	0.20	0.11
	0.164	0.20	-0.98	-0.04
	0.126	-0.10	-0.06	0.99

Table 3.8 Difference between the co-ordinates of ALS 9, ILS 4, and ILS 7.

Atom	Average Å x 10^3	Δx (Å x 10^3)			Δy (Å x 10^3)			Δz (Å x 10^3)		
		9-4	9-7	4-7	9-4	9-7	9-7	9-4	9-7	9-7
S1	3	-1	-5	-4	2	0	-2	-1	-3	-2
C2	10	-9	-2	7	6	1	-5	-7	-11	-4
C3	8	1	5	4	2	-2	-4	-17	0	-17
N4	8	0	15	15	1	1	1	-7	-5	2
C5	10	4	-14	-18	6	11	5	-4	4	8
C6	10	-3	0	3	-3	-2	1	3	12	9
C7	10	8	11	3	-1	27	26	-1	2	3
O8	9	3	7	-10	2	6	4	-6	-15	-9
C9	11	1	0	2	1	-11	-12	10	-12	-22
C10	12	10	18	8	9	2	-8	6	2	-4
C11	99	11	-4	-15	-8	12	19	-8	-5	-4
O12	7	8	-1	-9	-3	-1	2	5	-1	-6
O13	8	-3	-2	1	0	4	4	1	5	4
N14	8	0	15	15	1	1	1	-7	-5	2
Averages excluding S1										
	9	4.6	6.1	7.4	3.7	6.1	7.1	5.6	6.0	7.1

Table 3.9 Hydrogen peak heights (electrons $(\text{A}^{\circ})^3$)

Atom	ρ_3	ρ_6	$\rho_{1\,iso}$	$\rho_{7\ diff\ (iso)}$	$\rho_{7\ obs.\ (iso)}$
H30	.55	.40	.50	.40	1.2
H50	.35	.35	.50	.10	.9
H60	.3	.20	.35	.10	1.0
H90	.35	.25	.25	-.20	.7
H91	.40	.30	.30	.00	.9
H92	.35	.30	.40	.10	1.1
H100	.45	.40	.45	+.20	1.3
H101	.45	.30	.30	-.30	.7
H102	.20	.20	.10	.10	.9
H140	.30	.35	.40	.10	1.1
H141	.35	.40	.35	.10	1.1
H142	.30	.20	.35	.10	.9

Notes:

ρ_3 difference Fourier; full data heavy atom phases only.

ρ_6 and $\rho_{1\,iso}$ As above but only terms for which $\text{Sin}^2\theta \leq .75$. included.

$\rho_{7\ diff}$, $\rho_{7\ obs}$; Full data; contributions from hydrogen included in structure factor calculation.

Table 3.10

Agreement analysis from the structure factors output of ILS 7

Range of $F_{obs.}$ (electrons)	$\Sigma\|F_{obs.}\|$	$\Sigma\|F_{calc.}\|$	$\Sigma\|F_{diff.}\|$	R (%)
0 - 5	296	320	67	23
5 - 10	2855	2617	505	17
10 - 15	2736	2566	344	13
15 - 20	2185	2101	193	9
20 - 25	1410	1414	85	6
25 - 30	1298	1301	86	7
30 - 35	766	774	34	4
35 - 40	519	578	69	13
40 - 45	419	417	25	6
45 - 50	334	356	27	8
50 - 100	664	763	115	17
100 - 150	125	106	18	15
Range of $\sin^2\theta$.				
0 - 0.1	1783	1953	250	14
0.1 - 0.2	1805	1845	123	7
0.2 - 0.3	1699	1685	131	8
0.3 - 0.4	1740	1677	149	8
0.4 - 0.5	1673	1600	171	10
0.5 - 0.6	1352	1222	195	14
0.6 - 0.7	1242	1163	184	14
0.7 - 0.8	935	904	121	13
0.8 - 0.9	820	796	142	17
0.9 - 1.0	559	574	109	20
Totals	13618	13425	1580	11.6

CHAPTER 4

6-aminopenicillanic acid; results and conclusions.

RESULTS AND CONCLUSIONS: 6-AMINO-PENICILLANIC ACID

Fig. 4.1 shows sections of the final 3-D Fourier using positions derived from ILS 7, and fig. 4.2 the surroundings of the molecule projected on to the b c plane. Figs. 4.3 and 4.4 show the corresponding interatomic distances and angles. These are also shown in tables 4.1 and 4.2 together with those from ALS 9 and ILS 4, with their standard deviations. As a number of comparisons are made with the other penicillin structures their interatomic distances and angles are also given in tables 4.3 and 4.4.

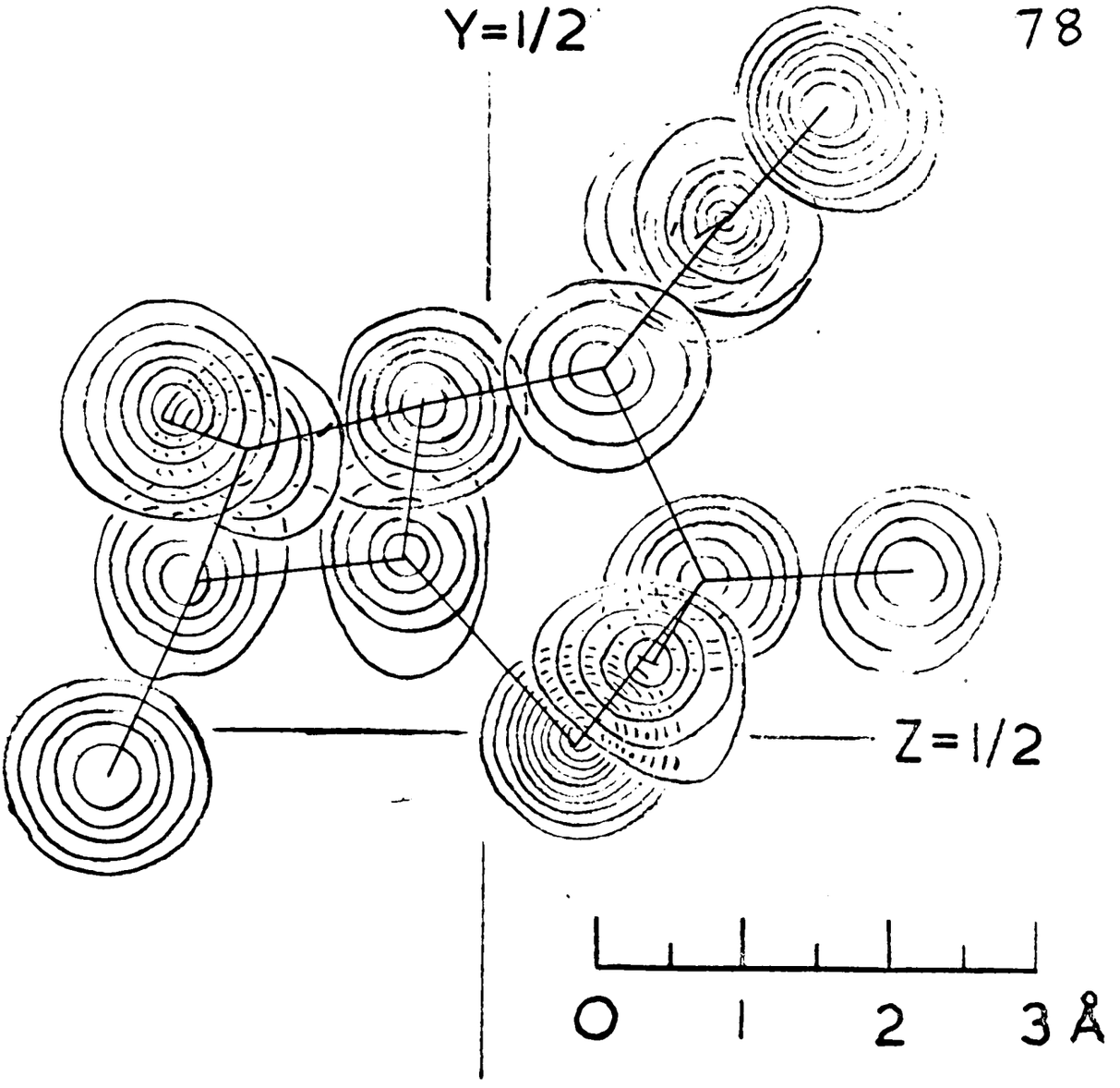


Figure 4.1.

6-aminopenicillanic acid; sections from the final observed Fourier contours at 1 and then every 2 electrons /A^{o3}, except for the sulphur atom where they are every 4 electrons /A^{o3}.

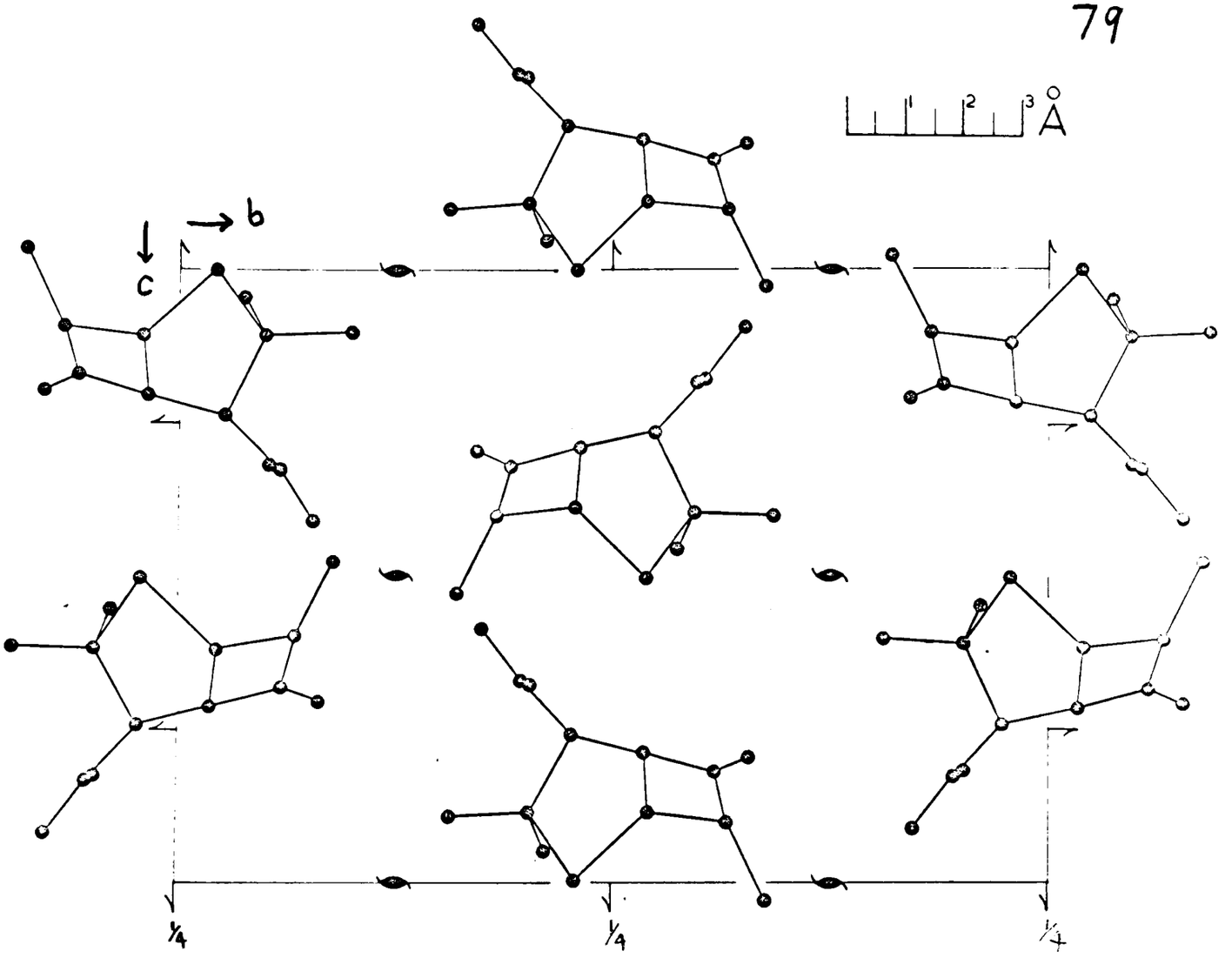


Figure 4.2. 6-aminopenicillanic acid projected on to 100 .

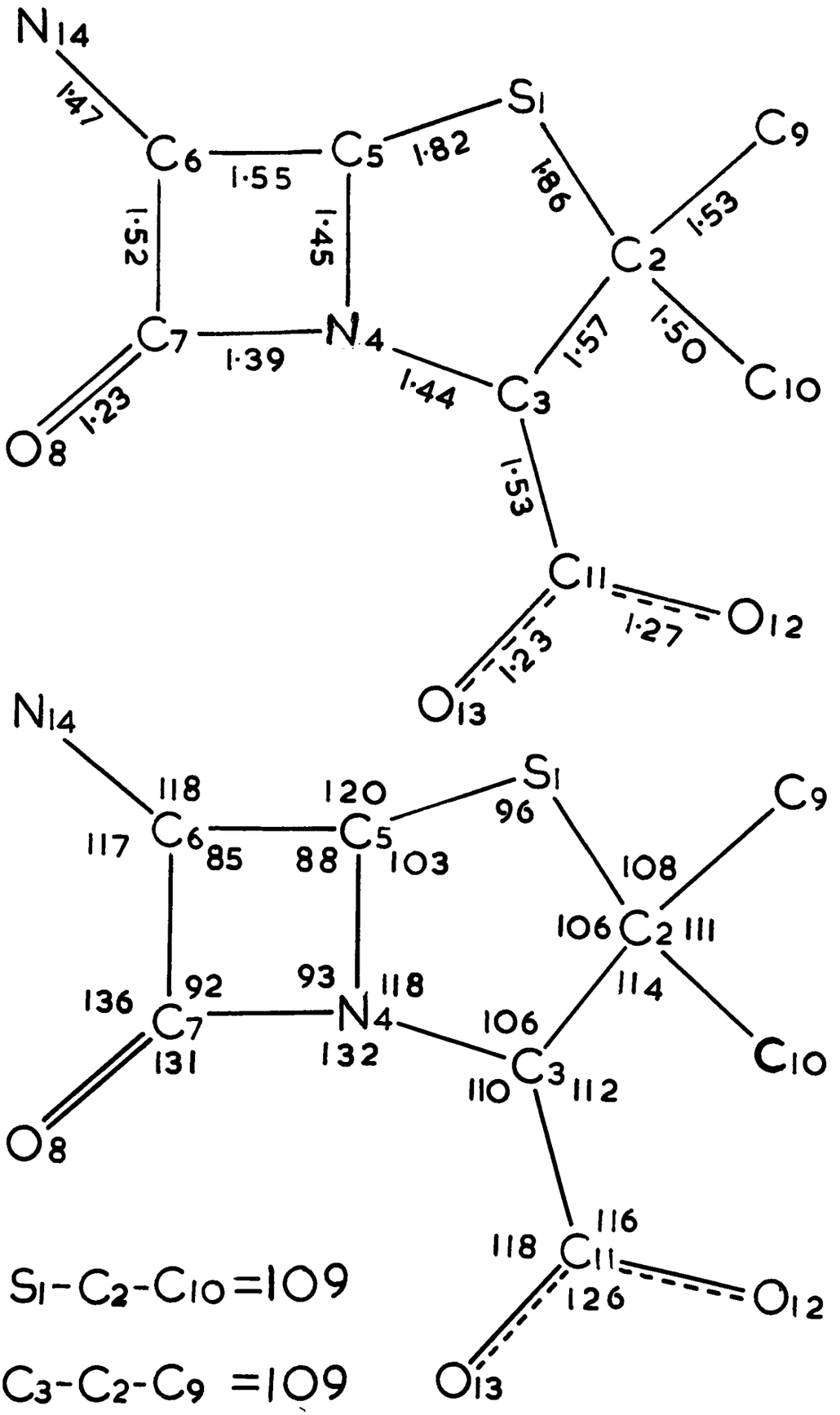


Fig. 4.3 6-APA interatomic distances (Å)

Fig. 4.4 6-APA interatomic angles (degrees)

Table 4.1 Interatomic Distances and their Standard Deviations: ALS 9, ILS 4 and ILS 7.

Bond	ALS 9 (Å)	ILS 4 (Å)	ILS 7 (Å)
S1 - C2	1.866 (.012)	1.869 (.012)	1.859 (.010)
S1 - C5	1.826 (.012)	1.822 (.012)	1.822 (.010)
C2 - C3	1.562 (.017)	1.556 (.016)	1.575 (.014)
C2 - C9	1.547 (.017)	1.534 (.018)	1.547 (.015)
C2 - C10	1.498 (.015)	1.505 (.017)	1.502 (.014)
C3 - N4	1.447 (.013)	1.442 (.014)	1.445 (.011)
C3 - C11	1.528 (.016)	1.540 (.015)	1.527 (.012)
C11- O12	1.280 (.014)	1.288 (.014)	1.273 (.011)
C11- O13	1.228 (.014)	1.212 (.014)	1.230 (.012)
N4 - C5	1.466 (.015)	1.471 (.015)	1.450 (.013)
N4 - C7	1.370 (.014)	1.372 (.015)	1.392 (.013)
C5 - C6	1.545 (.014)	1.555 (.016)	1.554 (.013)
C6 - C7	1.540 (.018)	1.530 (.017)	1.520 (.015)
C6 - N14	1.463 (.015)	1.470 (.015)	1.474 (.012)
C7 - O8	1.230 (.016)	1.232 (.016)	1.228 (.014)

Table 4.2 Interatomic Angles and their Standard deviations

ALS 9, ILS 4 and ILS 7

Bond	ALS 9	ILS 4	ILS 7
C2 - S1 - C5	95.8° (.5)	96.2° (.5)	95.6° (.5)
S1 - C2 - C3	105.9 (.7)	105.2 (.7)	105.7 (.6)
S1 - C2 - C9	107.7 (.8)	108.2 (.8)	107.5 (.7)
S1 - C2 - C10	109.5 (.8)	109.3 (.8)	109.4 (.7)
C3 - C2 - C9	108.9 (.9)	108.6 (.9)	108.9 (.8)
C3 - C2 - C10	114.5 (1.0)	114.1 (1.0)	113.8 (.9)
C9 - C2 - C10	109.9 (1.0)	111.0 (1.0)	111.2 (.8)
C2 - C3 - N4	105.9 (.9)	107.0 (.8)	106.0 (.7)
C2 - C3 - C11	112.0 (.8)	112.6 (.8)	111.8 (.7)
N4 - C3 - C11	109.2 (.8)	108.3 (.8)	109.8 (.7)
C3 - C11- O12	115.6 (1.0)	115.5 (1.0)	116.1 (.8)
C3 - C11- O13	118.5 (1.0)	118.9 (1.0)	118.2 (.8)
O12- C11- O13	125.9 (1.1)	125.6 (1.1)	125.7 (.9)
C3 - N4 - C5	118.6 (.8)	118.0 (.9)	118.0 (.7)
C3 - N4 - C7	131.9 (1.0)	132.1 (1.0)	132.5 (.8)
C5 - N4 - C7	93.6 (.9)	93.4 (.9)	93.5 (.7)
S1 - C5 - N4	102.4 (.7)	102.3 (.7)	103.4 (.6)
S1 - C5 - C6	119.9 (.8)	120.0 (.8)	120.2 (.7)
N4 - C5 - C6	88.2 (.8)	87.8 (.8)	88.1 (.7)
C5 - C6 - C7	84.2 (.8)	84.3 (.8)	84.6 (.7)
C5 - C6 - N14	116.9 (1,0)	117.2 (1.0)	118.4 (.8)
C7 - C6 - N14	119.3 (.9)	118.7 (.9)	117.3 (.8)
N4 - C7 - C6	92.0 (.9)	92.5 (.9)	91.7 (.8)
N4 - C7 - O8	133.3 (1.2)	132.9 (1.1)	131.7 (1.0)
C6 - C7 - O8	134.7 (1.0)	134.6 (1.1)	136.4 (1.0)

Table 4.3 Penicillin Bond Lengths (Å)

Bond	6 APA (a)	K Benz Pen.(b)	Pen V Free Acid (c)	P. Bromo Pen V Free Acid (d)	Ceph Cc (e)
S1 - C2	1.86	1.82	1.87	1.86	1.80
S1 - C5	1.82	1.85	1.82	1.80	1.80
C2 - C3	1.57	1.61	1.57	1.53	
C2 - C9	1.55	1.55	1.51	1.52	
C2 - C10	1.50	1.52	1.58	1.52	
C3 - N4	1.44	1.48	1.46	1.47	(1.42)
C3 - C11	1.53	1.51	1.54	1.51	
C11- O12	1.27	1.25	1.35	1.35	
C11- O13	1.23	1.24	1.21	1.21	
N4 - C5	1.45	1.45	1.52	1.47	1.48
N4 - C7	1.39	1.37	1.46	1.36	1.38
C5 - C6	1.55	1.56	1.58	1.55	1.54
C6 - C7	1.52	1.50	1.55	1.56	1.53
C6 - N14	1.47	1.48	1.44	1.42	1.44
C7 - O8	1.23	1.24	1.21	1.21	1.21

Notes. a) 6-APA final isotropic positions

b) J. S. Rollet and A. Vaciago's refinement of potassium benzyl penicillin (Pen G.) (personal communication)

c) phenoxymethyl penicillin (Pen V) Abrahamsson, Crowfoot Hodgkin and Maslen (10)

d) K. Watson (personal communication)

e) Final positions

Table 4.4 PENICILLIN BOND ANGLES

Bond	6 APA	K Benz Pen	Pen V Free Acid	P. Bromo Pen V Free Acid	Ceph Cc
C2 - S1 - C5	96°	97°	96°	96°	97°
S1 - C2 - C3	106	107	105	107	
S1 - C2 - C9	108	112	109	110	
S1 - C2 - C10	109	110	108	108	
C3 - C2 - C9	109	108	110	110	
C3 - C2 - C10	114	110	113	114	
C9 - C2 - C10	111	111	112	107	
C2 - C3 - N4	106	102	104	104	
C2 - C3 - C11	112	115	113	110	
N4 - C3 - C11	110	112	114	111	
C3 - C11- O12	116	120.6	115	124	
C3 - C11- O13	118	115.4	122	110	
O12- C11- O13	126	124	122	125	
C3 - N4 - C5	118	121	120	119	(119)
C3 - N4 - C7	132	127	129	123	131
C5 - N4 - C7	93	94	88	94	94
S1 - C5 - N4	103	102	103	106	(112)
S1 - C5 - C6	120	121	122	121	(115)
N4 - C5 - C6	88	88	92	89	87
C5 - C6 - C7	85	84	83	84	86
C5 - C6 - N14	118	115	115	114	121
C7 - C6 - N14	117	116	115	116	116
N4 - C7 - C6	92	94	96	93	90
N4 - C7 - O8	132	131	128	133	132
C6 - C7 - O8	136	135	136	134	137

General arrangement

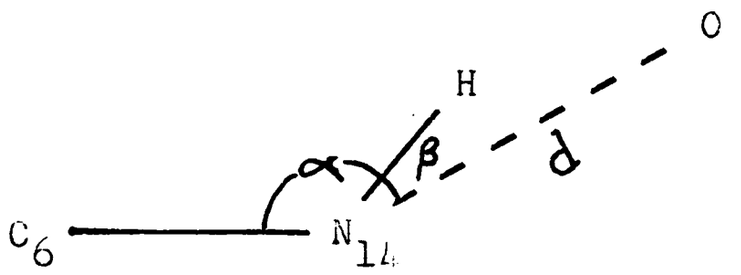
The molecules are grouped around the hydrogen bond system associated with the screw axes parallel to a. Each molecule is hydrogen bonded at one end to this system through its free amino group, and at the other end by its carboxylic acid group. The configuration of the two ring systems is in general similar to that reported by Crowfoot, Bunn, Roger-Low and Turner-Jones. (26), and Pitt (55) for potassium benzyl penicillin and by Abrahamsson, Crowfoot-Hodgkin and Maslen (10) for phenoxymethyl penicillin; i.e. N_{14} and the thiazolidine rings are on the same side of the roughly planar β-lactam ring. The carboxyl group is oriented at right angles to the plane of the five membered thiazolidine ring to which it is attached, and is trans to the β-lactam group.

In all the penicillin structures the thiazolidine ring has four atoms in a plane with one atom out of the plane. 6-APA differs from the others in that the out-of-plane atom is N_4 instead of C_3.

The hydrogen bond system

Fig. 4.5 shows this projected on the plane of the symmetry related carboxyl groups. The hydrogen bonds form a spiral flattened in the plane of the figure, joining atoms O_{12} and N_{14} on alternate molecules, so that O_{12} has two hydrogen bonds attached to it. The third hydrogen bond on N_{14} is directed towards O_{13}. The angles around N_{14} are shown in table 4.5 and fig. 4.5.

Table 4.5 Hydrogen bond distances and angular deviations



	α	β	d (Å)
$C_6 - N_{14} \cdots O_{12}$	111°	8°	2.72
$C_6 - N_{14} \cdots O_{12}$	82	40	2.64
$C_6 - N_{14} \cdots O_{13}$	107	24	2.85

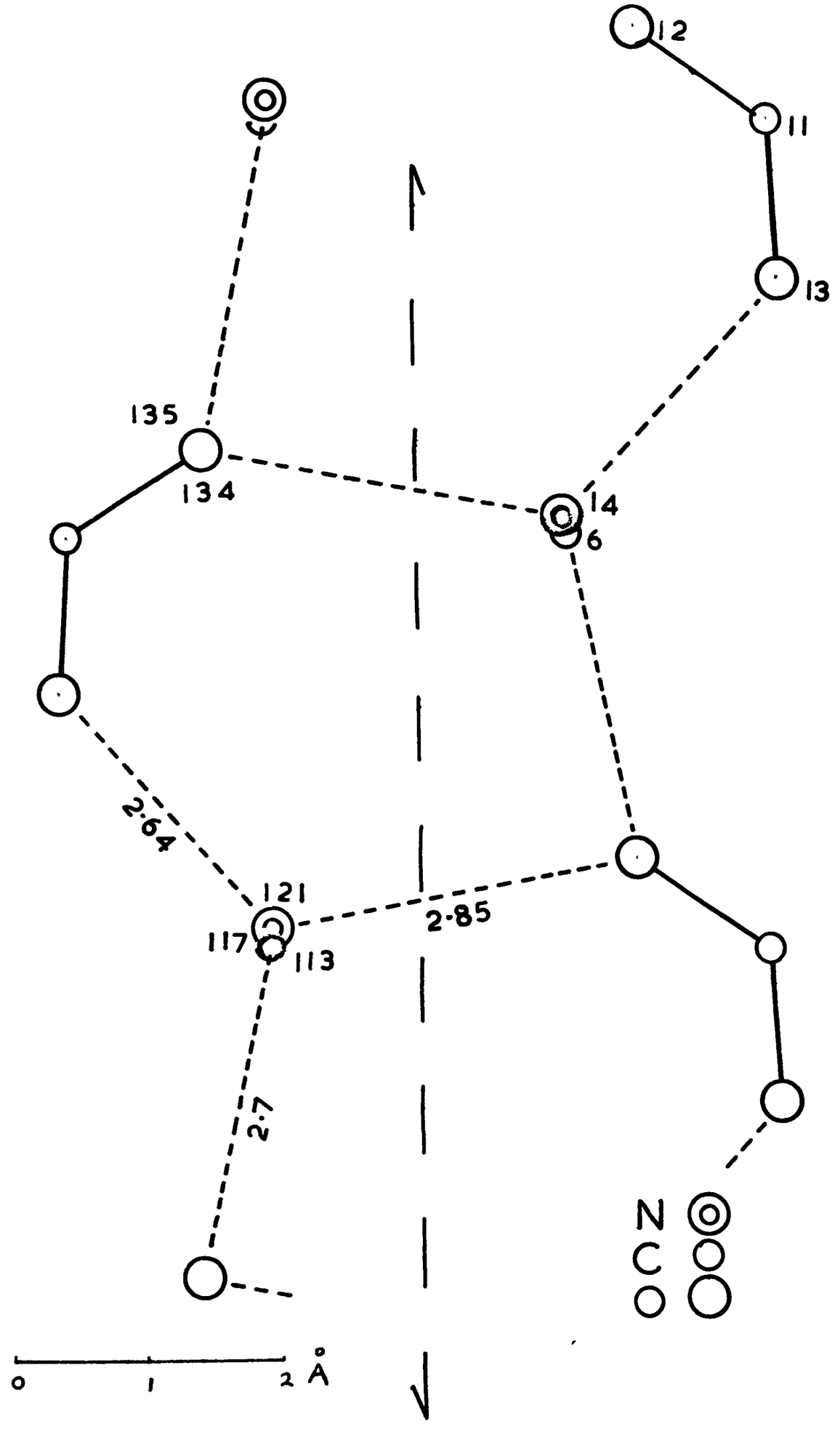


Fig. 4.5. 6-APA Hydrogen bond system projected on to the plane passing through the symmetry related nitrogen atoms. Distances in Å, angles in degrees.

The three nitrogen atoms that are hydrogen bonded to each carboxyl group are within 0.75 Å of the plane of this group, so that the hydrogen bond system is close to the plane of the carboxyl group. Considering the wide angular divergence of hydrogen bonds from their ideal directions that is found; (Pimmental and McClellan (54) quote hydrogen bond angles ranging from 90° to 164°) the packing of 6 APA results in a structure which is fairly close to ideal.

It is not clear from the bond lengths $C_{11} - O_{12}$ (1.27Å) and $C_{11} - O_{13}$ (1.23Å) whether the carboxyl group is ionized or not, though the bond lengths found here are nearer those accepted for an ionized carboxyl group (1.26Å) than those for a COOH group (1.23 and 1.36Å). Also it was possible to find peaks in appropriate positions for NH_3^+ hydrogens (p. 67), but not in the position necessary if there is a hydrogen attached to O_{12}. All these results point strongly to crystalline 6-APA being in the form of a zwitterion, though there remains a small possibility of the structure being unionized. In solution the carboxyl group has a pK of 2.3 and the amino group of 5.1 (13), and so it must

be unionised in neutral solution. These different results cannot really be compared as the environment in the solution is quite different from that in the crystal, where each amino group is surrounded by three carboxyl groups which are within 3 Å.

In his paper on Amino Acids and Peptides (35) Hahn uses Pauling's relation between bond length and bond order (53) to determine the bond order of resonant systems. He assumed that the total double-bond character of the two resonating bonds was 100%, and found that, by choosing reasonable values for the end points of his curve connecting double-bond character and bond length he could fit the experimental values fairly closely to his curve. He also found that using this definition of bond-order there was a linear relation between bond-order and the angle from a pure single bond to the resonant bond.

This gives a useful measure of the bond order, as well as providing a check on the accuracy of the determination. This method can be applied to carboxyl groups, carboxyl ions, and peptide linkages provided the appropriate end points are chosen. In Fig. 4.6 the results for 6-APA and ceph Cc are plotted in the

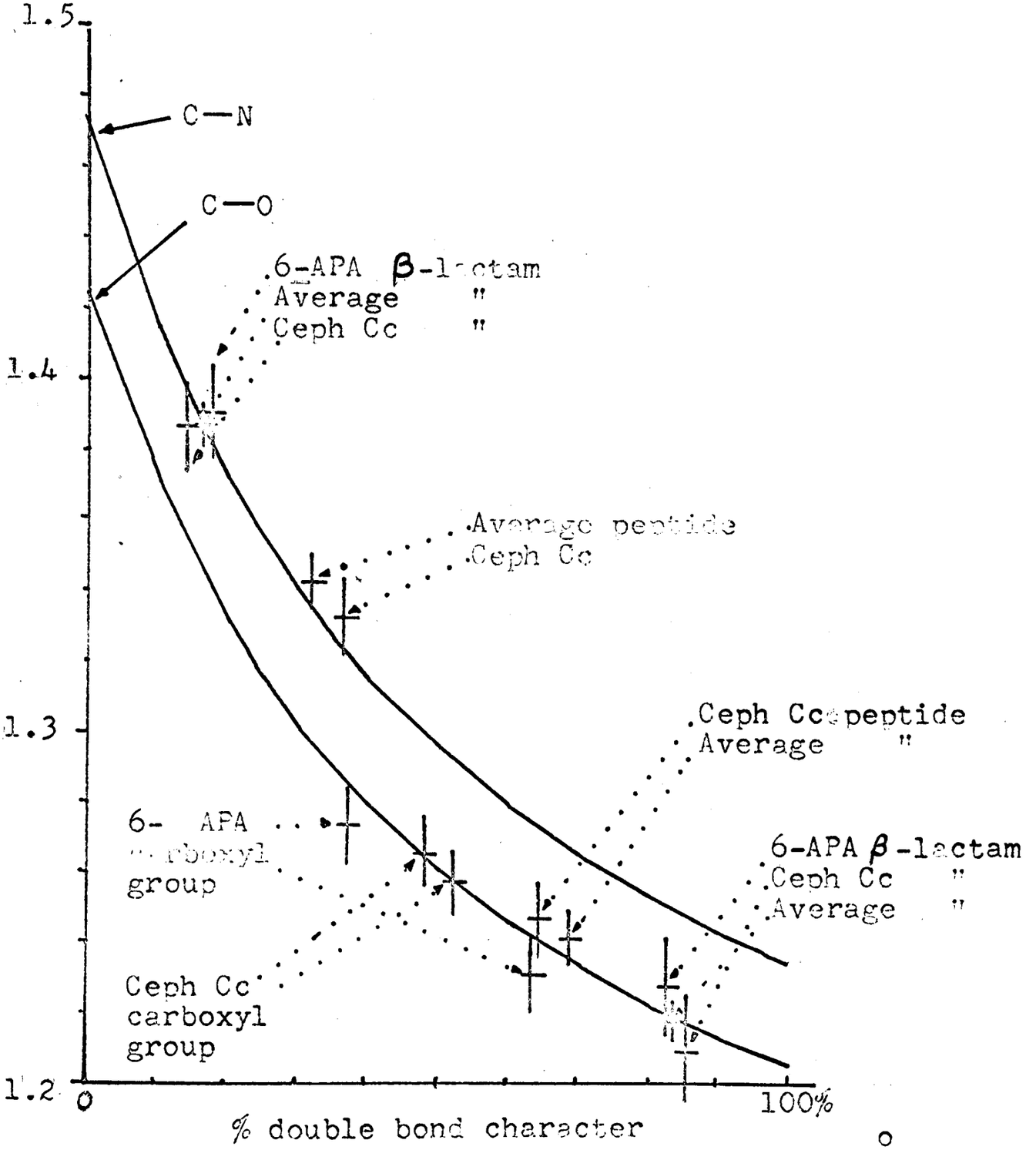


Figure 4.6. Bond order plot for C = N (1.233Å) to C - N (1.475Å) and C = O (1.205Å) to C - O (1.205). Pairs of resonanting bonds placed so that there is a total of 100% double bond character and the distance from the curve is the same for both bonds. Vertical lines give the calculated standard deviations.

Table 4.6 Bond orders derived from the bond order graph (figure 4.6) and from interatomic angles

Bond	Bond character derived from bond lengths			Bond order derived from bond angles		
	% double bond character	observed bond length (Å)	calculated bond length (Å)	% double bond character	observed bond angle	calculated bond angle
6-APA						
N4-C7	17.5	1.390	1.383			
C7-O8	82.5	1.288	1.220			
C11-O12	37	1.273	1.285	43	116.1°	116.3°
C11-O13	63	1.230	1.241	57	118.2	118.4
ceph Cc						
N5-C8	14	1.386	1.395	28	123.8	120.9
C8-O9	86	1.209	1.217	72	116.8	113.9
N18-C19	36	1.332	1.325	52	117.5	117.7
C19-O20	64	1.246	1.240	48	116.9	117.1
C25-O27	48	1.265	1.262	52	117.5	117.7
C26-O28	52	1.257	1.258	48	116.9	117.1
Average penicillin molecule						
β -lactam						
N-C	16	1.386	1.388			
C-O	84	1.217	1.219			
side chain peptide						
N-C	32	1.342	1.336			
C-O	68	1.241	1.235			

way described. Table 4.6 gives the values of the bond orders found in this way.

The values for the carboxyl group found by the bond-distance and bond-angle treatment agree, although the bond-distance results could be interpreted on the basis of the carboxyl group being unionized, it would mean that there was an unusually large degree of resonance.

The only non-hydrogen-bond contact of less than 3.25 Å is one of 3.03 Å between C_6 and O_{12}. This is not shown in fig. 4.5 but it lies directly behind the hydrogen bond between O_{12} and N_{14}, to which C_6 is bonded. It is not surprising that the shortest non-hydrogen-bond contact should be associated with the hydrogen-bond system, where the attractive intermolecular forces are strongest.

The Thiazolodine Ring

Figure 4.7 shows a projection of the molecule on to the best plane through S_1, C_2, C_3, and C_5. As mentioned on page 85 it is N_4 which is out of plane rather than C_3 as in the other four penicillins.

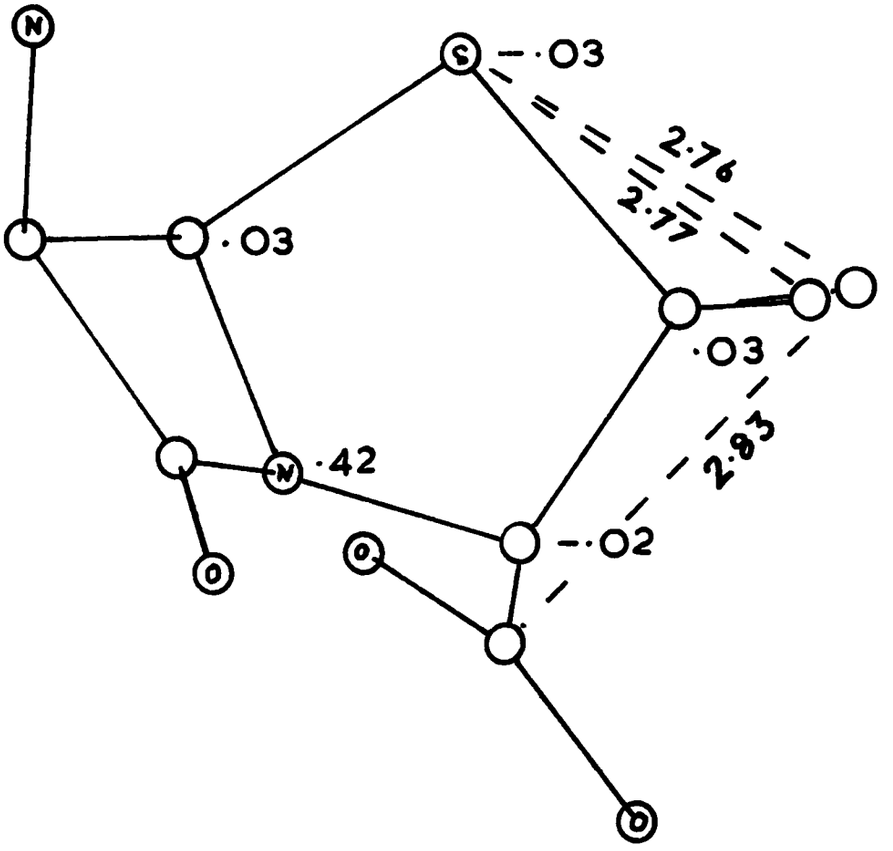
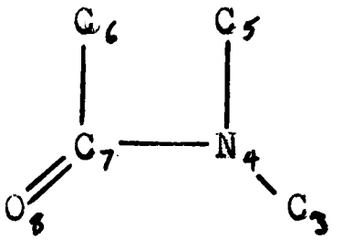


Figure 4.7. 6-aminopenicillanic acid projected on to the best plane through S_1 C_2 C_3 C_5. Deviations from the plane in Å units.

Crowfoot—Hodgkin, Bunn, Rogers-Low and Turner-Jones (26) suggested that this is probably due to the interactions between substituent groups and non-bonded electrons on the thiazolidine ring. Another possible cause may be the tendency for the group



to be as near as possible planar, as resonance would then be possible as in the ordinary amide linkage. That this has taken place to some extent is shown by the sums of angles around N_4 (343°) and around C_5 (311°). Fig. 4.8 shows a projection of part of the molecule along the bond $N_4 - C_5$. From the accompanying table (47) it can be seen that the dihedral angle α in the β-lactam ring is a function of the dihedral angle β in the thiazolidine ring. As the changes in α are much smaller than the corresponding changes in β, it would seem that the dihedral angle in the β-lactam ring is a result of the different configurations of the sulphur containing ring, the group

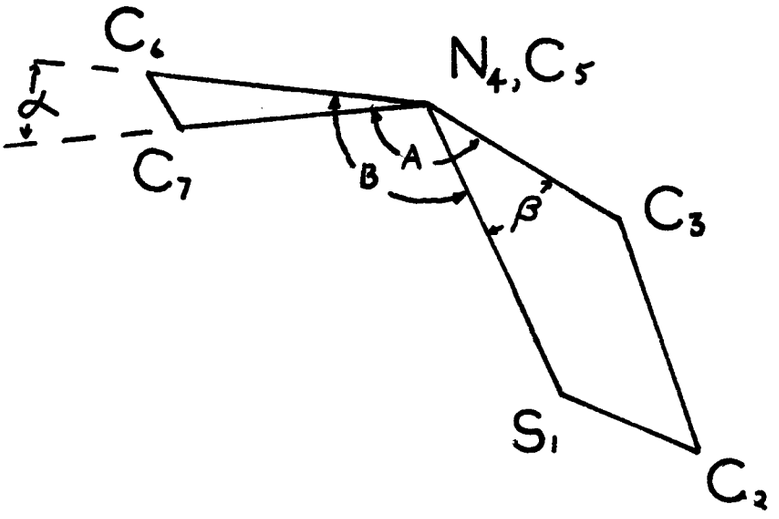


Figure 4.8 Projection of 6-aminopenicillanic acid ring system along the bond N_4-C_5.

Table 4.7 Dihedral angles of the penicillins along the bond C_4 C_5.

Penicillin	α	β	A	B
p-Bromo pen.V.	4°	9°	132°	127°
Pen.V.	7	22	144	128
Pen.G.	7	22	139	123
6-APA	11	32	142	120
Ceph. Cc.	13	40	142	115

For references see table 4.3 page

$S_1 - C_5 - C_6$ tending to swivel as a unit around the bond $N_4 - C_5$ relative to $C_3 - N_4 - C_7$.

The angle $C_3 - C_2 - C_{10}$ ($114.1^\circ \pm 1.0$), is some $4\frac{1}{2}^\circ$ larger than the equilibrium value. That this effect is real is shown not only by the standard deviations but also be the corresponding angles for Pen V, P. Bromo Pen V, and Pen G. which are 113°, 114.5°, 109.7° The value for Pen G. is undoubtedly in error in view of the very long distance of 1.61 Å found for the bond $C_2 - C_3$. The close contacts of C_{10} are shown in Fig. 4.7. The increase in the angle $C_3 - C_2 - C_{10}$, may well be due to repulsion between C_{10} and the π-electrons on C_{11}. Although C_{10} is nearer to S_1 (2.76Å) than to C_{11} (2.83Å), the non-bonded electrons on S_1 are pointing away from C_{10}, whereas the πelectrons on C_{11} are between it and C_{10}.

A remarkable feature of all the penicillins is the length of the C - S bonds, the weighted average values of these are 1.86 Å for $S_1 - C_2$ and 1.82 Å for $S_1 - C_5$ (excluding Ceph Cc) The calculated standard deviations for these bonds are 0.012 Å for 6 APA, 0.013 Å for p-bromo-pen V, 0.016 Å for pen V free acid, and .020Å

for Pen G. If these figures were correct the standard deviation of the mean would be .007 Å. However, the true standard deviations are bound to be larger than this, also there is no means of knowing what differences of chemical environment there are between the different molecules. In view of this a reasonable estimate of the true standard deviations of the mean would be about .02 Å. None of the bonds are significantly different from this, The value given for a single C - S bond by Sutton in Interatomic Distances (65) is 1.81 Å. Thus if the bond S_1 - C_2 is in fact 1.86 Å then this is noticeably longer than the accepted value; there does not seem to be any obvious reason for this difference, certainly there is no distortion of the bond angles of C_2. The absence of any lengthening of the C - S bonds in Ceph Cc suggests that the lengthening observed in the penicillins may be erroneous. The accuracy of the present penicillin structures is such that the evidence is highly suggestive of a length of between 1.84 - 1.87 Å for the bond S_1 - C_2, but is not sufficiently high to prove this conclusively.

The only other bonds in the thiazolidine ring which differ from their expected values are C_2 - C_{10} (1.50 Å) and C_2 - C_3 (1.57 Å), these differences are not sufficient to be significant in terms of the real standard deviations.

β-lactam ring

A projection of the molecule on to the plane of the β-lactam ring is shown in figure 4.9 The ring is not quite planar, the carbonyl group N_4,C_6,C_7, and O8 being very closely planar (the maximum deviation from the least squares plane through the carbonyl group is 0.02 Å), with C_5 0.14 Å out of the plane (on the same side as S_1). This can alternatively be regarded as a bending of the ring along the line N_4 - C_6. This bending is probably caused by the twisting of the molecule around the bond N_4 - C_5 as a result of the different configurations of the thiazolidine (or in the case of Ceph Cc the dihydrothiazine) ring (p.74) (Fig. 4.8). As any repulsion involving the non-bonded electrons on N_4 would tend to bend the ring in the opposite direction from that observed, it seems likely

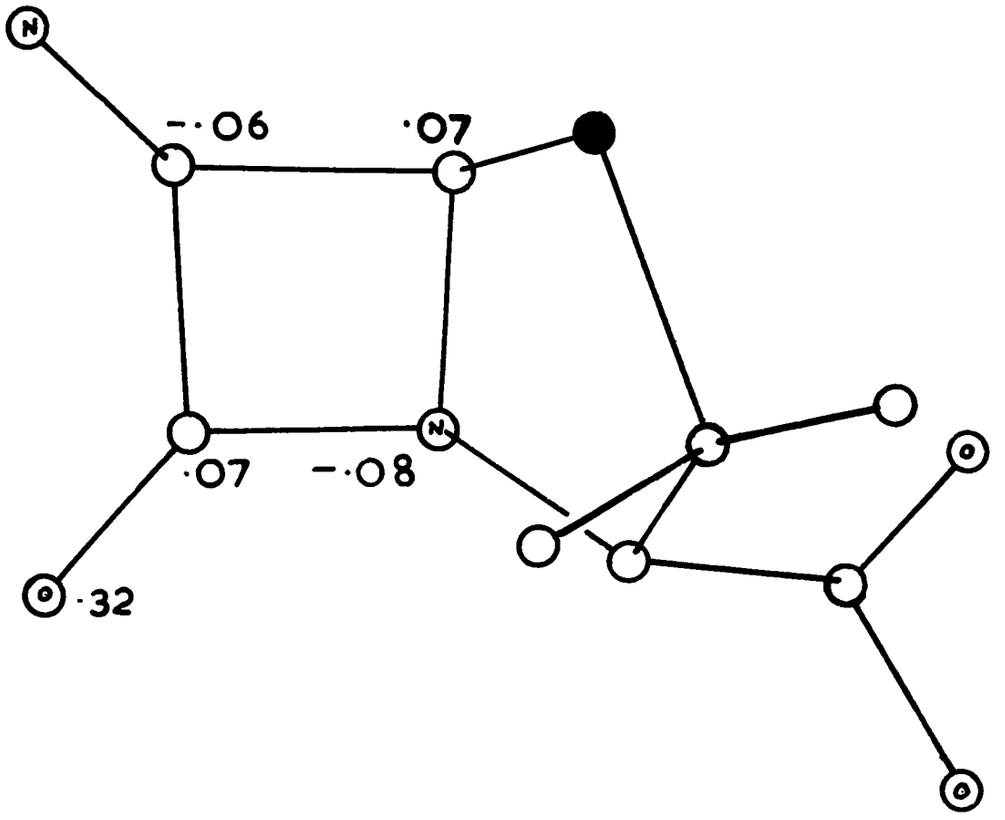


Figure 4.9.

6-aminopenicillanic acid projected on to the best plane through N_4 C_5 C_6 C_7. Deviations from the plane in Å units.

that this bending is indeed caused by the configuration of the larger ring.

The angles around N_4 are somewhat larger than those around C_5. This must be due to resonance in the β-lactam amide group. Woodward (69) has discussed the chemical consequences of incomplete resonance because of the non-planarity of the amide system in the fused β-lactam ring system. The bond distance $N_4 - C_7$, (1.39 Å) lies half-way between the limits of a single C - N bond and the value for a C - N bond involved in a peptide link of 1.33 Å given by Hahn (35) and confirmed by a number of more recent determinations (1.29 Å in N - methyl acetamide (41), 1.32 Å in β-glycylglycine (71), 1.32_5 in Diketopiperazine (30) and 1.33 Å in N - acetylglycine (28)). The length of the carbonyl bond $C_7 - O_8$ at 1.23 Å is much less sensitive to resonance, as it only varies between 1.20 for a pure double bond to 1.23 in a peptide link, the value for 6-APA, is within the expected range, as is also the average value for the penicillins (1.22 Å).

As can be seen from figure 4.6 and table 4.6 the β-lactam amide linkages and the side chain peptide linkages fall into two quite separate groups. The exact value of the amount of double bond resonance in

the two systems is not certain, but there can be no doubt that there is greater resonance in the ordinary peptide linkage than in the penicillin β-lactam linkage.

6-APA observed structure factors

scale

100 = 1 electron

order

l k h

* line convention

each * is followed by the values of l and k for the following group of reflections

Note:

A_{obs} and B_{obs} are printed rather than F_{obs} as this was more convenient for computing.

Columns

h A_{obs} B_{obs} A_{calc} B_{calc}

*	o	o		
	2	5159	o	6408
	4	-1305	o	1437
	6	996	o	946
*	o	1		
	1	o	-3127	o
	2	3754	o	4429
	5	o	2938	o
	7	o	1434	o
*	o	2		
	o	5547	o	8191
	1	o	2042	o
	2	2679	o	3357
	3	o	-2828	o
	5	o	1992	o
*	o	3		
	1	o	3834	o
	2	-2480	o	-2697
	3	o	1364	o
	4	-1683	o	-1812
	6	-1703	o	-1428
*	o	4		
	o	-5657	o	-6796
	1	o	2301	o
	2	2370	o	2415
	3	o	3296	o
	4	1265	o	1106
	*	o	2410	o
	6	-488	o	-431
*	o	5		
	2	-4103	o	-4074
	3	o	1763	o
	4	-3774	o	-3611
	6	-3008	o	-2820
*	o	6		
	o	1633	o	1503
	1	o	1364	o
	2	1544	o	1453
	3	o	1364	o
	4	787	o	441
	5	o	-936	o
	6	-1046	o	-826
*	o	7		
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	2	2081	o	2394
	3	o	508	o
	4	-2858	o	-2704
	5	o	-787	o
	6	-2151	o	-2177

*	o	8		
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	2	-1494	o	-1534
	5	o	-856	o
*	o	9		
	1	o	1056	o
	2	458	o	166
	3	o	-1623	o
	4	-1444	o	-1385
	5	o	-1026	o
	6	-1066	o	-1189
	7	o	-757	o
*	o	10		
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	1	o	876	o
	2	-2032	o	-2039
	3	o	1673	o
	5	o	737	o
	6	906	o	775
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	3	o	-1474	o
	5	o	-876	o
	6	-856	o	-737
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*	o	13		
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	3	o	-767	o
	5	o	-797	o
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	3	o	-866	o
	4	-1016	o	-1094
*	o	15		
	2	856	o	584
	3	o	-667	o
	4	418	o	601
*	o	16		
	o	-2081	o	-1777
	1	o	-1115	o
	3	o	-677	o
*	o	18		
	1	o	-1693	o
*	1	o		
	1	o	7887	o
	2	o	4362	o

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	6		0	1813	0	1639		
	7		0	847	0	893		
*	I	I						
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	1	5419		-555	6600	-676		
	2	1272		-1192	1466	-1374		
	3	2295		1913	2601	2168		
	4	1220		-403	1421	-469		
	6	-623		135	-442	96		
	7	-125		-513	-107	-437		
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	1	2153		2904	2451	3305		
	2	-306		2260	-337	2493		
	3	-143		-508	-153	-544		
	4	269		1712	280	1783		
	5	-1104		732	-1018	675		
	6	-1141		568	-914	455		
	7	-211		484	-127	291		
*	I	3						
	0		0	1783	0	1939		
	1	1599		-742	1531	-711		
	2		3	2101	4	2360		
	3	428		943	439	968		
	4	-617		730	-666	789		
	6	1336		148	1356	150		
	7	-352		-380	-191	-206		
*	I	4						
	0	-1434		0	-1406	0		
	1	1231		-1836	1351	-2014		
	2	3046		101	2946	98		
	3	-1583		543	-1780	610		
	4	-312		-426	-278	-379		
	5	-2848		-20	-2826	-20		
	6	-946		698	-773	570		
	7	-1704		-256	-1489	-223		
*	I	5						
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	2	513		3890	556	4210		
	3	1325		-1499	1263	-1428		
	4	67		493	87	638		
	5	326		-1396	346	-1482		
	6	688		-316	483	-222		
*	I	6						
	0	528		0	593	0		
	1	613		-759	562	-695		

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	3	-803		1457	-784	1422		
	4	1444		-25	1275	-22		
	5	-2419		-1578	-2401	-1565		
	6	259		549	234	496		
	7	-139		-215	-1555	-239		
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	0		0	857	0	907		
	1	-1005		2146	-952	2032		
	2	-509		1760	-511	1766		
	3	377		424	375	422		
	4	524		1399	488	1305		
	5	-123		-1439	-123	-1435		
	7	-162		-555	-192	-658		
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	0	4880		0	4602	0		
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	2	-182		-1090	-210	-1261		
	3	-1695		483	-1635	466		
	4	999		-776	892	-692		
	5	-696		366	-626	329		
	6	409		-540	429	-566		
	7	-733		-187	-618	-158		
*	I	9						
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	1	-2545		-272	-2366	-253		
	2	-169		1565	-153	1420		
	3	-2081		2	-2386	2		
	4	-103		2169	-109	2298		
	5	-882		-85	-1023	-99		
*	I	10						
	0	1823		0	1630	0		
	1	-692		-189	-576	-158		
	2	785		-3488	742	-3298		
	3	-1251		-592	-1191	-564		
	4	-864		-1243	-760	-1093		
	5	-799		1238	-809	1254		
	6	-277		-694	-206	-517		
*	I	11						
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	2	-353		590	-371	620		
	3	-1064		-97	-1037	-894		
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	6	-324		-931	-327	-939		

* I 13				
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2	-835	-841	-754	-759
4	-173	-593	-181	-618
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4	139	-966	133	-930
5	412	-419	436	-443
* I 15				
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1	-1249	-199	-1086	-173
2	117	-717	92	-562
3	-536	325	-572	347
4	-662	-218	-630	-208
* I 16				
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2	54	-745	41	-569
3	816	-260	888	-283
4	291	-544	294	-551
* I 17				
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2	437	-928	341	-726
* I 19				
0	0	-817	0	-805
* 2 0				
0	2659	0	2683	0
1	3645	0	4214	0
2	6633	0	6923	0
3	-1374	0	-1536	0
4	578	0	491	0
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7	757	0	716	0
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3	314	1511	332	1597
4	931	-1795	912	-1759
5	209	-758	307	-1112
6	-353	590	-358	597
7	216	-142	289	-190

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6	-433	148	-424	145
7	134	531	140	555
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2	-419	1023	-371	907
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6	412	-280	424	-289
7	-164	-678	-183	-756
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1	-778	1930	-779	1931
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3	-38	407	-46	494
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7	109	567	144	751
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7	177	-674	214	-816
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7	-87	-179	-96	-197
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7	160	-387	154	-372
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* 4 12				
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6	-637	161	-560	141
7	-12	478	-19	765
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7	156	-255	149	-243
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6	-366	157	-442	189
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4	-908	-777	-952	-815
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* 6 14				
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* 7 1				
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1	1923	-2453	1906	-2432
2	-1194	58	-1040	51
3	837	-1356	790	-1280
4	1193	169	1216	172
* 7 2				
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2	-1178	2068	-1203	2114
3	-556	753	-574	778
4	-1594	1400	-1525	1339
* 7 3				
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1	508	-2213	437	-1907
2	681	1776	609	1589
3	1265	-17	1184	-16
4	-809	1280	-667	1054
* 7 4				
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2	590	1555	540	1423
3	-1103	167	-977	148
4	808	986	798	973
5	-927	-689	-623	-463

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2	419	2646	437	2756
3	280	893	283	904
4	-650	1851	-610	1739

* 7 6				
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1	-849	-1747	-710	-1460
2	218	-503	274	-632
3	-1168	-762	-940	-613
4	662	543	613	503

* 7 7				
0	0	2579	0	2438
2	1055	1874	1076	1911
3	-972	-1068	-798	-877
4	78	1312	67	1124

* 7 8				
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2	-259	-900	-237	-824
3	-646	-606	-501	-470
4	247	-641	251	-651

* 7 9				
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2	230	1456	224	1418
3	-643	-825	-581	-746
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* 7 11				
1	-1294	-642	-972	-483
2	-937	726	-846	655
4	228	454	117	234

* 7 12				
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4	-444	-779	-516	-904

* 7 13				
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3	-664	272	-826	338

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* 7 15				
1	-832	451	-616	334
2	18	-378	26	-562

* 8 0				
0	1394	0	1347	0
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3	876	0	1067	0
4	697	0	83	0

* 1				
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1	-683	844	-512	632
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3	-1258	2263	-1150	2067
4	-477	-613	-402	-517
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* 8 2				
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5	-424	-778	-338	-620
6	789	-656	109	-91

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2	1114	-57	1002	-51
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5	590	1005	439	747
6	-799	169	-1007	213

* 8 4				
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1	-292	2141	-254	1863
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6	464	1326	336	961

* 8 5				
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3	-278	-618	-106	-237
4	-1448	138	-1413	135
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* 8 7				
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* 8 7				
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3	-664	-135	-611	-124
4	-526	-558	-539	-571
5	-393	-502	-313	-400
* 8 8				
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1	391	1524	321	1252
2	-683	625	-624	571
3	164	1164	175	1245
5	-205	624	-247	753
* 8 9				
1	-1416	-794	-1151	-645
2	-800	244	-461	140
3	416	-547	340	-448
4	-567	406	-352	252
5	548	-734	531	-711
* 8 10				
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1	-327	671	-290	595
2	-1138	331	-1083	315
3	-542	354	-698	455
4	-479	421	-227	199
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3	104	-930	95	-852
4	-503	-217	-478	-206
* 8 12				
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2	-933	-78	-757	-64
3	687	13	555	11
* 8 13				
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2	-148	945	-90	577
3	-147	-712	-164	-796
* 8 14				
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* 9 2				
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4	-153	700	-172	787
* 9 3				
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* 9 4				
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2	357	-876	125	-306
3	-1262	-606	-1114	-544
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2	100	760	118	899
3	221	858	308	1197
* 9 6				
1	88	-1222	33	-461
2	-141	1066	-105	798
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* 9 7				
1	-1350	-103	-965	-74
3	641	-90	708	-100
* 9 8				
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* 9 9				
1	-1243	231	-862	160
* 9 10				
2	188	-988	196	-1032
3	-85	672	-87	695
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* 9 12				
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* 9 13				
1	-1764	-174	-1478	-146
* 10 0				
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2	1514	0	1237	0
* 10 1				
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1	-1301	411	-962	304
2	757	-5	836	-5
3	67	1054	63	1001

* 10	2			
0	1105	0	1010	0
1	-162	1355	-126	1052
2	1386	584	1303	549
4	816	-38	974	-46
* 10	3			
0	0	598	0	440
1	-254	1330	-220	1152
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4	-726	-214	-612	-181
* 10	4			
1	386	1131	236	691
2	1136	258	948	216
3	21	1046	18	908
4	540	94	613	107
* 10	5			
0	0	-757	0	-611
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2	-1444	-343	-1318	-313
4	-805	-459	-1073	-612
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* 10	8			
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* 10	9			
1	565	-408	475	-343
2	-696	596	-540	462
3	357	-656	357	-656
* 10	10			
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1	-604	-204	-501	-169
2	-1111	316	-1157	329
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* 10	12			
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4	207	281	288	391
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0	727	0	505	0
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* 11	7			
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* 11	9			
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* 11	10			
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* 12	1			
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* 12	3			
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* 12	4			
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* I2	6			
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* I2	8			
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* I3	O			
I	O	827	O	603
* I3	I			
O	O	608	O	583
I	899	428	780	37I
* I3	2			
O	-757	O	-980	O
I	-650	-443	-34I	-232
* I3	3			
O	O	I046	O	I024
I	943	8I	I0I3	87

6 APA unobserved reflections

F^2 = ½ minimum observed intensity.

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*	0	2		
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	7	0	311	0	212
*	0	3		
	5	0	-369	0	-431
	7	0	-304	0	-364
*	0	4		
	7	0	296	0	99
*	0	5		
	1	0	-339	0	-65
	5	0	375	0	74
	7	0	280	0	275
*	0	6		
	7	0	-263	0	-164
*	0	7		
	7	0	-240	0	-159
*	0	8		
	1	0	460	0	148
	3	0	300	0	256
	4	416	0	201	0
	6	-326	0	-100	0
	7	0	-215	0	-309
*	0	10		
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*	0	11		
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*	0	12		
	1	0	603	0	160
	3	0	-356	0	-30
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	5	0	304	0	334
*	0	13		
	4	424	0	341	0
*	0	14		
	1	0	-618	0	-458
*	0	15		
	1	0	-594	0	-499
*	0	17		
	1	0	-480	0	-2
*	1	0		
	5	0	-465	0	-728
*	1	1		
	5	407	-226	522	-290
*	1	3		
	5	98	-462	119	-562
*	1	5		
	7	230	-272	140	-165

*	1	7		
	6	-435	-40	-340	-31
*	1	9		
	6	-80	379	-87	412
*	1	11		
	4	453	-16	176	-6
	5	348	243	93	65
	6	-5	-307	-3	-150
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	5	-360	141	-381	150
*	1	13		
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	5	339	20	423	25
*	1	14		
	0	-421	0	-74	0
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*	1	16		
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*	1	18		
	0	272	0	237	0
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*	2	6		
	4	350	142	307	124
*	2	8		
	6	-200	-114	-96	-54
*	2	9		
	0	0	-349	0	-403
*	2	12		
	1	240	118	241	119
*	3	0		
	7	0	-263	0	-363
*	3	5		
	7	-22	214	-28	264
*	3	7		
	1	-264	157	-113	67
*	3	8		
	0	-336	0	-371	0
*	3	9		
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*	3	11		
	5	28	-348	17	-217
*	3	13		
	0	0	-424	0	-242
	4	-342	-206	-72	-44
*	3	16		
	0	-359	0	-401	0

*	3	17				*	5	16	
	1	192	-230	170	-204		0	307	0	47	0
*	4	0					2	-209	-16	-118	-9
	3	-240	0	-113	0	*	6	0	
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	6	311	0	410	0	*	6	1	
	7	235	0	3	0		0	0	322	0	260
*	4	1				*	6	4	
	0	0	-249	0	-183		4	-153	197	-74	94
	6	195	237	278	338	*	6	5	
*	4	2					0	0	-359	0	-90
	1	71	134	35	67	*	6	6	
	5	-291	138	-236	112		4	197	-160	216	-175
	6	-168	-257	-187	-285	*	6	7	
*	4	6					5	-214	-85	-224	-89
	0	315	0	168	0	*	6	8	
	5	82	311	72	274		0	402	0	112	0
*	4	8					5	-181	125	-24	16
	5	75	298	86	339	*	6	12	
*	4	10					1	-93	231	-114	283
	5	13	-280	8	-174	*	7	1	
*	4	13					5	248	330	222	296
	0	0	-421	0	-181	*	7	2	
*	4	14					5	-293	287	-358	350
	3	-20	303	-3	45	*	7	3	
*	4	16					5	-37	406	-72	801
	0	-336	0	-50	0	*	7	7	
*	4	17					1	-342	168	-172	84
	0	0	-280	0	-251	*	7	10	
	1	185	-99	339	-181		3	-266	-199	-193	-144
*	5	0				*	7	11	
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	0	0	300	0	300	*	7	12	
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*	5	4					1	-340	-460	-223	-301
	0	-311	0	-85	0		3	211	-190	229	-206
*	5	5				*	7	13	
	7	-76	140	-101	185		1	-516	-110	-500	-107
*	5	9				*	7	14	
	4	-242	-201	-392	-325		1	369	-292	463	-367
	6	197	13	192	13	*	8	0	
*	5	10					1	-215	0	-307	0
	4	299	-73	337	-82		4	453	0	99	0
*	5	14					5	369	0	546	0
	2	-172	-226	-291	-383		6	-272	0	-127	0
*	5	15				*	8	2	
	0	0	-356	0	-173		4	256	290	207	234
	2	-249	14	-482	27		6	207	-163	113	-89

* 8 4				
4	18	384	29	615
5	-314	153	-359	175
* 8 5				
0	0	-344	0	-267
3	-170	-351	-111	-229
5	-308	142	-633	292
* 8 6				
4	86	383	90	399
* 8 7				
0	0	424	0	148
* 8 8				
4	47	384	42	340
* 8 9				
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* 8 11				
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2	334	148	253	112
* 8 12				
1	262	-246	216	-203
* 8 13				
1	-219	-241	-124	-136
* 9 0				
3	0	-353	0	-298
* 9 2				
0	-421	0	-192	0
3	147	-321	55	-121
* 9 3				
2	159	359	278	626
3	211	279	278	368
* 9 4				
0	424	0	235	0
* 9 6				
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* 9 7				
2	-30	374	-69	862
* 9 8				
0	-410	0	-754	0
1	-592	-49	-411	-34
2	77	354	109	502
* 9 9				
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2	-134	315	-222	521
3	-283	22	-351	28
* 9 10				
0	372	0	236	0
1	353	-407	155	-178
* 9 11				
2	-113	265	-108	254

* 9 12				
1	223	-379	129	-219
* 10 0				
1	617	0	112	0
3	329	0	576	0
4	381	0	153	0
* 10 1				
4	-153	-345	-135	-304
* 10 2				
3	86	-314	108	-393
* 10 3				
2	-189	324	-254	434
* 10 4				
0	-416	0	-326	0
* 10 5				
3	286	-113	262	-104
* 10 6				
2	282	212	259	194
* 10 7				
2	-335	27	-476	38
3	53	-271	40	-203
* 10 8				
0	-372	0	-26	0
2	-196	251	-463	595
* 10 9				
0	0	-349	0	-119
* 11 0				
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2	0	346	0	307
* 11 1				
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* 11 2				
1	-328	461	-211	296
2	55	335	82	505
* 11 3				
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4	-126	-220	-62	-108
* 11 6				
0	353	0	266	0
* 12 0				
1	482	0	211	0
* 12 1				
0	0	-336	0	-368
1	353	325	215	198
* 12 3				
1	458	68	682	101
* 12 4				
0	-311	0	-105	0

```
*   I2      5
    I      -326      -267       -50       -41
*   I2      7
    I      -339       -53      -242       -38
```

Chapter 5

Chemistry of cephalosporin C

and cephalosporin Cc.

CHAPTER V

Cephalosporin Cc Chemistry.

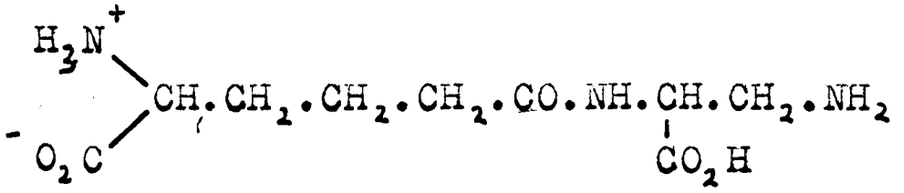
Abraham and Newton (7) have described the degradative chemistry of Cephalosporin C. The following account is derived from their paper.

Cephalosporin C ($C_{16}H_{21}O_8N_3S$) shows an infrared absorption bond at 5.62μ; this compares very closely with the corresponding bond in benzyl penicillin at 5.78μwhich is very characteristic of the fused β-lactam system.

The following degradation products could be obtained.

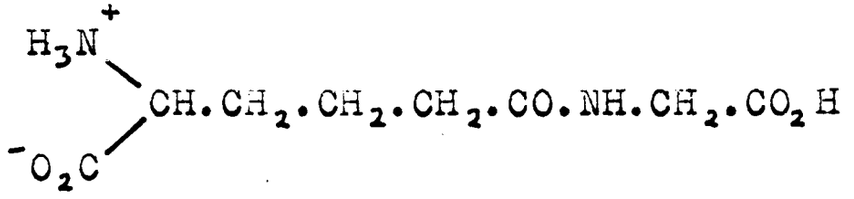
Vigorous hydrogenolysis followed by hydrolysis gave L-alanine, valine, glycine and D-αaminoadipic acid.

Hydrogenation with Raney nickel at room temperature followed by hydrolysis gave valine, alanine, glycine and the diaminodicarboxylic acid (I)



(I)

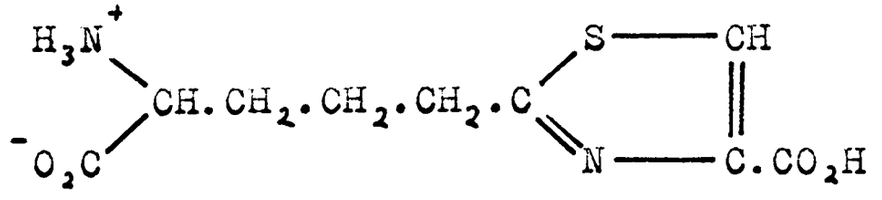
A small quantity of δ-amino-δcarboxy valeryl glycine (II)



(II)

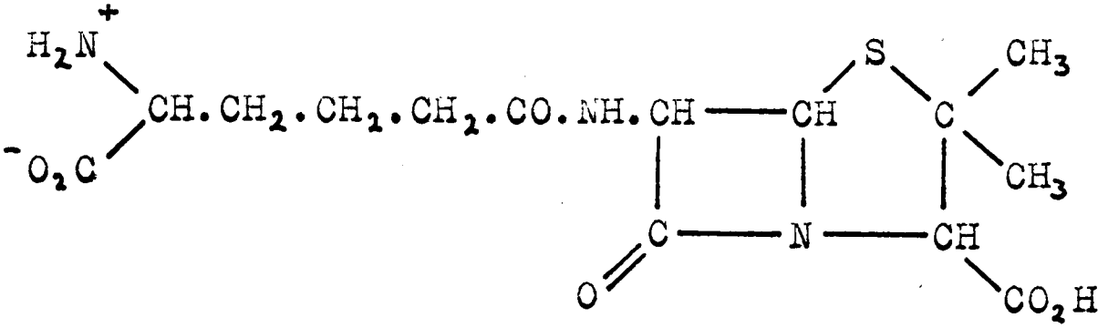
was formed on acid hydrolysis followed by oxidation of the neutral product with silver oxide.

On keeping cephalosporin C in neutral aqueous solution at 37°, it was possible to isolate 2- (D-4-amino-4-carboxy-butyl) thiazole-4-carboxylic acid.



(III)

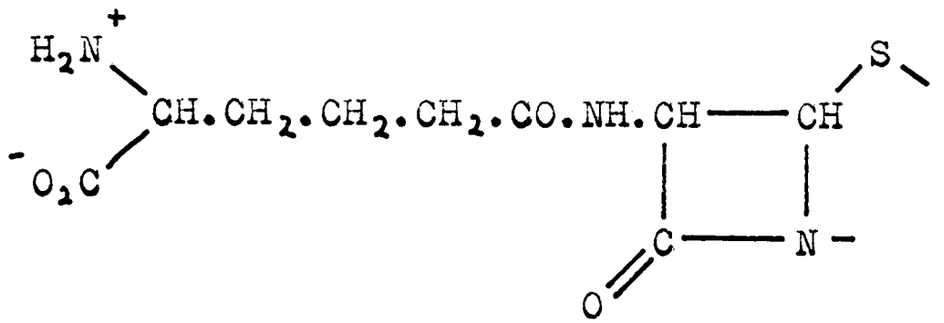
In many of these reactions cephalosporin C resembled cephalosporin N(IV) whose structure had already been determined by Newton and Abraham (52). In its biological action cephalosporin C resembles that of the penicillins in a number of ways.



(IV)

However it differed in a number of important ways. It did not give any penicillamine (D-β mercaptovaline) on hydrolysis. The valine obtained after treatment with Raney nickel was racemic, whereas D-valine was obtained from cephalosporin N.

From this evidence Abraham and Newton suggested that the cephalosporin C had the partial structure (V), which was identical with the corresponding portion of cephalosporin N, but that the remainder of the molecule was somewhat different.

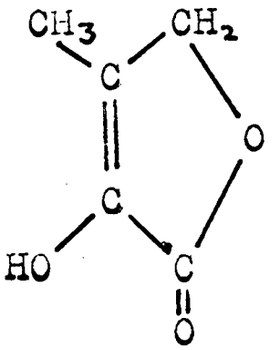


(V)

A number of other properties of cephalosporin C were important in deciding on the structure of the rest of the molecule.

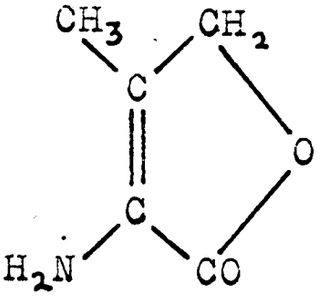
Cephalosporin C is a dibasic acid, and has an absorption maximum at 260 mμ in the ultraviolet. Nuclear magnetic resonance showed that there was no gem-dimethyl group as in the penicillins, though chemical analysis

indicated approximately one methyl group. One mole of acetic acid was liberated by the action of sulphuric acid, which could be assigned to a $CH_3.CO.O.C$- grouping in accord with the infrared spectrum. Treatment of cephalosporin C (or Cc) with Raney nickel followed by hydrolysis produced the enolic form (VI) of β- methyl-α-oxo-γ-butyrolactone.



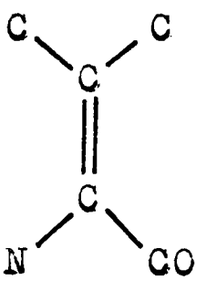
(VI)

If cephalosporin C was kept in N/10 HCl at room temperature a neutral antibiotic cephalosporin Cc, was obtained; (as the crystallography of this is the subject of this thesis its production and chemistry are dealt with more fully on p. 129). If cephalosporin Cc was treated with Raney nickel the lactone (VII) of αβdehydro-γ- hydroxyvaline was produced.

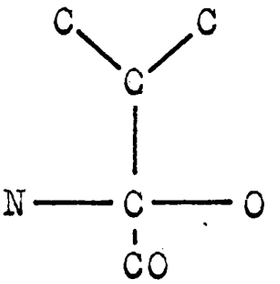


(VII)

The formation of DL-valine and α-oxoisovaleric acid $(CH_3)_2.CH.CO.CO_2H$. on hydrogenolysis suggested that they must have come from the skeletons (IX) or (X)



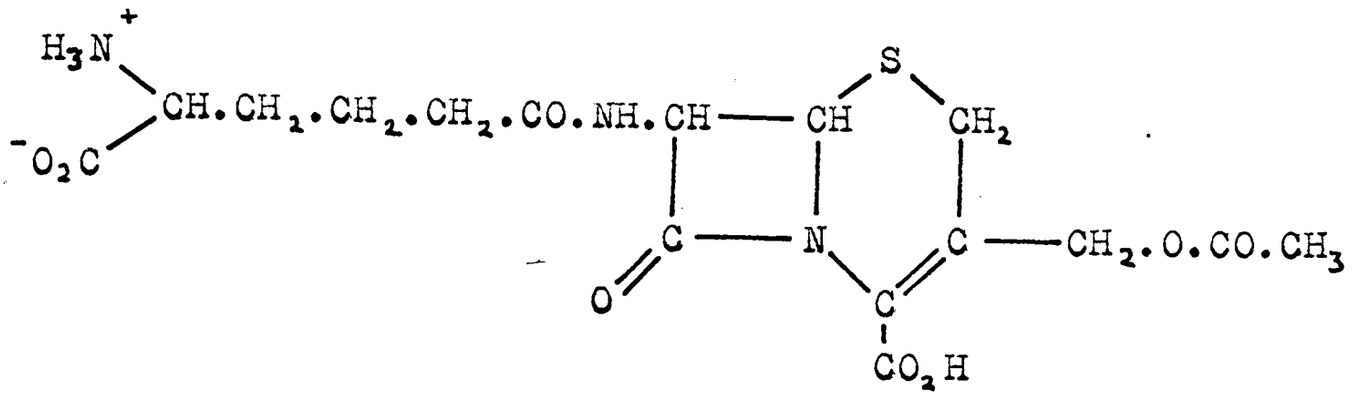
(IX)



(X)

Abraham and Newton had proposed (XI) as the structure of cephalosporin C, some time before all the degradative reactions had been worked out. This accounted for all the reactions mentioned here as well as others mentioned

in their paper.



(XI)

The double bond absorption at 260mμ was somewhat longer than the value in simple model compounds (223 mμ), but this may be due to the unusual properties of the β - lactam ring in the penicillins. The position of the double bond was determined by ozonolysis; also no other possible structure provided reasonable explanations for the known facts. The x-ray analysis of cephalosporin C by Hodgkin and Maslen (36) which was carried out at the

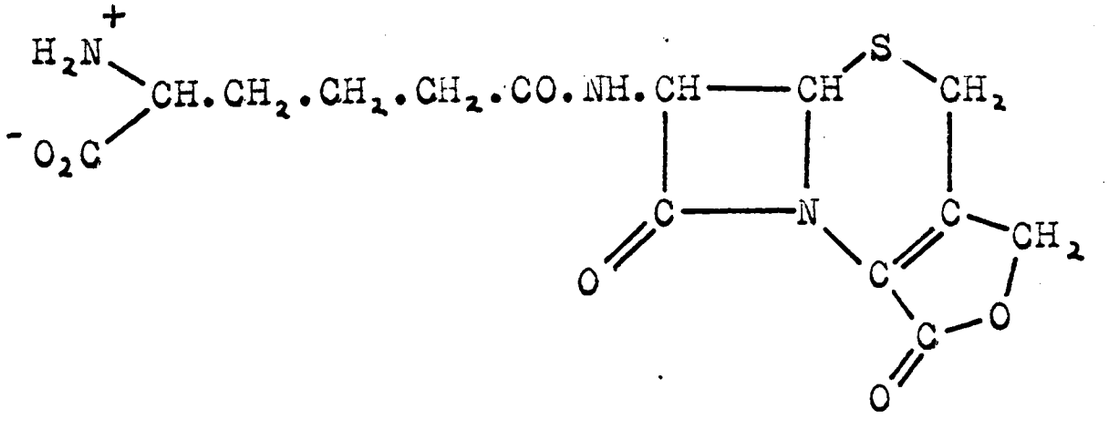
same time confirmed these conclusions (p 9).

Preparation of cephalosporin Cc.

Abraham and Newton prepared Ceph.Cc by keeping Ceph.c in acid solution for 18 hours at room temperature. About half the Cephalosporin C was converted to the lactone Ceph Cc. This was separated by chromatography, and the solution concentrated under vacuum. This crystallised on the addition of acetic acid.

Cephalosporin Cc showed about a tenth of the antibiotic activity of ceph C. The ultraviolet absorption bond at 260 mμ in ceph C is shifted to 257 mμ in ceph Cc. It has two ionizable groups with one pKa <2.4 and one at 9.6. In alkaline or neutral solutions ceph Cc was unstable, probably through fission of the β-lactam link.

In other respects it resembled Ceph c itself; its mode of formation and properties showing it to be deacetyl cephalosporin C lactone (XII)



(XII)

CHAPTER 6

Solution and refinement of the structure of cephalosporin Cc.

Crystallisation

The crystals supplied by Drs. Newton and Abraham were far too small for X-ray crystallographic investigation, so that it was necessary to grow larger crystals.

The original crystals had been obtained by acidifying with acetic acid a neutral aqueous solution of cephalosporin Cc. This method was not considered suitable for producing larger crystals as cephalosporin Cc is not stable in neutral solution (half life about 1 day). Instead it was decided to try to crystallise the Ceph Cc from an acidified aqueous solution, using a mixture of alcohol and acetic acid. First a method similar to that used by E. N. Maslen (36, 45) for ceph C was tried.

A small amount of an almost saturated solution of ceph Cc in water was put in the bottom of a number of small test tubes. To each of these was added an increasing quantity of precipitant. It was hoped that at the lowest concentration of precipitant there would be no crystallisation, and at the highest crystallisation would be rapid, but that somewhere in between would be the optimum concentration. In fact the best

crystals were obtained from the most concentrated solutions, but these were still too small for the collection of intensity data. After a number of attempts this approach was given up.

The next method tried was to crystallise the ceph Cc by diffusion. As only a small amount of ceph Cc was available it was decided to do this in thin walled capillary tubes of about 1mm. diameter. The solution of ceph Cc was introduced into one end of the horizontal tube, and a solution of alcohol and glacial acetic acid in the other end. The two ends were then sealed and the tube tipped up so as to allow the alcohol-acetic acid mixture to flow slowly down into the solution of Ceph Cc. After a number of trials, the best concentration that was found for the alcohol-acetic acid mixture was 90% methanol, 10% glacial acetic acid v.v. This method was rather erratic in its results but it did succeed about once in every five attempts. On these occasions the best crystals formed laths with the following dimensions:-

along a 1.0 mm.
along b 0.07mm.
along c 0.03mm.

The crystals showed parallel extinction.

Crystal Data

Formula $C_{14} H_{17} O_6 N_3 S , CH_3 CO_2H$

Molecular weight = 415.43

Laue symmetry mmm.

a = 5.04_5 ± 0.02 Å

b = 7.00_5 ± 0.02 Å

c = 54.6 ± 0.2 Å

V = 1931 Å

Dcalc = 1.428 gm/cm^{-3}

Z = 4

Fooo = 872 electrons

μ = 19 cm^{-1}

Systematic absences

h k l	none
h o o	h ≠ 2n only
o k o	k ≠ 2n only
o o l	l ≠ 2n only

So the space group can only be $P2_12_12_1$

CELL DIMENSIONS

Preliminary measurements were obtained from oscillation photographs. Because of the very long axis it was not possible to use the Straumanis technique with oscillation photographs because of the difficulty of indexing reflections.

Measurements were then made of the outer reflections on ordinary Weissenburg intensity photographs. The reflections used had values of θ between 60° and 75°.

Collection of Intensity Data

For the same reasons that applied in the case of 6-APA (p. 29) no correction was applied for absorption. All the reflections were recorded by multiple-film equi-inclination Weissenburg technique using filtered Cu $k\alpha$ radiation. As the crystals were small, exposures of about 10 days for each layer were necessary. Layers okl - 3kl on the main exis and hol - h5l on the subsidiary axis were measured. Two intensity strips were used for each axis, one for the inner and one for the outer layers. On the main axis spot-shape variations due to the shape of the crystal were not troublesome.

Each spot was measured at least once on the contracted side of the film and once on the expanded side, though more usually three or four independent measurements were made. The width of the spot was estimated visually by comparing its width with that of the spots on the intensity strip; a corresponding correction was made to the intensity. It was found that this gave quite consistent results with both contracted and expanded spots, even on the subsidiary axis.

The reflections extended right out to the limit of the copper sphere. However, because of the long (54.64 Å) c axis it was often impossible to index reflections on festoons with values of h higher than 5 or k higher than 6, thus in effect limiting the data to values of Sin θ less than 0.8 in a and b, but not in c.

A total of 1071 reflections were observed on the main axis, out of a possible 1900 on those layers measured, and on the subsidiary axis 1381 out of a possible 2081. All told 1520 reflections were measured out of a possible 2580 in the copper sphere. About 500 of those reflections that were not measured were those that were impossible to index, or did not occur on the

layers photographed; the remaining 500 were distributed at random throughout reciprocal space.

The different layers were scaled together using the methods described for 6-APA (p. 32). An average temperature factor of 2.7 Å^2 was obtained from HOL and OKL Wilson plots. This also provided an approximate value of the absolute scale.

Two-Dimensional Work

As there were two short (5.04 Å and 7.005 Å) axes it was decided to try and obtain some information from two-dimensional work. It was hoped that at least the positions of the heavy atoms might be found.

A Patterson series was calculated for each of the two projections using as terms F^2 sharpened by the function $(1/\hat{f}^2) \exp (K \ \text{Sin}^2 \theta)$. In this case, K was taken as 2 and $\hat{f}$ was the weighted mean of the scattering factor curves used throughout the calculations. (p. 49).

Atoms in general position for $P2_12_12_1$

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

give Harker vectors at

$\frac{1}{2}$+2x	2y	$\frac{1}{2}$
$\frac{1}{2}$	$\frac{1}{2}$+2y	2z
2x	$\frac{1}{2}$	$\frac{1}{2}$+2z

Attempts were made to find the S-S vectors in the two dimensional Pattersons; even after further sharpening it was not possible to find a sufficiently consistent set of peaks, and the attempt was given up. Later three-dimensional work showed that all the largest peaks were due to multiple superpositions of S to light-atom vectors.

Three-Dimensional Work

The F^2 were sharpened by the same function as was used for the two-dimensional Patterson (page 136), but using a value of 2.3 for K. This corresponded to the average value of B from the Wilson plots. The modification function was chosen for the reasons discussed in the section on 6-APA (Page 39). These modified F^2 were used as terms in a Patterson

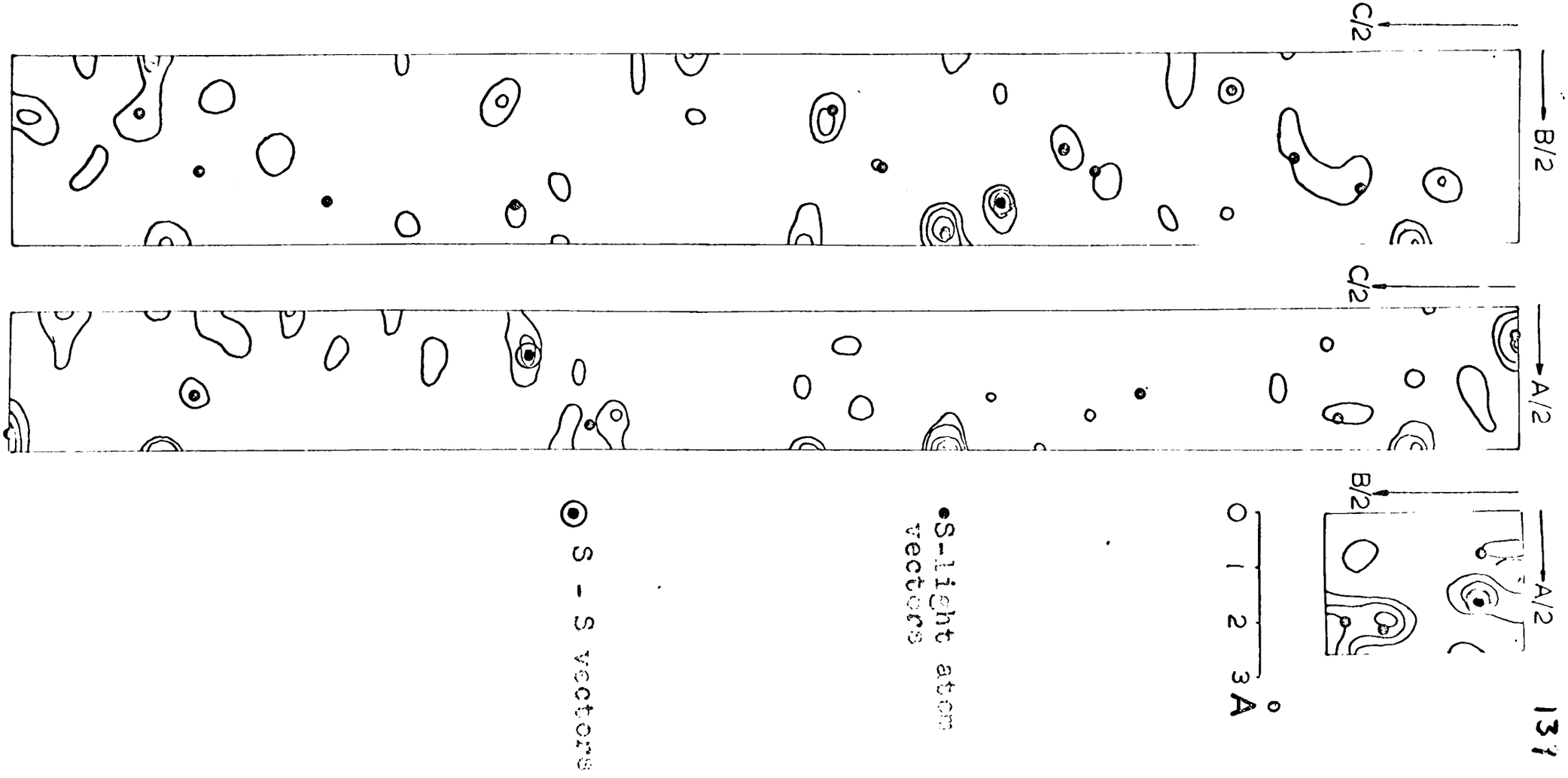
series.

The three unique S-S peaks lie in the Harker sections at $x = \frac{1}{2}$, $y = \frac{1}{2}$ $z = \frac{1}{2}$, (figs. 6.1, 6.2 and 6.3) with co-ordinates having the relationships given on page 137. From these Harker peaks it should be possible to determine the positions of the Sulphur atom. The choice of the correct S-S peaks is made easier by the fact that they must form a set consistent with these relationships. Many of the stronger peaks in the Harker section cannot represent a S-S peak, as there is no corresponding peak in any of the other sections.

The true peaks were identified as they were the only reasonably consistent set of peaks. On no Harker sections was the S-S vector the strongest peak, though on the section at $y = \frac{1}{2}$ it was the strongest peak in a general position. In order to try to confirm the choice of S-S peaks the Harker sections at $x = \frac{1}{2}$ and $y = \frac{1}{2}$ were recalculated using a value of $K = 5.5$ in the sharpening function, and later at the request of Sixten Abrahamsson the Harker section at $x = \frac{1}{2}$ was calculated with $K = 9.5$, in which only the outermost

Fig. 6.1, 6.2 and 6.3 Harker sections at $x = \frac{1}{2}$, $y = \frac{1}{2}$, $z = \frac{1}{2}$, (from left to right).

Contours at arbitrary intervals.



terms were of any importance. (All references to height of the Patterson function refer to these summations which are on an arbitrary scale). Contrary to Abrahamsson's experience with Ceph C. this did not significantly enhance the relative height of the S-S peaks, though later in the refinement the reason for this became clearer. The components of the temperature factor in the x and y planes, for the atoms in the α-amino acid group were about a quarter of those for the S atom. Thus for reflections with large values of h or k the contribution from each of C24, N25, C26, O27 and O28 were about the same as the contribution from S_1. These atoms would, therefore, be expected to give much stronger S-light atom vectors than the rest of the molecule, and to even give observable Harker peaks. The average value of the peaks corresponding to vectors between S and these atoms is 100 on an arbitrary scale, compared with an average value of 65 for the rest of the molecule and a height of 250 for the S-S peaks. The Harker peaks of this group of atoms all occurred in positive areas even though Fooo was omitted.

Only the two Harker sections at $x = \frac{1}{2}$ and $y = \frac{1}{2}$

could be used for determining the S position; the section at z = ½ is too small and too crowded. The S atom co-ordinates derived from the Patterson are given below and compared with the positions ultimately determined.

	Position of S calculated from Patterson	Position of S from final refinement	Difference
x (Å)	0.36	0.47	0.11
y (Å)	0.380	0.398	0.018
z (Å)	4.67	4.685	0.015

As the x and y co-ordinates could only be measured once, while the z co-ordinate could be measured twice, it was to be expected that the maximum error in z would be less then for the other two directions.

As in 6-amino-penicillanic acid (p. 42) the next stage of the refinement was the drawing of a superposition map from the Patterson. In order to obtain the function the whole Patterson was shifted without any rotation so as to superimpose the origin on one of the three related Harker peaks. The contours of the shifted Patterson were then copied on to the original Patterson

in coloured ink. This was repeated for the two other related peaks. Thus the final function was made up from four different coloured Pattersons.

The sum and minimum functions could then be easily estimated visually, the sum function at any point being obtained from the sum of the contours surrounding it, and the minimum function by finding the maximum contour surrounding the point that was present in all four colours.

The error in determining the x co-ordinate of the sulphur meant that the superpositions were based on Harker peaks at sections 2/16 and 10/16 instead of the true values of 3/16 and 11/16.

It was reasonable to assume that the relationships of the six- and four-numbered rings, and the first four atoms of the side chain would have a strong resemblance to the structure of ceph. C which had been solved by Hodgkin and Maslen (36). The five-membered ring had to be coplanar with the adjoining atoms of the six-membered ring if the proposed chemical structure was correct. In ceph C the short axis (5.0 Å) was fixed by the hydrogen bond between adjacent peptide linkages of the α amino adipic acid side chain. In view

of the similar cell dimensions it was likely that the peptide linkage would have a similar orientation to that in ceph C. (This was in fact the case).

Thus we began with a fairly good idea of the likely form of the ring section of the molecule. With this knowledge it was possible to decide on reasonable positions for the six-membered ring and for the peptide group. The probable distance of the oxygen of the amide carbonyl group from the sulphur atom was known from ceph C. There was only one strong peak at this distance, this had another strong peak next to it in the right position for the adjoining carbon atom. Both these peaks were strong as many of the contributary Patterson peaks occurred on special positions. The position found for the carbonyl group was consistent with the position of the six-membered ring. It was not possible to follow the α-amino adipic acid side chain beyond the carbonyl group. There were a number of heavy peaks with z co-ordinates of $\frac{1}{4}$, these were thought to be part of the α-amino acid grouping, as they were about the right distance from the ring system, and hydrogen bond formation would be expected around the screw axes.

The carboxyl group was in effect a mass of undefined electron density, and in particular the peak corresponding to C_{24} was not at all clear. So it was not possible to assign definite co-ordinates to any of the atoms of this group.

Table 6.1 gives an analysis of the errors in the positions during the early stages of the analysis. Only one false peak was picked at this stage, and the errors in the other peaks were surprisingly small.

It is interesting to compare the early stages of this work with the similar ones in the solution of ceph C by Hodgkin and Maslen (36). This crystallises in space-group C2, a = 38.88, b = 4.99, c = 25.65 Å, $\beta = 115^{\circ}25'$, with two molecules per asymmetric unit. These are approximately related to each other by a displacement of c/2. Some of the early work was on the mean molecule in which it was assumed that the relationship was exact. The S-S peak was one of the six heaviest peaks in the Harker section used to solve the structure; the sharpening function used was $\sin^4\theta e^{-(9\sin^2\theta)}$

In a later calculation using data sharpened to point atoms at rest the S-S vector was the highest

Table 6.1 Fourier Refinement Scheme

Stage	Atoms included	Average difference from ALS 3 (A)	Maximum difference from ALS 3 (A)
From superposition (input 1st 2.D trial	S_1 C_2 C_3 C_4 N_5 C_6 C_7 N_{18} C_{19} O_{20} O_{28} (1 erroneous)	0.14 (excluding erroneous atom)	0.29
Input 1st 3.D trial	S_1 C_4 C_{19} C_{20}	0.09	0.23
Input 2nd 2.D trial	All except C_6 and O_9	0.13	0.40
Input 2nd 3.D trial	S_1 C_3 C_4 O_{17} N_{18} C_{19} O_{20} C_{22} C_{23} N_{25} C_{26} O_{27} O_{28} C_{30} C_{31} O_{32} O_{33}	0.07 (excluding 0.17)	0.36
Input 3rd 3.D trial	All except O_{17}	0.05	0.23

Table 6.1 continued

From 3rd 3.D trial = input ILS.1	All	0.04	0.20

peak in the Harker section. The implication diagram plotted from the original Patterson showed better resolution than the corresponding stage for ceph Cc. This is partly due to the high temperature factor on the sulphur atom in ceph Cc, relative to the rest of the molecule. (p. 140). But it is probably also due to the differences in the space groups of the two structures. In C2 the Harker vectors have twice the weight of the non-Harker vectors, whereas in $P2_12_12_1$ they have 4 times the weight. However, in C2 there are two unique heavy-light atom vectors for each atom in a volume of $\frac{1}{2}$ that of the true asymmetric unit, and in $P2_12_12_1$ there are 4 unique heavy-light atom vectors in the same volume, the frequency of doubled peaks in the orthorhombic space group is much greater, so that though the S-S peak will be higher in relation to a true single peak there is liable to be a larger number of double and triple peaks in the orthorhombic case, so that the S-S peak will not in fact stand out any clearer and the superposition diagrams are more likely to be confused. Thus if it is assumed that a peak is double if there is another one within $\frac{3}{4}$ Å of it, and that only the mean

molecule for ceph C need be considered, there is the following probability distribution for the number of other peaks within $\frac{3}{4}$ Å .

Probability that there will be the stated number of peaks within $\frac{3}{4}$ Å of a given peak:

No. of peaks	0	1	2	3
ceph C	.67	.22	.075	.02
ceph Cc	.38	.23	.15	.09

These calculations are of course very approximate as the distribution of vector peaks is unlikely to be entirely random.

The same general relations can be looked at in another way. In ceph C there is sufficient information available to determine the x and y co-ordinates of each atom, but only |y| In ceph Cc it is possible in principle to find the positions of all the atoms, so that from the same amount of initial data one is trying to determine more information in the orthorhombic case.

Selection of Atoms

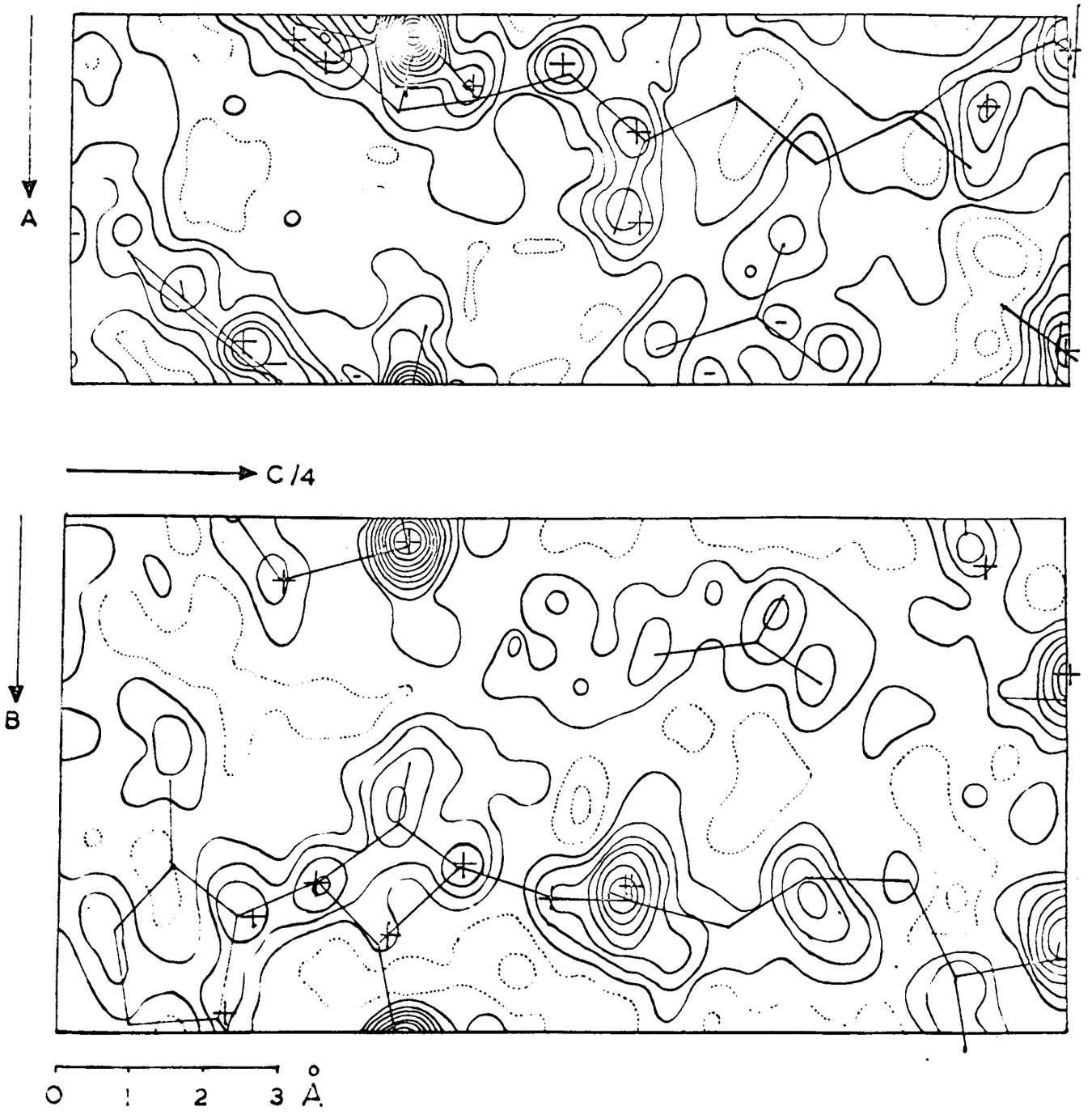
A cautious policy was followed in selecting atomic

positions for inclusion in the full three-dimensional calculations. Only those atoms which could be clearly seen as part of a chemical group and whose position could be measured from a well defined peak were used. A larger number of atoms were included in the two-dimensional calculations which provided a convenient check on the proposed structure.

The reason for this approach is that in a non-centrosymmetric structure a wrongly placed atom is likely to give a quite strong peak in an observed Fourier. An example of this occurred in the 2nd trial structure; O_{17} was accidentally given the wrong x co-ordinate, the resulting peak had a height of 8e A^3, which was higher than one other correctly placed oxygen atom, whereas the peak in the true position had a height of only 1.4 e A^3. Thus had the error not been obvious it might had remained undetected for some time. A similar incident occurred in the investigation of the structure of the vitamin B_{12} hexacarboxylic acid by Crowfoot-Hodgkin et al (37).

Projections

The positions determined from the Patterson were used to calculate projections down [010] and [100] . (figs. 6.4



+ Atoms included in structure factor calculations.

Figures 6.4 and 6.5. Cephalosporin Cc (010) and (100) projections from the first trial structure. Contours at intervals of 2 electrons / $Å^3$, zero and negative contours dotted.

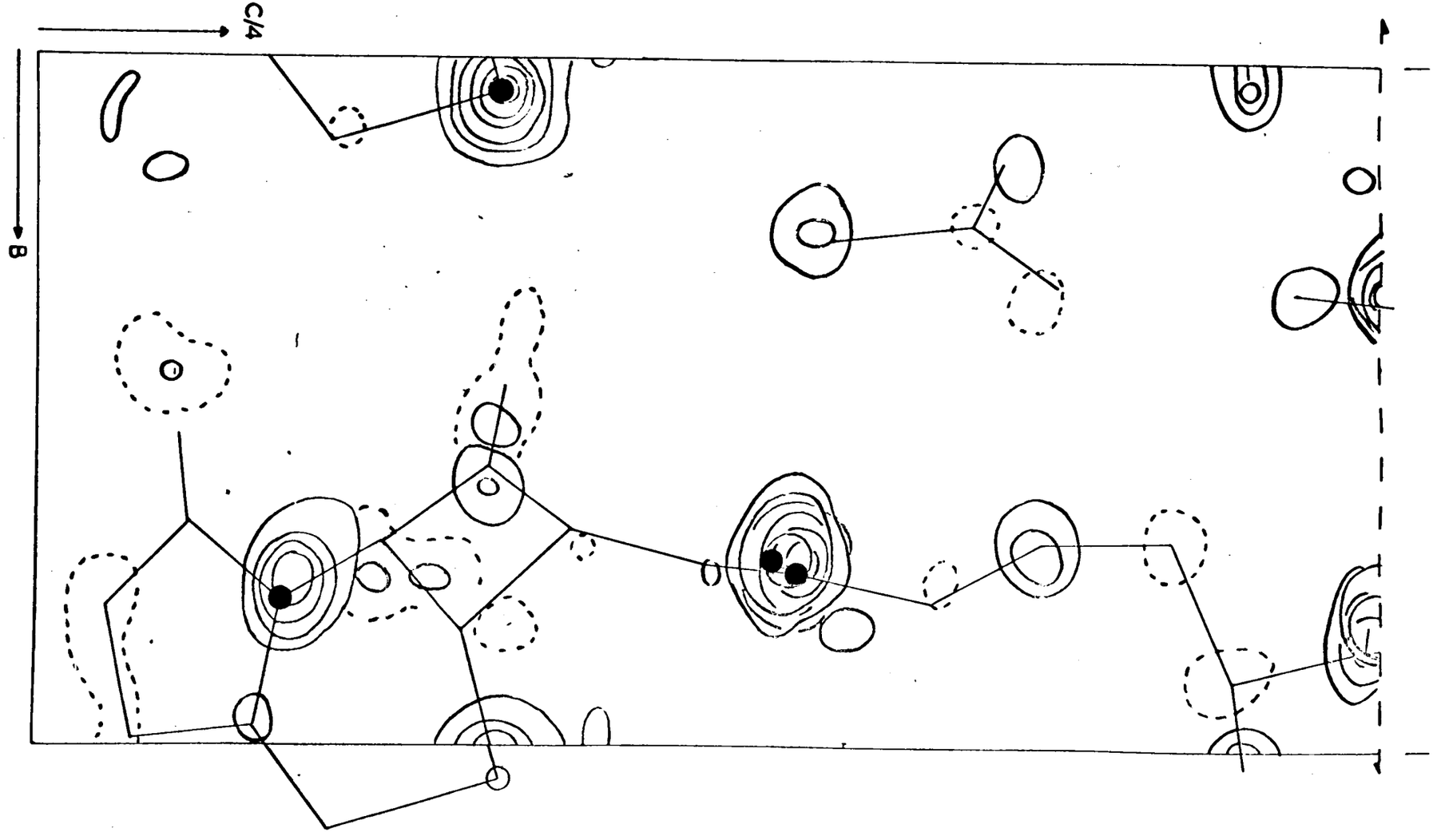
and 6.5). In these the area of the Fourier around the six-membered ring was consistent with the expected shape of the ceph Cc molecule. However, it was no more possible to follow α- aminoadipic acid side chain than it had been in the superposition map, even at its carboxylic acid end.

Only the four atoms S_1, C_4, C_{19}, and O_{20} were inserted in the first three-dimensional calculations. Their co-ordinates were obtained from the Patterson and from the projections. The resulting structure factors gave an R value of 63%. The agreement even for large reflections was not very good, though as only 20% of the total scattering matter had been included this was not very surprising. Of the centro-symmetric reflections about 80% had the same signs as in the final set of structure factors.

A three-dimensional Fourier (ρ_a, fig. 6.6) was then calculated; where the calculated structure factor was small the term was excluded from the summation.

The 2nd trial structure was derived with the help of a model, atomic positions were chosen which were as far as possible consistent with the electron density of the Fourier map, and gave reasonable bond distances and angles. The general shape of most of the molecule could be followed quite easily, even where individual atoms

Figure 6.6 First three dimensional Fourier, contours at intervals of 1 electron/A^3, except for atoms used in phasing where the level is 2e/A^3. 1st (dotted) contour usually omitted. Many small spurious peaks excluded.



were not properly resolved. However, the four-membered β-lactam ring could only be placed from chemical knowledge, as this area contained many false peaks at about 1.2 electrons / $\mathring{A}^3$ compared with a height of 0.6 electrons / $\mathring{A}^3$ at the true positions of O_9.

The γ-lactone ring was also poorly defined, x - co-ordinate of O_{11} being particularly bad.

Table 6.1 p. 145 shows the atoms which were inserted in the 2nd three-dimensionsl set of calculations.

O_{17} was inserted with the wrong x co-ordinate at a distance of 2.3 $\mathring{A}$ from its true position (see page 149 of this thesis). Apart from this the Fourier was very much clearer than the previous one; all the atoms were resolved and their positions could be quite accurately determined. The lowest of the true peaks had a height of 1.7 e $\mathring{A}^3$. All the false peaks had heights lower than this. As the peaks were distinct, R. A. Sparks's 19-point programme was used to calculate the centres of all the peaks (p. 50). If the atom had been included in the structure factor calculations the new position was obtained by applying an n - shift with n = 1.8 (64) to it. If the atom had not been included, the position

for the next round was simply the centre of the observed peak. The average errors in the resulting positions are shown in Table 6.1. An Agreement Analysis using Dr. Lai's programme, showed that there was no significant error in the overall value of B as deduced from the Wilson plots.

The bond distances at this stage were reasonable with an average deviation from the expected values of 0.1Å and a maximum deviation of 0.2Å.

The next stage in the refinement was the use of the new parameters to calculate a difference map. As the false position of O_{17} in the previous round gave the same general peak height as the true peaks (p.149) it was hoped that any gross errors would show up better in a difference map than in an observed map. O_{17} was left out of the calculation as the peak at the position it was thought to occupy only came to 1.4 $e/Å^3$ in the previous round. In this round there was a peak of height 3.1 $e/Å^3$ about 0.2Å away from the position originally chosen for O_{17} from the first three-dimensional Fourier.

This provided a measure of the expected peak height of any atom which had been missed in the structure-factor calculations. The only peaks higher than 1.2 $e/Å^3$ were those associated with atoms which needed shifting. From this it was clear that apart from O_{17} there were no atoms remaining in the structure that had not been included.

in the calculations. The possibility of some sort of disorder could not be ruled out at this stage, even though no evidence of it could be found.

The difference map was also used to work out shifts in the atomic co-ordinates. This was done using the formula

$$\Delta k = \frac{\left(\frac{\partial D}{\partial k}\right)_n}{2p\,(\rho_o)_n} \qquad \ldots\ldots\ldots 4.1\ (73)$$

where k is the co-ordinate of atom n,

$$\left(\frac{\partial D}{\partial k}\right)_n$$

is the slope of the difference map parallel to k, at the centre of the atom, $(\rho_o)_n$ is the peak height on the corresponding observed Fourier map, and p is the constant in the formula

$$\rho_r = Z\left(\frac{p}{\pi}\right)^{3/2} \exp(-pr) \ldots\ldots 4.2$$

which gives the electron density p of an atomic peak as a function of the distance r from the centre (20).
As the Δk given by equation 4.1 is the difference between the peaks in the observed and calculated Fouriers the values

given must be multiplied by the appropriate "n" ("n"-shift rule.)

In order to use equation 4.1 the correct values of ρ_o and p must be known. Only the difference map had been calculated for this round, the values of p and ρ_o were therefore obtained from the peaks in the previous Fourier(ρ_o) As at that stage only 18 out of 27 atoms had been included in the calculations, the peaks were not as sharp as they would be in the third trial structure in which all the atomic positions had been included. Because of this the value of p derived from the second trial structure must be lower by an unknown amount than the true value. So it was considered to be safer not to risk over-shifting, and so a n-shift of n=1 was applied.

No attempt was made to refine the individual temperature factors at this stage, as the results were liable to be misleading.

To check the progress of the refinement the bond distances and angles were calculated for the new positions. The average deviations of the bonds from estimates of their accepted values was 0.06 Å. There were 5 bonds with deviations greater than 0.1 Å, the largest being 0.15 Å.

Table 6.2 Progress of Refinement

Stage	Fourier	R(%)	Shifts (Å) Av.	Max.
12 Positions from superposition (2-D)	[100], [010] projections	65		
1st 3-D (4 atoms)	ρ_a	63		
2nd 3-D (17 atoms)	ρ_b	44		
3rd 3-D (27 atoms)	ρ_c	28		
positions from ρ_c. Input ILS 1.		23	.034	.135
ILS 2.		18.5	.016	.075
ILS 3		16.2	.015	.068
ILS 4	$\rho_{I4\ diff}$	16.0	.007	.038
ILS 5		15.6	.0035	.012
ALS 1		12.5	.0043	.021
ALS 2		11.0	.0058	.023
ALS 3	$\rho_{A3.\ obs.}$ $\rho_{A3\ diff}$	10.6	.0033	.013

The third three-dimensional Fourier provided a check that all possible atoms had been included in the structure.

Least squares refinement

As the largest indicated shift had been 0.1 Å it was unlikely that there were any errors greater than about 0.2 - 0.3 Å in the atomic co-ordinates. The bond distance calculation confirmed this; the largest deviation of a bond from its expected value being 0.15 Å.

As this accuracy is sufficient to enable the least-squares refinement procedure to be used successfully, it was decided to use this for the next stages. As the isotropic least-squares programme cannot absorb positional and other errors in the anisotropic thermal vibrations, (p. 60) it was decided to start with this. The weighting scheme was initially chosen to be scheme 1 with a value of F^* of 17.5 electrons (p 52). Later on as the agreement on the smaller structure factors improved the scheme was changed for the third round to scheme 3 with a = b = 7.5e. In the 1st round the average positional shift was 0.035 Å and the maximum shift 0.14 Å. These shifts were reasonable at this stage in the refinement.

The largest shifts were on atoms which had been difficult to define closely in the previous observed and difference Fourier maps. The distance and angle analysis showed that the bond distances were nearly all improved. The maximum deviation from the expected value was 0.1 Å and the average 0.04 Å compared with 0.15 and 0.06 for the structure derived from the difference Fourier.

The temperature factors at this stage varied between 5.8 $Å^2$ for O_{11} of the γ-lactone ring, and 1.1 $Å^2$ on N_{25} in the α-amino acid group. The low temperature factors associated with this latter group were attributed to the effect of the hydrogen-bond system in which this group was involved. At the γ-lactone end of the molecule there were no hydrogen bonds to prevent vibration, so that a somewhat higher temperature factor was reasonable.

The isotropic refinement was continued for 5 rounds till the positional shifts were negligible. At the end of round two, by assuming tetrahedral configuration of the carbon atoms and a C-H bond length of 1.05 Å it was possible to place all the hydrogen atoms apart from those attached to N_{25} and the acetic acid. It was possible to identify the hydrogens around N_{25} in

ρ_{I4} and those of the acetic acid in $\rho_{A3(diff)}$. These hydrogen atoms were finally placed by taking into account the peaks on the difference map and the expected molecular geometry. Table 6.3 gives the peak heights of the hydrogens in ρ_{A3obs} and ρ_{A3diff}. The hydrogens were placed at this early stage as previous experience with penicillanic acid had indicated that their presence made a difference in the final positions of the carbon atoms of up to 0.02 Å. (p. 59). By the fifth round the value of the average shift had fallen to a tenth of its value in the first round. The interatomic distances at the end of the 5th round were all within 0.04 Å of the accepted values in Sutton (65) so that by this stage any change in these differences was unlikely to be important as the local chemical environment of any bond is liable to change its length by a similar order of magnitude.

The temperature factors at the end of round six still had the same range of values as in the first isotropic round. If the high temperature factors were on isolated atoms these would have suggested a wrong structure, but as they increased fairly regularly from about 3.5 $Å^2$ in the β-lactam ring to 6.8 $Å^2$ on the

Table 6.3

Hydrogen peak heights in the final Fouriers (atoms included in the structure factor calculated)

Atom	Peak height on Fourier (electrons).	
	difference	observed
H20	-0.1	0.8
H21	0.2	0.5
H60	0.0	0.8
H70	0.2	0.9
H100	0.1	0.7
H101	0.1	0.6
H180	-0.1	0.9
H200	0.0	0.8
H201	0.0	0.7
H220	0.1	1.0
H221	0.0	0.7
H222	0.0	1.0
H230	0.2	0.6
H240	0.2	1.2
H250	0.0	0.8
H251	0.0	0.8
H252	0.0	0.9
atoms excluded from structure factor calculation.		
H300,	0.5	0.5
H301	0.5	0.6
H303	0.4	0.4
H330	0.3	0.7

oxygen atom O_{11} of the γ-lactone rings, it was certain that this was a real physical effect.

The structure factors calculated during round five were used to calculate a difference Fourier. This showed very clearly that at the ring end of the molecule there were very strong anisotropic thermal vibrations in the x y plane, which would account for the R value not falling below 0.16. The α-amino acid end of the adipic side chain showed no signs of anisotropic vibration. There were no peaks greater than 0.6 e A^{-3} that were not attributable to thermal vibrations.

As this difference map had shown the reality of the anisotropic vibrations, and the isotropic refinement had ceased to produce significant shifts, a further 3 rounds of anisotropic refinement were carried out. In the first two rounds the shifts were large with values up to 0.02 Å, and only in round three as the anisotropic temperature factors began to take effect did the shifts settle down, and drop in magnitude. The refinement was finished at this stage as any further shifts were unlikely to be significant. The final difference Fourier calculated had no peaks or dips of magnitude greater than

0.5 $e/\mathring{A}^3$. The final observed Fourier is shown in figure 6.7.

Tables 6.4 and 6.5 give the final positions and vibrations found for cephaloporin Cc. These correspond to the wrong absolute configuration which is also that in all the illustrations.

Table 6.6 gives the magnitudes and directions of the vibrational ellipsoids, and table 6.7 shows the agreement analysis for the final round of observed structure factors

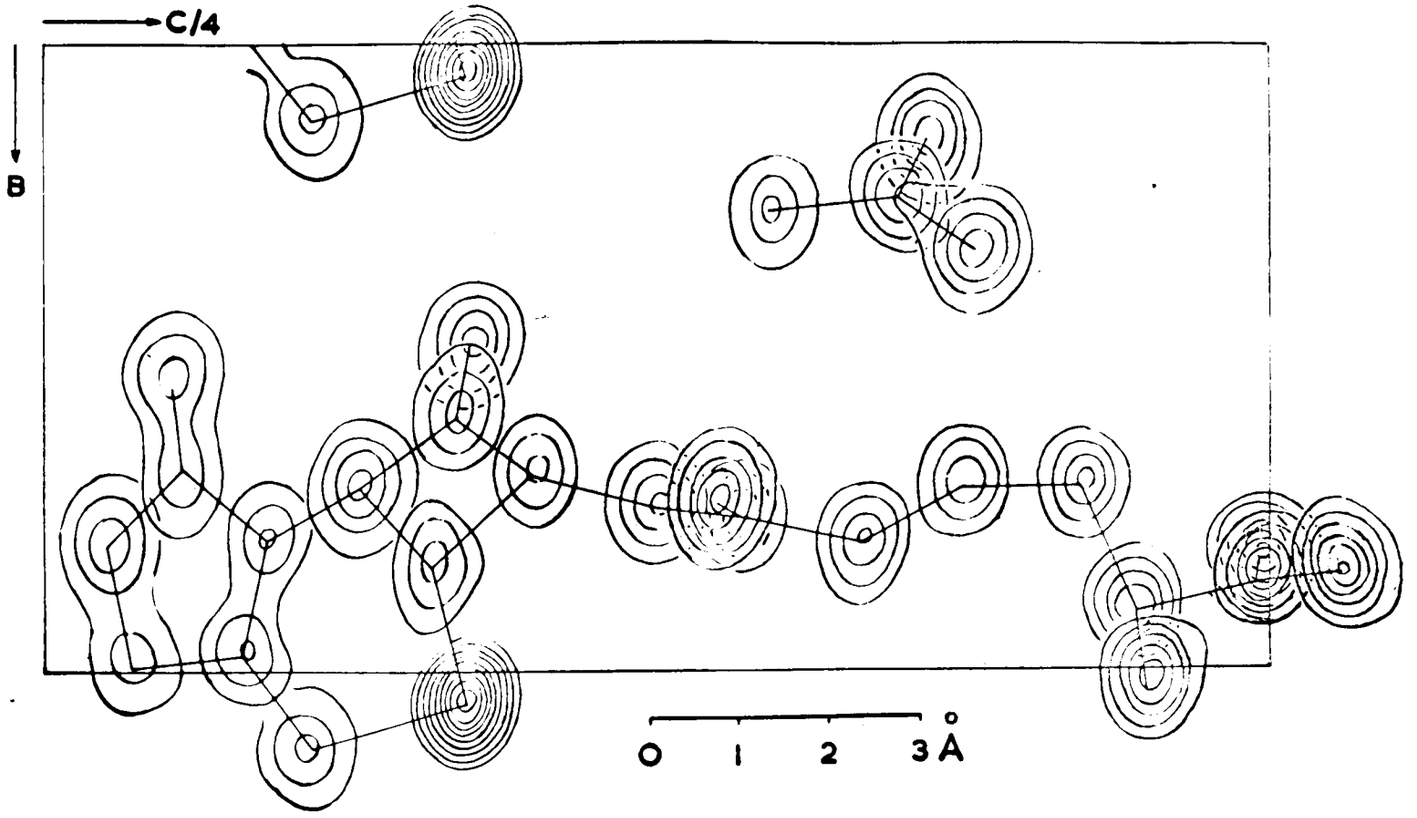


Figure 6.7

Cephalosporin Cc. sections from the final three dimensional Fourier. contours at intervals of 0.3 2 electrons /Å

Table 6.4 Cephalosporin Cc, final positions

Atom	x/a	y/b	3/c
S1	1.0945	1.0568	0.08569
C2	1.0282	1.1254	0.05449
C3	0.8998	0.9715	0.04000
C4	0.9234	0.7864	0.04493
N5	1.0773	0.7065	0.06413
C6	1.2504	0.8320	0.07892
C7	1.2258	0.6818	0.09927
C8	1.0024	0.5958	0.08397
O9	0.8249	0.4817	0.08678
C10	0.7348	0.9886	0.01780
O11	0.6361	0.7994	0.01284
C15	0.7614	0.6751	0.02803
O17	0.7212	0.5085	0.02627
N18	1.1556	0.7359	0.12390
C19	1.3335	0.7486	0.14185
O20	1.5737	0.7347	0.13688
C21	1.2297	0.7894	0.16683
C22	1.4094	0.7016	0.18707
C23	1.2769	0.6979	0.21194
C24	1.1892	0.8902	0.22291
N25	1.4180	1.0240	0.22542
C26	1.0677	0.8510	0.24804
O27	1.2239	0.8393	0.26610
O28	0.8236	0.8149	0.24863
C30	0.8937	0.2631	0.14768
C31	0.8058	0.2420	0.17366
O32	0.6159	0.1510	0.17971
O33	0.9555	0.3292	0.18948

Table 6.4 continued

Atom	x/a	y/b	z/c
H20	1.203	1.165	0.0458
H21	0.900	1.247	0.0544
H60	1.453	0.853	0.0718
H70	1.394	0.605	0.1000
H100	0.861	1.032	0.0027
H101	0.575	1.088	0.0210
H180	0.965	0.742	0.1285
H200	1.201	0.935	0.1696
H201	1.037	0.721	0.1680
H220	1.582	0.787	0.1883
H221	1.466	0.564	0.1823
H230	1.405	0.630	0.2244
H231	1.117	0.605	0.2114
H240	1.032	0.917	0.2118
H250	1.500	1.033	0.2082
H251	1.530	0.930	0.2390
H252	1.325	1.125	0.2375
H300	0.755	0.354	0.1373
H301	0.963	0.154	0.1418
H302	1.088	0.334	0.1460
H330	0.889	0.308	0.2012

Table 6.5 Cephalosporin Cc temperature factors

$$\exp- 10^{-5}(B_{11}h^2 + B_{22}k^2 + B_{33}l^2 + B_{23}kl + B_{13}hl + B_{12}hk)$$

Atom	B_{11}	B_{22}	B_{33}	B_{23}	B_{13}	B_{12}
S1	6969	2929	30	-73	82	414
C2	7044	3343	29	-9	24	1550
C3	6213	2579	28	-11	119	2289
C4	6203	3091	19	-73	-22	272
N5	4491	2682	18	-42	-17	139
C6	3319	2983	22	-167	35	753
C7	4439	3556	17	-108	-21	1167
C8	4438	2036	29	-140	33	1535
O9	5875	2879	30	-120	-18	-856
C10	7277	4138	46	118	-117	2703
O11	6620	5370	33	61	-175	913
C15	7344	4139	24	-78	-168	604
O17	8630	4290	40	-53	-323	-687
N18	3098	2122	18	-11	21	2155
C19	2919	2361	17	-35	-33	686
O20	2795	3427	25	-94	160	756
C21	2741	1651	16	-6	22	-555
C22	3517	1622	16	-32	10	1009
C23	2831	669	23	-26	4	-560
C24	1727	556	16	-17	-74	-722
N25	2057	1112	15	-27	-35	-154
C26	2657	910	12	-54	36	-649
O27	2102	1829	15	35	60	60
O28	1670	1281	26	63	39	-446
C30	8033	2450	21	-24	206	1425
C31	4319	866	24	40	128	783
O32	5943	2951	30	-10	68	-4727
O33	4561	2399	32	-128	174	-2567

Table 6.6 Root mean square vibrations in the three principal directions of the vibrational ellipsoid.

Atom	$\sqrt{(\overline{U_i})}$ Å	x	y	z
S1	0.301	-0.98	-0.160	-0.10
	0.273	-0.12	0.954	0.26
	0.208	0.13	-0.249	-0.95
C2	0.318	0.79	0.604	0.01
	0.269	-0.60	0.795	-0.05
	0.209	0.04	-0.039	-0.99
C3	0.308	0.82	0.547	0.11
	0.229	-0.45	0.781	-0.42
	0.196	0.32	-0.299	-0.89
C4	0.286	-0.82	-0.551	0.09
	0.275	-0.55	0.821	-0.10
	0.168	-0.02	-0.143	-0.98
N5	0.259	0.14	0.984	-0.10
	0.240	-0.98	0.146	0.01
	0.163	-0.03	-0.101	-0.99
C6	0.284	0.14	0.937	-0.31
	0.208	-0.94	0.043	-0.31
	0.162	0.27	-0.344	-0.89
C7	0.305	0.28	0.947	-0.15
	0.232	-0.95	0.276	-0.05
	0.156	0.00	-0.162	-0.98

Table 6.6 continued

Atom		x	y	z
C8	0.264	0.67	0.679	-0.29
	0.229	-0.63	0.331	-0.69
	0.170	0.37	-0.654	-0.65
O9	0.287	-0.70	0.680	-0.19
	0.263	-0.69	-0.626	0.34
	0.202	-0.11	-0.380	-0.91
C10	0.351	0.62	0.779	0.07
	0.291	0.62	-0.442	-0.64
	0.239	0.46	-0.442	0.76
O11	0.367	0.15	0.987	0.04
	0.297	0.93	-0.128	-0.32
	,0.213	0.31	-0.092	0.94
C15	0.328	0.50	0.844	-0.17
	0.304	-0.84	0.527	0.10
	0.183	-0.18	-0.092	-0.97
O17	0.347	-0.90	0.279	0.31
	0.326	0.21	0.952	-0.21
	0.224	-0.35	-0.124	-0.92
N18	0.258	0.58	0.810	0.00
	0.169	-0.47	0.341	-0.81
	0.157	0.65	-0.475	-0.58
C19	0.246	0.26	0.957	-0.11
	0.190	0.95	-0.277	-0.12
	0.160	-0.14	-0.074	-0.98
O20	0.297	0.09	0.982	-0.15
	0.219	-0.69	-0.048	-0.71
	0.153	0.71	-0.178	-0.67
C21	0.209	-0.50	0.860	-0.06
	0.180	-0.85	-0.508	-0.12
	0.154	-0.13	-0.008	0.99

Table 6.6 continued

Atom		x	y	z
C22	0.228	0.79	0.610	-0.04
	0.185	-0.59	0.754	-0.26
	0.152	0.12	-0.242	-0.96
C23	0.194	-0.93	0.250	-0.26
	0.186	-0.28	-0.051	0.95
	0.123	0.22	0.966	0.12
C24	0.170	-0.71	0.231	0.66
	0.147	-0.47	0.528	-0.70
	0.096	0.51	0.816	0.26
N25	0.169	-0.19	0.921	-0.33
	0.166	-0.88	-0.019	0.46
	0.143	-0.42	-0.386	-0.81
C26	0.194	-0.87	0.418	-0.23
	0.151	-0.47	-0.672	0.56
	0.122	0.08	0.609	0.78
027	0.214	0.06	0.985	0.15
	0.171	0.86	-0.133	0.49
	0.142	-0.50	-0.105	0.85
028	0.205	0.01	0.497	0.86
	0.175	-0.46	0.773	-0.43
	0.137	-0.88	-0.392	0.24
C30	0.330	0.95	0.243	0.17
	0.242	-0.20	0.957	-0.19
	0.170	0.21	-0.153	-0.96
C31	0.246	0.91	0.198	0.35
	0.179	0.37	-0.086	-0.92
	0.140	-0.15	0.976	-0.15
032	0.342	-0.72	0.690	-0.05
	0.215	0.08	0.173	0.98
	0.179	-0.68	-0.701	0.18
033	0.299	-0.64	0.657	-0.38
	0.202	-0.27	0.276	0.92
	0.190	0.71	0.700	-0.00

Table 6.7 Agreement analysis on the output from the final round of structure factors.

Range of $F_{obs.}$ (electrons)	$\Sigma\|F_{obs.}\|$	$\Sigma\|F_{calc.}\|$	$\Sigma\|F_{diff.}\|$	R
0 - 5	506	533	135	.27
5 - 10	3571	3514	555	.16
10 - 15	4028	3926	394	.10
15 - 20	3308	3242	335	.10
20 - 25	2613	2589	216	.08
25 - 30	2233	2207	175	.08
30 - 35	1576	1529	174	.11
35 - 40	1196	1187	77	.06
40 - 45	1053	1010	77	.07
45 - 50	988	993	93	.09
50 - 100	3398	3381	389	.11
100 - 150	729	709	39	.05
150 - 200	313	279	36	.11

Table 6.7 continued.

Range of $\sin^2\theta$	$\Sigma\lvert F_{obs.}\rvert$	$\Sigma\lvert F_{calc.}\rvert$	$\Sigma\lvert F_{diff.}\rvert$	R
0 - 0.1	4909	4908	612	0.12
0.1 - 0.2	4556	4408	426	0.09
0.2 - 0.3	4082	3974	371	0.09
0.3 - 0.4	3066	2942	278	0.09
0.4 - 0.5	3064	3048	250	0.08
0.5 - 0.6	2106	2110	232	0.11
0.6 - 0.7	1519	1485	198	0.13
0.7 - 0.8	1106	1100	130	0.12
0.8 - 0.9	719	721	109	0.15
0.9 - 1.0	390	405	92	0.24
TOTALS				
	25521	25105	2701	0.106

CHAPTER 7

Cephalosporin Cc;

Results and Conclusions.

CHAPTER 7

RESULTS AND CONCLUSIONS: CEPHALOSPORIN Cc.

The final bond distances and angles from ALS 3 are shown in figs 7.1 and 7.2. Figs 7.3 and 7.4 show a general view of the molecular structure projected on the (010) and (100) planes.

General Arrangement.

The polar α-amino acid grouping forms a hydrogen-bond system around the screw axes at $z = \frac{1}{4}$ and $\frac{3}{4}$. The dihydrothiazine and β-lactam ring systems at the other end of the molecule form a roughly planar group which is inclined at an angle of about 30° to the c axis and parallel to the b axis. The two neighbouring γ-lactone groups are displaced by the screw axis parallel to a that lies between them, allowing the closest possible approach (fig 7.5). An unusual feature of the crystals is the acetic acid of crystallisation, which seems to be essential as it was not possible to remove it by drying under vacuum at 100° C (7). That the acetic acid was very difficult to remove is hardly surprising considering the important part it plays in the hydrogen

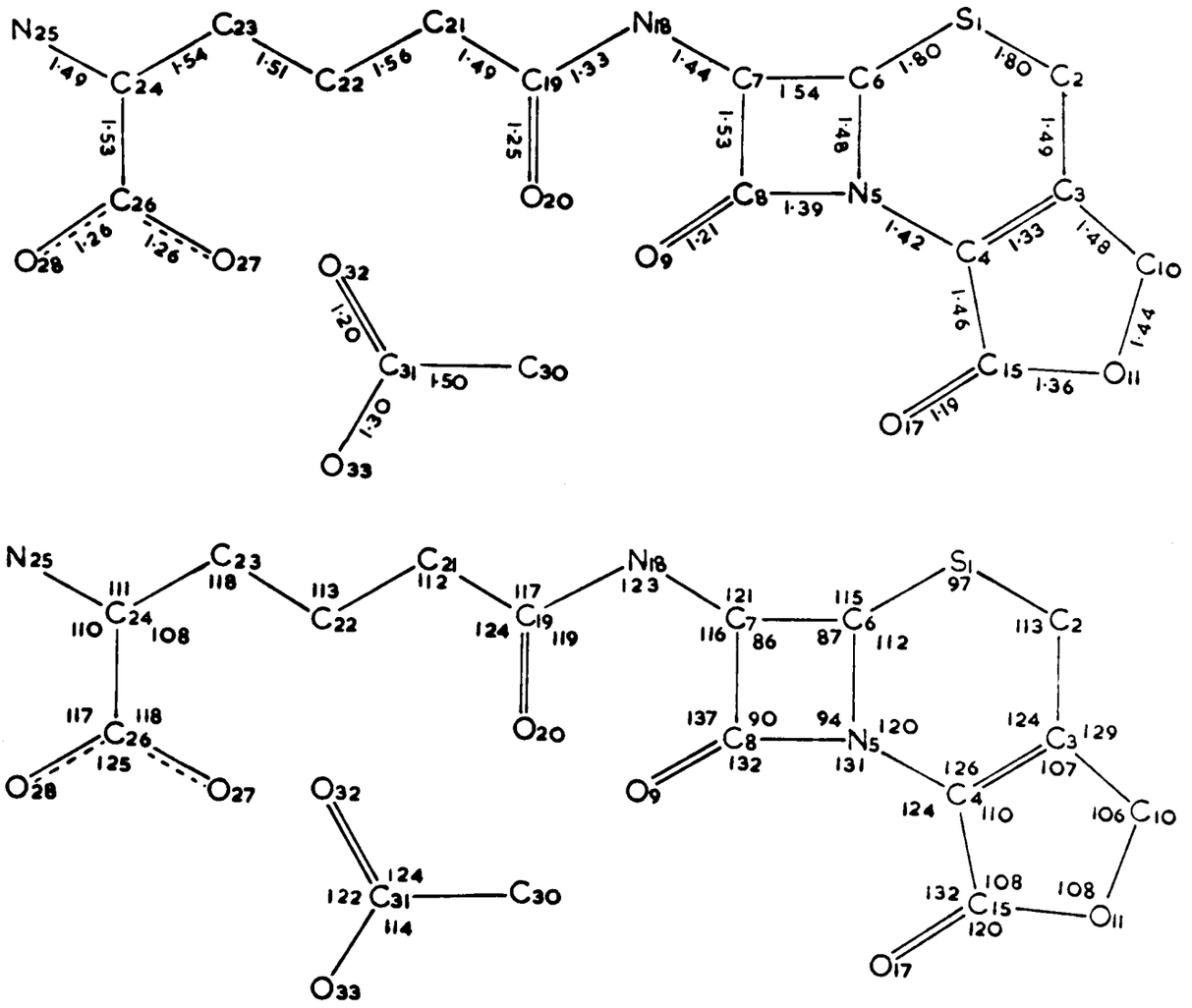


Figure 7.1 and 7.2

Schematic diagram of cephalosporin Cc showing interatomic distances (Å) and angles (degrees).

Table 7.1 Interatomic distances and their standard deviations

Bond	Distance (Å)	Standard deviations
S1-C2	1.802	0.011
S1-C2	1.798	0.012
C2-C3	1.486	0.019
C3-C4	1.329	0.019
C3-C10	1.476	0.019
C4-N5	1.419	0.014
C4-C15	1.459	0.018
N5-C6	1.479	0.014
N5-C8	1.385	0.013
C6-C7	1.535	0.015
C7-C8	1.527	0.015
C7-N18	1.442	0.010
C8-O9	1.209	0.015
C10-O11	1.441	0.023
O11-C15	1.358	0.018
C15-O17	1.188	0.021
N18-C19	1.332	0.011
O19-O20	1.245	0.011
C19-C21	1.489	0.010
C21-C22	1.556	0.011
C22-C23	1.514	0.010
C23-C24	1.539	0.011
C24-N25	1.493	0.010
C24-C26	1.528	0.009
C26-O27	1.265	0.008
C26-O28	1.257	0.009
C30-C31	1.494	0.012
C31-O32	1.197	0.013
C31-O33	1.300	0.011

Table 7.2 Interatomic Angles and their Standard Deviations

Bond	Angles	Standard Deviations
C2-S1-C6	96.9°	0.5°
S1-C2-C3	113.0	0.9
C2-C3-C4	124.0	1.1
C2-C3-C10	128.6	1.3
C4-C3-C10	107.2	1.2
C3-C4-N5	125.6	1.1
C3-C4-C15	110.0	1.1
N5-C4-C15	124.3	
C4-N5-C6	119.5	0.9
C4-N5-C8	130.5	1.0
C6-N5-C *	93.7	0.7
S1-C6-N5	111.9	0.7
S1-C6-C7	114.5	0.7
N5-C6-C7	86.6	0.8
C6-C7-C8	86.1	0.7
C6-C7-N18	121.0	1.1
C8-C7-N18	115.6	0.8
N5-C8-C7	90.3	0.8
N5-C8-09	132.1	0.9
C7-C8-09	137.5	0.9
C3-C10-011	105.9	1.3
C10-011-C15	108.2	1.1
C4-C15-011	107.7	1.3
C4-C15-017	132.1	1.3
011-C15-017	120.0	1.2
C7-N18-C19	122.6	0.8

Table 7.2 continued

Bond	Distance (Å)	Standard Deviations
N18-C19-C20	119.3	0.7
N18-C19-C21	116.7	0.7
O20-C19-C21	123.8	0.7
C19-C21-C22	111.7	0.7
C21-C22-C23	112.7	0.7
C22-C23-C24	117.4	0.7
C23-C24-C25	111.2	0.6
C23-C24-C26	107.9	0.6
N25-C24-C26	109.8	0.5
C24-C26-C27	117.5	0.6
C24-C26-C28	116.8	0.5
O27-C26-O28	125.2	0.6
O30-C31-O32	123.5	0.9
O30-C31-O33	114.3	0.9
O32-C31-O33	122.0	0.7

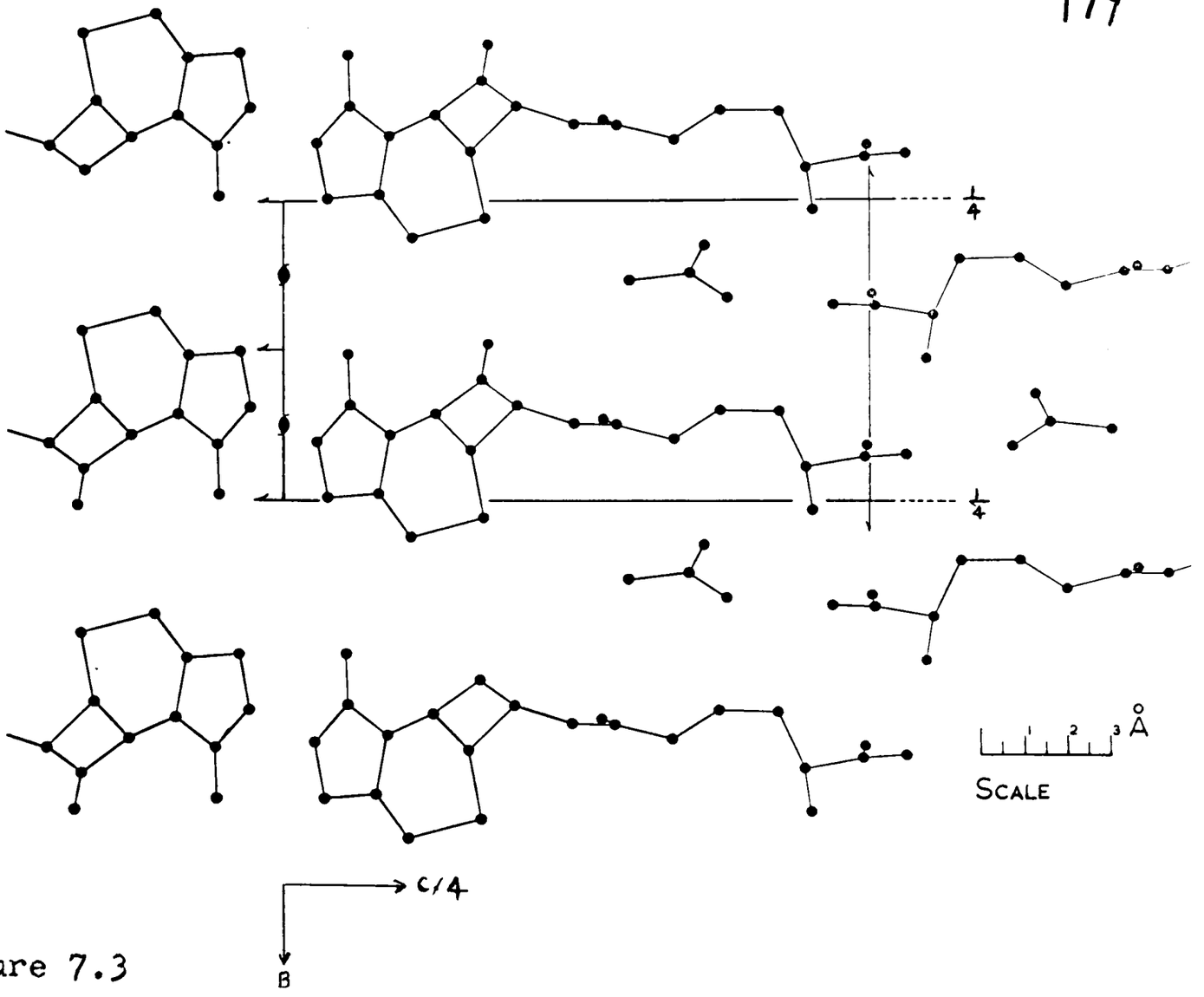


Figure 7.3

Projection of cephalosporin Cc on to the [100] plane.

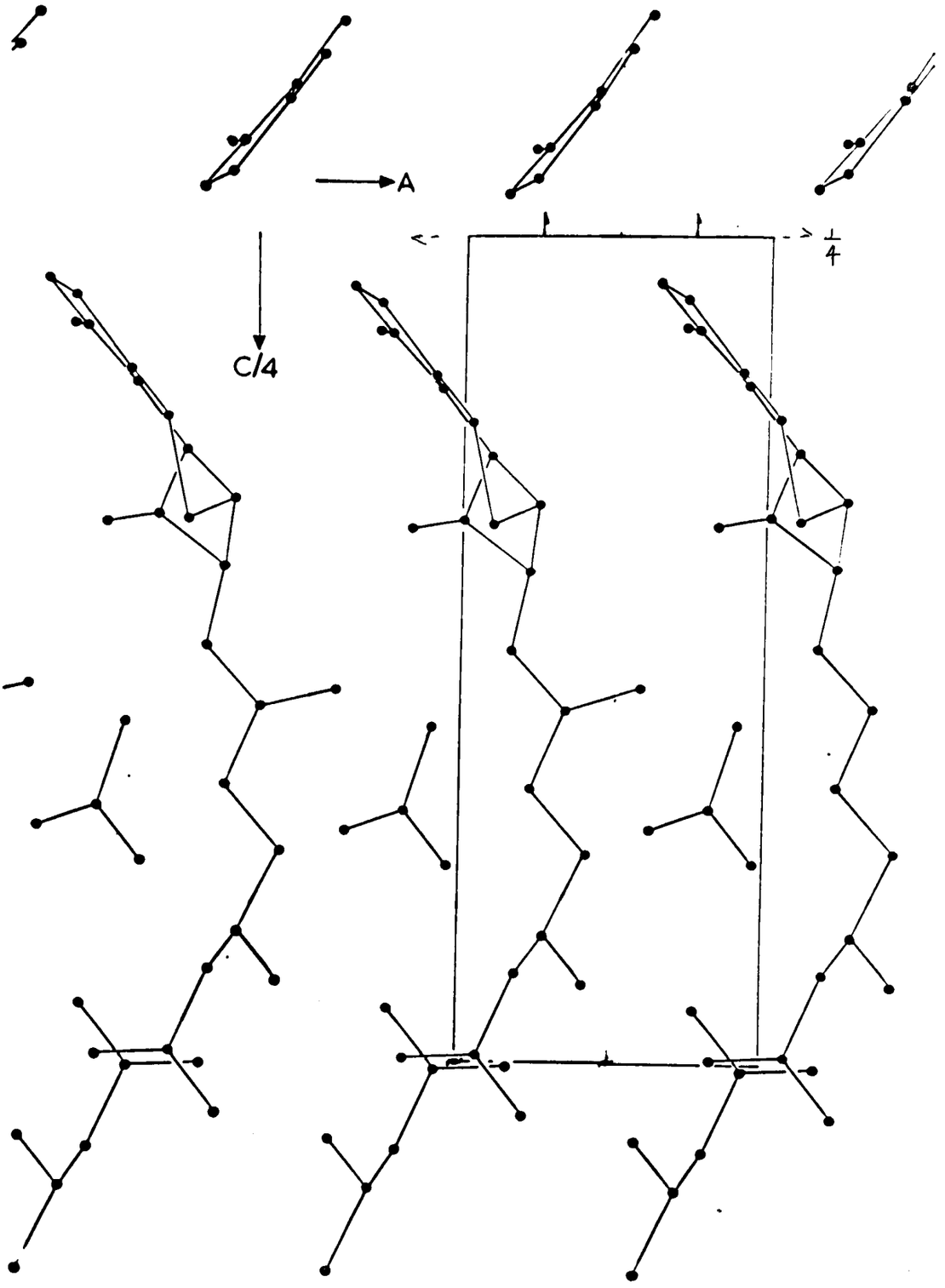


Figure 7.4

Projection of cephalosporin Cc on to the [010] plane.

bond system (p183). The reason for its inclusion in the crystals is not so obvious, but it is possible to propose a qualitative explanation for this. The highly polar zwitterion group in the α-amino adipic acid side chain is bound to crystallise in close proximity to the same group in other molecules. However this end of the molecule is very much thinner than the more bulky ring system, so that any regular packing becomes very unlikely. Adding an acetic acid molecule to the crystal, will equalise the size of the two ends of the molecule and so make the packing easier. In cephalosporin C the sodium ion fills up the necessary space to even out the size of the molecule.

Short Contacts.

Table 7.3 shows all the intermolecular distances under 5.25Å. Those involved in hydrogen bonding are underlined.

TABLE 7·3

The term s/x. y. z. refers to the relation of the 2nd atom to its unique position. S. gives the symmetry relation and x, y, z specify the number of Cell translations in any given direction. The symmetry relations are.

1/ =	x	y	z
2/ =	$\frac{1}{2}$ - x	- y	$\frac{1}{2}$ + z
3/ =	$\frac{1}{2}$ + x	$\frac{1}{2}$ - y	- z
4/ =	- x	$\frac{1}{2}$ + y	$\frac{1}{2}$ - z

Vector	symmetry	distance(Å^{O})
C_2 - O_9	1/0 1 0	3.22
O_{11}- O_{11}	3/0 1 0	2.97
O_{11}- O_{11}	3/$\bar{1}$ 1 0	2.97
O_{11}- C_{15}	3/$\bar{1}$ 1 0	2.91
N_{18}- O_{20}	1/$\bar{1}$ 0 0	3.03
N_{25}- O_{27}	4/3 0 0	2.89
N_{25}- O_{28}	1/1 0 0	2.82
N_{25}- O_{28}	4/2 0 0	2.76
N_{25}- O_{32}	1/1 1 0	2.84
O_{27}- O_{28}	1/1 0 0	3.18
O_{27}- O_{33}	4/2 0 0	2.59

The distances between the two γ-lactone groups on neighbouring molecules are at the lower limit for ordinary van der Waals contacts, there is however no possibility of hydrogen bonding in this part of the molecule.

The Hydrogen Bond System.

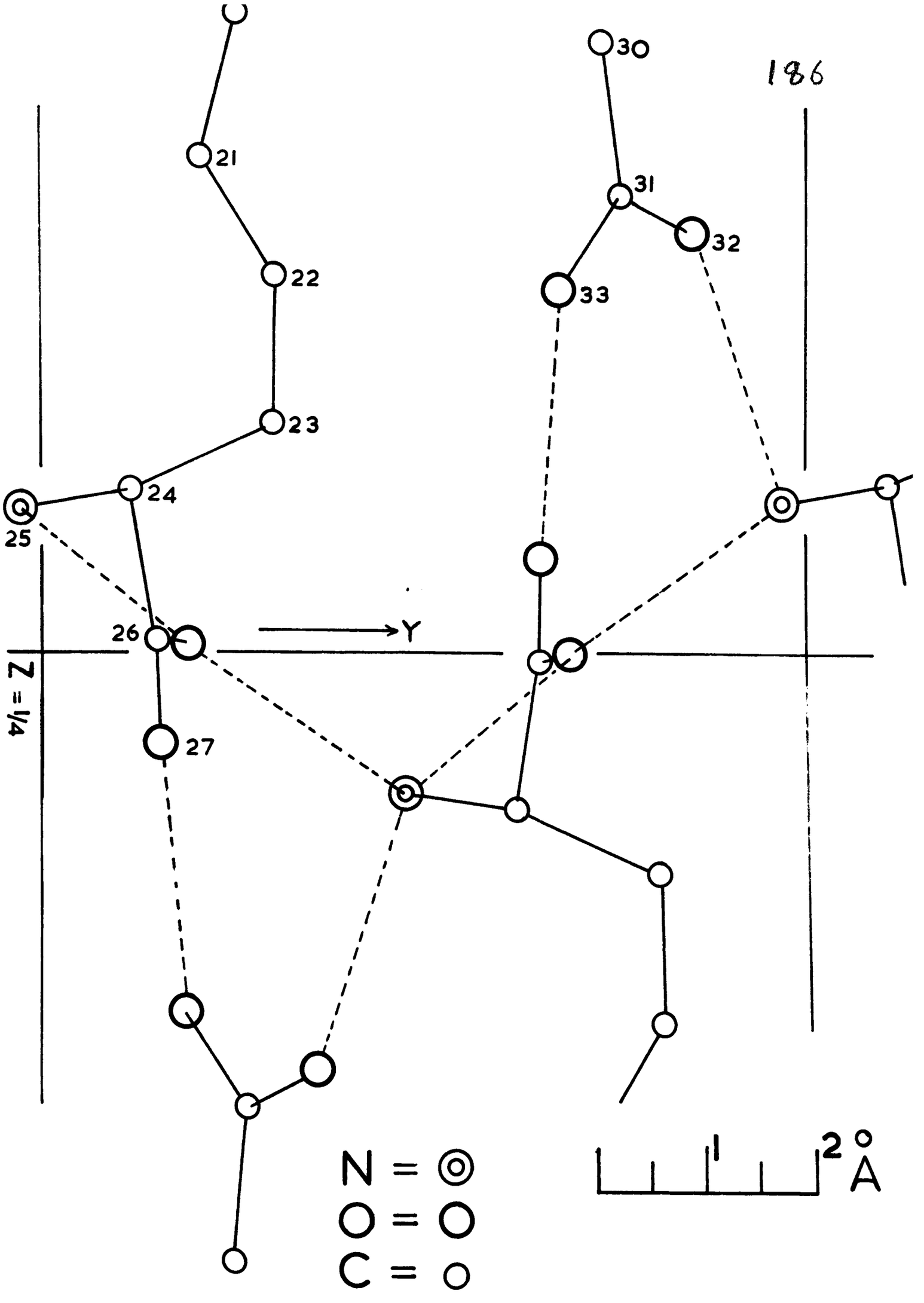
The molecule is held together by a hydrogen bond system that forms a continuous spiral around the screw axes at $z = \frac{1}{4}$ and $\frac{3}{4}$. This is shown looking down the axis in figure 7.6 and across it in figures 7.5 and 7.7. The angles between the hydrogen bonded atoms are shown in table 7.4.

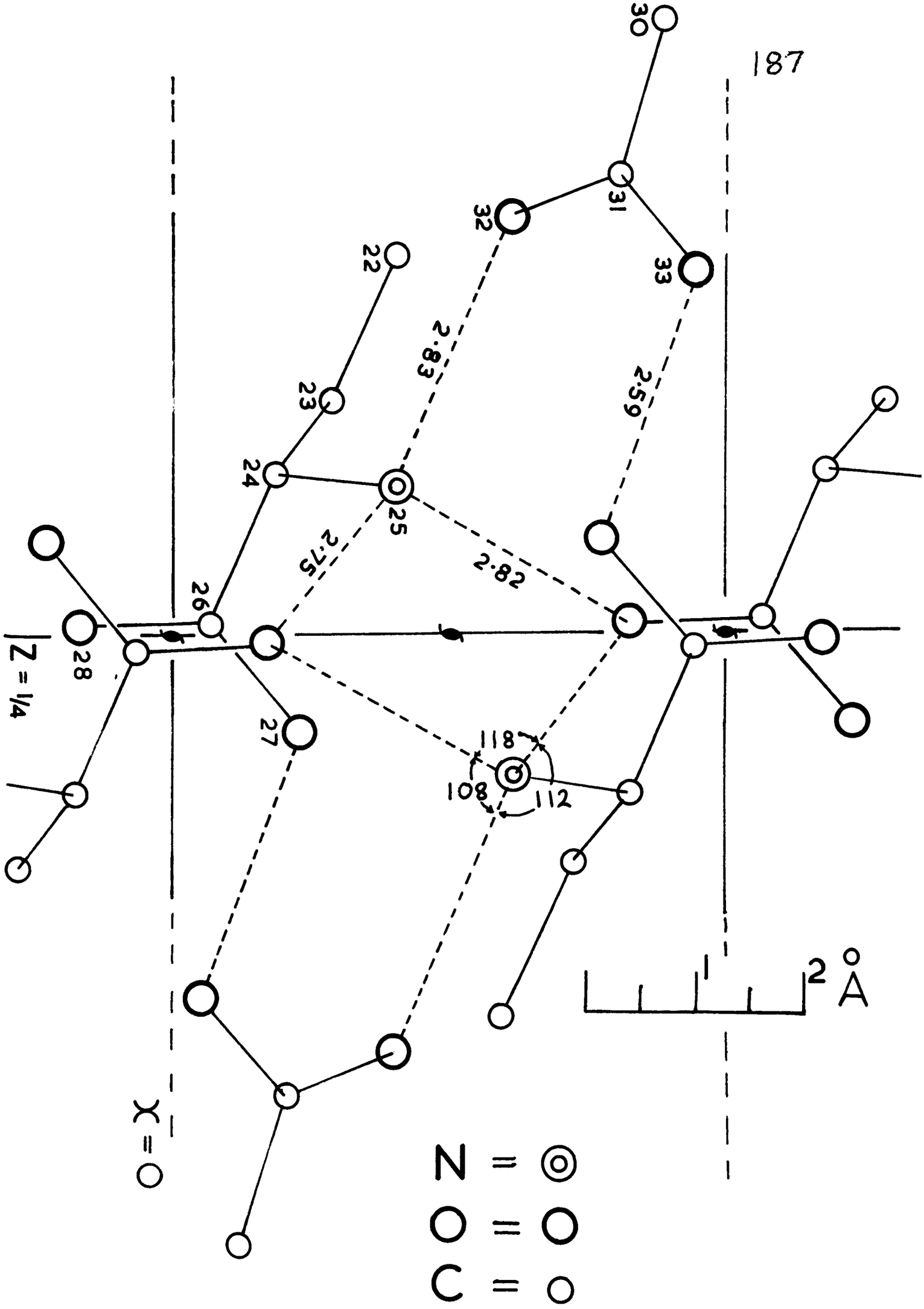
It is interesting that O_{27}(4/003) which is immediately opposite N_{25} along the line of the bond C_{24}- N_{25} is only 2.89Å away from N_{25} even though because of its position it cannot be hydrogen bonded (C_{24}- N_{25} ... O_{27} = 167°). This would suggest that in this case the ionic forces between oppositely charged atoms are the same order of magnitude as the purely covalent forces.

One oxygen (O_{32}) of the acetic acid is hydrogen bonded to N_{25}, the hydrogen being attached to the nitrogen, and the other oxygen (O_{33}) is ~~attached~~ hydrogen bonded to O_{27} on another molecule. In this case the hydrogen must be attached to the acetic acid group. In agreement with this the bond C_{31}- O_{32} is short (1.20Å) and C_{31}- O_{33} is long (1.30Å). The only other hydrogen bond in the crystal is one between N_{18} and O_{20} in the peptide linkage. This is shown in fig 7.11. The hydrogen bond is directed along the direction of the bond C_{19} O_{20} and passes directly through the hydrogen attached to N18.

Figures 7.5, 7.6 and 7.7

Projections of the hydrogen bond system down [100], [010], [001]. Distances in Å units, angles in degrees.


21
22
23
24
25
26
27
30
31
32
33
Y
Z = 1/4
N = ◎
O = O
C = ○
1
2 Å


30
31
32
33
22
23
24
25
26
27
28
2·83
2·59
2·75
2·82
118
108
112
Z = 1/4
X = 0
1
2 Å
N = ◎
O = O
C = ○

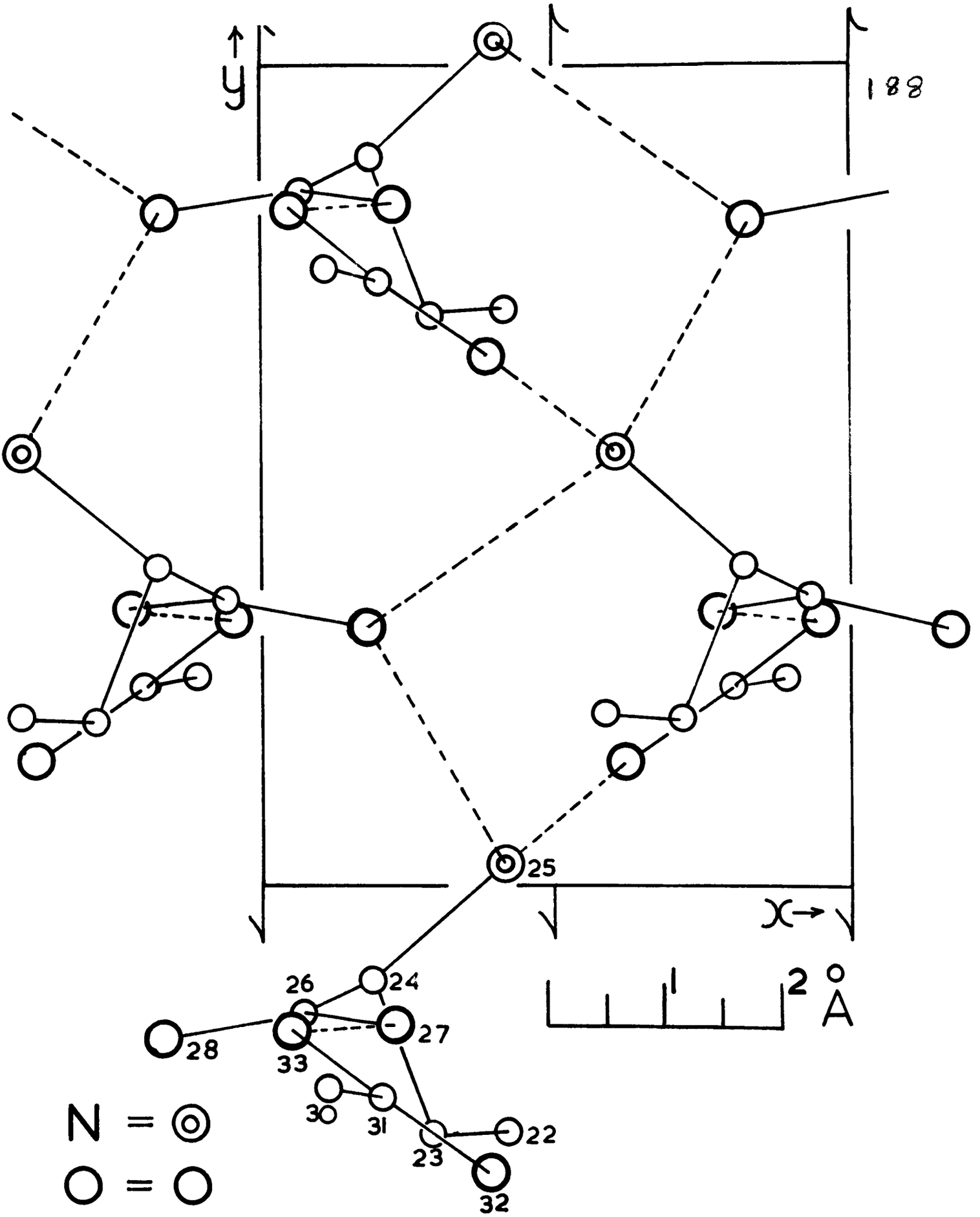
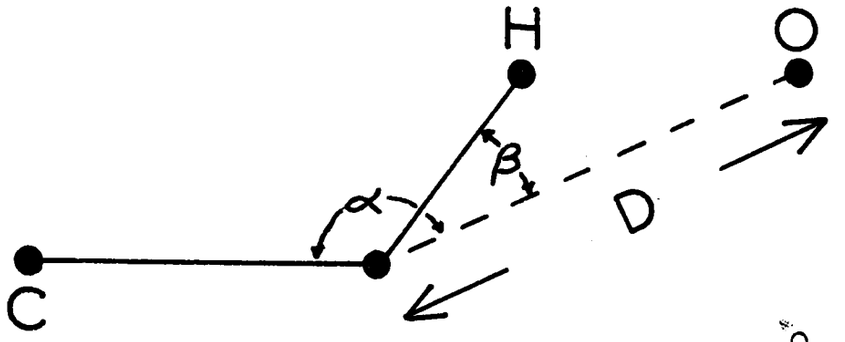

y
188
25
x
1
2 Å
26
24
28
27
33
30
31
22
23
32
N = ◎
O = O
C = O

Table 7.4 Hydrogen bond distances and angles



Bond	α	β	D (Å)
C24 - N25 .....028 (1)	106°	18°	2.82
C24 - N25 .....028 (4)	99	7	2.77
C24 - N25 032	113	15	2.83
C31 - 033 027	116	10	2.59
C24 - N25 .....027*	167		2.89

* Non hydrogen bond

The Dihydrothiazine ring (fig. 7.8)

Five of the atoms of the ring form a plane, with the sulphur lying 0.87 Å below it. This conclusively proves that the double bond in the ring system (p.128) is between C_3 and C_4, no other position is possible. Thus as C_2, C_3, C_4 and N_5 are planar, the angular strain that would result if the ring were entirely planar must be taken up by pushing S_1 and or C_6 out of the plane. This can happen either with the S_1 on the same side of the plane of the double bond as the β-lactam (below) or above it on the opposite side. In both Ceph. C and Ceph. Cc the S atom is in the former position. A possible reason for this may be found from experiments with flexible models. If the S is below the plane of the ring, then the three ring systems are more extended than in the opposite configuration, thus the distance from C_2 to N_{18} is 4¼ Å as measured from the model (actual distance 4 3/4 Å) in the former case, and 3 1/4 Å with S, above the plane of the ring. The exact conformation adapted cannot be critical as in cepalosporin C, S_1 and C_6 are both out of the plane of the ring, whereas in cephalosporin Cc only S_1 is noticeably out of the plane.

δ-Lactone-ring

Figure 7.9 shows the projection of this ring on to the best least-squares plane through C_2, C_3, C_4, N_5, C_{10} O_{11}, C_{15}, O_{17}. The calculated standard deviations in this part of the ring are between 0.01 and 0.02 Å, so that it is unlikely that the largest deviations from the plane are erroneous. These are probably caused by van der Waals forces; there are a number of short contacts

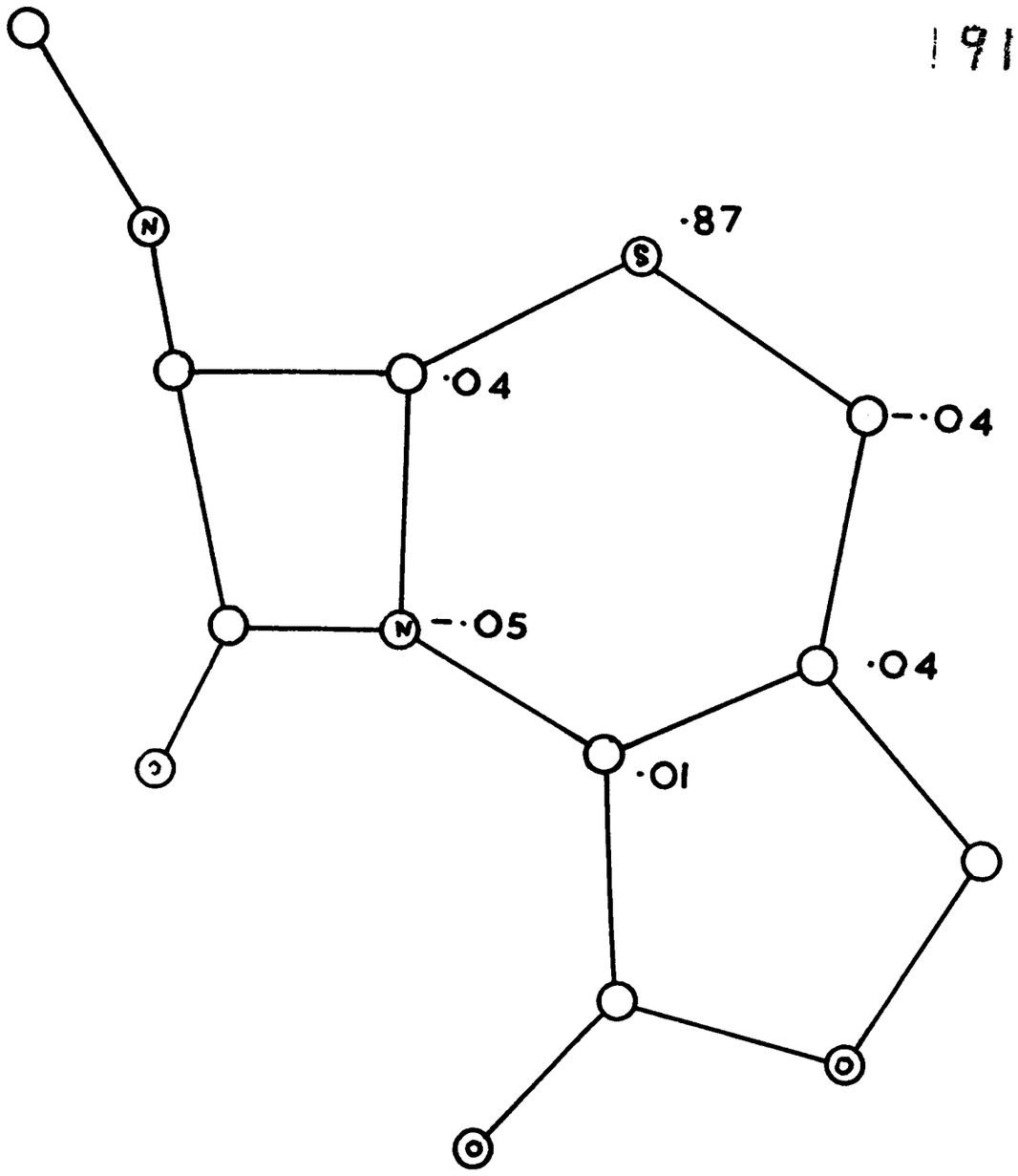


Fig. 7.8 cephalosporin Cc. Projection of part of the molecule on to the best plane through C_2, C_3, C_4 and N_5. Deviations from the plane in Å units.

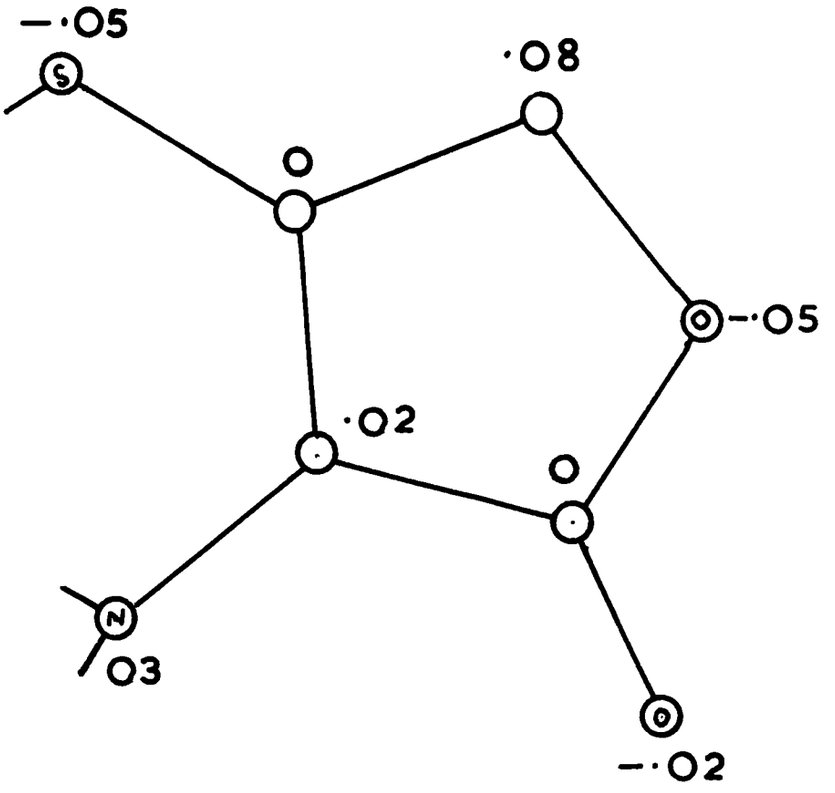


Fig. 7.9 Cephalosporin Cc. Projection of part of the molecule on to the best plane through S_1, C_2, C_3, N_4, C_{10}, O_{11}, C_{15}, and O_{17}. Deviations from the plane in $\mathring{A}$ units.

between the two γ-lactone groups in neighbouring molecules (table 7.3 p.182), in particular there is a contact of 2.93 Å between C_{15} and O_{11} which would twist the γ-lactone ring in the way found.

In agreement with other γ-lactone structures (34, 39, 40, 57) the bond $C_{11}-C_{15}$ (1.36±.02Å) which is next to the C=O group is significantly shorter than the bond $C_{10}-O_{11}$ (1.44± .02Å) which is identical to the normally accepted length (1.44Å) for this bond.

All the bonds surrounding the double bond C_3-C_4 are somewhat shorter than the accepted value of 1.52-1.54Å for a C-C single bond, consistent with the shortening of bonds involving sp^2 hybridization when compared with those involving sp^3 (29) hybridization.

The β-Lactam group

The projection of the region of the molecule around the β-lactam ring onto the best plane through the 4 members of this ring is shown in figure 7.10. The equivalent projection for 6-APA is superimposed on this. The correspondence of the two four-membered rings is too close for any differences to be shown on the drawing. For the five bonds of the β-lactam ring, the maximum difference is 0.03Å and the average 0.015Å. The corresponding figures for the angles excluding those to S_1 and C_4 are 1.5° and 2.6°. The largest differences are the angles between the β-lactam ring and N_{18}. (See tables 4.3 and 4.4 p. 83).

These figures show that any differences between the β-lactam rings in the two molecules are less than the errors in the analysis. The only significant differences are the angles between the β-lactam ring

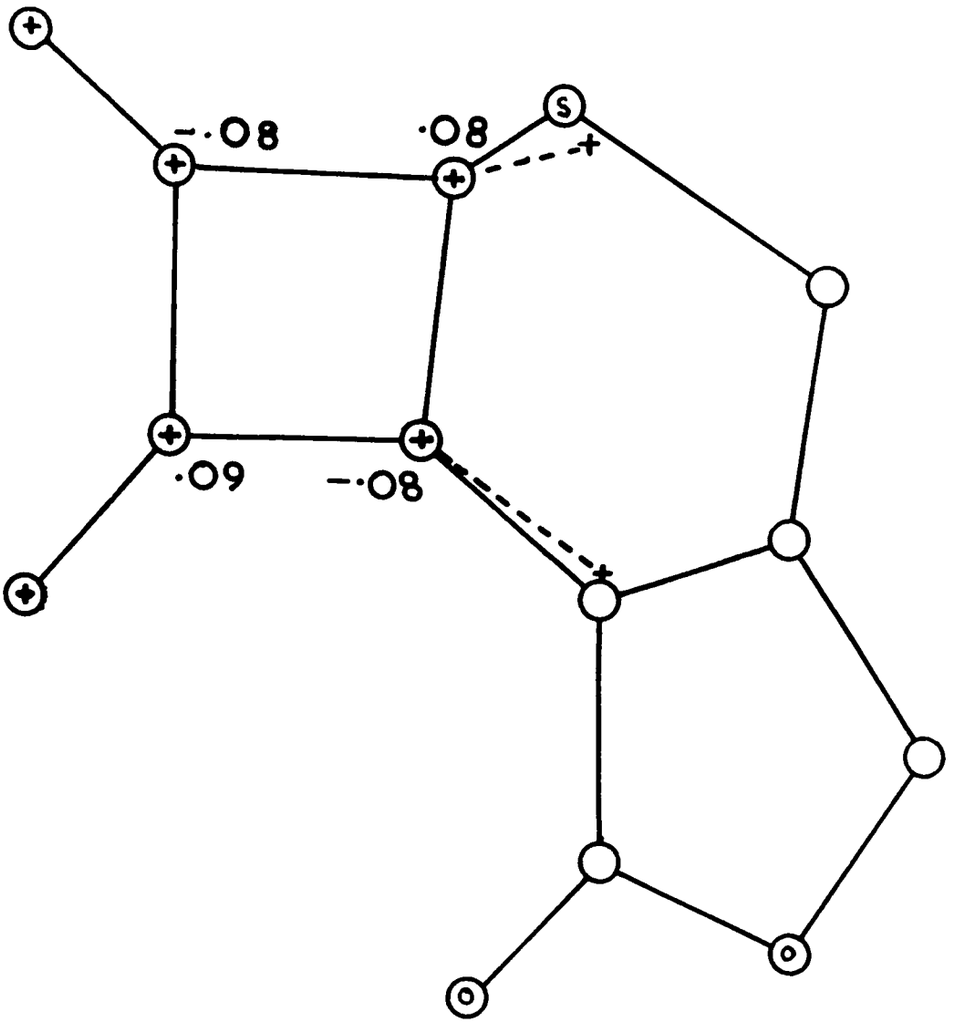


Fig. 7.10 Projection of part of the molecule of cephalosporin Cc on to the best plane through N_5, C_6, C_7 and C_8. The lactam ring of 6-APA is shown superimposed on this. Deviations from the plane in Å units.

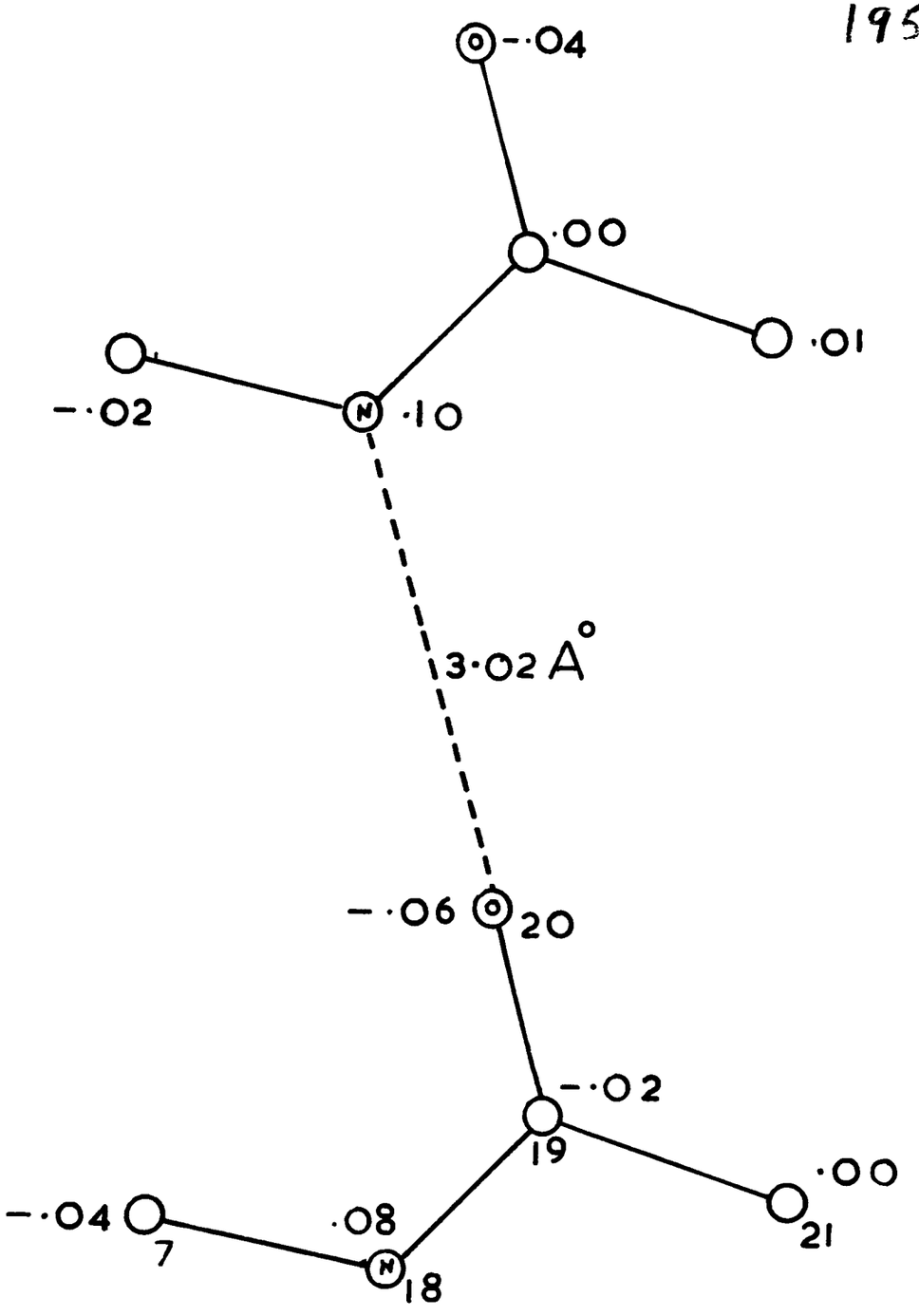
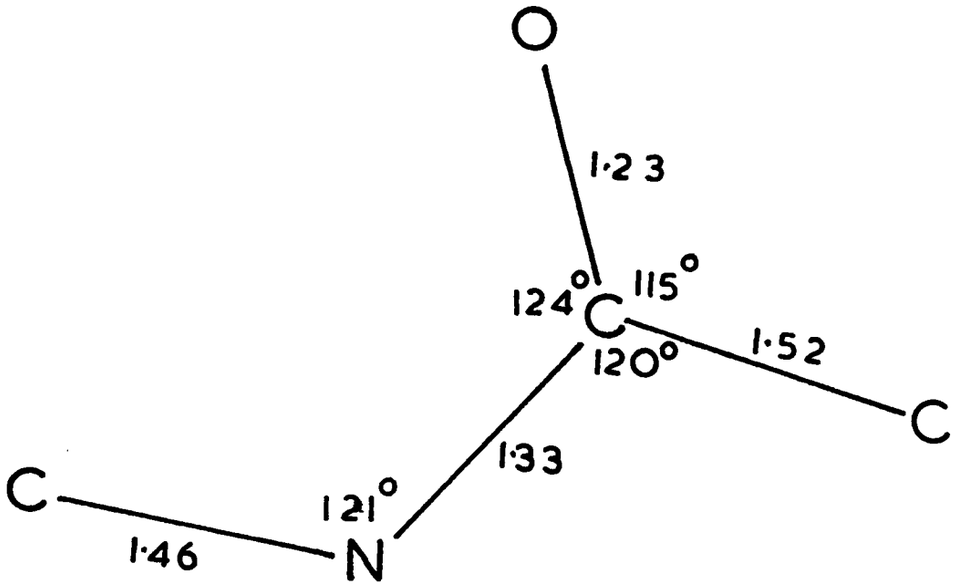


Fig. 7.11 Cephalosporin Cc projection of the peptide units of two neighbouring molecules on to the best plane through them both. Deviations from the plane in Å units.

Figure 7.12 Average value of peptide bonds quoted by Hahn (35).



and the dihydrothiazine and thiazolidine rings, the ring angles being more acute in the latter case.

As the structure of the β-lactam ring in 6-APA has already been discussed on page 98 it will not be dealt with further here.

The peptide linkage

Figure 7.11 shows this projected on to the best plane through two neighbouring groups. As can be seen the whole system including the hydrogen bond is very nearly planar. The average values of bond lengths and angles found by Hahn are shown in figure 7.12. The bond lengths of ceph. Cc are in very close agreement with these, however the angle $N_{18}-C_{14}-O_{20}$ is very much smaller than the average. This difference is sufficiently large to be significant and may well be due to the hydrogen bond between N_{18} and O_{20}.

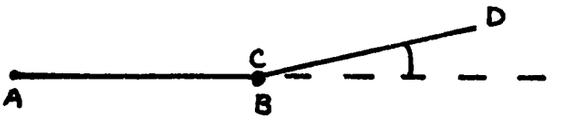
The α-amino adipic acid side chain

All the bonds are staggered, as would be expected. The bond $C_{19}-C_{21}$ is shortened to 1.49Å, this may be compared with a small shortening to 1.52 Å found by Hahn[5] for the average of this bond. The two hydrogens on C_{21} are directed away from the carbonyl group. The rest of the C-C bonds are alternately long and short though the differences are never as much as two standard deviations from the expected values, so it is very doubtful if this pattern is significant.

The bond angles at C_{21} (112°) and C_{22} (113°) are close to the average values found by Wright and Marsh

for L-lysine hydrochloride. (70) The angle at C_{23} is however noticeably larger (117.5°), this feature was also found by Leung and Marsh (42) in leucyl prolyl glycine, and by Wright and Marsh in L-lysine hydrochloride. They suggested that this may be due to interaction between the γ-carbon and the groups hydrogen bonded to the α-amino group. In ceph. 3c the nearest atoms to the γ-carbon (C_{22}) are the oxygen O_{32} of the acetic acid (3.34Å) and N_{25} in the same molecule. (3.08Å, C_{22}-H_{250}=2.63Å) None of these contacts appear to be sufficiently short to account for the increased angle, as there are contacts of the same order of magnitude elsewhere in the side chain.

The dihedral angles of the side chain are as follows:



N_{18} C_{19} C_{21} C_{22}	29°
C_{19} C_{21} C_{22} C_{23}	12°
C_{21} C_{22} C_{23} C_{24}	120°
C_{22} C_{23} C_{24} C_{26}	1°
C_{23} C_{24} C_{26} O_{27}	93°

At the two ends of the chain where it meets a planar group the tetrahedral carbon adopts a configuration with one bond at right angles to the planar group. Elsewhere the configuration is close to that for a staggered chain.

The NH_3^+ group is 0.82Å out of the plane of the carboxyl group. This is ionized, and as can be seen from figure 4.6, the bond distances and angles are in very close agreement with those found by Hahn for a CO_2^- group.

Acetic acid

This is unionized, with the hydrogen attached to O_{33}. The bond lengths are in reasonable agreement with those found by Jones and Templeton for acetic acid (38). Though the c-c bond is three calculated standard deviations from the accepted value, yet, in terms of the realistic standard deviations, this difference is probably within the expected range.

Cephalosporin Cc observed structure factors

scale

100 = 1 electron

order

k l h

* line convention

each * is followed by the values of k and l for the following group of reflections

columns

h A_{obs} B_{obs} A_{calc} B_{calc}

Note:

A_{obs} and B_{obs} are printed instead of F_{obs} as this was more convenient for computing.

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	3	0	-2635	0	-2434
	4	0	720	0	753
	5	0	2018	0	2072
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	3	892	0	937	0
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1	-633	1092	-651	1740
2	1403	1140	1540	1170
3	-622	-171	-707	-113
• 4 23				
0	1391	0	1300	0
1	-2547	-1441	-2303	-1350
2	-301	-1158	-300	-1227
4	-141	134	-135	90
• 4 24				
0	674	0	604	0
1	626	1547	700	1740
2	-1049	-407	-1507	-271
4	202	651	163	450
• 4 25				
0	-2070	0	-1670	0
1	-1170	501	-1357	740
2	-555	1230	-400	1040
4	612	-1127	400	-1440
• 4 26				
0	-1105	0	-1307	0
1	-192	542	-145	547
2	1732	-1764	1600	-1603
4	-767	-549	-799	-650
• 4 27				
0	-4011	0	-4459	0
1	102	663	143	530
2	-165	-1754	-100	-1703
4	-776	210	-913	446
• 4 28				
0	-703	0	-559	0
1	391	-70	504	-65
2	200	-911	219	-707
• 4 29				
0	-1009	0	-1006	0
1	630	54	610	53
2	-504	249	-510	255
4	420	511	306	465
• 4 30				
0	661	0	605	0
1	524	-3040	492	-2656
2	1050	61	1090	63
• 4 31				
0	652	0	791	0
1	314	202	374	312
2	1437	1153	1550	1243
4	-534	-212	-402	-159
• 4 32				
0	-907	0	-973	0
1	-354	209	-317	205
2	30	1335	49	1207
• 4 33				
0	1422	0	1564	0
1	566	-455	505	-452
4	190	-437	127	-263
• 4 34				
0	1029	0	1006	0
1	-1036	226	-1026	224
2	-909	240	-640	225
• 4 35				
0	2421	0	2563	0
1	-2477	2456	-2561	2640
2	203	-719	102	-731
• 4 36				
1	-461	1000	-492	1037
2	-1100	-32	-722	-21
• 4 37				
0	-2527	0	-2543	0
1	-673	-645	-751	-743
2	-339	779	-201	600
• 4 38				
0	-1917	0	-2075	0
1	192	711	242	695
2	98	-1997	76	-1545
• 4 39				
1	352	-689	403	-769
• 4 40				
0	-630	0	-652	0
1	-468	-1051	-516	-1115
• 4 41				
0	-677	0	-901	0
1	306	-543	432	-940
2	-108	-451	-120	-501
• 4 42				
0	1197	0	1128	0
1	1142	-150	1055	-144
2	-578	966	-475	611
• 4 43				
0	1561	0	1350	0
1	633	126	633	166
2	-43	659	-39	776
• 4 44				
1	-1668	330	-1705	337
2	639	237	677	251
• 4 45				
0	666	0	466	0
1	-1133	-1554	-1069	-1473
2	370	-697	563	-766
• 4 46				
0	1233	0	1117	0
1	630	157	673	216
2	53	1079	30	727
• 4 47				
1	156	1109	134	940
2	-343	560	-349	557

4	48			
0	964	0	1037	0
1	5	1079	5	1151
2	-576	-734	-564	-717
4	49			
0	-1733	0	-1738	0
1	-460	351	-435	338
2	-451	-908	-422	-849
4	50			
1	-171	-635	-213	-792
2	369	-131	369	-131
4	51			
1	506	514	596	605
2	-39	375	-54	521
4	52			
0	-515	0	-689	0
2	-63	356	-69	391
4	54			
1	762	-622	897	-733
2	396	416	312	328
4	55			
0	766	0	811	0
2	521	264	346	175
4	56			
1	-258	492	-189	360
2	-611	185	-672	203
4	57			
0	-500	0	-144	0
1	-111	456	-139	530
2	608	-285	646	-303
4	58			
0	668	0	747	0
1	-372	242	-230	150
4	59			
0	668	0	679	0
4	60			
0	-902	0	-941	0
1	198	530	279	747
4	61			
0	-778	0	-943	0
5	0			
1	0	-1876	0	-1598
5	1			
1	-572	-1070	-489	-914
2	1054	-467	1021	-452
5	2			
0	0	-1607	0	-2015
1	-1801	1395	-1475	1143
2	-784	155	-799	158
5	3			
0	0	811	0	829
1	429	552	394	506
5	4			
0	0	716	0	635
1	-265	-1540	-254	-1476
2	-490	452	-415	383
5	5			
0	0	-1947	0	-2098
1	-255	-2704	-232	-2457
2	1530	-211	1660	-229
5	6			
0	0	-1062	0	-900
1	1610	878	1667	999
2	1694	971	1756	1887
5	7			
0	0	-2153	0	-2436
1	1285	1484	677	1013
2	-1895	109	-1156	97
5	8			
0	0	1613	0	1905
1	90	-460	92	-469
2	272	-1774	291	-1901
5	9			
0	0	1826	0	928
1	-895	1506	-783	1317
2	1198	1056	1360	1200
5	10			
1	1732	-469	1525	-413
2	-406	1842	-393	1783
5	11			
0	0	2759	0	2735
2	-812	-1150	-760	-1075
5	12			
1	-285	870	-275	837
2	-970	-358	-762	-281
5	13			
0	0	2679	0	3098
1	-1164	-537	-1108	-511
2	-790	1425	-708	1278
5	14			
0	0	1513	0	1311
1	-1917	-255	-1881	-250
2	64	1241	60	1178
5	15			
0	0	-1695	0	-1543
1	-406	-997	-391	-961
2	-544	-638	-461	-542
5	16			
0	0	-1392	0	-1404
1	-1781	-1093	-1684	-1033
2	953	-17	856	-15
5	17			
0	0	-3090	0	-2883
1	-1006	82	-991	81
2	-667	125	-652	122
5	18			
1	164	-633	144	-553
2	1632	-1268	1602	-1245

5	19			
0	0	457	0	423
2	599	-510	367	-313
5	20			
0	0	572	0	19
1	1706	-1157	1537	-1042
2	-1306	-495	-1241	-470
5	21			
0	0	-1302	0	-1299
1	516	1337	549	1423
2	-667	169	-532	151
5	22			
0	0	-652	0	-686
1	497	395	440	350
2	-298	-1361	-266	-1216
5	23			
0	0	1664	0	1440
1	703	-1036	703	-1037
2	-519	-817	-348	-548
5	24			
1	-880	-341	-810	-314
2	-807	704	-788	682
5	25			
0	0	660	0	614
1	832	746	974	873
2	154	1045	140	947
5	26			
0	0	1923	0	2083
1	-327	1190	-324	1179
2	-834	-614	-841	-619
5	27			
1	29	-1663	27	-1557
5	28			
1	447	-1137	492	-1250
5	29			
0	0	-1099	0	-1128
1	955	-107	942	-106
5	30			
0	0	1687	0	1765
1	-909	1138	-1092	1368
5	31			
1	-1236	-318	-976	-347
5	32			
0	0	1236	0	1298
1	1611	-1059	1730	-1137
5	33			
0	0	822	0	526
5	34			
0	0	-1201	0	-1283
1	425	1347	448	1420
5	35			
0	0	1606	0	1642
1	-735	489	-720	479
2	-666	470	-510	361
5	36			
1	-470	902	-483	926
2	-539	204	-700	265
5	37			
1	519	643	591	733
2	974	-509	960	-501
5	38			
0	0	-672	0	-801
1	-683	14	-843	757
2	-85	-643	-90	-681
5	39			
1	-66	-1038	-69	-1083
2	-1405	-116	-1325	-110
5	40			
2	1371	610	1276	568
5	41			
2	1095	-353	1044	-337
5	42			
1	196	507	146	379
5	43			
0	0	-1841	0	-2131
1	782	1016	801	1041
2	-945	288	-970	296
5	44			
0	0	-928	0	-1004
1	803	-39	903	-44
5	45			
0	0	1415	0	1486
5	47			
2	-114	612	-74	394
5	48			
0	0	-811	0	-659
2	-471	136	-627	181
5	51			
0	0	-1012	0	-1181
2	701	-323	591	-272
5	52			
2	205	732	175	625
5	53			
2	370	-392	354	-376
6	0			
2	-986	0	-645	0
6	1			
0	-1663	0	-1817	4
1	-1092	-170	-1237	-193
6	2			
2	-946	-752	-867	-689
6	3			
1	362	-861	347	-824
6	4			
0	3226	0	3526	0
2	-396	-1801	-377	-1714
6	6			
0	-1179	0	-1328	0
2	1215	472	1486	577

6	7			
0	1475	0	1513	0
1	1010	-1144	1067	-1211
2	300	1108	342	1309
6	8			
2	-552	420	-562	436
6	9			
1	-1213	1076	-1410	1250
2	-177	-1290	-209	-1370
6	11			
2	1159	390	1140	364
6	12			
1	-3	1110	-3	1144
2	-959	726	-975	744
6	13			
2	-1335	-371	-1343	-373
6	14			
0	-1941	0	-2141	0
1	575	147	736	109
6	15			
0	-1751	0	-1059	0
1	-181	-1175	-215	-1305
6	16			
2	690	-365	849	-305
6	17			
1	708	1124	858	1044
2	-1066	200	-1020	199
6	18			
1	565	-182	743	-240
2	1330	900	1045	708
6	19			
1	126	-1252	133	-1297
2	255	640	249	078
6	20			
1	-750	-372	-1027	-509
2	-125	953	-99	753
6	21			
0	1189	0	1392	0
1	-343	1277	-382	1424
6	22			
0	-1187	0	-1224	0
6	23			
1	-808	-198	-911	-223
2	594	-995	360	-603
6	24			
1	-407	543	-457	533
6	25			
1	412	-6	498	-7
2	-419	-832	-368	-730
6	26			
1	351	-477	454	-454
2	-77	-1026	-60	-892
6	27			
1	-682	-836	-750	-919
6	28			
2	887	176	588	117
6	29			
1	803	20	952	23
2	247	1653	196	1313
6	30			
1	-502	5	-1006	9
6	31			
2	133	-858	132	-851
6	32			
2	640	375	545	320
6	33			
1	-791	924	-918	1073
2	-353	233	-291	192
6	34			
1	-99	528	-130	693
2	-565	-141	-544	-136
6	35			
1	574	-479	760	-654
6	36			
2	72	-553	68	-522
6	37			
2	-934	-557	-832	-496
6	38			
1	340	-366	355	-382
2	-1177	-396	-1219	-411
6	39			
1	488	15	462	14
6	40			
2	538	569	558	591
6	41			
1	215	-526	257	-031
2	66	580	67	595
6	42			
1	-480	-416	-589	-510
6	43			
2	688	-39	723	-41
6	44			
2	181	1072	163	961
6	45			
1	-495	488	-656	040
6	46			
1	-50	380	-68	517
6	51			
1	273	-350	476	-609
7	9			
2	-822	-332	-735	-297
7	11			
2	472	1307	428	1188
7	15			
2	1384	-48	1349	-47
7	16			
2	-96	-844	-93	-815
7	17			
2	-536	-496	-488	-452
7	18			
2	194	557	211	606
7	21			
2	-679	-433	-500	-319
7	23			
2	239	500	231	463
7	24			
2	-908	-354	-822	-350
7	26			
2	402	337	399	252
7	27			
2	611	-151	632	-156
7	30			
2	545	-305	624	-235
7	32			
2	-453	301	-407	271

Cephalosporin Cc. unobserved reflections

F_o^2 = ½ minimum observed intensity.

*	0	2			
	4	-249	0	-165	0
	5	-258	0	-46	0
*	0	5			
	5	0	-258	0	-34
*	0	6			
	4	-253	0	-480	0
*	0	7			
	4	0	-253	0	-40
*	0	9			
	5	0	253	0	269
*	0	11			
	4	0	253	0	203
	5	0	-253	0	-342
*	0	12			
	4	253	0	214	0
	5	-253	0	-281	0
*	0	14			
	4	-258	0	-94	0
*	0	15			
	5	0	-249	0	-507
*	0	16			
	5	249	0	106	0
*	0	17			
	5	0	-249	0	-91
*	0	19			
	3	0	-230	0	-187
*	0	22			
	5	239	0	559	0
*	0	23			
	4	0	262	0	135
*	0	24			
	4	262	0	25	0
	5	-234	0	-188	0
*	0	25			
	4	0	262	0	207
*	0	26			
	4	-262	0	-28	0
*	0	27			
	5	0	224	0	160
*	0	28			
	5	-219	0	-60	0
*	0	31			
	2	0	230	0	147
	3	0	253	0	463
*	0	32			
	0	374	0	643	0
	5	203	0	359	0
*	0	34			
	4	-253	0	-21	0
*	0	35			
	1	0	224	0	222
*	0	36			
	5	185	0	163	0
*	0	39			
	1	0	-239	0	-439
*	0	40			
	5	-159	0	-43	0
*	0	41			
	1	0	249	0	266
*	0	43			
	4	0	219	0	141
*	0	45			
	3	0	253	0	332
*	0	46			
	3	-249	0	-321	0
	4	203	0	39	0
*	0	47			
	3	0	-244	0	-227
*	0	49			
	2	0	258	0	250
*	0	50			
	2	-258	0	-106	0
	4	-173	0	-197	0
*	0	51			
	2	0	-253	0	-12
	3	0	-224	0	-90
*	0	52			
	3	219	0	383	0
*	0	53			
	3	0	214	0	112
*	0	54			
	2	239	0	341	0
*	0	55			
	2	0	-234	0	-142
	3	0	-197	0	-230
*	0	57			
	3	0	-179	0	-93
*	0	58			
	1	234	0	1	0
*	0	62			
	2	179	0	118	0
*	1	1			
	4	335	-229	316	-216
*	1	3			
	4	-359	189	-592	312
*	1	4			
	4	322	-247	341	-261
	5	82	404	108	530

```
*   I    7
    4   46    406    110    972
    5  196   -359    131   -239
*   I    8
    5 -113    393    -73    255
*   I    9
    4  190    362    306    583
*   I   10
    1   75    189    148    370
*   I   12
    4  -72   -408    -42   -238
    5  269    300    366    408
*   I   13
    0    0   -191      0    -41
*   I   14
    5  301   -264    370   -324
*   I   15
    5   55   -397     38   -274
*   I   18
    5 -193   -341   -198   -351
*   I   20
    5 -327   -205   -365   -229
*   I   22
    5 -356   -132   -148    -55
*   I   24
    4 -278   -319   -187   -214
    5  365     65    225     40
*   I   26
    5  -43    359    -23    188
*   I   28
    5  343     76    453    100
*   I   29
    5   40    343     36    314
*   I   30
    1   56   -327     14    -81
*   I   32
    5  322     42    365     48
*   I   33
    4   36   -407     34   -386
*   I   34
    2 -321   -302   -329   -309
    5 -243   -192   -232   -183
*   I   35
    2 -168   -416   -101   -251
    4  223   -336    219   -330
*   I   37
    5 -273     56   -201     41
*   I   38
    0    0   -374      0    -57
    4 -385    -57   -542    -80
*   I   39
    5  197    166    209    176
*   I   40
    3  270   -321     75    -89
    5 -119    213   -185    331
*   I   41
    4 -264   -255   -281   -271
*   I   42
    0    0    398      0     76
    2 -335   -355   -153   -163
*   I   44
    3  134    386    147    426
*   I   45
    4  -11   -331     -7   -229
*   I   46
    2   87    487     64    355
*   I   47
    3 -131   -369    -23    -64
    4  309     30    282     27
*   I   51
    1 -100    408   -116    474
*   I   53
    1 -273    308   -236    266
*   I   54
    1  136   -383    134   -377
*   I   59
    2  -47   -386    -60   -499
*   I   60
    2  257   -268     92    -96
*   I   61
    0    0   -352      0   -285
    1 -336    -37   -273    -30
*   I   63
    2 -224   -137    -88    -54
*   I   64
    0    0    310      0    221
    2  142   -193    327   -444
*   I   67
    0    0    253      0    345
*   2    0
    4 -446      0   -321      0
*   2    3
    5  391   -193    470   -231
*   2    4
    4 -444    -47   -181    -19
*   2    5
    3  194    -37    514    -98
*   2    7
    4 -117   -433    -92   -340
```

*	2	8				*	2	53			
	5	7	-433	9	-593		0	444	0	284	0
*	2	9				*	2	56			
	5	-400	-159	-279	-111		0	423	0	572	0
*	2	11				*	2	60			
	5	368	219	245	146		0	-377	0	-431	0
*	2	12				*	2	61			
	4	-443	88	-725	144		0	-361	0	-120	0
*	2	13				*	2	62			
	2	-98	298	-3	9		0	-345	0	-86	0
*	2	14				*	3	0			
	4	192	411	151	323		6	83	0	341	0
*	2	15				*	3	1			
	4	-410	195	-184	87		3	-216	-113	-289	-152
*	2	17					5	-151	-69	-408	-187
	4	267	-371	451	-627	*	3	2			
*	2	18					3	182	163	52	47
	5	-277	-304	-280	-307		4	-146	-149	-285	-290
*	2	19					6	-49	-67	-205	-282
	5	-68	403	-79	466	*	3	4			
*	2	26					4	34	-206	114	-682
	3	-271	-67	-686	-170	*	3	6			
	4	-382	246	-644	416		5	147	90	413	251
*	2	29				*	3	7			
	2	311	-294	282	-267		5	-13	-172	-28	-369
*	2	31				*	3	9			
	1	158	192	437	529		4	103	-188	203	-372
	4	433	86	265	53		6	72	-40	129	-72
*	2	32				*	3	11			
	4	391	-199	196	-100		0	0	-197	0	-216
*	2	38					5	-141	100	-353	251
	0	417	0	517	0		6	37	-57	92	-140
	3	-306	7	-395	9	*	3	12			
*	2	40					4	32	212	79	530
	3	-15	306	-16	324	*	3	13			
*	2	43					2	199	-78	182	-71
	0	446	0	254	0		3	262	-8	327	-10
*	2	45				*	3	14			
	0	452	0	723	0		0	0	-209	0	-701
	3	-295	10	-176	6	*	3	15			
*	2	46					5	-137	-105	-326	-250
	3	-198	-208	-189	-198	*	3	16			
*	2	48					0	0	214	0	440
	0	-457	0	-471	0	*	3	17			
	3	242	139	239	138		0	0	-214	0	-421
*	2	49				*	3	19			
	0	457	0	133	0		3	153	233	116	178
*	2	50				*	3	20			
	0	454	0	610	0		4	-221	-37	-427	-71
	2	-91	-462	-54	-272		5	11	-165	12	-175

*	3	25			
	0	0	-244	0	-38
	5	159	4	42	1
*	3	26			
	5	-41	153	-8	31
*	3	27			
	2	24	257	47	505
*	3	28			
	0	0	253	0	301
*	3	29			
	5	-146	-40	-134	-37
*	3	31			
	0	0	-266	0	-97
*	3	32			
	4	113	-188	170	-284
*	3	33			
	0	0	275	0	512
	3	-103	289	-86	239
*	3	34			
	1	-238	145	-429	262
*	3	35			
	0	0	279	0	514
	5	49	117	70	167
*	3	36			
	0	0	283	0	769
	1	-263	-104	-364	-144
*	3	38			
	0	0	-291	0	-8
*	3	39			
	2	-5	283	-2	124
*	3	40			
	0	0	295	0	204
*	3	46			
	1	240	171	475	338
*	3	48			
	2	-129	-233	-215	-386
*	3	50			
	0	0	-287	0	-8
	3	212	111	476	248
*	3	52			
	0	0	279	0	567
*	3	53			
	2	-182	148	-385	312
*	3	54			
	0	0	271	0	242
	3	-26	171	-46	306
*	3	55			
	0	0	-262	0	-510
	1	156	199	379	482

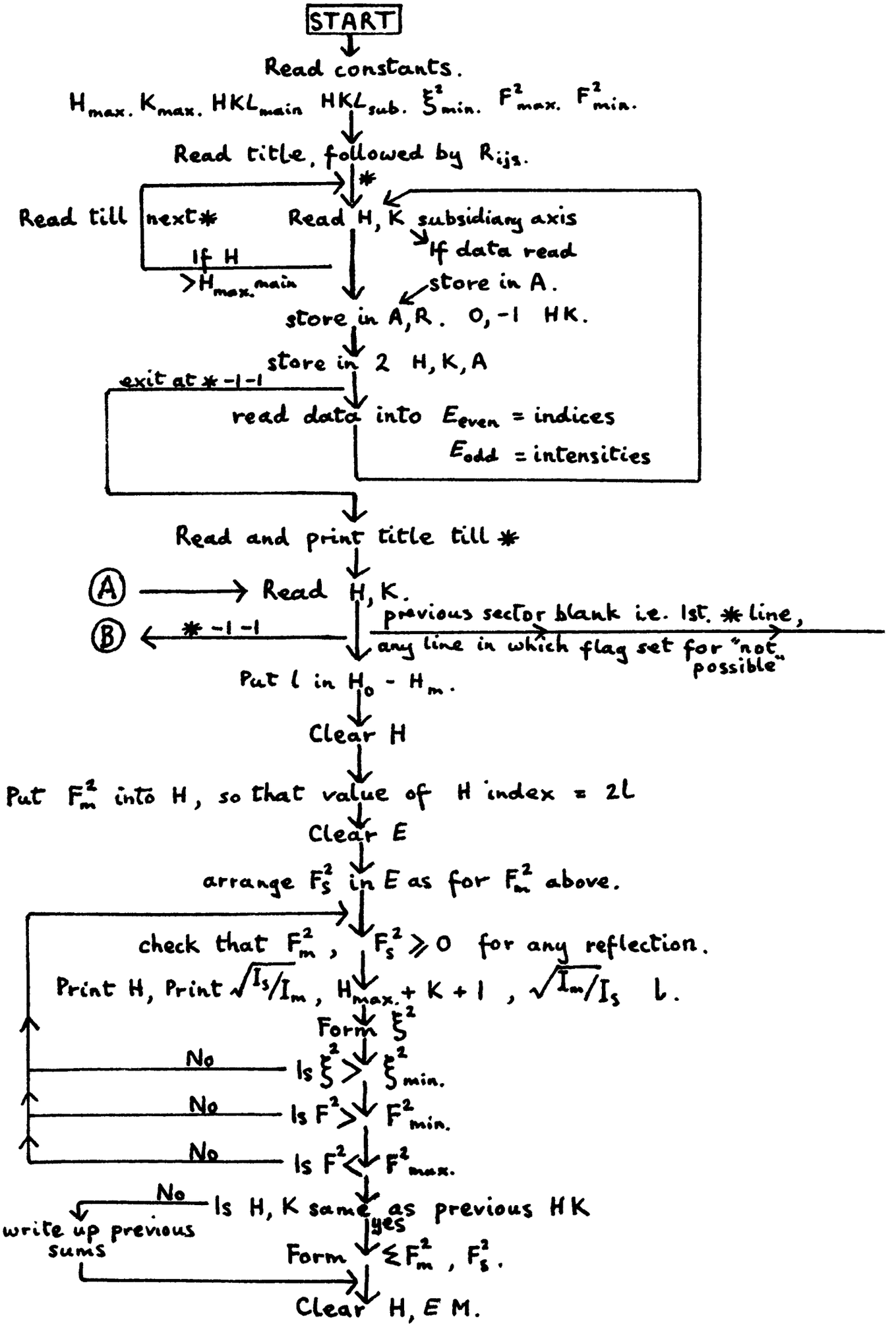
*	3	56			
	1	180	165	119	109
	2	-201	-75	-333	-124
	3	-52	-134	-212	-545
*	3	57			
	1	-234	4	-354	7
	2	180	-94	180	-93
*	3	58			
	0	0	-239	0	-395
*	3	59			
	0	0	230	0	430
*	3	60			
	1	-20	-208	-18	-186
*	3	61			
	0	0	209	0	26
	1	171	-85	112	-56
*	3	62			
	0	0	-197	0	-126
	1	-99	150	-239	363
*	3	63			
	1	90	-139	240	-372
*	4	0			
	5	0	279	0	425
*	4	1			
	3	16	-310	38	-713
	5	-258	-107	-91	-38
*	4	3			
	5	107	-258	21	-51
*	4	5			
	5	80	-267	190	-634
*	4	7			
	5	-270	52	-498	95
*	4	9			
	4	267	-203	343	-260
	5	150	-226	57	-85
*	4	11			
	5	117	-244	30	-63
*	4	12			
	5	78	-255	57	-185
*	4	13			
	4	29	330	37	420
*	4	16			
	3	-67	-318	-101	-483
	4	113	308	48	132
*	4	19			
	4	-317	-70	-491	-109
*	4	21			
	0	244	0	551	0
*	4	24			
	0	262	0	604	0

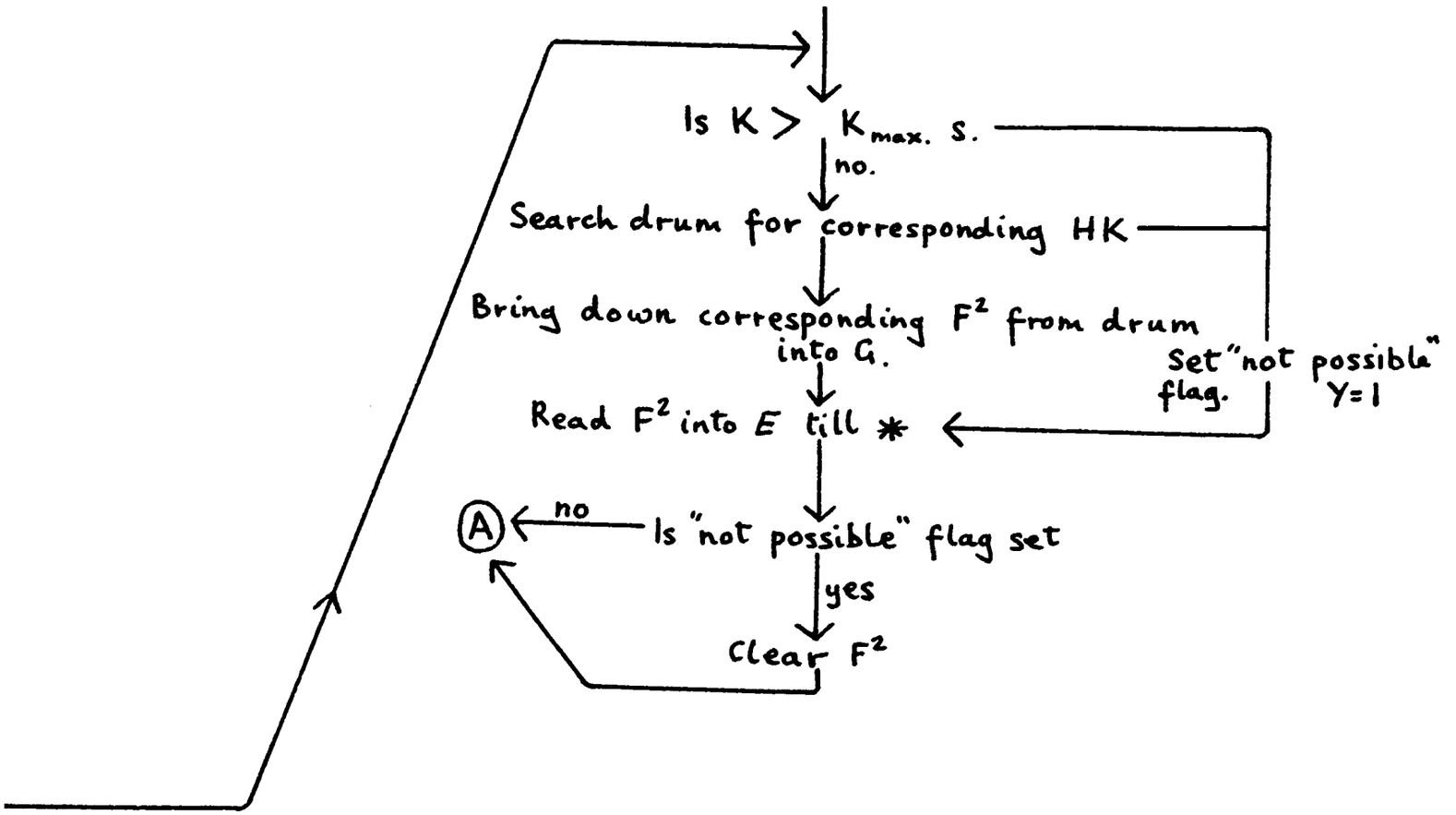
*	4	28				*	5	3							
	4	258	-159	281	-173		2	-338	329	-114	111				
*	4	30				*	5	7							
	4	133	259	74	145		3	-321	-257	-274	-219				
*	4	32				*	5	10							
	3	271	-191	24	-17		0	0	-234	0	-528				
	4	142	245	173	297	*	5	12							
*	4	33					0	0	253	0	271				
	2	-230	244	-250	265	*	5	18							
*	4	36					0	0	-314	0	-58				
	0	-325	0	-200	0	*	5	19							
*	4	39					1	-152	310	-118	240				
	0	-332	0	-113	0	*	5	24							
	2	219	249	34	38		0	0	361	0	18				
*	4	40				*	5	27							
	2	-55	-327	-78	-467		0	0	383	0	210				
	3	-209	219	-38	40		2	-338	-359	-425	-451				
*	4	42				*	5	28							
	3	-274	-85	-321	-99		0	0	389	0	584				
*	4	43					2	322	373	456	529				
	3	258	117	186	85	*	5	29							
*	4	44					2	28	-490	16	-286				
	0	335	0	133	0	*	5	30							
*	4	47					2	454	-179	384	-152				
	0	325	0	834	0	*	5	31							
	3	247	31	177	22		0	0	-403	0	-630				
*	4	49					2	-74	-480	-17	-112				
	3	62	-216	79	-278	*	5	32							
*	4	50					2	163	-455	172	-481				
	0	-310	0	-264	0	*	5	33							
*	4	51					1	-247	-333	-71	-96				
	0	306	0	333	0		2	474	82	682	117				
*	4	52				*	5	34							
	1	-284	-42	-243	-36		2	357	315	256	226				
*	4	53				*	5	36							
	0	-291	0	-36	0		0	0	417	0	360				
	1	99	-261	74	-196	*	5	37							
	2	-36	-246	-81	-551		0	0	417	0	381				
*	4	54				*	5	39							
	0	-283	0	-197	0		0	0	-414	0	-292				
*	4	55				*	5	40							
	1	-222	-140	-192	-121		0	0	-414	0	-438				
*	4	56					1	-344	-221	-227	-146				
	0	-262	0	-259	0	*	5	41							
*	4	59					0	0	412	0	529				
	1	-121	-170	-197	-278		1	-396	88	-228	51				
*	5	0				*	5	42							
	2	471	0	222	0		0	0	409	0	10				
	3	0	-409	0	-528		2	-309	-284	-147	-135				

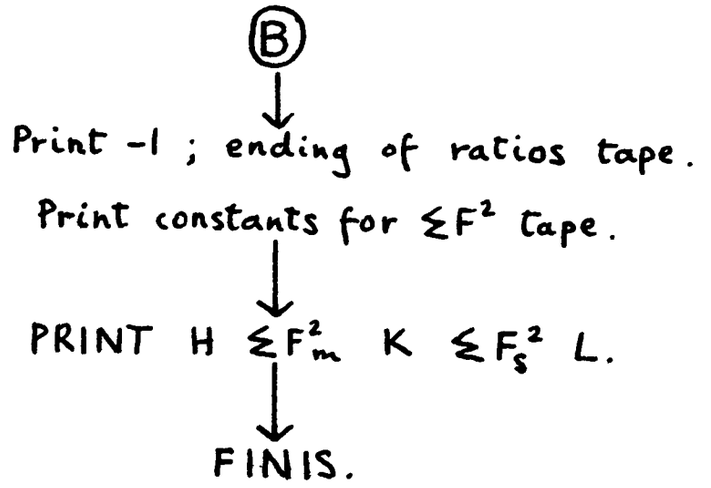
*	5	44				*	6	13	
	2	-277	-289	-167	-174		1	-283	97	-360	124
*	5	45				*	6	14	
	1	-222	-130	-147	-86		2	-415	-261	-492	-309
	2	301	-241	262	-209	*	6	15	
*	5	46					2	487	-35	652	-47
	1	-239	84	-459	162	*	6	16	
	2	-111	357	-159	509		0	486	0	858	0
*	5	47					1	237	182	212	163
	0	0	377	0	21	*	6	17	
	1	223	98	248	109		0	-486	0	-54	0
*	5	49				*	6	18	
	0	0	358	0	244		0	-486	0	-828	0
	2	-80	318	-89	354	*	6	19	
*	5	50					0	-486	0	-998	0
	0	0	-348	0	-68	*	6	20	
	2	223	221	91	91		0	486	0	669	0
*	6	0				*	6	21	
	0	481	0	1262	0		2	-391	275	-77	54
	1	0	-299	0	-343	*	6	24	
*	6	1					2	-424	199	-337	158
	2	-185	-459	-167	-414	*	6	27	
*	6	2					2	160	-430	19	-50
	0	-481	0	-1208	0	*	6	28	
	1	207	215	449	466		1	183	-221	269	-325
*	6	3				*	6	30	
	0	481	0	720	0		2	261	356	46	62
	2	-460	182	-184	73	*	6	31	
*	6	4					1	143	240	239	403
	1	221	-201	479	-434	*	6	32	
*	6	5					1	-199	195	-394	385
	0	-481	0	-651	0	*	6	35	
	1	-232	188	-259	210		2	403	-24	246	-14
	2	424	250	372	219	*	6	36	
*	6	6					1	257	-51	325	-64
	1	113	-277	319	-784	*	6	37	
*	6	8					1	242	89	234	86
	0	481	0	321	0	*	6	39	
*	6	9					2	-45	362	-32	254
	0	483	0	1324	0	*	6	40	
*	6	10					1	148	-188	108	-138
	0	-483	0	-28	0	*	6	42	
	2	-315	-379	-148	-179		2	216	-243	32	-35
*	6	11				*	6	43	
	0	-483	0	-849	0		1	211	61	338	98
	1	260	-147	396	-223	*	6	44	
*	6	12					1	186	94	94	47
	0	483	0	638	0	*	6	47	
*	6	13					1	114	-146	216	-276
	0	483	0	1077	0						

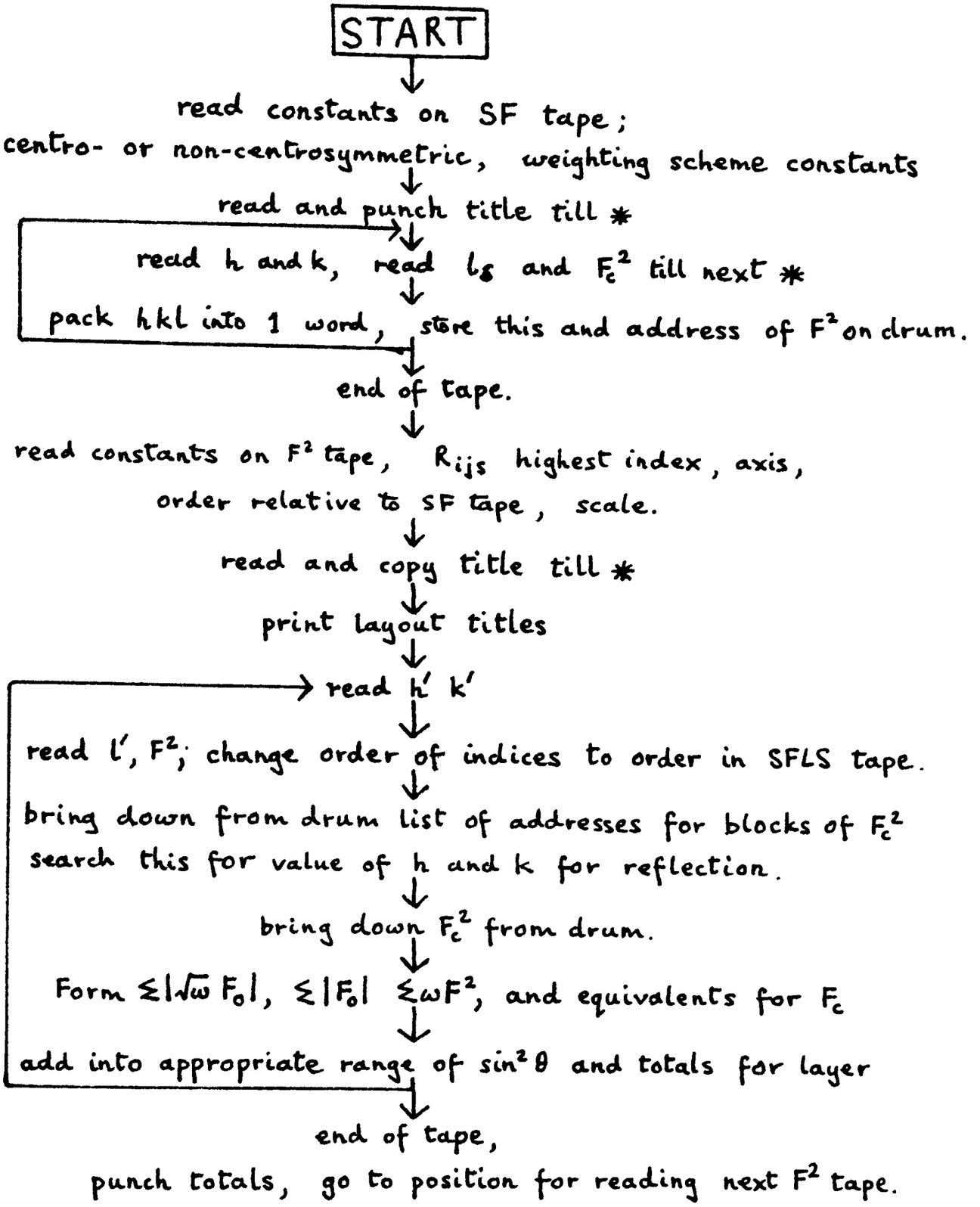
```
*    6    48
  I       120      -124        65       -67
*    6    49
  I      -158       -13      -555       -45
*    6    50
  I       143        13       548        49
```

Flow Chart for Intensity Ratios.


START
Read constants.
$H_{max.}$ $K_{max.}$ HKL_{main} $HKL_{sub.}$ $\xi^2_{min.}$ $F^2_{max.}$ $F^2_{min.}$
Read title, followed by R_{ijs}.
*
Read till next*
Read H, K subsidiary axis
If data read
If H > $H_{max.}$ main
store in A.
store in A, R. 0, -1 HK.
store in 2 H, K, A
exit at * -1 -1
read data into E_{even} = indices
E_{odd} = intensities
Read and print title till *
A
Read H, K.
B
* -1 -1
previous sector blank i.e. 1st. * line,
any line in which flag set for "not possible"
Put l in H_0 - H_m.
Clear H
Put F^2_m into H, so that value of H index = 2l
Clear E
arrange F^2_s in E as for F^2_m above.
check that F^2_m, $F^2_s \geqslant 0$ for any reflection.
Print H, Print $\sqrt{I_s/I_m}$, $H_{max.}$ + K + 1, $\sqrt{I_m/I_s}$ l.
Form ξ^2
No
Is $\xi^2 > \xi^2_{min.}$
No
Is $F^2 > F^2_{min.}$
No
Is $F^2 < F^2_{max.}$
No
Is H, K same as previous HK
yes
write up previous sums
Form $\leqslant F^2_m$, F^2_s.
Clear H, E M.


Is K > K max. s.
no.
Search drum for corresponding HK
Bring down corresponding F² from drum into G.
Set "not possible" flag. Y=1
Read F² into E till *
A
no
Is "not possible" flag set
yes
Clear F²


B
Print -1 ; ending of ratios tape.
Print constants for ΣF² tape.
PRINT H ΣF²m K ΣF²s L.
FINIS.



Flow Diagram for Agreement Analysis on Original Data.

Dihedral Angles

Flow Chart.

START

Read and Print title.

Read constants ; a b c α β γ , $N_x \rightarrow \frac{1}{N_x}$, $N_y \rightarrow \frac{1}{N_y}$, $N_z \rightarrow \frac{1}{N_z}$
$l_{min.}$ $l_{max.}$ scalefactor for output.

Transform cell dimensions so that the new β is the largest angle.
Record arrangement.

Form non-zero terms of Bond matrix .(Am. Min. 31 31 1946).

$$\begin{vmatrix} a\sin\beta & v_1 & 0 \\ 0 & v_2 & 0 \\ a\cos\beta & \cos\alpha & c \end{vmatrix}$$

where $v_1 = \dfrac{\cos\gamma - \cos\alpha\cos\beta}{\sin\beta}$

$$v_2 = \frac{\sqrt{1 + 2\cos\alpha\cos\beta\cos\gamma - (\cos^2\alpha + \cos^2\beta + \cos^2\gamma)}}{\sin\beta}$$

b = unity.

Read atom symbol and number (if no number put number = -1)
and store on drum in 2000 →

Read x y z → $\frac{x}{N_x}$, $\frac{y}{N_y}$, $\frac{z}{N_z}$.
put in positions indicated by transformation of cell dimensions.

multiply by matrix, to give orthogonal co-ordinates, store
in A from 3 onwards.

If * read

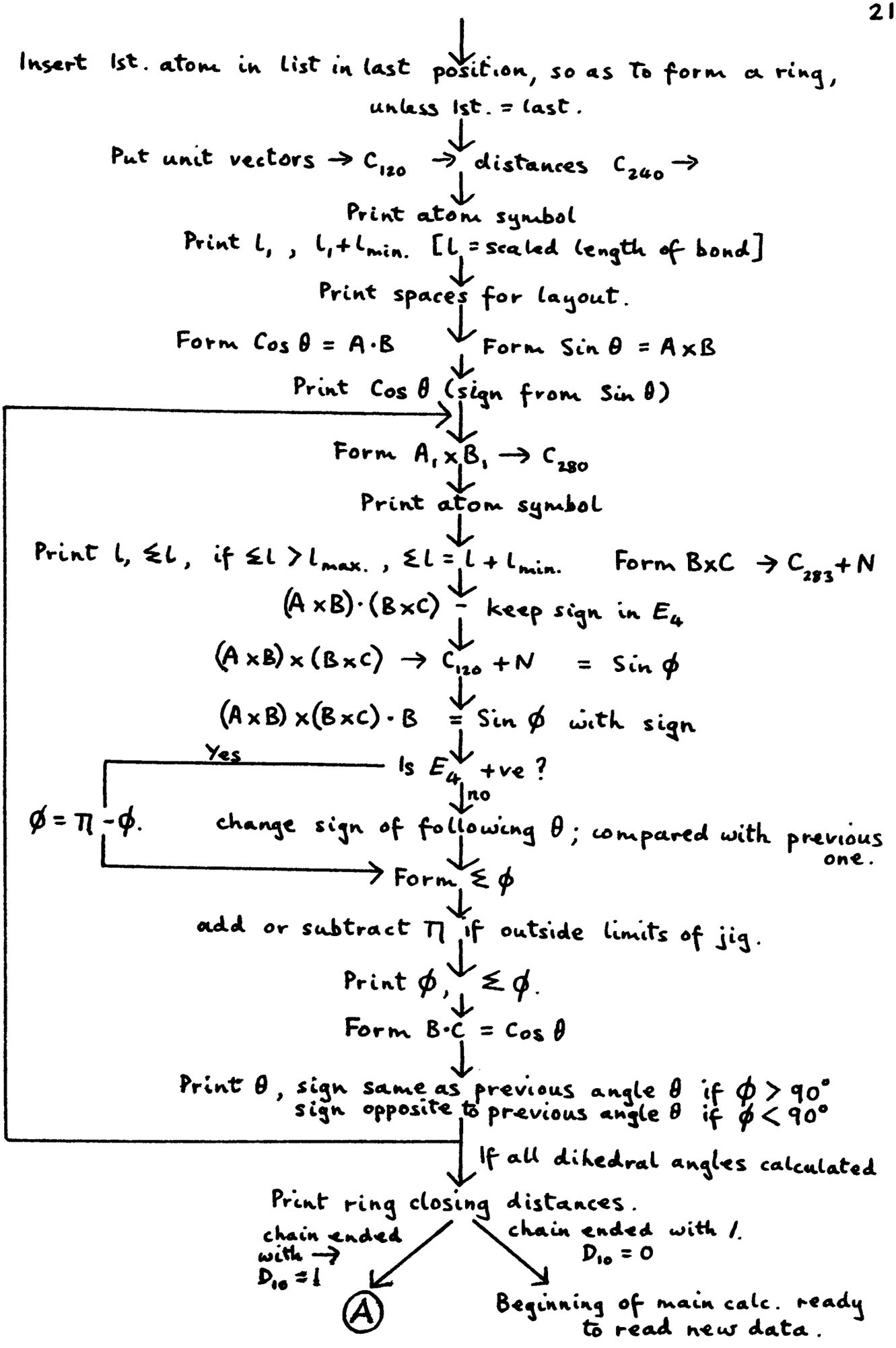
(A) →

Read position of atom in chain = A.

store in drum from 1800 →

A = 3A, and bring down co-ordinates in corresponding
position on drum. Put in C_0 → in fast store.

If → or / read, if / set
$D_{10} = 0$ - end of calc.


Insert 1st. atom in list in last position, so as to form a ring, unless 1st. = last.
Put unit vectors → C_{120} → distances C_{240} →
Print atom symbol
Print l, , $l_1 + l_{min.}$ [l = scaled length of bond]
Print spaces for layout.
Form Cos θ = A·B
Form Sin θ = A×B
Print Cos θ (sign from Sin θ)
Form $A_1 \times B_1 \rightarrow C_{280}$
Print atom symbol
Print l, Σl, if $\Sigma l > l_{max.}$, $\Sigma l = l + l_{min.}$
Form B×C → $C_{283} + N$
(A×B)·(B×C) - keep sign in E_4
(A×B)×(B×C) → $C_{120} + N$ = Sin φ
(A×B)×(B×C)·B = Sin φ with sign
Is E_4 +ve ?
Yes
no
φ = π - φ.
change sign of following θ; compared with previous one.
Form Σφ
add or subtract π if outside limits of jig.
Print φ, Σφ.
Form B·C = Cos θ
Print θ, sign same as previous angle θ if φ > 90°
sign opposite to previous angle θ if φ < 90°
If all dihedral angles calculated
Print ring closing distances.
chain ended with → $D_{10} = 1$
chain ended with /. $D_{10} = 0$
A
Beginning of main calc. ready to read new data.

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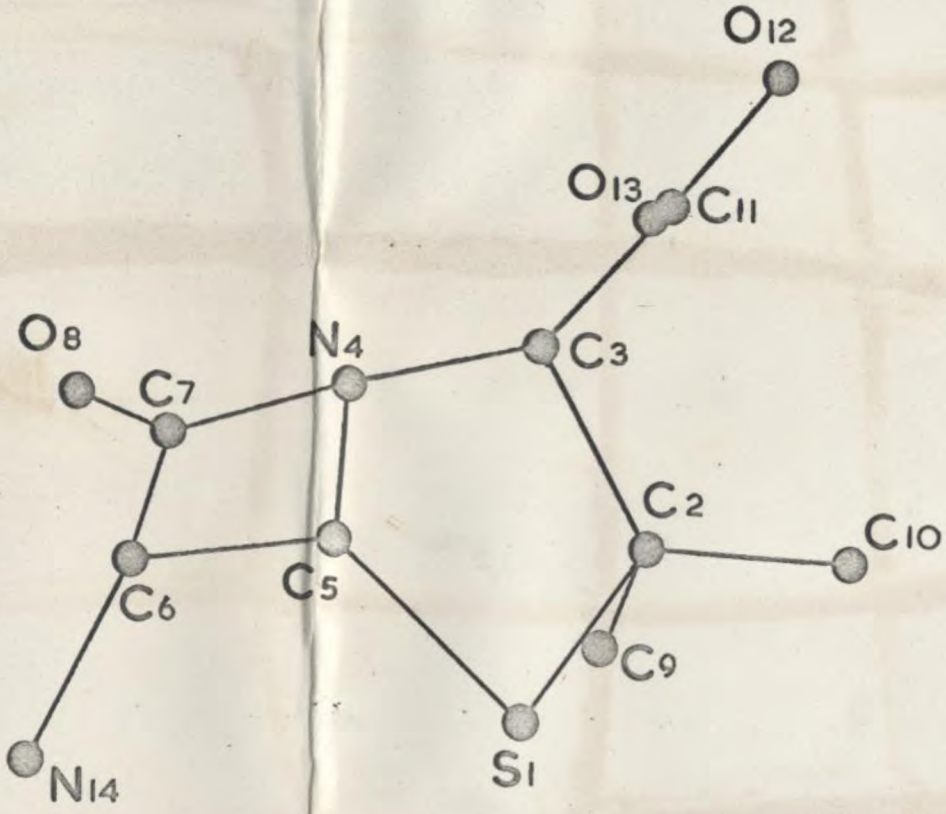
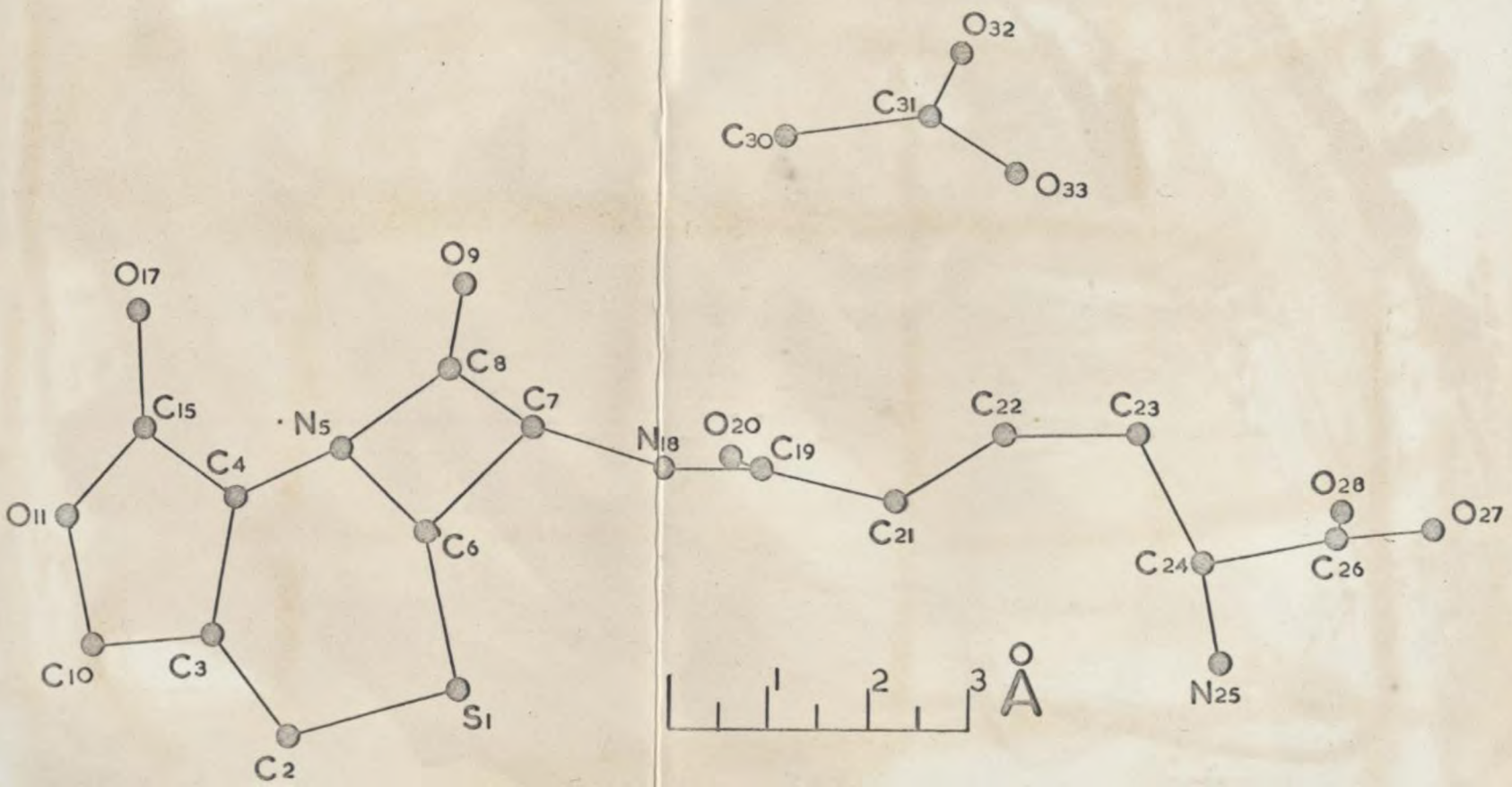
6-aminopenicillanic acid projection on to the [100] pplane



Cephalosporin Cc., projection on to the [100] plane.



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Note

PEN are reports of the Penicillin Production Committee of the Ministry of Supply, CPS report of the Committee on Penicillin Synthesis of the Medical Research Council. Merck, Squibb etc. are reports by these firms to the Office of Scientific Research and Development (N.Y.)