

STUDIES IN HETEROCYCLIC CHEMISTRY

a thesis presented by

DAVID GEORGE TAYLOR GREIG

in partial fulfilment of the award of

the degree of

DOCTOR OF PHILOSOPHY

Whiffin Laboratory

Imperial College

London, S.W.7.

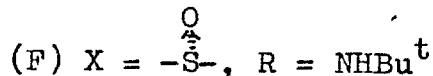
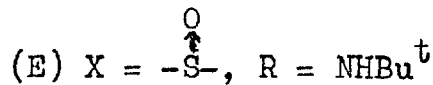
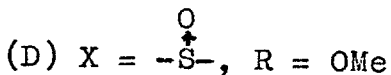
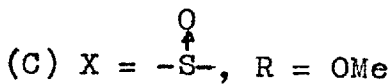
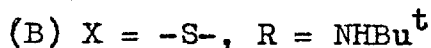
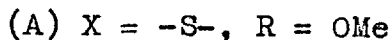
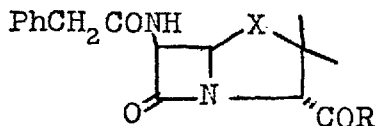
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Abstract

The content of this thesis has been divided into six chapters.

Chapter 1. This contains a brief summary of the historical developments of the penicillins and the cephalosporins and an outline of the published method of conversion of a penicillin into a cephalosporin.

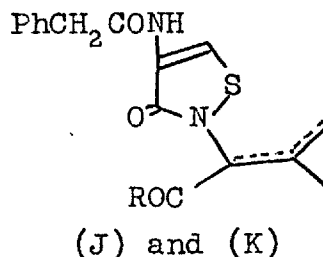
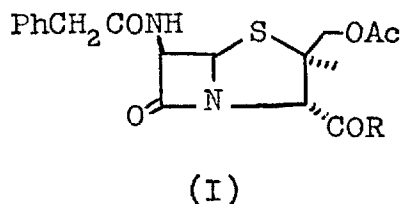
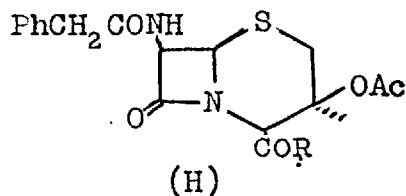
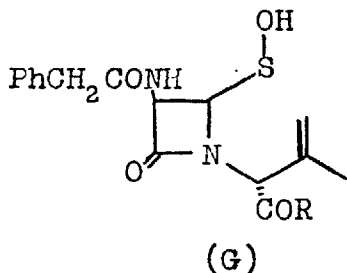
Chapter 2. The preparations of the two isomeric sulphoxides of both benzylpenicillin methyl ester (A) and benzylpenicillin t-butylamide (B), using sodium metaperiodate for the 1β -sulphoxides (C) and (E) and iodobenzene dichloride for the 1α -sulphoxides (D) and (F), are described. A study of internal nuclear Overhauser effects (N.O.E.) in (A), (B), (C), (D) and (E) enabled the assignment of the methyl signals in



the n.m.r. spectra of these compounds and gave the conformation of the thiazolidine ring in solution. Using chemical shift differences between sulphides and sulphoxides, aromatic solvent-induced shifts and hydrogen bonding studies the configuration of the metaperiodate produced sulphoxides was shown to be (S)(β -sulphoxide) and the isomer prepared with iodobenzene dichloride to be (R)(α -sulphoxide). A mechanistic rationale for the formation of the two sulphoxides is presented.

Chapter 3. The thermal isomerisation of a benzylpenicillin- α -sulphoxide to a $l\beta$ -sulphoxide is described and the mechanism discussed. The evidence, from a deuterium incorporation study, for the existence of the sulphenic acid intermediate (G) is given. The rearrangements of benzylpenicillin- $l\beta$ -sulphoxide t-butylamide and trichloroethyl ester in toluene-acetic anhydride systems have been investigated and the rearrangement products assigned as (H), (I), (J) and (K). The oxidation of the cepham (H) in the t-butylamide series with sodium metaperiodate gave solely the $l\alpha$ -sulphoxide, as shown by n.m.r. data and confirmed by an X-ray

crystallographic analysis of the *p*-bromobenzyl analogue.



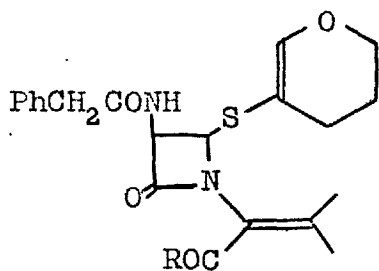
The oxidations of the 2 β -acetoxymethylpenams (I) in the trichloroethyl ester series proceeded in a manner directly analogous to those of the unsubstituted penicillins (Chapter 1). Thermal isomerisation of the 2 β -acetoxymethylpenam-1 α -sulphoxide caused inversion of both the sulphoxide and 2-acetoxymethyl functions, the mechanism is discussed. None of the 2-acetoxymethylpenams could be rearranged into a cephalosporin molecule. The stereochemistries of the 2-acetoxymethylpenam compounds were deduced from a consideration of the n.m.r. spectral characteristics, and differences between the penam and cepham products are discussed. A speculation as to the mechanism has been

presented.

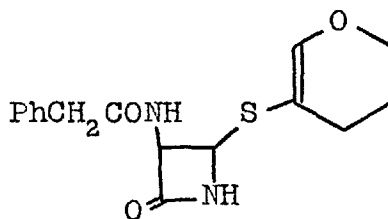
Chapter 4. This contains a summary of the reactions which have been carried out to trap the sulphenic acid intermediate (G).

Chapter 5. Dihydropyran was known (Ch. 4) to react with a penicillin sulphoxide to give an adduct. As a result of investigations into the chemistry of the adduct the previously proposed structure was shown to be erroneous and the structure revised to (L). Two methods for the controlled degradation of the adduct to the β -lactam compound (M) are described.

Chapter 6. This contains the experimental details to Chapters 2, 3 and 5.



(L)



(M)

FOR MY PARENTS

forsan et haec olim meminisse juvabit.

Virgil, Aeneid i. 203.

Acknowledgements

I wish to thank Professor D. H. R. Barton, F.R.S. for allowing me to work in his research group and for providing guidance and encouragement at all times.

It is a pleasure to acknowledge the contribution of Dr. P. G. Sammes who made many useful suggestions during the course of the experimental work, and who read the proofs of this thesis.

I thank the Science Research Council for the award of a C.A.P.S. scheme grant in conjunction with Glaxo (Greenford) Ltd., and the latter for the supply of penicillins used in this study.

Technical assistance was given skilfully by Mr. A. P. Coleman and his staff. N.m.r. spectra were run by Mrs. I. Boston and Mr. P. Jenkins, and Mr. P. Jenkins is specially thanked for his skill and patience in conjunction with the N.O.E. studies described in this thesis. Mr. K. I. Jones and his staff are thanked for micro-analyses.

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CHAPTER 1

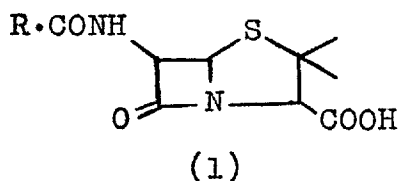
HISTORICAL SURVEY

The penicillins and cephalosporins are closely related classes of compounds which are members of the large family of microbial metabolites known as antibiotics. One member of the penicillin group (Penicillin G) was the first such antibiotic to be put into clinical use, and, in spite of the subsequent discovery of many other antibiotics, the penicillins continue to be used extensively in the treatment of bacterial infections.

(i) Penicillins

The name penicillin was given by Fleming in 1929¹ to an antibacterial substance produced by a mould of the genus penicillium, however it was not until 1940² that the material was isolated in solid, though inhomogeneous, form and its in vivo activity demonstrated. A joint British-American programme was set up during the second world war period to arrange for large scale production of penicillin and to investigate the chemistry of the molecule in the hope that it could be synthesised. Although a rational synthesis was not achieved until 1957³⁻⁵ the fermentation processes were developed to such an extent that penicillin emerged as a relatively cheap antibacterial agent. The results of this joint programme were published in an extensive monograph in 1949.⁶

It was shown during this period that the nucleus of all the penicillins was a bicyclic system containing a β -lactam fused to a thiazolidine ring, and that the different penicillins varied only in the nature of the side chain substituent R (1).^{7,8}

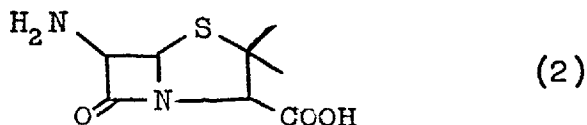


R = PhCH₂-, Penicillin G

R = EtCH=CHCH₂-, Penicillin F

In all only six natural penicillins have been isolated from the fermentation media, and of these only Penicillin G proved therapeutically useful. About fifty penicillins have been prepared by biosynthetic methods⁹ where a suitable side chain precursor is fed to the penicillin producing mould, and of these Penicillin V ((1) R = phoxymethyl) proved to be of use since it could be administered orally. The availability in 1960 of the penicillin nucleus, 6-aminopenicillanic acid (6APA)(2), by direct fermentation¹⁰ and by enzymatic cleavage of the natural penicillins¹¹⁻¹⁴, together with methods for acylating this^{5,15}, has led to the production of many thousands of new semisynthetic penicillins, some of which show advantages over the

natural materials and have found extensive use in medicine.¹⁶



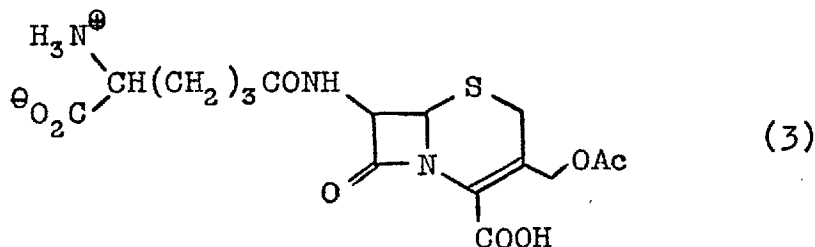
The early investigations into the development, chemistry and structural determinations of the penicillins and the methods for the preparation of the semisynthetic drugs have been extensively reviewed¹⁷ and will not be considered further here.

(ii) Cephalosporins

The history of cephalosporin extends back to 1945^{17A} when Professor Giuseppe Brotzu began to search the sewers of Sardinia for some form of organism that was a natural enemy of the bacteria which cause gastro-intestinal diseases. He found a fungus of the cephalosporium type which produced an antibiotic of low toxicity. A culture of this fungus was obtained by Lord Florey at Oxford, and his group were able to isolate a number of antibiotic substances from it.^{18,19} One of these proved to be Penicillin N[⊗] ((1), R = α -amino-adipyl), and during the subsequent investigations into this material a second, minor, component was found and named

[⊗] This compound was originally named Cephalosporin N but was revised when the structure was deduced.

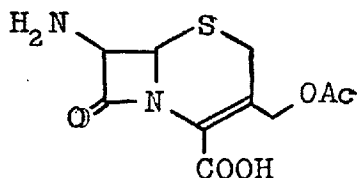
Cephalosporin C.²⁰ The structure of the cephalosporin was shown^{21,22} to be very similar to that of the penicillins in that it again consisted of a bicyclic unit containing a β -lactam, however in this case it was fused to a dihydrothiazine ring (3). For the Cephalosporin C the side chain was the α -aminoadipyl group, and this is the only cephalosporin compound which has been found in fermentation media.



Whilst the natural penicillins possess the advantages of high activity against many pathogenic bacteria and low toxicity towards the host, they suffer the disadvantages of being unstable in acid and sensitive to cleavage of the β -lactam (i.e. inactivation) by penicillinase, an enzyme produced by certain strains of bacteria. Some of the new semisynthetic penicillins did show greater acid stability, penicillinase resistance and an improved antibacterial spectrum, but no single new drug showed all three features. Although the antibacterial activity of the Cephalosporin C was too low for therapeutic use the material was relatively stable in acid and was resistant to penicillinase and in

addition was active against some penicillin resistant organisms. It was argued, by analogy to the penicillin case, that modification of the side chain of the cephalosporin might lead to a more useful antibiotic. The microbiological methods which had proved satisfactory for the manufacture of 6APA were not successful for the preparation of the analogous 7-aminocephalosporanic acid (7ACA)(4), however this material has been isolated in good yield by the chemical cleavage of Cephalosporin C.^{23,24} The semisynthetic cephalosporins did show the desired higher activity in conjunction with the other advantages, and some have found a place in medical practice.

Again the chemistry, syntheses and modifications of the cephalosporins have been reviewed²⁵ and will not be considered further here.

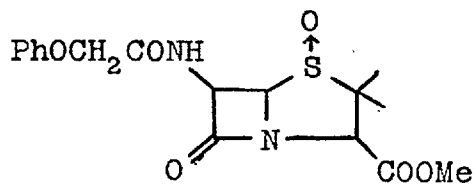


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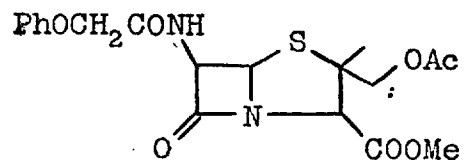
(iii) Conversion of penicillin to cephalosporin

The structural similarity between the penicillins and cephalosporins and the fact that both are produced in the cephalosporin fermentation has prompted investigations into the utilisation of the readily available penicillin for the preparation of cephalosporin antibiotics.

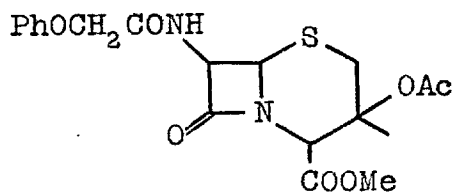
The most successful investigations into this conversion have come from a study of the reactions of the penicillin sulphoxides. Workers at the Lilly Research Laboratories reported²⁶ that treatment of phenoxymethylpenicillin sulphoxide methyl ester (5) with acetic acid at reflux led to the formation of a mixture of new β -lactam containing compounds. Careful purification gave two isomeric substances (6) and (7), which were shown to be a 2-acetoxymethylpenam derivative (6)(major component) and a 3-acetoxycepham derivative (7)(minor component) respectively. The two isomeric isothiazolones (9) and (10) were also isolated from the reaction mixture, and isothiazolone (10) was formed as the sole product when the sulphoxide (5) was heated at reflux in pyridine. Mild base treatment of the 3-acetoxycepham derivative (7) resulted in the loss of the elements of acetic acid to give the deacetoxycephalosporinate (8). This same compound was obtained in ~15% yield when the sulphoxide (5) was refluxed in xylene containing a



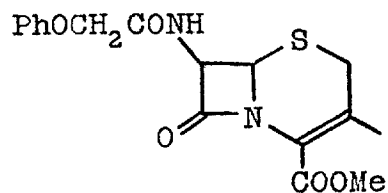
(5)



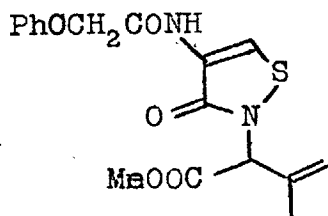
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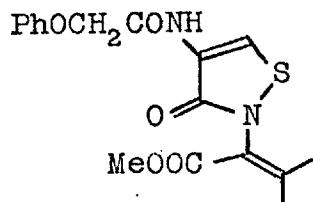
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(8)

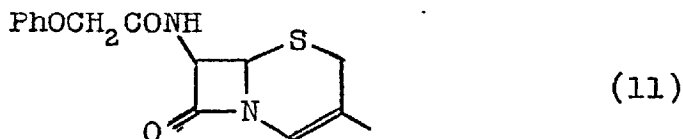


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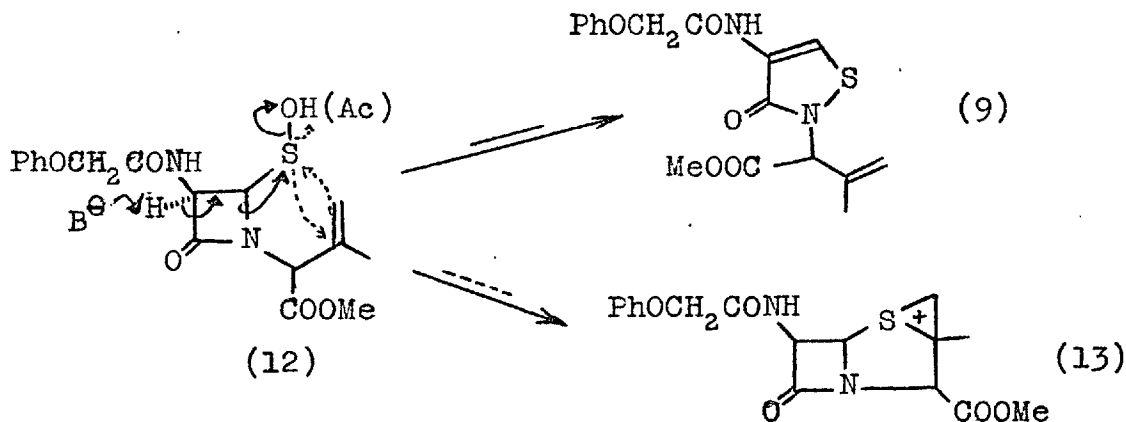


(10)

catalytic amount of p-toluenesulphonic acid.²⁷ Attempts to rearrange the free acid form of the sulphoxide gave, as the only β -lactam product, the decarboxylated cephem (11).

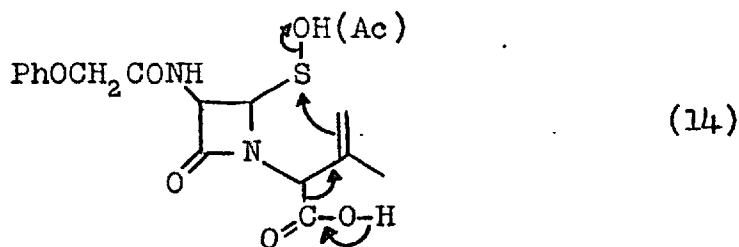


It was suggested that these rearrangements proceeded via the initial formation of a sulphenic acid (12) which then either collapsed to the isothiazolone or to the episulphonium ion (13) by the mechanisms indicated.

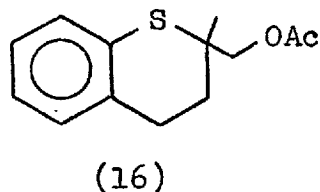
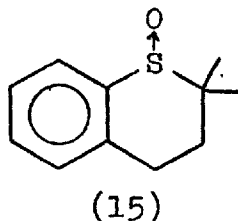


Formation of the acetoxy products (6) and (7) then occurred through attack of acetate ion at the primary or tertiary centre of this episulphonium ion.

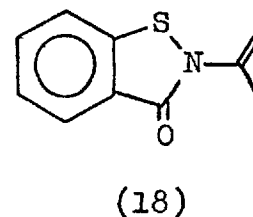
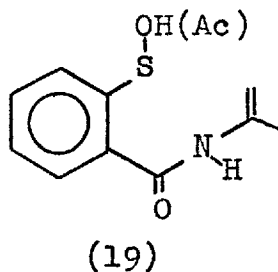
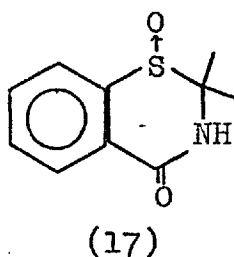
The formation of the decarboxylated material from the penicillanic acid sulphoxide was rationalised in terms of a concerted process as shown in (14).



The generality of this reaction of 2,2-dimethyl substituted cyclic sulphoxides has been demonstrated by a study of the 2,2-dimethylthiochroman-1-oxide (15)²⁸ where the 2-acetoxymethyl-2-methylthiochroman (16) was isolated after treatment with acetic anhydride.

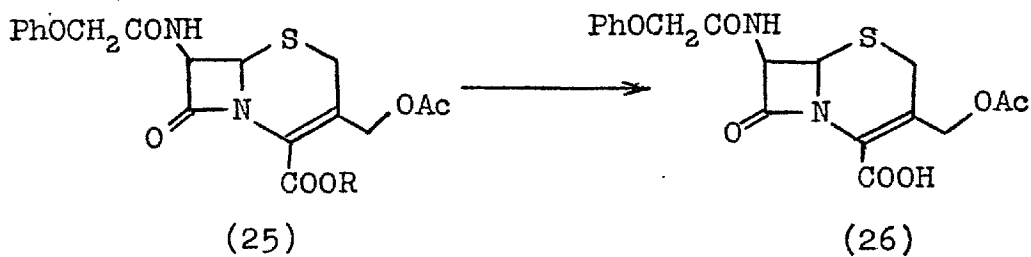
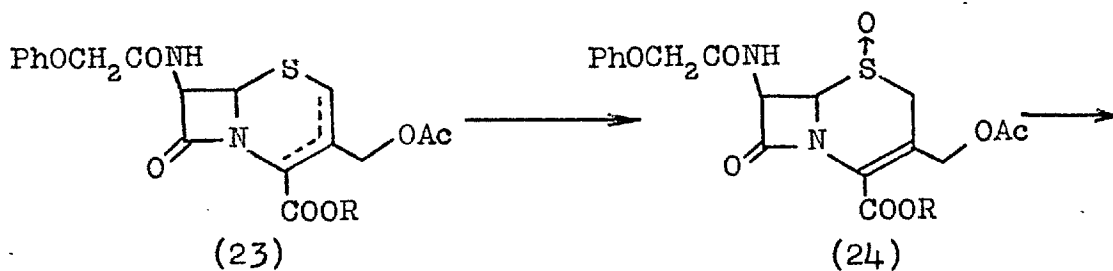
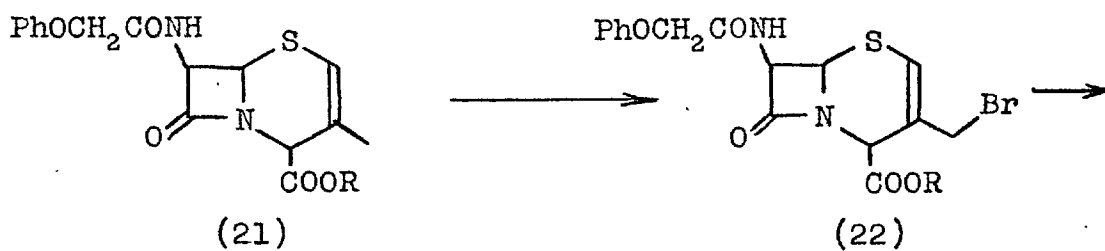
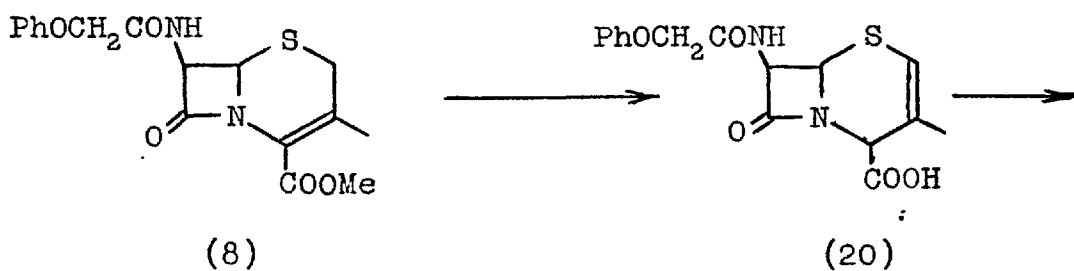


Also the nitrogen containing cyclic sulphoxide (17) reacted with acetic anhydride in toluene to give the cyclic sulphenamide (18).²⁹ This was presumed to arise from the sulphenyl acetate (19) which was trapped internally by the nitrogen atom in preference to acylation of the double bond.

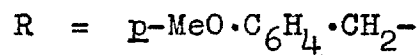


Recently the Lilly group have published³⁰ a method for the functionalisation of the deacetoxycephalosporinate (8) by the reaction sequence shown in Scheme 1.

The methyl ester (8) was hydrolysed to the 7-phenoxyacetamido-3-methyl-2-cephem-4-carboxylate (20) (Δ^2 -deacetoxycephalosporin V) by controlled hydrolysis with sodium hydroxide in pyridine-water. This acid was converted into its p-methoxybenzyl ester (21) using dimethylformamide dineopentyl acetal and p-methoxybenzyl alcohol. This ester interchange process was carried out in order to permit facile removal of the ester function at the end of the sequence, and the double bond isomerisation because the Δ^3 -isomer had proved resistant to allylic bromination. Allylic bromination of the Δ^2 -ester (21) with N-bromosuccinimide led to the bromination product (22) which was not purified but treated immediately with sodium acetate in acetone to give a mixture of the Δ^2 - and Δ^3 -isomers of Cephalosporin V (23). As these isomers could not be separated easily at this stage the Δ^2, Δ^3 -sulphide mixture was converted into the Δ^3 -sulphoxide (24) by oxidation with m-chloroperbenzoic acid. Reduction of this sulphoxide with acetyl chloride-sodium dithionite in dimethylformamide and cleavage of the ester with trifluoroacetic acid gave the 7-phenoxyacetamido-3-acetoxymethyl-3-cephem-4-carboxylate



Scheme 1.



(Cephalosporin V)(25) identical in all respects to authentic material.

Certain other studies have been published by the Lilly group, but as these parallel to some extent the studies described in this thesis they will be discussed at the relevant places in the following sections.

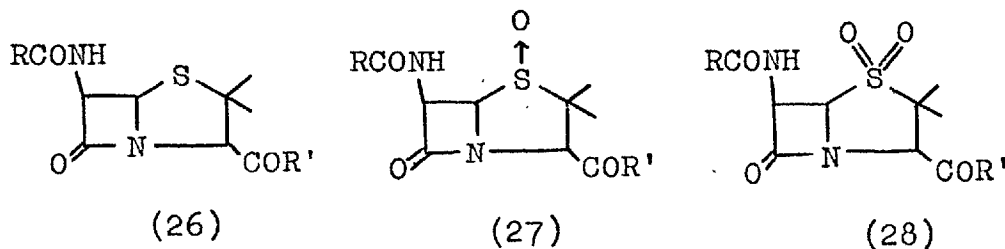
CHAPTER 2

STEREOCHEMISTRY OF THE PENICILLIN

SULPHOXIDES

Stereochemistry of the Penicillin Sulphoxides

It has long been known that treatment of a penicillin (26) with sodium metaperiodate in buffered aqueous conditions gives a single sulphoxide (27), which can be further oxidised to a sulphone (28) with stronger oxidising agents.³¹⁻³³



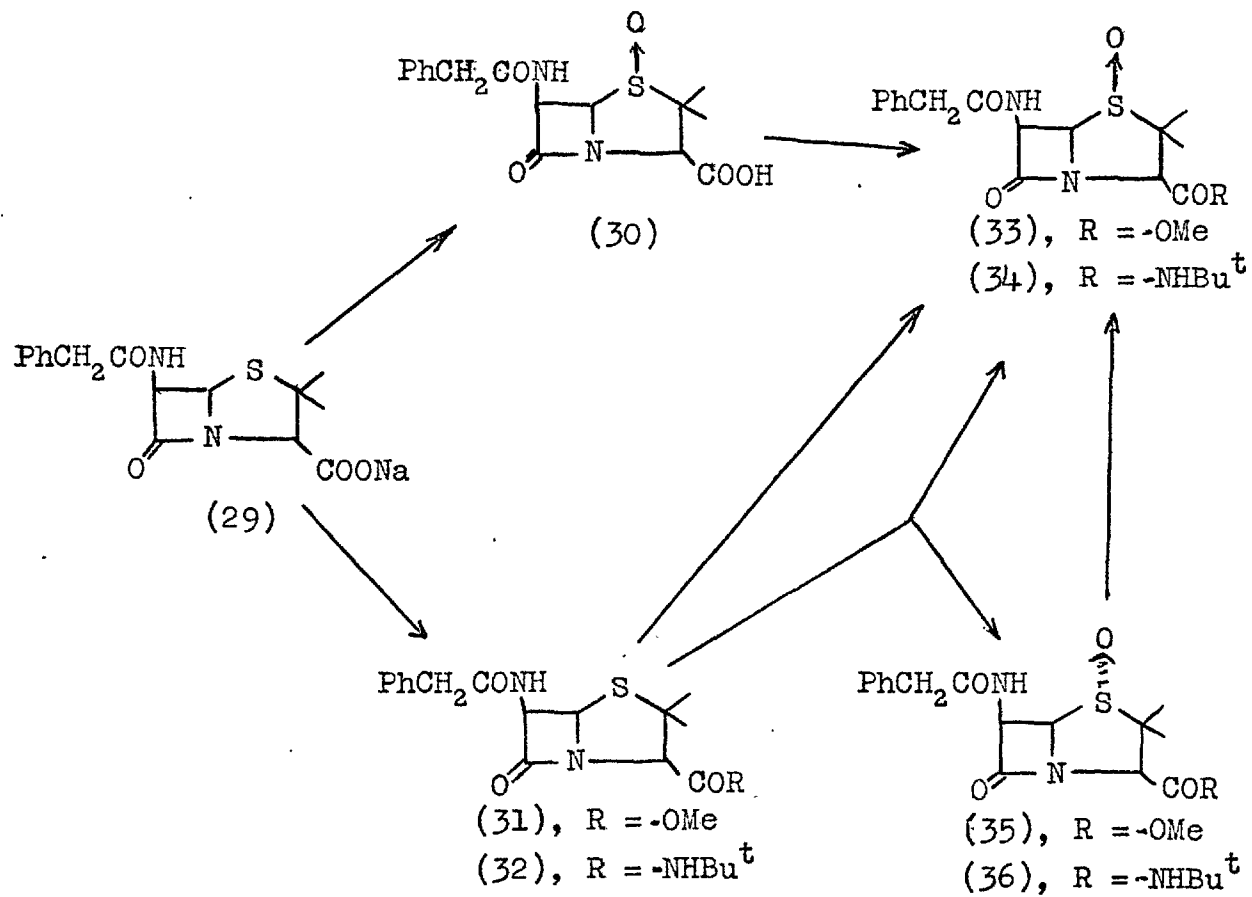
Theoretically two sulphoxide isomers are possible; these are the S-sulphoxide where the oxygen atom is on the β -face of the molecule and the R-sulphoxide where the oxygen is on the α -face of the molecule. The terminologies 1β -sulphoxide and 1α -sulphoxide respectively can be used to describe these. Prior to 1969 there was no reference in the literature to this possibility for the penicillin molecule, although sulphoxide isomers had been reported for other cases.³⁴⁻³⁶ The isomeric penicillin sulphoxide was prepared in these laboratories in 1967^{37, 38} by treatment of a penicillin ester or amide with iodobenzene dichloride in pyridine-water at -40°C , and subsequently by the Lilly group³⁹ by isomerisation of the known sulphoxide of phenoxymethylpenicillin methyl ester by irradiation with u.v. light.

However no details of the photosensitiser energy requirements or yields for this reaction were given.

In order that a study of the reactions of the penicillin sulphoxides be meaningful it was first necessary to establish the absolute stereochemistries of the molecules.

The two isomeric sulphoxides of benzylpenicillin methyl ester and benzylpenicillin t-butylamide (33), (34), (35) and (36) were prepared from benzylpenicillin sodium salt (29) by the reactions shown in Scheme 2.

Benzylpenicillin sodium salt (29) was treated with sodium metaperiodate³² in aqueous buffer to give the sulphoxide acid (30) as the sole product. This acid was converted to the methyl ester sulphoxide (33) by methylation with diazomethane or to the t-butylamide (34) by treatment with triethylamine, ethyl chloroformate and t-butylamine.⁴⁰ Alternatively the free acid of benzylpenicillin was converted into its methyl ester (31) or t-butylamide (32) by methods analogous to those above and these sulphides then oxidised. Oxidation with sodium metaperiodate in buffered aqueous dioxan gave, as the sole products, the oxides (33) and (34) identical to those described above. When iodobenzene dichloride in aqueous pyridine at -40°C



Scheme 2.

was used a mixture of two sulphoxides formed with both the methyl ester and t-butylamide, although in the latter case degradation occurred and the yields were lower than for the methyl ester. The less polar of these sulphoxides were identical to those already described (33) and (34), the more polar being assigned as the new sulphoxides (35) and (36). These new sulphoxides (35) and (36) on heating in benzene isomerised completely to the other isomers (33) and (34) respectively. (An explanation of the mechanism of formation of the sulphoxides and of the isomerisation will be found after the discussion of the assignments of stereochemistry).

The technique of nuclear magnetic resonance spectroscopy was considered to be the best method to enable elucidation of the configurations and conformations of these two sulphoxides.

(i) Nuclear Magnetic Resonance Studies

The n.m.r. spectra of the penicillins are relatively simple and unequivocal assignments can be made for the 3, 5 and 6 protons from their splitting patterns⁴¹ i.e. the 3-H is uncoupled and appears as a singlet, the 5-H is a doublet coupled only to the 6-H with a coupling

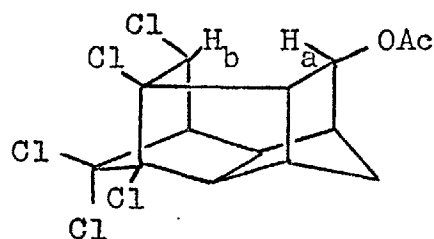
constant of 4-5Hz, characteristic of cis protons of a β -lactam⁴², the 6-H is a double doublet coupled both to the 5-H and the side chain amide proton. It is not possible to make unequivocal assignments for the geminal methyl groups at C-2 from chemical shift information alone as both of these appear as singlets.

(a) Nuclear Overhauser Effects

A study of Internal Nuclear Overhauser Effects⁴³ in the penicillin sulphides and sulphoxides enabled the assignment of these methyl signals and in addition gave the conformations of the thiazolidine ring of the penicillin nucleus.

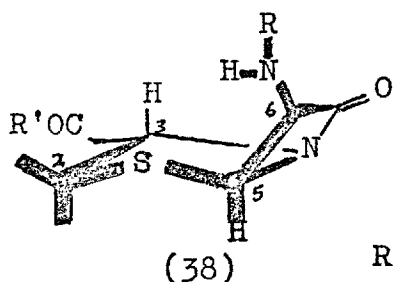
This effect arises because the intramolecular dipole-dipole interaction, which is the principal mechanism of relaxation in organic compounds, is of very short range and varies as the inverse sixth power of the internuclear distance. Thus if the intermolecular contributions to the nuclear relaxation processes are minimised by dissolving the compound in a magnetically inert solvent and also by removing all paramagnetic impurities from the solution, the dominant relaxation process will then be by magnetic dipole-dipole interactions between neighbouring nuclei within the same molecule. Saturation of the n.m.r. signal

from one of the nuclei can lead to intensity changes in the signals of the neighbouring nuclei, the maximum enhancement for dipolar interactions being 50% of the integrated signal intensity. Thus in the original example of this effect⁴⁴, the protons H_a and H_b of the half cage acetate (37) are held so close that enhancement of either proton signal could be observed on irradiation of the other proton.



(37)

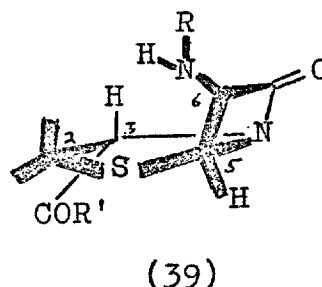
Inspection of models of the penicillin molecule indicated that the thiazolidine ring can exist in two conformations (38) and (39); these can be conveniently designated by the terminologies "Sulphur UP" and "Sulphur DOWN" respectively.



(38)

R = PhCH₂CO-

"Sulphur UP"



(39)

"Sulphur DOWN"

The spatial arrangements of the substituents of the penicillin molecule indicated that there should be finite differences in the N.O.E. observed for each of the conformations (38) and (39), and therefore that this technique could be used to differentiate between the two conformations. Thus in conformation (38) the 2β -methyl group is located close in space to the 3-H and the 2α -methyl group close to the 5-H. Hence irradiation of the 2β -methyl signal should cause an increase in the observed integral intensity of the 3-H, and irradiation of the 2α -methyl signal should enhance the 5-H, although to a lesser extent since the internuclear distance is greater. In conformation (39) the 3-H is again sufficiently close to the 2β -methyl group to be enhanced by irradiation of this, however the change in spatial orientation of the 2α -methyl group means that this is now closer to the 3-H than to the 5-H. Thus no effect to the 5-H should occur on irradiation of the 2α -methyl signal but a small enhancement of the 3-H might be observed.

Irradiation of the low field methyl signal of the benzylpenicillin t-butylamide (32) and its less polar sulphoxide (34) caused an 8-13% increase in the observed integral intensity of the 3-H signal, and irradiation

of the high field methyl signal caused a 6-10% increase in the integral intensity of the 5-H. Hence for these two molecules the low field methyl signal can be assigned to the 2β -methyl group and the high field signal to the 2α -methyl group. Also in both cases the conformation of the thiazolidine ring must be as in (38), i.e. with the "Sulphur UP".

With the benzylpenicillin methyl ester (31) the position was complicated by the proximity of the two methyl signals ($\tau 8.54(6)$ and $\tau 8.57(5)$), however irradiation of the methyl signal at $\tau 8.54(6)$ caused a 20% increase in the integral intensity of the 3-H, whereas irradiation at $\tau 8.57(5)$ had no significant effect. This result was taken to indicate that the low field signal was due to the 2β -methyl group and the conformation as in (39), i.e. with the "Sulphur DOWN".

In both the sulphoxides of benzylpenicillin methyl ester (33) and (35) irradiation of the low field methyl signal caused an increase in the 3-H integral and irradiation of the high field methyl signal enhanced the 5-H integral. Thus in both these molecules the low field signal was assigned to the 2β -methyl group, the high field signal to the 2α -methyl group and the ring conformation as in

(38), i.e. with the "Sulphur UP".

A meaningful result could not be obtained for the more polar sulphoxide of benzylpenicillin t-butylamide (36), presumably due to the thermal isomerisation taking place at the probe temperature during the time required for the investigation (ca. 6hrs.)

The results of this N.O.E. study are summarised in Table 1.

(b) Chemical Shift Studies

The N.O.E. results gave, in addition to the assignments of the methyl signals, the conformations of the thiazolidine rings of the penicillins studied, but they gave no information on the configurations of the sulphoxides. These were deduced from a consideration of the chemical shift differences between the corresponding protons in the sulphides and sulphoxides.

The chemical shifts of the protons of the various penicillins are shown in Table 2., and the shift differences between the sulphides and the two sulphoxides for both series are shown in Table 3.

The conformations given by the N.O.E. study are such

Table 1. Nuclear Overhauser Effect Results

COMPOUND	METHYL IRRADIATED	OBSERVATION ^a	CONCLUSION ^b
(32)	Low field High field	+8% to 3-H 0% to 5-H 0% to 3-H +6% to 5-H	
(34)	Low field High field	+13% to 3-H 0% to 5-H 0% to 3-H +10% to 5-H	
(31) ^c	Low field High field	+20% to 3-H +5% to 5-H +6% to 3-H +7% to 5-H	
(33) ^d	Low field High field	+21% to 3-H +12% to 5-H	
(35)	Low field High field	+9% to 3-H -1.8% to 5-H +8% to 3-H -1.8% to 5-H	

For footnotes see next page.

Footnotes to Table 1.

a. Results quoted are for ~10% w/v solutions in CDCl_3 with T.M.S. as internal reference and are based on the measurement of 15-20 integrals. The figures are expected to be accurate to better than $\pm 3\%$.

b. The conclusion is expressed in the form of a Newman projection along the C2-C3 bond. (L) refers to the low field methyl signal and (U) to the high field methyl signal.

c. The close proximity of the methyl resonances of this molecule may have caused the secondary irradiation to overlap the other methyl signal and so increase the error.

d. This result is based on the measurement of the change in ratio of the 3-H to the 5-H, others are based on the ratio of the 3-H or 5-H to the benzylic $-\text{CH}_2-$ signal which was used as internal reference.

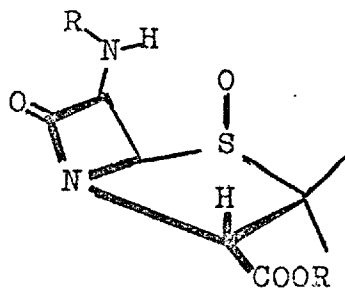
COMPD.	2 β -Me	2 α -Me	3-H	5-H ^a	6-H ^b	N-H	CH ₂ Ph	Ph	-OMe or -NHBu ^t
(31)	8.55	8.57	5.61	4.47 J=4	4.39 J=4, 9	3.70 J=9	6.30	2.69	6.26
(33)	8.33	8.82	5.37	4.99 J=4	4.00 J=4, 10	2.94 J=10	6.47	2.76	6.26
(35)	8.37	8.71	5.61	5.30 J=4	4.74 J=4, 7	3.18 J=7	6.46	2.76	6.27
(32)	8.37	8.49	5.95	4.65 J=4	4.29 J=4, 9	3.52 J=9	6.40	2.69	8.63
(34)	8.26	8.80	5.60	5.08 J=5	4.01 J=5, 10	2.72 J=10	6.42	2.74	8.65
(36)	8.44	8.65	5.85	5.40 J=4	4.80 J=4, 9	3.50 J=9	6.42	2.69	8.65

Table 2. N.m.r. spectral parameters of the penicillins and sulphoxides.
As τ values in CDCl₃ with T.M.S. as internal reference.
a. appears as a doublet. b. appears as a double doublet.

J values in Hz.

	Methyl Esters		t-Butylamides	
	(33)-(31)	(35)-(31)	(34)-(32)	(36)-(32)
2 β -Me	-0.22	-0.18	-0.11	+0.07
2 α -Me	+0.25	+0.14	+0.31	+0.16
3-H	-0.24	0.00	-0.36	-0.09
5-H	+0.52	+0.83	+0.43	+0.75
6-H	-0.39	+0.35	-0.28	+0.51
N-H	-0.80	-0.58	-0.80	-0.02

Table 3. Chemical shift differences between the sulphides and sulphoxides. Positive values indicate upfield shifts (i.e. shielding), negative values downfield shifts (deshielding). All values in p.p.m.



(40)

that in all the sulphoxides studied the thiazolidine ring existed in conformation (38), i.e. with the "Sulphur UP". Hence in a β -sulphoxide the sulphur-oxygen bond would be co-parallel with the proton at position 3, i.e. in a pseudo-axial orientation (40), and therefore a syn-axial effect⁴⁵⁻⁴⁸ should be observed for this oxide. This should result in deshielding of the 3-H of the penicillin nucleus, also the 6-H and the 2 β -methyl group should be deshielded since both are in a similar geometrical relationship to the sulphur atom (see molecular models).

Considering first the t-butylamides in which there is no conformational difference between the sulphide (32) and sulphoxide (34); inspection of the results in Table 3. shows that the 3-H, 6-H and 2 β -methyl protons are deshielded (-0.36, -0.28 and -0.11p.p.m. respectively), whereas the 5-H and 2 α -methyl protons are shielded (+0.43 and +0.31 p.p.m. respectively) between the sulphide (32) and sulphoxide (34). This was taken to be a clear indication that the sulphoxide (34) was the β -sulphoxide, and, by inference, that the sulphoxide (36) was the α -sulphoxide.

In the methyl ester series there is a conformational

difference between the sulphide (31) and the sulphoxides (33) and (35), however it is reasonable to assume that the n.m.r. chemical shifts of the benzylpenicillin methyl ester (31) in the two conformations would not differ much, and therefore the above arguments can be applied. The deshielding of the 3-H, 6-H and 2 β -methyl protons was observed between the sulphide (31) and the sulphoxide (33), but not between the sulphide (31) and the sulphoxide (35) (see Table 3.), this being taken to indicate that the compound (33) was the β -sulphoxide and (35) was the α -sulphoxide.

The large upfield shift of the 5-H in the α -sulphoxides (35) and (36) relative to the sulphides (31) and (32) is possibly due to the shielding effects associated with the trans-orientated lone pair on the sulphur atom. A similar effect has been reported for the thiane-1-oxides.^{49,50}

Further evidence for the assignment of the β -configuration to the sulphoxides (33) and (34) and the α -configuration to the sulphoxides (35) and (36) came from a consideration of the chemical shift differences of the side chain amide protons. Models indicated that in the β -sulphoxides this proton should be sufficiently close to the sulphoxide oxygen atom to permit hydrogen bonding. Such an interaction

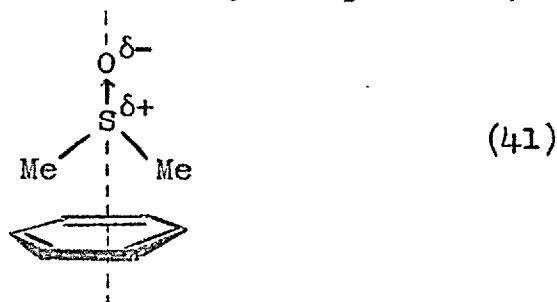
would predict a downfield shift of this proton, and that it would exchange with D_2O very much more slowly than would the starting sulphide.⁵¹ The results shown in Table 3. show that there is a large downfield shift for the sulphoxides (33) and (34) (0.80 p.p.m. in both cases), and additionally the exchange with D_2O of the methyl ester sulphoxide (33) required 2 days, whereas the sulphide (31) exchanged completely in 10 minutes. This again indicated the above assignment of stereochemistry.

(c) Solvent Shift Studies

Aromatic solvent-induced shifts provide further confirmation for the assignment of the metaperiodate oxidation products (33) and (34) as β -sulphoxides and the other, more polar, sulphoxides (35) and (36) as α -sulphoxides.

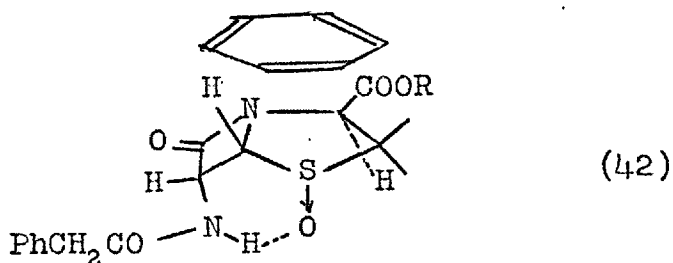
It is well known that interactions between solvents and polar solutes can cause finite differences in chemical shifts. These differences, relative to the chemical shifts in an "inert" solvent, are referred to as solvent shifts⁵², and it has been demonstrated that the shifts caused by aromatic solvents can be useful in elucidating certain structural features in the solute molecule.⁵³ This utility

arises because the protons situated in the vicinity of the solvent molecule experience a large screening effect as a result of the large anisotropy in the magnetic susceptibility associated with the aromatic system. When experimental conditions are used where the contributions to the solvent shift from the solvent's bulk magnetic susceptibility, from its magnetic anisotropy, from Van der Waals interactions⁵⁴ and from the reaction field⁵⁵ set up by the solute are minimised then any shift arising must be due to specific interactions between the solute and solvent. It has been proposed⁵⁶ that this interaction is due to a local dipole-induced dipole interaction leading to a transient 1:1 complex between the solute and solvent. Ledaal⁵⁷ has suggested that the geometry of the benzene-solute complex in solutes containing any polar function can be rationalised in terms of one common model. This presumes that the dipole axis of the polar function in the solute molecule is located along the sixfold axis of symmetry of the benzene ring, with the positive end nearest and the negative end farthest away. This geometry is illustrated for dimethyl sulphoxide (41)



It must be emphasised that the nature of this complex can only be approximated since five parameters are required⁵⁸ to unambiguously fix its geometry, nevertheless good qualitative results have been obtained using this model.⁵⁷

Applying this model to the penicillin case the formation of the complex with the β -sulphoxide would be from the less hindered α -face of the molecule (42), whereas in the α -sulphoxide the approach would be from the more crowded β -face.



Thus a large upfield shift for the 5-H and the 2 α -methyl protons would be predicted in the β -sulphoxide relative to the sulphide, but predictions could not be made for the α -sulphoxide since the solvent molecule would be prevented from taking up this form of association by the substituents of the β -face of the molecule. The solvent shifts between deuteriochloroform and hexadeutero-

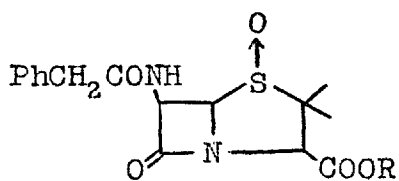
Group	Sulphoxide (33)	Sulphoxide (36)
2 α -Me	+0.64	+0.09
2 β -Me	+0.41	+0.24
3-H	-0.07	-0.05
5-H	+0.99	+0.34
6-H	+0.04	+0.88

Table 4. Solvent shifts in the isomeric methyl ester sulphoxides.

Positive values indicate upfield shifts, negative values downfield shifts; i.e. values as $\nu_{C_6D_6} - \nu_{CDCl_3}$.

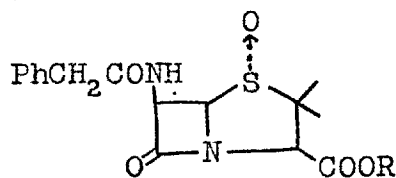
benzene are shown in Table 4. The large upfield shifts are observed for the sulfoxide (33) but not for (35), this again indicates the β -configuration for sulfoxide (33).

Thus the results from the chemical shift difference studies, the hydrogen bonding studies and the solvent shift studies showed that the sulfoxide produced by metaperiodate oxidation had the β -configuration (33) and (34), and the more polar isomer from the iodobenzene dichloride reaction had the α -configuration (35) and (36).



(33), R = -OMe

(34), R = -NHBu^t



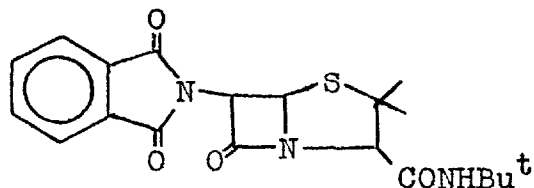
(35), R = -OMe

(36), R = -NHBu^t

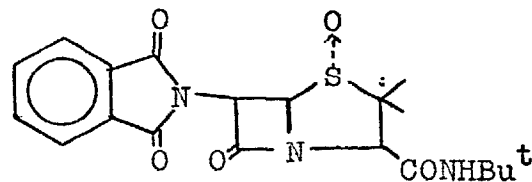
(ii) Mechanistic Considerations

The preferential formation of the β -sulfoxide by the normal oxidising agents can be rationalised in terms of reagent approach control by hydrogen bonding between the oxidising agent and the $\delta\beta$ -side chain amide proton. For the $\delta\beta$ -phthalimidopenicillanate as its

t-butylamide (43), in which there is no β -amide proton, the major product from oxidation with metaperiodate was the α -sulphoxide (44).^{59,60} Again n.m.r. chemical shifts were useful in establishing this stereochemistry.

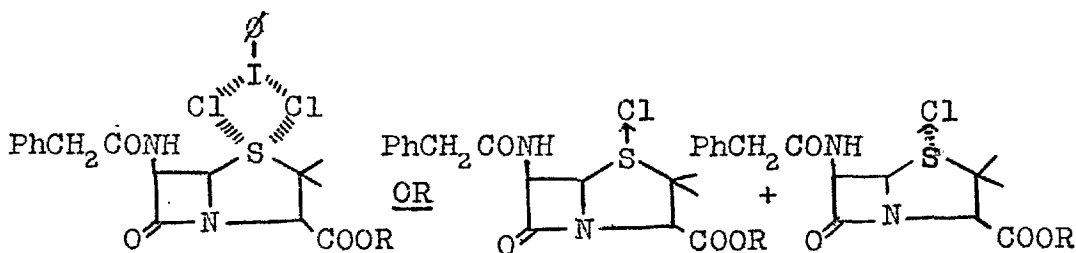


(43)



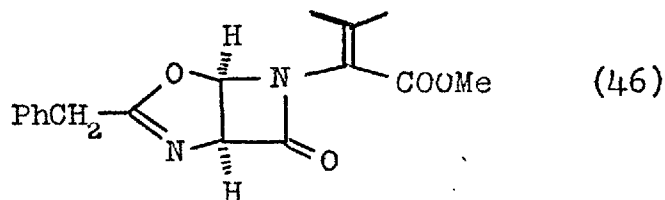
(44)

The mechanism of the oxidation with iodobenzene dichloride must proceed by a two step process in which the intermediate, either a complex or a sulphonium chloride (45), is capable of forming on either face of the molecule such that hydrolysis can proceed with inversion about the sulphur to give both sulphoxide isomers.^{61,62}



(45)

More direct participation of the side chain can not be ruled out as one of the minor products which has been isolated from the reaction was the oxazoline (46).³⁸ This material is also formed by reaction of the penicillin sulphide (31) with t-butylhypochlorite and triethylamine.⁶³



(iii) Comparisons with Published Results

Subsequent to the initiation of these investigations the Lilly group have published similar studies on the phenoxymethyl analogue of the sulphoxide (33)⁶⁴ and have confirmed that this has the β -configuration. They studied the Nuclear Overhauser Effects in this series to assign the signals of the n.m.r. spectra and used these results to calculate, using the McConnell point dipole approximation, the chemical shift differences between the sulphide and sulphoxide. A reasonable agreement was obtained between the measured differences and those calculated for the β -sulphoxide. In addition an X-ray analysis of the sulphoxide acid confirmed the β -configuration of the sulphoxide.

CHAPTER 3

THE REARRANGEMENTS OF PENICILLIN SULPHOXIDES

The Rearrangements of Penicillin Sulphoxides

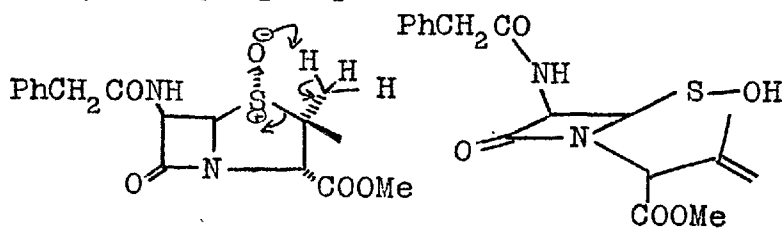
(1) α -Sulphoxide to β -Sulphoxide

The suggestion by Morin et al.²⁶ that the sulphenic acid was an intermediate in the catalysed rearrangements of a penicillin β -sulphoxide led us to predict that this sulphenic acid might also be the intermediate in the isomerisation of an α -sulphoxide to a β -sulphoxide.

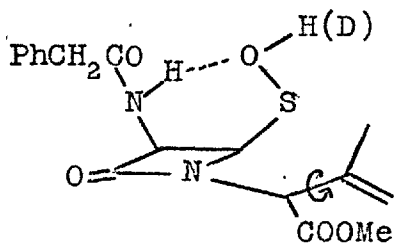
A stereospecific electrocyclic ring opening of the thiazolidine ring of the penicillin molecule via a cis-proton abstraction from the 2 α -methyl group by the 1 α -sulphoxide oxygen atom would lead to the sulphenic acid (47). This could then invert to the configuration (48) which would be stabilised by hydrogen bonding between the side chain amide proton and the sulphoxide oxygen atom. Analogous hydrogen bonding was found to occur in the penicillin β -sulphoxides (vide ut supra). Ring closure could then occur after rotation about the C2-C3 bond to locate the double bond cis to the sulphenic acid function.

In order to test this hypothesis the isomerisation was carried out in the presence of a deuterium source (Bu^tOD) and the incorporation of deuterium investigated.⁶⁵

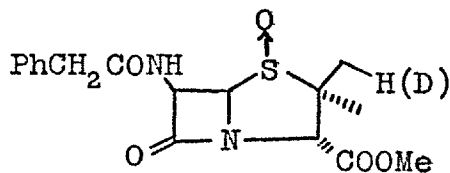
When the benzylpenicillin α -sulphoxide methyl ester (35) was heated at reflux in benzene containing 18% v/v deuterated *t*-butanol there was a 46% incorporation of one atom excess of deuterium into the 2 β -methyl group of the isolated β -sulphoxide. When neat deuterated *t*-butanol was used the incorporation was 60%. In both cases the extent of deuterium incorporation was determined from the mass spectrum, after normalisation of any deuterium incorporated into the side chain amide group. The position of incorporation was given by the n.m.r. spectrum where the integral intensity of the methyl signal at τ 8.33 was reduced by an amount corresponding to the deuterium incorporated. The results from the Nuclear Overhauser Effect investigations had shown that this signal was due to the 2 β -methyl group.



(47)



(48)



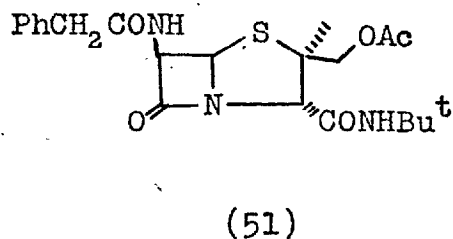
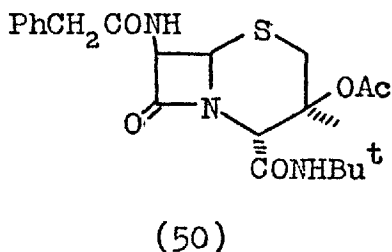
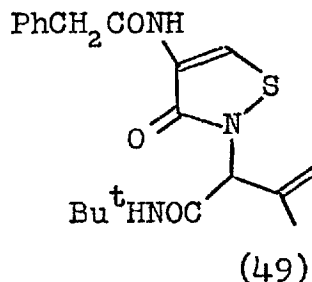
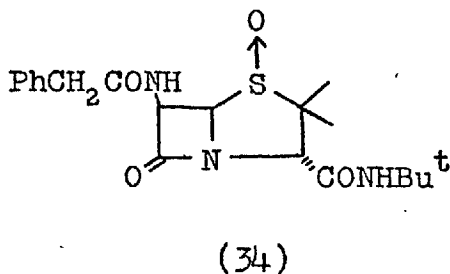
This result was consistent with the proposed mechanism, the exchange taking place at the sulphenic acid stage prior to the ring closure.

(ii) Benzylpenicillin β -sulphoxide t-butylamide in Acetic Anhydride ^{*}

When benzylpenicillin β -sulphoxide t-butylamide (34) was heated at reflux in acetic anhydride for 1hr. it was totally destroyed and a mixture of products formed. Purification of this mixture by column chromatography gave, as the first material eluted, the crystalline isothiazolone (49). The subsequent fractions contained apparently only one component, by t.l.c. analysis, and from this the 3β -acetoxy- 4α -t-butylcarbamoyl- 3α -methyl- 7β -phenylacetamido-cepham[‡] (50) crystallised.

^{*} As the configurations of some of the products from the rearrangement reactions were deduced from a detailed study of their n.m.r. spectra a separate section dealing with these assignments has been given (c.f. pg.). Prior to this the structures have been quoted.

[‡] This material is hereinafter called the 3β -acetoxy-cepham-t-butylamide.



The assignment of the isothiazolone structure (49) was supported by its elemental analysis and mass spectrum. The unsaturated chromophore was indicated by the u.v. spectrum (λ_{\max} 298 μ ., $\text{Log}_{10} \epsilon$ 4.07) and the olefinic protons appeared in the n.m.r. spectrum at 1.75 (1H s) and 4.89 (2H m).

The structure of the 3 β -acetoxycepham-t-butylamide was similarly supported by elemental analysis and its mass spectrum. The presence of the β -lactam function was confirmed by the infrared absorption at 1780 cm^{-1} and by the characteristic pattern of the β -lactam protons in the n.m.r. spectrum. The acetate function was indicated

by the infrared absorption (1740 and 1220cm.^{-1}) and the n.m.r. spectrum ($\tau 8.22$ (3H s)). The methylene protons appeared as an AB quartet in the n.m.r. spectrum, and the relevance of this will be discussed subsequently.

When the rearrangement was carried out in an acetic anhydride-toluene system under conditions of high dilution the formation of the isothiazolone (49) was suppressed and treatment of the concentrated reaction mixture with ether gave a t.l.c. homogeneous solid. Examination of the 100MHz. n.m.r. spectrum of this solid showed it to be a mixture of the 3β -acetoxycephem-t-butylamide (50) and another β -lactam component. Repeated crystallisation of this mixture gave the 3β -acetoxycephem-t-butylamide pure, however the component was non-crystalline and could not be freed from the last traces of cepham. This second component was presumed, and subsequently proved (cf. sect. 3(9)), to be the 2β -acetoxymethyl- 3α -t-butylcarbamoyl- 2α -methyl- 6β -phenylacetamido-penam (51).[‡]

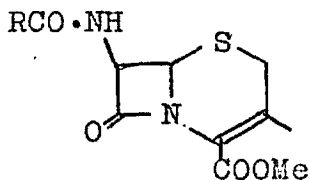
[‡] Hereinafter called the 2β -acetoxymethylpenam t-butylamide.

If a more concentrated solution was used and sodium acetate added then the isothiazolone (49) could be crystallised directly from the reaction mixture, and was the only product isolated.

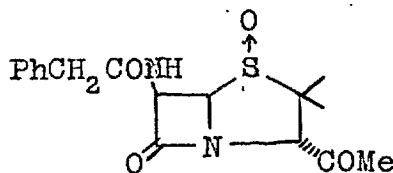
(iii) Attempted Preparation of Benzylpenicillin

Methyl Ketone

It was decided at this stage to attempt to investigate the effect of the enolisability of the proton at position 3 of the penicillin nucleus on the rearrangement reaction. This arose from the report by Morin *et. al.*²⁶ that small quantities of the Δ^3 -cephem (52) could be isolated from the acetic anhydride rearrangement in the methyl ester series. None of the corresponding cephem compound was found in the rearrangement of the *t*-butylamide, in which the 3-proton is less enolisable.



(52)



(53)

The methyl ketone (53) was considered to be a suitable modification to study and therefore attempts were made to prepare this. The route chosen consisted of converting the penicillanic acid to the diazoketone (54) and reducing this to the methyl ketone (53).

As the sulphoxide was required for the rearrangement reaction this was chosen as starting material for the diazoketone preparation since the sulphoxides are known to be more stable towards acidic reagents. The conventional route from an acid to a diazoketone involves conversion to the acid chloride, which is then treated with diazomethane to give the diazoketone (Scheme 3).⁶⁶

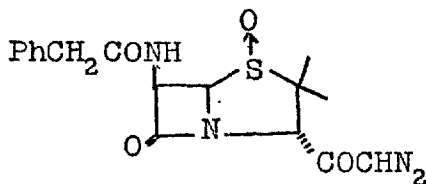


Scheme 3

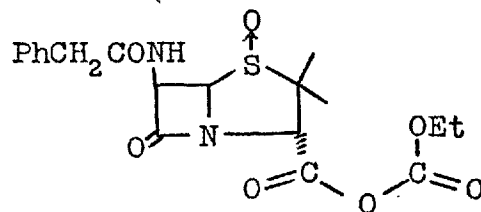
Treatment of benzylpenicillin β -sulphoxide acid with thionyl chloride in pyridine,⁶⁷ followed by treatment with diazomethane gave the methyl ester (33) as the sole product. The infrared spectrum of the crude reaction mixture had a weak absorption at 2120cm^{-1} corresponding to the diazoketo-group, but none of this could be isolated

from the reaction mixture. Phosphorus trichloride⁶⁸ was also tried for the preparation of the acid chloride but this led to total destruction of the β -lactam ring.

It was then considered that the mixed ethoxyformic anhydride⁴⁰ (55) should react with diazomethane to give the desired diazoketone.



(54)

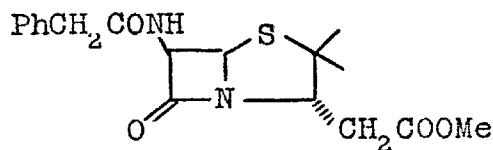


(55)

In the initial experiments, using the literature conditions for the formation of the mixed ethoxyformic anhydride, the reaction with diazomethane led to mixtures of the methyl ester (33) and the diazoketone (54). These were separated by fractional crystallisation. After a series of experiments it was found that treatment of the sulphoxide acid at 0°C with a two mole excess of triethyl-

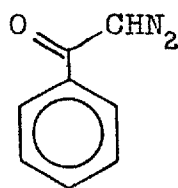
amine for ten minutes followed by a four mole excess of ethyl chloroformate for thirty minutes, and then a large excess of diazomethane gave diazoketone containing only trace quantities of the methyl ester. The structure of the diazoketone was supported by the elemental analysis, by the infrared spectrum which showed the β -lactam (1780cm.^{-1}) and diazoketo (2120cm.^{-1})⁶⁹ absorptions and by the n.m.r. spectrum which confirmed the β -lactam and had a singlet proton at $\tau 4.1$ due to the diazoketo function.

Photolysis of this diazoketone in methanol-benzene gave, as a non-crystalline foam, a sample of the homopenicillin sulphoxide methyl ester (56). This was characterised by only its infrared and n.m.r. spectra. Further experiments along these lines were not undertaken since Russian workers⁷⁰ had prepared the homopenicillinic acid by a similar route and had shown that it had negligible biological activity.

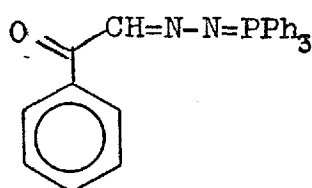


(56)

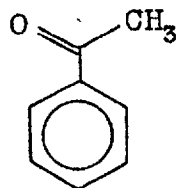
As the known methods of reduction of diazoketones involve the use of strong mineral acid solutions⁷¹, and the penicillins are known to be unstable towards such conditions, milder methods were sought. In order to investigate the reactions of the diazoketo group the model compound diazoacetophenone (57) was prepared from benzoyl chloride and diazomethane⁷² and this used for study.



(57)



(58)



(59)

A number of reagents and conditions were investigated and these are summarised in Table 5. As can be seen only those reagents involving the use of strong mineral acid solutions gave appreciable reduction to acetophenone. One experiment was carried out on the penicillin derived diazoketone using the mildest conditions which were successful for the model compound ($\text{CrCl}_2/\text{HCl}(15\%)$) but this led to total destruction of the β -lactam ring.

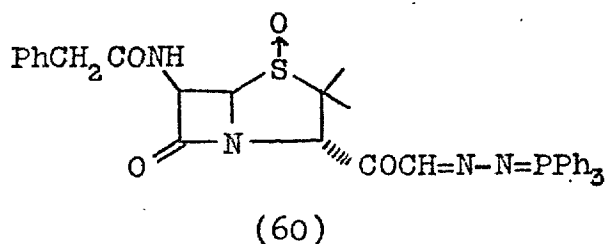
Bestmann^{73,74} had reported that treatment of a

<u>Reagent</u>	<u>Reaction time</u>	<u>Result</u>
Zn/AcOH/PhH	30min.	No acetophenone
Zn/AcOH/EtOAc	30min.	No acetophenone
Zn/AcOH/H ₂ O	30min.	No acetophenone
AcOH	30min.	No Reaction
CrCl ₂ /HCl (30%)	15min.	50% acetophenone
Cr(OAc) ₂ /AcOH	60min.	No reaction
HCl (30%) aq.	5min.	<i>w</i> -Chloro- acetophenone
SnCl ₂ /HCl	60min.	"
CrCl ₂ /HCl (1%)	60min.	"
CrCl ₂ /HCl (5%)	60min.	"
HCl/Et ₂ O	5min.	"
CrCl ₂ /HCl (20%)	15min.	Acetophenone
CrCl ₂ /HCl (15%)	15min.	Acetophenone
CrCl ₂ /HCl (10%)	15min.	<i>w</i> -Chloro- acetophenone.

Table 5. Attempted reductions of Diazoacetophenone.
(All reactions carried out at room temperature with stirring. The reactions with chromous chloride were in an atmosphere of dry nitrogen)

diazoketone with triphenylphosphine gave a "phosphazine" and that this could be reduced under mild basic conditions to the methyl ketone. Reaction of diazoacetophenone with triphenylphosphine in ether led to the formation of the phosphazine (58). This, when treated first with methanol-water then with 0.1N sodium hydroxide, gave acetophenone (59) in reasonable yield.

The benzylpenicillin diazoketone sulphoxide gave, when treated with triphenylphosphine, the phosphazine (60) but this decomposed when treated as above.



At this stage these investigations were discontinued in favour of a study of the rearrangements of benzylpenicillin β -sulphoxide trichloroethyl ester.

(iv) Benzylpenicillin β -sulphoxide trichloroethyl
Ester in Acetic Anhydride *

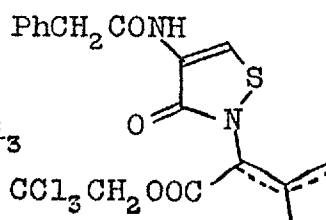
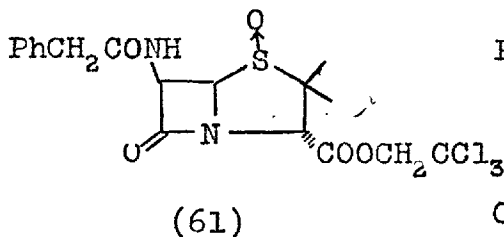
It was hoped that a further rearrangement of the 2-acetoxymethylpenam compound (cf. pg. 52) could lead to the cephalosporin having the acetoxymethyl moiety at position 3. The trichloroethyl ester was selected since this is more enolisable than the t-butylamide, and hence may ring expand more readily, and also because it was known⁷⁵ that it could be readily removed from a cephalosporin compound were it to be made. The rearrangement of benzylpenicillin β -sulphoxide trichloroethyl ester with acetic anhydride was investigated to obtain the desired 2-acetoxymethylpenam trichloroethyl ester.[≠]

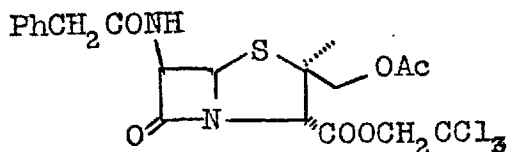
When the benzylpenicillin β -sulphoxide tce. (61) was refluxed in toluene-acetic anhydride until the disappearance of starting material (25hrs.) a dark tarry oil was obtained after the removal of solvents. Purification of this reaction mixture by column chromatography gave firstly the isothiazolone (62), which had elemental analysis

* See footnote pg. 50

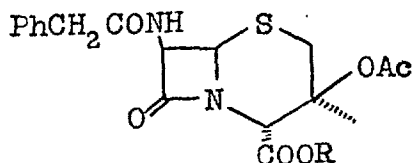
≠ Trichloroethyl ester abbreviated to tce.

corresponding to this molecular formula and similar spectral properties to the t-butylamide analogue. The subsequent fractions contained a mixture of the 2 β -actoxymethylpenam tce. (63) and the 3 β -acetoxycephame tce. (64), the proportion of the latter being given as ca. 10% by n.m.r. spectroscopy. These materials were separated by prep. t.l.c. the less polar material being the penam (63) and the more polar the cepham (64). The structure of the cepham was inferred from the spectral properties, these being very similar to those of the t-butylamide analogue, and confirmed by conversion into the t-butylamide. The 3 β -acetoxycephame tce. was treated with zinc in acetic acid-water to yield the acid (65), which was not isolated but treated directly with triethylamine, ethyl chloroformate and t-butylamine to give the 3 β -acetoxycephame t-butylamide (50), identical with that produced by rearrangement of the benzylpenicillin β -sulphoxide t-butylamide.





(63)

(64), R = $-\text{CH}_2\text{CCl}_3$ (65), R = $-\text{H}$

In contrast to the t-butylamide series the 2 β -acetoxy-methylpenam tce. (63) was the major product and was crystalline whereas the 3 β -acetoxycepham tce. was a non-crystalline foam.

When acetic anhydride was used as reagent and solvent the reaction was very much more rapid, being complete in ca. 15min., but purification was more difficult presumably due to the presence of decomposition products. The n.m.r. spectrum of the mixed acetoxy fraction isolated from this reaction indicated approximately 20% of the unwanted 3 β -acetoxycepham tce. If sodium acetate was added to the reaction in neat acetic anhydride the only isolable product was the conjugated isothiazolone (66), which could be crystallised directly from the reaction mixture.

The structure of the conjugated isothiazolone (66) was supported by the elemental analysis and the spectroscopic similarities between this and the non-conjugated isomer (62), the differences being consistent with the different double bond positions. The relationship between the two isothiazolones (66) and (62) was demonstrated by treating the non-conjugated isomer with triethylamine which isomerised it to the conjugated isomer (62).

After a study of the rearrangement reaction (some values of the effect of concentration, time, etc. are shown in Table 6.) it was found that using a 3% w/v solution of the starting β -sulphoxide in 30% v/v acetic anhydride in toluene at reflux for 5hrs. were the best conditions for the reaction. After removal of the toluene and acetic anhydride the unreacted starting material was removed by trituration with ether and the products isolated by column chromatography. The 2-acetoxymethylpenam isolated in this way contained less than 5% of the 3-acetoxycepham isomer (as estimated by n.m.r. spectroscopy) and was used for the subsequent reactions without further purification.

<u>Penicillin.</u>	<u>Time.</u>	<u>Acetic</u> <u>Anhydride.</u>	<u>Toluene.</u>	<u>Recovered</u> <u>S. Mat.</u>	<u>β-Lactam</u> <u>Prod.</u>	<u>Isothia-</u> <u>zolones.</u>
(g.)	(hrs.)	(ml.)	(ml.)	(g.)	(g.)	(g.)
20	2.5	250	1,500	16	2.0	0.9
20	4	250	1,500	15.2	2.4	1.0
20	5	350	1,000	14.5	3.5	1.2
20	5	250	650	13	3.6	1.8
*20	5	150	500	12	4.5	1.3

Table 6. The rearrangements of the benzylpenicillin-1 β -sulphoxide trichloroethyl ester, in acetic anhydride-toluene.

*Repetitions of these conditions were reproducible to $\pm 10\%$.

(v) Second Oxidations

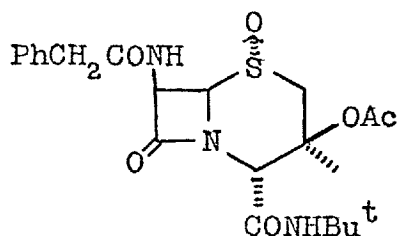
(a) t-Butylamide Series

Oxidation of the 3 β -acetoxycepham t-butylamide (50) with sodium metaperiodate gave a single sulphoxide formulated as the 3 β -acetoxycepham-1 α -sulphoxide t-butylamide (67). The elemental analysis and mass spectrum substantiated the molecular formula and the infrared and n.m.r. spectra confirmed the presence of the β -lactam and acetate functions [1780 and 1740 cm.^{-1} respectively and τ 5.05 (1H d, $J=4\text{Hz.}$), τ 4.71 (1H dd, $J=4, 8\text{Hz.}$) and τ 8.22 (3H s) respectively].

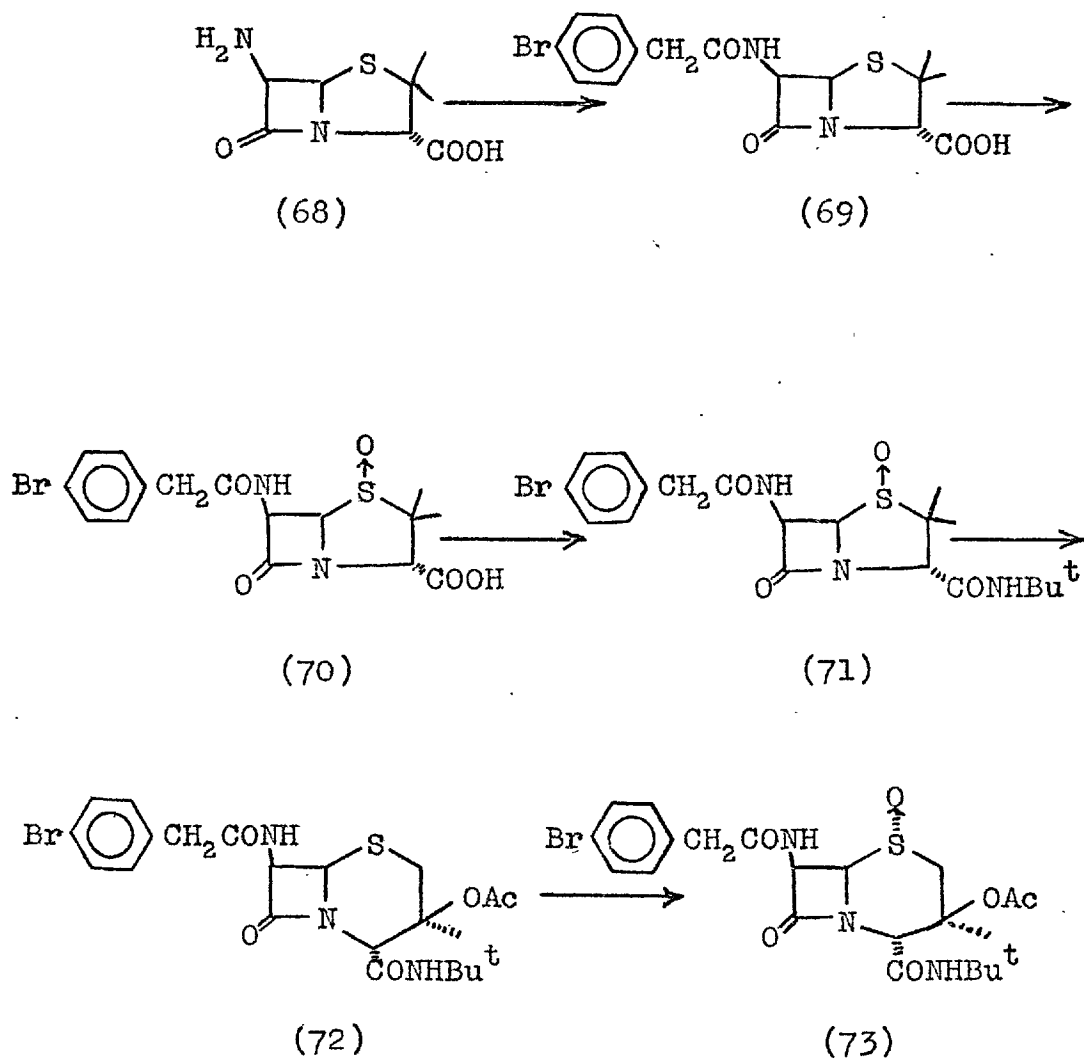
In addition the shift induced in the proton at position 4 of the cepham nucleus on the introduction of this sulphoxide bond was +0.31p.p.m. which is comparable with the shift of +0.51p.p.m. between the penam sulphide and its α -sulphoxide; the shift between the penam sulphide and its β -sulphoxide is to lower field. An inspection of models indicated that the spatial orientations of the 3-H of the penam and the 4-H of the cepham relative to the sulphur atom were approximately the same. Thus the anisotropy of the sulphoxide bond would have a similar effect in both molecules and the above comparability of chemical shift differences was taken to indicate that the sulphoxide bond of the 3-acetoxycepham t-butylamide

sulphoxide was in the α -orientation. The configuration of the acetoxy function was inferred from a Nuclear Overhauser Effect study in which a 5-6% increase in the integral intensity of the 4-H on irradiation of the 3-methyl group in either the sulphide (50) or the sulphoxide (65). As a small coupling of ca. 1Hz. was observed between the 4-H and the higher field proton at position 2, the six-membered ring must have a conformation such that there is a planar zig-zag arrangement between these protons.⁷⁶ Such a conformation would place the 4-H relatively close to a 3β -methyl group and further from a 3α -methyl group. The observed low Nuclear Overhauser Effect is consistent with the methyl group having the 3α -orientation, hence the acetoxy function must have the 3β -orientation.

In order to confirm these proposed stereochemistries the *p*-bromobenzyl analogue of the above α -sulphoxide (73) was synthesised by the reactions shown in Scheme 4. and the crystal structure determined.^{65,77}



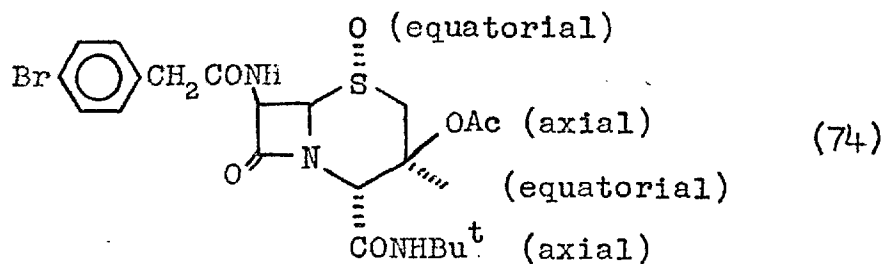
(67)



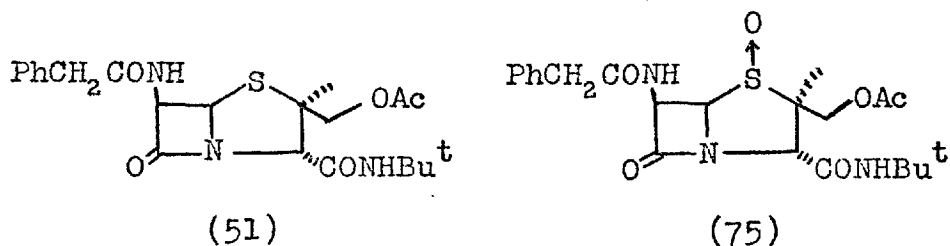
Scheme 4.

p-Bromobenzylpenicillin (69) was prepared by the reaction of 6-aminopenicillanic acid (68) with p-bromophenylacetyl chloride⁷⁸ and this oxidised to its β -sulphoxide (70). After converting to the t-butylamide (71) the penam was rearranged to the cepham (72) using the optimum conditions for the rearrangement. The cepham was oxidised with sodium metaperiodate to the (-)-3 β -acetoxy-4 α -t-butylcarbamoyl-3 α -methyl-7 β -(p-bromophenyl)acetamido-cepham-1 α -oxide (73). Crystals of this material were obtained from dichloromethane and the crystal structure determined⁷⁷ from 3-dimensional X-ray diffraction data. These crystals had the unit cell data:-
 $C_{22}H_{28}O_6N_3BrS$, $M = 542.4$, space group P1 (No. 1), $a = 9.123$, $b = 12.135$, $c = 12.525\text{\AA}$, $\alpha = 93^\circ 49'$, $\beta = 93^\circ 52'$, $\gamma = 91^\circ 45'$, $U = 1380\text{\AA}^3$, $Z = 2$, $D_m = 1.30 \pm 0.01$ (flotation), $D_c = 1.30 \text{ g.cm.}^{-3}$

The data obtained showed two molecules per unit cell and that these had the stereochemistry as predicted by the n.m.r. results. The structure of the molecule is shown schematically in (74).



Oxidation of the 2 β -acetoxymethylpenam t-butylamide (51) with sodium metaperiodate gave the β -sulphoxide of this (75). As stated previously the penam could not be freed from the last traces of the 3 β -acetoxyccepham isomer (50) at the sulphide stage, however the cepham- α -oxide (67) was very much more polar than the penam- β -oxide (75) and therefore the mixture was easily separated into the two components by column chromatography.



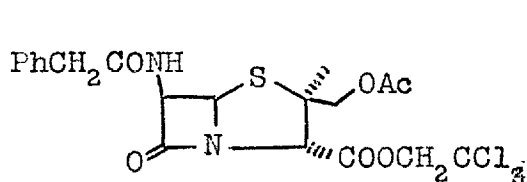
The structure of the 2 β -acetoxymethylpenam-t-butylamide β -sulphoxide was supported by the elemental analysis and mass spectrum; the presence of the β -lactam was shown by both the infrared absorption (1775cm.^{-1}) and the characteristic pattern of the β -lactam protons in the n.m.r. spectrum. The acetate function was indicated by the infrared absorption (1725cm.^{-1}) and the n.m.r. spectrum ($\tau 7.90$ (3H s)).

The reasoning which led to the stereochemical

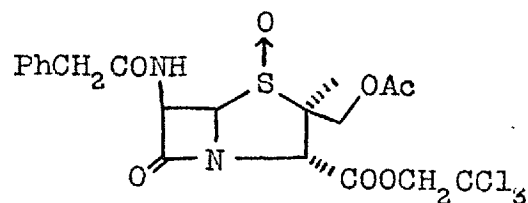
assignments and to the differentiation between the penam and cepham molecules will be considered subsequently.

(b) Trichloroethyl Ester Series

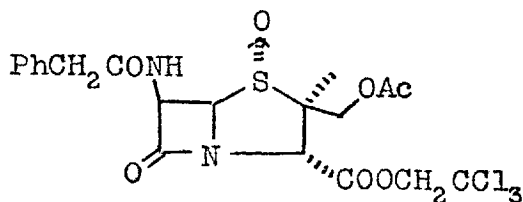
In the trichloroethyl ester series the oxidations of the 2 β -acetoxymethylpenam (63) were entirely analogous to the oxidations of the 2,2-dimethylpenam methyl ester (cf. Chapter 2). Thus treatment with sodium metaperiodate in buffered aqueous dioxan gave a single sulphoxide isomer which was assigned as the 1 β -sulphoxide (76).



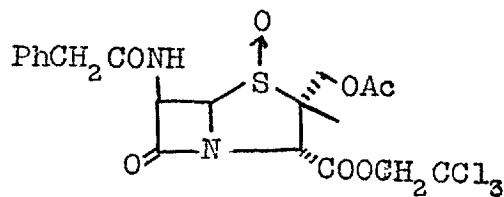
(63)



(76)



(77)



(78)

This same compound (76) was formed when m-chloro-perbenzoic acid was used as oxidant, but in this case the reaction was much more rapid and a higher yield of the sulphoxide was obtained.

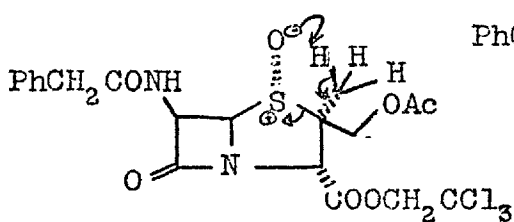
The use of iodobenzene dichloride in pyridine-water at -40°C gave a mixture of the two isomeric sulphoxides (76) and (77), the new sulphoxide (77) being assigned as the α -sulphoxide.

The structures of both these compounds were supported by their elemental analyses; their infrared absorptions ((76) at 1775 and 1720cm.^{-1} , (77) at 1780 and 1740cm.^{-1}) indicated the presence of the β -lactam and acetate functions, these being confirmed by the characteristic absorptions in the n.m.r. spectra (Table 7.).

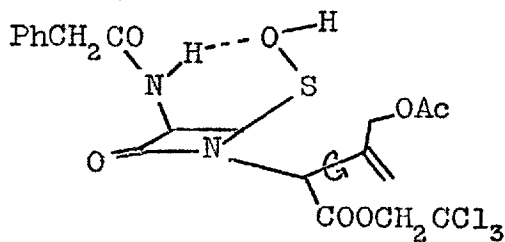
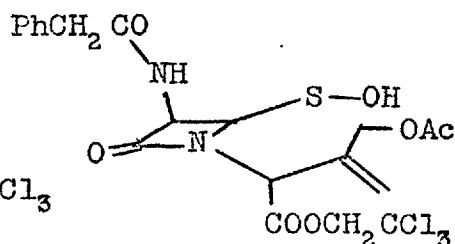
t-Butyl hypochlorite in pyridine-water at -40°C ⁷⁹ was also found to give the mixture of sulphoxides, but with this the proportion of the α -sulphoxide was greater than with the iodobenzene dichloride.

Further evidence was obtained here for the mechanism of isomerisation of an α -sulphoxide to a β -sulphoxide. In the 2 β -acetoxymethylpenam- α -sulphoxide tce. (77)

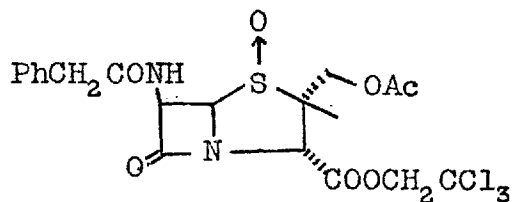
there is available the methyl group cis to the sulphoxide oxygen, but in this molecule the 2 β -methyl group is labelled by the acetoxy function. Thus a mechanism as previously described of electrocyclic cis ring opening, formation of the hydrogen bonded stabilised form of the sulphenic acid (79), rotation about the C2-C3 bond and cis ring closure should lead to inversion of both the sulphoxide and 2-acetoxymethyl functions.



(77)



(79)



(78)

When the 2 β -acetoxymethylpenam-1 α -sulphoxide tce. (77) was heated at reflux in toluene it isomerised rapidly to a β -sulphoxide which proved to have the inverted configuration at C2 (78).

(vi) The Stereochemistries of the Penam Products

As the conclusions reached in Chapter 2. for the 2,2-dimethyl penicillins and their sulphoxides are required for a consideration of the stereochemistries of the 2-acetoxymethyl penicillins they are summarised here:-

(A) The pseudo syn-axial effect in the β -sulphoxides results in deshielding of the protons at positions 3 and 6 of the penicillin nucleus relative to the sulphide.

(B) The trans-orientated lone pair on the sulphur atom in the α -sulphoxide causes a large upfield shift in the proton at position 5 relative to the sulphide.

(C) In the β -sulphoxide the side chain amide proton appears at lower field than in the sulphide, this being due to hydrogen bonding.

(D) The Nuclear Overhauser Effects showed that the low field methyl signal could be assigned to the 2 β -methyl protons and the high field signal to the 2 α -methyl protons.

The origins of the acetoxymethylpenam derivatives are summarised in Table 7., and the n.m.r. spectral data given in Table 8.

The sulphoxide configuration in the compounds (75), (76), (77) and (78) were deduced by application of the conclusions (A), (B) and (C) above to the chemical shift differences of the various protons between these sulphoxides and the corresponding sulphides. These differences are shown in Table 9.

As can be seen from Table 9. the 3-H and 6-H are deshielded in the sulphoxides (75), (76) and (78) but in (77) the 6-H is shielded. Also in compounds (75), (76) and (78) the side chain amide proton is shifted to lower field indicating that it is hydrogen bonded to the sulphoxide oxygen atom. These results were taken to indicate that the sulphoxides (75), (76) and (78) were all β -sulphoxides, and that the sulphoxide (77) was the α -sulphoxide. The large upfield shift of the 5-H in the sulphoxide (77) relative to the sulphide (63) was also consistent with this having the α -orientation. Further the very close correspondance of these shift differences for the 2-acetoxymethyl penicillins and the 2,2-dimethyl penicillins (cf. Table 3.) gave added confirmation to these

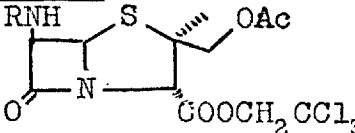
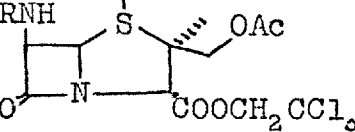
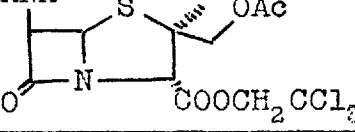
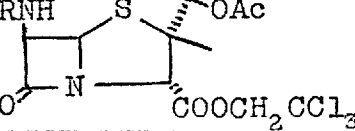
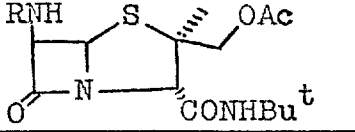
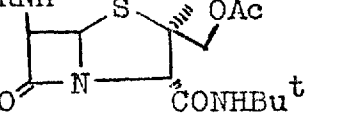
COMPOUND (R = PhCH ₂ CO-)	ORIGIN	PAGE REF.
(63) 	By rearrangement of benzylpenicillin-1β-sulphoxide trichloroethyl ester in acetic anhydride-toluene.	60-61
(76) 	By oxidation of (63) with metaperiodate, <i>m</i> -chloroperbenzoic acid or the less polar isomer from the oxidation with iodobenzene dichloride.	70-71
(77) 	The more polar isomer from the oxidation of (63) with iodobenzene dichloride.	71
(78) 	By thermal isomerisation of (77)	72-73
(51) 	By rearrangement of benzylpenicillin-1β-sulphoxide <i>t</i> -butylamide in acetic anhydride-toluene.	52
(75) 	By oxidation of (51) with metaperiodate.	69

Table 7.

CMPD.	2 β -Me	2 α -Me	3-H	5-H ^a	6-H ^b	N-H	-CH ₂ Ph	-Ph	Acetate		Ester -CH ₂ -	Amide -CMe ₂
									-CH ₃	-CH ₂ -		
(61)	8.27	8.72	5.18	5.05 J=4	4.00 J=4, 10	2.92 J=10	6.43	2.72	-	-	5.02 5.40 ^c	-
(63)	-	8.48	5.25	4.41 J=4	4.32 J=4, 11	3.20 J=11	6.38	2.70	7.98	5.67 ^d 6.32	5.22	-
(76)	-	8.73	5.18	5.05 J=4	3.99 J=4, 9	2.92 J=9	6.48	2.70	7.94	5.26 ^d 5.52	5.30	-
(77)	-	8.62	5.25	5.21 J=4	4.82 J=4, 11	3.20 brd.	6.42	2.70	8.00	5.40	5.22	-
(78)	8.30	-	5.20	4.98 J=4	3.94 J=4, 9	2.92 J=9	6.44	2.70	7.98	5.43 ^d 6.02	5.16 ^c 5.35	-
(51)	-	8.45	5.78	4.49 J=4	4.28 J=4, 9	3.37 J=9	6.38	2.66	8.02	5.70 ^d 6.08	-	8.65
(75)	-	8.77	5.61	5.05 J=5	3.95 J=5, 10	2.80 brd.	6.41	2.70	7.90	5.28 ^d 5.40	-	8.65

Table 8. N.m.r. spectral parameters of the 'Acetoxypenicillins'. As τ values in CDCl₃ with T.M.S. as internal reference.

a. appears as a doublet, b. appears as a double doublet, c. and d. AB quartets ($J_c=12$, $J_d=12-12.5$). J values in Hz.

	(76)-(63)	(77)-(63)	(78)-(63) [*]	(75)-(51)
2 β -CH ₂	-0.60	+0.60	-	-0.55
2 α -Me	+0.25	-0.14	-	+0.32
3-H	-0.07	0.00	-0.05	-0.17
5-H	+0.64	+0.80	+0.57	+0.56
6-H	-0.33	+0.50	-0.38	-0.33
N-H	-0.28	0.00	-0.28	-0.57

Table 9. Chemical shift differences between the sulphides and sulphoxides for the 'acetoxy-penicillins'. Positive values indicate upfield shifts (i.e. shielding) and negative values downfield shifts. All values in p.p.m.

^{*}As this is a comparison of the 2 α -acetoxy-methyl-1 β -sulphoxide to the 2 β -acetoxymethyl-sulphide figures for the protons on the carbon atoms attached to C2 can not be given.

stereochemistries and indicated that the sulphoxide oxygen atom in the two series of compounds had the same stereochemical arrangement relative to the ring system.

Also, as can be seen from Table 9., the methyl signals in the compounds (75) and (76) are shielded relative to their respective sulphides (51) and (63). The Nuclear Overhauser Effects in the 2,2-dimethyl penicillins had shown that in these the 2α -methyl group was shielded relative to the sulphide. Hence in the 2-acetoxymethylpenicillins (51), (63), (75) and (76) the methyl group must be in the 2α -orientation, and therefore the acetoxymethyl function must have the 2β -orientation. The deshielding of the acetoxymethylene protons in these compounds was consistent with the 2β -orientation.

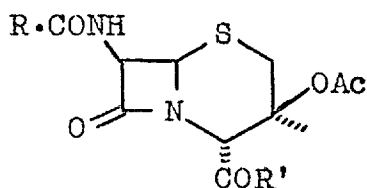
Further evidence for this assignment of stereochemistry came from a consideration of the positions of the methyl signals in the n.m.r. spectra of the two isomeric 2-acetoxymethylpenam- β -sulphoxides (76) and (78) and the 2,2-dimethylpenam- β -sulphoxide (61) in the trichloroethyl ester series. The introduction of the acetoxy moiety at C2 would be predicted to have only

a slight effect on the n.m.r. spectrum of the remainder of the molecule. This is borne out by the virtual superimposability of the 3-H, 5-H, 6-H and NH in the n.m.r. spectra of these three compounds (cf. Table 8.). The conclusion (D) above enabled the assignment of the methyl signals of the 2,2-dimethylpenam- β -sulphoxide tce. as the 2 α -methyl group at τ 8.72 and the 2 β -methyl group at τ 8.27. The methyl signal of the compound (76) was at τ 8.73, and that of (78) was at τ 8.30. If the introduction of the acetoxy function has little effect on the resonance positions of the methyl groups then the very close agreement between the positions of the methyl absorptions in the 2,2-dimethylpenicillin and those in the 2-acetoxymethyl compounds leads to the conclusion that the methyl group of (76) must have the 2 α -orientation and that of (78) the β -orientation.

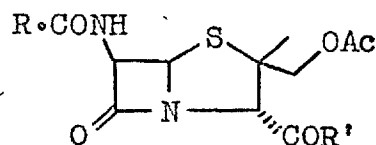
Thus the material produced in the rearrangement of the penicillin- β -sulphoxides in acetic anhydride had the 2-acetoxymethyl function on the β -face of the molecule, and thermal isomerisation of the 1 α -sulphoxide of this gave the isomeric 2 α -acetoxymethylpenam derivative.

(vii) The differentiation between 3-Acetoxycephams
and 2-Acetoxyethylpenams

The 3-acetoxycephams (general formula 80) and the 2-acetoxyethylpenams (general formula 81) from the rearrangement of penicillin sulphoxides in toluene-acetic anhydride are isomeric and contain very similar structural features.



(80)



(81)

The infrared spectra of both series of compounds show the side chain amide group (ca. 3400, 1680 and 1520 cm.^{-1}), the strained β -lactam (ca. 1780 cm.^{-1}) and the acetate function (1720 and 1250 cm.^{-1}). Also both series show, in the n.m.r. spectra, the two β -lactam protons with their characteristic splitting, the proton α -to the ester or amide function, one methyl group, the methyl of the acetate and two methylene protons (in the ring for (80) or α -to the acetate in (81)). In the majority of cases these methylene protons

appear as an AB quartet in both the penam and cepham series.

Due to these close similarities the initial assignment of structures was difficult, however the studies on the various compounds of the two series have revealed certain characteristic differences which can be used for differentiation.

In the solution spectra of the sulphides of the cepham (80) and penam (81) the β -lactam absorption of the former appeared at ca 10cm.^{-1} lower frequency than that of the latter. This probably reflects the greater strain inherent in the β -lactam fused to the five-membered thiazolidine ring than fused to a six-membered ring.⁴¹

In the n.m.r. spectra of the sulphides the AB quartet of the cepham (80) ring protons appeared at slightly higher field than that of the acetoxymethylene protons in the penam (81). Also in all the derivatives studied in which these protons appear as an AB quartet the coupling constant in the cephams (80) is 14-15Hz. whereas that in the penams (81) is 12-13Hz. Additionally

in the cepham derivatives there was a long range coupling of ca. 1Hz. between the proton α -to the carboxyl function and the 2β -ring proton. This reflects the geometry of the six-membered ring where there must be a planar zig-zag arrangement between these protons.⁷⁶

Possibly a clearer differentiation comes from a consideration of the rotations of the compounds. In all the 2-acetoxymethylpenams studied the rotation ($[\alpha]_D$) was greater than $+130^\circ$ whereas in the cephams they were less than $+20^\circ$.

(viii) Mechanistic Considerations

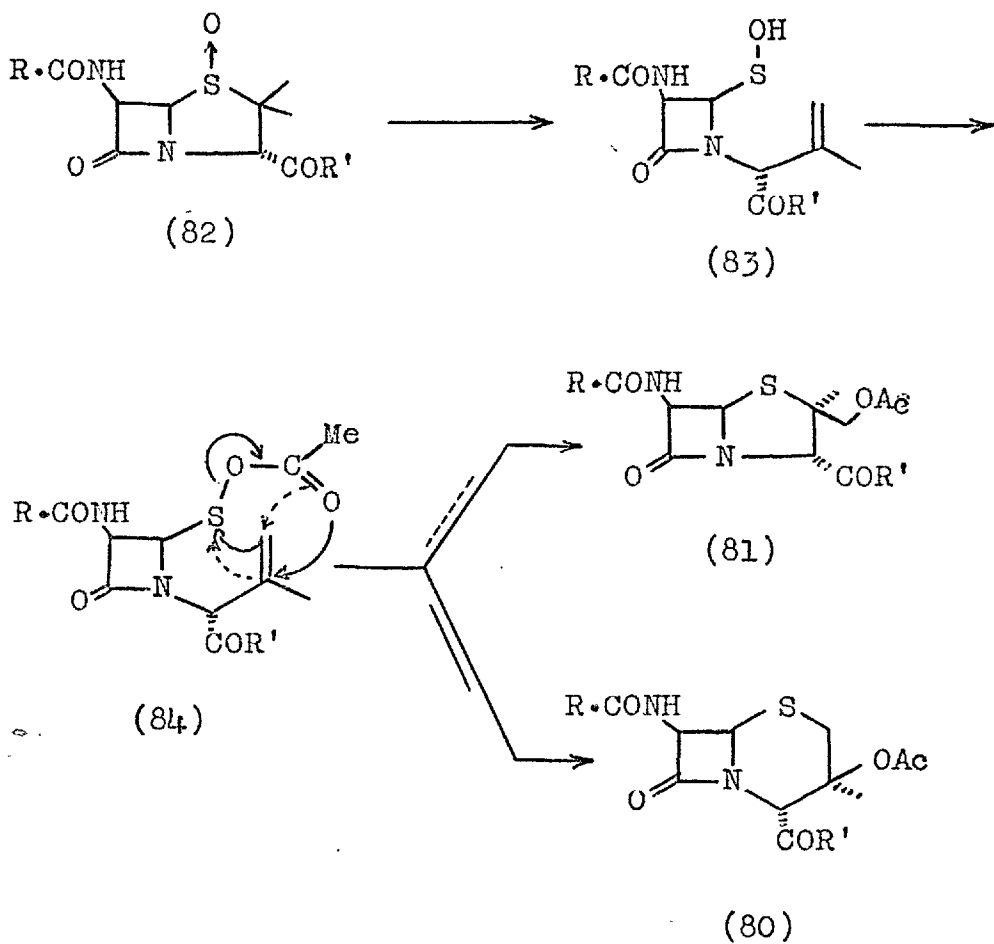
The evidence accumulated in this study indicated that the 3-acetoxycepham (80) and the 2-acetoxymethylpenam produced in the rearrangement of a penicillin sulphoxide in toluene-acetic anhydride were such that the acetoxy group was substituted on the β -face of the molecule only. Therefore any mechanism must account for this observed stereospecificity.

The initial stage in the reaction must be the thermal opening of the sulphoxide (general formula 82) to the sulphenic acid (general formula 83) by a cis-proton

abstraction from the 2β -methyl group by the 1β -sulphoxide oxygen. This is directly analogous to the first stage in the thermal isomerisation of a penicillin- α -sulphoxide to its β -isomer. This sulphenic acid must then form a second intermediate since it itself could not account for the stereospecificity due to the rotation possible about the C2-C3 bond. Initially it was thought that the sulphenyl acetate (84) could form, the acetate of which could then attack the double bond with accompanying ring closure to give the two acetoxy compounds with the acetate functions on the β -face of the molecule (Scheme 5.). The steric control in this sequence would arise from hydrogen bonding between the side chain amide proton and the oxygen attached to sulphur to hold the acetate function on the β -face of the molecule.

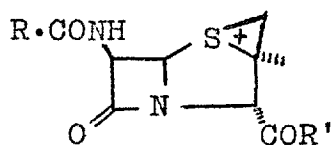
Evidence against this mechanism was found from experiments with O^{18} labelled penicillin- β -sulphoxide.⁸⁰ Iodosobenzene labelled with 3% O^{18} was prepared and used to oxidise the benzylpenicillin-*t*-butylamide to its β -sulphoxide and this labelled sulphoxide rearranged in toluene-acetic anhydride. The 3-acetoxycepham isolated from this reaction was unlabelled, moreover a mass

spectral analysis of the reaction mixture after 2hrs. showed that the 3-acetoxycepham product contained no label whereas the unreacted starting sulfoxide retained its label.

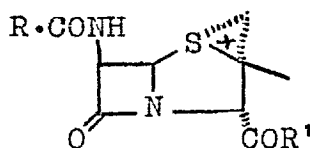


Scheme 5.

Morin et al.²⁶ explained the stereospecificity of the reaction in terms of the formation of a β -episulphonium ion (85) from the sulphenyl acetate (84), this being opened by attack of acetate anion to give the observed products. No rationale for the specific formation of the β -episulphonium ion was given. If, as seems probable from the previously discussed studies, the acetate of the sulphenyl acetate is held on the β -face of the molecule by hydrogen bonding then a cis elimination of acetate ion must be invoked to account for the formation of a β -episulphonium ion. It seemed to us that this explanation was unsatisfactory as rotation about C2-C3 would give a conformation where the more favourable trans elimination of acetate could occur to give the α -episulphonium ion (86)



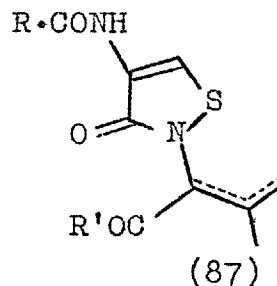
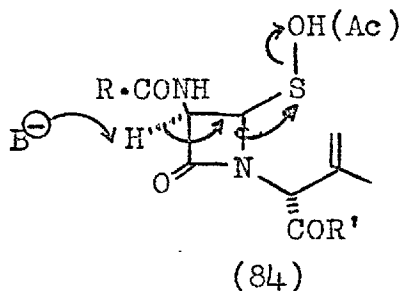
(85)



(86)

Hence some other explanation had to be sought and if the episulphonium ion is an intermediate then there are two possibilities. Firstly, that both the episulphonium ions are formed but that only the β -ion gives the acetoxy-products; secondly, that there is some reason which directs that only the β -ion is formed.

The principal side product from the rearrangement reactions was the isothiazolone (general formula 87) and in the first instance it was thought that this could arise from the sulphenyl acetate (84) by the mechanism depicted below.



However an inspection of models of the two isomeric episulphonium ions showed that the configuration of the α -ion (86) was such that this could collapse to the isothiazolone (87) in preference to the β -isomer. In the α -episulphonium ion attack of the nitrogen electrons

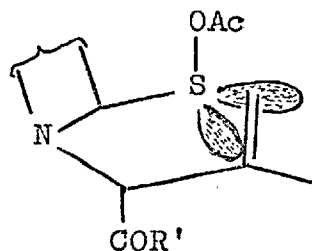
on the sulphur atom would be in a trans sense, whereas in the β -episulphonium ion it would be in a cis sense and the former would be the more favourable process. Thus it is reasonable to assume that if the α -ion is formed under the reaction conditions it collapses rapidly and preferentially to the isothiazolone, leaving the β -isomer to give the acetoxy products.

This alone can not be a full explanation for the reaction mechanism since the proportion of the isothiazolone in the reaction mixture was less than that of the acetoxy compounds whereas the mechanism outlined above would predict a greater proportion of the isothiazolone because of the apparent favourability of the processes leading to it.

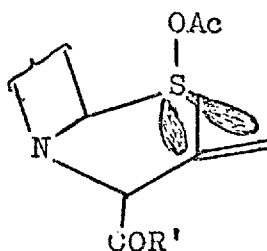
A study of a model of the intermediate sulphenyl acetate (84) revealed that the relative orientations of the π -electron cloud of the double bond and the d-orbitals of the sulphur atom could provide a reason for the preferential formation of the β -episulphonium ion.

When the double bond is in the β -orientation the electrons of the π -bond and the sulphur d-orbitals can be represented by (88), and when in the α -orientation

by (89).



(88)

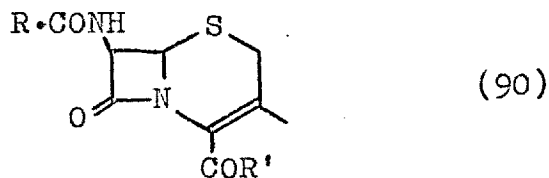


(89)

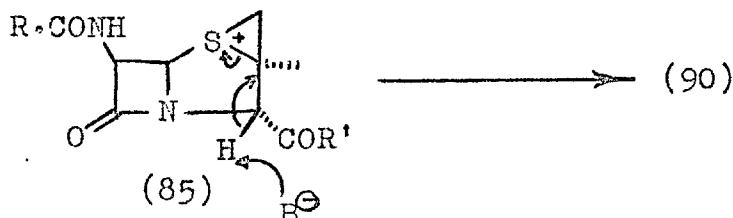
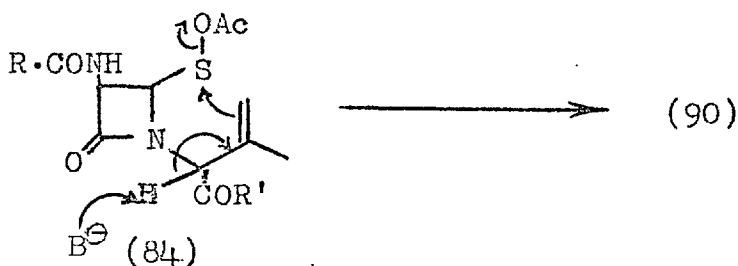
In the configuration as shown in (88) the double bond is orthogonal to the sulphur d-orbitals which might permit a relatively close approach of the sulphur atom to the double bond and hence allow an interaction to give the β -episulphonium ion. In the alternative configuration (89) the filled sulphur d-orbitals are directly opposed to the π -electron cloud of the double bond and this arrangement could result in a repulsion between the functions and so reduce the probability of

interaction. Indeed this repulsive force might even be sufficiently great to exclude completely the formation of the α -episulphonium ion, even if at first analysis the trans-elimination process appeared the more favourable.

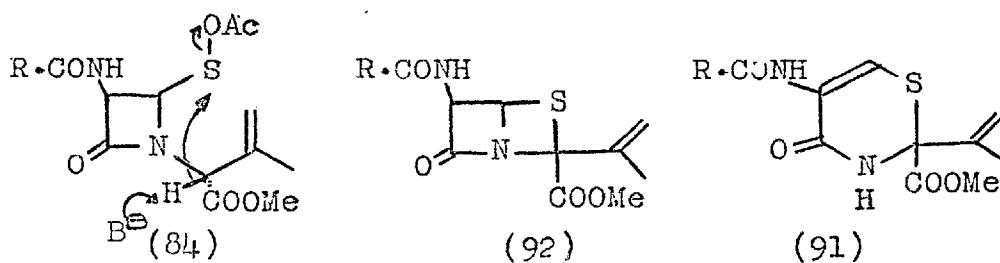
Another product which has been isolated from the catalysed rearrangement of penicillin β -sulphoxides is the deacetoxycephalosporin (general formula 90).²⁷ The



formation of this can be rationalised from the sulphenyl acetate (84) and from the β -episulphonium ion (85) as depicted below.



The Lilly group also reported²⁶ the isolation of small quantities of the compound (91) from the rearrangement of phenoxymethylpenicillin β -sulphoxide in acetic anhydride. They formulated that this arose by loss of the 3-H of the intermediate sulphenyl acetate (84) to give the bicyclic compound (92), which collapsed to the compound (91).

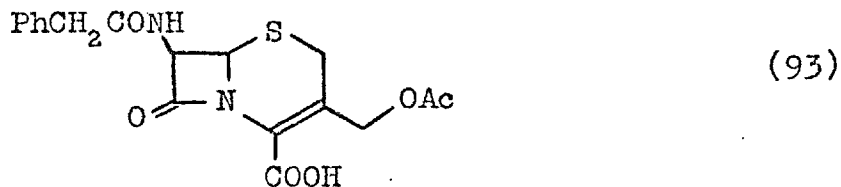


The widely varying proportions of the products obtained from the rearrangements in the different media does not rule out the formation of other intermediates in which the catalyst and/or solvents are directly involved.

(ix) Attempted Ring Expansion of the 2-Acetoxyethylpenam

The 2-acetoxyethylpenam sulphoxide was considered to be a suitable precursor for the synthesis of the Cephalosporin C derivative (93) by a second rearrangement

reaction. As the initial step in the reaction is a stereospecific cis proton abstraction from the methyl group at C2 by the sulphoxide oxygen atom only two of the 2-acetoxymethylpenam sulphoxides are suitable, i.e. the 2 β -acetoxymethylpenam-1 α -sulphoxide (77) and the 2 α -acetoxymethylpenam-1 β -sulphoxide (78), both of which were preparable in the trichloroethyl ester series (cf. pg. 75).



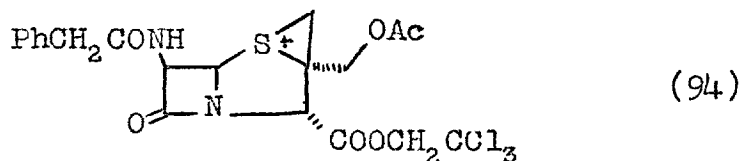
The mode of preparation of the of the sulphoxide (78) confirmed the existence of the sulphenic acid in this series, this being necessary for the reaction.

A number of reactions were carried out on both the sulphoxides (77) and (78) using a variety of catalysts and solvents and the reaction mixture monitored for the cephalosporin nucleus. In no case did the ultraviolet spectrum, n.m.r. spectrum or t.l.c. show any trace of the desired product. In practically every case the

t.l.c. showed that there were no definite products but appeared as one smear from origin to solvent front.

The conditions used are summarised in Table 10.

As stated above the sulphenic acid (79) was known to form in this series, and was presumably formed in these rearrangement reactions. Thus there must have been some factor which prevented the formation of the episulphonium ion (94).

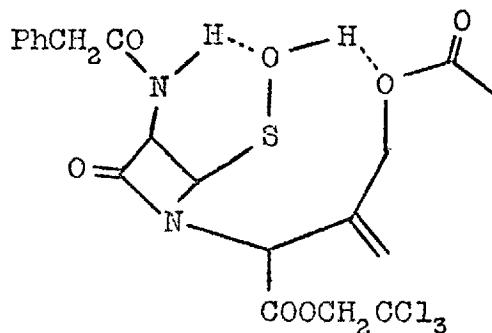


Since the only difference between this series and the unsubstituted penicillins, where the ring expansion can occur in high yields, is the presence of the acetoxy-methyl function it is logical to assume that it is this which is hindering the reaction. It is conceivable that the acetoxy function is being held on the β -face of the molecule by hydrogen bonding to the sulphenic acid function

<u>Starting Material</u>	<u>Solvent</u>	<u>Catalyst</u>	<u>Time (hrs.)</u>
(77)	Dioxan	Pyridine phosphate	2
"	M.I.B.K.	"	2.5
"	"	"	18
"	"	Phosphoric acid	2.5
"	Toluene	Tosyl acid	4
"	Toluene	Acetic anhydride	3
"	-----	Acetic Anhydride -----	0.5
(78)	Dioxan	Pyridine phosphate	2
"	M.I.B.K.	"	2.5
"	"	Phosphoric acid	2.5
"	Toluene	Tosyl acid	3
"	Toluene	Acetic anhydride	3
"	-----	Acetic Anhydride -----	0.5

Table 10. Conditions for the attempted ring expansions of the acetoxypenicillins. (M.I.B.K. = Methyl-isobutyl ketone).

(95). This means that the configuration is analogous to that previously described (89) where the double bond was also in the α -orientation. Thus the molecule might not be in a suitable form to allow the formation of the second intermediate and would react by general decomposition.



(95)

At this stage these investigations were discontinued but the author still feels that the ring expansion of the 2 α -acetoxymethylpenam-1 β -sulphoxide should occur given the correct combination of catalyst, solvent, conditions, 6-side chain and 3- protecting group.

CHAPTER 4

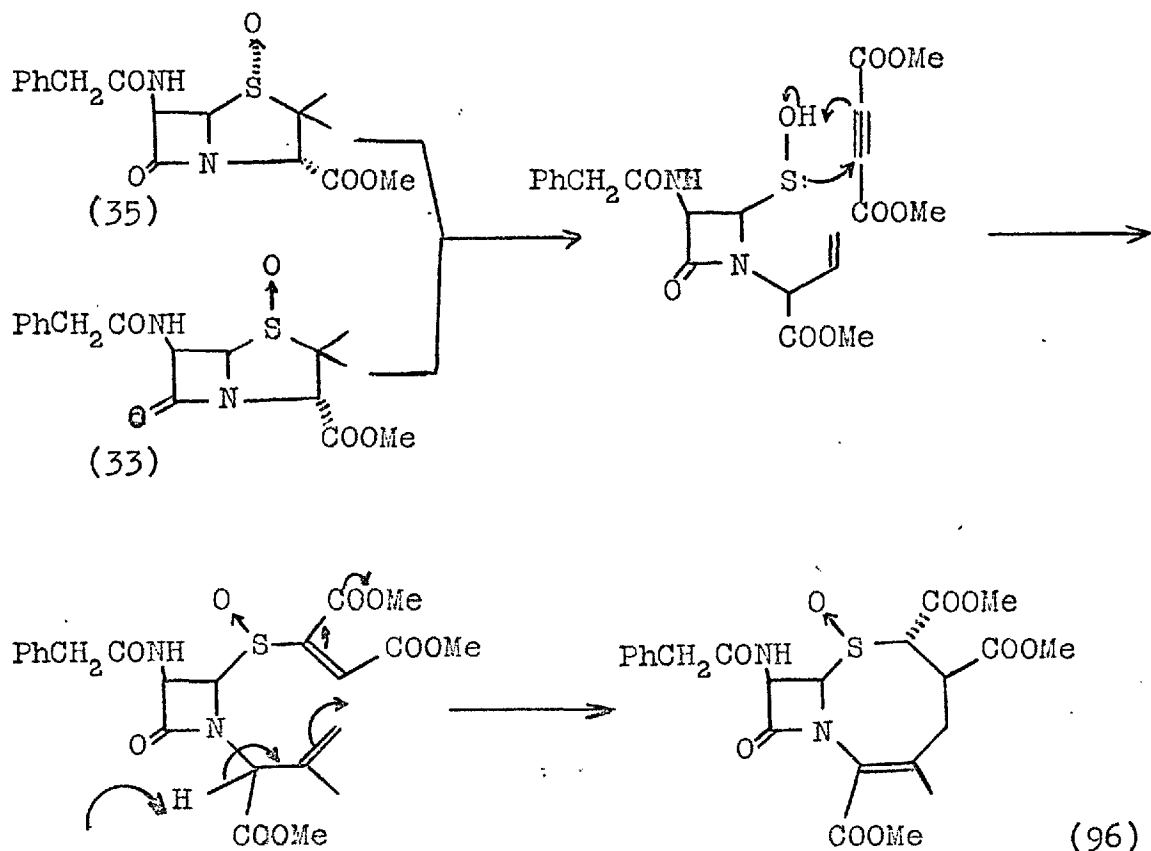
THE SULPHENIC ACID INTERMEDIATE

The Sulphenic Acid Intermediate

The sulphenic acid moiety has been postulated throughout these studies and some evidence for its existence has been presented in the form of the deuterium labelling experiments and the thermal isomerisation of the 2 β -acetoxymethylpenam- α -sulphoxide trichloroethyl ester (77). Because the observed rotation about the C2-C3 bond during these reactions requires a relatively long-lived intermediate more standard trapping experiments⁸¹ have been carried out.⁸² As these are of relevance to the material to be presented in Chapter 5, a brief summary is given here.

(a) Dimethylacetylenedicarboxylate⁸²

Both benzylpenicillin methyl ester α - and β -sulphoxides (35) and (33) reacted with the reagent on refluxing in benzene solution to give mainly one product. The structure of this material was tentatively assigned as (96) on the basis of its spectral properties. This was formulated as arising by the mechanism indicated (Scheme 6.)

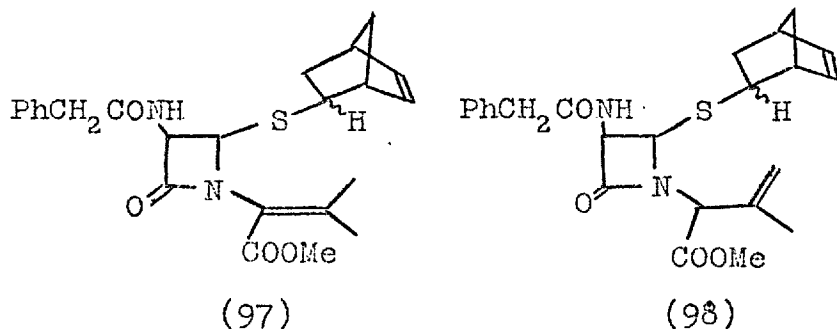


Scheme 6.

(b) Norbornadiene

Again both the α - and β -sulfoxides (35) and (33) reacted to give one major fraction by t.l.c. analysis. The reaction with the α -sulfoxide was complete in 5hrs. whereas the β -isomer required 18hrs. A careful examination of this t.l.c. fraction revealed the presence of two components corresponding to the $\alpha\beta$ -unsaturated ester (97)

and the $\beta\gamma$ -unsaturated ester (98). The latter could not be isolated in a pure state because of its facile isomerisation to the former, however treatment of the reaction mixture with a mild base caused complete isomerisation to the $\alpha\beta$ -unsaturated ester (97) which could be crystallised directly from the reaction mixture.

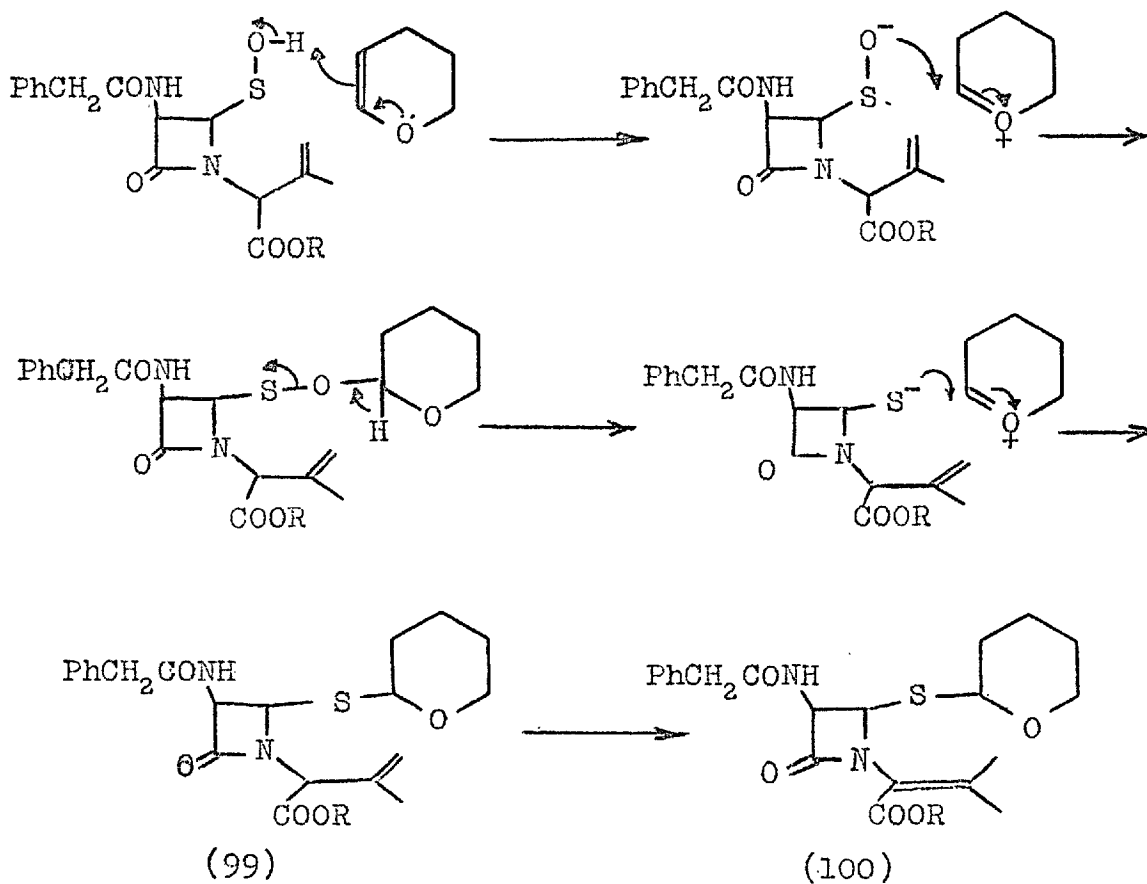


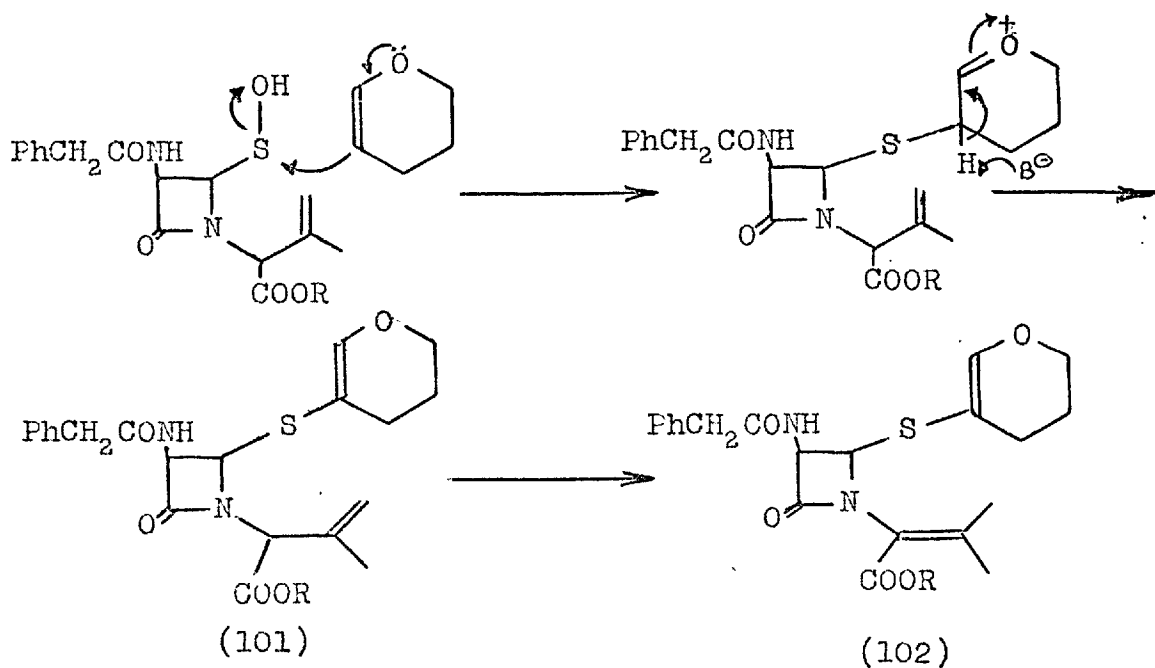
The assignment of these structures was supported by the elemental analysis and the spectral properties⁸² and in addition the presence of the sulphoxide function was confirmed by reduction to a sulphide with phosphorus tribromide in dimethyl formamide.

An analogous reaction was found to occur with the benzylpenicillin-1 β -sulphoxide trichloroethyl ester.

(c) Dihydropyran

Dihydropyran reacted with the β -sulphoxides in the methyl ester (33) and trichloroethyl ester (61) series to give mixtures of $\beta\gamma$ -unsaturated and $\alpha\beta$ -unsaturated adducts. Initially it was thought that these adducts had the structures (99) and (100) arising by the mechanism indicated (Scheme 7.)⁸², but were subsequently reassigned as (101) and (102) (Scheme 8.) on the basis of their spectral properties. Details of this reassignment will be presented in the next chapter.

Scheme 7.

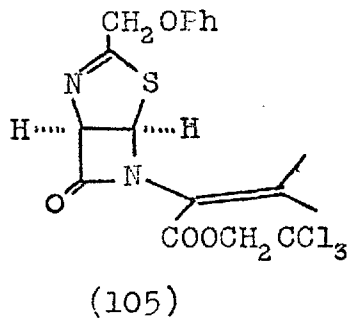
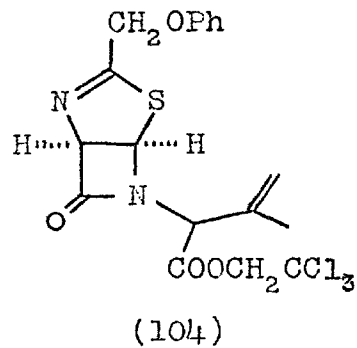
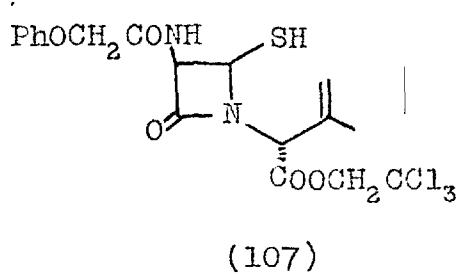
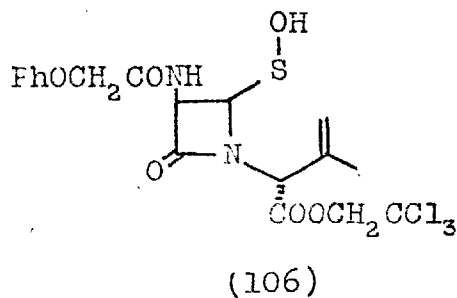
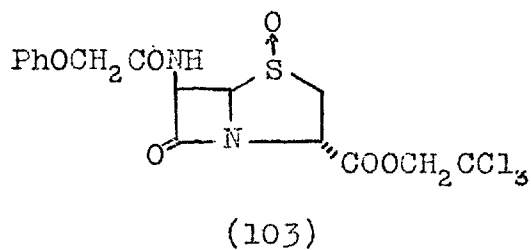


Scheme 8.

(d) Reduction of the Sulphenic Acid⁸³

Cooper and Jose⁸³ have also demonstrated the existence of the sulphenic acid intermediate by a reductive method. Penicillin V sulfoxide trichloroethyl ester (103) was heated at reflux in benzene containing trimethyl phosphite to give two products (104) and (105). The route of formation of (104) was considered to be the initial opening of the sulfoxide to the sulphenic acid (106) which was reduced to the thiol (107) by the trimethyl phosphite.

This thiol then underwent a condensation with the amino-side chain to give the thiazoline ring (104). The compound (105) presumably arose by isomerisation of (104).

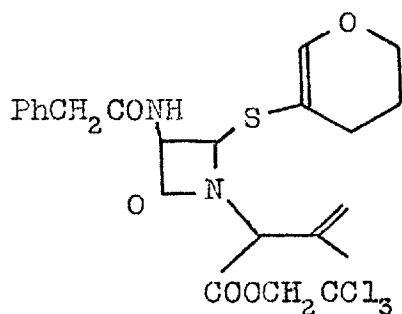


CHAPTER 5

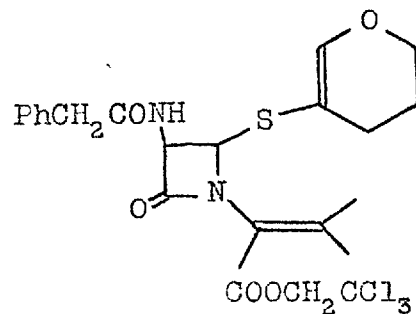
STUDIES ON THE PENICILLIN-DIHYDROPYRAN ADDUCT

Studies on the Penicillin-Dihydropyran Adduct

The benzylpenicillin- β -sulphoxide trichloroethyl ester (61) was heated at reflux in dihydropyran containing a trace of aluminium tribromide for 20hrs. This gave, as indicated by t.l.c. analysis, a product containing mainly the $\beta\gamma$ -unsaturated ester adduct (101) and only trace quantities of the $\alpha\beta$ -unsaturated ester adduct (102).



(101)



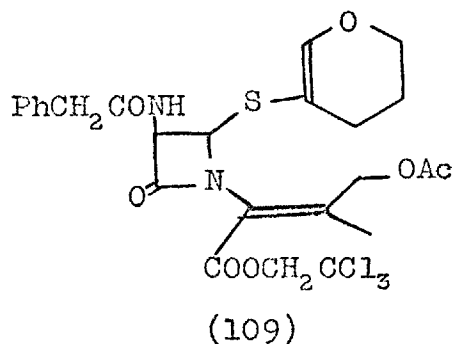
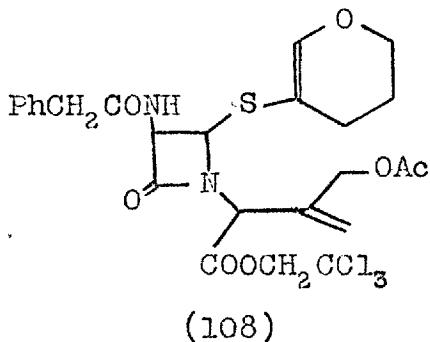
(102)

The isolation of the adducts was greatly facilitated when it was found that the differential solubilities of the materials involved could be used for purification instead of the extensive chromatography previously required.⁸¹ After removal of most of the dihydropyran in vacuo the reaction mixture was poured into petrol and stirred for two hours. This removed the remaining dihydropyran and dihydropyran polymers and left the unreacted starting material and products as an oily syrup.

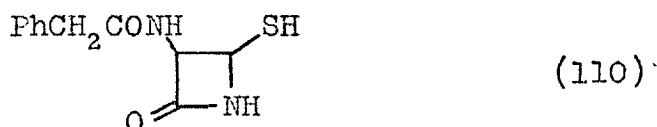
The petrol was removed by decantation and the residue triturated with ether which precipitated the unreacted starting material and left the adduct in solution.

Removal of the ether solvent gave the adduct (101) as a sticky foam which could be isomerised to the $\alpha\beta$ -unsaturated ester adduct (102) either by treatment with triethylamine in ethyl acetate at room temperature or with pyridine in benzene at reflux. The $\alpha\beta$ -unsaturated adduct (102) was crystallised from ether-petrol.

Preliminary trapping experiments were also carried out on the 2-acetoxymethylpenam trichloroethyl ester sulphoxides (76), (77) and (78). Both the α -sulphoxide (77) and the β -sulphoxide (78) reacted with dihydropyran to give the same adducts (108) and (109). These were not fully purified but characterised only by their spectral characteristics, these being very similar to those of the dimethyl penicillin adducts (101) and (102). There was little evidence for reaction of the β -sulphoxide (76) with dihydropyran.



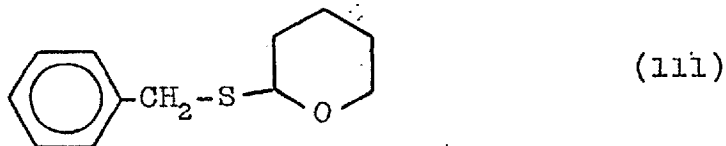
The dimethyl penicillin adducts (101) and (102) were considered to be potentially useful for the synthesis of the parent β -lactam compound, 3(N-phenylacetyl)amino-4-mercaptoazetidin-2-one (110). This material is of importance as the parent for a whole range of new β -lactam antibiotics.



In order to carry out this controlled degradation two problems had to be solved, viz. the removal of the protecting group on the sulphur and the cleavage of the isopropenyl function.

At the outset of these studies the structures assumed for the adducts were (99) and (100), in which easy removal of the tetrahydropyran function was envisaged. However the investigations into the removal of the isopropenyl side chain led to the reassignment of these structures as (101) and (102). Removal of the dihydropyran ring from these was not expected to be as facile as for the tetrahydropyran moiety. Indeed the adducts proved resistant

to the action of acid, trityl fluoroborate and mercuric chloride^{84,91}, reagents which cleaved readily the simple hemithioketal (111)



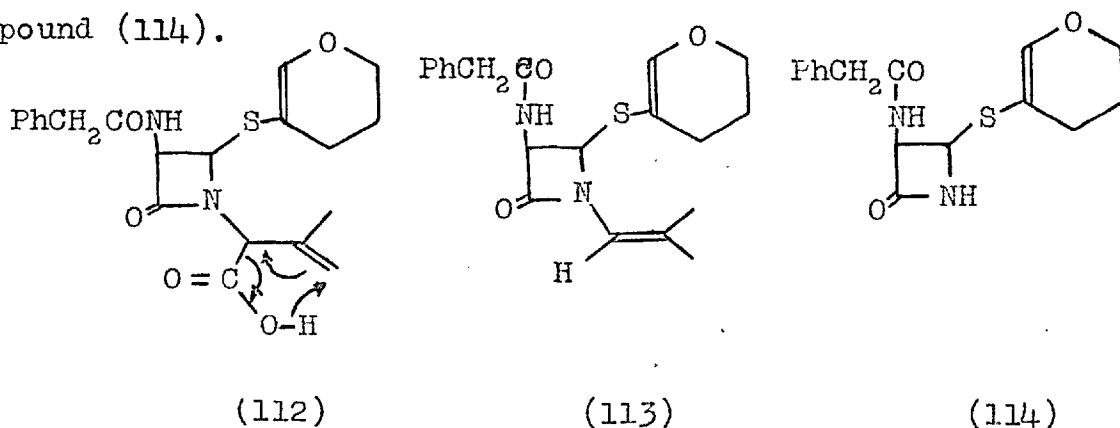
The stability of the penicillin-dihydropyran adducts towards hydrolysis corroborates the reassigned structures.

Approaches to the removal of the isopropenyl side chain

Inspection of the structures of the adducts (101) and (102) shows the presence of the double bond in the isopropenyl side-chain and in (102) this is in conjugation with the ester function. Oxidative methods (e.g. ozone) could theoretically be used to cleave this double bond which could give an intermediate which should collapse with removal of the isopropenyl side-chain. These reagents were ruled out because of the virtual certainty of oxidising the sulphide to the sulphoxide or sulphone.

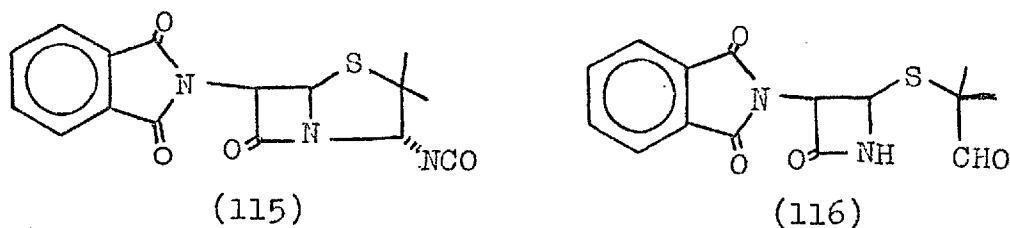
Three alternative routes for the removal of this side chain were considered possible and were investigated.

Firstly it was felt that the acid (112) could thermally decarboxylate to the enamine (113) by the mechanism indicated and that this could then be hydrolysed to the β -lactam compound (114).

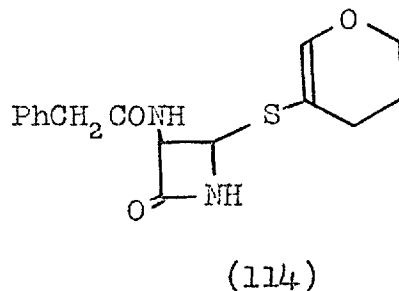
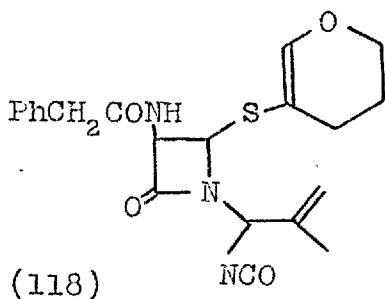
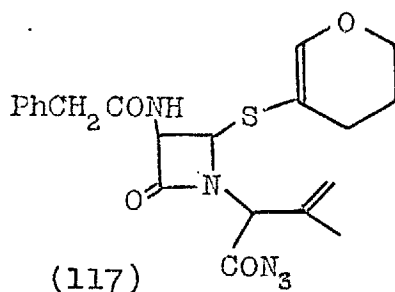
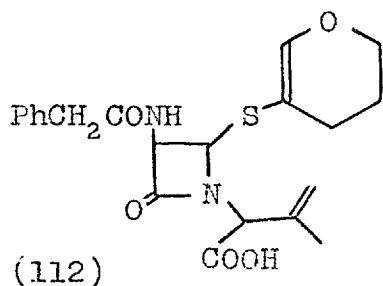


The trichloroethyl ester function was readily removed from the $\beta\gamma$ -unsaturated ester adduct by treatment with zinc and acetic acid in water⁷⁵ to give the acid (112). However, when this was heated at reflux in xylene the β -lactam ring was rapidly destroyed, as evidenced by the disappearance of the infrared band at ca. 1770cm.^{-1} .

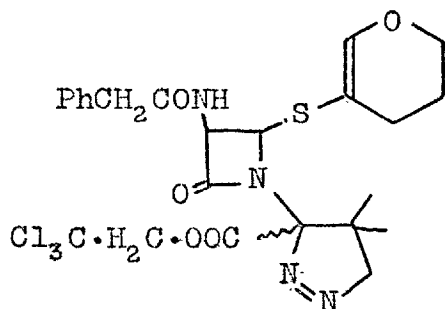
The second approach considered was based on the report by Sheehan⁸⁵ that treatment of phthalimidopenicillin isocyanate (115) with dilute acid led to cleavage of the C3-C4 bond to give the aldehyde (116).



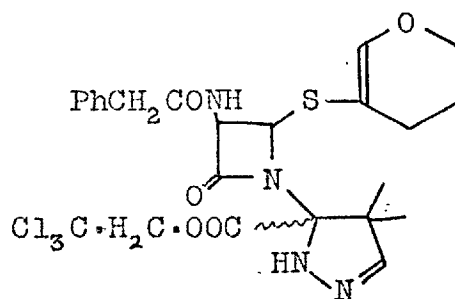
An analogous process on the acid adduct (112) should therefore lead to the β -lactam compound (114) since the thiazolidine ring of the penicillin nucleus has been opened at the S1-C2 bond in the formation of the adduct. Thus the β -unsaturated acid adduct (112) was treated with ethyl chloroformate and sodium azide to give the azide (117) which rearranged completely to the isocyanate (118) during the work-up procedure. This isocyanate was not rigorously purified but showed a characteristic absorption at 2270cm.^{-1} due to the isocyanate function.⁸⁶ Direct treatment with dilute aqueous acid gave, after purification by preparative t.l.c., a very low yield a compound having identical infrared and n.m.r. spectra to those of the required authentic β -lactam (114), prepared subsequently by the method described below.



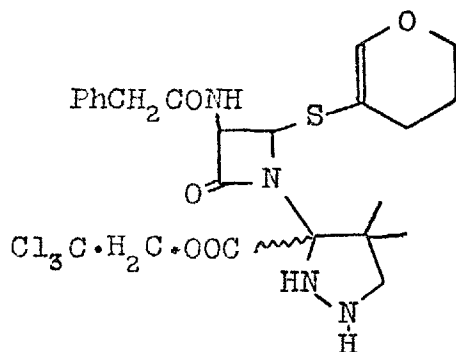
As a third approach to the isopropenyl side chain removal the introduction of an internal nucleophile was attempted. Addition of diazomethane to the $\alpha\beta$ -unsaturated ester should lead to a Δ^1 -pyrazoline (119), which could theoretically be isomerised to the Δ^2 -pyrazoline (120) or reduced to the pyrazolidine (121).



(119)



(120)



(121)

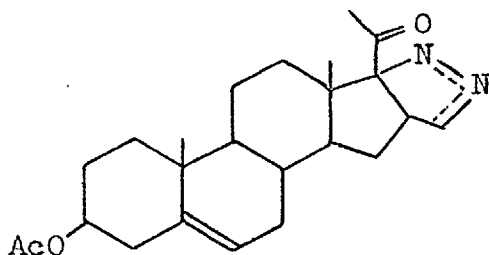
It was hoped that either the Δ^2 -pyrazoline or the pyrazolidine would collapse readily to the desired β -lactam compound (114).

When the $\alpha\beta$ -unsaturated ester adduct (112) was treated with diazomethane in ether two products were formed in an approximately 10/1 ratio. Both of these materials analysed for the addition of one molecule of diazomethane to the ester adduct. The position of the addition was clearly shown to be to the double bond of the isopropenyl side chain by the upfield shift of the methyl signals in the n.m.r. spectra. Also the appearance of a two proton AB quartet centred at 5.5 in the n.m.r. spectra of both compounds indicated that they were both Δ^1 -pyrazolines. It seemed probable that these two compounds were stereoisomeric about the addition of the diazomethane to the double bond, however the absolute stereochemistries of the two materials were not deduced.

The isomerisation of Δ^1 -pyrazolines to Δ^2 -pyrazolines has been well documented for steroid molecules where it has been reported that treatment with either strong base (KOH)⁸⁷ or acid (HCl)⁸⁸ caused the isomerisation. Several methods are known for the reduction of pyrazolines⁸⁹ but these involve the use of conditions which were considered to be too vigorous for the β -lactams involved here.

In order to test the isomerisation and reduction

a sample of the known $16\alpha,17\alpha$ -(3,1-(1-pyrazolino))-5-pregnen- 3β -ol-20-one acetate (122)⁹⁰ was prepared and the reactions of this investigated.



(122) Δ^1 -pyrazoline

(123) Δ^2 -pyrazoline

The isomerisation to the Δ^2 -pyrazoline (123) was found to occur very readily with hydrochloric acid or sodium hydroxide, but not with milder acids or bases such as *p*-toluene sulphonic acid or triethylamine. Also no mild conditions giving reduction could be found. Reagents tried included chromous acetate in acetic acid, hydrazobenzene, sodium borohydride, zinc in acetic acid, diimide, benzyl thiol and sodium dithionite.

As the reduction did not appear feasible, isomerisation was considered the only possibility. Treatment of the major penicillin derived pyrazoline (119A) with hydrochloric acid in either a two phase or one phase system gave no isomerisation and treatment with sodium hydroxide caused

destruction of the β -lactam ring. It was then felt that although the β -lactam would not withstand the sodium hydroxide it might be stable towards a less nucleophilic strong base. Therefore the major pyrazoline (119A) was suspended in t-butanol and potassium t-butoxide added. This caused an immediate reaction to give, after extraction with ethyl acetate, only one product which proved to be the desired β -lactam compound (114). In the infrared spectrum the trichloroethyl ester function was absent, but the material showed the absorptions of the β -lactam (1770cm.^{-1}), amide (3400 , 1670 and 1520cm.^{-1}) and dihydropyran (1620 and 1150cm.^{-1}) groups. The n.m.r. spectrum showed only the dihydropyran ring, side chain amide and β -lactam ring, confirming the removal of the isopropenyl side chain. The elemental analysis was substantiated by the accurate mass measurement (m/e 318.10337 , M^+ for $C_{16}H_{18}N_2O_3S$, 318.10381).

This same compound was also formed from the minor pyrazoline (119B), although the reaction was slower and accompanied by some degradation.

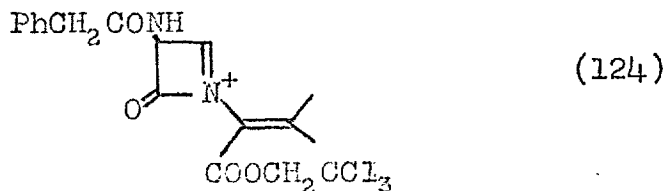
The Structures of the Penicillin-Dihydropyran Adducts

The initially proposed structures for the penicillin-dihydropyran adducts (99) and (100) were based mainly on the elemental analyses and n.m.r. spectra of the two adducts and the two sulphoxides of the $\alpha\beta$ -unsaturated ester adduct.⁸² There were, however, a number of factors which arose and placed a certain degree of doubt as to the validity of these structures.

Firstly, in the n.m.r. spectra of all the adducts studied the single proton at the point of attachment of the sulphur to the pyran ring appeared at ca. 3.50. This is considerably lower than the proton of the model compound (111) where this appears at τ 5.2.⁹¹

Secondly, in the infrared spectra there was an absorption at 1620cm.^{-1} which could not be explained satisfactorily in terms of the tetrahydropyranyl structures.

Thirdly, in the mass spectra both the pyran ring and the sulphur were lost on electron impact to give the ion (124) as the highest molecular weight ion. For the tetrahydropyranyl structures the pyran-sulphur bond would be predicted to cleave first.



Finally, the by-product, valerolactone (cf. Scheme 8.), could not be detected in the reaction mixture.

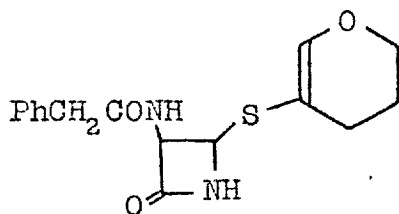
The number of protons in the dihydropyran ring could not be determined accurately from the n.m.r. spectra as the methyl signals appear in the same region as those of the pyran ring. This gives a large integral where the limits of error are such that either the tetrahydropyranyl or dihydropyranyl structures can be accounted for. However, in the two pyrazolines the methyl signals were shifted to higher field leaving the pyran protons clear. The integral over this region did not correspond to the six protons required for the tetrahydropyranyl structure. Moreover, in the β -lactam compound (114), where the two methyl groups were absent the integral corresponded to four protons.

This latter observation, together with the mass measurement on the β -lactam compound (114) prompted the reassignment of structures to the vinyl sulphides (101) and (102).

The previously unexplained features could be completely accounted for in terms of this structure.

The position of the vinylic proton of dihydropyran itself ($\tau 3.6$)⁹² was very close to that of the singlet proton in the adducts. The infrared absorption at 1620cm.^{-1} could be assigned to the vinylic ether/thioether unit.⁹³ The mass spectral fragmentation is consistent with the structure.

Thus the structure of the β -lactam compound isolated from the degradation of the penicillin-dihydropyran adducts is correctly formulated as (114).



(114)

CHAPTER 6

EXPERIMENTAL

....compounded of many simples,
extracted from many objects....

As You Like It.

Unless otherwise stated the following conditions apply to the experiments described in this section.

Infrared spectra were recorded as nujol mulls or as solutions in chloroform or bromoform on a Unicam S.P.200 spectrometer, the phase employed is indicated in the text. Ultraviolet spectra were taken on a Unicam S.P.800 spectrometer and are for ethanol solutions.

N.m.r. spectra were obtained for deuteriochloroform solutions on either a Varian A60 or HA100 spectrometer and the following notations are used to describe the spectra:-

- s singlet.
- d doublet.
- dd double doublet.
- t triplet.
- q quartet.
- m multiplet.
- b broad.
- AB AB quartet.

Thin layer chromatography was carried out on Merck GF₂₅₄ silica plates, with the solvents indicated in the text. Preparative thin layer chromatography was on lmm.

thick Merck GF₂₅₄ silica plates with the solvents indicated.

Column chromatography was on Merck silica gel with the solvents indicated.

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected.

Reagents.

t-Butyl hypochlorite.- Prepared by the action of chlorine on an alkaline solution of t-butanol.⁹⁷

Diazomethane.- Prepared by the action of alkali on nitrosomethylurea. The ether solution of diazomethane was decanted from the aqueous layer, dried over potassium hydroxide pellets and used without distillation.⁹⁵

Iodobenzene dichloride.- Prepared by the direct combination of iodobenzene and chlorine in chloroform.⁹⁶

Certain of the experiments described here have also been carried out by other students in these laboratories. This arose from the history of penicillin chemistry at Imperial College which had left some questions unanswered, the solution of these necessitating the repetition of some previous work. This repetition led to the solution of most of the outstanding problems and to a complete revision of the structures of some of the materials isolated from certain of the experiments.

Where experiments have been repeated from previous work or by other students the following notations are used:-

(F.C.), F. Comer, postdoctoral research fellow 1966-1967.

(G.L.), G. Lucente, postdoctoral research fellow
1968-1969.

(M.V.T.), M. V. Taylor, postgraduate student 1968-

Nuclear Overhauser Effect Investigations

The spectra were recorded using a Varian HA100 spectrometer in the frequency sweep mode with tetramethylsilane as internal reference. Sample concentrations were of the order of 10% w/v and the solutions were thoroughly degassed by the vacuum freeze-thaw method. The secondary irradiation at the methyl resonance position was increased in 10mV. increments until the maximum effect was observed. The region under study was integrated five times alternately with and without power for three cycles, this means that the peaks of interest were integrated a total of fifteen times. The final results were computed from the average of these measurements and are expected to be accurate to better than $\pm 3\%$.

As stated at the beginning of this thesis special acknowledgement must be given to Mr. P. N. Jenkins for running the spectra necessary for this study.

Benzylpenicillin-1 β -sulphoxide acid (30)³².- Benzylpenicillin sodium salt (14.24g.) in water (300ml.) and pH 6.8 phosphate buffer (80ml.) was treated with sodium metaperiodate (10.04g.) in water (300ml.) and the solution stirred at room temperature for three hours. The reaction mixture was acidified to pH 2 with phosphoric acid (20% v/v) and extracted with ethyl acetate (400ml.). The organic layer was washed with water, dried and the solvent removed in vacuo at ca. 25°C until the onset of crystallisation, when the solution was removed from the vacuum and cooled to 0°C for 5-10min. The crystals of the benzylpenicillin-1 β -sulphoxide acid so isolated (11.6g.) were sufficiently pure for the subsequent reactions. A sample of the material was recrystallised from ethyl acetate to give colourless prisms, m.p. 160-163°; $\nu_{\text{max}}^{\text{nujol}}$ 3380, 1785, 1700, 1525, 1020 cm.^{-1} ; τ 1.46 (1H s), 2.72 (5H bs), 4.00 (2H bs), 5.00 (1H d J=5Hz.), 5.40 (1H s), 6.40 (2H s), 8.30 (3H s), 8.75 (3H s).

Benzylpenicillin-1 β -sulphoxide methyl ester (33).-

Method A. Benzylpenicillin-1 β -sulphoxide acid (1.0g.) in methylene chloride was treated with diazomethane in methylene chloride until the evolution of nitrogen ceased. The

excess diazomethane was destroyed with acetic acid until the solution was pale yellow and then the solvents removed in vacuo. The resulting solid was crystallised from ethyl acetate to give the ester (0.9g.), m.p. 124-126°; $[\alpha]_D^{22} +202^\circ$ (c 1.1 dioxan); $\nu_{\max}^{\text{nujol}}$ 3350, 1780, 1740, 1670 cm.^{-1} ; τ 2.7 (5H bs), 2.94 (1H d, J=10Hz.), 4.05 (1H dd, J=5, 10Hz.), 5.08 (1H d, J=5Hz.), 5.43 (1H s), 6.26 (3H s), 6.47 (2H s), 8.35 (3H s), 8.63 (3H s).

Method B. Benzylpenicillin methyl ester (31) (1.0g.), prepared by treatment of benzylpenicillinic acid with diazomethane, in dioxan was treated with sodium metaperiodate (2.0g.) in water (20ml.). Dioxan was then added to give a homogeneous solution which was stored at room temperature for three days. The reaction mixture was extracted with ethyl acetate (3 x 20ml.), the organic layer being washed with water, dried and the solvent removed in vacuo. Recrystallisation from ethyl acetate gave the ester (0.5g.) as above.

Benzylpenicillin-t-butylamide (32) (F.C.).- Benzylpenicillin sodium salt (356mg.) in chloroform (100ml.) was treated at 0°C with triethylamine (0.153ml.) and ethyl chloroformate (0.100ml.) with stirring for 30 min. The cooling bath

was removed, t-butylamine (0.115ml.) added and the solution stirred at room temperature for a further one hour. After washing with water, 0.5N HCl, water and drying the solvent was removed in vacuo to give the benzylpenicillin-t-butylamide (340mg.) as an inhomogeneous solid. Crystallisation from methylene chloride-ether-petrol gave colourless prisms, m.p. 193-196° (dec.); $[\alpha]_D^{23} +207^\circ$ (c 0.8 chloroform); $\nu_{\max}^{\text{nujol}}$ 3370, 3330, 1775, 1690, 1660, 1550, 1530 cm.^{-1} ; τ 2.96 (5H bs), 3.52 (1H d, J=9Hz.), 3.67 (1H s), 4.29 (1H dd, J=4, 9Hz.), 5.65 (1H d, J=4Hz.), 6.40 (2H s), 8.37 (3H s), 8.49 (3H s), 8.65 (9H s); O.R.D. (c 1.07mg./5ml. dioxan) $[\theta]_{400} +9,900$, $249 +39,500$, $220 -17,000$, $213 +2,470$, $a_{220}^{249} +565$. (Found. C, 61.58; H, 6.92; N, 10.75; S, 8.52; $\text{C}_{20}\text{H}_{27}\text{N}_3\text{O}_3\text{S}$ requires C, 61.68; H, 6.99; N, 10.57; S, 8.22).

Benzympenicillin-1 β -sulphoxide t-butylamide (34) (F.C.).-

Method A. Benzympenicillin-1 β -sulphoxide acid (6.3g.) in methylene chloride was treated with triethylamine (2.0ml.), ethyl chloroformate (1.6ml.) and t-butylamine (2.1ml.) as described above for the preparation of benzympenicillin t-butylamide. Crystallisation from methylene chloride gave the benzympenicillin-1 β -sulphoxide-t-butylamide

(5.4g.) as colourless plates, m.p. 165-167° (dec.); $[\alpha]_D^{22} +197^\circ$ (c 0.9 chloroform); $\nu_{\max}^{\text{nujol}}$ 3300, 1785, 1695, 1670, 1550, 1525, 1018 cm.^{-1} ; τ 2.70 (5H bs), 3.52 (1H b), 4.01 (1H dd, J=5, 10Hz.), 5.08 (1H d, J=5Hz.), 5.60 (1H s), 6.42 (2H s), 8.26 (3H s), 8.65 (9H s), 8.80 (3H s); O.R.D. (c 1.14mg./5ml. dioxan) $[\phi]_{400} +7,830$, $_{244} +38,300$, $_{223} +10,200$, $_{213} +33,750$, $_{223}^{244} +281$. (Found. C, 59.00; H, 6.60; N, 9.97; S, 7.71: $\text{C}_{20}\text{H}_{27}\text{O}_4\text{N}_3\text{S}$ requires C, 59.24; H, 6.71; N, 10.37; S, 7.89).

Method B. Benzylpenicillin t-butylamide (100mg.) in aqueous dioxan (20ml. dioxan and 20ml. pH 6-8 phosphate buffer) was treated with excess sodium metaperiodate (214mg.) and stored at room temperature for three days. The solution was extracted with ethyl acetate (3 x 10ml.), the extract being washed with water, dried and the solvent removed in vacuo. Crystallisation from methylene chloride gave the benzylpenicillin-1 β -sulphoxide t-butylamide (62mg.) as colourless plates, m.p. 166-168°, a mixed m.p. with the material from method A above showed no depression.

Benzylpenicillin-1 α -sulphoxide methyl ester (35) (G.L.).-
Benzylpenicillin methyl ester (696mg.) in pyridine (6.5ml.) and water (1.6ml.) was cooled to -30°C, iodobenzene

dichloride (1.19g.) in pyridine (5ml.) at this temperature was added and the solution stirred for one hour. During this time the temperature was kept below -25°C . The reaction mixture was poured into water (15ml.) and extracted with ethyl acetate (3 x 10ml.). The combined organic layers were washed with phosphoric acid (20% v/v) until free from pyridine, then with water. After drying and removal of solvents in vacuo the residue was purified by column chromatography (silica, EtOAc/PhH 1/2). Eluted first was the benzylpenicillin- β -sulphoxide methyl ester, identical in all respects to that described above, followed by the benzylpenicillin- α -sulphoxide methyl ester (340mg.) which gave colourless plates from ethyl acetate, m.p.

$124-125^{\circ}$; $[\alpha]_{\text{D}}^{22} +153^{\circ}$; $\nu_{\text{max}}^{\text{nujol}}$ 3250, 1790, 1748, 1670, 1220cm.^{-1} ; τ 2.76 (5H bs), 3.18 (1H d, $J=7\text{Hz.}$), 4.79 (1H dd, $J=4, 7\text{Hz.}$), 5.38 (1H d, $J=4\text{Hz.}$), 5.67 (1H s), 6.27 (3H s), 6.46 (2H s), 8.41 (3H s), 8.76 (3H s). (Found. C, 56.0; H, 5.7; N, 7.7; S, 9.7: $\text{C}_{17}\text{H}_{20}\text{N}_2\text{O}_5\text{S}$ requires C, 56.0; H, 5.5; N, 7.7; S, 9.5).

Benzylpenicillin- α -sulphoxide t-butylamide (36) (F.C.).-

Benzylpenicillin t-butylamide (300mg.) in pyridine (5ml.) and water (0.5ml.) was cooled to -30°C and treated with

iodobenzene dichloride (780mg.) in the manner described for the methyl ester analogue. Purification by prep. t.l.c. gave as the less polar material the benzylpenicillin- 1β -sulphoxide t-butylamide (42mg.) and the benzylpenicillin- 1α -sulphoxide t-butylamide (20mg.) as a non-crystalline foam, $[\alpha]_D^{22} +142^\circ$ (c 0.8 chloroform); τ 2.74 (5H bs), 3.50 (1H d, $J=9\text{Hz.}$), 4.80 (1H dd, $J=4.5, 9\text{Hz.}$), 5.40 (1H d, $J=4.5\text{Hz.}$), 5.85 (1H s), 6.45 (2H s), 8.44 (3H s), 8.65 (12H bs). (Found. C, 59.27; H, 6.65; N, 10.15; S, 8.02: $\text{C}_{27}\text{H}_{27}\text{N}_3\text{O}_4\text{S}$ requires C, 59.24; H, 6.71; N, 10.37; S, 7.89).

Isomerisation of Benzylpenicillin- 1α -sulphoxide methyl ester to the β -isomer (G.L.).- The benzylpenicillin- 1α -sulphoxide methyl ester (100mg.) was heated at reflux in dry toluene (40ml.) for 30min. The solvent was removed in vacuo and the residue purified by column chromatography (silica, EtOAc/PhH 1/2) to give the benzylpenicillin- 1β -sulphoxide methyl ester (78mg.) identical (m.p., mixed m.p., i.r. and n.m.r.) to that described above.

Isomerisation of the benzylpenicillin- α -sulphoxide t-butylamide to the β -isomer.- The benzylpenicillin- α -sulphoxide t-butylamide (20mg.) was heated at reflux in dry toluene for 30min. Removal of the solvent in vacuo followed by purification by prep. t.l.c. (EtOAc/PhH 1/2) gave the benzylpenicillin- β -sulphoxide t-butylamide (10mg.) identical in all respects to the material described above.

Isomerisation of the benzylpenicillin- α -sulphoxide methyl ester in the presence of deuterium[‡].- a). The benzylpenicillin- α -sulphoxide methyl ester (284mg.) was heated at reflux, under a stream of dry nitrogen, in benzene (5ml.) containing deuterated t-butanol (1.1ml.) (assay >95%) for three hours. The residue obtained after removal of solvents was dissolved in methanol and this solution allowed to stand overnight at room temperature. Prep. t.l.c. separation (PhH/Me₂CO 95/5 three elutions) gave the β -sulphoxide (110mg.) and unreacted α -sulphoxide (40mg.).

In the n.m.r. spectrum of this β -sulphoxide the ratio of the areas of the methyl signals at C2 (τ 8.35/8.63)

[‡] With Drs. P. G. Sammes and G. Lucente.

was 0.85/1, indicating a 46% incorporation of deuterium.

b). The benzylpenicillin-1 α -sulphoxide methyl ester (273mg.) was heated at reflux in deuterated t-butanol (3ml.) for three hours. Purification as above gave a sample of the β -sulphoxide (41mg.) where the ratio of the methyl signals at C2 was 0.80/1, indicating a 60% incorporation of deuterium.

c). In a control experiment the benzylpenicillin-1 β -sulphoxide methyl ester was heated at reflux in deuterated t-butanol under the same conditions as above. The n.m.r. spectrum of the recovered material had signals of equal area for the methyl groups at C2.

The Rearrangement of the Benzylpenicillin-1 β -sulphoxide t-butylamide in Acetic Anhydride-Toluene (F.C.).- Benzylpenicillin-1 β -sulphoxide t-butylamide (1.0g.) in dry toluene (300ml.) and acetic anhydride (10ml.) was heated at reflux for 7hrs. The toluene and acetic anhydride were removed in vacuo and the residue taken up in ethyl acetate (30ml.). This solution was washed with 50% sat. sodium bicarbonate solution until free from acetic anhydride, then with water. After drying and removal of the solvent

in vacuo the residue was treated with ether and allowed to stand overnight to crystallise. Four recrystallisations from ethyl acetate gave the 3-acetoxycepham t-butylamide (50) (200mg.), m.p. 255-257° (dec.); $[\alpha]_D^{22} +14.6^\circ$ (c 0.58 chloroform); $\nu_{\text{max}}^{\text{nujol}}$ 3340, 3305, 1788, 1740, 1663, 1560, 1520 cm.^{-1} ; τ 2.66 (5H bs), 3.58 (1H s), 3.77 (1H d, J=9Hz.), 4.40 (1H dd, J=4, 9Hz.), 4.67 (1H d, J=4Hz.), 5.51 (1H s), 6.14 and 6.76 (2H AB, J=14Hz.), 8.22 (3H s), 8.45 (3H s), 8.68 (9H s); O.R.D. (c 1.18mg./5ml. dioxan) $[\phi]_{400} +375$, $343 +1,180$, $304 +1,450$, $275 +4,650$, $256 +4,070$, $236 +8,050$, $222 -2,740$, $217 -965$, $a_{256}^{275} +6$, $a_{222}^{236} +108$. (Found. C, 58.78; H, 6.24; N, 9.01; S, 7.34: $\text{C}_{22}\text{H}_{29}\text{N}_3\text{O}_5\text{S}$ requires C, 59.65; H, 6.53; N, 9.39; S, 7.14).

Examination of the 100MHz. n.m.r. spectrum of the concentrated mother liquors and, indeed, of the material first crystallised from the ether indicated the presence of the above compound and a second component. This second component was assigned as the 2 β -acetoxymethylpenam t-butylamide (51) and could not be isolated in a pure state. τ 2.66 (5H bs), 3.37 (1H d, J=9Hz.), 3.67 (1H s), 4.28 (1H dd, J=4, 9Hz.), 4.49 (1H d, J=4Hz.), 5.78 (1H s), 5.70 and 6.08 (2H AB, J=12Hz.), 6.38 (2H s), 8.02 (3H s), 8.45 (3H s), 8.68 (9H s).

The Rearrangement of the Benzylpenicillin-1 β -sulphoxide t-butylamide in Acetic Anhydride-Toluene containing Sodium Acetate (F.C.).- Benzylpenicillin-1 β -sulphoxide t-butylamide (3.39g.) and sodium acetate (35mg.) were heated at reflux in toluene (150ml.) containing acetic anhydride (6ml.) for 7hrs. The toluene and acetic anhydride were removed in vacuo, the residue being taken up in ethyl acetate and washed with 50% sat. sodium bicarbonate and water. After drying and removal of the ethyl acetate in vacuo the residue was treated with ether and allowed to stand overnight to crystallise. Recrystallisation from ethyl acetate gave the isothiazolone (49) (463mg.) as colourless plates, m.p. 200-202°; $[\alpha]_D^{22}$ -13°; $\nu_{\max}^{\text{nujol}}$ 3340, 3280, 1700, 1690, 1670, 1632, 1618, 1600, 1540, 1370 cm^{-1} ; λ_{\max} 298nm. ($\text{Log}_{10} e$ 4.04); τ 1.75 (1H s), 2.66 (5H bs), 3.97 (1H b), 4.52 (1H b), 4.89 (2H b), 6.26 (2H s), 8.30 (3H s), 8.69 (9H s). (Found. C, 61.60; H, 6.42; N, 10.82; S, 7.82; $\text{C}_{20}\text{H}_{25}\text{N}_3\text{O}_3\text{S}$ requires C, 62.00; H, 6.51; N, 10.86; S, 8.27). T.l.c. examination of the reaction mixture and of the mother liquors from the crystallisations indicated that the only products formed in this reaction were the isothiazolone as above and polar decomposition products.

The Rearrangement of Benzylpenicillin-1 β -sulphoxide in Acetic Anhydride (F.C.).- Benzylpenicillin-1 β -sulphoxide t-butylamide (3.4g.) was heated at reflux in acetic anhydride (15ml.) for one hour, at which time the solution was black. The acetic anhydride was removed in vacuo, toluene being added to aid the process, and the black tarry residue taken up in ethyl acetate. After washing with 50% sodium bicarbonate, water and drying the residue was purified by column chromatography (silica, EtOAc/PhH 1/3) to give, as first eluted, the isothiazolone (49) (85mg.) identical to the above. The subsequent fractions contained a t.l.c. homogeneous solid from which the 3-acetoxycepham t-butylamide (120mg.) could be crystallised.

Benzylpenicillin-1 β -sulphoxide diazoketone (54).- Benzylpenicillin sodium salt (3.56g.) in chloroform (150ml.) at 0°C was treated with triethylamine (1.57ml.) and ethyl chloroformate (1.0ml.) with stirring for 30min. This solution was then treated with diazomethane (from 20g. nitrosomethylurea) in methylene chloride and the stirring continued for a further 2hrs. at 0°C. The excess diazomethane was destroyed by the addition of acetic acid and the solvents removed in vacuo to give a dark viscous oil,

which was purified by prep. t.l.c. to give the crude diazoketone (1.5g.) which contained some methyl ester.

Fractional crystallisation afforded the pure diazoketone, m.p. 171-172° (dec.); $n_{D_{20}}^{20}$ 3300, 2120, 1780, 1670, 1610, 1520, 1280, 1020 cm.^{-1} ; τ 2.73 (5H bs), 4.00 (1H dd, $J=4, 9\text{Hz.}$), 4.10 (1H s), 5.00 (1H d, $J=4\text{Hz.}$), 5.50 (1H s), 6.42 (2H s), 8.25 (3H s), 8.75 (3H s). (Found. C, 54.4; H, 5.06; N, 14.82; S, 8.51: $\text{C}_{17}\text{H}_{18}\text{N}_4\text{O}_4\text{S}$ requires C, 54.5; H, 4.87; N, 14.98; S, 8.55).

When the above reaction was carried out using a two mole excess of triethylamine and a four mole excess of ethyl chloroformate only trace quantities of the methyl ester were formed and the diazoketone could be crystallised directly from the reaction mixture after washing and removal of some of the solvent in vacuo.

Photolysis of Benzylpenicillin-1 β -sulphoxide diazoketone.-

Benzylpenicillin-1 β -sulphoxide diazoketone (100mg.) in benzene (9.5ml.) and methanol (0.5ml.) was irradiated using a 125W. medium pressure u.v. lamp for one hour. The apparatus was of quartz and stirring was by means of a stream of dry nitrogen which was bubbled through the

solution. After removal of the solvents in vacuo and purification by prep. t.l.c. (EtOAc/PhH 1/2) a non-crystalline foam of the homopenicillin methyl ester sulphoxide (56) (35mg.) was obtained, $\nu_{\max}^{\text{CHCl}_3}$ 3400, 1780, 1730, 1670, 1600, 1500, 1020 cm^{-1} , τ 2.70 (5H bs), 4.10 (1H dd, J=5, 10Hz.), 5.18 (1H d, J=5Hz.), 5.60 (1H t, J=8Hz.), 6.28 (3H s), 6.40 (2H s), 7.50 (2H d, J=8Hz.), 8.50 (3H s), 8.55 (3H s).

Diazoacetophenone (57)⁷².- Benzoyl chloride (1.7g.) in ether (4ml.) was added dropwise during 10min. to a stirred cooled (0-5°C) solution of diazomethane and triethylamine (1.25g.) in ether (10ml.). The solution was stirred at 0°C for five hours after which time the triethylamine hydrochloride was filtered off, the excess diazomethane destroyed with acetic acid and the solvents removed in vacuo. The resulting oily solid was crystallised from petrol (b. 60-80°C) to give the diazoacetophenone (0.9g.) as yellow needles, m.p. 44.5-45.4° (rep. 45-46°)⁷², $\nu_{\max}^{\text{CHCl}_3}$ 2120, 1620, 1575, 1455, 1375, 1140 cm^{-1} .

The conditions used for the attempted reductions are summarised in Table 5. pg. 58.

Rearrangement of Benzylpenicillin-1 β -sulphoxide
 $\beta\beta\beta$ -trichloroethyl ester in Acetic Anhydride-Toluene
to Complete Reaction.- Benzylpenicillin-1 β -sulphoxide
 $\beta\beta\beta$ -trichloroethyl ester (10g.) was heated at reflux in
toluene (300ml.) and acetic anhydride (200ml.) in an
atmosphere of dry nitrogen for 25hrs. At this time no
starting material was apparent by t.l.c. analysis. The
toluene and acetic anhydride were removed in vacuo and
the residue dissolved in ethyl acetate and this solution
washed with 50% sodium bicarbonate until free from the
last traces of acetic anhydride, then with water. The
dark oil obtained after drying and removal of the solvent
in vacuo was purified by column chromatography (silica,
EtOAc/PhH 1/7). First eluted was the non-conjugated
isothiazolone (62) (1.5g.) which gave colourless needles
from ethyl acetate, m.p. 171-172°; $[\alpha]_D^{21} -14.4^\circ$ (c 1.0
chloroform); $\nu_{\max}^{\text{nujol}}$ 3360, 1750, 1670, 1645, 1530, 1240,
1190 cm^{-1} ; λ_{\max} 296nm. ($\log_{10} \epsilon$ 4.03); τ 1.45 (1H s),
2.10 (1H b), 2.73 (5H bs), 4.32 (1H s), 4.78 (2H b),
5.10 and 5.42 (2H AB, $J=11\text{Hz.}$), 6.33 (2H s), 8.18 (3H s).
(Found. C, 46.6; H, 3.9; N, 5.5; S, 6.9; Cl, 22.7;
 $\text{C}_{18}\text{H}_{17}\text{N}_2\text{O}_4\text{S}_2\text{Cl}_3$ requires C, 46.6; H, 3.67; N, 6.04; S, 6.91;
Cl, 22.78).

The subsequent fractions contained a mixture of the two acetoxy compounds (2.8g.), a sample (1g.) of this was further separated by prep. t.l.c. (Me₂CO/PhH 1/9, 4 elutions) by removal of the leading and tailing quarters of the broad band. The less polar material was the 2β-acetoxymethylpenam βββ-trichloroethyl ester (63) (140mg.) which gave colourless plates from methylene chloride-ether, m.p. 118-119°, $[\alpha]_D^{22} +192^\circ$ (c 0.93 chloroform); $\nu_{\max}^{\text{nujol}}$ 3300, 1780, 1745, 1660, 1550, 1230cm.⁻¹; τ 2.72 (5H bs), 3.20 (1H b), 4.32 (1H dd, J=4, 11Hz.), 4.41 (1H d, J=4Hz.), 5.52 (2H s), 5.67 and 6.32 (2H AB, J=12Hz.), 6.38 (2H s), 7.98 (3H s), 8.48 (3H s). (Found. C, 45.9; H, 4.23; N, 5.32; S, 6.15; Cl, 20.63: C₂₀H₂₁N₂O₆SCl₃ requires C, 45.90; H, 4.01; N, 5.35; S, 6.11; Cl, 20.32).

The more polar material (20mg.) was the 3-acetoxycepham βββ-trichloroethyl ester (64) which was a non-crystalline foam, $\nu_{\max}^{\text{CHCl}_3}$ 3400, 1770, 1725, 1670, 1500cm.⁻¹; τ 2.70 (5H bs), 3.92 (1H d, J=9Hz.), 4.40 (1H dd, J=4, 9Hz.), 4.70 (1H d, J=4Hz.), 5.18 (1H s), 6.50 and 6.72 (2H AB, J=14Hz.), 6.35 (2H s), 8.22 (3H s), 8.39 (3H s). $[\alpha]_D^{22} +35^\circ$ (c 0.9 chloroform) (This value may be somewhat high due to the presence of a trace quantity of the penam isomer).

The zone remaining on the t.l.c. plate was removed to give 600mg. of the mixture.

Rearrangement of Benzylpenicillin-1 β -sulphoxide

$\beta\beta\beta$ -trichloroethyl ester in Acetic Anhydride.- Benzylpenicillin-1 β -sulphoxide $\beta\beta\beta$ -trichloroethyl ester (2.0g.) in acetic anhydride (40ml.) was heated at reflux for 15min., at which time the solution was deep red. The acetic anhydride was removed in vacuo, toluene being added to the concentrated solution to aid removal of the acetic anhydride. The black tar so obtained was taken up in ethyl acetate and washed with 50% sat. sodium bicarbonate, water then dried and the solvent removed in vacuo. The residue was purified by column chromatography (silica, EtOAc/PhH 1/6). First eluted was the non-conjugated isothiazolone (62) (108mg.) identical to that described above. The subsequent fractions contained the mixture of 2 β -acetoxymethylpenam and 3 β -acetoxycephams $\beta\beta\beta$ -trichloroethyl esters (400mg.) and the n.m.r. spectrum of this mixture indicated a greater proportion of the cepham compound than was produced with toluene-acetic anhydride as described above. (ca. 20% against ca. 10%).

Rearrangement of Benzylpenicillin-1 β -sulphoxide
 $\beta\beta\beta$ -trichloroethyl ester in Acetic Anhydride containing
Sodium Acetate.- Benzylpenicillin-1 β -sulphoxide $\beta\beta\beta$ -
trichloroethyl ester (2.0g.) was heated at reflux in
acetic anhydride (25ml.) containing sodium acetate (0.3g.)
for 4min., at which time the solution was black. The
acetic anhydride was removed in vacuo, the residue taken
up in ethyl acetate and this solution washed with 50%
sat. sodium bicarbonate, water and dried. The black
tarry oil obtained after removal of the ethyl acetate in
vacuo was allowed to stand in ether overnight during which
time the conjugated isothiazolone (66) separated and was
filtered off. Recrystallisation from ethyl acetate gave
colourless plates (580mg.), m.p. 205-207°; $[\alpha]_D^{22}$ ca. +1°
(c 0.92 chloroform); $\nu_{\max}^{\text{nujol}}$ 3300, 1715, 1650, 1590, 1530cm.⁻¹;
 λ_{\max} 295nm. (Log₁₀ e 4.10); τ 1.40 (1H s), 1.58 (1H b),
2.69 (5H bs), 5.36 (2H s), 6.28 (2H s), 7.66 (3H s), 8.10
(3H s). (Found. C, 46.5; H, 3.66; N, 6.11; S, 7.01;
Cl, 22.9: C₁₈H₁₇N₂O₄SCl₃ requires C, 46.6; H, 3.67;
N, 6.04; S, 6.91; Cl, 22.78).

The t.l.c. of the mother liquors indicated mainly polar
materials.

2 β -Acetoxymethylpenam $\beta\beta\beta$ -trichloroethyl ester (63).-

Benzylpenicillin-1 β -sulphoxide $\beta\beta\beta$ -trichloroethyl ester (20g.) was heated at reflux in toluene (1500ml.) and acetic anhydride (250ml.) for four hours, at which time the solution was lime-green in colour. The solvents were removed in vacuo during which process the unreacted β -sulphoxide crystallised. When the solvents ceased to distill off the residue was slurried with ether and the unreacted β -sulphoxide filtered off and washed with ether (recovery typically 14-15g.). The ethereal solution was concentrated in vacuo, the last traces of acetic anhydride being removed in high vacuum, to give a sticky foam. This was purified (without washing) by column chromatography (silica, EtOAc/PhH 1/5) to give, as first eluted, the non-conjugated isothiazolone (62) (typically 1.0g.) and subsequently the 2 β -acetoxymethylpenam $\beta\beta\beta$ -trichloroethyl ester (63) (typically 3.5-4.0g.). The n.m.r. of the penam product showed that it contained $\ll 10\%$ of the cepham isomer (64) and therefore it was used for the subsequent reactions without further purification. The physical constants are as above.

Conversion of the 3-Acetoxycepham $\beta\beta\beta$ -trichloroethyl Ester to the t-Butylamide analogue.- The 3-acetoxycepham $\beta\beta\beta$ -trichloroethyl ester (50mg.) in acetic acid (9ml.) and water (1ml.) was treated with zinc dust (100mg.) and the suspension stirred at room temperature for one hour. The reaction mixture was filtered and the filtrate evaporated to dryness in vacuo. The resulting cream solid was dissolved in water and acidified to pH 2 with 20% v/v phosphoric acid and extracted with methylene chloride. The organic layer was washed with water, dried and the solvent removed in vacuo to give an oil which had an infrared spectrum consistent with an acid (3100 and 1710 cm.^{-1}). This oil was not purified but was taken up in methylene chloride, cooled to 0°C, triethylamine (0.015ml.) and ethyl chloroformate (0.010ml.) added and the solution stirred at 0°C for 30min. The cooling bath was removed, t-butylamine (0.015ml.) added and stirring continued for one hour at room temperature. The solution was washed with water, 0.5N hydrochloric acid, water, dried and the solvent removed in vacuo. Purification by prep. t.l.c. gave the 3-acetoxycepham t-butylamide (10mg.), this sample having identical i.r., n.m.r. and m.p. to the authentic material.

Isomerisation of the $\beta\gamma$ -Unsaturated Isothiazolone

$\beta\beta\beta$ -Trichloroethyl Ester (62).- The $\beta\gamma$ -unsaturated isothiazolone $\beta\beta\beta$ -trichloroethyl ester (100mg.) in ethyl acetate (15ml.) was treated with triethylamine (0.1ml.) and the solution stirred at room temperature for one hour. The resulting solution was washed with phosphoric acid (20ml. of 20% v/v), water, dried and the solvent removed in vacuo to give the $\alpha\beta$ -unsaturated isothiazolone $\beta\beta\beta$ -trichloroethyl ester (66) (90mg.). Recrystallisation from ethyl acetate gave a sample (63mg.) which was identical (m.p., mixed m.p., i.r., n.m.r. and u.v.) to the material described above.

3-Acetoxycepham-1 α -sulphoxide t-butylamide (67)(F.C.).-

The 3-acetoxycepham t-butylamide (700mg.) in dioxan (20ml.) was treated with sodium metaperiodate (2.5g.) in the minimum of water and the solution stored at room temperature in the dark for 3 days. The reaction mixture was poured into water (100ml.) and extracted with ethyl acetate (3 x 20ml.). The combined organic layers were washed with water, dried and the solvent removed in vacuo to give a foam (530mg.). A sample was further purified by prep. t.l.c. (EtOAc/PhH 1/1) to give the pure

3-acetoxyccepham-1 α -sulphoxide t-butylamide as a non-crystalline solid, m.p. 115-120°; $[\alpha]_D^{22} -32.9^{\circ*}$; $\nu_{\max}^{\text{nujol}}$ 3550, 3360, 1775, 1745, 1675, 1540, 1220, 1030cm.⁻¹; τ 2.71 (5H bs), 3.31 (2H b), 4.71 (1H dd, J=4, 8Hz.), 5.01 (1H d, J=4Hz.), 5.34 (1H s), 6.14 (2H s), 6.39 (2H s), 8.07 (3H s), 8.38 (3H s), 8.65 (9H s); O.R.D. (c 1.41mg./5ml. dioxan) $[\phi]_{400} -840$, $236 +7,560$, $222 -5,600$, $217 -11,100$, $a_{222}^{236} +132$. (Found. C, 57.12; H, 6.15; N, 8.97; S, 6.58: C₂₂H₂₉N₃O₆S requires C, 57.01; H, 6.31; N, 9.07; S, 6.91).

p-Bromobenzylpenicillin (69).- p-Bromopenylacetyl chloride was prepared from p-bromophenylacetic acid by heating at reflux in thionyl chloride for one hour. The volatile materials were removed in vacuo and the acid chloride used as isolated.

To a solution of 6-aminopenicillanic acid (4.3g.) in potassium hydrogen carbonate (140ml. of 4%) and acetone (120ml.), was added dropwise, with stirring and ice bath cooling, a solution of the acid chloride (5g.) in acetone (20ml.) over a period of 20min. After an additional 30min. stirring at 0°C the acetone was removed in vacuo

* Concentration for rotation (c 0.55 chloroform)

and the aqueous phase extracted with ether (200ml.) and these extracts discarded. The remaining aqueous phase was layered with fresh ether (100ml.) and acidified to pH 2 with phosphoric acid (20% v/v) and the ether layer removed. The aqueous layer was reextracted with ether, the ether layers being combined, washed with water (100ml.), dried and the solvent volume reduced to ca. 50ml. in vacuo. The p-bromobenzylpenicillin was isolated as its triethylamine salt by adding triethylamine (2.8ml.) to this ether solution, removing the ether in vacuo and any excess triethylamine under high vacuum. Crude yield 7.4g., $\nu_{\max}^{\text{CHCl}_3}$ 3300, 1780, 1720, 1670 cm.^{-1} , τ 2.7 (4H AA'BB' pattern), 3.75 (1H d, J=5Hz.), 4.4 (2H m), 5.70 (1H s), 6.42 (2H s), 6.92 (6H q, J=7Hz.), 8.42 (3H s), 8.47 (3H s), 8.75 (9H t, J=7Hz.).

p-Bromobenzylpenicillin-1 β -sulphoxide acid (70).-

p-Bromobenzylpenicillin triethylamine salt (7.3g.) in water (150ml.) and pH 6.8 phosphate buffer (45ml.) was treated with sodium metaperiodate (3.9g.) in water (150ml.) and the solution stirred at room temperature for 3 hours. The reaction mixture was acidified to pH 2 with phosphoric acid (20% v/v) and extracted with ethyl acetate (400ml.), the organic extract being washed with water, dried and the

solvent removed in vacuo to give the p-bromobenzylpenicillin-1 β -sulphoxide acid (4g.), $\nu_{\max}^{\text{CHCl}_3}$ 3350, 1780, 1720, 1680, 1520, 1460 cm^{-1} .

p-Bromobenzylpenicillin-1 β -sulphoxide t-butylamide(71).-

p-Bromobenzylpenicillin-1 β -sulphoxide acid (2.0g.) in methylene chloride (50ml.) was treated with triethylamine (0.665ml.) and ethyl chloroformate (0.50ml.) at 0°C for 30 min. The cooling bath was removed, t-butylamine (0.575ml.) added and the solution stirred at room temperature for a further hour. After washing with water, 0.5N HCl, water and drying the solvent was removed in vacuo to give a white powder. Recrystallisation from ethyl acetate gave the p-bromobenzylpenicillin-1 β -sulphoxide t-butylamide (1.93g.) as colourless plates, m.p. 183-184.5°; $[\alpha]_D^{22} +196^\circ$ (c 0.78 chloroform), $\nu_{\max}^{\text{nujol}}$ 3350, 1780, 1685, 1650, 1540, 1520 cm^{-1} ; τ 2.75 (4H AA'BB' pattern), 3.7 (2H b), 4.00 (1H dd, J=5, 10Hz.), 5.10 (1H d, J=5Hz.), 5.62 (1H s), 6.45 (2H s), 8.25 (3H s), 8.62 (9H s), 8.78 (3H s). (Found. C, 49.8; H, 5.38; N, 8.61; Br, 16.4: $\text{C}_{20}\text{H}_{26}\text{N}_3\text{O}_4\text{SBr}$ requires C, 49.9; H, 5.39; N, 8.42; Br, 16.63).

3 β -Acetoxy-4 α -t-butylcarbamoyl-3 α -methyl-7 β -(p-bromo-phenyl)acetamido-cepham (72).- p-Bromobenzylpenicillin-1 β -sulphoxide t-butylamide (1.2g.) in dry toluene (300ml.) and acetic anhydride (10ml.) was heated at reflux for 7 hrs. The toluene and acetic anhydride were removed in vacuo and the residue taken up in ethyl acetate (30ml.). This solution was washed with 50% sat. sodium bicarbonate until free from the last traces of acetic anhydride, then with water. After drying and removal of the solvent in vacuo the residue was treated with ether and allowed to stand overnight to crystallise. The crystals so formed (300mg.) were used for the oxidation without further purification. The 3 β -acetoxy-4 α -t-butylcarbamoyl-3 α -methyl-7 β -(p-bromo-phenyl)acetamido-cepham isolated had $[\alpha]_D^{22} +34.1^\circ$ (c 0.37 chloroform); $\nu_{\max}^{\text{CHCl}_3}$ 3400, 1770, 1730, 1680, 1510 cm^{-1} , τ 2.75 (4H AA'BB' pattern), 3.65 (2H b), 4.35 (1H dd, J=4,9Hz.), 4.70 (1H d, J=4Hz.), 5.50 (1H s), 6.21 and 6.82 (2H AB, J=14Hz.), 6.42 (2H s), 8.20 (3H s), 8.45 (3H s), 8.63 (9H s). The rotation of this compound when compared to that of the rigorously purified benzylpenicillin analogue indicates that this sample may have been contaminated with a small quantity of the 2 β -acetoxyethylpenam isomer.

3 β -Acetoxy-4 α -t-butylcarbamoyl-3 α -methyl-7 β -(p-bromophenyl)acetamido-cepham-1 α -oxide (73).— The crystalline material from above (250mg.) in dioxan (100ml.) and pH 6.8 buffer (40ml.) was treated with sodium metaperiodate (1.5g.) in water (10ml.) and the solution stored at room temperature in the dark for seven days. The reaction mixture was then diluted with an equal volume of water and extracted with methylene chloride (2 x 50ml.) and ethyl acetate (50ml.). The organic layers were washed with water, dried and the solvents removed in vacuo to give an oily residue (210mg.) which was purified by prep. t.l.c. to give the 3 β -acetoxy-4 α -t-butylcarbamoyl-3 α -methyl-7 β -(pbromophenyl)acetamido-cepham-1 α -oxide (130mg.) as a non crystalline foam. Crystallisation from methylene chloride gave colourless plates (44mg.), m.p. 227-228°; $[\alpha]_D^{22}$ -45° (c 0.1 chloroform); $\nu_{\max}^{\text{nujol}}$ 3320, 1775, 1740, 1670, 1550, 1215 cm.^{-1} ; τ 2.65 (4H AA'BB' pattern), 3.7 (1H d, J=9Hz.), 4.7 (1H dd, J=4, 9Hz.), 5.0 (1H d, J=4Hz.), 5.3 (1H s), 6.2 (2H s), 6.42 (2H s), 8.01 (3H s), 8.34 (3H s), 8.63 (9H s). (m/e 541 and 543. M^+ for $C_{22}H_{28}N_3O_6$ SBr (Br = 79.9) at 542.4)

2 β -Acetoxymethylpenam-1 β -sulphoxide t-butylamide (75)
(F.C.).- The solid isolated from the rearrangement of the
benzylpenicillin-1 β -sulphoxide t-butylamide (i.e. the
mixture of the 2 β -acetoxymethylpenam and 3-acetoxycepham
derivatives) (582mg.) in dioxan (100ml.) and pH 6.8 buffer
(80ml.) was treated with sodium metaperiodate (1.5g.) in
water (10ml.) and the solution stored at room temperature
for six days. The reaction mixture was diluted with an
equal volume of water and extracted with ethyl acetate
(3 x 30ml.) and these extracts washed with water, dried
and the solvent removed in vacuo. Purification of the
residue by prep. t.l.c. gave unreacted starting material
(132mg.), the 3-acetoxycepham-1 α -sulphoxide t-butylamide
(73) (286mg.) as described above and the 2 β -acetoxymethyl-
penam-1 β -sulphoxide t-butylamide (104mg.) as a non-
crystalline solid foam, $[\alpha]_D^{22} +172^\circ$ (c 0.2 chloroform);
 $\nu_{\text{max}}^{\text{CHCl}_3}$ 3550, 3360, 1795, 1745, 1670, 1520, 1225, 1040 cm.^{-1} ;
 τ 2.70 (5H bs), 2.80 (1H b), 3.47 (1H b), 3.95 (1H dd, J=
5, 10Hz.), 5.05 (1H d, J=5Hz.), 5.28 and 5.40 (2H AB, J=?)[‡],
5.61 (1H s), 6.41 (2H s), 7.90 (3H s), 8.65 (9H s), 8.77
(3H s); O.R.D. (c 1.13mg./5ml. dioxan) $[\theta]_{400} -2,260$,

[‡]The J value for this quartet could not be determined as
the high and low field lines were 'lost' in the instrument
noise.

247 +15,800, 228 +3,480, 217 +29,500, α_{228}^{247} +123.

(Found. C, 56.65; H, 6.32; N, 8.81: $C_{22}H_{29}N_3O_6S$ requires C, 57.01; H, 6.31; N, 9.07).

2 β -Acetoxymethylpenam-1 β -sulphoxide $\beta\beta\beta$ -trichloroethyl Ester (76).- Method A.- The 2 β -acetoxymethylpenam $\beta\beta\beta$ -trichloroethyl ester (1.0g.) in dioxan (15ml.) was treated with sodium metaperiodate (2.5g.) in water (20ml.), and sufficient dioxan to give a homogeneous solution added. The reaction mixture was stored at room temperature for five days then poured into water (40ml.) and extracted with ethyl acetate (3 x 10ml.). The oil obtained after drying and removal of the ethyl acetate in vacuo was purified by prep. t.l.c. to give recovered starting sulphide (220mg.) and the 2 β -acetoxymethylpenam-1 β -sulphoxide $\beta\beta\beta$ -trichloroethyl ester (120mg.) as a non-crystalline solid foam, $[\alpha]_D^{22}$ +184° (c 0.9 chloroform); $\nu_{\max}^{CHBr_3}$ 3300, 1775, 1760, 1725, 1670, 1540, 1230cm.⁻¹; τ 2.65 (5H bs), 2.92 (1H d, J=9Hz.), 3.99 (1H dd, J= 4, 9Hz.), 5.05 (1H d, J=4Hz.), 5.18 (1H s), 5.30 (2H s), 5.26 and 5.52 (2H AB, J=12Hz.), 6.48 (2H s), 7.94 (3H s), 8.73 (3H s). (Found. C, 44.70; H, 4.03; N, 5.18; S, 5.70: $C_{20}H_{21}N_2O_7SCl_3$ requires C, 44.51; H, 3.89; N, 5.18; S, 5.93).

Method B.- The 2 β -acetoxymethylpenam $\beta\beta\beta$ -trichloroethyl ester (1.0g.) in ethyl acetate (15ml.) was treated with m-chloroperbenzoic acid (380mg.) and the solution stirred at room temperature for two hours. The reaction mixture was washed with 50% sat. sodium bicarbonate, water, dried and the ethyl acetate removed in vacuo. Purification by column chromatography (silica, EtOAc/PhH 1/2) gave the 2 β -acetoxymethylpenam-1 β -sulphoxide $\beta\beta\beta$ -trichloroethyl ester (890mg.) identical to that described above.

2 β -Acetoxymethylpenam-1 α -sulphoxide $\beta\beta\beta$ -trichloroethyl Ester (77).- Method A.- The 2 β -Acetoxymethylpenam $\beta\beta\beta$ -trichloroethyl ester (2.0g.) in pyridine (40ml.) and water (5ml.) was cooled to -35° to -40°C, iodobenzene dichloride (2.5g.) in pyridine (20ml.) at this temperature was added and the solution stirred at a temperature below -30°C for 30min. The reaction mixture was poured into phosphoric acid (250ml. of 20% v/v) and extracted with ethyl acetate (3 x 25ml.). The combined extracts were washed with phosphoric acid until free from pyridine, then with water. The aqueous washings were re-extracted with ethyl acetate (2 x 15ml.) and these similarly washed. After drying the total combined organic layers the solvent

was removed in vacuo and the resulting foam purified by column chromatography (silica, EtOAc/PhH 1/2 followed by EtOAc/PhH 1/1). First eluted was the 2 β -acetoxymethylpenam-1 β -sulphoxide $\beta\beta\beta$ -trichloroethyl ester (76) (0.6g.)

identical to that described above. The subsequent fractions contained the 2 β -acetoxymethylpenam-1 α -sulphoxide

$\beta\beta\beta$ -trichloroethyl ester (0.7g.) which was isolated as a non-crystalline foam, $[\alpha]_D^{22} +137^\circ$ (c 0.9 chloroform); $\nu_{\max}^{\text{CHBr}_3}$ 3300, 1780, 1745, 1670, 1520 cm.^{-1} ; τ 2.73 (5H bs), 3.20 (1H b), 4.82 (1H dd, $J=4, 11\text{Hz.}$), 5.21 (1H d, $J=4\text{Hz.}$), 5.25 (1H s), 5.22 (2H s), 6.42 (2H s), 8.00 (3H s), 8.62 (3H s). (Found. C, 45.02; H, 4.08; N, 5.20:

$\text{C}_{20}\text{H}_{21}\text{N}_2\text{O}_7\text{SCl}_3$ requires, C, 44.51; H, 3.89; N, 5.18).

Method B.- The 2 β -acetoxymethylpenam $\beta\beta\beta$ -trichloroethyl ester (3.0g.) in pyridine (50ml.) and water (3ml.) at -40 to -50°C was treated with *t*-butyl hypochlorite (1.5ml.) and the solution stirred at this temperature for 5min. The reaction was stopped by the addition of sodium dithionite and pouring into water. After washing with phosphoric acid, water, drying and removal of the solvent in vacuo the residue was purified by column chromatography

as described for Method A above. The first fractions contained the 2 β -acetoxymethylpenam-1 β -sulphoxide $\beta\beta\beta$ -trichloroethyl ester (0.5g.) and the subsequent fractions the 2 β -acetoxymethylpenam-1 α -sulphoxide $\beta\beta\beta$ -trichloroethyl ester (1.5g.). For both products the physical constants were identical to those described above.

2 α -Acetoxymethylpenam-1 β -sulphoxide $\beta\beta\beta$ -trichloroethyl Ester (78).- The 2 β -acetoxymethylpenam-1 α -sulphoxide $\beta\beta\beta$ -trichloroethyl ester (100mg.) was heated at reflux in dry toluene (200ml.) for 30min. The solvent was removed in vacuo and the residue purified by prep. t.l.c. (EtOAc/PhH 1/1) to give the 2 α -acetoxymethylpenam-1 β -sulphoxide $\beta\beta\beta$ -trichloroethyl ester (80mg.) as a non-crystalline foam, $[\alpha]_D^{22} +181^\circ$ (c 1.0 chloroform); $\nu_{\text{max}}^{\text{CHBr}_3}$ 3300, 1770, 1745 (broad), 1670, 1540, 1230 cm^{-1} ; τ 2.70 (5H bs), 3.94 (1H dd, J=4, 9Hz.), 4.98 (1H d, J=4Hz.), 5.20 (1H s), 5.16 and 5.35 (2H AB, J=12Hz.), 5.43 and 6.02 (2H AB, J=12.5Hz.), 6.44 (2H s), 7.98 (3H s), 8.30 (3H s). (Found. C, 44.69; H, 4.07; N, 5.21; S, 6.08; Cl, 19.88: $\text{C}_{20}\text{H}_{21}\text{N}_2\text{O}_7\text{SCl}_3$ requires C, 44.50; H, 3.89; N, 5.18; S, 5.97; Cl, 19.76).

Attempted ring expansion of the 2 β -acetoxymethylpenam-
1 α -sulphoxide trichloroethyl ester.- The sulphoxide (200mg.)
and p-toluenesulphonic acid (25mg.) were heated at reflux
in dry toluene (200ml.) for four hours. The solution was
washed with 50% sat. sodium bicarbonate, water and the
solvent volume reduced to ca. 5ml. in vacuo. The t.l.c.
of this solution was a smear from origin to solvent front.
Attempted purification by removal of lcm. bands from a
prep. t.l.c. plate gave no definite products but the top
two zones showed u.v. absorption at 298nm. (isothiazolone ?).
None of the zones isolated showed the u.v. absorption at
ca. 265nm. due to the cephalosporin nucleus.

Other reactions were carried out along these lines
using the catalysts and solvents described in Table 10.,
but none of these showed any sign of containing the
cephalosporin nucleus.

Reaction of Benzylpenicillin-1 β -sulphoxide trichloroethyl ester with dihydropyran (G.I.,M.V.T.).- The benzylpenicillin-1 β -sulphoxide trichloroethyl ester (10g.) was heated at reflux for 14hrs. in dihydropyran (60ml.) which contained a small quantity of aluminium tribromide (ca. 100mg.). The volume of dihydropyran was reduced to ca. 20ml. in vacuo and this poured into petrol (150ml., b. 60-80°). After shaking vigorously for 10min. the petrol was removed by decantation and the residual oil treated similarly with a further batch of fresh petrol. The oil obtained after removal of this petrol by decantation was triturated with ether (30ml.) which gave as a precipitate the unreacted starting sulphoxide (1.9g.). Removal of the ether in vacuo gave the $\beta\beta\beta$ -trichloroethyl α -isopropenyl- α -[3' β -phenylacetamido-4' β -S-(3'',4''-dihydro-2''H-pyranyl--5''-thio)-azetidin-2'-on-1'-yl] acetate, the $\beta\gamma$ -unsaturated ester adduct, (101) (8.5g.) as a non-crystalline sticky foam

$\nu_{\text{max}}^{\text{CHCl}_3}$ 3450, 1760(broad), 1670, 1620, 1260 cm.^{-1} ; τ 2.72 (5H bs), 3.35 (1H b?), 4.80 (2H m), 5.10 (1H s), 5.22 (2H s), 6.10 (2H m), 6.42 (2H s), 8.60 (3H s), 8.1-8.4 (4H m). The n.m.r. spectrum indicated that this material contained approx. 5% of the $\alpha\beta$ -unsaturated isomer. A sample

was further purified by prep. t.l.c. for analysis. (Found. C, 49.7, H, 4.62, N, 4.91. $C_{23}H_{25}N_2O_5SCl_3$ requires C, 50.4, H, 4.60, N, 5.11).

Isomerisation of the $\beta\gamma$ -unsaturated ester adduct (G.L., M.V.T.).- Method A.- The $\beta\gamma$ -unsaturated ester adduct (5g.) in benzene (100ml.) and pyridine (5ml.) was heated at reflux for 30 min. The solution was washed free from pyridine with 2N hydrochloric acid, then with water and the solvent removed in vacuo the resulting oil was crystallised from ether-petrol to give the $\beta\beta\beta$ -trichloroethyl $\beta\beta$ -dimethyl- α -[3' β -phenylacetamido-4'-S-(3'',4''-dihydro-2''H-pyran-5''-thio)-azetidin-2'-on-1'yl] acrylate, the $\alpha\beta$ -unsaturated ester adduct, (102)(4.2g.), m.p. 109-110°; $[\alpha]_D^{22} +61^\circ$ (c 1.2 chloroform); $\nu_{\max}^{CHCl_3}$ 3400, 1760, 1740, 1670, 1620, 1520, 1260 cm^{-1} ; τ 2.7 (5H bs) 3.38 (1H b), 4.60 (1H dd, J=4, 9Hz.), 4.65 (1H d, J=4Hz.), 4.95 and 5.37 (2H AB, J=12Hz.), 6.1 (2H m), 6.42 (2H s), 7.65 (3H s), 7.82 (3H s), 8.0-8.3 (4H m). (Found. C, 50.01; H, 4.78; N, 4.87; $C_{23}H_{25}N_2O_5SCl_3$ requires C, 50.40; H, 4.60; N, 5.11).

Method B.- The $\beta\gamma$ -unsaturated ester adduct (2.0g.) in ethyl acetate (15ml.) was treated with triethylamine (0.5ml.)

and the solution stored at room temperature overnight. Working up as for Method A above gave the $\alpha\beta$ -unsaturated ester adduct identical in all respects to that described above.

The $\beta\gamma$ -unsaturated acid adduct (112).- The $\beta\gamma$ -unsaturated trichloroethyl ester adduct (3.0g.) in acetic acid (15ml.) and water (2ml.) was treated with excess zinc (5g.) and the slurry stirred at room temperature for 30min. The zinc was removed by filtration through cotton wool and the solvent volume reduced to ca. 5ml in vacuo. The concentrate was poured into water and extracted with ether (3 x 10ml.), the combined ether layers being back extracted with sodium bicarbonate. This aqueous layer was layered with fresh ether (30ml.) and acidified to pH 2 with phosphoric acid (20% v/v). The separated ether layer was washed with water, dried and the solvent removed in vacuo to give the acid as an amorphous, hygroscopic, solid (1.1g.), $\nu_{\text{max}}^{\text{CHCl}_3}$ 3450, 3400, 3300, 1760, 1720(broad), 1670, 1625, 1530, 1510, 1400, 1280 cm^{-1} ; n_D^{20} 1.40 (1H s), 2.7 (5H bs), 3.20 (1H d, J=9Hz.), 3.42 (1H b), 4.45 (1H dd, J=4, 9Hz.), 4.8-4.9 (3H m), 5.20 (1H s), 6.10 (1H m), 6.32 (2H s), 8.12 (7H b). This material was further

characterised by methylation to give the methyl ester identical in all respects to that previously isolated from the reaction of benzylpenicillin-1 β -sulphoxide methyl ester with dihydropyran.⁸²

The β -lactam compound (114) by the isocyanate route.-
 The $\beta\gamma$ -unsaturated acid adduct (850mg.) in tetrahydrofuran (5ml.) was treated with triethylamine (0.28ml.) and ethyl chloroformate (0.28ml.) and the solution stirred at 0°C for 5min. Sodium azide (135mg.) in water (2ml.) was added and the whole stirred at 0°C for 45min. The solution was poured into water (25ml.) and extracted with ethyl acetate, these extracts being washed with water, dried and the solvent removed in vacuo to give the isocyanate (118) directly. The Curtius rearrangement presumably took place on the removal of the ethyl acetate, $\nu_{\text{max}}^{\text{CHCl}_3}$ 3400, 2260, 1775, 1670, 1620, 1510, 1100cm.⁻¹. This isocyanate in tetrahydrofuran (5ml.) was treated with 5% aqueous formic acid (0.5ml.) and the solution stirred at room temperature for 5min. The reaction mixture was poured into water and extracted with ethyl acetate, the organic layer being washed with water, dried and the solvent removed in vacuo. Purification of the residue by prep. t.l.c. gave the β -phenylacetamido-4 β -S-(3',4'-dihydro-2'H-pyranyl-5'-thio)-azetidin-2-one, the β -lactam compound, (114) (20mg.)

as an inhomogeneous solid, $\nu_{\text{max}}^{\text{CHCl}_3}$ 3400, 1760, 1620, 1110 cm^{-1} , τ 2.7 (1H bs), 3.2 (1H b), 3.45 (1H bs), 3.52 (1H s), 4.58 (1H dd, $J=4.5, 10\text{Hz.}$), 5.30 (1H d, $J=4.5\text{Hz.}$), 6.20 (2H m), 6.42 (2H s), 8.10 (4H m). The close similarities between this compound and the authentic β -lactam compound (vide infra) led to the assignment of structure.

Reaction of the $\alpha\beta$ -unsaturated ester adduct (102) with diazomethane.— The adduct (5g.) in ether was treated with diazomethane (from 20g. nitrosomethylurea) in ether (60ml.) and the solution stored at 0°C for 6 days. The excess diazomethane was destroyed by the addition of acetic acid and the solvents removed in vacuo. Purification of the residual foam by prep. t.l.c. (EtOAc/PhH 1/1) gave two products. The less polar, minor, component (119B) (35mg.) had $\nu_{\text{max}}^{\text{CHCl}_3}$ 3400, 1770, 1760(sh), 1670, 1620, 1550, 1100 cm^{-1} , τ 2.7 (5H bs), 3.40 (1H s), 4.30 (1H dd, $J=4, 10\text{Hz.}$), 4.60 (1H d, $J=4\text{Hz.}$), 5-5.5 (4H m), 6.1 (2H m), 6.42 (2H s), 8-8.4 (4H m), 8.65 (3H s), 9.00 (3H s). (Found. C, 48.81; H, 4.69; N, 9.65; $\text{C}_{24}\text{H}_{27}\text{N}_4\text{O}_5\text{SCl}_3$ requires C, 48.86; H, 4.86; N, 9.50).

The more polar, major, component (N.B. t.l.c. r.f. identical

to that of the starting ester) (119A)(2.7g.) had
 $\nu_{\max}^{\text{CHCl}_3}$ 3400, 1760(broad), 1670, 1620, 1510, 1100 cm^{-1} ,
 τ 2.7 (5H bs), 3.74 (1H d, $J=9\text{Hz.}$), 4.43 (1H dd, $J=4, 9\text{Hz.}$),
 4.88 (1H d, $J=4\text{Hz.}$), 5.33 and 5.52 (2H AB, $J=12\text{Hz.}$), 5.28
 and 5.72 (2H AB, $J=17\text{Hz.}$), 6.25 (2H m), 6.38 (2H s),
 8-8.3 (4H m), 8.66 (3H s), 9.04 (3H s). (Found. C, 48.88,
 H, 4.65, N, 9.52, S, 5.46, Cl, 17.86. $\text{C}_{24}\text{H}_{27}\text{N}_4\text{O}_5\text{SCl}_3$
 requires C, 48.86, H, 4.61, N, 9.50, S, 5.43, Cl, 18.03).
 These two materials were assigned as the two isomeric
 4,4-dimethyl-5 β -(trichloroethoxycarbonyl)-5 β -(3' β -phenyl-
 acetamido-4' β -S-(3'',4''-dihydro-2''H-pyran-5''-thio)-
 -azetidin-2'-on-1'-yl)- Δ^1 -pyrazolines.

Reaction of the major pyrazoline (119B) with potassium
 t-butoxide.- The pyrazoline (100mg.) in t-butanol (2ml.)
 was treated with potassium t-butoxide (30mg.) in t-butanol
 (2ml.) and the mixture shaken for ca. 2min. During this
 time the solution went deep red and then colourless again.
 As soon as the solution lost its red colour it was poured
 into water and extracted with ether (3 x 5ml.), the
 combined ether layers being washed with water, dried and
 the solvent removed in vacuo to give the 3 β -phenylacet-
 amido-4 β -S-(3',4'-dihydro-2'H-pyran-5'-thio)-azetidin-

-2-one, the β -lactam compound (114) (40mg.) as a foam, which was crystallised from ether, m.p. 130-134°, $\nu_{\text{max}}^{\text{CHCl}_3}$ 3400, 1760, 1620, 1100 cm^{-1} , τ 2.7 (5H bs), 3.2 (1H d, $J=10\text{Hz.}$), 3.45 (1H b), 3.52 (1H s), 4.58 (1H dd, $J=4.5, 10\text{Hz.}$), 5.30 (1H d, $J=4.5\text{Hz.}$), 6.20 (2H m), 6.42 (2H s), 8.10 (4H m). (Found, C, 60.03; H, 5.67; N, 9.17; $\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_3$ requires C, 60.40; H, 5.70; N, 8.84).

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