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THE UNIVERSITY OF ALBERTA

STUDIES ON 2β-(SUBSTITUTED METHYL)PENAMS

BY

SUCHETA BETAGERI

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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The undersigned certify that they have read, and recommend to the faculty of graduate studies and research for acceptance, a thesis entitled STUDIES ON 2β-(SUBSTITUTED METHYL)PENAMS submitted by SUCHETA BETAGERI in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Pharmaceutical Sciences (Medicinal Chemistry).

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TO THE MEMORY
OF MY SON
CHIRAG

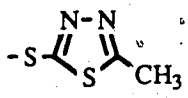
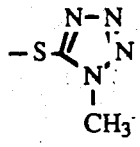
ABSTRACT

This research involved studies on 2β-(substituted methyl)penams **18**. Reactions were attempted towards the synthesis of 2β-(fluoromethyl)penams **18** (X=F) using metal fluorides. A series of structurally modified penicillins was synthesized and tested for antimicrobial activity. Three penam sulfones **19**, were synthesized as potential β-lactamase inhibitors.

The 2β-(chloromethyl)penams **18** (X=Cl) having different substituents at C-6 and ester groups at C-3 reacted with silver fluoride under various reaction conditions and gave 3β-fluorocephams **22** as the only identifiable fluorine containing products. The reaction of 2β-(bromomethyl)penam **18** (X=Br) and silver fluoride followed the same pattern but proceeded faster. The reaction of the 2β-(halomethyl)penam **18** (X=Cl, Br) and the silver fluorides was dependent on the solvent, time and temperature. The reaction with other metal fluoride such as sodium fluoride, potassium fluoride, cobaltic fluoride, cupric fluoride and ferric fluoride was unsuccesful. The ^{19}F, ^{13}C and ^{1}H NMR spectral date of the 3β-fluorocephams **22**, their sulfoxides **36** and sulfones **40** are presented.

A series of structurally modified benzylpenicillins, phenoxymethylpenicillins, ampicillins and piperacillins with chloro, bromo, 2-mercapto-5-methyl-1,3,4-thiadiazole and 5-mercapto-1-methyl-1,2,3,4-tetrazole at the C_2-β-methyl carbon was synthesized. The minimum inhibitory concentrations

(MICs) of these 2β-(substituted methyl)pemans **18** (R, R' and X substituents identified as below) were determined against a range of Gram +ve and Gram -ve bacteria by the agar dilution method. The details of the synthesis and comparative antimicrobial activity are reported.

R	X	R'
$C_6H_5CH_2CONH$	Cl	CH_2CCl_3
$C_6H_5OCH_2CONH$	Br	Na
$C_6H_5CHCONH$ (NH$_2$)		
$C_6H_5CHCONH$ (NH–CO–N(C$_2$H$_5$)–CO)		

A class of penam sulfones, the 6,6-dihydro-2β-(substituted methyl)penam sulfones **19** (R=H) having 2-mercapto-5-methyl-1,3,4-thiadiazole at C_2-β-methyl carbon was synthesized and oxidation studies carried out using various oxidizing agents such as hydrogen peroxide/glacial acetic acid, *m*-chloroperbenzoic acid and potassium permanganate. Various oxidation products were isolated and characterized.

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I wish to extend my gratitude to Dr. R. G. Micetich for his supervision of this project. His advice, guidance and encouragement throughout the study is sincerely appreciated.

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I wish to thank my husband, Guru, friends and members of my family for the various form of help, support and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

α	Alpha
AC	L-α-Aminoadipyl-L-Cysteine
ACV	δ-(L-α-Aminoadipyl)-L-Cysteinyl-D-Valine
Ala	Alanine
6-APA	6-Aminopenicillanic Acid
ASIS	Aromatic Solvent Induced Shifts
ATP	Adenosine Triphosphate
β	Beta
°C	Degree celsius
$CDCl_3$	Deuterated Chloroform
CTT	2-Chloro-1,2,2-trifluorotriethylamine
δ	Delta
D	With reference to D-glyceraldehyde
DMF	Dimethylformamide
DMSO	Dimethyl Sulfoxide
DPO	2,5-Diphenyloxazole
E	Enzyme
e.g.	Example
E. S	Enzyme-Substrate Complex
E. S'	Modified Enzyme-Substrate Complex
g	Gram
Glu	Glutamic acid
Gly	Glysine
h	Hour

IR	Infra Red
Lys	Lysine
L	With reference to L-glyceraldehyde
m	Meta
MBC	Minimum Bactericidal Concentration
mg	Milligram
MIC	Minimum Inhibitory Concentration
min	Minutes
mol. wt	Molecular Weight
m.mol	Millimole
Mur Nac	N-Acetyl Muramic Acid
NMR	Nuclear Magnetic Resonance
π	pi
P	Product
p	para
PBP	Penicillin Binding Proteins
PMR	Proton Magnetic Resonance
PNB	*p*-Nitrobenzyl
PST	Piperdino sulfur trifluoride
SAR	Structure Activity Relationship
SDS	Sodium dodecyl sulfate
TMS	Tetramethylsilane
UDP	Uridine 5'-diphosphate
Unsym	Unsymmetrical
YTR	Code name refering to Taiho Pharmaceutical C
-ve	Negative
+ve	Positive

I. INTRODUCTION

A. *ANTIBIOTICS*

Antibiotics are substances produced by micro-organisms or produced wholly or partly by chemical synthesis, which in low concentrations inhibit the growth of other organisms[1]. Thus, the three major sources of antibiotics are (a) micro-organisms (b) synthesis and (c) semisynthesis. Even though many antibiotics are produced by synthetic or semisynthetic routes, the fermentation process still is the most important primary route for the production of antibiotics.

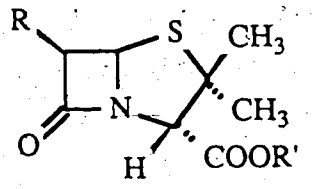
Some of the important classes of antibiotics include[2], β-lactam: compounds containing a four membered β-lactam ring fused to a thiazolidine ring in the penicillins and to a dihydrothiazine ring in the cephalosporins; aminoglycosides: containing one or more amino sugars, such as glucosamine or neosamine, with glycosidic linkages to a basic (amino or guanidino) 6-membered carbon ring, e.g., streptidine or streptamine; macrolides: which are hydroxylated macrocyclic lactones containing 12 to 20 carbon atoms in the primary ring, e.g., erythromycin; polypeptides: which differ from each other in their mechanism of action and antibacterial spectrum, e.g., bacitracin, polymyxin B sulfate; tetracyclines: have a perhydronaphthacene skeleton, quinolones: which have the common structural feature, 1-alkyl-1,4-dihydro-4-oxo-quinoline carboxylic acid; sulfonamides and miscellaneous antibiotics.

The advantages of β-lactam antibiotics include a broad spectrum of activity, low toxicity, easy availability and low cost.

B. *β-LACTAM ANTIBIOTICS*

Classes of β-Lactams

β-Lactams are 4-membered cyclic amides derived from 3-aminopropanoic acids; the first member was synthesized by Staudinger, in 1907[3]. As a class they consist of penicillins **1**, that contain a thiazolidine ring fused to the β-lactam ring, cephalosporins **2**, that contain a dihydrothiazine ring fused to the β-lactam ring and the "non-classical" β-lactam antibiotics such as clavulanic acid **3**, thienamycin **4**, nocardicin **5**, and the monobactams **6**.



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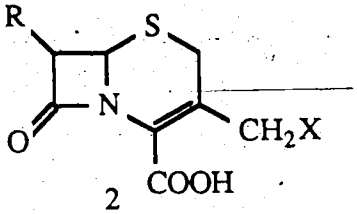

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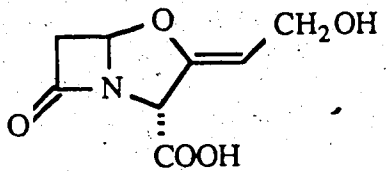

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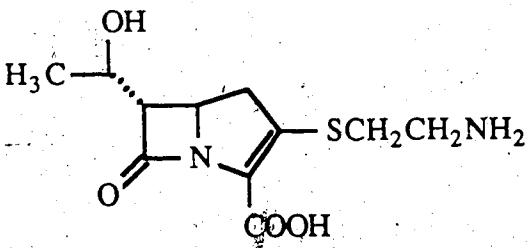

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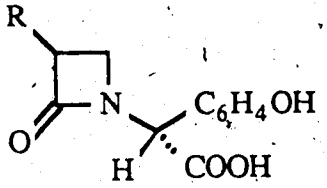

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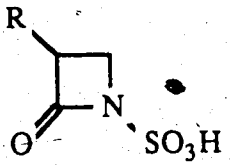

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Sources of β-Lactams

Penicillin **1**, was accidentally discovered by Alexander Fleming[4]. Penicillin is produced by a variety of Penicillia[5], including strains of *Penicillium chrysogenum* [6]. Later, a number of diverse fungi other than penicillia were reported to synthesize a variety of β-lactam metabolites. A few examples include the eukaryotic species of *Aspergillus* [7], *Malbranchea*[8], *Cephalosporium*[9], *Emericellopsis*[10], *Epidermophyton* and *Trichophyton*[11]. Although *Penicillium notatum* produced only a few milligrams of penicillin

per liter, with advances in the art of submerged fermentation and successful mutagenesis, strains producing close to 30 g/L of penicillin G have been developed[12]. The preferred procedure for preparing a fungal culture for the fermentation process consisted of propagating a cell mass, either conidia, arthrospores or vegetative mycelium; from a master source and dispensing a constant amount with or without substituting another menstrum for extracellular fluid, into a number of vials. The vials as a group were frozen either rapidly (in the lyophilization process) or at a sustained rate (under liquid nitrogen refrigeration)[13].

Fermentation Process

The process of fermentation begins with the inoculum or seed development stage on a laboratory scale, followed by a pilot plant inoculum development step. A typical seed medium provides a good source of nitrogen and carbon; contains sucrose or glucose sugars and corn-steep liquor or corn-steep protein. $CaCO_3$ is included as a buffer while other inorganic salts are required to assure maximal vegetative development. The cycle time, air flow rate, pH plateau, dissolved O_2 levels, CO_2, NH_3, respiratory quotient and residual carbon and N_2 concentrations are monitored during this step[14]. The industrial production of penicillin by fermentation is carried out by a submerged culture method. Various fermentation media have been used for the manufacture of penicillin; the most common one containing glucose or molasses, corn-steep liquor, $CaCO_3$, side chain precursors, surface active agents and mineral salts[15]. Addition of side chain precursors such as phenylacetic acid for benzyl penicillin (penicillin G) and phenoxyacetic acid for phenoxymethyl penicillin (penicillin V) is the standard procedure to increase the total production of penicillins and to direct the fermentation

towards a single penicillin. Although, penicillins are made by many species of fungi, *Penicillium chrysogenum* remains as the choice for the industrial production of penicillin[16].

Biosynthesis of Penicillins

The initial reaction of penicillin biosynthesis appears to be the condensation of L-cysteine and L-α-aminoadipic acid to form the dipeptide, L-α-aminoadipyl-L-cysteine[17], AC, (Figure 1). The reaction requires ATP and proceeds by the activation of the δ-carboxyl group of α-aminoadipic acid which then reacts with the α-amino group of cysteine to form the dipeptide, AC, the reaction being catalysed by AC synthetase[18]. The incorporation of L-valine into the dipeptide, proceeds by the activation of the carboxyl group of the cysteinyl residue of AC, which then condenses with L-valine and leads to δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine (ACV). In this reaction, there is inversion of configuration of L-valine to D-valine, catalysed by "ACV synthetase". The cyclization of LLD-ACV to isopenicillin N was first observed using a lysed protoplast preparation from *Cephalosporium acremonium* by Fawcett, *et al.*,[19]. Meesschaert, *et al.*,[20] observed this same cyclization using cell-free extracts of *Penicillim chrysogenum*. This is an oxidative step involving the removal of four protons and the closure of the two ring systems, and is catalysed by the enzyme cyclase. 6-Aminopenicillanic acid (6-APA) is the major penicillin derivative accumulating in precursor-free fermentation of penicillin-producing fungi[21], and is presumably obtained by the hydrolysis of isopenicillin N, catalysed by penicillin acyltransferase, which is intracellular and present in all penicillin-producing fungi. Conversion of isopenicillin N to penicillin N is catalysed by the enzyme epimerase which

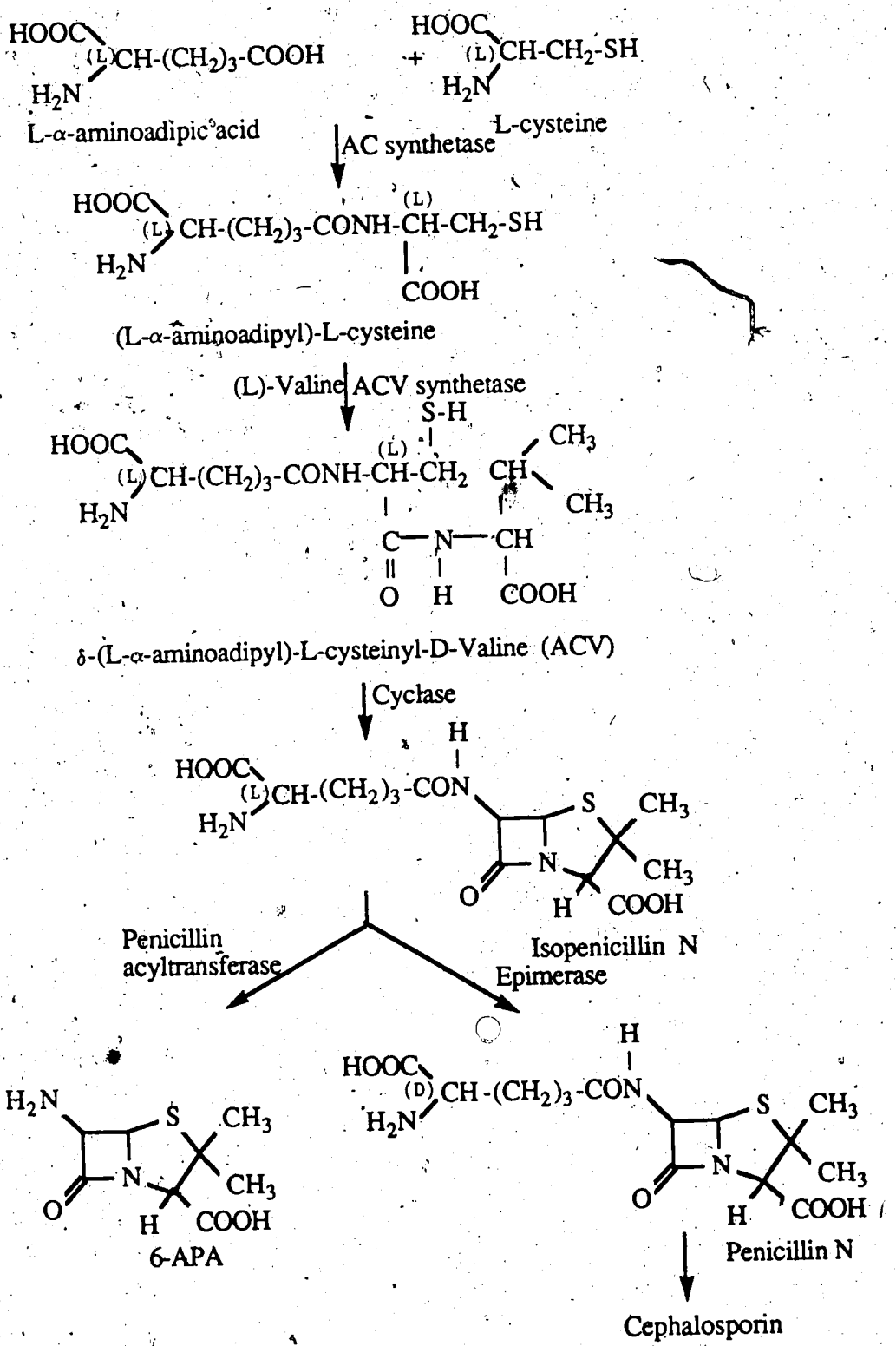


Figure 1. Pathway of Penicillin Biosynthesis.

isomerizes the L-aminoadipic acid side chain to the D-configuration. This was discovered for the first time in fresh cell-free extracts of *Cephalosporium acremonium*. This enzyme, epimerase, was found in only those organisms producing cephalosporins and not in penicillin-producing organisms. Cephalosporins are produced only from penicillin N, thus confirming epimerase activity in these organisms[22]. Chemical modifications of the 6-APA obtained by the biosynthetic route has yielded a number of semi-synthetic derivatives as clinically useful antibiotics.

C. *MODE OF ACTION OF PENICILLINS*

Bacterial Cell Wall

The bacterial cell wall is a highly complex structure consisting of multiple classes of polymers such as peptidoglycans, teichoic acids, polysaccharides and proteins[23]. Of all the constituents of the cell wall, peptidoglycans are of particular importance for their integrity is required for the maintainance of cell shape in bacteria and imparts the rigidity necessary to protect the bacteria from osmotic pressure[24]. The peptidoglycans also known as murein or mucopeptides, possess a heteroglycan backbone of alternating residues of N-acetylglucosamine and N-acetylmuramic acid, which are substituted by peptide chains and cross-linked to give a mesh like character to the peptidoglycan[25]. The synthesis of peptidoglycans proceed in three steps[26]. The first step is the synthesis of the peptidoglycan precursor, uridine 5'-diphosphate (UDP)-N-acetylmuramyl-pentapeptide, catalyzed on the inside of the cell by the cytoplasmic enzymes. This step is followed by the transfer of N-acetylmuramyl-pentapeptide and N-acetylglucosamine to a lipid carrier, in the membrane, forming a subunit of the glycan polymer. The membrane-

bound disaccharide pentapeptide is modified to include substitution of the carboxyl group of glutamic acid or diaminopimelic acid residues or attachment of a peptide side chain as in *S. aureus*. In the last stage the modified disaccharide-pentapeptide residue is transferred to a glycan acceptor on the outside of the cell to form the linear peptidoglycan which is finally cross-linked in a reaction catalyzed by the enzyme transpeptidase. The cross-bridge is formed between the carboxyl group of the penultimate D-alanine in the peptidoglycan of one chain and an amino group in a nearby chain, (Figure 2), with the release of D-alanine, catalysed by transpeptidase. This last step of peptidoglycan synthesis is the penicillin sensitive reaction.

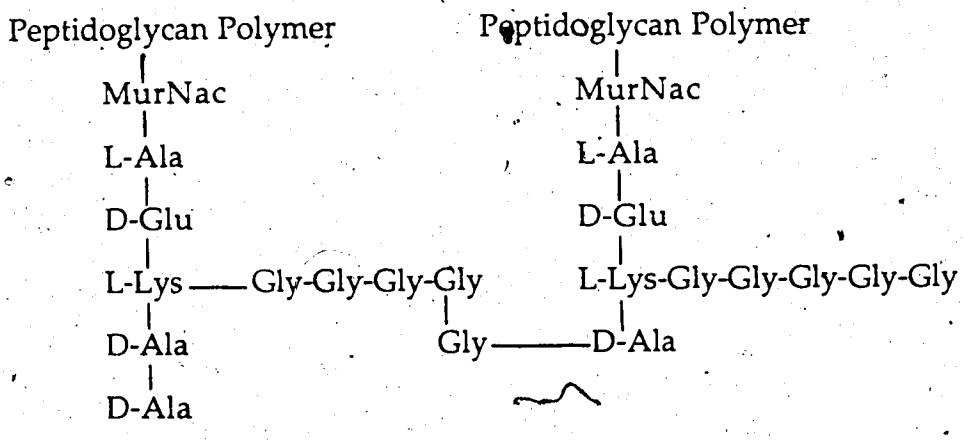


Figure 2. Transpeptidation in *Staphylococcus aureus*

The penicillins and cephalosporins show bactericidal effects because they disrupt the bacterial cell-wall synthesis by inhibiting the enzyme i.e., the transpeptidase [the Penicillin Binding Proteins, (PBPs)], that catalyzes the cross-linking reaction of D-alanyl peptides on peptidoglycan strands of the growing cell wall.

$$\text{X-D-Ala-D-Ala-COOH} + \text{Y-NH}_2 \longrightarrow \text{X-D-Ala-CONHY} + \text{D-Ala-COOH}$$

Transport of β-Lactams Through The Cell Wall

To inhibit the growth of micro-organisms the antibiotic has to acheive an inhibitory concentration at its target site. First, the antibiotic must cross the outer membrane by passive diffusion through channels formed by the "porin" proteins. These channels do present a barrier to free access to the intermembrane space (the periplasm). Recent studies by Nikaido, *et al.*,[27] have revealed that the wild type *Escherichia coli* K-12 produces two types of porins, OmpF (protein 1a) and OmpC (protein 1b). Rates of penetration of hydrophilic, uncharged solutes through the *Escherichia coli* channel are dependent on the size of the solute; bulky side chains at C-6 in penicillins and at C-7 in cephalosporins led to slower rates of penetration. With monoanionic β-lactams, the penetration rate depends on the hydrophobicity and electrical charges of the molecule. An additional negative charge drastically reduced the penetration rate through porin channels, whereas an additional positive charge significantly accelerated the penetration. The zwitterionic compounds penetrated rapidly[27]. The antibiotic then crosses the cell wall (the cross-linked peptidoglycan network) which presents no further barrier, because the "holes" in the network are large enough to allow the free passage of the antibiotic molecule. The β-lactam must then cross the periplasm on its way to the targets, which are the inner membrane enzymes that are responsible for the biosynthesis of the cell wall, (Figure 3)[28]. The nature of the bacterial cell wall viz., the outer membrane lipopolysaccharide, cytoplasmic lipid bilayer, and molecular weight and the charge present on the antibiotic molecule considerably affect the transport of the antibiotic to the target site[29].

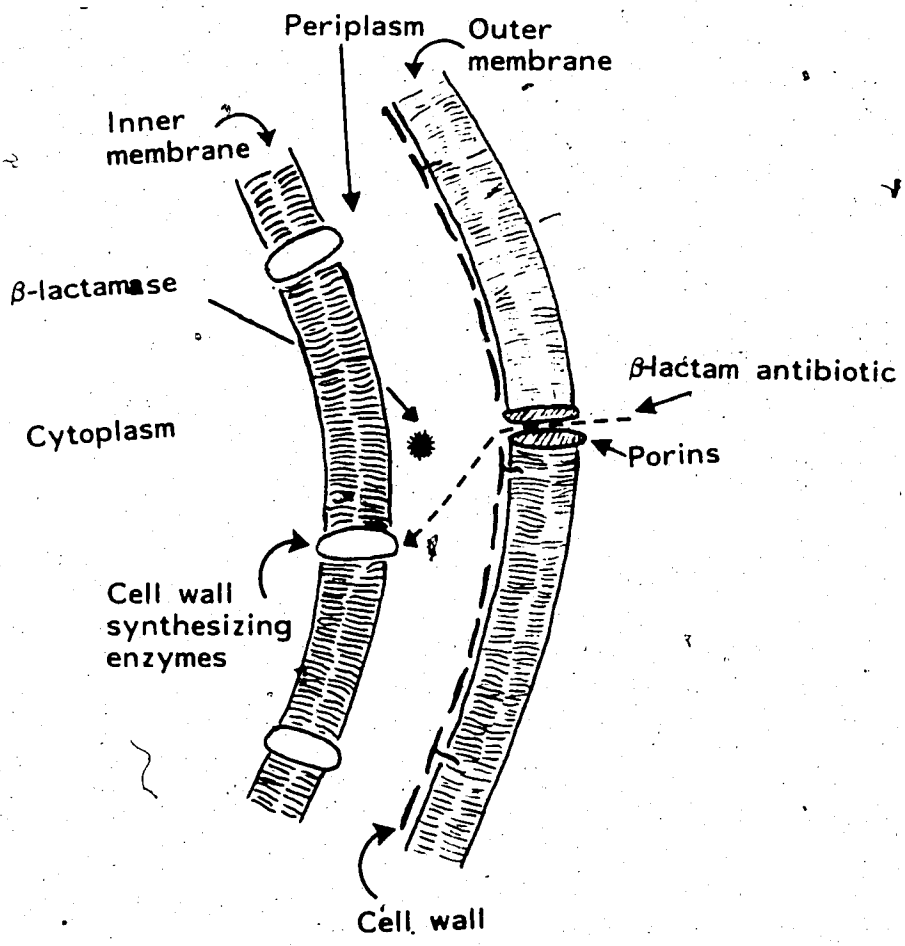


Figure 3. Section Through the Cell Envelope of Gram -ve Bacterium, Illustrating the Transport of Beta-Lactam Antibiotic.

Mechanism of Antibacterial Action

Penicillin was hypothesized to be an analog of the terminal D-alanyl-D-alanine in the peptidoglycan chain. The CO-N bond in the highly strained β-lactam ring would correspond to the peptide bond cleaved during transpeptidation. The transpeptidase would react with penicillin to split β-lactam ring and form a penicilloyl-enzyme complex which is stable and hence transpeptidase would be inactivated[24], interfering with the cell-wall synthesis. Subsequent studies have shown that several proteins in the bacterial membrane bind specifically to penicillin and these are called penicillin binding proteins (PBPs) and this suggests the possibility of multiple targets for penicillin action[30].

Penicillin Binding Proteins

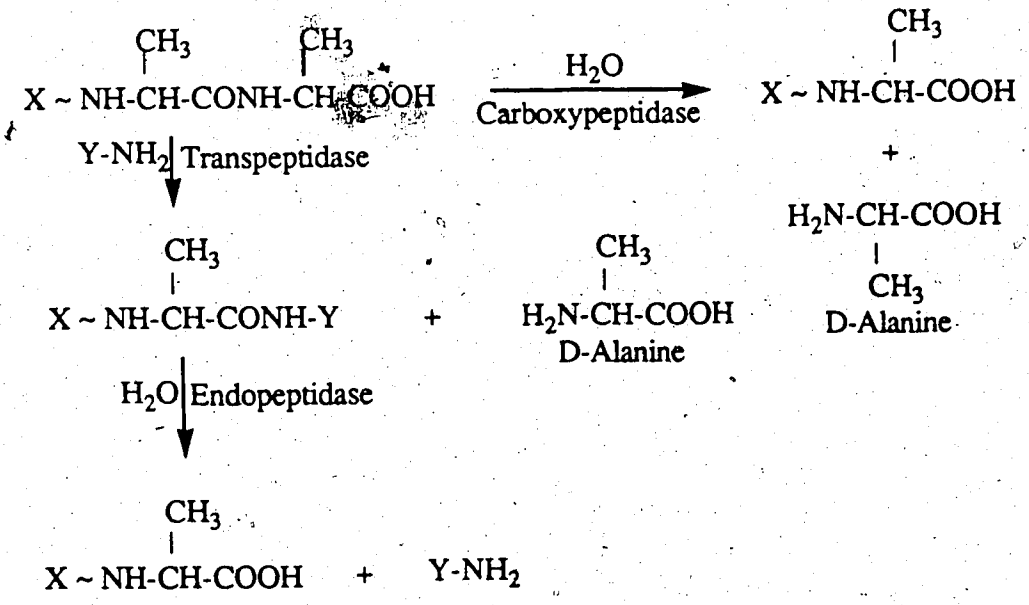


Fig. 4: Model Reactions catalyzed by D,D-Carboxypeptidase, Transpeptidase and Endopeptidase.

Carboxypeptidase catalyzes the removal of the carboxy terminal D-alanine and endopeptidase hydrolyzes the transpeptide bond. (Figure 4). The

transpeptidase, carboxypeptidase and endopeptidase reactions are penicillin-sensitive enzyme reactions and were detected in a large number of other bacterial species[31]. Blumberg and Strominger[24] have reported that penicillin forms covalent complexes with these enzymes. This, together with the earlier localization of cell-bound penicillin molecules to the bacterial plasma membrane, led to the introduction of radioactive penicillin for the visualization of bacterial proteins capable of covalently binding penicillins[32].

The procedure developed by Spratt[33] for *Escherichia coli* membranes is as follows: [^{14}C] benzylpenicillin or [^{14}C] mecillinam was bound to purified cell envelopes, prepared by sonication and differential centrifugation[34], the inner membranes were selectively solubilized with Sarkosyl NL-97, and the binding proteins separated on sodium dodecyl sulfate/polyacrylamide slab gels and detected by fluorography by incorporating the scintillant 2,5-diphenyloxazole (DPO) into the gel matrix and exposing the dried gel to Kodak RP Royal X-ray film at -70 ^{o}C[35]. This procedure resulted in the detection of a number of bacterial proteins capable of binding penicillins. The molecular size of the PBP, the relative and absolute amount of penicillin bound by them and the stability of the penicilloyl complexes varies from one bacterium to another. The number of PBPs and their molecular sizes are reproducible characteristics of bacterial species[33].

The binding reactions are specifically prevented by preincubation of the membranes with the non-radioactive penicillin or by denaturation of the proteins. Hydroxylamine treatment results in the release of radioactivity, in the form of penicilloic acid from the protein complexes. Their molecular weight ranges from 90,000 to 12,000 and are numbered in the order of decreasing molecular weight on the SDS-gel[36]. They are present in amounts

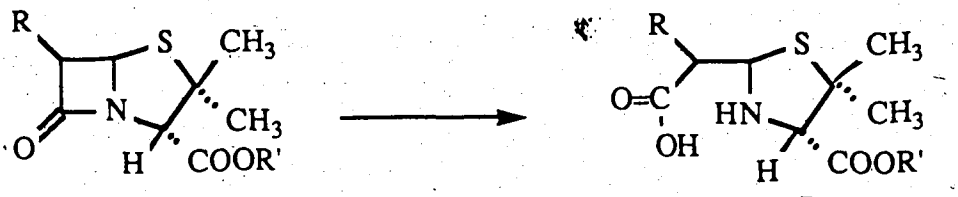
ranging from a few molecules to a several thousand. Several PBPs have been identified as carboxypeptidases and transpeptidases and their active sites seem to be oriented towards the outer surface of the plasma membrane[37]. Individual PBPs of a bacterium may have widely different selective affinities for structurally different β-lactam antibiotics[33]. Spratt[33] established the specific correlation between morphological effects and the inhibition of specific PBPs, by demonstrating that the selective inactivation of PBPs by an appropriate β-lactam, would result in specific morphological changes, in *E. coli* - β-lactam antibiotics that bind preferentially to binding protein 1 (molecular weight 91,000) inhibits cell elongation and causes cell lysis; β-lactam antibiotics that bind preferentially to binding protein 2 (molecular weight 66,000) specifically results in the production of ovoid cells; β-lactam antibiotics that specifically bind to binding protein 3 (molecular weight 60,000) inhibits cell division. In E. coli PBP 1A and PBP 1B are transglycosylase/transpeptidases involved in cell elongation, PBP 2 are transpeptidases that may initiate peptidoglycan insertion at new growth sites and PBP 3 are transglycosylase/transpeptidase required specifically for formation of the cross-wall in cell division[38a]. Similar effects were observed in several members of *Enterobacteriaceae* [36,38b]. Filament formation was observed in the Gram +ve *Clostridium perfringens* [39]. Even though mutants lacking specific PBPs 4, 5 and 6 showed no morphological effects and grew normally[38], in *E. coli* mutants lacking specific PBPs, PBP 4 seemed to be identical to the carboxypeptidase IB endopeptidase and as a secondary transpeptidase in maturation of peptidoglycan, while PBPs 5 and 6 were associated with major carboxypeptidase activity[40,41]. These PBPs (4, 5 and 6) perform less vital physiological functions.

D. *BACTERIAL RESISTANCE*

There are several ways in which a bacterial population can become resistant[42]. The target enzymes may become less susceptible to acylation and inactivation. Changes in the outer membrane permeability governed by porins may limit the access of the antibiotic to the periplasm so that the enzyme activity remains high enough to allow cell growth and division. Alterations in the activities of other enzymes responsible for the lysis of the cells, whose cell-wall synthetic apparatus has been blocked by the β-lactam, may lead to cell stasis rather than cell lysis and finally, the appearance of β-lactamases may result in the hydrolytic destruction of the antibiotic in the periplasm[43]. The last type of resistance is the most common.

E. *β-LACTAMASES*

β-Lactamases are the bacterial enzymes present in the bacterial of cell-wall that catalyze the hydrolysis of the cyclic amide bond of the β-lactam containing molecule. When the β-lactam ring of the penicillin **1**, is hydrolysed by the β-lactamases, antibiotically inactive penicilloic acid **7**, is produced in stoichiometric proportions.



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The majority of β-lactamases produced by Gram +ve organisms are inducible enzymes which appear in quantity only in the presence of an inducer[44]. They are extracellular, i.e., they are excreted into the environment.

Gram -ve organisms produce both inducible and constitutive enzymes which, with few exceptions, are cell-bound[45].

Action of β-Lactamases

The specific mechanism of hydrolysis may vary according to the substrate and the source of the enzyme. Initially, the reaction involves the formation of an enzyme-substrate complex (E.S) in which no chemical bonds are formed. This complex may undergo further modification[46] (E.S') and finally the release of the product results in a simple ring opened β-lactam.

$$E + S \rightleftharpoons (E.S) \rightleftharpoons (E\text{-}S') \longrightarrow E + P$$

β-Lactamases lack a rigid tertiary structure and are hence often described as floppy enzymes[47]. Koshland's concept[48] of "induced fit" explains the binding of the enzyme-substrate. This states that the formation of the enzyme-substrate complex is caused by the substrate's ability to induce a conformational change in the enzyme; the proper alignment of an active site amino acid, a single homologous serine, in this case, catalyzes the hydrolysis.

Classes of β-Lactamases

Different schemes have been proposed[45,49,50] for the classification of β-lactamases based on biochemical and genetic data. β-Lactamases of *S. aureus* have been serologically divided into four types, viz.; A, B, C and D, while those of *B. cereus* fall into two classes, viz.; I (mol. wt 30,000) and II (mol. wt 22,000). Those of aerobic Gram -ve bacteria are classified, with respect to the location of their structural genes in the cell, into chromosomal and extrachromosomal.

According to Richmond, *et al.*,[51] β-lactamases can be arbitrarily classified into four main classes:

Class I: Enzymes predominantly active against cephalosporins.

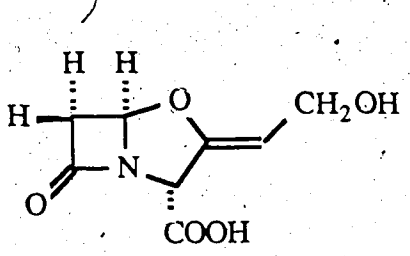
Class II: Enzymes predominantly active against penicillins.

Class III: Enzymes with approximately equal activity against penicillins and cephalosporins but sensitive to cloxacillin.

Class IV: Enzymes with approximately equal activity against penicillins and cephalosporins, but resistant to cloxacillin.

F. *β-LACTAMASE INHIBITORS*

Protection of β-lactam antibiotics from inactivation by β-lactamases can be achieved by incorporation of structural features which decrease susceptibility. Work has been done in two main areas: (1) the design of new β-lactams with increased stability to enzymatic hydrolysis, and (2) the development of inhibitors that bind to or inactivate β-lactamases, thereby affording protection for β-lactam substrates[52]. The promising β-lactamase inhibitors generally contain a β-lactam ring. They consist of two types: (1) the penicillins and cephalosporins which bind, at least to some extent reversibly, to the enzyme and subsequently are converted via the normal hydrolytic sequence to penicilloic acid or cephalosporonic acids, and their decomposition products, usually at a very slow rate[53]. (2) The second type act as "suicide" or "mechanism-based" reagents i. e., they are recognized by the enzyme as potential substrates resulting in the diversion from the normal course of the hydrolytic reaction, to inhibition and/or inactivation of the enzyme[54].



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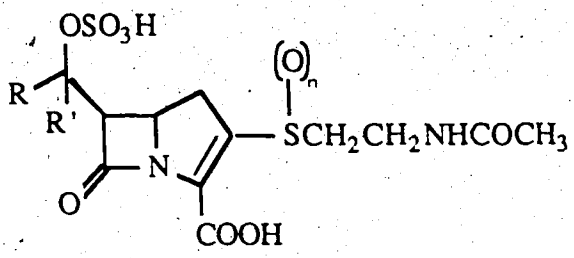

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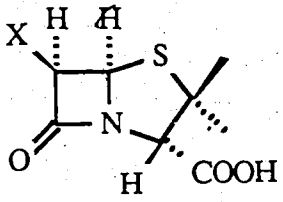

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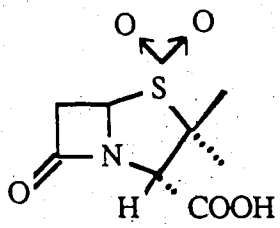


10

All these mechanism-based inhibitors of β-lactamases fall into two classes: (a) those that contain a hetero atom at the 1-position which may act as a leaving group from C-5 and (b) those, such as carbapenems, that do not[43]. The utility of such β-lactamase inhibitors started with the discovery of clavulanic acid[55] **3**. Other inhibitors resulting from research in this area include the carbapenems[56] **8**, halopenicillanic acids[57] **9** and penicillanic acid sulfones[58] **10**.

Mode of Action

The interaction of the penicillanic acid sulfones **10**, with the β-lactamases resulting in the inhibition of the enzyme is represented in Figure 5. Three distinct processes occur. First, the enzyme catalyzes the hydrolytic opening of the β-lactam ring. Second, at pH 8, the sulfone ring opens and the enzyme is converted into a transiently inhibited state. Third, further reactions ultimately lead to irreversible inactivation of the enzyme. In the hydrolytic reaction the active-site serine hydroxyl group attacks the β-lactam carbonyl to form a tetrahedral intermediate **A**, which then collapses to the

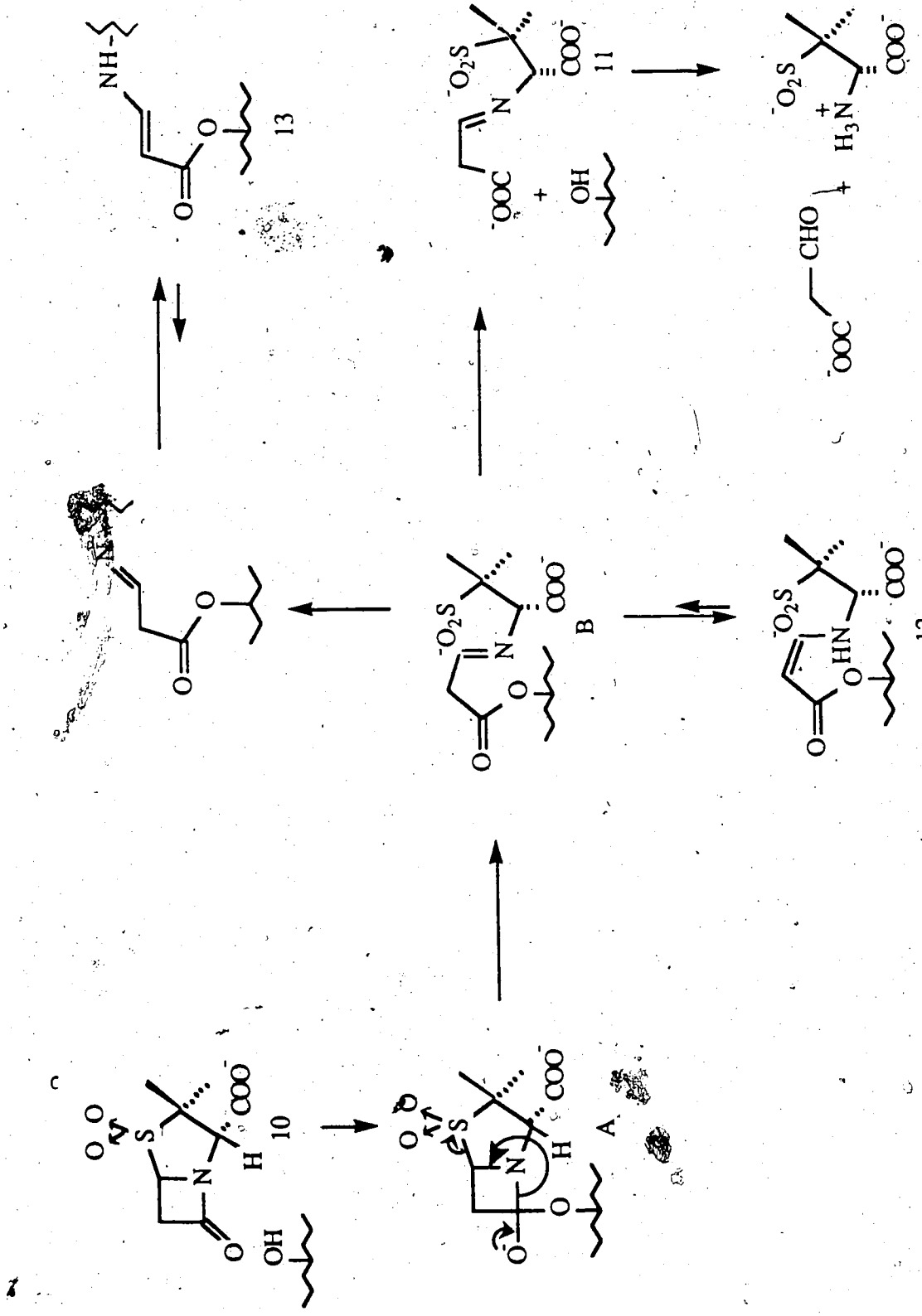


Fig. 5. Interaction of penicillanic acid sulfone, (10), with Beta-lactamases

acyl enzyme **B**, the deacylation of which liberates the imine **11**, which undergoes spontaneous hydrolysis to give the sulfinate of penicillamine and malonsemialdehyde[59]. The second reaction is the inhibition of the enzyme with the tautomerization of imine **B** to the enamine **12**[60]. The inactivation of the enzyme is due to the transimination reaction of **B** by an enzyme lysine residue giving **13**, an inactivated enzyme, in which two active site residues have been linked[61].

Methods of Detection

β-Lactamases can be detected by one of the following methods[62]:

(1) Acidimetric Method: β-Lactamase catalyzed hydrolysis leads to the formation of at least one extra carboxyl group. The generation of the extra carboxyl group can be detected with pH indicators, e.g., phenol red or bromocresol purple. Filter strips[63], capillary tubes[64] and the use of membrane filters[65] are different methods available for the detection of the extra carboxyl group formed after the hydrolysis of the β-lactam ring.

(2) Iodometric Method: The products of β-lactamase hydrolysis act as reducing agents, removing iodine from its starch complex resulting in the loss of color intensity. Filter strips[66], tubes[67] and plate[68] methods are available for the detection of β-lactamase hydrolyzed reducing agents by the iodometric assay.

(3) Microbiological Method: On hydrolysis by β-lactamases, β-lactam antibiotics lose all antibacterial activity and so the enzymes can be detected by microbiological assay techniques; the clover leaf[69] and double disc[70] methods are available.

(4) Chromogenic Method: Hydrolysis of certain β-lactam containing molecules by β-lactamases, leads to the formation of products having an

absorption spectrum in the visible range different from that of the parent compound. Thus the presence of β-lactamase can be detected by color changes in the solution. Nitrocefin[71], a cephalosporin, is the commonly used chromogenic reagent, that shows an absorption peak at 386 nm. Hydrolysis of nitrocefin by β-lactamases leads to the appearance of an absorption peak at 482 nm. N-(2-Furyl)acryloylpenicillin[72], is another reagent which shows diminution of absorption at 330 nm, after hydrolysis by β-lactamases.

G. *PENICILLINS*

After the large scale isolation of 6-aminopenicillanic acid (6-APA) by Batchelor, *et al.*,[73] efforts were directed towards producing structurally modified penicillins having:

(a) a greater degree of intrinsic activity and a wider spectrum than that possessed by penicillin G, along with acid stability and oral absorbability comparable to that of penicillin V

(b) a low degree of deleterious binding to serum proteins

(c) reduced allergenicity and

(d) resistance to microbial β-lactamases[74].

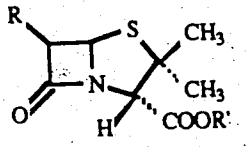
Classification

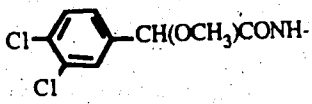
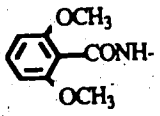
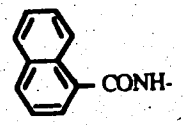
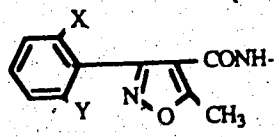
Numerous penicillin derivatives were synthesized and these may be divided into five main groups[75]

1. Those resembling benzyl penicillin, (Table 1,1-4).
2. Compounds mainly for use in staphylococcal infections (Table 1,5-11).
3. Penicillins related, or giving rise, to ampicillin, (Table 2).
4. Compounds resembling (in antimicrobial spectrum), or giving rise, to carbenicillin, (Table 3)
5. Pivmecillinam (Figure 6)

TABLE 1

Structure of Penicillins (1); R'=H.



		R
1.	Benzylpenicillin	$C_6H_5CH_2CONH-$
2.	Phenoxymethylpenicillin	$C_6H_5OCH_2CONH-$
3.	Azidocillin	$C_6H_5CH(N_3)CONH-$
4.	Clometocillin	
5.	Methicillin	
6.	Naficillin	
7.	Isoxazolylpenicillins	

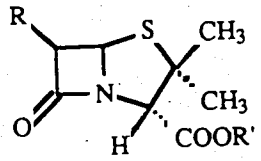
	X	Y
8.	H	H
9.	Cl	H
10.	Cl	Cl
11.	Cl	F

1 - 4: Conventional penicillins.

5 - 11: Antistaphylococcal penicillins.

TABLE 2.

Structures Of Penicillin (1) Resembling or Giving Rise to Ampicillin



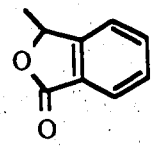
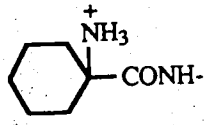
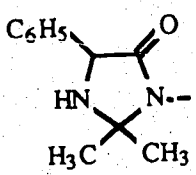
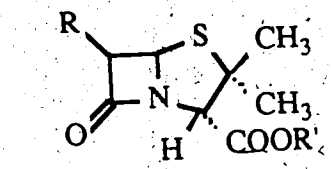
	Name of penicillin	R	R'
1.	Ampicillin	$C_6H_5CH(NH_3)^+CONH$-	H
2.	Bacampicillin	$C_6H_5CH(NH_3)^+CONH$-	$CH(CH_3)OCOOC_2H_5$
3.	Pivampicillin	$C_6H_5CH(NH_3)^+CONH$-	$CH_2OCOC(CH_3)_3$
4.	Talam pillin	$C_6H_5CH(NH_3)^+CONH$-	
5.	Amoxycillin	$HO.C_6H_4CH(NH_3)^+CONH$-	H
6.	Epicillin	$C_6H_5CH(NH_3)^+CONH$-	H
7.	Methampicillin	$C_6H_5CH(N:CH_2)CONH$-	H
8.	Cyclacillin		H
9.	Hetacillin		H

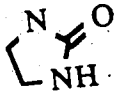
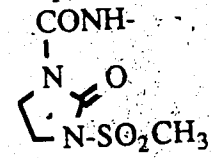
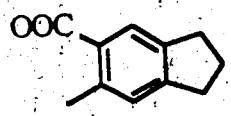
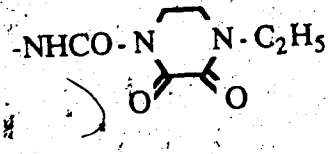
TABLE 3

Structure of Penicillins (1) resembling or giving rise to carbenicillin



R=YCHCONH- R'=H

X

Name of Penicillin	X	Y
1. Carbenicillin	COO-	C_6H_5-
2. Azlocillin	CONH- 	C_6H_5-
3. Mezlocillin	CONH- 	C_6H_5-
4. Carfecillim	$-COOC_6H_5$	C_6H_5-
5. Carindacillin		$-C_6H_5$-
6. Ticarcillin	COO-	
7. Pipercillin		$-C_6H_5$

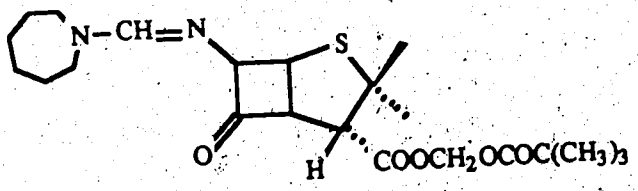


Figure 6. Structure of Pivmecillinam.

Requirements for Antibacterial Activity

The biological activity of this class of antibiotics is due to the chemical reactivity of the β-lactam amide bond; hindered amide resonance in the β-lactam ring is due to the pyramidal character of the β-lactam nitrogen atom. The pyramidalization is caused by the strain of the ring fusion in the case of penicillin or electron delocalization through enamine resonance outside the β-lactam ring as in cephalosporins[76].

The 3-dimensional aspects, although not clearly understood, may also play an important role in the biochemical processes involved. Conformational studies can reveal that precise geometrical requirements for the recognition of the antibiotics by the enzymes could exist. A structure exactly conforming to the precise geometrical requirements of the enzymes will possess antibiotic activity whereas a structure which does not conform will be unable to modify its geometry to adjust to these requirements and will have no antibacterial activity[77].

The presence of an amide bond does not assure a high degree of potency since the grouping adjacent to the amide carbonyl greatly influences the level of intrinsic activity. Maximal antistaphylococcal activity is obtained when the neighboring group is methylene as in benzyl penicillin (penicillin G). Thus for maximal intrinsic activity, the substituent at the C-6 position of penicillanic acid must be a β-substituted acetamido group[74,78].

Spectrum of Activity

Penicillin G is the antibiotic of choice for most infections caused by Gram +ve cocci, including *Staphylococci*. It is used in streptococcal pneumonia, streptococcal and meningococcal meningitis, group A streptococcal infections, streptococcal endocarditis, syphilis and gonorrhea.

Many clinical strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* have however developed total resistance to penicillin G.

Efforts to stabilize the molecule against the action of staphylococcal penicillinases led to oxacillin, nafcillin and methicillin. Methicillin is now the preferred antibiotic for treatment of infections caused by *Staphylococcus aureus*.

Development of acid-stable and orally absorbed penicillin derivatives like penicillin V and ampicillin appear to have solved the problem of chemical in-stability and poor oral absorption.

Development of ampicillin led to its wide use in pediatrics to treat infections caused by *Haemophilus influenzae, Neisseria meningitidis* and *Streptococcus pneumoniae;* while carbenicillin led to the expansion of activity to include Gram -ve organisms like *Enterobacteriaceae* and *Pseudomonas*.

Piperacillin and azlocillin have excellent *in vitro* activity against *Pseudomonas aeruginosa, Klebsiella* species, *Enterococci* and a few penicillinase-producing strains of *Staphylococcus aureus* [79].

Testing of Susceptibility to Antimicrobial Agents

An antimicrobial susceptibility test is the determination of the least amount of an antimicrobial chemotherapeutic agent that will inhibit the growth of micro-organisms [Minimum Inhibitory Concentrations (MICs)] *in vitro* [80]. For standardized, routine *in vitro* testing the environmental conditions must be optimal for the growth of organisms and the inoculum used must represent a good cross-section of the population. Variations in results are introduced by factors such as inoculum size, media, protein-binding and incubation time. Determination of the MICs has become a useful

reference point in evaluating the efficacy of antimicrobial agents by *in vitro* tests. Among the numerous methods available to determine the susceptibility of micro-organisms, the agar dilution method and the broth dilution method are most widely used.

The principle for the diffusion method is dependent upon the inhibition of the growth of a micro-organism, on an inoculated agar plate, by the antimicrobial agent that diffuses from a depot in the medium. The zone of inhibition is dependent upon (a) the diffusibility of the agent and (b) the degree of susceptibility of the organism. Thus the diameter of the zone of inhibition is related to the MIC.

The principle behind the dilution method is the inhibition of growth of the test organism by an antimicrobial agent incorporated into a broth or agar media that is optimal for growth and does not neutralize the agent used. The agent may be serially double-diluted, in sequence, in test tubes or a specific amount of the agent may be added to each tube in ascending or descending order. The advantage of this method over the diffusion method is that it permits the determination of both the MIC and the minimum bactericidal concentration (MBC). The MBC is determined by sub-culturing the broth from the tube that shows gross inhibition of growth onto an appropriate media. The susceptibility is defined in specific μg/ml[80].

H. *PENICILLIN-1-SULFOXIDES*

Benzylpenicillin sulfoxide methyl ester[81] was one of the early derivatives of penicillin prepared but was found to be inactive[82]. Free acid forms of several penicillin sulfoxides were made with the hope that they might possess a higher degree of activity and also exhibit desirable properties not shown by the conventional penicillins. Although, the sulfoxides of

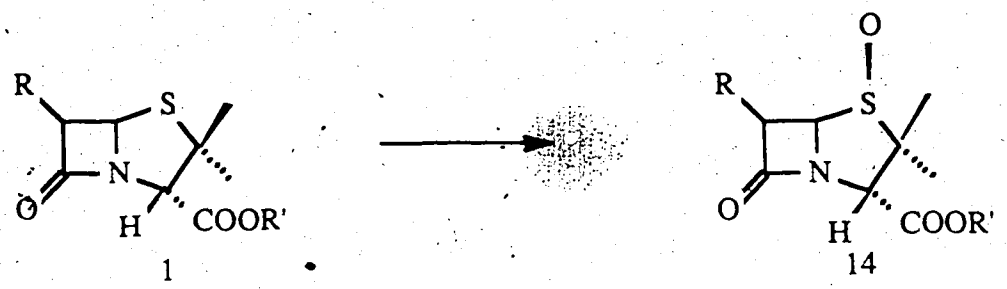
penicillins were inactive they possessed stability to acidic and basic conditions superior to the parent penicillins[81]. Since the report by Morin, *et al.*,[83] describing the acidic rearrangement of penicillin sulfoxide to the cephalosporins, investigations into the chemistry of penicillin sulfoxides has increased. A review by Cooper and Spry[84] outlines the utility of penicillin sulfoxide as an economic starting material for the synthesis of more inaccessible cephalosporins. The reason for the importance of penicillin sulfoxide is that the thiazolidine ring can be quantitatively opened under relatively mild conditions without the loss of stereochemical integrity of carbons 5 or 3 in the starting penicillin. Penicillin sulfoxide is in thermal equilibrium with an unstable ring open sulfenic acid olefin. Electrophilic agents can be added to the double bond to form the cephalosporin ring system or 2-(substituted methyl)penams. The ring opening and the addition reactions are stereospecific[85].

Methods of Oxidation

A number of methods are available for the oxidation of penicillins to their sulfoxides. Some of the oxidizing agents used are sodium metaperiodate, *m*-chloroperbenzoic acid, peracetic acid, hydrogen peroxide/ acetic acid, iodobenzene dichloride and ozone.

Using Sodium Metaperiodate:

Treatment of a solution of benzyl-6β-phenylacetamidopenicillanate **1a**, or benzyl-6β-phenoxyacetamidopenicillanate **1b**, in dioxane and phosphate buffer (pH 6.8) with a solution of sodium metaperiodate gave the benzyl-6β-phenylacetamidopenicillanate-1β-oxide **14a**, and benzyl-6β-phenoxyacetamidopenicillanate-1β-oxide **14b**, in 27% and 57% yields respectively[86].



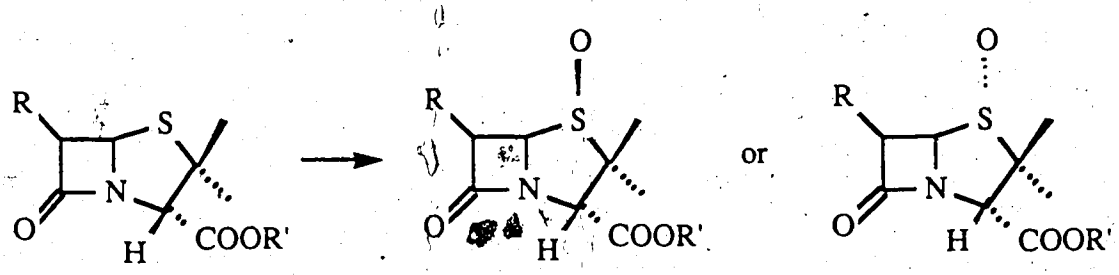
1 14

a. $R=C_6H_5CH_2CONH-$ $R'=-CH_2C_6H_5$

b. $R=C_6H_5OCH_2CONH-$ $R'=-CH_2C_6H_5$

Using *m*-Chloroperbenzoic acid:

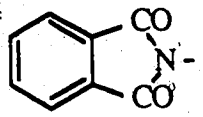
The reaction of a solution of 2,2,2-trichloroethyl-6β-phenylacetamido-penicillanate **1c**, or 2,2,2-trichloroethyl-6β-phenoxyacetamidopenicillanate **1d**, in chloroform with a solution of 85% *m*-chloroperbenzoic acid in chloroform in an ice-water bath gave the β-sulfoxides of penicillins **14c**, and **14d**, in 84% and 94% yields respectively[87], while the reaction of methyl-phthalimidopenicillanate **1e**, under similar conditions gave the methyl-phthalimidopenicillanate-1α-sulfoxide **15e**, as the major product[88].



1 14 15

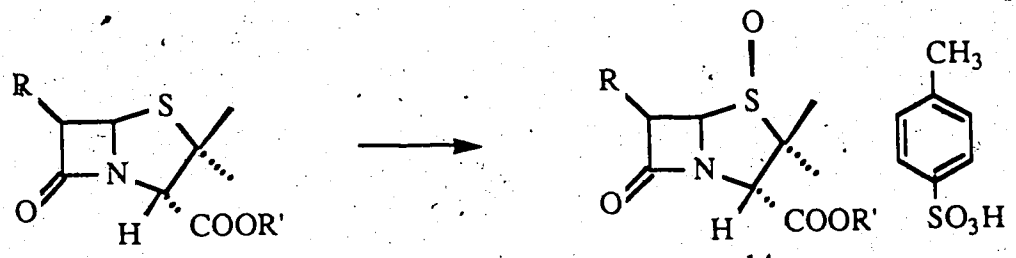
c. $R=C_6H_5CH_2CONH-$ $R'=-CH_2CCl_3$

d. $R=C_6H_5OCH_2CONH-$ $R'=-CH_2CCl_3$

e. R=  $R'=-CH_3$

Using Peracetic acid:

To a stirred cold suspension of 6-aminopenicillanic acid **1f**, p-toluene sulfonic acid was added followed by peracetic acid. After one hour a white precipitate of 6-aminopenicillanic acid-β-sulfoxide p-toluenesulfonate **14f**, was obtained in 63% yield. Under similar conditions, ampicillin **1g** gave ampicillin sulfoxide **14g**[89].



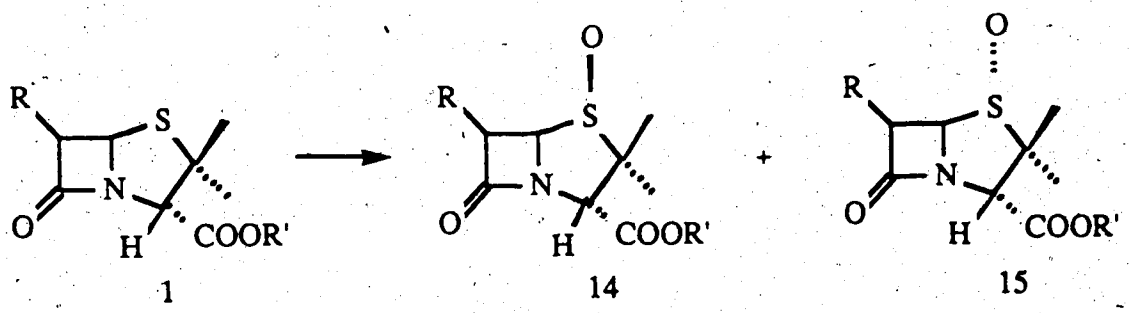
1 14

f. . . R=NH_2 R'=H

g. R=$C_6H_5CHCONH$- R'=H
|
NH_2

Using Iodobenzene dichloride:

The oxidation of methyl-6β-phenylacetamidopenicillanate **1h**, by iodo benzene dichloride in aqueous pyridine gave the β- and α- sulfoxides **14h**, and **15h** respectively, in approximately a 1:1 ratio[90].

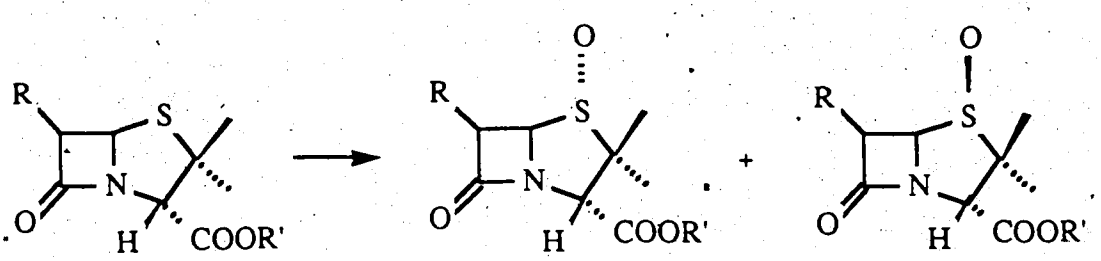


1 14 15

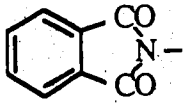
h. R=$C_6H_5CH_2CONH$- R'=-CH_3

Using Ozone:

Bubbling ozone into a cooled solution of 6-aminopenicillanic acid **1i**, in water, in an ozonizer, for 3 h followed by lyophilization gave the β- and α-sulfoxides **14i** and **15i**, (98% yield) in the ratio 4:1 respectively, while a solution of phenoxyacetamidopenicillanic acid **1j**, in 1:1 acetone-water gave the two sulfoxides β- and α- sulfoxides **14j**, and **15j**, in the ratio 1:1; and the oxidation of the bulky β-phthalimidopenicillanic acid **1k**, gave only α-sulfoxide **15k**[91].



1 15 14

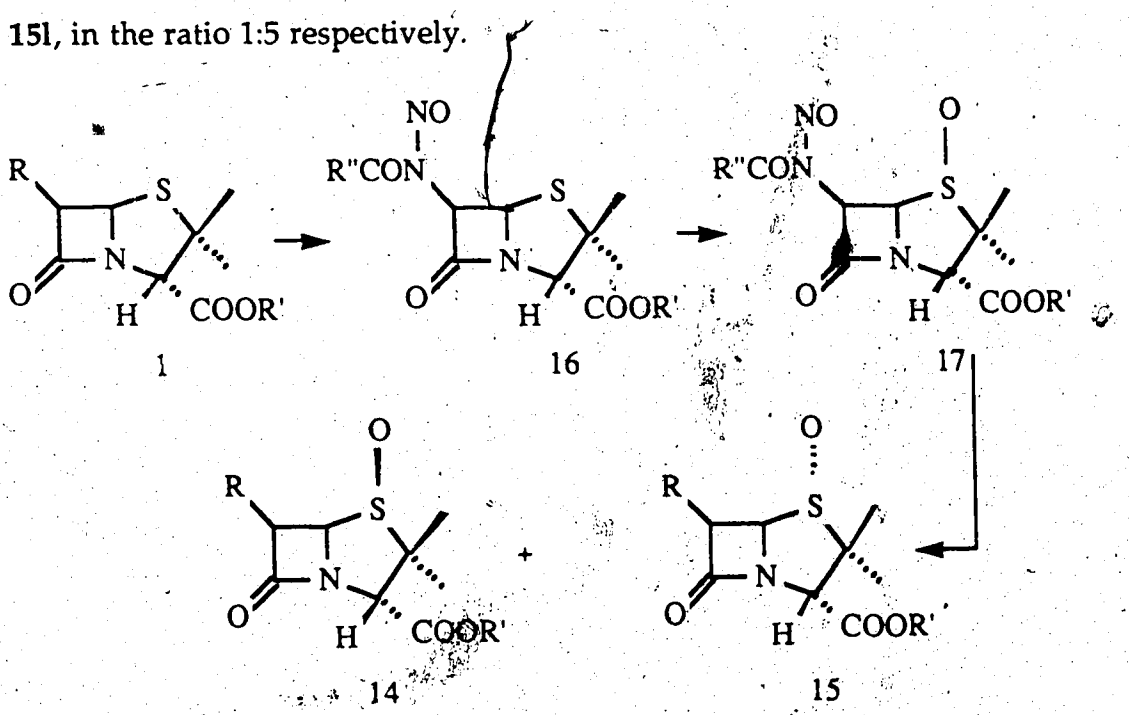
R		
i. NH_2	1	4
j. $C_6H_5OCH_2CONH$-	1	1
k. 	1	0

R'=H

The preferential formation of the β-sulfoxides **14**, by most oxidizing agents, which tends to oxidize normal sulfides under steric approach control, suggests that a powerful directing influence must be present in the penicillin molecules. The β-sulfoxides **14**, although more sterically hindered,were the result of hydrogen bonding between the 6β-amido proton with the oxidants or the stabilization by an intra 6β-amido proton-sulfoxide hydrogen bond[88,90].

Preparation of α-Sulfoxides

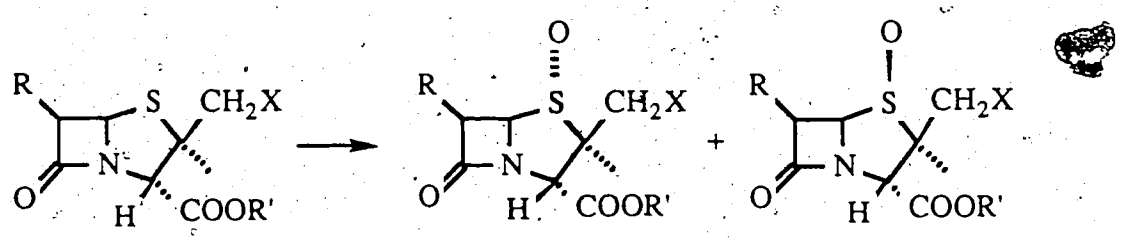
Blocking the NH- group in the C-6 position of penicillins, with an easily removable group followed by peracid oxidation results in the predominant formation of α-sulfoxides due to the attack from the less hindered α- side. The N-nitroso group was selected by Uye, *et. al.*,[92] since preparation of N-nitrosopenicillin and removal of the N-nitroso functionality occur in good yields[93]. Benzhydryl-phenoxyacetamido-penicillanate **11**, was converted into its N-nitroso derivative **16**, using nitrogen tetroxide, and the product oxidized immediately with *m*-chloroperbenzoic acid to the unstable N-nitrosopenicillin sulfoxide **17**, which on treatment with zinc and acetic acid at 0 °C gave the β- and α- sulfoxides **14**l and **15**l, in the ratio 1:5 respectively.



l. R=R"CONH- R'=-$CH(C_6H_5)_2$ R"=$C_6H_5OCH_2$-

The methyl-6-di(phenylacetylamino)penicillanates **18**, (X=H), on oxidation with *m*-chloroperbenzoic acid in methylene chloride at 0 °C, gave a mixture (58:42) of the 1α-, **15**, and the 1β- sulfoxide **14**, while the 2β-

(chloromethyl)derivative **18**, (X=Cl), on oxidation gave exclusively (100%) of the 1α-derivative **15**, (X=Cl)[94].



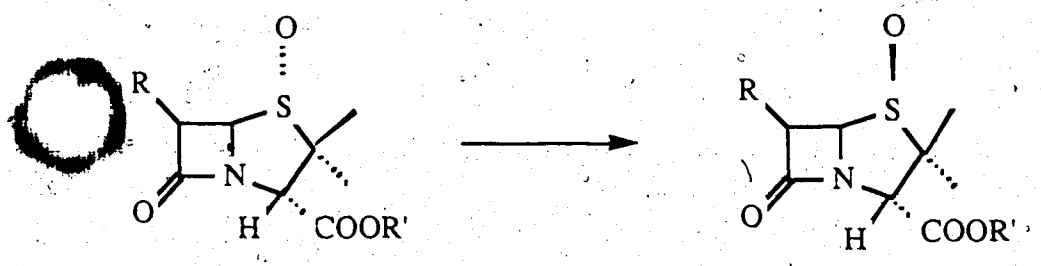
18 15 14

R=$(C_6H_5CH_2CO)_2N$- R'=-CH_3 X=H

R=$(C_6H_5CH_2CO)_2N$- R'=-CH_3 X=Cl

Interconversion of Penicillin Sulfoxides

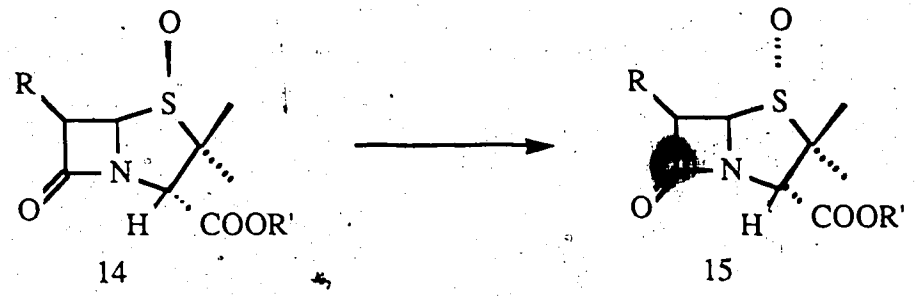
The methyl-phenylacetamidopenicillanate-1α-sulfoxide **15h**, in refluxing benzene was converted into the β-sulfoxide **14h**[90].



15 14

h. R=$C_6H_5CH_2CONH$- R'=-CH_3

Irradiation of the methyl-acetamidopenicillanate-1β-sulfoxide **14m**, or the methyl-phenoxyacetamidopenicillanate-1β-sulfoxide **14n**, in acetone gave a single product viz., the α-sulfoxide of the respective penicillins **15m**, **15n**, confirmed by spectrophotometric and chemical methods[95].



14 15

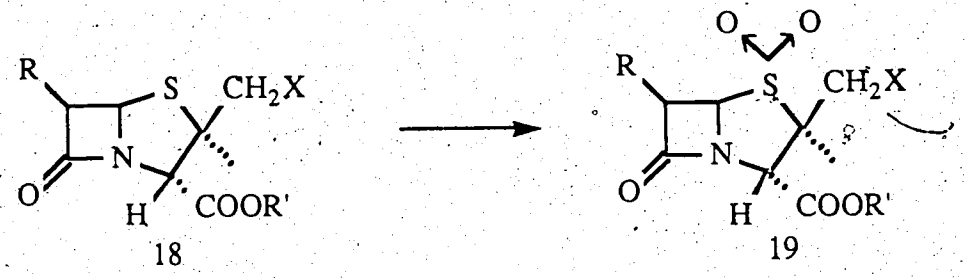
m. R=CH_3CONH- R'=-CH_3

n. R=$C_6H_5OCH_2CONH$- R'=-CH_3

I. *PENICILLIN SULFONES*

The first penicillin sulfone to be reported as a β-lactamase inhibitor exhibiting synergy with appropriate β-lactams against a variety of bacteria was 6-desaminopenicillanic acid sulfone **10** (CP 45899)[58]. Similar to the well known β-lactamase inhibitor, clavulanic acid[55] **3**, it lacks an acyl-amino side chain and has a weak C-5 to sulfur bond, which is the driving force for the fragmentation occurring after formation of an acyl intermediate with β-lactamases[48]. However, the spectrum of inhibition of β-lactamases is different from that of clavulanic acid. The 6α-chloropenicillanic acid sulfone efficiently and irreversibly inactivates extracellular penicillinase from *Staphylococcus aureus* [96]. Facile epimerization at the 6α- position would be expected to play a role in the inactivation process. Both the 6β-bromo and the 6β-iodopenicillanic acids have been shown to exhibit synergistic activity with ampicillin comparable to that of clavulanic acid and greater than that of sulbactam[97]. The discovery of penicillanic acid sulfones[58] as β-lactamase inhibitors led to the oxidation of other penicillin derivatives to their sulfones followed by testing for β-lactamase inhibition. Some of the recently developed inhibitors include sulfones of the 2β-(substituted

methyl)penicillins **19**, which are usually prepared by the oxidation of the respective penicillins **18**, with potassium permanganate[98,114] or *m*-chloroperbenzoic acid[99] as oxidizing agents.



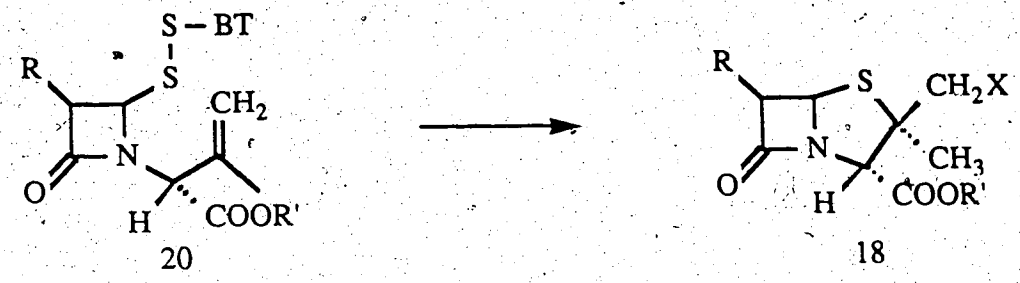
18 **19**

R=H R'=Na, K X=Cl, Br, N_3, OH

J. 2-(SUBSTITUTED METHYL)PENAMS

Reactions of the penicillin sulfoxide **14**, with heteroaromatic thiols are known to readily produce the dithioazetidinones **20**[100]. Thus, treatment of penicillin sulfoxide **14**, with 2-mercaptobenzothiazole in refluxing toluene for 4 h gave the crystalline *unsym*-azetidinone disulfide **20**, which is a very useful intermediate in the synthesis of numerous penicillin and cephalosporin derivatives.

Treatment of the *unsym*-azetidinone disulfide **20a**, or **20b**, with chlorine or cupric chloride, in methylene chloride, afforded the 2β-(chloromethyl)penams **18**, (X=Cl), the configuration of which was confirmed by internal Nuclear Overhauser Effect (NOE) and solvent-induced shifts. Bromine or cupric bromide under similar conditions gave the 2β-(bromomethyl)penams[100] **18** (X=Br).



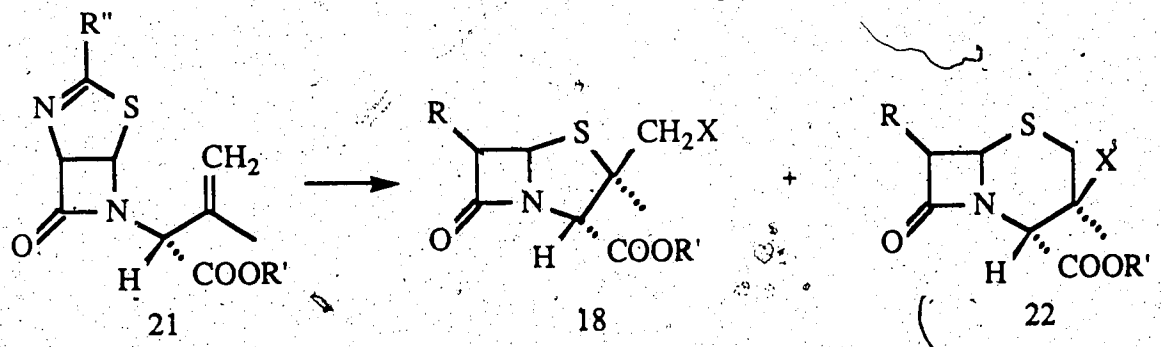
20 18

a. R=$C_6H_5CH_2CONH$- R'=-CH_3 X=Cl BT=

b. R=$C_6H_5OCH_2CONH$- R'=-CH_2CCl_3 X=Br

The conversion of azetidinone disulfides **20**, and thiazoline azetidinones[101] **21**, to the 3-iodocephams **22**, (X=I), and the 2β-(iodomethyl)penams **18**, (X=I), has been reported[102].

The thiazoline azetidinone **21**, obtained in 80% yield by the reaction of penicillin sulfoxide esters **14f**, with trimethylphosphite in refluxing benzene for 30 h, on reaction with iodine in a suitable organic solvent and in presence of moisture in 1-3 h gave the 2β-(iodomethyl)penam **18**, (X=I), in 80% yield which was then converted to the 3-iodocephams **22**, (X=I), with time.

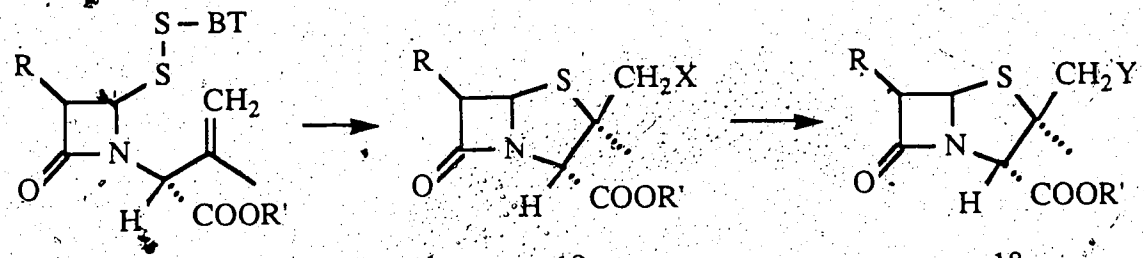


21 18 22

R=R"CONH- R=$C_6H_5OCH_2$ R'=-$CH(C_6H_5)_2$ X=I

Reaction of the *unsym*-azetidinone disulfide **20**, with chloroacetic acid and silver acetate gave, after chromatography, 35-40% of the 2β-(chloroacetoxymethyl)penam **18**, (X=$OCOCH_2Cl$), along with 30% of 3-chloroacetoxycepham **22**, (X=$OCOCH_2Cl$), and 8% cephem **26**. Treatment of

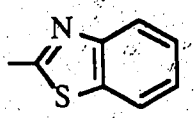
the penam **18**, (X=$OCOCH_2Cl$) with thiourea in ethanol at 60 °C for 30 mins gave 74-84% yield of crude 2β-(hydroxymethyl)penam **18**, (Y=OH), the structure of which was confirmed by physical data[103].



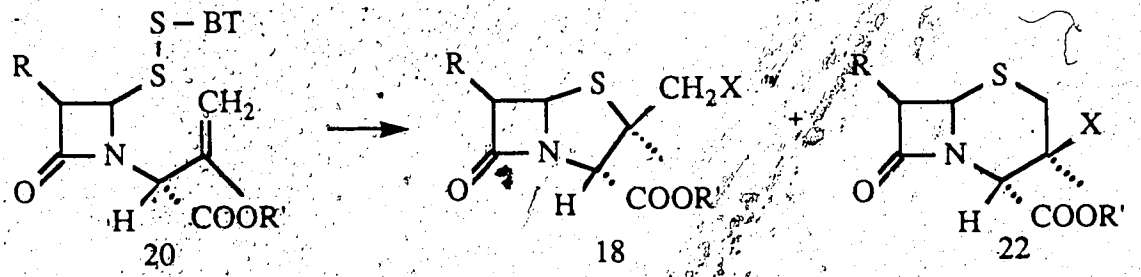
20 **18** **18**

c. R=$C_6H_5OCH_2CONH$- X=$OCOCH_2Cl$ BT=

R'=-$CH_2C_6H_4$-p-NO_2 Y=OH



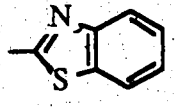
The *unsym*-azetidinone disulfide **20**, on treatment with silver oxide, acetic acid and iodine for 15 mins at room temperature or silver acetate, glacial acetic acid and iodine for 1 h is reported to afford the 2β-(acetoxymethyl)penam **18**, (X=$OCOCH_3$), and 3-acetoxycepham **22**, (X=$OCOCH_3$) in the ratio 2.5:1[104].



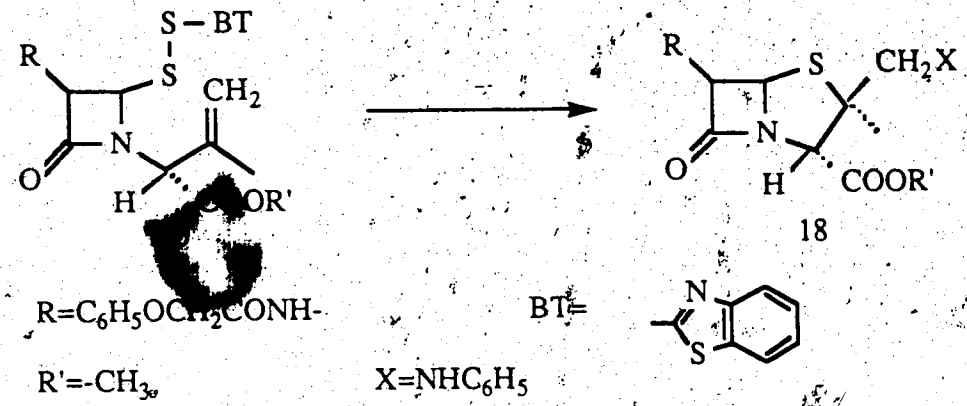
20 **18** **22**

d. R=$C_6H_5OCH_2CONH$- X=$OCOCH_3$ BT=

R'=-CH_3

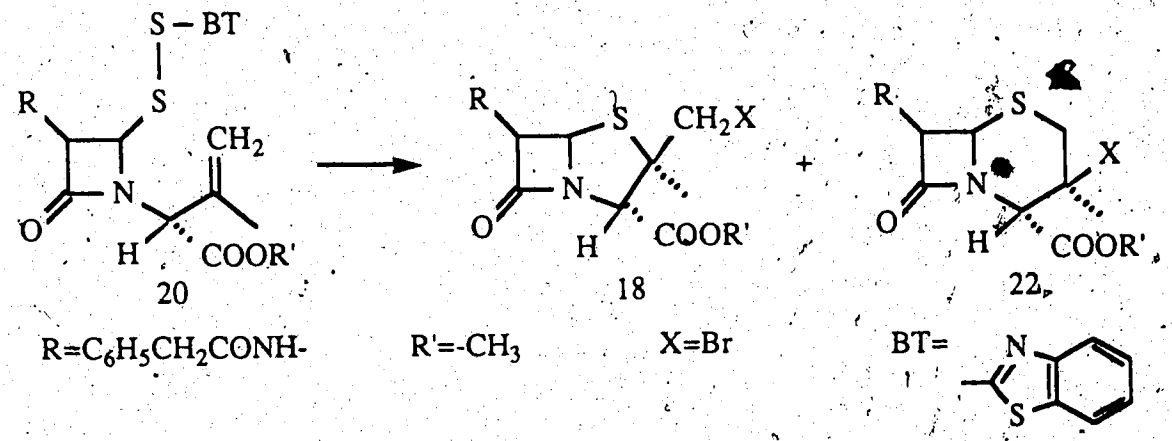


The reaction of *unsym*-azetidinone disulfide **20**, with aniline in ethyl acetate in the presence of silver acetate is reported to afford the 2β-(anilinomethyl)penam **18**, (X=NHC_6H_5), in 90% yield[105].



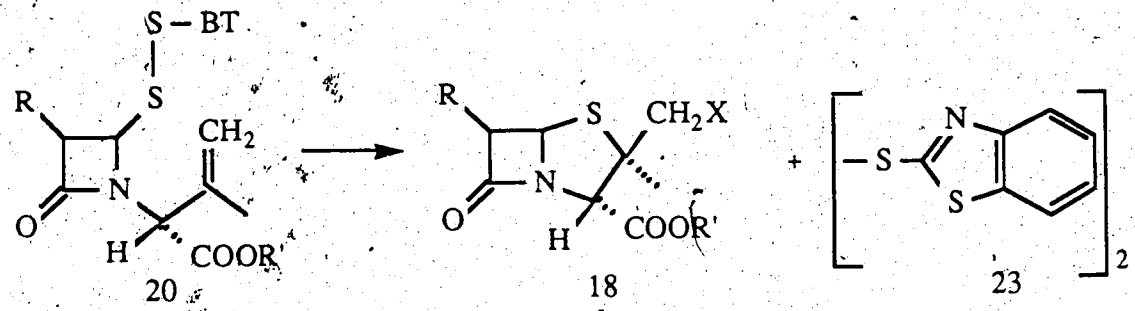
An electrochemical S-S bond cleavage of the *unsym*-azetidinone disulfide **20**, derived from benzylpenicillin led to 2β-(halomethyl)penams **18**, (X=Cl, Br), and 3-halocephams **22**, (X=Cl, Br).

A solution of the *unsym*-azetidinone disulfide **20**, and magnesium bromide in acetonitrile, tetrahydrofuran and water when electrolyzed at 10 mA/cm^2 at 23-25 °C, after 35 mins gave 52% of 2β-(bromomethyl)penam **18**, (X=Br), and 44% of 3-bromocepham **22**, (X=Br). Other alkali metal salts, NaBr and KBr or HBr afforded the above mixture in 46-73% yields[106].

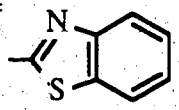


Electrolysis of the *unsym*-azetidinone disulfide **20**, in acetonitrile under 8 mA constant current, in presence of monochloroacetic acid and stoichiometric amounts of tetramethylammonium bromide in water, yielded

exclusively 2β-(bromomethyl)penam **18**, (X=Br), accompanied by 2-benzothiazolyl disulfide[107] **23**.

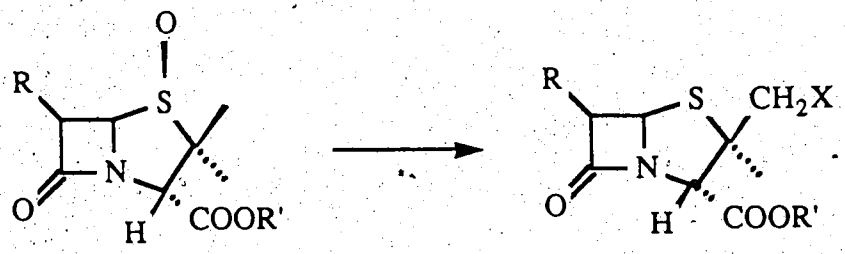


e.R=$C_6H_5CH_2CONH$- R'=-CH_2CCl_3 X=Br BT=



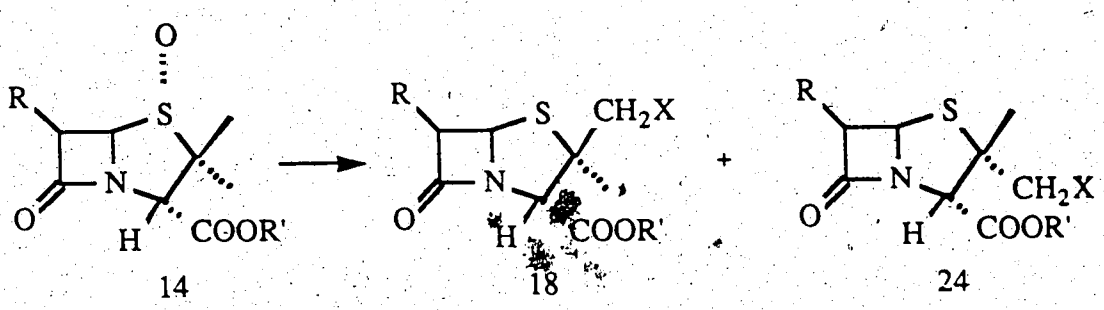
c.R=$C_6H_5OCH_2CONH$- R'=-$CH_2C_6H_4$-p-NO_2

A toluene solution of the benzylpenicillin-1β-sulfoxide ester **14o**, (or an analog having a secondary amide side chain) on reflux with one equivalent of acyl halide (or aryl halide) and pyridine, for 1-2 h, gave the 2β-(chloromethyl)penam **18**, (X=Cl), in 30-40% yield, depending upon the C-6 amide chain and the acyl halide employed[108].

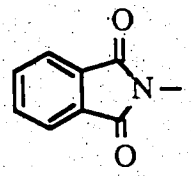


o. R=$C_6H_5CH_2CONH$- R'=-$CH(C_6H_5)_2$ X=Cl

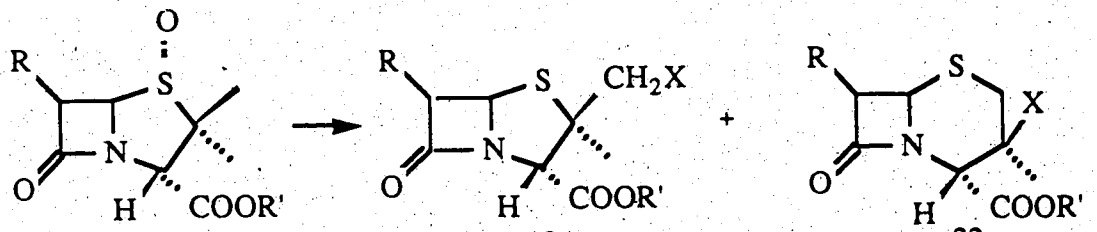
An identical reaction with the phthalimidopenicillin-1α-sulfoxide methyl ester **15e**, as substrate, yielded a mixture of two β-lactam derivatives viz., the 2β-(chloromethyl)penam **18**, (X=Cl), and 2α-(chloromethyl)penam **24**, (X=Cl), in 67% yield in the ratio 53:47.



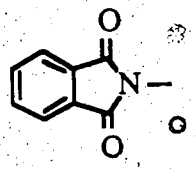
14 18 24

R=  R'=-CH_3 X=Cl

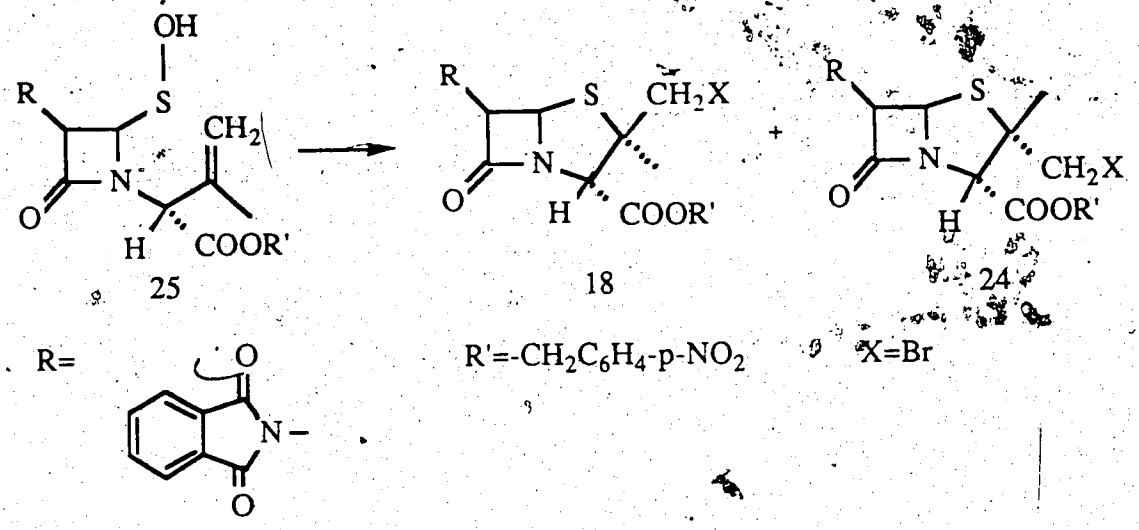
The sulfenic acid, prepared by the thermolysis of penicillin sulfoxide **15e**, was converted to the sulfenyl chloride with thionyl chloride and triethylamine in carbon tetrachloride. This highly reactive intermediate instantly cyclized to two stable products viz., 2β-(chloromethyl)penam **18**, (X=Cl), and 3-chlorocepham **22**, (X=Cl), in the ratio 3:4[109].



15 18 22

R=  R'=-CH_3 X=Cl

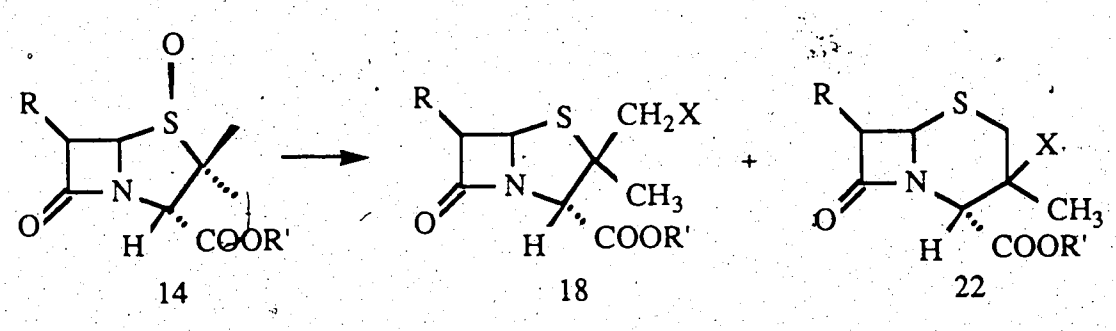
Slow addition of the *p*-nitrobenzylsulfenic acid[110] **25**, obtained from the penicillin sulfoxide **14**, to a cold solution of phosphorus tribromide in methylene chloride, followed by reflux for 1 h gave a mixture of 2β-(bromomethyl)penam **18**, (X=Br), and 2α-(bromomethyl)penam **24**, (X=Br), in the ratio 1:1[111].



25 18 24

R= R'=-$CH_2C_6H_4$-p-NO_2 X=Br

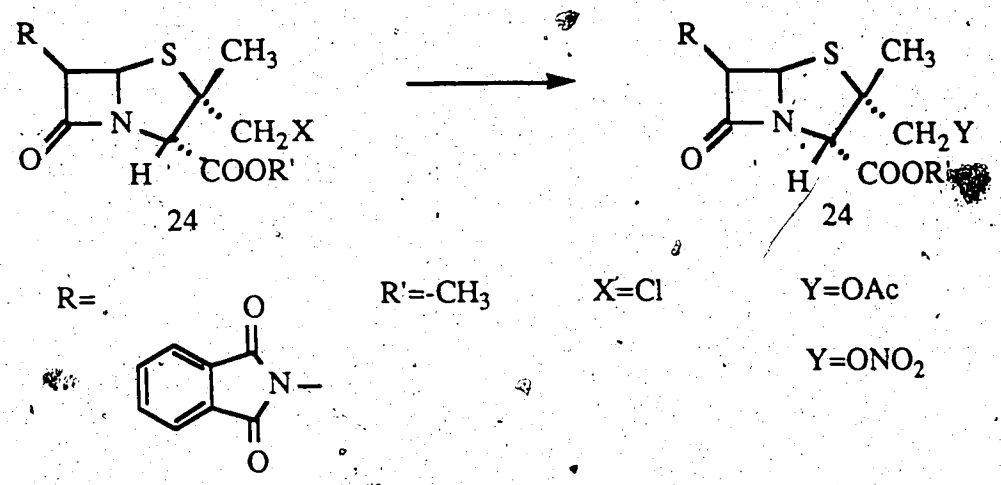
The penicillin sulfoxide **14**, on reflux with acetic anhydride gave a mixture of 2β-(acetoxymethyl)penam **18**, (X=OAc), and 3-acetoxycepham **22**, (X=OAc), in 30 mins in the ratio 1:2 in 60% yield[112].



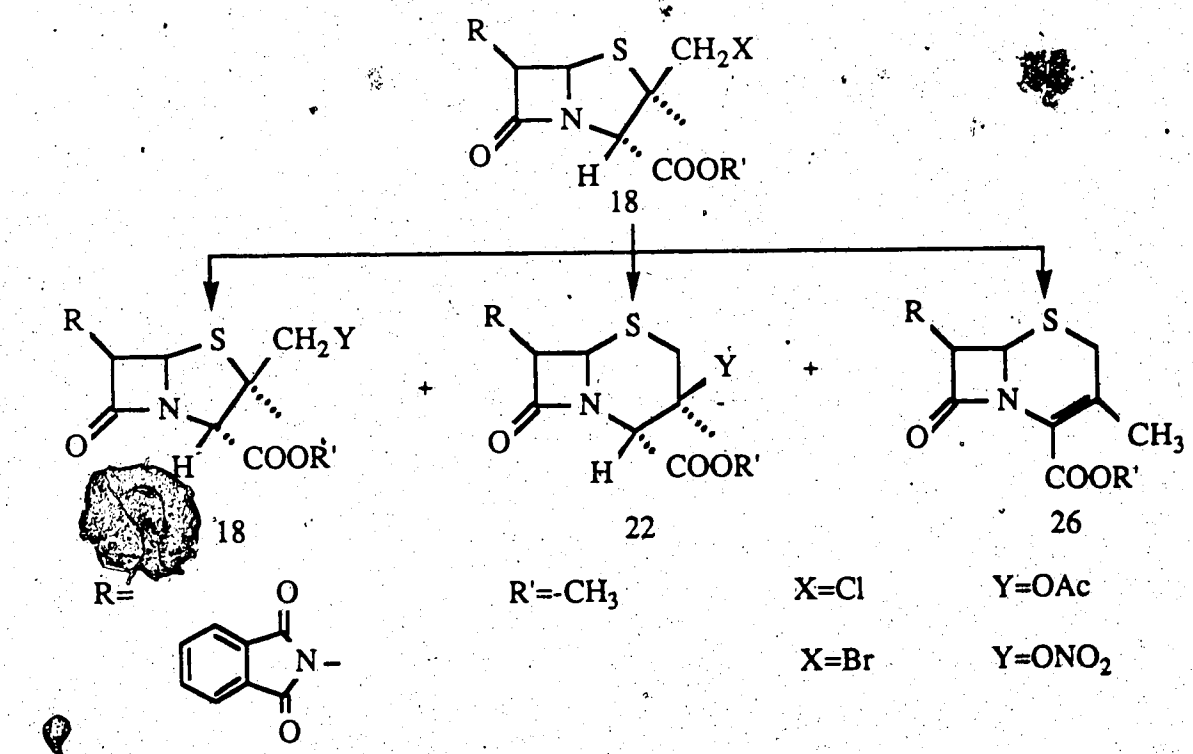
14 18 22

R=$C_6H_5OCH_2CONH$- R'=-CH_3 X=OAc

The 2α-(chloromethyl)penam **24**, (X=Cl), when treated with silver acetate and acetic acid gave 2α-(acetoxymethyl)penam **24**, (Y=OAc), while with silver nitrate and acetone the 2α-(nitrooxymethyl)penam **24**, (Y=ONO_2) was obtained[111].

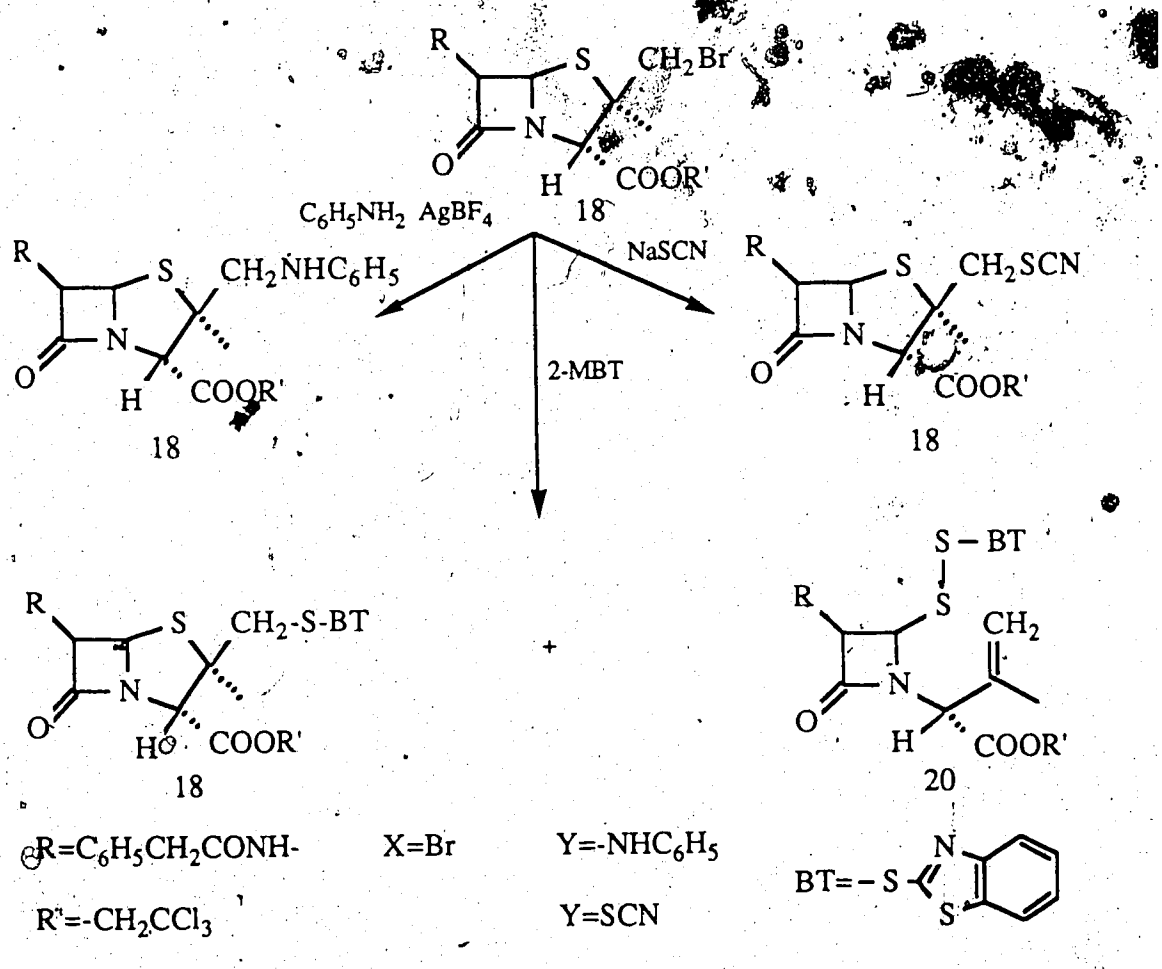


The 2β-(chloromethyl)penam **18**, (X=Cl), or 2β-(bromomethyl)penam **18**, (X=Br), with silver acetate and acetic acid or with silver nitrate and acetone gave 2β-(acetoxymethyl)penam **18**, (Y=OAc), 3-acetoxycepham **22**, (Y=OAc), and ceph-3-em **26**, in the ratio 3:3:1, or 2β-(nitrooxy)penam **18**, (Y=ONO_2), 3-nitrooxycepham **22**, (Y=ONO_2), and ceph-3-em **26**, in the ratio 5:15:1[110].

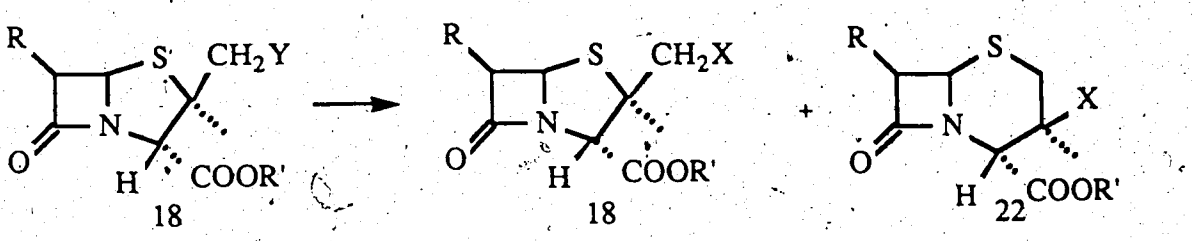


The 2β-(bromomethyl)penam **18**, on treatment with aniline in the presence of silver fluoroborate gave 2β-(anilinomethyl)penam **18**, as the

major product; with sodium thiocyanate it gave only 2β-(thiocyanomethyl)penam **18**; With 2-mercaptobenzothiazole in the presence of a base it gave 2β-(mercaptobenzothiazomethyl)penam **18**, (the normal substitution product) and the disulfide **20**, which was produced by the nucleophilic attack of thiol on the sulfur of the episulfonium ion[113].



Treatment of the 2β-(anilinomethyl)penam **18**, (Y=NHC_6H_5), with BF_3OEt_2, in methanol gave, in 80% yield, a mixture of 2β-(methoxymethyl)penam **18**, (X=OCH_3), and 3-methoxycepham **22**, (X=OCH_3), while a similar reaction with HCl yielded the 2β-(chloromethyl)penam **18**, (X=Cl), in quantitative yield[105].

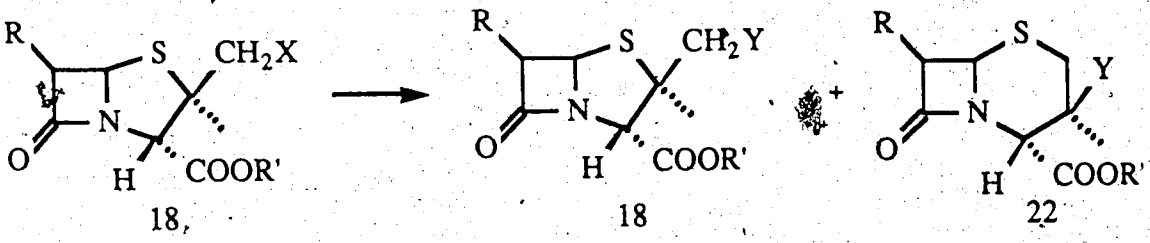


18 18 22

R=$C_6H_5OCH_2CONH$- Y=-NHC_6H_5

R'=-CH_3 X=OCH_3, Cl

The 2β-(chloromethyl)penam **18**, (X=Cl), on treatment with sodium azide in aqueous dimethylformamide for 3 h gave a 2:1 mixture of 6,6-dihydro-2β-(azidomethyl)penam **18**, (Y=N_3), and the isomeric 7,7-dihydro-3β-azidocepham **22**, (Y=N_3)[114].

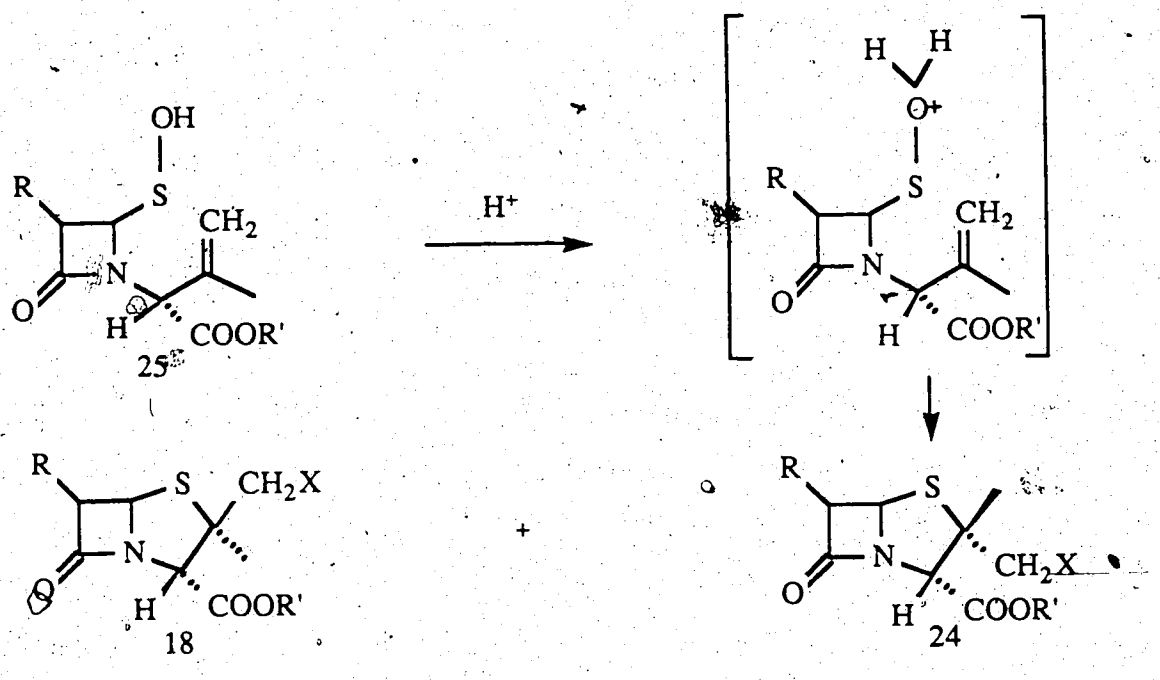


18, 18 22

R=H X=Cl

R'=-$CH(C_6H_5)_2$ Y=N_3

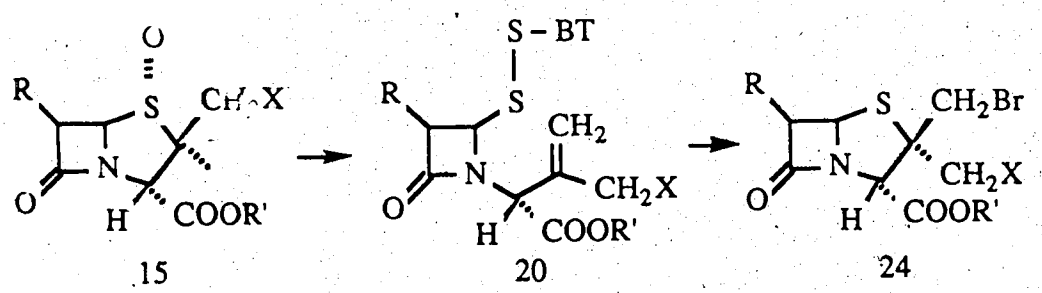
The sulfenic acid **25**, could also be protonated thereby changing the "S" from a good nucleophile to an electrophile. Nucleophilic displacement at "S" by the double bond gave 2β-(hydroxymethyl)penam **18**, (X=OH), and 2α-(hydroxymethyl)penam **24**, (X=OH)[115].

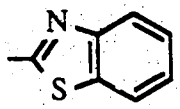


R=$C_6H_5OCH_2CONH$- R'=-CH_2CCl_3 X=OH

Thus the 2-(substituted methyl)penicillins **18**, can act as intermediates for introducing other nucleophilic groups into the penicillin nucleus.

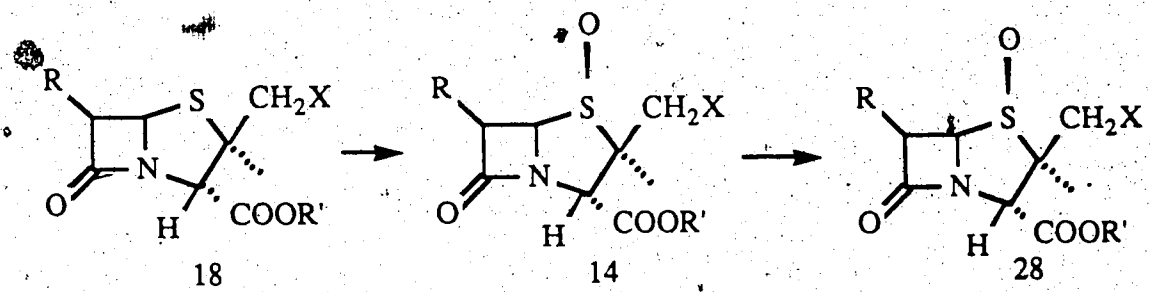
The disulfide **20** (X=OAc), obtained from 2β-(acetoxymethyl)penam-1α-sulfoxide **15**, (X=OAc), on treatment with molecular bromine gave the 2β-(bromomethyl)-2α-(acetoxymethyl)penam **24**, (X=OAc), while the disulfide **20**, (X=Cl), obtained from 2β-(chloromethyl)penam-1α-sulfoxide **15**, (X=Cl), under similar conditions, gave the 2β-(bromomethyl)-2α-(chloromethyl)penam **24**, (X=Cl)[116].



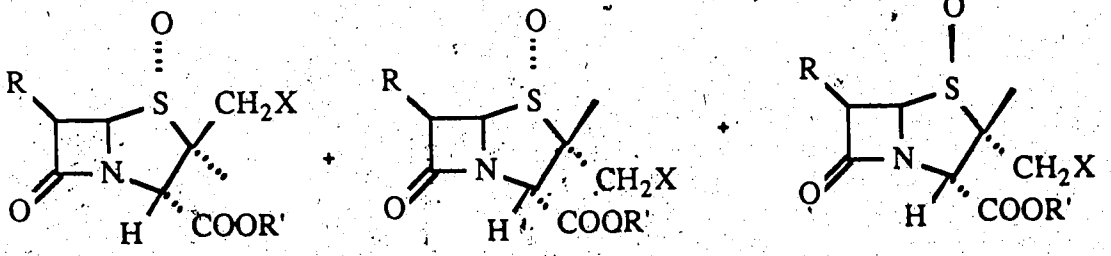
$R=C_6H_5OCH_2CONH-$ X=Cl BT= 

$R'=-CH(C_6H_5)_2$ X=OAc

The 2β-(acetoxymethyl)penam **18**, (X=OAc), was obtained by the oxidation of methyl-acetamidopenicillanate followed by acetic anhydride rearrangement. The oxidation of **18**, (X=OAc), with peracid, gave the 2β-(acetoxymethyl)penam-1β-sulfoxide **14**, (X=OAc), which on irradiation by the procedure of Archer[117], gave the α-sulfoxides of 2β-(acetoxymethyl)penams **27**, and 2α-(acetoxymethyl)penams **29**, and β-sulfoxides of 2β-(acetoxymethyl)penams **28** and 2α-(acetoxymethyl)penams **30**.



18 **14** **28**

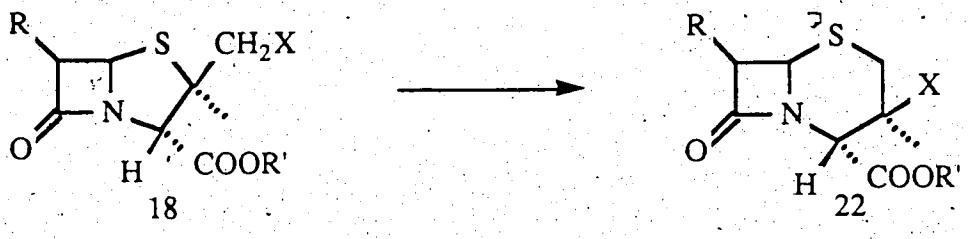


27 **29** **30**

$R=CH_3CONH-$ $R'=CH_3$ X=OAc

The 2β-(substituted methyl)penams **18**, (X=Cl, Br), are converted into their isomeric cephams **22**, (X=Cl, Br), by ring expansion. Some typical examples include:

(1) Conversion of the 2β-(bromomethyl)penam **18**, (X=Br), in DMF at room temperature (overnight) to 3-bromocepham **22**, (X=Br), and conversion of 2β-(chloromethyl)penam **18**, (X=Cl), by refluxing in benzene in presence of pyridine to 3-chlorocepham **22**, (X=Cl)[100].

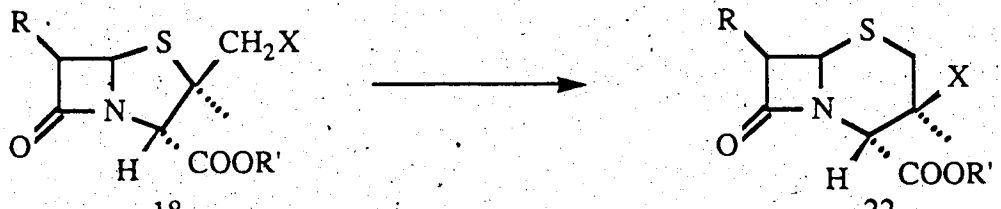


18 **22**

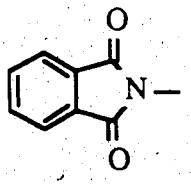
$R=C_6H_5CH_2CONH-$ R'=-CH_3 X=Br

$R=C_6H_5OCH_2CONH-$ R'=-CH_2CCl_3 X=Cl

(2) Conversion of the 2β-(chloromethyl)penam **18**, (X=Cl), to 3-chlorocepham **22**, (X=Cl), by heating in DMSO at 100 °C for 1 h[108].



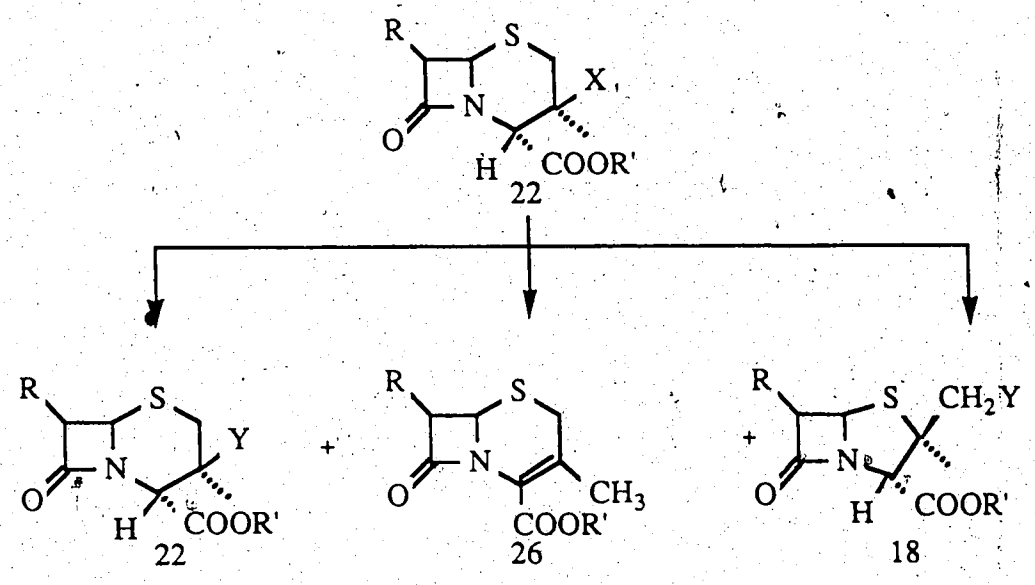
18 **22**

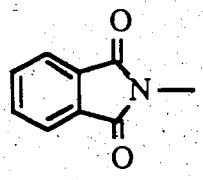
R=  R'=CH_3 X=Cl

The ring contraction reactions converting the cephams **22**, into penams **18**, is also cited in the literature[109-111].

Treatment of 3-chlorocepham **22**, (X=Cl), with silver acetate in acetic acid for 5 mins gave a mixture of 3β-acetoxycepham **22**, (Y=OAc), ceph-3-em **26**, and 2β-(acetoxymethyl)penam **18**, (Y=OAc), in almost quantitative yields, in the ratio 3:1:3, while treatment of 3-hydroxycepham **22**, (X=OH), with

thionyl chloride, triethylamine and carbon tetrachloride gave a mixture of 3β-chlorocepham **22**, (X=Cl), ceph-3-em **26**, and 2β-(chloromethyl)penam **18**, (Y=Cl), in the ratio 4:1:1.

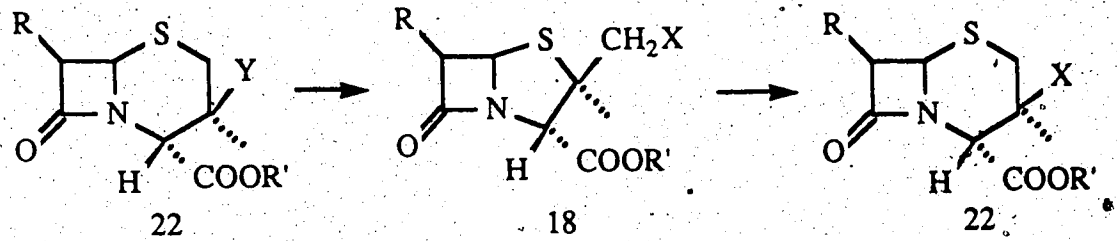


R=  R'=CH_3

a. X=Cl, Y=OAc

b. X=OH, Y=Cl

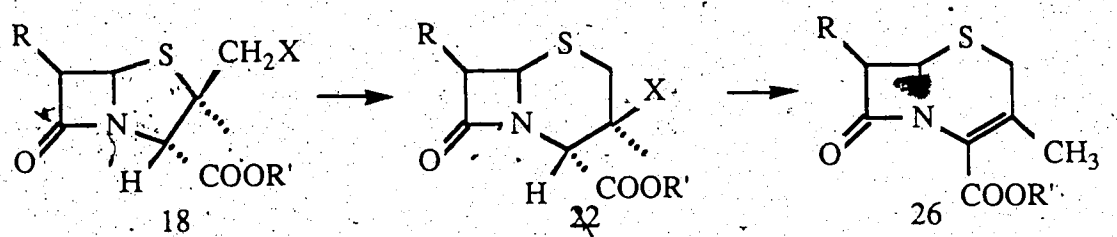
The *p*-nitrobenzyl ester of phenoxyacetamido-3β-hydroxycepham **22**, (Y=OH), when refluxed with thionyl chloride and DMF in boiling benzene for 30 mins gave the corresponding 2β-(chloromethyl)penam **18** (X=Cl), which rearranged to 3β-chlorocepham **22** (X=Cl), on standing at room temperature[111].

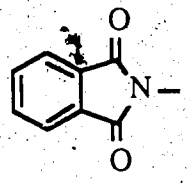


$R=C_6H_5OCH_2CONH-$ Y=OH

$R'=-CH_2C_6H_4$-p-NO_2 X=Cl

The cephems **26**, are prepared from the 2β-(chloromethyl)penam**18** (X=Cl), cepham chloride **22** (X=Cl), by warming at 130 °C in DMSO in presence of a weak base such as urea, with the elimination of HCl[108].



R=  $R'=CH_3$ X=Cl

K. *FLUORO-ORGANIC COMPOUNDS*

The unusual properties numerous compounds acquire on introduction of fluorine are varied and extend from extreme stabilization in fluorine-containing polymers and blood-substituents to alterations in the activity of pharmacologically and phytomedicinally active compounds. One physical peculiarity of the fluorine atom, its magnetic moment, suggests a new approach to the decyphering of metabolic processes[119]. The importance of monofluorinated molecules with distinctive and highly interesting biological activities is continually reflected in the literature[120]. Today, fluorine containing medicinals are represented in diverse areas such as the anticancer agents[121], e.g., 5-fluorouracil, 5-fluoro-2'-deoxy-β-uridine and N-tetrahydrofuranyl-5-fluorouracil; antiviral agents[122], e.g., 5-substituted-(2-fluoro-β-D-arabinofuranosyl)pyrimidines; inhalation anaesthetics[123], e.g.,

halothane, enflurane, isoflurane, sevoflurane, synthane and aliflurane; anti-inflammatory drugs[124], e.g., paramethasone, dexamethasone, flurbiprofen an arylalkanoic acid, and sulindac and the salicylic acid analog diflunisal; antibiotics[124], e.g., flucloxacillin, 5-fluorocytosin, fludalanine and mefloquine; and CNS agents[125], e.g., fluphenazine HCl, flupenthixol decanoate, haloperidol, penfluridol, flurazepam, fenfluramine, progabide. Other applications include the use of bendroflumethiazide as a diuretic and antihypertensive agent[124] and flecainide acetate as an antiarrhythmic drug[126]. Perfluorochemicals have been used as superior oxygen carrying blood substituents[127]. Radio-opaque fluorocarbons e.g., fluorocarbon monobromides, have been shown to have limited but useful application for contrast enhancement of X-rays[128]. Fluorine-18 is a positron emitting radioisotope of fluorine which is being increasingly used in positron emission tomography[129].

The incorporation of fluorine in drug molecules as a means of increasing therapeutic efficacy is based on several considerations including[124]

(1) Fluorine, mimics hydrogen with respect to steric requirements at enzyme receptor sites. The Van der Waal's radius for fluorine is 1.35 A° while it is 1.2 A° for hydrogen.

(2) The strong electron-withdrawing effect of fluorine can significantly influence reactivity and stability of functional groups and the reactivity of neighboring reaction centers.

(3) The substitution of hydrogen by fluorine at or near a reactive site frequently causes inhibition of metabolism because of the high C-F bond energy.

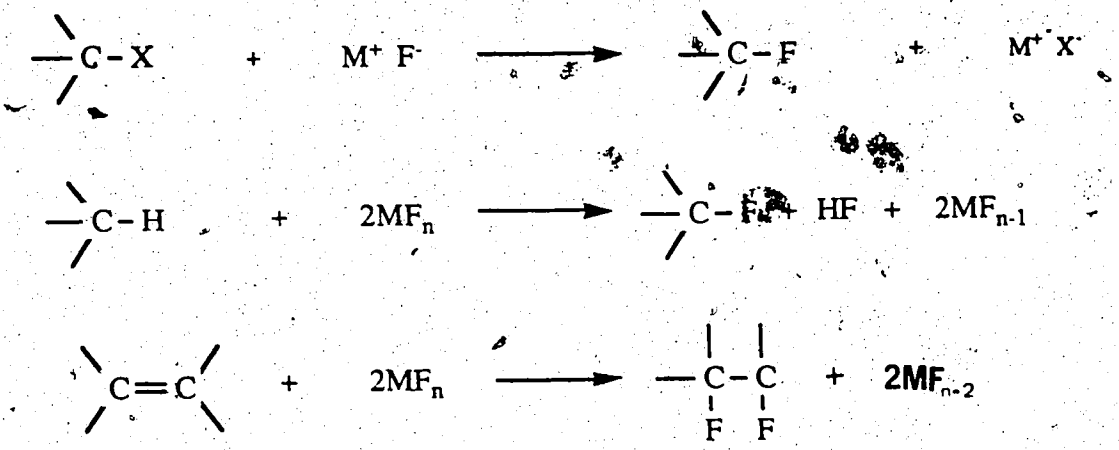
(4) The replacement of hydrogen by fluorine usually increases lipid solubility, thereby enhancing the rate of absorption and transport of drugs *in vivo*. In

many cases, this factor may be the most significant in improving pharmacological activity.

(5) Sometimes, as in the case of 5-fluorouracil, the presence of fluorine instead of hydrogen actually blocks an essential biochemical reaction i. e., the fluorine behaves as a deceptor group.

The realization of the importance of fluorinated compounds has led to a drastic increase in the availability of synthetic methods and reagents for fluorination of organic compounds. In highly fluorinated compounds all or most of the hydrogen atoms initially bonded to carbon atoms have been replaced by fluorine, while in low fluorinated compounds, only some of the hydrogen atoms are replaced by fluorine. As a rule, specific interaction of a fluorinated compound with a living organism is only possible when not all the carbon-bonded hydrogen atoms have been replaced by halogens. The most elegant method of fluorination is the incorporation of a fluorine atom into the compound in question at the end of the conventional synthesis[119].

Fluoro-organic compounds may be synthesized by the direct reaction of hydrocarbon-type organic derivatives with metallic fluorides. The metallic fluoride may be used to effect the exchange of fluorine for functional groups, frequently other halogen atoms (Cl, Br or I), hydrogen and unsaturated linkages. In another process, all substituents on a carbon skeleton can be replaced by fluorine, and unsaturation removed, to give highly fluorinated products and eventually fluorocarbons. Metallic fluorides may be classified into two groups according to which reaction they promote. In exchange reactions, with alkali metal fluorides, metals form derivatives in more than one valency state and the metal fluoride is converted into another salt by lower valence fluorides. In exhaustive fluorination, high valency metal fluorides are reduced to a lower valency fluoride as the reaction proceeds.



Some of the metal fluorides which bring about exchange are antimony pentafluoride, silver fluoride, potassium fluoride, mercuric fluoride, antimony trifluoride and mercurous fluoride. The most important high valency metal fluoride is cobalt trifluoride. Silver difluoride, manganese trifluoride, cerium tetrafluoride and lead tetrafluoride are some examples of this group which are used to a lesser extent[130].

Fluorination by halogen exchange is by far the most widely used method of synthesizing organic fluorine compounds in the laboratory and industry. Alkali metal fluorides are frequently very effective fluorinating agents for halogen exchange fluorinations and are used in the preparation of compounds possessing a functional group and only one halogen atom. A large number of inorganic fluorides have been studied as reagents for fluoride exchange reactions[131]; examples include,

(a) Anhydrous hydrogen fluoride (AHF) which causes substitution of only very reactive atoms such as benzylic halogen atoms; converts benzotrichloride to benzotrifluoride[132], substitutes fluorine for chlorine atoms in diphenyldichloromethane[133] and triphenylchloromethane[134].

(b) Antimony trifluoride is similar to AHF. Benzylic[135] and allylic[136] halogen atoms, halogens in acid chlorides[137], trichloromethylsulphides[138],

alkylchlorosilanes[139] and organic phosphorous compounds containing a P-Cl bond[140] are a few examples where an exchange reaction of chloride to fluoride is mediated by antimony trifluoride. Antimony pentafluoride is a powerful fluorinating agent used for the complete replacement of halogen atoms by fluorine[141].

(c) Alkali metal fluorides: Potassium fluoride is the most versatile alkali metal fluoride used for fluorination by halogen exchange. It is useful for the replacement of isolated halogen atoms in the alkane series[142] and also in compounds containing a functional group such as in ethers[143] and alcohols[144].

Mercurous fluoride is used for fluorination of alkyl halides, especially iodides and bromides as well as esters. The disadvantage is that it promotes the removal of hydrogen halide from an alkyl halide with the formation of olefins[145].

Mercuric fluoride is a powerful fluorinating agent which does not cause elimination of hydrogen halide from an organic fluoride and is useful for replacing a single halogenoalkane by fluorine[146].

Because of the ready availability of metal fluorides, the halogen (Cl, Br or I) exchange reaction by fluoride continues to enjoy great popularity. This simple method can also prove successful with complicated derivatives of natural products such as prostaglandins[147], sugars[148] and nucleosides[149] in which case a tosyl group is exchanged.

Some simple examples of halogen exchange reactions using metal fluorides are as follows:

$$CH_3(CH_2)_5Cl \xrightarrow{KF/Glycol,\ 175\text{-}185\ ^\circ C} CH_3(CH_2)_5F \qquad 150$$

$$CHBr_2Cl \xrightarrow{HgF_2,\ 40\ ^\circ C} CHBrClF \qquad 151$$

Methyl fluoride can be obtained in 80% yield by fluorinating methyl iodide with mercuric fluoride[152]. The same reaction using silver fluoride was studied much earlier by Moissan and Meslans[153].

Fluorination of $CBrCl_2CHBrCl$ in presence of a catalyst prepared from $SbCl_3$, $SbCl_5$ and HF proceeds exclusively on trihalogenomethyl group[154].

$$CBrCl_2CHBrCl \xrightarrow{HF/Sb\ catalyst,\ 90\text{-}120\ ^\circ C} CF_3CHBrCl$$

Another compound of potential interest as a non-inflammable anaesthetic is 2-bromo-1,1,1,2-tetrafluoroethane, which was prepared in 50% yield by either of the fluorinations[155]:

$$CF_3CHBr_2 \xrightarrow{HF/Sb_3ClF,\ 110\ ^\circ C/250\ psi} CF_3CHBrF$$

$$CBrF_2CHBrF \xrightarrow{HF/SbF_3/SbCl_5,\ 110\ ^\circ C/250\ psi} CF_3CHBrF$$

On treatment of 1,2-dibromo-1,1,2-trifluoroethane with silver fluoride in a sealed tube, fluorination occured on the monobromo methyl group.

$$CBrF_2CH_2Br \xrightarrow{AgF,\ 120\ ^\circ C} CBrF_2CH_2F \qquad 156$$

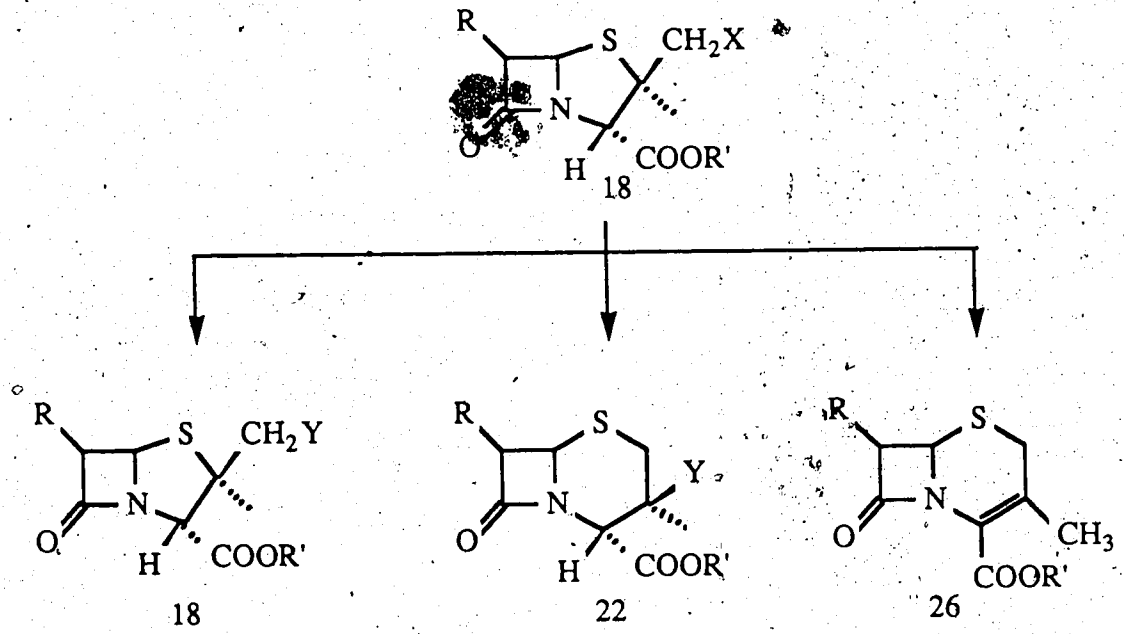
Numerous methods for the preparation of fluoroalkane by halogen exchange reaction using metal fluorides are given in reference # 131.

Reagents other than metal fluorides are also available for the introduction of fluorine into organic molecules. However, since the fluorinations studies were based on the use of metal fluorides, I have limited the discussion to this class of compounds.

Fluorinated β-Lactam Antibiotics

In spite of the availability of a number of methods for the fluorination of organic compounds and natural products and the knowledge about the usefulness of this class of medicinal compounds, very little is known about the fluorination of β-lactam antibiotics. Only a few examples of β-lactams with fluorine in the molecule are known.

Von Daehne, *et al.*,[157] have reported the reaction of 6β-bromo-2β-(bromomethyl)penam **18** (X=Br), with nucleophilic agents to provide a mixture of 6β-bromo-2β-(fluoromethyl)penam **18** (Y=F), 7β-bromo-3β-fluorocepham **22** (Y=F), and 7β-bromo-ceph-3-em **26**, although no details of the reaction are provided.



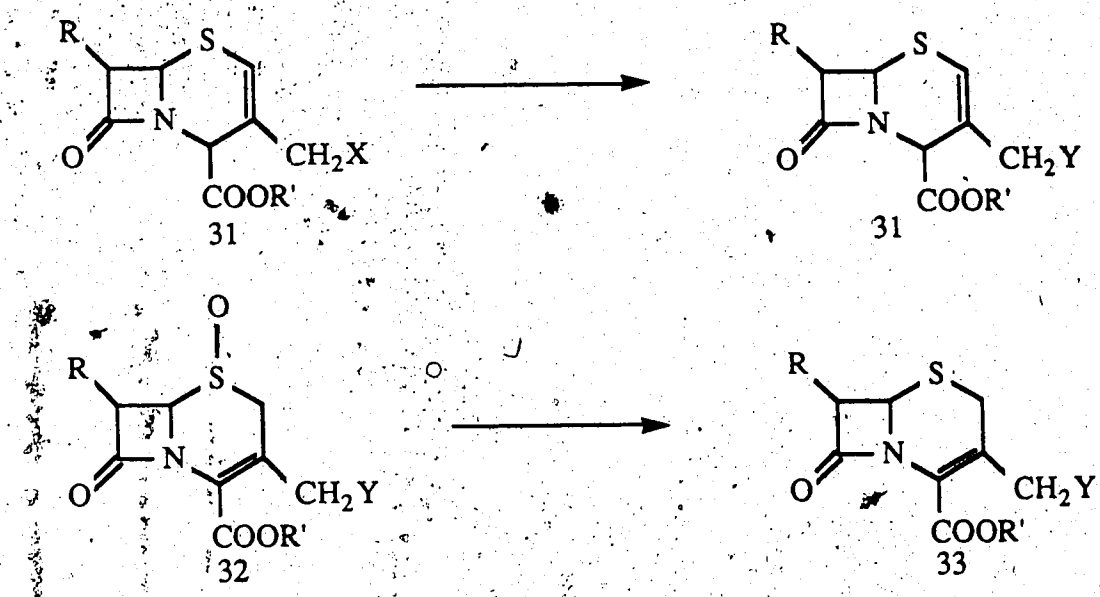
R=Br X=Br

R'=-CH_2OAc, H, K Y=F

Muller, *et al.*,[158] have reported the preparation of 3-fluoromethyl-3-cephems and 3-difluoromethyl-3-cephems using 2-chloro-1,1,2-trifluoro-

triethylamine (CTT)[159] or piperidino sulfur trifluoride (PST)[160] as fluorinating agents, while 3-fluoro-3-cephem has been disclosed in the patent literature[161].

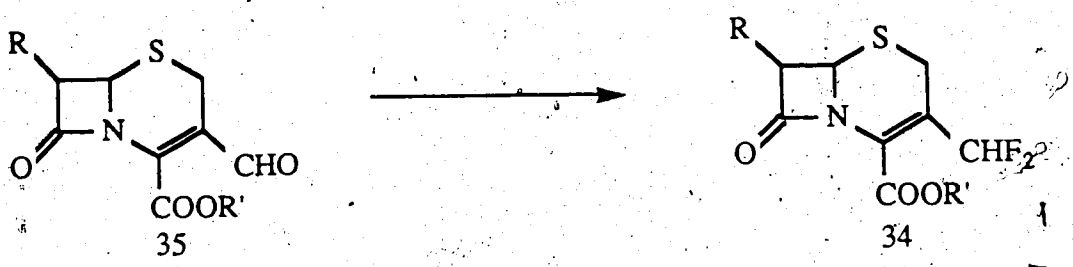
The alcohol **31** (X=OH), was converted with CTT in 1,2-dichloromethane to the 3-fluoromethyl-Δ^2-cephem ester **31** (Y=F), 73%, which on oxidation with *m*-chloroperbenzoic acid gave 3-fluoromethyl-Δ^3-cephem sulfoxide **32**; deoxygenation with PCl_3 in DMF gave the required 3-fluoromethyl-Δ^3-cephem ester **33** (68%)[158].



R=$C_6H_5CH_2CONH$- X=OH

R'=-$CH(C_6H_5)_2$ Y=F

For the preparation of 3-difluoromethyl-Δ^3-cephem **34**, the readily available aldehyde **35**, was treated in dioxane with 7 equivalents of PST at room temperature for 1.5 hrs, when crystalline **34**, was isolated in 45% yield[158].



35 34

$R=C_6H_5CH_2CONH-$

$R'=-CH(C_6H_5)_2$

Flucloxacillin[162] **13**, a narrow spectrum penicillinase stable antibiotic is another example of a β-lactam antibiotic with fluorine in the molecule.

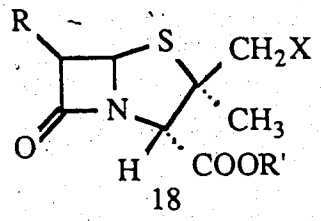
13

R'=Na

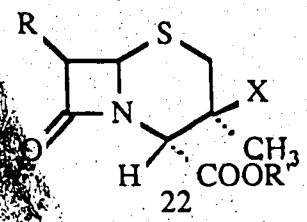
The reaction of metal fluorides with *unsym*-azetidinone disulfides **20**, and 2β-(halomethyl)penicillins **18**, (X=Cl, Br), leading to 3β-fluoro-3α-methylcephams **22**, (X=F), has been recently reported by Micetich, *et al.*,[163].

II. OBJECTIVES

The main objective was to study the synthesis and properties of 2β-(substituted methyl)penams **18**, the isomeric 3β-substituted cephams **22**, and their oxidation products - the sulfoxides and the sulfones.

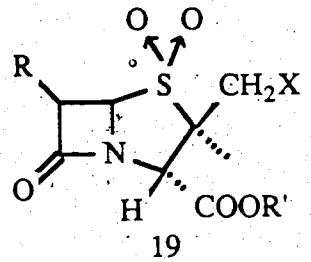


18



22

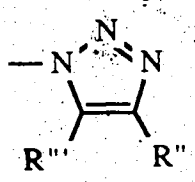
An active β-lactamase inhibitor, the 6,6-dihydropenicillanic acid sulfone[58] **19**, (X=H, R'=H), is being marketed by Pfizer. The 6,6-dihydro-2β-(chloro-, bromo- and azidomethyl)penam sulfones **19a**, and the YTR class of compounds **19b**, are known to possess good β-lactamase inhibitory activity[114, 164-169]. Among the 2-(halomethyl)penams the 2β-(chloro- and bromomethyl)penams **18**, (X=Cl, Br), have been fairly extensively studied[100,111,170]. The 2β-(iodomethyl)penams **18**, (X=I), have also been described[102,171]. In contrast the 2β-(fluoromethyl)penams **18**, (X=F), are relatively unknown. As the 6,6-dihydro-2β-(halomethyl)penam sulfones **19**, (X=Cl, Br), proved to have β-lactamase inhibitory activity, my objective was directed towards the investigation of methods for the synthesis of 2β-(fluoromethyl)penams **18**, (X=F), by studying the fluorination of *unsym*-azetidinone disulfides **20**, and the 2β-(halomethyl)penams **18**, (X=Cl, Br), with the metal fluorides.



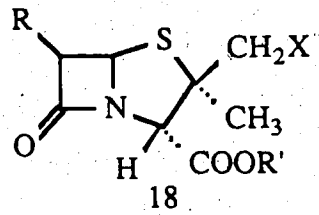
19

R=H

R'=Na

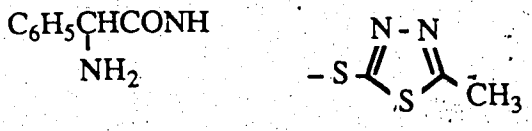
19	X
a	Cl, Br, N_3
b	

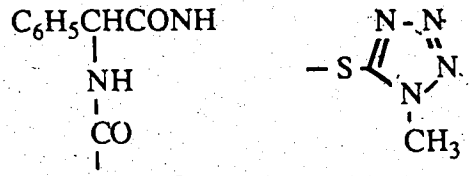
Although the synthesis and characteristics of 2β-(halomethyl)penams 18, (X=Cl, Br, I), analogs of benzylpenicillin and phenoxymethylpenicillins have been reported[100,102,111,170,171], comparative antibacterial activities (MICs) of these penicillin derivatives is not available. Broad spectrum antibacterial activity of cephalosporins with heterocyclic ring substituents at C-3 has been reported[172]. My other objective was to synthesize a series of 2β-(substituted methyl)penams 18, which had various substituents at C-6 such as [R=$C_6H_5CH_2CONH$, $C_6H_5OCH_2CONH$, $C_6H_5CH(NH_2)CONH$], at C-2 [X=Cl, Br, 2-mercapto-5-methyl-1,3,4-thiadiazole and 5-mercapto-1-methyl-1,2,3,4-tetrazole] and a suitable ester at C-3 [R'=CH_2CCl_3] which could be subsequently hydrolyzed and the acid converted to the sodium salt.

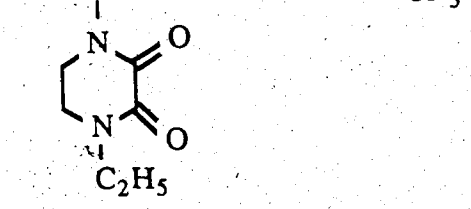


18

R	X	R'
$C_6H_5CH_2CONH$	Cl	CH_2CCl_3
$C_6H_5OCH_2CONH$	Br	Na

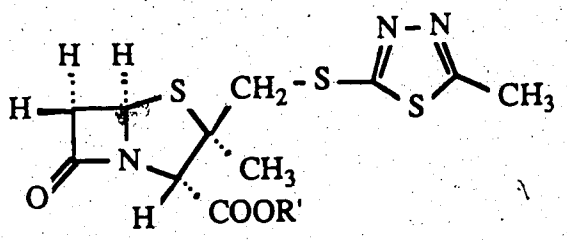






The compounds thus prepared were to be tested for antibacterial activity (MIC determination) against selected strains of Gram +ve and Gram -ve bacteria, to obtain data that would be useful for SAR studies, based on variations at the C-6 and C-2 β-methyl positions of the penicillins.

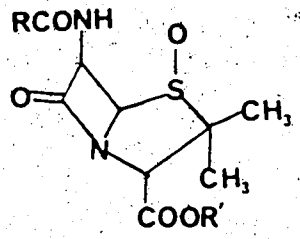
The 6,6-dihydro-2β-(triazolylmethyl)penicillin sulfone **19b**, (YTR 830, R'=Na, R"=R'"=H), the most effective β-lactamase inhibitor now known, is under preclinical testing in combination with a variety of antibiotics. The other objective was to prepare the 6,6-dihydropenicillins with 2-mercapto-5-methyl-1,3,4-thiadiazole at C-2 β-methyl and carry out a systematic oxidation study on this compound.



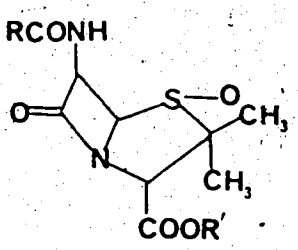
III. RESULTS AND DISCUSSION

A. *OXIDATION*

The oxidation of penicillin **1**, to its sulfoxide could theoretically lead to two isomers viz., penicillin β-sulfoxide **14**, and penicillin α- sulfoxide **15**.



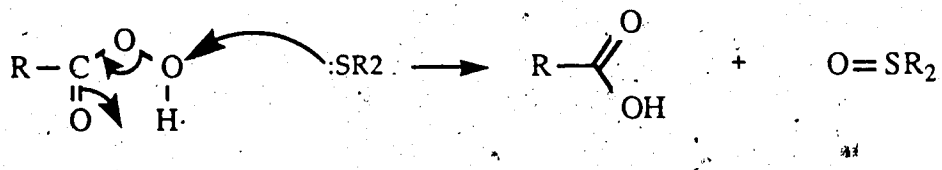
14



15

However, several modified penicillins **1**, with varying substituents at C-6 such as [R=$C_6H_5CH_2CONH$-, $C_6H_5OCH_2CONH$- and CH_3CONH-] and at C-3 [R'=CH_3, CH_2CCl_3], under various oxidation conditions gave exclusively one sulfoxide isomer viz., the β-sulfoxide **14**, the configuration of which was assigned by NMR and X-ray crystallography studies[173]. The β-configuration, although sterically hindered, was the result of the directing influence of the 6β-amido proton, either through hydrogen bonding with the oxidants (reagent approach control) or through thermodynamic control in which the β-configuration was stabilized by an internal 6β-amido proton-sulfoxide hydrogen bond. In the absence of the 6β-amido proton, steric control would be the major directing influence in the oxidation[88].

The proposed mechanism[174] of sulfide oxidation by peracid is as follows:



The hydrogen bonding and subsequent bond displacement existed in an especially favored form with the peracid itself and no accessory hydrogen-bonding agent was necessary. The oxidation proceeded by a nucleophilic attack of the sulfur atom on the peracid. Extending this mechanisms to penicillins and from considerations of steric hindrance, the β-sulfoxide would seem to be the least likely product. The investigations of Henbest and coworkers[175] have shown that the stereochemistry of epoxidation was influenced by the presence of hydrogen-bonding functions e.g., hydroxyl, in positions close to the reaction sites. The epoxidation of 3-benzamidocyclohexene with peracetic acid leads to the *cis*-epoxide due to the directing effect of the benzamido proton[176]. It was postulated that in non-polar solvents oxidation occurred by the nucleophilic attack of the double bond on the peracid hydrogen bonded to the hydroxyl group. Application of this argument of "reagent approach control" to the oxidation of penicillin to its β-sulfoxide would require that the peracid be initially hydrogen bonded to the amido proton, (Figure 7).

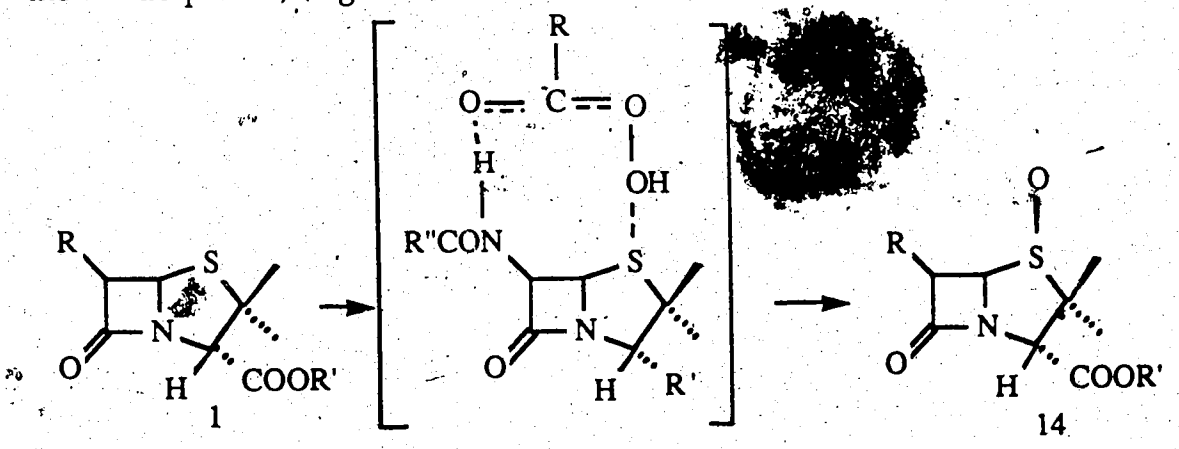


Figure 7. The Mechanism of Oxidation of Penicillin with Peracid.

Theoretically, it would still be possible for either of the sulfur lone pair of electrons to attack the hydroxyl oxygen of the peracid. However, inspection of the non-bonded interactions for both transition states indicated that the

product with the β-configuration (the stereochemistry *cis* to the amide side chain) represented a transition state with a lower energy barrier, hence of higher stability[173].

An alternative explanation was that no matter which isomer was initially formed, under the reaction conditions employed, isomer equilibration may have occurred so that the final product was governed by thermodynamic factors, i.e., the formation of an intramolecular hydrogen bond between the amido proton and the sulfoxide oxygen atom. This hydrogen bond explained the inability of phenoxymethylpenicillin-1β-sulfoxide **14**, [R=$C_6H_5OCH_2CONH$-] to isomerize to the α-isomer.

The oxidation of penicillin **1**, to penicillin-1β-sulfoxide **14**, by hydrogen peroxide and acetic acid or hydrogen peroxide in an aprotic solvent was a one step interaction of the sulfide with the peroxide-solvent complex in which a series of electronic displacements led to hydrogen exchange and oxygen transfer[177], (Figure 8 and Figure 9). Even in this case, an intramolecular hydrogen bond between the amido proton at C-6 and the sulfoxide oxygen atom stabilized the β-isomer.

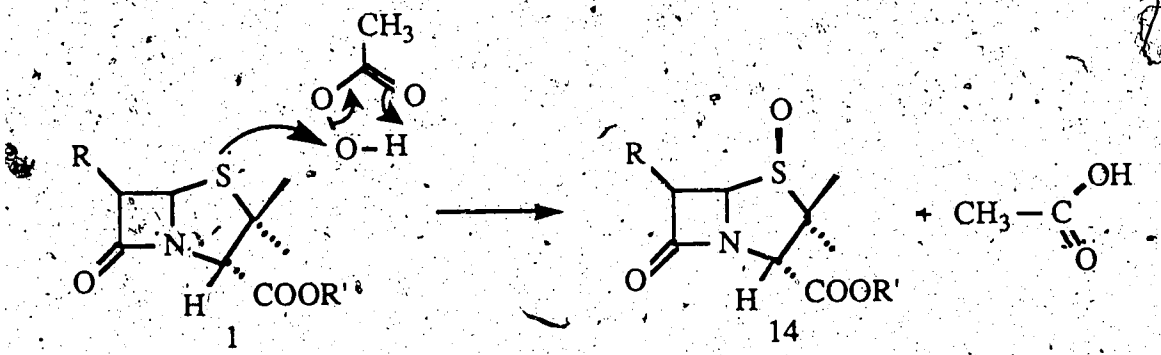
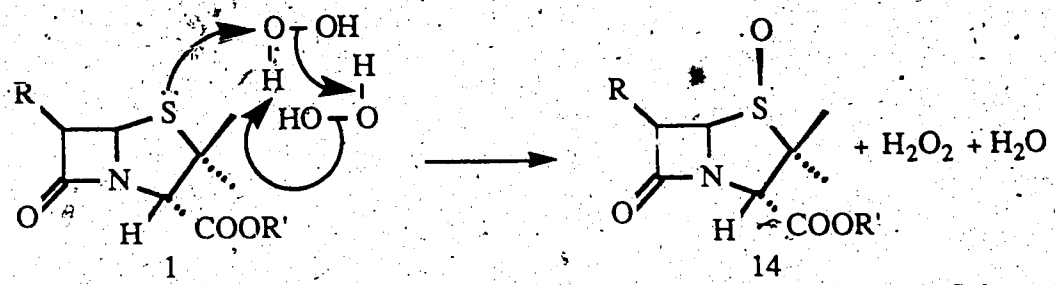


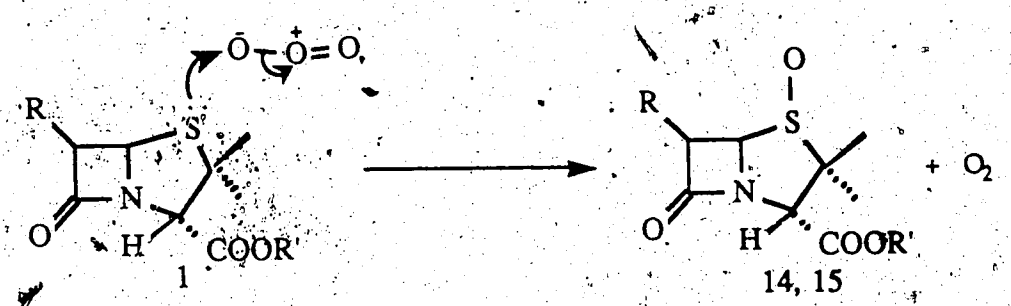
Figure 8: The Mechanism of Oxidation of Penicillin with H_2O_2/Glacial Acetic Acid



1 14

Figure 9: The Mechanism of Oxidation of Penicillin with H_2O_2 in Aprotic Solvent

Various penicillins **13**, exhibited a steric effect on the approach of the ozone molecule which determined the stereochemistry of the resulting sulfoxide[91]. Figure 10 represents the reaction mechanism of ozone oxidation of penicillin **13**.



1 14, 15

Figure 10. The Mechanism of Oxidation of Penicillin with Ozone.

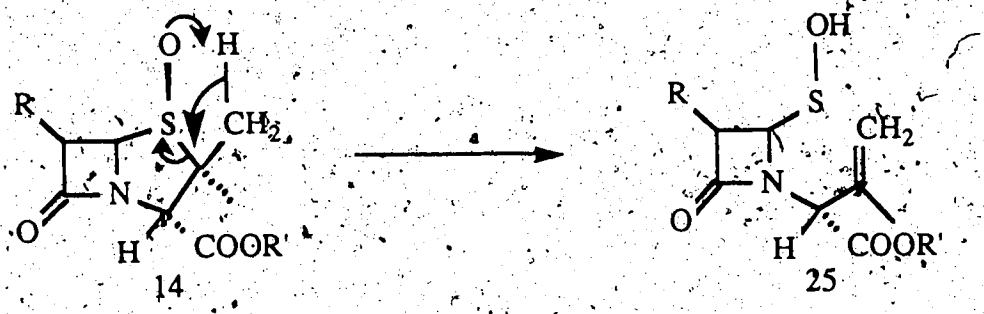
The varied proportions of the two isomers, penicillin-1β-sulfoxide **14**, and penicillin-1α-sulfoxide **15**, in the ratio 4:1, 1:1 and only 0:1 formed with varying substituents at C-6 [R= NH_2, $C_6H_5OCH_2CONH$- and phthalimido], respectively might have been the result of a β- to α- conversion via the sulfenic acid, with the driving force being the release of strain between two bulky β-groups[118].

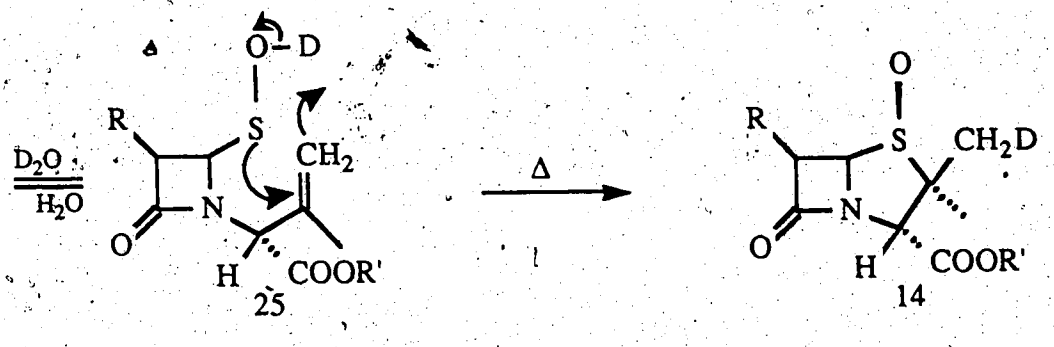
The oxidation of penicillin **1**, [R=$C_6H_5CH_2CONH$-], by iodobenzene dichloride in aqueous pyridine gave the α- and β- sulfoxides in approximately 1:1 ratio. The reaction proceeded by a two step process in which the intermediate, either a complex or a sulfonium chloride, was capable of

being hydrogen bonded to the amide side chain, the hydrolysis of which led to the inversion about sulfur[178,179] giving the mixture of the two isomers[107].

Morin, *et al.*,[83] proposed the intermediacy of the sulfenic acid **25**, in the rearrangement of penicillin-1β-sulfoxide **14**, [R=$C_6H_5OCH_2CONH$-]. The inversion of methyl penicillin-1α-sulfoxide **15**, [R=CH_3], to the β-sulfoxide **14**, [R=CH_3], in refluxing benzene reported by Archer, *et al.*,[95] also cited the sulfenic acid **25**, as a possible intermediate.

On heating a solution of penicillin-1β-sulfoxide **14**, in benzene containing a large excess of D_2O for 24 h, the recovered sulfoxide had an average of one deuterium atom located only in the C-2 β-methyl group as shown by NMR, while phthalimidopenicillin-1α-sulfoxide gave deuterium incorporation only in the C-2 α-methyl group[180]. These results indicated the existence of a thermal equilibrium between the sulfoxide **14**, **15** and sulfenic acid **25**. When this equilibrium was established in the presence of D_2O, hydrogen-deuterium exchange occurred in the sulfenic acid **25**, with consequent deuterium incorporation into the methyl group, either α-methyl or β-methyl of the sulfoxide, (Figure 11).





$R=C_6H_5CH_2CONH-$ $R'=CH_3$

Figure 11: The Deuterium Incorporation in Penicillin Sulfoxide

Cooper, *et al.*,[101,181] have suggested the formation of a sulfenic acid **25**, as a reversible thermal six-electron sigmatropic rearrangement. This was possible as the hydrogen atom involved in transfer from the methyl group to the sulfoxide oxygen had a symmetrical electron distribution (*s* orbital). Thus effective overlap could be [illegible] between the oxygen *p* orbitals and the hydrogen atom in the forward sense and the carbon-carbon double bond π orbitals and the hydrogen atom in the reverse sense. The deuteration was stereospecific. The readdition of sulfenic acid **25**, to the olefin gave the deuterium to the methyl group *cis* to the sulfoxide and the configuration could be controlled by two possible factors. Firstly, the thermodynamic stability of the product since, in all cases, the recovered sulfoxides had the same stereochemistry as the starting sulfoxides. Secondly, due to the restricted conformation of the intermediate sulfenic acid **25**, (because of hydrogen bonding to the amido proton), the ring closure occurred on the β-face of the molecule, while in the case of phthalimido side chain with no amido proton at C-6, the steric effects dominated and ring closure occurred on the α- face[180].

A single step reaction of the potassium salts of phenylacetamido or phenoxyacetamido penicillin **1** [$R=C_6H_5CH_2CONH$ and $C_6H_5OCH_2CONH$, R'=K], in water and acetone, with peracetic acid as oxidizing agent and

benzophenone hydrazone for esterification gave the β-sulfoxides of the respective penicillins in 80 - 90% yields. The structures of these penicillin sulfoxides **14**, were confirmed by comparision of the NMR data with that available in the literature. The unequivocal assignments of protons H-3 (singlet), H-5 (doublet) and H-6 (quartet) could be made. However, as both geminal methyl protons (2α-CH_3 and 2β-CH_3) appeared as singlets, the assignment of these methyl protons to specific signals in the NMR spectrum could not be based on chemical shift information alone.

X-ray studies[173,182] of several salts of benzyl penicillanic acid have established the conformation of the thiazolidine ring of sulfide and sulfoxide as *a* and *b* respectively in Figure 12.

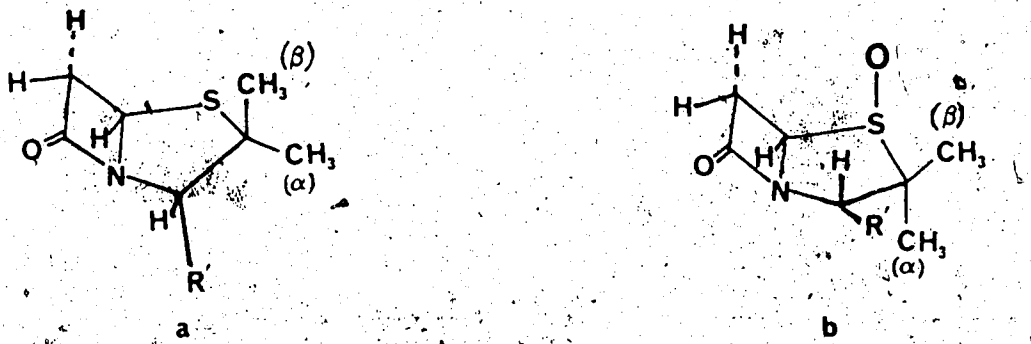


Figure 12. The Possible Thiazolidine Conformations

From internal Nuclear Overhauser Effect (NOE) studies[183,184] Cooper, *et al.*,[173] have determined which protons in conformationally rigid systems are proximal to each other, thus ascertaining the relative spatial proximity of different protons in the penicillins, which allowed a complete chemical shift assignment of the methyl proton signals. Irradiation of the low field methyl peak increased the intensity of H-3 by 21% for sulfide and 26% for sulfoxide respectively, with no effect was observed on H-5. Irradiation of the high field methyl peak, resulted in 7% increase in intensity for H-3 in the sulfide and had no effect on H-3 in the sulfoxide. On the other hand, the H-5 increased in

intensity by 14% for the sulfoxide. Accordingly, the low field methyl signal in the NMR spectrum was assigned to the 2β-methyl protons. Because of their proximity only the 2β-methyl protons could relax H-3. Consequently, the high field methyl signal was assigned to the 2α-methyl protons. In solution, definite differences existed between the conformation of the thiazolidine ring of the sulfide and the sulfoxide. Upon irradiation of the 2α-methyl protons, the H-5 signal was enhanced in the sulfoxide but not in the sulfide. Only in conformation (Figure 12) could the H-5 be relaxed by the 2α-methyl protons. Thus, the sulfide and sulfoxide apparently existed in the same conformation as that in the crystal state[173].

Aromatic solvent induced shifts (ASIS)[185] were used to study the conformation of the sulfoxide isomers. Ledaal[186] proposed a model to rationalize the geometry of benzene-solute collision complexes involving solutes containing any polar functional group. According to this model, the dipole axis of the polar functional group in the solute molecule was located along the six fold axis of symmetry of the benzene system with the positive end of the polar function near it and the negative end farther away from it. Assuming, the formation of such a complex between benzene molecules and the S–O bond of the penicillin molecule, the geometry of association for the isomeric penicillin sulfoxides **14** and **15** should be different. For the β-sulfoxide **14**, the benzene association would take place from the α- face of the solute molecule, i. e, from the positively polarised end of the S–O bond (Figure 13). The geometry of this complex and the anisotropy of the aromatic system strongly shielded the 2α-methyl and H-5 protons and the H-3 , H-6 and 2β-methyl protons were only marginally affected, while for the α-sulfoxide **15**, the complexation of benzene with the penicillin molecule was from the β face, leading to a strong shielding of the 2β-methyl and the H-3 protons.

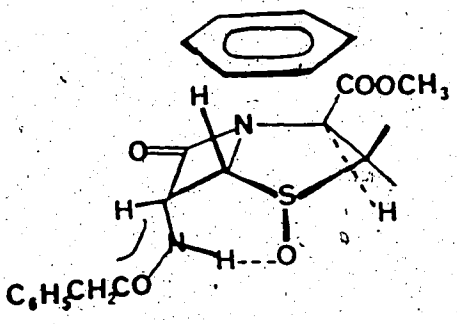


Figure 13. The Solvation of Penicillin Sulfoxide.

Hydrogen bonding studies also helped to establish the conformation of the two sulfoxide isomers. The formation of hydrogen bonds has been shown generally to cause a downfield shift[187] of the resonance signal of the proton involved, and the use of DMSO as a solvent for studying the NMR spectra of compounds containing hydroxyl groups has also been well established[188]. With penicillin sulfides **1**, the -NH proton was shifted downfield by 1.21 ppm in DMSO with respect to $CDCl_3$, due to the solute-solvent hydrogen bonding i. e., the formation of a -NH- - -OS bond. The penicillin β-sulfoxide **14**, showed a downfield shift of -NH proton with respect to penicillin in $CDCl_3$ because of the intramolecular hydrogen bonding or by anisotropic shielding but experienced no downfield shift on changing the solvent to DMSO, for the intramolecular hydrogen bond between the -NH and S–O would not be affected by the external hydrogen bonding agent viz., DMSO. This study confirmed the conformation of the penicillin sulfoxide to be β when an amido side chain was present at C-6[173].

The penicillin sulfoxides **14**, required as starting material for the synthesis of 2β-(substituted methyl)penams **18**, were prepared using either *m*-chloroperbenzoic acid or hydrogen peroxide/glacial acetic acid or peracetic acid as oxidizing agent. The oxidation with 1:1 mole equivalents of *m*-

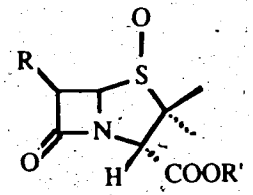
chloroperbenzoic acid was carried out for 1.5 h in methylene chloride at 0 °C. The hydrogen peroxide/glacial acetic acid oxidation was carried out in methylene chloride at room temperature for 24 h and the peracetic acid oxidation took about 4 - 5 h for completion: ^{1}H NMR data of penicillin sulfoxides **14**, used as starting material are summarized in Table 4.

B. *ESTERIFICATION*

Esterification of the carboxyl group at C-3 of penicillin protected the carboxyl group from undesirable side reactions and facilitated the process of purification. Several groups such as 2,2,2-trichloroethyl[189], *p*-nitrobenzyl[190-192], trimethylsilyl[193] and benzhydryl[194], which can be removed under mild conditions without ring cleavage, have been extensively used in the laboratory. Woodward, *et al.*,[189] have proven the usefulness of 2,2,2-trichloroethyl ester as a protecting group in the synthesis of cephalosporin C. This ester survived the working conditions of the rearrangement and subsequent side-chain cleavage reactions and could be removed without damage to the β-lactam ring using zinc and acetic acid and thus was chosen as a suitable protecting group. The *p*-nitrobenzyl esters were easily prepared by treating the corresponding acid with *p*-nitrobenzyl bromide in the presence of a base at temperatures between 0 °C and 20 °C. The p-nitrobenzyl esters were stable to acidic and basic conditions and could be selectively removed by catalytic or chemical reduction. Benzhydryl esters[194] could be prepared using reactive intermediates like diphenyl diazomethane arising from the oxidation of benzophenone hydrazone with HgO.

TABLE 4

^{1}H NMR Data of Penicillin Sulfoxides **14**.



R	R'	PMR Chemical shifts (δ) ppm
$C_6H_5CH_2CONH$	$CH(C_6H_5)_2$	0.9(3H, s, C_2-C<u>H</u>$_3$); 1.7(3H, s, C_2-C<u>H</u>$_3$); 3.63(2H, s, $C_6H_5C\underline{H}_2$); 4.76(1H, s, C_3-<u>H</u>); 5.0(1H, d, J=4 Hz, C_5-<u>H</u>); 6.06(1H, dd, J=4 Hz, 10 Hz, C_6-<u>H</u>); 6.4(1H, d, J=10 Hz, N<u>H</u>); 7.03(1H, s, C<u>H</u>$(C_6H_5)_2$); 7.36(15H, m, $(C_6\underline{H}_5)_2$, $C_6\underline{H}_5$).
$C_6H_5CH_2CONH$	CH_2CCl_3	1.27(3H, s, C_2-C<u>H</u>$_3$); 1.77(3H, s, C_2-C<u>H</u>$_3$); 3.6(2H, s, $C_6H_5C\underline{H}_2$); 4.71(2H, s, C<u>H</u>$_2CCl_3$); 4.76(1H, s, C_3-<u>H</u>); 5.0(1H, d, J=4.5 Hz, C_5-<u>H</u>); 6.1(1H, dd, J=4.5 Hz, 9 Hz, C_6-<u>H</u>); 7.23(1H, d, J=9 Hz, N<u>H</u>), 7.37(5H, m, $C_6\underline{H}_5$).
$C_6H_5OCH_2CONH$	$CH(C_6H_5)_2$	0.93(3H, s, C_2-C<u>H</u>$_3$); 1.73(3H, s, C_2-C<u>H</u>$_3$); 4.6(2H, s, O<u>CH</u>$_2$); 4.76(1H, s, C_3-<u>H</u>); 5.0(1H, d, J=5 Hz, C_5-<u>H</u>); 6.2(1H, dd, J=5 Hz, 10 Hz, C_6-<u>H</u>); 7.06(1H, s, C<u>H</u>$(C_6H_5)_2$); 7.43(15H, m, $(C_6\underline{H}_5)_2$, $C_6\underline{H}_5$); 8.4(1H, d, J=10 Hz, N<u>H</u>).

Table 4(cont.)

$C_6H_5OCH_2CONH$	CH_2CCl_3	1.43(3H, s, C_2-C<u>H</u>$_3$); 1.83(3H, s, C_2-C<u>H</u>$_3$); 4.56(2H, s, OC<u>H</u>$_2$); 4.76(1H, s, C_3-H); 4.86(2H, s, C<u>H</u>$_2$CCl$_3$); 5.13(1H, d, J=5 Hz, C_5-<u>H</u>); 6.16(1H, dd, J=5 Hz, J=10 Hz, C_6-<u>H</u>); 7.16(1H, s, C<u>H</u>(C_6H_5)$_2$); 8.33(1H, d, J=10 Hz, N<u>H</u>).
H	$CH(C_6H_5)_2$	1.13(3H, s, C_2-C<u>H</u>$_3$); 1.56(3H, s, C_2-C<u>H</u>$_3$); 3.3(1H, dd, J=1.5 Hz, 16.5 Hz, C_6-<u>H</u>); <u>H</u>); 3.53(1H, dd, J=4.5 Hz, 16.5 Hz, C_6-<u>H</u>); 4.5(1H, s, C_3-<u>H</u>); 4.56(1H, dd, J=4.5 Hz, 1.5 Hz, C_5-<u>H</u>); 7.0(1H, s, C<u>H</u>(C_6H5)$_2$); 7.33(10H, m, (C_6<u>H</u>$_5$)$_2$).
$C_6H_5CHCONH$	CH_2CCl_3	1.24(3H, s, C_2-C<u>H</u>$_3$); 1.74(3H, s, C_2-C<u>H</u>$_3$); 4.70, 4.76(2H, ABq, J=12.06 Hz, C_3-C<u>H</u>$_2$CCl3); 4.62, 5.04(2H, ABq, J=12.06, C<u>H</u>$_2$CCl$_3$); 4.82(1H, s, C_3-<u>H</u>); 5.0(1H, d, J=4.34 Hz, C_5-<u>H</u>); 5.52(1H, d, J=12 Hz, C_6H_5C<u>H</u>); 6.08(1H, dd, J=4.34 Hz, 9.77 Hz, C_6-<u>H</u>); 6.78(1H, d, J=9.77 Hz, N<u>H</u>); 7.42(5H, m, C_6<u>H</u>$_5$); 7.82(1H, d, J=12 Hz, C_6H_5CHN<u>H</u>).
$C_6H_5CHCONH$	CH_2CCl_3	1.26(3H, s, C_2-C<u>H</u>$_3$); 1.74(3H, s, C_2-C<u>H</u>$_3$); 1.22(3H, t, J=6.86 Hz, N-CH_2C<u>H</u>$_3$); 3.58(2H, q, J=6.86 Hz, N-C<u>H</u>$_2$$CH_3$); [3.6(2H, m,); 4.02(1H, m,); 4.16(1H, m,), NC<u>H</u>$_2$C<u>H</u>$_2$N]; 4.68, 5.04(2H, ABq, J=11.24 Hz, C<u>H</u>$_2$CCl$_3$); 4.76(1H, s, C_3-<u>H</u>); 5.06(1H, d, J=4.47 Hz, C_5-<u>H</u>); 5.52(1H, d, J=5.75 Hz, C_6H_5C<u>H</u>); 6.04(1H, dd, J=4.5 Hz, J=9 Hz, C_6-<u>H</u>); 7.42(5H, m, C_6<u>H</u>$_5$); 7.6(1H, d, J=9 Hz, N<u>H</u>); 10.0(1H, d, J=5.75 Hz, C_6H_5CHN<u>H</u>)

C. *FORMATION OF UNSYM-AZETIDINONE DISULFIDES*

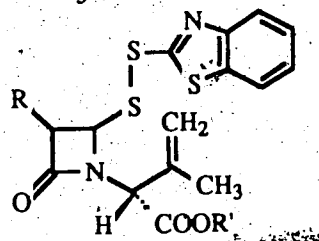
Dithioazetidinones[100] **20**, easily obtained by the reaction of penicillin sulfoxides **14**, with heteroaromatic thiols, were the key intermediates for preparing the 2β-(halomethyl)penams **18**. Treatment of penicillin sulfoxide **14**, with 2-mercaptobenzothiazole in refluxing toluene for 4 h gave the crystalline *unsym*-azetidinone disulfides **20**. ^{1}H NMR data of *unsym*-azetidinone disulfides **20**, used as intermediates for the synthesis of 2β-(halomethyl)penams **18**, are summarized in Table 5. With halogenating agents *unsym*-azetidinone disulfides **20**, gave 2β-(halomethyl)penams **18**, 3-halocephams **22** and ceph-3-em **26**, depending on the reaction conditions and the substituents at the C-6 position[100]. The reaction of *unsym*-azetidinone disulfides **20**, in methylene chloride with cupric chloride for 1.5 ÷ 4 h gave the 2β-(chloromethyl)penams **18**, (X=Cl), and with cupric bromide gave the 2β-(bromomethyl)penams **18**, (X=Br). The *unsym*-azetidinone disulfides **20**, 2β-(chloromethyl)penams **18**, (X=Cl), and 2β-(bromomethyl)penams **18**, (X=Br), were used as substrates for fluorination reactions with metal fluorides.

D. *FLUORINATION STUDIES*

The β-lactamase inhibitory activities of 2β-(substituted methyl)penam 1,1-dioxides **19a**, [R=H, R'=Na, X=Cl, Br, N_3) and the YTR class of compounds **19b**, are well documented[114,164-169]. We have investigated methods for the synthesis of 2β-(fluoromethyl)penam 1,1-dioxides **19c** (X=F), since the fluoromethyl substituent in an organic molecule can function as an irreversible enzyme inhibitor[195].

TABLE 5

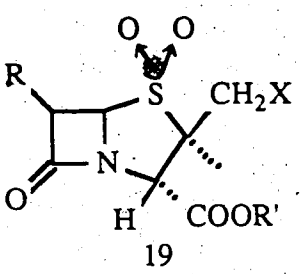
^{1}H NMR Data of *Unsym*-Azetidinone Disulfides 20,



R	R'	PMR Chemical Shifts (δ) ppm
$C_6H_5CH_2CONH$	$CH(C_6H_5)_2$	1.96(3H, s, $=C\underline{H}_3$); 3.7(2H, s, $C_6H_5C\underline{H}_2$); 4.96(1H, s, N-C<u>H</u>); 5.06(2H, s, $C\underline{H}_2$); 5.2(1H, dd, J=4 Hz, 8 Hz, C_3-<u>H</u>); 5.56 (1H, d, J=4 Hz, C_4-<u>H</u>); 7.33(15H, m, $CH(C_6\underline{H}_5)_2$, $C_6\underline{H}_5$); 6.46(1H, d, J=8 Hz, N<u>H</u>).
$C_6H_5CH_2CONH$	CH_2CCl_3	2.0(3H, s, $=C\underline{H}_3$); 3.73(2H, s, $C_6H_5C\underline{H}_2$); 4.76, 4.8(2H, ABq, J=12 Hz, $C\underline{H}_2CCl_3$); 5.13(2H, s, $C\underline{H}_2$); 5.23(1H, s, N-C<u>H</u>); 5.43(1H, d, J=4 Hz, C_4-H); 5.6(1H, ABq, J=4 Hz, C_3-<u>H</u>); 7.03(1H, d, J=9 Hz, N<u>H</u>); 7.36(5H,m, $C_6\underline{H}_5$).
$C_6H_5OCH_2CONH$	$CH(C_6H_5)_2$	2.0(3H, s, $=C\underline{H}_3$); 4.60(2H, s, $OC\underline{H}_2$); 5.0(1H, s, N-C<u>H</u>); 5.16(2H, s, $C\underline{H}_2$); 5.53(1H, dd, J=5 Hz, 10 Hz, C_3-<u>H</u>); 5.66(1H, d, J=5 Hz, C_4-<u>H</u>); 6.96(1H, s, $C\underline{H}(C_6H_5)_2$); 7.33(15H, m, $CH(C_6\underline{H}_5)_2$, $C_6\underline{H}_5$).

Table 5 (cont.)

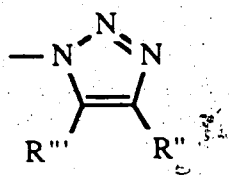
$C_6H_5OCH_2CONH$	CH_2CCl_3	2.06(3H, s, $=C\underline{H}_3$); 4.56(2H, s, $OC\underline{H}_2$); 4.76(2H, d, J=4 Hz, $C\underline{H}_2CCl_3$); 5.16(1H, s, N-C$\underline{H}$); 5.2(2H, s, $C\underline{H}_2$); 5.53(1H, dd, J=4 Hz, 10 Hz, C_3-$\underline{H}$); 5.76(1H, d, J=4 Hz, C_4-$\underline{H}$); 7.36(5H, m, $C_6\underline{H}_5$).
H	$CH(C_6H_5)_2$	1.86(3H, s, $=C\underline{H}_3$); 3.16(1H, dd, J=2 Hz, 13.5 Hz, C_3-$\underline{H}$); 3.4(1H, dd, J=4.5 Hz, 13.5 Hz, C_3-$\underline{H}$); 4.86(2H, s, $C\underline{H}_2$); 5.0(1H, s, N-C$\underline{H}$); 5.3(1H, dd, J=2 Hz, 4.5 Hz, C_4-$\underline{H}$); 6.93(1H, s, C$\underline{H}(C_6H_5)_2$); 7.3(10H, m, $(C_6\underline{H}_5)_2$).
$C_6H_5CHCONH$	CH_2CCl_3	1.93(3H, s, $=C\underline{H}_3$); 4.63(4H, s, $C\underline{H}_2CCl_3$); 5.06(2H, s, $C\underline{H}_2$); 5.16(1H, s, N-C$\underline{H}$); 5.53(1H, d, J=10.5 Hz, $C_6H_5C\underline{H}$); 5.4(1H, d, J=4.5 Hz, C_4-$\underline{H}$); 5.73(1H, dd, J=5.73 Hz, 10.5 Hz, C_3-$\underline{H}$); 6.66(1H, d, J=10.5 Hz, N$\underline{H}$); 7.33(5H, m, $C_6\underline{H}_5$).
$C_6H_5CHCONH$	CH_2CCl_3	1.2(3H, t, J=7.46 Hz, N-$CH_2C\underline{H}_3$); 1.96(3H, s, $=C\underline{H}_3$); 3.52(2H, q, J=7.46 Hz, N-$C\underline{H}_2CH_3$); [3.62(2H), 3.9(1H), 4.06(1H), $NC\underline{H}_2C\underline{H}_2N$]; 4.64, 4.8(2H, ABq, J=11.53 Hz, $C\underline{H}_2CCl_3$); 5.16(1H, s, N-C$\underline{H}$); 5.14(2H, s, $C\underline{H}_2$); 5.52(1H, d, J=4.75 Hz, C_4-$\underline{H}$); 5.74(2H, m, C_3-$\underline{H}$, $C_6H_5C\underline{H}$); 7.36(5H, m, $C_6\underline{H}_5$); 8.12(1H, d, J=9 Hz, N$\underline{H}$); 10.12(1H, d, J=9 Hz, CON$\underline{H}$CH).



19

R=H

R'=Na

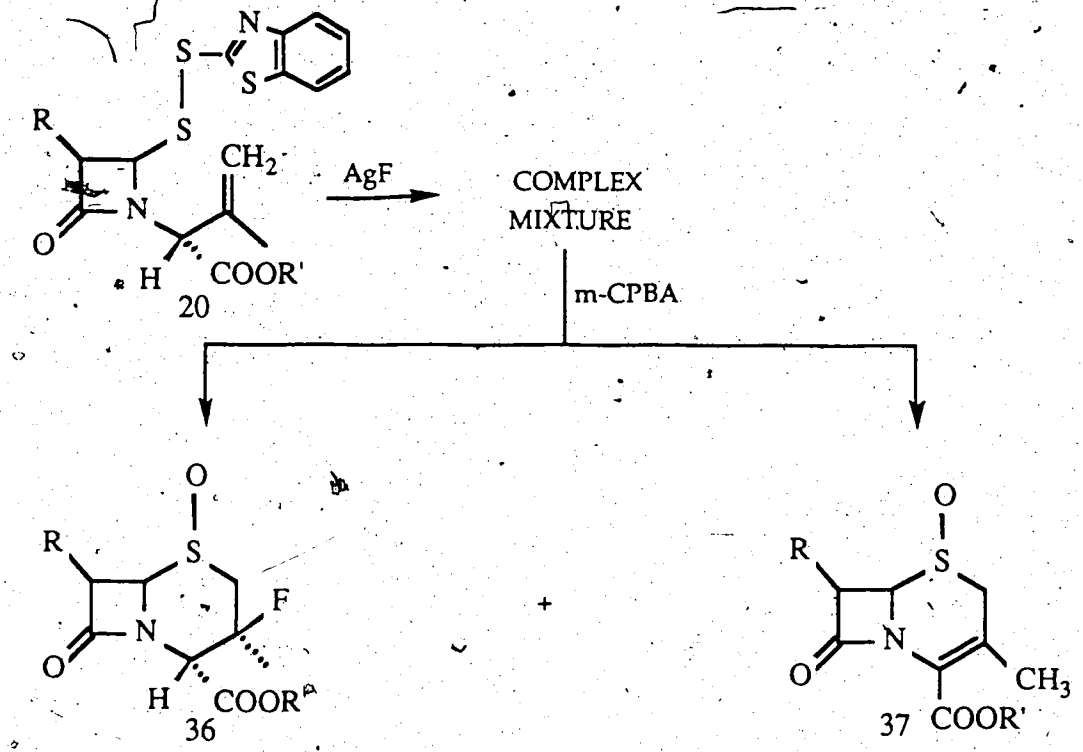
19	X
a	Cl, Br, N_3
b	
c	F

The reaction of silver fluoride and mercuric fluoride with *unsym*-azetidinone disulfide **20f**, under varying reaction conditions was studied. Displacement reactions of 2β-(halomethyl)penams **18**, (X=Cl, Br), with various substituents at C-6 [R=$C_6H_5CH_2CONH$, $C_6H_5OCH_2CONH$, Br_2 and α-Br) and C-3 [R'=$CH(C_6H_5)_2$, CH_2CCl_3, $CH_2C_6H_4$-*p*-NO_2 and CH_2OAc] with metal fluorides such as silver fluoride, sodium fluoride, potassium fluoride, mercuric fluoride, ferric fluoride, cupric fluoride and cobaltic fluoride in methylene chloride, acetonitrile, dimethylformamide and ethyl acetate at different temperatures and reaction time were investigated.

Reac[illegible]*unsym*–Azetidinone Disulfides 20, With Metal Fluorides.

[illegible]ion of 1 mole of *unsym*-azetidinone disulfide **20f**, [illegible]ONH, R'=$CH(C_6H_5)_2$], with 1.05 mole equivalents of silver [illegible]ene chloride at room temperature for 48 h gave a complex [illegible]xidation with a mixture of hydrogen peroxide (2.4 moles) [illegible]ic acid (8 moles) followed by purification over a silica gel column did not produce the desired 2β-(fluoromethyl)penam-1β-oxide, or any fluorine containing cepham derivative. Under identical reaction conditions with 2 mole equivalents of silver fluoride similar results were obtained.

The reaction of 1 mole equivalent of *unsym*-azetidinone disulfide **20f**, with 2 mole equivalents of silver fluoride in methylene chloride at room temperature for 120 h, gave a difficult-to-separate reaction mixture, (Scheme 1), which was oxidized with 1.1 mole equivalents of *m*-chloroperbenzoic acid. The silica-gel chromatographic purification of the oxidized product by gradient elution using ethyl acetate and hexane did not lead to a good separation of the compounds. The fraction that showed a mixture of two β-lactam containing compounds from the NMR spectrum, was purified on a preparative silica-gel chromatography plate using chloroform and ethyl acetate (1:2) as the solvent system. Two clear bands, observed under a UV lamp were cut out, extracted with ethyl acetate, filtered, concentrated and dried. Crystallization from a mixture of ethyl acetate and hexane gave the 3β-fluoro-3α-methylcepham 1-oxide **36**, the structure of which was confirmed by comparing the ^{1}H and ^{13}C NMR spectral data of the compound with authentic samples of the 3β-chloro- and 3β-bromocepham sulfoxides which were preparaed by literature methods. In addition the ^{19}F NMR spectrum establishes the cepham structure. The other compound isolated was given the structure ceph-3-em 1-oxide **37**, as it was identical in its ^{1}H NMR spectrum to that of an authentic sample obtained by oxidizing a sample of ceph-3-em **26**, under established conditions. [^{1}H NMR data: 2.2(3H, s, C_3-C$\underline{H}_3$); 3.24, 3.64(2H, ABq, J=18 Hz, C_2-C$\underline{H}_2$); 4.54(1H, d, J=4 Hz, C_6-$\underline{H}$); 4.62(2H, s, OC$\underline{H}_2$); 6.16(1H, dd, J=4 Hz, 10.5 Hz, C_7-$\underline{H}$); 6.98(1H, s, C$\underline{H}$(C_6H_5)$_2$); 7.3(15H, m, $C_6\underline{H}_5$, ($C_6\underline{H}_5$)$_2$; 7.98(1H, d, J=10.5 Hz, N$\underline{H}$)].



Scheme I

The reaction of 1 mole of the *unsym*-azetidinone disulfide **20f**, with 2 mole equivalents of silver fluoride was carried out in methylene chloride at room temperature for 24 h. The crude reaction mixture was oxidized with hydrogen peroxide (30%, 2.4 moles)/glacial acetic acid (8 moles) and purified on a preparative silica-gel plate with ethyl acetate and hexane (2:3) as solvent system. The three bands observed under a UV lamp were cut out, extracted with ethyl acetate, concentrated and dried. The only pure product isolated was given the structure ceph-3-em 1-oxide **37**, by comparision with an authentic sample.

As the reaction did not proceed to yield the 2β-(fluoromethyl)penam **18**, (X=F), and because of the difficulty in the separation of the 3β-

fluorocepham **22**, (X=F) and its low yield, a reaction under the same conditions was closely monitered by working up an aliquot periodically and checking the ^{1}H NMR spectrum. After 4 and 8 h, only the starting material **20f**, was recovered; after 24 h and up to 96 h two more β-lactam containing compounds, increasing in quantity with time, were present along with a decreased amount of starting material **20f**. After 120 h, when the ^{1}H NMR of the aliquot showed no starting material, the reaction mixture was worked up, oxidized and purified as before to isolate the same two compounds viz., the 3β-fluorocepham 1-oxide **36**, and ceph-3-em-1-oxide **37**, in very low yields.

The reaction of *unsym*-azetidinone disulfide **20f**, with silver fluoride in methylene chloride was repeated under various reaction conditions. At low temperature ((0 °C) the reaction did not proceed well, only starting material **20f**, being recovered, while at reflux temperatures, after 2 h, mostly starting material **20f**, and very little 3-fluorocepham **22**, (X=F), were present; after 5 h, 30% of 3β-fluorocepham **22**, (X=F), 10% of ceph-3-em **26**, and 60% of starting material **20f**, were present. Addition of crown ether to the reaction mixture did not help to speed up the reaction, as about 10% of 3β-fluorocepham **22**, (X=F), was isolated after 5 h.

From the reaction of the *unsym*-azetidinone disulfide **20f**, with mercuric fluoride in methylene chloride at room temperature for 24 h or at reflux temperature for 4 h, only the starting material was recovered. A similar reaction with *unsym*-azetidinone disulfide **20g** [R=H, R'=$CH_2C_6H_4$-*p*-NO_2] or **20h** [R=H, $CH(C_6H_5)_2$] or **20i** [R=$C_6H_5CH_2CONH$, R'=$CH(C_6H_5)_2$] and silver fluoride failed to give the desired products.

Thus, the reaction of various *unsym*-azetidinone disulfides **20**, with silver fluoride or mercuric fluoride gave 3β-fluorocepham **22**, (X=F), as the only fluorine containing product that could, in turn, undergo

dehydrofluorination as confirmed by the isolation of ceph-3-em **26** in most of the reactions. The results of the reaction of the *unsym*-azetidinone disulfide **20f** and **20g**, with metal fluorides in methylene chloride are summarized in Table 6.

Due to the long reaction time, low yields, problem of purification and inconsistent results, the displacement reaction of 2β-(halomethyl)penicillins **18**, (X=Cl, Br), by fluoride using metal fluorides was tried.

Reaction of 2β-(Chloromethyl)penams 18, (X=Cl), with Metal Fluorides

In the reaction of 1 mole benzhydryl-6β-phenylacetamido-2β-(chloromethyl)penicillanate **18**, (X=Cl), with 2 mole equivalents of silver fluoride in methylene chloride at room temperature after 4 h 75% of 3β-fluorocepham **22**, (X=F), was formed. The amount of the 3β-fluorocepham **22**, (X=F), formed remained constant in the next 48 h, at the end of which 13.5% of 3β-chlorocepham **22**, (X=Br), and 11.5% of ceph-3-em **26**, were present. In a similar reaction of 2,2,2-trichloroethyl-6β-phenoxyacetamido-2β-(chloromethyl)penicillanate **18**, (X=Cl), with silver fluoride, 62.5% of 3β-fluorocepham **22**, (X=F), was present after 24 h to 144 h. The reaction of benzyhydryl-6β-phenoxyacetamido-2β-(chloromethyl)penicillanate **18**, (X=Cl), with 2 mole equivalents of silver fluoride gave 86% of 3β-fluorocepham **22**, (X=F), 10% of 3β-chlorocepham **22**, (X=Br), and 4% of ceph-3-em **26**. The 3β-chlorocepham **22**, (X=Br), formed initially underwent dehydrochlorination with a gradual increase in the amount of ceph-3-em **26**, formed during the reaction. The results of displacement reactions of 2β-(chloromethyl)penams **18**, (X=Cl), with varied substituents at C-6 and C-3, using 2 mole equivalents of silver fluoride in methylene chloride at room temperature at various time intervals are summarized in Table 7.

TABLE 6

Relative Percentage* of Products Formed During the Reaction of *Unsym*-Azetidinone Disulfides with Metal Fluorides in CH_2Cl_2.

C. #	Reaction Conditions			Products (%)		
	Metal Fluorides	Reaction Time (h)	Reaction Temp (^oC)	Starting Material 20	3β-Fluorocepham 22 (X=F)	Ceph-3-em 26
20f	AgF	7	0	100	-	-
20f	AgF	4	25	95	5	-
		24		80	20	-
		48		60	40	-
		72		40	50	10
		96		20	60	20
		120		-	80	20
20f	AgF	4	Reflux	60	30	10
20f	HgF_2	2	25	100	-	-
		24		100	-	-
20f	HgF_2	2	Reflux	100	-	-
		4		100	-	-
20f	CuF_2	24	25	100	-	-
20f	ZnF_2	24	25	100	-	-
20g	AgF	72	25	100	-	-

* These percentages are calculated from the NMR spectrum of the crude product. In every case the yield of the crude product was better than 90%.

TABLE 7

Relative Percentage* of Products Formed During the Reaction of 2β-(Chloromethyl)penams **18** (X=Cl), with Silver Fluoride at Room Tempertaure in CH_2Cl_2

R	R'	Rx. T. (h)	3β-Fluorocepham **22** (X=F)	3β-Chlorocepham **22** (X=Cl)	Ceph-3-em **26**	Starting Material **18**
$C_6H_5CH_2CONH$	$CH(C_6H_5)_2$	4	75	---	---	25
		24	75	---	5.0	20
		48	75	13.5	11.5	---
$C_6H_5OCH_2CONH$	CH_2CCl_3	24	62.5	35	2.5	---
		48	62.5	32	5.5	---
		72	62.5	29	8.5	---
		144	62.5	25	12.5	---
$C_6H_5OCH_2CONH$	$CH(C_6H_5)_2$	24	86	10	4	---
		144	86	6	8	---
H	$CH(C_6H_5)_2$	48	85	---	15	---
H	PNB	48	85	---	15	---

Table 7 (cont.)

$C_6H_5CHCONH-$ $\quad\quad\vert$ $\quad\quad NH$ $\quad\quad\vert$ $\quad\quad CO$ $\quad\quad\vert$ $\quad\quad OCH_2C_6H_5$	$CH_2C_6H_5$	48	85	—	15	—

* These percentages are calculated from the NMR spectrum of the crude product. In every case the yield of the crude product was better than 90%.

Thus, in all the reactions of 2β-(chloromethyl)penams **18**, (X=Cl), with silver fluoride, the only fluorine containing product isolated was the 3β-fluorocepham **22**, (X=F).

Reaction of 2β-(Bromomethyl)penams 18, (X=Br), with Metal Fluorides

In the reaction of benzhydryl-6β-phenoxyacetamido-2β-(bromomethyl)-penicillanate **18**, (X=Br), with 2 mole equivalents of cupric fluoride, cobaltic fluoride, ferric fluoride or sodium fluoride at room temperature in methylene chloride after 24 h, the starting material **18**, (X=Br), along with 10 - 15% of 3β-bromocepham **22**, (X=Br), were isolated. No trace of any fluorinated compound was detected. With mercuric fluoride after 2 h the 3β-fluorocepham **22**, (X=F), and the starting material 2β-(bromomethyl)penams **18**, (X=Br), were obtained in equal proportions (1:1). When silver fluoride was used to bring about the exchange reaction, in 2 h, the major product 3β-fluorocepham **22**, (X=F), was isolated in over 90% yield.

Thus the metal fluorides like cobaltic fluoride, cupric fluoride, ferric fluoride and sodium fluoride failed to react with the 2β-(bromomethyl)-penam **18**, (X=Br). The 3β-bromocepham **22**, (X=Br), obtained during the reaction was the result of the ring expansion of the 2β-(bromomethyl)penam **18**, (X=Br). The mercuric fluoride reacted with 2β-(bromomethyl)penams **18**, (X=Br), at a slower rate compared to silver fluoride. The reaction of silver fluoride with 2β-(bromomethyl)penam **18**, (X=Br), with various substituents at C-6 was tried. The only major fluorine containing product isolated in all the cases was the 3β-fluorocepham **22**, (X=F). The results of the reaction of 2β-(bromomethyl)penam **18**, (X=Br), with various metal fluorides are summarized in Table 8.

TABLE 8

Relative Percentage* of Products Formed During the Reaction of 2β-(Bromomethyl)penams 18, (X=Br), with Metal Fluorides at Room Temperature in CH_2Cl_2

R	R'	Metal Fluoride	Reaction Time (h)	Starting Material	3β-Fluoro-cepham 22	3β-Bromo-cepham 22
$C_6H_5OCH_2CONH$	$CH(C_6H_5)_2$	NaF	48	90	---	10
		KF	48	90	---	10
		FeF_3	48	88	---	12
		CoF_3	48	85	---	15
		CuF_2	48	85	---	15
		HgF_2	2	50	50	---
		AgF	2	---	95	5
Br_2	$CH(C_6H_5)_2$	AgF	2	---	70	30
α-Br	$CH(C_6H_5)_2$	AgF	4	---	95	5

* These percentages are calculated from the NMR spectrum of the crude product. In every case the yield of the crude product was better than 90%.

To study the rate of reaction of 2β-(bromomethyl)penam **18**, (X=Br), with silver fluoride, the reaction was performed at different temperatures for various time intervals. The results are summarized in Table 9. As expected, at 25 °C the reaction was complete in 2 h, while at 0 °C in 2 h, only 75% of the reaction occurred. At reflux temperature in methylene chloride, after 2 h, a 67% yield of 3β-fluorocepham **22**, (X=F), along with a 33% yield of 3-bromocepham **22**, (X=Br), resulted. It appeared that under reflux the 2β-(bromomethyl)penam **18**, (X=Br), converted fairly rapidly to the 3β-bromocepham **22**, (X=Br), presumably via the episulfonium ion **39**, and that this compound was relatively stable and unreactive to silver fluoride.

The solvent was also an important factor in this reaction. The reaction proceeded in acetonitrile at a slower rate than in methylene chloride, about 80% of the 3β-fluorocepham **22**, (X=F), being isolated in 24 h. In DMF there was no observable reaction, only about 20% of the 3β-fluorocepham **22**, (X=F), was isolated in 24 h.

The 3β-fluorocepham **22**, (X=F), was oxidized to the sulfoxide using 1 mole equivalent of *m*-chloroperbenzoic acid for 2 h in methylene chloride at 0 °C. The sulfone was obtained using 2 mole equivalents of potassium permanganate (Scheme II). The ^{19}F and ^{1}H NMR data of the 3β-fluorocepham **22**, (X=F), its sulfoxide **36**, and sulfone **40**, are summarized in Table 10.

TABLE 9

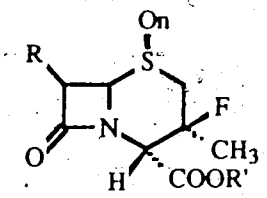
Relative Percentage* of Products Formed During the Reaction of 2β-(Bromomethyl)penan **18**, [X=Br, R=$C_6H_5OCH_2CONH$, R'=$CH(C_6H_5)_2$] with 2 Equivalents of AgF at Various Temperatures in CH_2Cl_2

Temp	Time (h)	Starting Material **18** (X=Cl)	3β-Fluorocepham **22** (X=F)	3β-Bromocepham **22** (X=Br)
0 °C	1	50	50	---
	2	25	75	---
	4	---	95	5
25 °C	2	---	95	5
Reflux	2	---	67	33

* These percentages are calculated from the NMR spectrum of the crude product. In every case the yield of the crude product was better than 90%.

TABLE 10

Physical Constants and Spectroscopic Data of 3β-Fluoro-3α-methylcepham **22**, (X=F), its Sulfoxide **36**, and Sulfone **40**.



22, n=0

36, n=1

40, n=2

C. #	R	R'	m. p.	$^{19}F(\delta)$ ppm	PMR Chemical Shifts (δ) ppm
22	$C_6H_5OCH_2CONH$	$CH(C_6H_5)_2$	103	16.55	1.26(3H, d, J_{H-F}=21 Hz, C_3-C<u>H</u>$_3$); 2.76(1H, dd, [ABF], J_{H-H}=15.4 Hz, J_{H-F}=6.8 Hz, C_2-C<u>H</u>$_2$); 3.38(1H, dd, [ABF], J_{H-F}=33.5 Hz, J_{H-H}=15.4 Hz, C_2-C<u>H</u>$_2$); 4.84(1H, d, J_{H-F}=12.9 Hz, C_4-<u>H</u>); 5.3(1H, d, J=4.7 Hz, C_6-<u>H</u>); 5.78(1H, dd, J=4.7 Hz, 10.98 Hz, C_7-<u>H</u>); 7.54(1H, d, J=10.98 Hz, N<u>H</u>); 7.3(15H,m, C_6<u>H</u>$_5$, (C_6<u>H</u>$_5$)$_2$).
36	$C_6H_5OCH_2CONH$	$CH(C_6H_5)_2$	96	23.07	1.34(3H, d, J_{H-F}=21 Hz, C_3-C<u>H</u>$_3$); 3.28(1H, dd, [ABF], J_{H-F}=37.7 Hz, J_{H-H}=12.56 Hz, C_2-C<u>H</u>$_2$); 3.54(1H, d, J_{H-H}=12.56 Hz, C_2-C<u>H</u>$_2$); 4.72(1H, d, J_{H-F}=13.8 Hz, C_4-<u>H</u>); 4.92(1H, d, J=4.32 Hz, C_6-<u>H</u>); 5.78(1H, dd,

Table 10 (cont.)

					J=4.32 Hz, 8.6 Hz, C_7-H); 7.3(15H, m, C_6H_5, $(C_6H_5)_2$); 8.66(1H, d, J=8.65 Hz, NH).
40	$C_6H_5OCH_2CONH$	$CH(C_6H_5)_2$	185	16.22	1.32(3H, d, J_{H-F}=21 Hz, C_3-CH_3); 3.28(1H, dd, [ABF], J_{H-H}=14.7 Hz, J_{H-F}=3.9 Hz, C_2-CH_2); 3.52(1H, dd, [ABF], J_{H-F}=32.5 Hz, J_{H-H}=14.7 Hz, C_2-CH_2); 4.9(1H, d, J_{H-F}=12.6 Hz, C_4-H); 5.1(1H, d, J=3.95 Hz, C_6-H); 6.16(1H, dd, J=3.95 Hz, 9.49 Hz, C_7-H); 7.3(15H, m, C_6H_5, $(C_6H_5)_2$); 8.36(1H, d, J=9.49 Hz, NH).
22	H	$CH_2C_6H_4NO_2$	127	15.67	1.42(3H, d, J_{H-F}=21 Hz, C_3-CH_3); 2.8(1H, dd, [ABF], J_{H-H}=15.1 Hz, J_{H-F}=4.2 Hz C_2-CH_2); 2.98(1H, dd, J=1.8 Hz, 15.12 Hz, C_7-H); 3.42 (1H, dd, [ABF], J_{H-F}=31 Hz, J_{H-H}=15.1 Hz, C_2-CH_2); 3.44(1H, dd, J=5.4 Hz, 15.12 Hz, C_7-H); 4.76(1H, d, J_{H-F}=12.4 Hz, C_4-H); 5.04(1H, d, J=5.47 Hz, C_6-H).

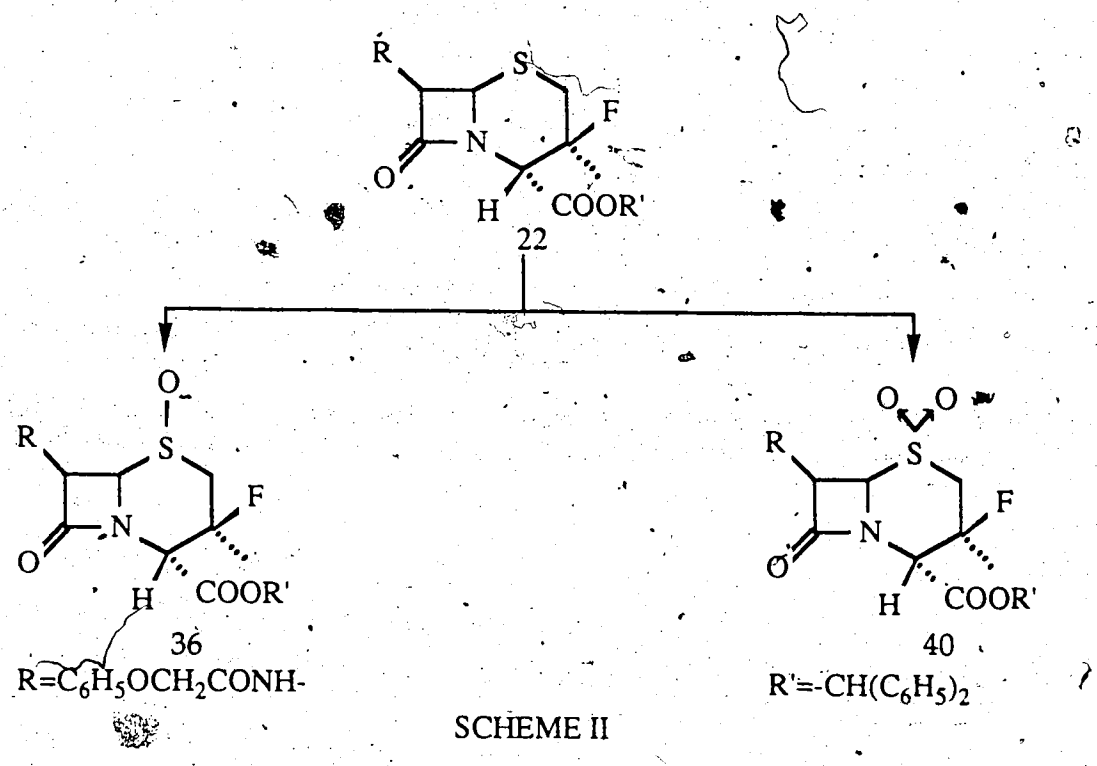
Table 10 (cont.)

22	H	$CH(C_6H_5)_2$	121	16.19	1.26(3H, d, J_{H-F}=21 Hz, C_3-$C\underline{H}_3$); 2.7(1H, dd, [ABF], J_{H-H}=14.9 Hz, J_{H-F}=4.7 Hz, C_2-$C\underline{H}_2$); 2.96(1H, dd, J=1.86 Hz, 14.9 Hz, C_7-$\underline{H}$); 3.36(1H, dd, [ABF], J_{H-F}=31.9 Hz, J_{H-H}=14.9 Hz, C_2-$C\underline{H}_2$); 3.38(1H, dd, J=4.53 Hz, 14.9 Hz, C_7-$\underline{H}$); 4.82(1H, d, J_{H-F}=11.53 Hz, C_4-$\underline{H}$); 5.0(1H, d, J=3.95 Hz, C_6-$\underline{H}$); 7.3(10H, m, $(C_6\underline{H}_5)_2$).
40	H	$CH_2C_6H_4NO_2$	220	3.0[a]	1.56(3H, d, J_{H-F}=21 Hz, C_3-$C\underline{H}_3$); 3.56(1H, dd, [ABF], J_{H-F}=57 Hz, J_{H-H}=4.75 Hz, C_2-$C\underline{H}_2$); 3.64(1H, dd, J=5.14 Hz, 10.29 Hz, C_7-$\underline{H}$); 3.8(1H, dd, [ABF], J_{H-F}=26.3 Hz, J_{H-H}=14.8 Hz, C_2-$C\underline{H}_2$); 4.1(1H, dd, J=5.7 Hz, 16.01 Hz, C_7-$\underline{H}$); 5.04(1H, d, J_{H-F}=13.72 Hz, C_4-$\underline{H}$); 5.14(1H, d, J=3.43 Hz, C_6-$\underline{H}$).

All NMR spectra were recorded in $CDCl_3$ as solvent and TMS as internal standard.

a DMSO used as solvent for NMR spectra.

ABF ia an AB quartet with H-F coupling.



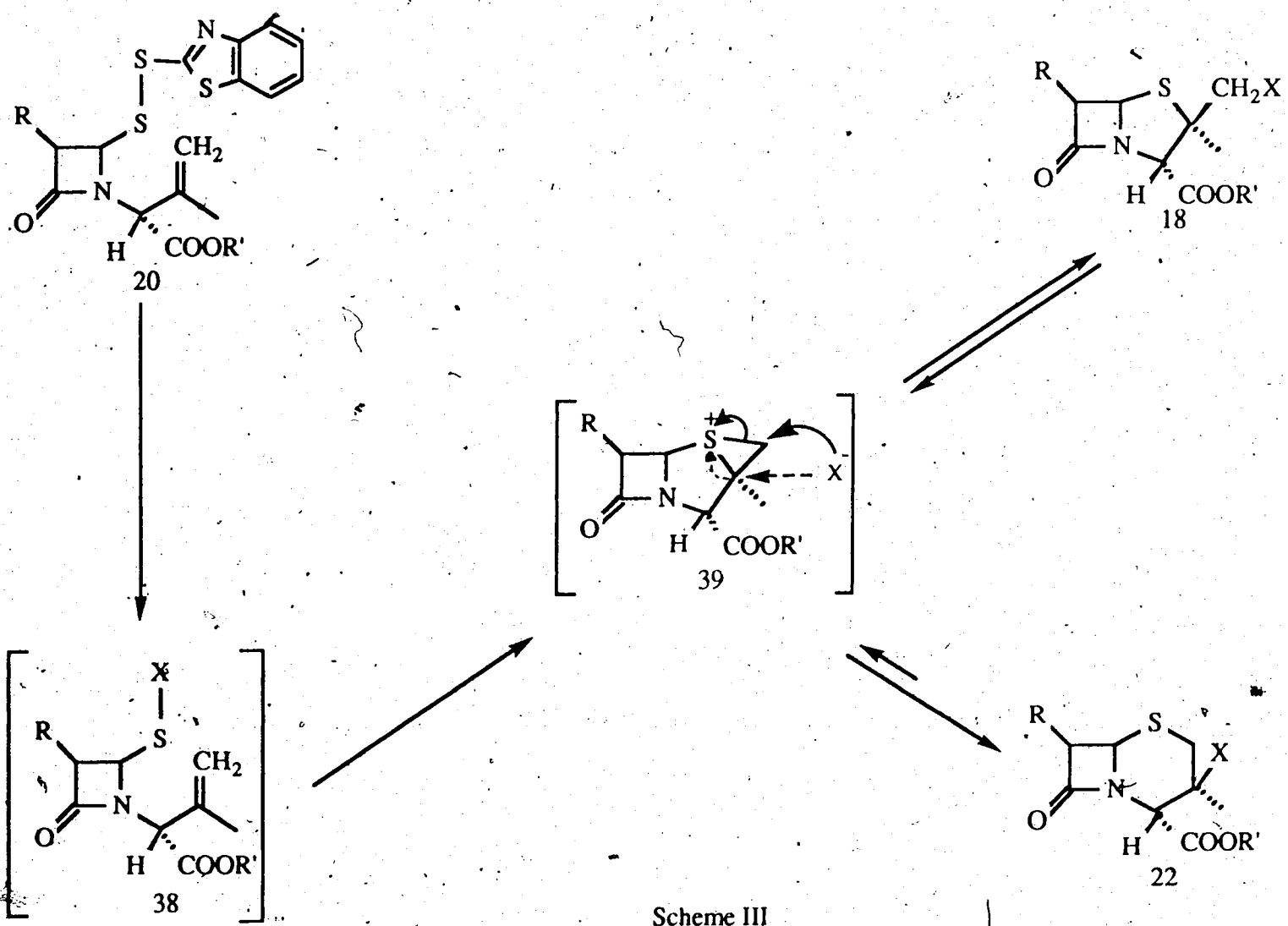
SCHEME II

In the reaction of *p*-nitrobenzyl-6,6-dihydro-2β-(bromomethyl)-penicillanate **18**, (X=Br), with mercuric fluoride in methylene chloride at room temperature for 1 h, 66% of 3β-fluorocepham **22**, (X=F), and 33% of 3β-bromocepham **22**, (X=Br), were readily formed. After 5 h, 20% of 3β-bromocepham **22**, (X=Br), and 20% of ceph-3-em **26**, were present along with 60% of 3β-fluorocepham **22**, (X=F). So, as the reaction proceeded, the conversion of 2β-(bromomethyl)penam **18**, (X=Br), to 3β-bromocepham **22**, (X=Br) and to the 3β-fluorocepham **22** (X=F), occurred along with the dehydrofluorination and dehydrobromination of the cephams **22**, to the ceph-3-em **26**. At 0 °C, a similar result was obtained, but at -20 °C there was no observable reaction, only starting material being recovered.

Mechanism

Scheme III summarizes the mechanism of these reactions. The *unsym*-azetidinone disulfides **20**, were presumably converted to azetidinone sulfenyl halides **38**, which transform to the thiranium halide **39**. In the case of nucleophilic displacement reactions of 2β-(halomethyl)penams **18**, the intermediate thiiranium ion **39**, was presuambly involved. This reactive thiiranium intermediate **39**, could then follow one of the rearrangement pathways - attack of the anion at the *tertiary* - carbon leading to the thermodynamically stable 3β-substituted cepham **22**, or attack of the anion at the *primary* - carbon producing the kinetic product, 2β-(substituted methyl) penams **18**. With halides (X=Cl, Br or I) the products formed in this reaction were dependent on the experimental conditions. Thus, particularly in the case of iodo- and bromo-compounds, the formation of the 2β-(halomethyl)penams **18**, (X=I, Br), were favored by short reaction times and low temperatures. When *unsym*-azetidinone disulfide **20g**, [R=H], was reacted with cupric bromide in methylene chloride at room temperature for 4 h, an about 1:1 mixture of 2β-(bromomethyl)penam **18**, (X=Br), and 3β-bromocepham **22**, (X=Br), were formed, while the same reaction run at 0 °C, gave only 2β-(bromomethyl)penam **18**, (X=Br), as the sole product[114]. The 2β-(halomethyl)penams **18**, (X=Cl, Br, I), were also readily converted to 3β-halocephams **22**, *via* the same thiiranium intermediate **39**. In the case of 2β-(halomethyl)penams **18**, (X=Br or I), the conversion to 3β-halocephams **22**, occurred with time even in the solid state[100,102,111].

Thus, the ease of preparation of the 2β-(halomethyl)penams **18**, (X=Cl, Br or I), followed the sequence Cl> Br> I, paralleling the increase in the ionic character of the C–X bond from C–Cl to C–I. Fluorine is an atypical halogen and forms essentially covalent bonds with carbon. It would appear that the


R
S
S
N
S
CH2
O
N
H
COOR'
20
R
S
CH2X
O
N
H
COOR'
18
R
S
X
O
N
H
COOR'
39
X
R
S
CH2
O
N
H
COOR'
38
R
S
X
O
N
H
COOR'
22

Scheme III

difference in character favours a preferential attack of the F^- at *tertiary* - carbon, resulting in the formation of the 3β-fluorocepham **22**, (X=F). Thus, with cupric bromide or iodine, the reaction with *unsym*-azetidinone disulfides **20**, for a short reaction time or at low temperature resulted in the preferential formation of 2β-(halomethyl)penams **18**, (X=Cl, I). These factors had no effect on the reaction of 2β-(halomethyl)penam **18**, (X=Cl or Br), or of *unsym*-azetidinone disulfide **20f** and **20g**, with silver fluoride since only the 3β-fluorocepham **22**, (X=F), was detected in these reactions.

In the reaction of silver fluoride with *unsym*-azetidinone disulfides **20g**, or silver fluoride and mercuric fluoride with 2β-(halomethyl)penams **18**, (X=Cl and Br), only one identifiable fluorine containing product **22**, (X=F), was isolated. The ^{19}F NMR spectrum, (Figure 14), showed a complex multiplet at δ 16.55, expected of the 3β-fluorocepham **22**, (X=F), rather than the 2β-(fluoromethyl)penam **18**, (X=F). In the ^1H NMR spectrum (Figure 15), the 3α-CH_3 signal of compound **22**, (X=F), appeared as a doublet at δ 1.36. Although the position was more in keeping with the C_2-CH_3 of a penicillin, the splitting of the 3α-CH_3 group due to the adjacent fluorine was 21 Hz, characteristic of a CH_3-C-F group[196]. In addition, the splitting pattern of the C_2-CH_2 group in the δ 2.76 and δ 3.38 region was also that expected of the C_2-CH_2 of 3β-fluorocepham **22**, (X=F), rather than the C_2-CH_2F of 2β-(fluoromethyl)penam **18**, (X=F). The ^{19}F NMR and ^1H NMR spectra of the sulfoxide **36**, and sulfone **40**, of 3β-fluorocepham **22**, (X=F), followed the same pattern.

Table 11 summarizes the ^{13}C data on the 2β-(halomethyl)penams **18**, (X=Cl, Br), and the isomeric 3β-halocephams **22**, (X=Br, F). The C_2-CH_2 carbon of the penams**18**, appeared at δ 52.69 when X was Cl and at δ 42.23 when X was Br, whereas in the cephams **22**, the C_2-CH_2 carbon appeared at δ 37.44 when X was Br, and in the case of the fluoro compound the C_2-CH_2 carbon

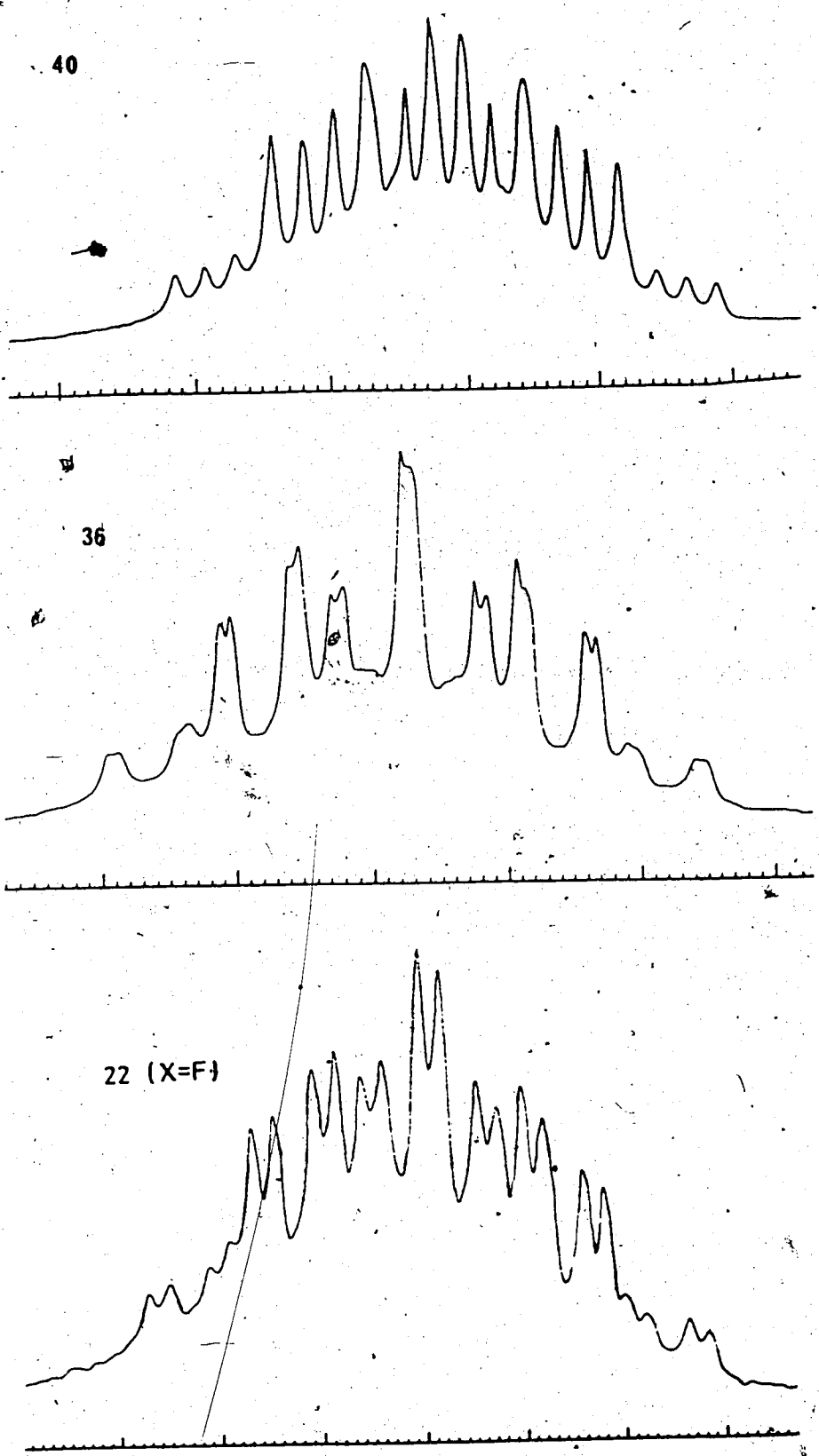


Figure 14. ^{19}F NMR Spectra of 3β-Fluorocepham (22, X=F), its Sulfoxide (36) and Sulfone (40)

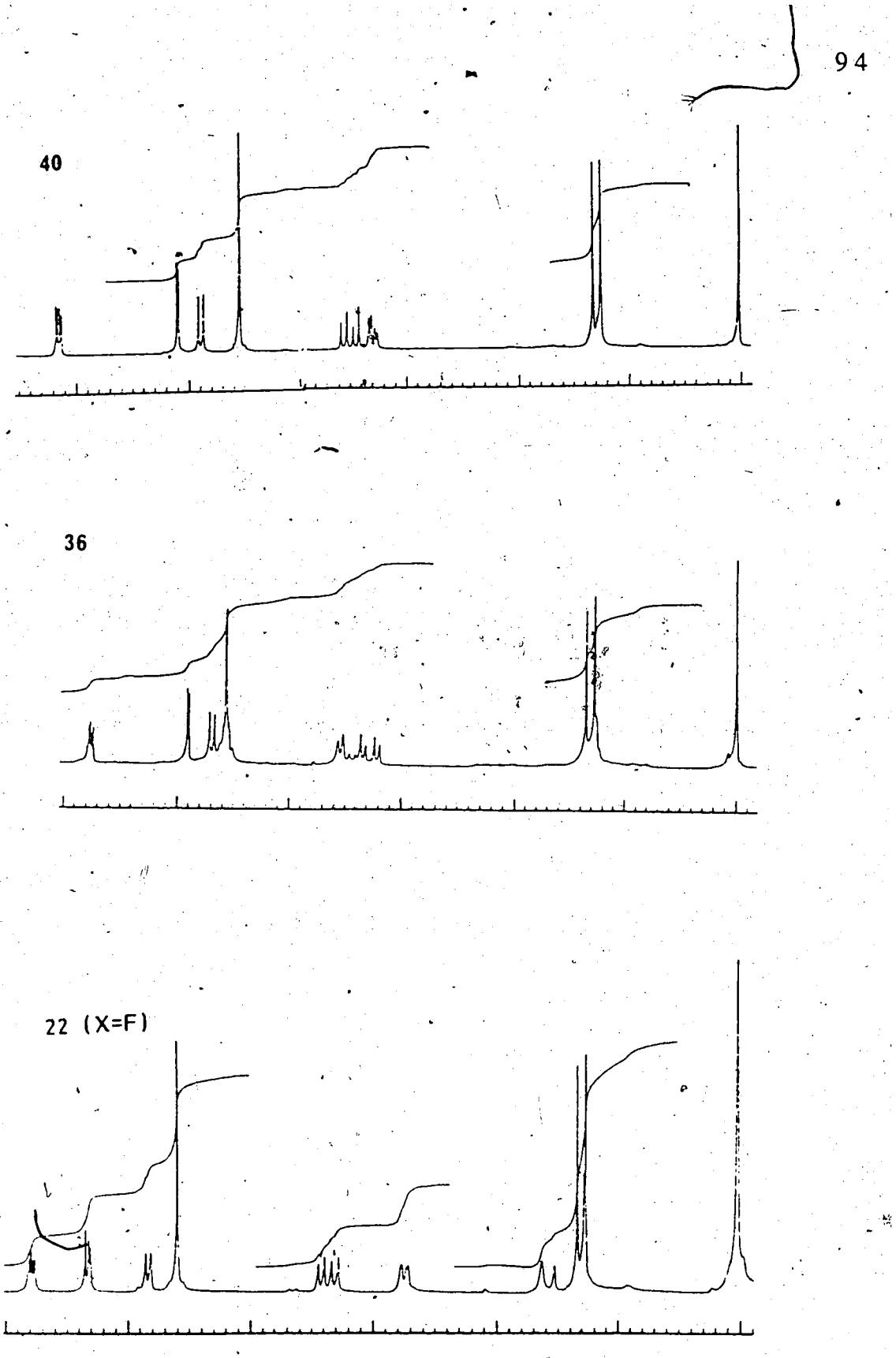
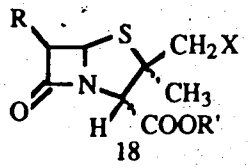


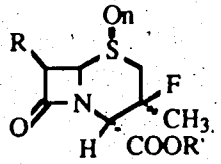
Figure 15. ^{1}H NMR Spectra of 3β-Fluorocepham (22, X=F), its Sulfoxide (36) and Sulfone (40)

TABLE 11

^{13}C Chemical Shift Data of 2β-(Halomethyl)penams **18**, (X=Cl, Br), 3β-Bromocepham **22**, (X=Br), 3β-Fluorocepham **22**, (X=F), its Sulfoxide **36** and Sulfone **40**.



18 X=Cl, Br



22, n=0
36, n=1
40, n=2

2β-(Halomethyl)penams			3β-Halocephams				
Carbon #	**18** (X=Cl)	**18** (X=Br)	Carbon #	**22** (X=Br)	**22** (X=F)	**36**	**40**
2	69.29	68.83	2	37.44	32.03 32.34	54.78 55.07	56.65 56.96
3	68.14	68.31	3	58.39	83.60 85.75	122.81 123.44	92.98 95.52
5	65.14	65.80	4	54.07	57.89 58.24	56.70 57.05	56.36 56.65
6	59.48	59.56	6	58.84	53.59	73.63	67.95
2-CH_3	21.70	22.75	7	61.75	58.61	59.64	59.09
2-CH_2Cl	52.69	42.23	3-CH_3	30.22	23.78 24.09	23.74 24.04	23.58 23.89

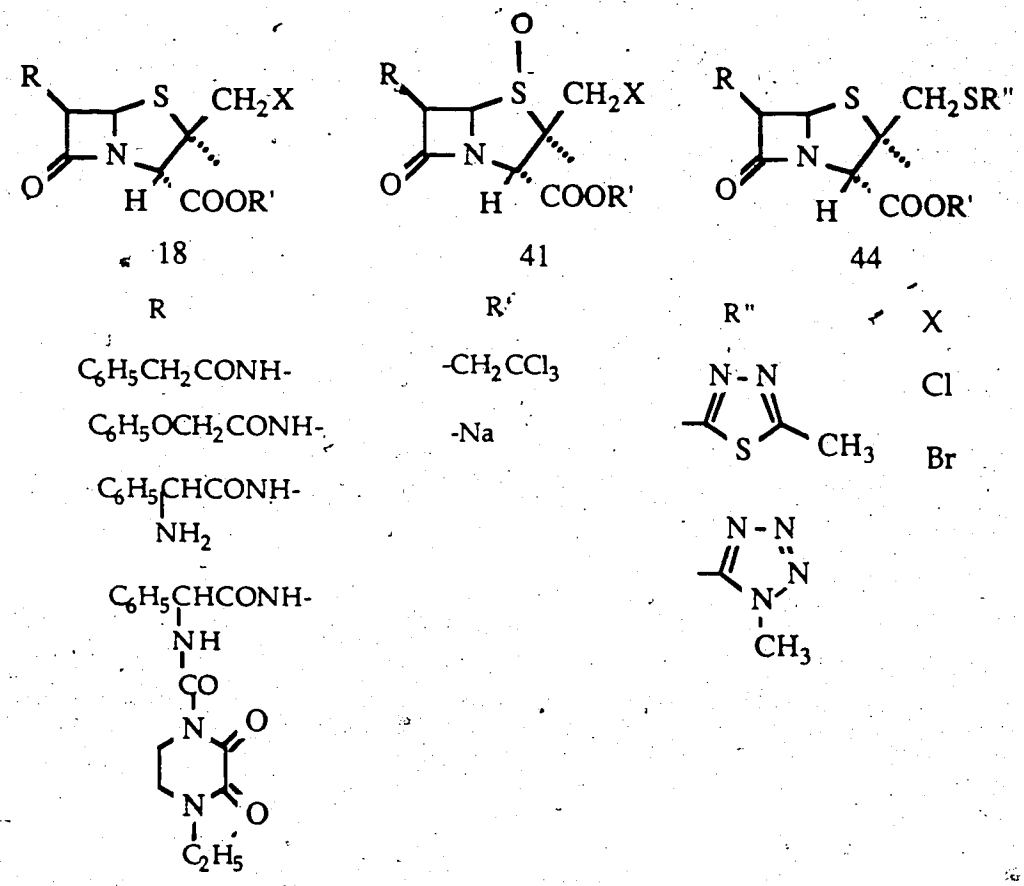
a. The characterization of the methyl, methylene, methine and *tert*-carbons was on the basis of J (Mod) experiments.
b. All spectra were recorded in $CDCl_3$ as solvent and TMS as internal standard.
c. Only the carbon atoms affected by the halogen are listed.

appeared as a doublet at δ 32.03 and δ 32.34. In addition the *tertiary* C_3-carbon appeared as a doublet at δ 83.60 and δ 85.75, the *secondary* C_4-carbon as a doublet at δ 57.89 and δ 58.24 and the C_3-CH_3 carbon as a doublet at δ 23.78 and δ 24.09. With 3β-bromocepham **22**, (X=Br), these signals were found as singlets at δ 58.39 for *tertiary* C_3-carbon, δ 54.07 for the *secondary* C_4-carbon and δ 30.22 for the C_3-CH_3 carbon.

When the 3β-fluorocepham sulfoxide **36** was treated with pyridine for 30 min at room temperature, the ceph-3-em-1β-oxide **37**, was obtained in 35% yield. This product could have arisen only by dehydrofluorination of the 3β-fluorocepham-1β-oxide **36**. It was unlikely for the 2β-(fluoromethyl)penam-1β-oxide to isomerize to the 3β-fluorocepham-1β-oxide **37** under these conditions. The data thus indicated that the fluorine containing product isolated in all the reactions of metal fluorides with *unsym*-azetidinone disulfides **20**, or 2β-(halomethyl)penams **18**, (X=Cl, Br) was the 3β-fluoro- 3α-methylcepham **22**, (X=F).

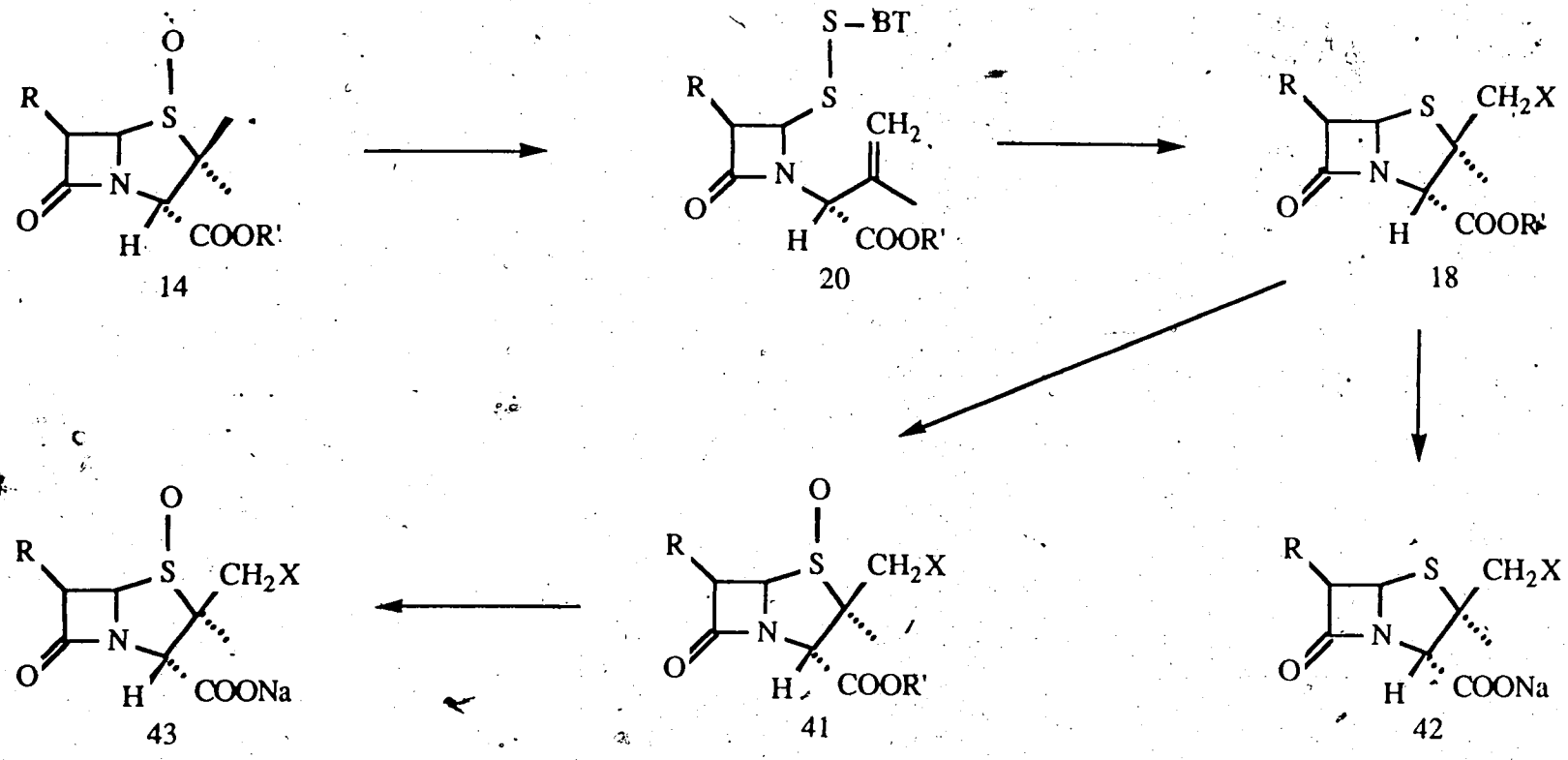
E. *2β-(SUBSTITUTED METHYL)PENAMS*

The development of resistance to β-lactam antibiotics by bacteria led to the search for novel β-lactamase-stable β-lactams. In the process, numerous penicillins were synthesized with modifications at C-6 and C-2 and the penicillin nucleus itself. On the same lines we have synthesized penicillins with various substituents at C-6 [R=$C_6H_5CH_2CONH$-, $C_6H_5OCH_2CONH$-, $C_6H_5CH(NH_2)CONH$-] and at C-2 such as chloromethyl, bromomethyl, (5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl and (1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl to test for antibacterial activity.



Synthesis of 2β-(Halomethyl)penams 18, (X=Cl, Br), and Their Sulfoxides

Scheme IV represents the route for the synthesis of 2β-(halomethyl)-penams **18**, (X=Cl, Br) and their sulfoxides **41**. The 2,2,2-trichloroethyl-penicillanate-1β-oxide **14**, [R'=CH_2CCl_3], obtained in over 90% yield by the oxidation of 2,2,2-trichloroethyl-penicillanate **1**, with *m*-chloroperbenzoic acid, on reflux with 1.1 mole equivalents of 2-mercaptobenzothiazole in toluene for 5 h, gave the *unsym*-azetidinone disulfide **20c** and **20d**, in quantitative yields. The reaction of the *unsym*-azetidinone disulfide **20b**, with 1.1 mole equivalents of cupric chloride in methylene chloride at room temperature for 5 h gave the 6β-phenoxyacetamido-2β-(chloromethyl)penam **18**, (X=Cl), while the disulfide **20c**, with 1.1 mole equivalents of cupric chloride at room temperature gave 6β-phenylacetamido-2β-



R=$C_6H_5CH_2CONH$-

R=$C_6H_5OCH_2CONH$-

R'=CH_2CCl_3

BT=

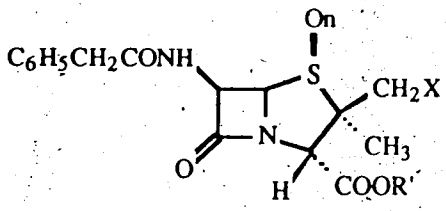
Scheme IV

(chloromethyl)penam **18**, (X=Cl), in 45 min. A similar reaction of the *unsym*-azetidinone disulfide **20b** and **20e**, with 1.1 mole equivalents of cupric bromide in methylene chloride at 0 °C gave 6β-phenoxyacetamido-2β-(bromomethyl)penam **18**, (X=Br), and 6β-phenylacetamido-2β-(bromomethyl)penam **18**, (X=Br), in 4 h and 45 min respectively.

Oxidation of the 2β-(halomethyl)penams **18**, (X=Cl, Br) with 1.1 mole equivalent of *m*-chloroperbenzoic acid in methylene chloride at 0 °C for 2 h yielded the 2β-(halomethyl)penam-1β-oxides **41**, (X=Cl, Br). Purification on a silica-gel column by gradient elution using a mixture of ethyl acetate and hexane as the solvent system gave the pure sulfoxides **41**, in ~ 60% yield. The 2,2,2-trichloroethyl group of the 2β-(halomethyl)penams **18**, (X=Cl, Br) and their corresponding sulfoxides **41**, could be removed by reductive cleavage with zinc and acetic acid in DMF at 0 °C for 3 h. The acids thus obtained were converted to their corresponding sodium salts by dissolving the acids in ethyl acetate and adjusting the pH of the solution to 7.0 by dropwise addition of sodium-2-ethylhexanoate in ethanol. Table 12 summarizes the ^{1}H NMR data of esters and sodium salts of 6β-phenylacetamido-2β-(halomethyl)penams **18**, (X=Cl, Br) and **42**, (X=Cl, Br) and their corresponding sulfoxides **41**, (X=Cl, Br) and **43**, (X=Cl, Br). Table 13 summarizes the ^{1}H NMR data of esters and sodium salts of 6β-phenoxyacetamido-2β-(halomethyl)penams **18**, (X=Cl, Br) and **42**, (X=Cl, Br), and their corresponding sulfoxides **41**, (X=Cl, Br) and **43**, (X=Cl, Br).

TABLE 12

^{1}H NMR Data of 6β-Phenylacetamido-2β-(halomethyl)penams and Their Sulfoxides.



18 and 42, n=0
41 and 43 n=1

C #	R'	X	PMR Chemical Shifts (δ) ppm
18	CH_2CCl_3	Cl	1.6(3H, s, C_2-C<u>H</u>$_3$); 3.32(2H, s, C_2-C<u>H</u>$_2$); 3.66(2H, s, C_6H_5C<u>H</u>$_2$); 4.76, 4.82(2H, ABq, J=10.95 Hz, C<u>H</u>$_2CCl_3$); 5.02(1H, s, C_3-<u>H</u>); 5.64(2H, m, C_6-<u>H</u>, C_5-<u>H</u>); 6.24 (1H, d, J=8.03 Hz, N<u>H</u>): 7.7(5H, m, C_6<u>H</u>$_5$).
18	CH_2CCl_3	Br	1.62(3H, s, C_2-C<u>H</u>$_3$); 3.22, 3.28(2H, ABq, J=10.52 Hz, C_2-C<u>H</u>$_2$); 3.66(2H, s, C_6H_5C<u>H</u>$_2$); 4.74, 4.8(2H, ABq, J=11.84 Hz, C<u>H</u>$_2CCl_3$); 5.08(1H, s, C_3-<u>H</u>); 5.64(2H, m, C_6-<u>H</u>, C_5-<u>H</u>); 6.1(1H, d, J=8.45 Hz, N<u>H</u>); 7.34(5H, m, C_6<u>H</u>$_5$).
41	CH_2CCl_3	Cl	1.42(3H, s, C_2-C<u>H</u>$_3$); 3.60, 3.66(2H, ABq, J=15.06 Hz, C_6H_5C<u>H</u>$_2$); 4.08, 4.3 (2H, ABq, J=11.72 Hz, C_2-C<u>H</u>$_2$); 4.7, 4.98(2H, ABq, J=11.72 Hz, C<u>H</u>$_2CCl_3$);

Table 12 (cont.)

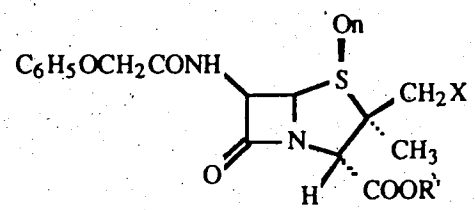
			5.02(1H, d, J=4.9 Hz, C_5-H); 6.1(1H, dd, J=4.9 Hz, 10.35 Hz, C_6-H); 7.06(1H, d, J=10.35 Hz, NH); 7.3(5H, m, C_6H_5).
41	CH_2CCl_3	Br	1.44(3H, s, C_2-CH_3); 3.56, 3.64(2H, ABq, J=15.22 Hz, $C_6H_5CH_2$); 3.84, 4.12 (2H, ABq, J=11.84 Hz, C_2-CH_2); 4.72, 4.98(2H, ABq, J=11.84 Hz, CH_2CCl_3); 4.82(1H, s, C_3-H); 5.02(1H, d, J=5.35 Hz, C_5-H); 6.1(1H, dd, J=5.35 Hz, 9.64 Hz, C_6-H); 7.08(1H, d, J=9.64 Hz, NH); 7.32(5H, m, C_6H_5).
42	Na	Cl	1.38(3H, s, C_2-CH_3); 2.62, 3.54(2H, ABq, J=14.14 Hz, C_2-CH_2); 3.7(2H, s, $C_6H_5CH_2$); 4.16(1H, s, C_3-H); 5.26(1H, d, J=3.4 Hz, C_5-H); 5.42(1H, d, J=3.4 Hz, C_6-H); 7.36(5H, m, C_6H_5).
42	Na	Br	1.6(3H, s; C_2-CH_3); 3.34, 3.58(2H, ABq, J=15 Hz, C_2-CH_2); 3.54, 3.68(2H, ABq, J=15 Hz, $C_6H_5CH_2$); 4.4(1H, s; C_3-H); 5.3(1H, d, J=3.5 Hz, C_5-H); 5.4(1H, d, J=3.5 Hz, C_6-H); 7.36(5H, m, C_6H_5).
43	Na	Cl	1.34(3H, s, C_2-CH_3); 3.66(2H, s, $C_6H_5CH_2$); 3.98, 4.02(2H, ABq, J=12 Hz, C_2-CH_2); 4.26(1H, s, C_3-H); 5.26(1H, d, J=3.5 Hz, C_5-H); 5.9(1H, d, J=3.5 Hz, C_6-H); 7.36 (5H, m, C_6H_5).

Table 12 (cont.)

43	Na	Br	1.3(3H, s, C_2-C<u>H</u>$_3$); 3.6(2H, s, $C_6H_5C\underline{H}_2$); 3.82, 3.88(2H, ABq, J=12 Hz, C_2-C<u>H</u>$_2$); 4.34(1H, s, C_3-<u>H</u>); 5.18(1H, d, J=4.8 Hz, C_5-<u>H</u>); 5.76(1H, d, J=4.8 Hz, C_6-<u>H</u>): 7.26(5H, m, $C_6\underline{H}_5$).

TABLE 13

^{1}H NMR Data of 6β-Phenoxyacetamido-2β-(halomethyl)penam and Their Sulfoxides.



18 and 42 n=0
41 and 43 n=1

C #	R'	X	PMR Chemical shifts (δ) ppm
18	CH_2CCl_3	Cl	1.66(3H, s, C_2-C<u>H</u>$_3$); 3.52(2H, s, C_2-C<u>H</u>$_2$); 4.58, 4.64(2H, ABq, J=14.13 Hz, C<u>H</u>$_2$CCl$_3$); 4.82(2H, s, OC<u>H</u>$_2$); 5.12(1H, s, C_3-<u>H</u>); 5.72(1H, d, J=3.21 Hz, C_5-<u>H</u>); 5.78(1H, dd, J=3.21 Hz, 10.28 Hz, C_6-<u>H</u>); 6.96(1H, d, J=10.28 Hz, N<u>H</u>); 7.36(5H, m, C_6<u>H</u>$_5$).
18	CH_2CCl_3	Br	1.73(3H, s, C_2-C<u>H</u>$_3$); 3.53(2H, s, C_2-C<u>H</u>$_2$); 4.66(2H, s, OC<u>H</u>$_2$); 4.76(2H, s, C<u>H</u>$_2$CCl$_3$); 5.23(1H, s, C_3-<u>H</u>); 5.8(2H, m, C_5-<u>H</u>, C_6-<u>H</u>); 7.3(5H, m, C_6<u>H</u>$_5$); 7.73(1H, d, J=10 Hz, N<u>H</u>).
41	CH_2CCl_3	Cl	1.48(3H, s, C_2-C<u>H</u>$_3$); 4.12, 4.34(2H, ABq, J=12 Hz, C_2-C<u>H</u>$_2$); 4.82(1H, s, C_3-<u>H</u>); 4.78, 5.08(2H, ABq, J=14 Hz, C<u>H</u>$_2$CCl$_3$); 4.60(2H, s, OC<u>H</u>$_2$); 5.12(1H, d, J=4.84 Hz,

Table 13 (cont.)

			C_5-H); 6.22(1H, dd, J=4.84 Hz, 10.44 Hz, C_6-H); 7.36(5H, m, C_6H_5); 8.22(1H, d, J=10.44 Hz, NH).
41	CH_2CCl_3	Br	1.46(3H, s, C_2-CH_3); 4.02, 4.2(2H, ABq, J=11.27 Hz, C_2-CH_2); 4.56(2H, s, OCH_2); 4.72, 4.98(2H, ABq, J=12.02 Hz, CH_2CCl_3); 4.92(1H, s, C_3-H); 5.12(1H, d, J=4.55 Hz, C_5-H); 6.16(1H, dd, J=4.55 Hz, 10.33 Hz, C_6-H); 7.32(5H, m, C_6H_5); 8.2(1H, d, J=10.33 Hz, NH).
42	Na	Cl	1.32(3H, s, C_2-CH_3); 2.52, 3.48(2H, ABq, J=14.28 Hz, C_2-CH_2); 4.1(1H, s, C_3-H); 4.52(2H, s, OCH_2); 5.22(1H, d, J=3.96 Hz, C_5-H); 5.36(1H, d, J=3.96 Hz, C_6-H); 7.3(5H, m, C_6H_5).
42	Na	Br	1.54(3H, s, C_2-CH_3); 3.14, 3.42(2H, ABq, J=14.7 Hz, C_2-CH_2); 4.46(1H, s, C_3-H); 4.58(2H, s, OCH_2); 5.28(1H, d, J=4.11 Hz, C_5-H); 5.38(1H, d, J=4.11 Hz, C_6-H); 7.2(5H, m, C_6H_5).
43	Na	Cl	1.28(3H, s, C_2-CH_3); 4.02(2H, s, C_2-CH_2); 4.3(1H, s, C_3-H); 4.6(2H, s, OCH_2); 5.32(1H, d, J=4.5 Hz, C_5-H); 5.92(1H, d, J=4.5 Hz, C_6-H); [6.92(2H), 7.0(1H), 7.28(2H), C_6H_5)].

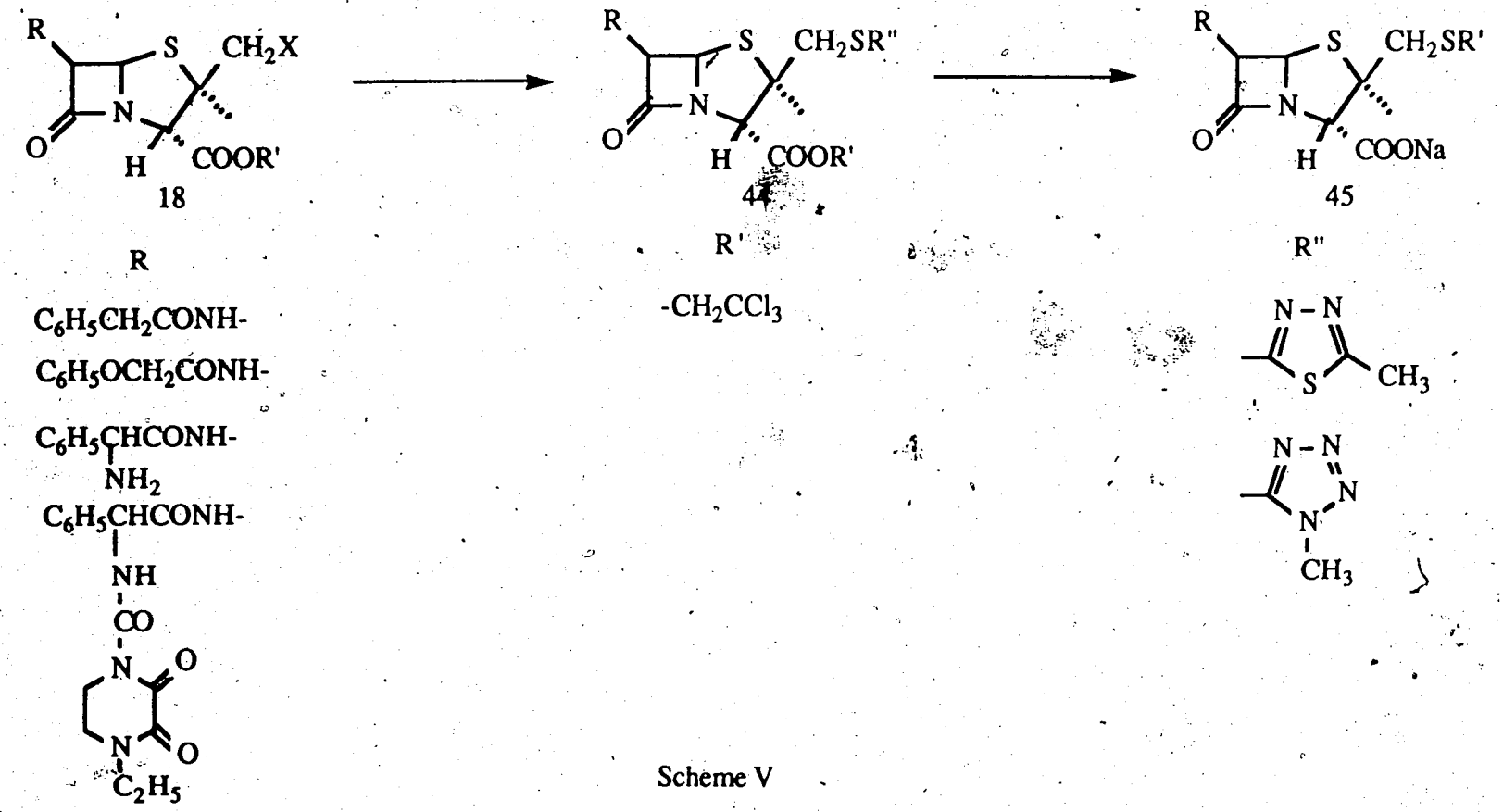
Table 13 (cont.)

43	Na	Br	1.26(3H, s, C_2-C<u>H</u>$_3$); 3.86, 4.02(2H, ABq, J=11.08 Hz, C_2-C<u>H</u>$_2$); 4.36(1H, s, C_3-<u>H</u>); 4.58(2H, s, OC<u>H</u>$_2$); 5.2(1H, d, J=4.44 Hz, C_5-<u>H</u>); 5.88(1H, d, J=4.4 Hz, C_6-<u>H</u>); 7.36(5H, m, C_6<u>H</u>$_5$).

Synthesis of 2β-[(5-Methyl-1,3,4-thiadiazol-2-yl)thiomethyl]penams and 2β-[(1-Methyl-1,2,3,4-tetrazol-5-yl)thiomethyl]penams.

Scheme V represents the route for the synthesis of the sodium salts of 2β-(substituted methyl)penams **45**, where R'' was 5-methyl-1,3,4-thiadiazol-2-yl or 1-methyl-1,2,3,4-tetrazol-5-yl. The coupling reaction of 2,2,2-trichloroethyl-2β-(chloromethyl)penicillanates **18** (X=Cl), having substituents such as phenylacetamido, phenoxyacetamido, D-2-amino-2-phenylacetamido and D-2-[(4-ethyl-2,3-dioxo-piperazin-1-yl)carbonylamino]-2-phenylacetamido at C-6 with 2-mercapto-5-methyl-1,3,4-thiadiazole and 5-mercapto-1-methyl-1,2,3,4-tetrazole were carried out by refluxing in acetone, containing phosphate buffer (pH 6.8) and sodium bicarbonate. The trichloroethyl group was removed by reductive cleavage using zinc and acetic acid in DMF at 0 °C for 3 h and the acid was converted to the corresponding sodium salt by adjusting the pH to 7.0 with sodium-2-ethylhexanoate. Table 14 summarizes the ^{1}H NMR data of the 2,2,2-trichloroethyl esters and sodium salts of 6β-phenylacetamidopenicillin and 6β-phenoxyacetamidopenicillins with substituents such as 2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl] and 2β-[(1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl] at C-2. Table 15 summarizes the ^{1}H NMR data of 2,2,2-trichloroethyl esters and sodium salts of ampicillin with 2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl] and 2β-[(1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl] at C-2 and Table 16 summarizes the ^{1}H NMR data of the 2,2,2-trichloroethyl esters and sodium salts of piperacillin with 2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl] and 2β-[(1-methyl-1,2,3,4-tetrazol-5-yl)thio- methyl] at C-2.

In the ^{1}H NMR spectra of the β-lactams, the proton at C-5 of penicillin and C-6 of cephalosporin gave rise to the β-lactam doublet [$J_{H\text{-}H}$(*cis*)=4-5 Hz, $J_{H\text{-}H}$(*trans*)=1.5-2 Hz], while the protons at C-6 and C-7 of the penicillins and



R

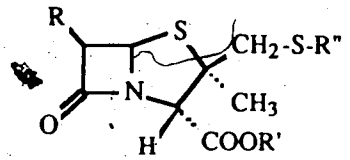
$C_6H_5CH_2CONH-$

$C_6H_5OCH_2CONH-$

Scheme V

TABLE 14

^{1}H NMR Data of 2β-(Substituted methyl)penams.



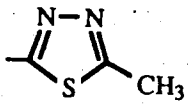
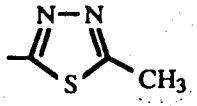
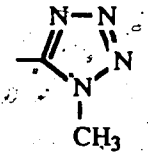
R	R'	R"	PMR Chemical Shifts (δ) ppm.
$C_6H_5CH_2CONH$	CH_2CCl_3		1.63(3H, s, C_2-C<u>H</u>$_3$); 2.66(3H, s, >C-C<u>H</u>$_3$); 3.76(2H, s, $C_6H_5C\underline{H}_2$); 3.53, 4.06 (2H, ABq, J=13.5 Hz, C_2-C<u>H</u>$_2$); 4.76(2H, s, C<u>H</u>$_2CCl_3$); 4.9(1H, s, C_3-<u>H</u>); 5.7(1H, d, J=4 Hz, C_5-<u>H</u>); 5.86(1H, dd, J=4 Hz, 10 Hz, C_6-<u>H</u>); 7.3(5H, m, $C_6\underline{H}_5$); 7.63(1H, d, J=10 Hz, N<u>H</u>);
$C_6H_5CH_2CONH$	Na		1.46(3H, s, C_2-C<u>H</u>$_3$); 2.5(3H, s, >C-C<u>H</u>$_3$); 3.44, 3.52(2H, ABq, J=13.12 Hz, C_2-C<u>H</u>$_2$); 3.56, 3.64(2H, ABq, J=14.37 Hz, $C_6H_5C\underline{H}_2$); 4.4(1H, s, C_3-<u>H</u>); 5.3(1H, d, J=3.75 Hz, C_5-<u>H</u>); 5.42(1H, d, J=3.75 Hz, C_6-<u>H</u>); 7.2(5H, m, $C_6\underline{H}_5$).
$C_6H_5CH_2CONH$	CH_2CCl_3		1.68(3H, s, C_2-C<u>H</u>$_3$); 3.96(3H, s, N-C<u>H</u>$_3$); 3.68, 3.94(2H, ABq, J=14.17 Hz, C_2-C<u>H</u>$_2$); 3.8, 3.86(2H, ABq, J=14.62 Hz, $C_6H_5C\underline{H}_2$); 4.80, 4.88(2H, ABq, J=12.40 Hz, C<u>H</u>$_2CCl_3$); 4.9(1H, s, C_3-<u>H</u>); 5.74(1H, d, J=4.19 Hz, C_5-<u>H</u>);

Table 14 (cont.)

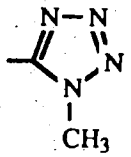
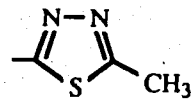
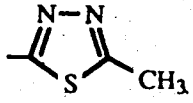
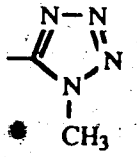
			5.98(1H, dd, J=4.19 Hz, 9.1 Hz, C_6-H); 7.34(5H, m, $C_6H_5CH_2$); 8.26(1H, d, J=9.1 Hz, NH).
$C_6H_5CH_2CONH$	Na		1.48(3H, s, C_2-CH_3); 3.58(2H, s, C_2-CH_2); 3.66(2H, s, $C_6H_5CH_2$); 3.86(3H, s, N-CH_3); 4.42(1H, s, C_3-H); 5.32(1H, d, J=3.12 Hz, C_5-H); 5.48(1H, d, J=3.12 Hz, C_6-H); 7.22(5H, m, C_6H_5).
$C_6H_5OCH_2CONH$	CH_2CCl_3		1.7(3H, s, C_2-CH_3); 2.66(3H, s, ⟩-CH_3); 3.76, 4.03(2H, ABq, J=15 Hz, C_2-CH_2); 4.63(2H, s, CH_2CCl_3); 4.83(2H, s, OCH_2); 5.0(1H, s, C_3-H); 5.73 (1H, d, J=4 Hz, C_5-H); 5.86(1H, dd, J=4 Hz, 10 Hz, C_6-H); 7.2(5H, m, C_6H_5); 7.73(1H, d, J=10 Hz, NH).
$C_6H_5OCH_2CONH$	Na		1.5(3H, s, C_2-CH_3); 2.48(3H, s, ⟩-CH_3); 3.50, 3.58(2H, ABq, J=14.12 Hz, C_2-CH_2); 4.46(1H, s, C_3-H); 4.56(2H, s, OCH_2); 5.4(1H, d, J=4.03 Hz, C_5-H); 5.56(1H, d, J=4.03 Hz, C_6-H); 7.2(5H, m, C_6H_5).
$C_6H_5OCH_2CONH$	CH_2CCl_3		1.7(3H, s, C_2-CH_3); 3.88(3H, s, N-CH_3); 3.8, 3.98(2H, ABq, J=13.23 Hz, C_2-CH_2), 4.66(2H, s, CH_2CCl_3); 4.86(2H, s, OCH_2); 5.0(1H, s, C_3-H); 5.76

Table 14 (cont.)

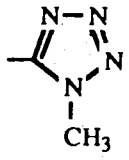
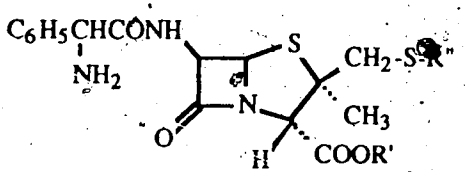
$C_6H_5OCH_2CONH$	Na		(1H, d, J=4.41 Hz, C_5-H); 5.88(1H, dd, J=4.41 Hz, 11.03 Hz, C_6-H); 7.2(5H, m, C_6H_5); 7.98(1H, d, J=11.03 Hz, NH).
			1.48(3H, s, C_2-CH_3); 3.58(2H, s, C_2-CH_2); 3.78(3H, s, N-CH_3); 4.44(1H, s, C_3-H); 4.58(2H, s, OCH_2); 5.38(1H, d, J=4 Hz, C_5-H); 5.56(1H, d, J=4 Hz, C_6-H); [6.84(2H), 6.96(1H), 7.24(2H), C_6H_5].

TABLE 15

^{1}H NMR Data of 2β-(Substituted methyl)ampicillin Derivatives



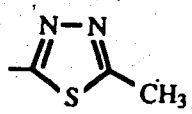
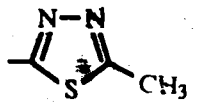
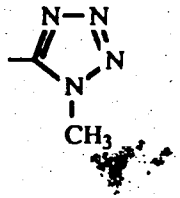
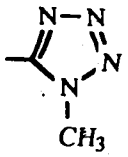
R'	R"	PMR Chemical Shifts (δ) ppm.
CH_2CCl_3		1.62(3H, s, C_2-C<u>H</u>$_3$); 2.76(3H, s, >C<u>H</u>$_3$); 3.58, 4.14(2H, ABq, J=14.45 Hz, C_2-C<u>H</u>$_2$); 4.72(2H, s, C<u>H</u>$_2$CCl$_3$); 4.82(2H, s, C_3-C<u>H</u>$_2$CCl$_3$); 4.88(1H, s, C_3-<u>H</u>); 5.68(1H, d, J=3.73 Hz, C_5-<u>H</u>); 5.98(2H, m, C_6-<u>H</u>, C_6H_5C<u>H</u>); 6.76(1H, d, J=7.12 Hz, N<u>H</u>); 7.3(5H, m, C_6<u>H</u>$_5$); 8.44(1H, d, J=9.71 Hz, CHN<u>H</u>).
Na		1.82(3H, s, C_2-CH$_3$); 2.7(3H, s, >C<u>H</u>$_3$); 3.7, 3.82(2H, ABq, J=13.56 Hz, C_2-C<u>H</u>$_2$); 4.4(1H, s, C_3-<u>H</u>); 4.82(1H, s, C_6H_5C<u>H</u>); 5.44(2H, m, C_5-<u>H</u>, C_6-<u>H</u>); 7.3(5H, m, C_6<u>H</u>$_5$); 3.46(2H, br s, N<u>H</u>$_2$).
CH_2CCl_3		1.64(3H, s, C_2-C<u>H</u>$_3$); 3.96(3H, s, N-C<u>H</u>$_3$); 3.68, 3.86(2H, ABq, J=14.01 Hz, C_2-C<u>H</u>$_2$); 4.62, 4.78(2H, ABq, J=11.38 Hz, C<u>H</u>$_2$CCl$_3$); 4.82, 4.86(2H, ABq, J=9.6 Hz, C_3-C<u>H</u>$_2$CCl$_3$); 4.9(1H, s, C_3-<u>H</u>); 5.7(1H, d, J=3.86 Hz, C_5-<u>H</u>); 5.7(1H, d, J=7.07 Hz, C_6H_5C<u>H</u>); 6.04(1H, dd,

Table 15 (cont.)

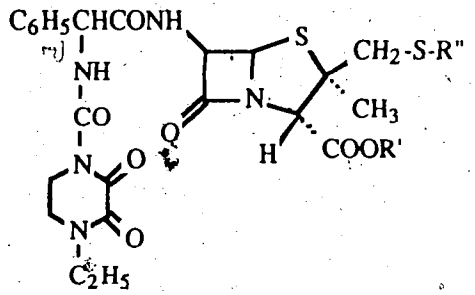
J=3.86 Hz, 9.65 Hz, C_6-H); 6.7(1H, d, J=7.07 Hz, C_6-NH); 7.3(5H, m, C_6H_5); 8.6(1H, d, J=9.65 Hz, CHNH).

Na 

1.82(3H, s, C_2-CH_3); 3.64, 3.78(2H, ABq, J=13.30 Hz, C_2-CH_2); 4.0(3H, s, N-CH_3); 4.42(1H, s, C_3-H); 4.82(1H, s, C_6H_5CH); 5.46(2H, m, C_5-H, C_6-H); 7.42(5H, m, C_6H_5); 3.42(2H, br s, NH_2).

TABLE 16

^{1}H NMR Data of 2β-(substituted methyl)piperacillin Derivatives.



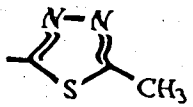
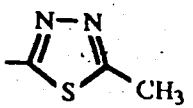
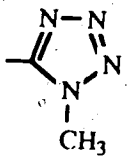
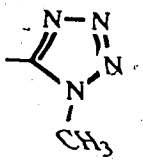
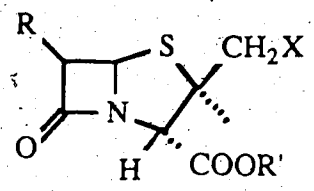
R'	R"	PMR Chemical Shifts (δ) ppm
CH_2CCl_3		1.18(3H, t, J=7 Hz, N-CH_2CH_3); 1.58(3H, s, C_2-CH_3); 2.78(3H, s, >C-CH_3); 3.68, 4.04(2H, ABq, J=12 Hz, C_2-CH_2); 3.54(4H, m, N-CH_2CH_3, CH_2-N-C_2H_5); 3.94, 4.18(2H, m, CO-N-CH_2); 4.82(2H, dd, J=18 Hz, 12 Hz, CH_2CCl_3); 4.94(1H, s, C_3-H); 5.66(1H, d, J=4 Hz, C_5-H); 5.92(1H, dd, J=9 Hz, 4 Hz, C_6-H); 6.02(1H, d, J=7 Hz, C_6H_5CH); 7.3(5H, m, C_6H_5); 8.22(1H, d, J=9 Hz, C_6-NH); 9.02(1H, d, J=7 Hz, C_6H_5CHNH).
Na		1.06(3H, t, J=6 Hz, N-CH_2CH_3); 1.4(3H, s, C_2-CH_3); 2.6(3H, s, >C-CH_3); 3.4(4H, m, N-CH_2CH_3, $CH_2NC_2H_5$); 3.48(2H, s, C_2-CH_2); 3.58(2H, m, CO-N-CH_2); 4.38(1H, s,

Table 16 (cont.)

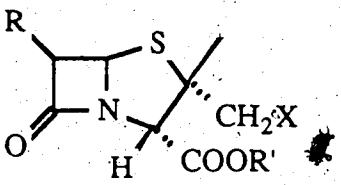
		C_3-H); 5.34(1H, d, J=4.5 Hz, C_5-H); 5.4(1H, s, C_6H_5CH); 5.46(1H, d, J=4.5 Hz, C_6-H); 7.3(5H, m, C_6H_5).
CH_2CCl_3		1.18(3H, t, J=6 Hz, N-CH_2CH_3); 1.58(3H, s, C_2-CH_3); 3.52(4H, m, N-CH_2CH_3, $CH_2NC_2H_5$); 3.74(2H, s, C_2-CH_2); 3.96(4H, s, N-CH_3; 1H, CO-N-CH_2); 4.08(1H, m, CON-CH_2); 4.78, 4.84(2H, ABq, J=11.16 Hz, CH_2CCl_3); 4.92(1H, s, C_3-H); 5.64(1H, d, J=4.18 Hz, C_6H_5CH); 5.84(1H, d, J=4.88 Hz, C_5-H); 5.86(1H, d, J=9.06 Hz, 4.88 Hz, C_6-H); 7.26(5H, m, C_6H_5); 8.42(1H, d, J=9.06 Hz, C_6-NH); 9.88(1H, d, J=4.18 Hz, C_6H_5CHNH).
Na		1.04(3H, t, J=6 Hz, N-CH_2CH_3); 1.38(3H, s, C_2-CH_3); 3.32(4H, m, N-CH_2CH_3, $CH_2NC_2H_5$); 3.30, 3.36(2H, ABq, J=12 Hz, C_2-CH_2); 3.52(2H, m, CO-N-CH_2); 3.86(3H, s, N-CH_3); 4.36(1H, s, C_3-H); 5.34(1H, d, J=4 Hz, C_5-H); 5.4(1H, s, C_6H_5CH); 5.44(1H, d, J=4 Hz, C_6-H); 7.3(5H, m, C_6H_5).

cephalosporins located vicinal to the protons at C-5 and C-6 of penicillins and cephalosporins respectively, and to the amido proton gave rise to the quartet [$J_{H\text{-}H}$(*cis*)=4-5 Hz, $J_{H\text{-}H}$(*trans*)=1.5-2 Hz and $J_{NH\text{-}H}$=8-11 Hz], confirmed by the addition of D_2O to the solution of penicillins and cephalosporins in a deuterated organic solvent, followed by shaking and observing the collapse of the quartet to a doublet[197,198]. It was also observed that the ring junction β-lactam proton at C-5 of penicillins or C-6 of cephalosporins generally resonated at a higher field than the neighboring proton at C-6 and C-7[199]. Epimerization at C-6 in penicillins or C-7 in cephalosporins giving the *trans* β-lactam system, resulted in a large upfield shift (~0.7-0.8 ppm) for the two protons. The presence of two methyl singlets correspond to the 2α- (δ 1.23-1.66) and 2β- (δ 1.49-1.84) geminal methyl groups and a one proton singlet (δ 4.38-3.92) to H-3[199,200].

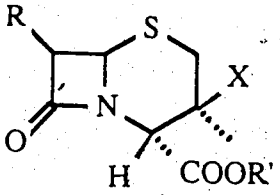
In the ^1H NMR spectra of 2β-(substituted methyl)penams **18**, 2α-(substituted methyl)penams **24**, and 3-substituted cephams **22**, (a) a methyl singlet (b) an AB system due to the methylene group (c) a one proton singlet of the C-3 or C-4 and (d) the pattern of resonances characteristic of the two methine proton resonances of the azetidinone ring were observed[201]. The geminal coupling constant observed for methylene protons in penams were in the range of 11.5-12.0 Hz, while it was 14-14.5 Hz in the cephams. The chemical shift of the methyl protons of the 2β-(substituted methyl)penams **18** and the 2α-(substituted methyl)penams **24**, helped to distinguish between the two. The chemical shifts of the 2β-methyl protons were at uniformly lower field than those of the 2α- analogs and the shifts of H-5 and H-6 were consistently similar for the 2α-analog with slight variations (< 0.1ppm) for the 2β-analogs.



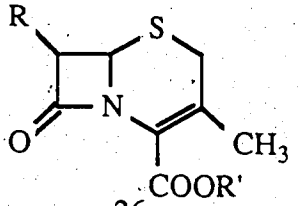
18



24



22



26

In the upfield region of the ^{13}C NMR spectra six carbon resonances were observed, which could be assigned to non-protonated, methine, methylene and methyl carbons. The penam **18**, and the cepham **22**, systems could be easily distinguished through the chemical shift of the methylene carbons. In penams **18**, the methylene carbon was directly attached to "X" (halogen) and was therefore deshielded to a degree largely dependent upon the electronegativity of this substituent. Whereas in the cephams **22**, the methylene carbon was linked to the ring sulfur which had a small effect and therefore resonated in the chemical shift range 35 ± 4 ppm, while those of the penams **18**, were found below 42 ppm. The identification of the resonances due to substituents R at C-6 or C-7 and R' at C-3 or C-4 in penams **18**, or cephams **22**, respectively presented no problem. On the basis of the chemical shift the carbonyl carbons could be identified, even though they do not help in distinguishing the two systems. The methyl carbon of penams **18**, cephams **22**, or cephems **26**, resonated between 20-30 ppm. In cephams **22**, the non-protonated carbon C-3 was directly attached to "X" but because of the varying deshielding effects of "X" the chemical shift of the C-3 resonances showed

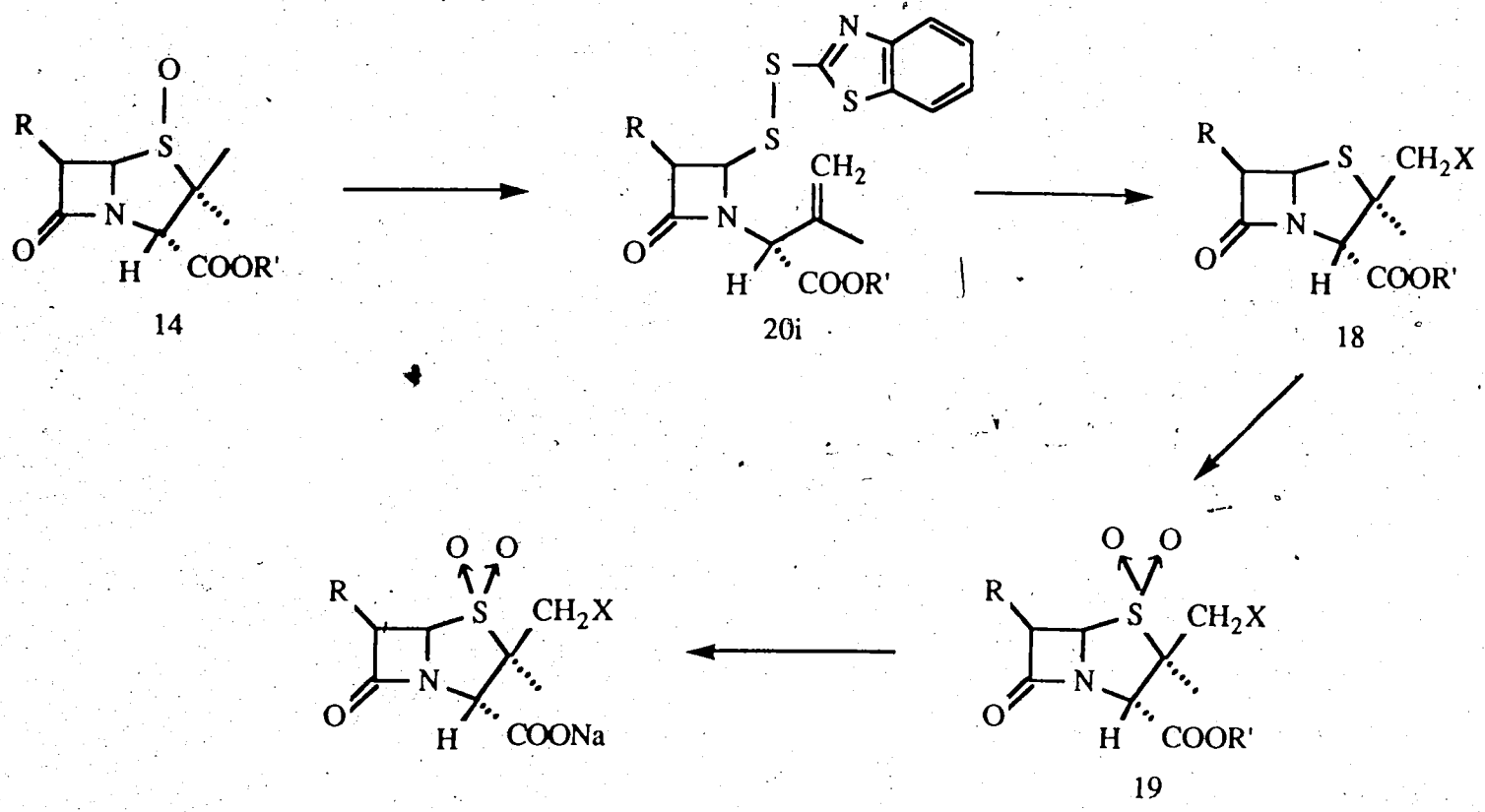
wider variations than that observed for C-2 of the penams **18**. The C-6 or C-7 substituted by "R" showed relatively constant shifts in penams **18**, cephams **22**, and cephem **26**, while the other two methine carbons, C-5 or C-6 and C-3 or C-4, resonated at lower field in the penams **18**, than in the cephams **22**, thus helping in distinguishing the methine carbons of the thiazolidine ring of penam **18**, and thiazine ring of the cepham **22**. In cephems **26**, two resonances in the range typical of olefinic carbons made their identification easy[201].

F. *PENAN SULFONES AS β-LACTAMASE INHIBITORS.*

The sodium and potassium salts of 6,6-dihydro-2β-(halomethyl)penam sulfones **19a**, (X=Cl, Br), have been found to be active β-lactamase inhibitors and this effect has been extended to 6,6-dihydro-2β-(azidomethyl)- and -2β-(triazolylmethyl)penam sulfones **19a, b**[114,164-169]. The 6,6-dihydro-2β-(triazolylmethyl)penam sulfone **19b**, is the most effective β-lactamase inhibitor known. The effect of another heterocyclic ring system such as 2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl] at C-2 and different oxidation states of the side chain sulfur of the 6,6-dihydropenam sulfones **49**, **50** and **51**, were investigated in order to study the variations on β-lactamase inhibitory activity.

6,6-Dihydro-2β-(halomethyl)penam Sulfones

A class of penicillin sulfones were synthesized as potential β-lactamase inhibitors. Scheme VI represents the route for the synthesis of the sodium salts of 6,6-dihydro-2β-(halomethyl)penam sulfones **19**, (X=Cl, Br). The *p*-nitrobenzyl-6,6-dihydropenicillanate-1β-oxide **14**, [R=H], was obtained by the oxidation of *p*-nitrobenzyl-6,6-dihydropenicillanate **13**, [R=H], with 1.1 mole



R= H

R'=$CH_2C_6H_4NO_2$

R'=$CH(C_6H_5)_2$

Scheme VI

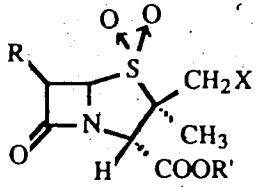
equivalents of *m*-chloroperbenzoic acid for 2 h in methylene chloride at 0 °C. The penam sulfoxide **14**, thus obtained on reflux with 2-mercaptobenzothiazole in toluene for 2 h gave the *unsym*-azetidinone disulfide **20i**, which on treatment with 1.1 mole equivalents of cupric chloride in methylene chloride at room temperature for 4 h led to *p*-nitrobenzyl-6,6-dihydro-2β-(chloromethyl)penicillanate **18**, (X=Cl, R=H). The reaction of the *unsym*-azetidinone disulfide **20i**, with cupric bromide in methylene chloride was carried out at 0 °C for 4 h; *p*-nitrobenzyl-6,6-dihydro-2β-(bromomethyl)penicillanate **18**, (X=Br, R=H), was obtained exclusively. The sulfone of 2β-(halomethyl)penams could be obtained by oxidation with 2.2 mole equivalents of potassium permanganate in water and glacial acetic acid mixture. The *p*-nitrobenzyl group was removed by catalytic hydrogenation in THF/water on palladium-carbon (10%) catalyst. The acid thus obtained was treated with sodium bicarbonate yielding the sodium 6,6-dihydro-2β-(halomethyl)penicillanate-1,1-dioxides **19i**, (X=Cl, Br, R'=Na). Table 17 summarizes the ^{1}H NMR data of the 6,6-dihydro-2β-(halomethyl)penicillanate-1,1-dioxides.

Oxidation Studies on the Benzhydryl-6,6-Dihydro-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl]penicillanate

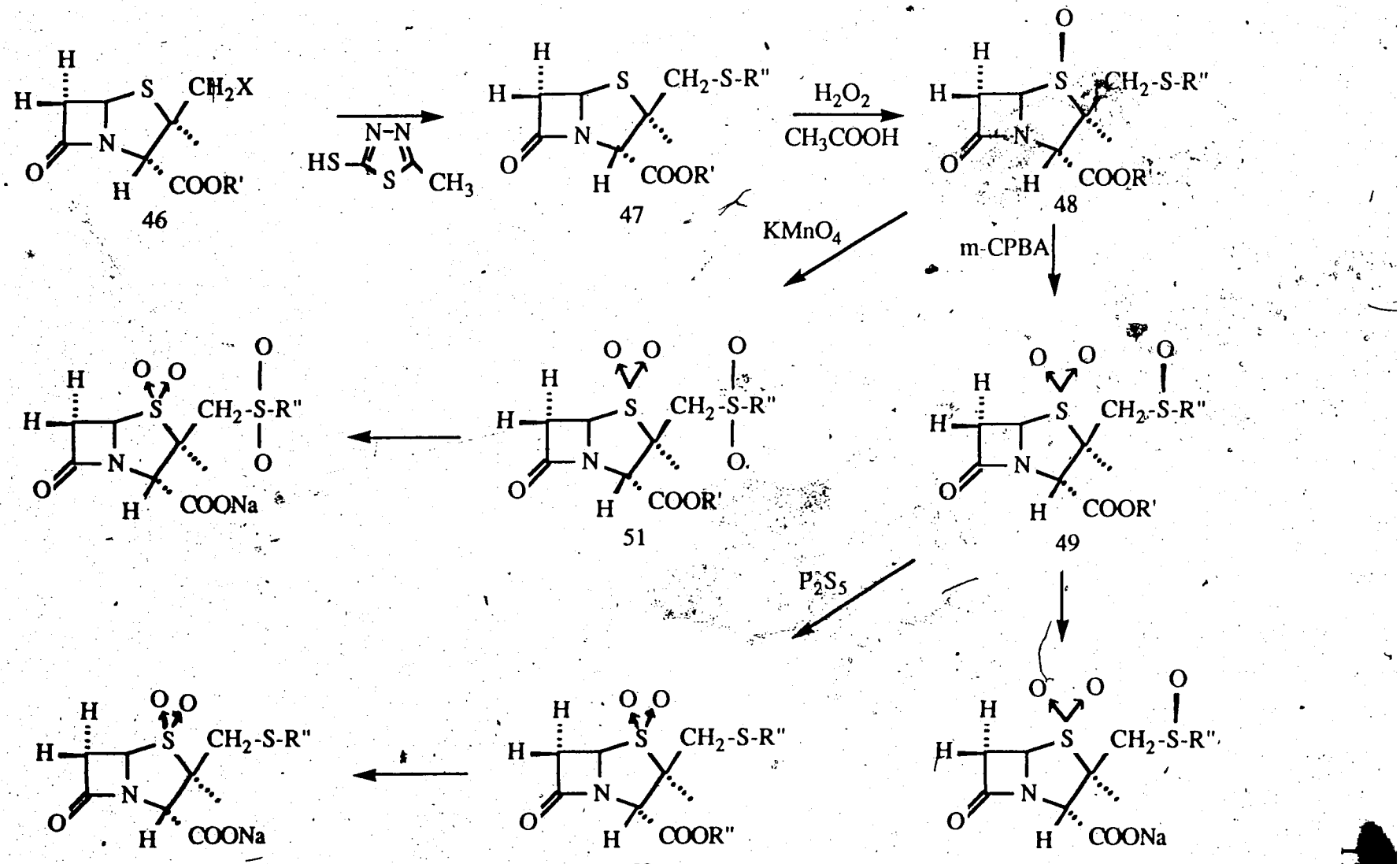
Benzhydryl-6,6-dihydro-2β-(chloromethyl)penicillanate **46**, obtained as shown in the Scheme VI on reflux with 2-mercapto-5-methyl-1,3,4-thiadiazole in acetone for 5 h in presence of sodium bicarbonate and phosphate buffer (pH 6.8) gave the crude benzhydryl-6,6-dihydro-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)]penicillanate **47**, which was purified on a silica-gel column by gradient elution using a mixture of ethyl acetate and hexane to obtain the pure compound **47**, in 35% yield (Scheme VII). Oxidation of this compound **47**, with hydrogen peroxide (2.4 moles) and glacial acetic acid

TABLE 17

^{1}H NMR Data of 2β-(Halomethyl)penam Sulfones **19**.



R	R'	X	PMR Chemical Shifts (δ) ppm
H	$CH_2C_6H_4NO_2$	Cl	1.53(3H, s, C_2-C<u>H</u>$_3$); 3.56(2H, br s, C_6-C<u>H</u>$_2$): 3.83, 4.13(2H, ABq, J=16.5 Hz, C_2-C<u>H</u>$_2$); 4.76(2H, br s, C_3-<u>H</u>, C_5-<u>H</u>); 5.33(2H, s, C<u>H</u>$_2C_6H_4NO_2$); 7.6, 8.33(4H, 2 d, C_6<u>H</u>$_4NO_2$).
H	$CH_2C_6H_4NO_2$	Br	1.53(3H, s, C_2-C<u>H</u>$_3$); 3.54(2H, br s, C_6-C<u>H</u>$_2$); 3.73, 3.93(2H, ABq, J=13 Hz, C_2-C<u>H</u>$_2$); 4.76(2H, br s, C_3-<u>H</u>, C_5-<u>H</u>); 5.33(2H, s, C<u>H</u>$_2C_6H_4NO_2$); 7.6, 8.3(4H, 2 d, C_6<u>H</u>$_4NO_2$).
H	Na	Cl	1.52(3H, s, C_2-C<u>H</u>$_3$); 3.32(1H, dd, J=1.5 Hz, 9 Hz, C_6-<u>H</u>); 3.58(1H, dd, J=3 Hz, 9 Hz, C_6-<u>H</u>); 4.02, 4.16(2H, ABq, J=15 Hz, C_2-C<u>H</u>$_2$); 4.22(1H, s, C_3-<u>H</u>); 4.92(1H, dd, J=1.5 Hz, 3 Hz, C_5-<u>H</u>).
H	Na	Br	1.54(3H, s, C_2-C<u>H</u>$_3$); 3.34(1H, dd, J=1.5 Hz, 9 Hz, C_6-<u>H</u>); 3.6(1H, dd, J=3 Hz, 9 Hz, C_6-<u>H</u>); 3.9, 4.02(2H, ABq, J=12 Hz, C_2-C<u>H</u>$_2$); 4.26(1H, s, C_3-H); 4.94(1H, dd, J=1.5 Hz, C_5-<u>H</u>).


H
S
CH2X
N
O
H
COOR'
46
N-N
HS
S
CH3
CH2-S-R"
47
H2O2
CH3COOH
48
KMnO4
m-CPBA
49
P2S5
51
50
COONa
R'=-CH(C6H5)2

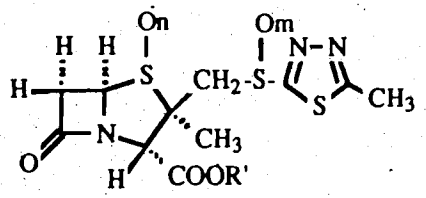
Scheme VII

(8 moles) in methylene chloride at room temperature for 24 h gave the benzhydryl-6,6-dihydro-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)]penicillanate-1β-oxide **48**, in over 50% yield. This penicillin sulfoxide **48**, was further oxidized with 2 mole equivalents of *m*-chloroperbenzoic acid in methylene chloride at room temperature for 6 h, purified on a silica-gel column by gradient elution using a mixture of ethyl acetate and hexane. During this reaction the thiomethyl "S" was oxidized to sulfoxide and the thiazolidine ring "S" was oxidized to the sulfone, and gave the compound benzhydryl-6,6-dihydro-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfinylmethyl]penicillanate-1,1-dioxide **49**. The oxygen on the thiomethyl "S" could be removed using P_2S_5 in combination with pyridine in methylene chloride for deoxygenation at room temperature for 24 h. The product **50**, was purified on a preparative silica-gel chromatography plate using a mixture of ethyl acetate and toluene (2:3) as the solvent system to obtain the benzhydryl-6,6-dihydro-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl]penicillanate-1,1-dioxide **50**, in 25% yield.

The benzhydryl-6,6-dihydro-2β-[(5methyl-1,3,4-thiadiazol-2-yl)thiomethyl]penicillanate-1β-oxide **48**, on oxidation with 3 mole equivalents of potassium permanganate in glacial acetic acid and water at room temperature for 4 h led to the oxidation of the thiazolidine ring "S" to sulfone and thiomethyl "S" to the dioxide and thus benzhydryl-6,6-dihydro-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfonylmethyl]penicillanate-1,1-dioxide **51**, was obtained in 90% yield. The benzhydryl ester of penam sulfones **49**, **50** and **51**, with the thiomethyl "S" as sulfoxide, sulfide and sulfone state thus obtained were converted to the corresponding sodium salts using sodium bicarbonate after conversion to the acid by catalytic hydrogenation over palladium-carbon (10%) catalyst in a mixture of ethyl acetate and water at room temperature for 3 h at 50 p.s.i. Table 18 summarizes the ^1H NMR data and Table 19

TABLE 18

^{1}H NMR Data of the Benzhydryl-6,6-Dihydro-2β-(substituted methyl)penam 47, its Sulfoxide and Sulfone.



	n	m
47	0	0
48	1	0
50	2	0
49	2	1
50	2	2

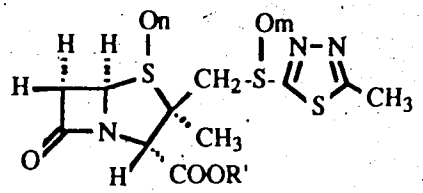
C #	PMR Chemical Shifts (δ) ppm
47	1.34(3H, s, C_2-C<u>H</u>$_3$); 2.74(3H, s, >C-C<u>H</u>$_3$); 3.2(1H, dd, J=2.3 Hz, 15.33 Hz, C_6-<u>H</u>); 3.6(1H, dd, J=4.23 Hz, 15.33 Hz, C_6-<u>H</u>); 3.82, 4.02(1H, ABq, J=13.75 Hz, C_2-C<u>H</u>$_2$); 5.38(1H, dd, J=1.58 Hz, 4.75 Hz, C_5-<u>H</u>); 6.98(1H, s, C<u>H</u>(C_6H_5)$_2$); 7.34(10H, s, (C_6<u>H</u>$_5$)$_2$).
48	1.08(3H, s, C_2-C<u>H</u>$_3$); 2.68(3H, s, >C-C<u>H</u>$_3$); 3.26(2H, m, C_6-C<u>H</u>$_2$); 4.02, 4.32(2H, ABq, J=13.93 Hz, C_2-C<u>H</u>$_2$); 4.78(1H, s, C_3-<u>H</u>); 4.88(1H, dd, J=2.23 Hz, 3.5 Hz, C_5-<u>H</u>); 6.94(1H, s, C<u>H</u>(C_6H_5)$_2$); 7.32(10H, m, (C_6<u>H</u>$_5$)$_2$).
50	1.28(3H, s, C_2-C<u>H</u>$_3$); 2.74(3H, s, >C_3-<u>H</u>); 3.44(1H, dd, J=2.27 Hz, 16.34 Hz, C_6-<u>H</u>); 3.48(1H, dd, J=4.30 Hz, 16.34 Hz, C_6-<u>H</u>); 4.02, 4.24(2H, ABq, J=14.46 Hz, C_2-C<u>H</u>$_2$); 4.6(1H, dd, J=2.24 Hz, 4.30 Hz, C_5-<u>H</u>); 4.88(1H, s, C_3-<u>H</u>); 6.96(1H, s, C<u>H</u>(C_6H_5)$_2$); 7.34(10H, m, (C_6<u>H</u>$_5$)$_2$).

Table 18 (cont.)

49	1.54(3H, s, C_2-C<u>H</u>$_3$); 2.84(3H, s, $\rangle$-C<u>H</u>$_3$); 3.42(1H, dd, J=1.73 Hz, 16.16 Hz, C_6-<u>H</u>); 3.54(1H, dd, J=4.62 Hz, 16.16 Hz, C_6-<u>H</u>); 3.62, 3.74(2H, ABq, J=14.14 Hz, C_2-C<u>H</u>$_2$); 4.68(1H, dd, J=1.73 Hz, J=4.62 Hz, C_5-<u>H</u>); 5.2(1H, s, C_3-<u>H</u>); 6.98(1H, s, C<u>H</u>(C_6H_5)$_2$); 7.36(10H, m, (C_6<u>H</u>$_5$)$_2$).
51	1.52(3H, s, C_2-C<u>H</u>$_3$); 2.94(3H, s, $\rangle$-C<u>H</u>$_3$); 3.46(1H, dd, J=1.76 Hz, 16.56 Hz, C_6-<u>H</u>); 3.6(1H, dd, J=4.40 Hz, J=16.56 Hz, C_6-<u>H</u>); 4.12, 4.2(2H, ABq, J=15.15 Hz, C_2-C<u>H</u>$_2$); 4.66(1H, dd, J=1.76 Hz, J=4.40 Hz, C_5-<u>H</u>); 5.24(1H, s, C_3-<u>H</u>); 6.98(1H, s, C<u>H</u>(C_6H_5)$_2$); 7.38(10H, m, (C_6<u>H</u>$_5$)$_2$).

TABLE 19

^{13}C NMR Data of Benzhydryl-6,6-Dihydro-2β-(substituted methyl)penam 47, its Sulfoxide and Sulfone.



	n	m
47	0	0
48	1	0
50	2	0
49	2	1
50	2	2

Carbon #	Compound #				
	47	**48**	**50**	**49**	**51**
2-CH_3	15.48	14.80	15.61	15.81	15.96
$\rangle CH_3$	23.62	15.52	17.53	17.72	17.44
	70.38	79.14	66.28	64.42	64.25
C_2-CH_2	47.55	36.58	39.60	60.49	57.51
3	61.22	64.91	61.91	61.23	61.44
5	67.30	70.55	62.57	62.44	62.97
6	46.35	36.30	36.48	40.44	40.97
	117.89	138.83	138.62	138.52	138.66
	139.07	138.95	165.64	169.64	165.35

a. The characterisation of the methyl, methylene, methine and *tert*-carbon was on the basis of J (Mod) experiments
b. All spectra were recorded in $CDCl_3$ as solvent and TMS as internal standard.

summarizes the ^{13}C NMR data of the 6,6-dihydro-2β-(substituted methyl)penams **47 - 51**.

G. ANTIMICROBIAL TESTING.

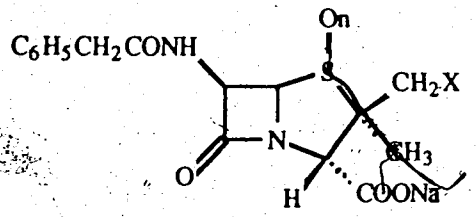
The 2β-(substituted methyl)penams were tested for their antimicrobial activity by the agar plate dilution method using selected Gram +ve (strains of *Staphylococcus, Streptococcus,* and *Bacillus*) and Gram -ve (strains of *Klebsiella, Pseudomonas, Enterobacter* and *Citrobacter*) bacteria. The principle of the agar plate dilution method was the inhibition of growth on the surface of the agar by the antimicrobial agent incorporated into the medium[202].

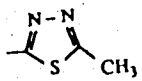
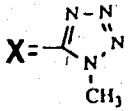
Benzylpenicillin (penicillin G), phenoxymethylpenicillin (penicillin V), ampicillin and piperacillin were used as references to test their derivatives with chloromethyl, bromomethyl, [(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl] and [(1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl] substituents at the C-2 β-position, for their antimicrobial activity. To determine the MIC, the drug was incorporated into the liquified medium (45 - 50 °C), mixed and allowed to solidify. A series of plates were prepared with increasing concentrations of the drug, the bacterial culture spot inoculated on each plate and incubated overnight (18 h, 37 °C). The plates were read for MIC, which represented the lowest concentration of antimicrobial agent at which complete inhibition occurred; a very hazy, barely visible or single colony was disregarded.

The MICs of the penicillin derivatives are tabulated in the Tables 20-22. Among the 6β-phenylacetamido-2β-(halomethyl)penams **42**, (X=Cl, Br), the 2β-(chloromethyl)penams **42**, (X=Cl), had lower MIC values than the 2β-(bromomethyl)penams **42**, (X=Br), against Gram +ve organisms, although

TABLE 20

MICs (μg/ml) of 6β-Phenylacetamido-2β-(substituted methyl)penams.

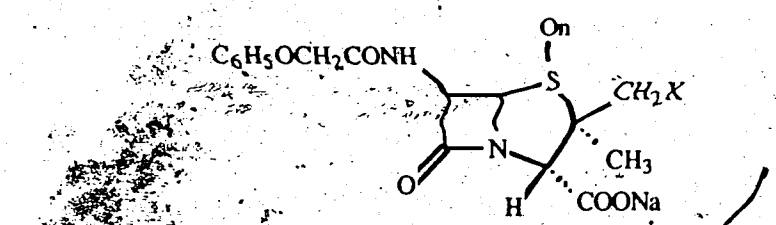


Organism Name	X=H n=0	X=Cl n=0	X=Br n=0	X=  n=0	X=  n=0	X=Cl n=1	X=Br n=1
Gram +ve							
S. aureus	0.12	7.42	12.5	0.23	0.46	14.84	53.12
B. subtilis ATCC 21332	0.06	0.43	0.78	0.46	0.92	3.71	6.61
B. cereus ATCC 14579	0.48	3.71	6.25	0.46	3.71	3.71	6.64
M. luteus ATCC 9341	0.12	3.71	6.25	0.11	0.23	3.71	6.64
S. epidermidis ATCC 12228	0.06	3.71	6.25	0.92	0.92	3.71	6.64
S. aureus ATCC 29213	0.06	1.85	3.12	0.11	0.23	7.42	26.56
S. faecalis ATCC 29212	0.12	7.42	12.5	0.11	0.23	118.75	212.5
S. aureus ATCC 12600	0.12	7.42	6.25	0.23	0.23	14.84	26.56

Gram -ve							
C. freundii	62.5	>475	>425	475	475	>475	>425
P. mirabilis	3.90	>475	>425	475	475	>475	>425
P. vulgaris ATCC 13315	0.976	>475	425	29.68	14.84	118.75	212.50
E. coli ATCC 25922	62.5	>475	425	475	475	>475	>425
C. diversus	62.5	>475	425	>475	>475	>475	>425
M. morganii	500	>475	425	>475	>475	>475	>425
S. marcescens ATCC 8100	500	>475	425	475	237.5	>475	>425
E. cloacae ATCG 23355	31.25	>475	425	475	>475	>475	>425
P. rettgeri	15.62	>475	425	>475	475	>475	>425
K. pneumoniae	125	>475	425	>475	>475	>475	>425
P. aeruginosa ATCC 27853	500	>475	425	475	475	>475	>425

TABLE 21

MICs (μg/ml) of 6β-Phenoxyacetamido-2β-(substituted methyl)penams



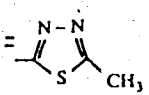
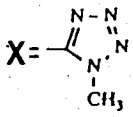
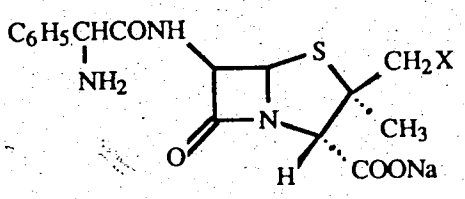
Organism Name	X=H n=0	X=Cl n=0	X=Br n=0	X=Cl n=1	X=Br n=1	X =  n=0	X=  n=0
Gram +ve							
S. aureus	0.39	59.37	56.25	29.68	56.25	0.23	0.46
B. subtilis ATCC 21332	31.25	14.84	7.03	29.68	28.12	0.058	0.05
B. cereus ATCC 14579	>0.06	475	450	475	225	0.11	0.16
M. luteus ATCC 9341	>125	59.37	28.12	7.42	56.25	0.23	0.16
S. epidermidis ATCC 12228	0.19	14.84	28.12	7.42	56.25	0.23	0.46
S. aureus ATCC 29213	0.19	29.68	28.12	14.84	112.5	0.23	0.23
S. faecalis ATCC 29212	0.78	118.75	56.25	118.75	225	0.92	1.85
S. aureus ATCC 12600	0.12	59.37	56.25	29.68	56.25	0.11	0.46

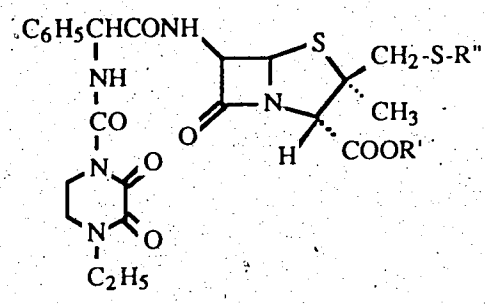
Table 21 (cont.)

Gram -ve							
C. freundii	31.25	475	450	475	450	475	475
P. mirabilis	7.81	237.5	450	475	450	237.5	475
P. vulgaris ATCC 13315	0.39	237.5	450	475	450	118.75	237.5
E. coli ATCC 25922	31.25	475	450	475	450	475	475
C. diversus	31.25	475	450	475	450	475	475
M. morganii	125	475	450	475	450	475	475
S. marcescens ATCC 8100	>125	475	450	475	225	475	475
E. cloacae ATCC 23355	475	450	475	450	475	475	
P. rettgeri	>125	475	450	475	450	237.5	475
K. pneumoniae	62.5	475	450	475	450	237.5	475
P. aeruginosa ATCC 27853	125	475	450	475	450	237.5	475

TABLE 22

MICs of 2β-(Substituted methyl) Ampicillin and Piperacillin Derivatives.





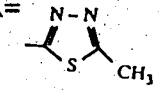
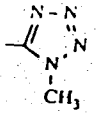
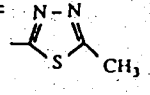
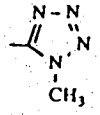
Organism name	X=H	X= 	X= 	X=H	X= 	X= 
Gram +ve						
S. aureus	0.12	3.51	3.51	1.95	1.06	6.64
B. subtilis ATCC 21332	0.06	14.06	0.87	1.95	1.06	1.66
B. cereus ATCC 14579	3.90	7.03	14.06	7.81	2.12	13.28
M. luteus ATCC 9341	0.12	3.51	1.75	3.90	2.12	26.56
S. epidermidis ATCC 12228	0.24	7.03	7.03	0.97	0.26	1.66
S. aureus ATCC 29213	0.12	3.51	3.51	0.48	0.53	1.66
S. faecalis ATCC 29212	0.48	3.51	3.51	1.95	2.12	6.64
S. aureus ATCC 12600	0.12	3.51	3.51	3.90	0.53	0.83

Table 22 (cont.)

Gram -ve						
C. freundii	31.25	>225	>250	3.90	8.5	53.12
P. mirabilis	7.81	>225	>250	0.48	2.12	6.64
P. vulgaris ATCC 13315	0.39	225	>225	0.12	1.06	0.41
E. coli ATCC 25922	31.25	>225	>225	1.95	4.25	53.12
C. diversus	31.25	>225	>225	3.90	8.5	26.56
M. morganii	125	>225	>225	0.48	1.06	26.56
S. marcescens ATCC 8100	125	>225	>225	3.90	8.5	26.56
E. cloacea ATCC 23355	15.62	>225	112.50	0.97	2.12	6.64
P. rettgeri	125	112.50	225	0.24	1.06	3.32
K. pneumoniae	62.5	>225	>225	7.81	8.5	26.56
P. aeruginosa ATCC 27853	125	>225	>225	3.90	8.5	53.12

their MICs against Gram -ve organisms did not vary much. The MICs of the 2β-((halomethyl)penam sulfoxides **43**, (X=Cl, Br), were not lower than the MICs of the 2β-(halomethyl)penams **42**, (X=Cl, Br), and so the oxidation of the thiazolidine ring 'S' did not lead to improved antibacterial activity. However, the 2β-(chloromethyl)penam sulfoxides **43**, (X=Cl), had lower MICs than the 2β-(bromomethyl)penam sulfoxides **43**, (X=Br), against Gram +ve organisms. The MICs of the 2β-(thiomethyl)derivatives of penicillins **45**, were lower than the MICs of the 2β-(halomethyl)penams **42**, (X=Cl, Br), or their sulfoxides **43**, and thus, have better antibacterial activity against Gram +ve organisms. The MICs of the 2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl]penams and 2β-[(1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl]penams **45**, did not show considerable variation. On comparision of the MICs of the reference, penicillin G, with its 2β-(substituted methyl)derivatives **42**, **43**, and **45**, it was found that the MICs of penicillin G against the selected organisms were much lower which indicated that the introduction of varied substituents such as chloro, bromo, [(5-methyl-1,3,4-thiadiazol-2-yl)thio] and [(1-methyl-1,2,3,4-tetrazol-5-yl)thio] at C-2 β-methyl or oxidation of the thiazolidine 'S' to the sulfoxide did not improve the antimicrobial activity.

The MICs of the 6β-phenoxyacetamido-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl]penams **45**, and 2β-[(1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl]penams **45**, were very similar and lower than the MICs of 6β-phenoxyacetamido-2β-(halomethyl)penams **42**, (X=Cl, Br), and their sulfoxides **43**, (X=Cl, Br), and thus have better antibacterial activity. The MICs of the 2β-(halomethyl)penams **42**, (X=Cl, Br), and their sulfoxides **43**, (X=Cl, Br), showed variations among the organisms tested.

MICs of ampicillin and piperacillin derivatives with heterocyclic ring systems at C-2 β-methyl were lower than that of the 6β-phenylacetamido-2β-

(substituted methyl)penams **42** and **45**, and the 6β-phenoxyacetamido-2β-(substituted methyl)penams **42**, and **45**. The MICs of the ampicillin and piperacillin derivatives with heterocyclic ring systems at C_2-β-methyl were comparable with the MICs of ampicillin and piperacillin used as references without any significant improvement in antibacterial activity.

IV. EXPERIMENTAL

^1H, ^{13}C and ^{19}F NMR spectra were recorded on a Bruker AM-300 spectrometer, chemical shifts were recorded in δ values relative to tetramethylsilane and hexafluorobenzene, respectively, as internal standards. IR (KBr) spectra were recorded on a Nicolet DX-FTIR spectrometer for all the compounds prepared. Satisfactory microanalyses (C, H and N, within ± 0.4% of the calculated value) were obtained on all compounds reported. Microanalyses were performed at Department of Chemistry, University of Alberta. Melting points were determined on a Thomas Hoover Capillary Melting Point Apparatus.

A. *PENICILLIN SULFOXIDES*

Significant IR signals are 1795 - 1805 cm^{-1} (β-lactam C=O), 1750 - 1760 cm^{-1} (ester C=O), and 1685 - 1695 cm^{-1} (amide C=O).

Benzhydryl-6β-(phenylacetamido)penicillanate-1β-oxide, [14o, R=$C_6H_5CH_2CONH$, R'=$CH(C_6H_5)_2$].

To a suspension of the potassium salt of 6β-(phenylacetamido)-penicillin (37.2 g, 0.1 mole) in a mixture of $CHCl_3$ (143.6 ml), water (86 ml) and acetone (6.99 ml) in an ice-salt bath (0 °C), 40% peracetic acid(20.9 g, 0.11 mole) was added over a 30 min period, and the mixture stirred for another 15 min. Benzophenone hydrazone (21.56 g, 0.11 mole) and 1% KI solution (6.03 ml) were added, followed by peracetic acid (20.9 g, 0.11 mole) and 10% H_2SO_4 (31.1 ml) at a rate to maintain the temperature at 0 °C. The stirring was continued for another 45 min and the reaction mixture was allowed to warm up to +12 °C in the next half an hour. The $CHCl_3$ layer was separated; the aqueous portion extracted with $CHCl_3$, and the combined organic extracts

washed sequentially with water, saturated $NaHCO_3$ solution, distilled water and brine, and dried over anhydrous Na_2SO_4, filtered, concentrated and dried. The crude product was purified by gradient silica-gel column chromatography using ethyl acetate/hexane as eluant. The purified product was obtained as a white solid, yield: 43.89g (85%), m. p. 140 - 143 °C, ^1H NMR data: Table 4.

Benzhydryl-6β-(phenoxyacetamido)penicillanate-1β-oxide, [14l, R=$C_6H_5OCH_2CONH$, R'=$CH(C_6H_5)_2$].

The above procedure was followed with the potassium salt of 6β-(phenoxyacetamido)penicillin (38.8 g, 0.1 mole) in $CHCl_3$ (143.6 ml) water (86 ml) and acetone (6.9 ml), and benzophenone hydrazone (21.56 g, 0.11 mole), KI solution (6.03 ml) peracetic acid (41.8 g, 0.22 mole) and 10% H_2SO_4 (31.1 ml). The product was a white powder, yield: 46.2 g (87%), , m. p. 118 - 121 °C, ^1H NMR data: Table 4.

2,2,2-Trichloroethyl-6β-(phenylacetamido)penicillanate-1β-oxide, [14c, R=$C_6H_5CH_2CONH$, R'=CH_2CCl_3].

To a solution of 2,2,2-trichloroethyl-6β-(phenylacetamido)penicillanate 1c, (23.25 g, 0.05 mole) in CH_2Cl_2 (300 ml) was added 30% H_2O_2 (13.6 g, 0.12 mole) and glacial acetic acid (24 g, 0.4 mole) and the mixture stirred at room temperature for 24 h. The reaction mixture was washed sequentially with aqueous of $NaHCO_3$, distilled water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and the filtrate concentrated, and purified by gradient silica-gel column chromatography using ethyl acetate/hexane as eluants. The product obtained was an off-white solid, yield: 9.62 g (40%), , m.p. 158 - 161 °C, ^1H NMR data: Table 4.

2,2,2-Trichloroethyl-6β-(phenoxyacetamido)penicillariate-1β-oxide [14d, R=$C_6H_5OCH_2CONH$, R'=CH_2CCl_3].

In a manner similar to that described above, the title compound was prepared from 2,2,2-trichlorοethyl-6β-(phenoxyacetamido)penicillanate **1d**, (24.05 g, 0.05 mole) in CH_2Cl_2 (300 ml), H_2O_2 (13.6 g, 0.12 mole) and glacial acetic acid (24 g, 0.4 mole). The product was obtained as an off-white solid, yield: 12.48 g (50%), m. p. 135 - 138 °C, ^1H NMR data: Table 4.

2,2,2-Trichloroethyl-3-[N-(2,2,2-trichloroethyloxycarbonyl)-D-α-phenylglycyl-amido]penicillanate-1β-oxide [14, R=$C_6H_5CH(NHCOOCH_2CCl_3)CONH$, R'=$CH_2CCl_3$]

To a cooled solution of 2,2,2-trichloroethyl-3-[N-(2,2,2-trichloroethyl-oxycarbonyl)-D-α-phenylglcylamido]penicillanate (51 g, 0.078 mole) in CH_2Cl_2 (500 ml), *m*-chloroperbenzoic acid (15.9 g, 0.078 mole) was added portionwise over a period of 30 min. The stirring was continued under cooling for another 30 min, and the reaction mixture was filtered. The filtrate was washed sequentially with aqueous $NaHCO_3$ solution, distilled water and brine, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated and dried; purified by silica-gel column chromatography using ethyl acetate/CH_2Cl_2 (10:90) as eluants to give a white solid, yield: 26 g (50%), m. p. 130 - 133 °C, ^1H NMR data: Table 4.

2,2,2-Trichloroethyl-3-{D-2-phenyl-2-[(4-ethyl-2,3-dioxo-piperazin-1-yl)-carbonylamino]acetamido}penicillanate-1β-oxide [14, R=$C_6H_5CH(NHCO$-N N-C_2H_5)$CONH$, R'=CH_2CCl_3].

The procedure for the preparation of **14c**, was followed with a solution of 2,2,2-trichloroethyl-3-{D-2-phenyl-2-[(4-ethyl-2,3-dioxo-piperazin-1-yl)-carbonylamino]acetamido}penicillinate (19.09 g, 0.029 mole) in CH_2Cl_2 (200 ml) and 30% H_2O_2 (7.96 g, 0.07 mole) and glacial acetic acid (14.06 g, 0.232

mole) to give a white solid, yield: 8.8 g (45.2%), 185 - 188 °C, ^{1}H NMR data: Table 4.

B. *UNSYM-AZETIDINONE DISULFIDES*

Significant IR signals are 1770 - 1780 cm^{-1} (β-lactam C=O), 1750 - 1760 cm^{-1} (ester C=O).

Benzhydryl-3-phenylacetamido-4-(2'-benzothiazolyldithio)-2-azetidinon-1-yl isopropenyl acetate, [20i, R=$C_6H_5CH_2CONH$, R'=$CH(C_6H_5)_2$].

To a solution of benzhydryl-6β-(phenylacetamido)penicillanate-1β-oxide **14o**, (5.15 g, 0.01 mole) in toluene (90 ml), 2-mercaptobenzothiazole (1.84 g, 0.011 mole) was added and the mixture heated to reflux for 4 h. The residue after concentration was suspended in ether and the 2-mercaptobenzothiazole which separated was collected by filteration. The ether solution was evaporated and the residue crystallized from ethyl acetate and hexane to give a pale yellow solid, yield: 4.98 g (75%), m.p. 130 - 133°C, ^{1}H NMR data: Table 5.

In a similar manner to that described above the following compounds were prepared.

Benzhydryl-3-phenoxyacetamido-4-(2'-benzothiazolyldithio)-2-azetidinon-1-yl isopropenyl acetate, [20f, R=$C_6H_5OCH_2CONH$, R'=$CH(C_6H_5)_2$].

The benzhydryl-6β-(phenoxyacetamido)penicillanate-1β-oxide (10.62 g, 0.02 mole) and 2-mercaptobenzothiazole (3.679 g, 0.022 mole) in toluene (180 ml) was refluxed until reaction was complete. The product was obtained as a pale yellow solid, yield: 10.06 g (74%), m.p. 135 - 137 °C, ^{1}H NMR data: Table 5.

2,2,2-Trichloroethyl-3-phenylacetamido-4-(2'-benzothiazolyldithio)-azetidinon-1-yl isopropenyl acetate, [20e, R=$C_6H_5CH_2CONH$, R'=CH_2CCl_3].

The 2,2,2-trichloroethyl-6β-(phenylacetamido)penicillanate-1β-oxide (4.8 g, 0.01 mole) and 2-mercaptobenzothiazole (1.839 g, 0.011 mole) in

toluene (90 ml) was refluxed until reaction was complete. The product was obtained as a pale yellow solid, yield: 4.53 g (72%), m. p. 129 - 131 °C, ^{1}H NMR data: Table 5.

2,2,2-Trichloroethyl-3-phenoxyacetamido-4-(2'-benzothiazolyldithio)-2-azetidinon-1-yl isopropenyl acetate, [20b, R=$C_6H_5OCH_2CONH$, R'=CH_2CCl_3).

The 2,2,2-trichloroethyl-6β-(phenoxyacetamido)penicillanate-1β-oxide (14.92 g, 0.03 mole) and 2-mercaptobenzothiazole (5.52 g, 0.033 mole) in toluene (270 ml) was refluxed until reaction was complete. The product was obtained as a pale yellow solid, yield: 15.5 g (80%), m. p. 168 - 171 °C, ^{1}H NMR data: Table 5.

Benzhydryl-3,3-dihydro-4-(2'-benzothiazolyldithio)-2-azetidinon-1-yl isopropenyl acetate, [20h, R=H, R'=$CH(C_6H_5)_2$].

The benzhydryl-6,6-dihydropenicillanate-1β-oxide (13.4 g, 0.035 mole) and 2-mercaptobenzothiazole (6.439 g, 0.0385 mole) in toluene (250 ml) was refluxed for 2 h 45 min. The product was obtained as a pale yellow solid, yield: 17.11 g (92%), m.p. 140 - 142 °C, ^{1}H NMR data: Table 5.

***p*-Nitrobenzyl-3,3-dihydro-4-(2'-benzothiazolyldithio)-2-azetidinon-1-yl isopropenyl acetate, [20g, R=H, R'=$CH_2C_6H_4$-*p*-NO_2].**

The *p*-nitrobenzyl-6,6-dihydropenicillanate-1β-oxide (3.48 g, 0.01 mole) and 2-mercaptobenzothiazole (1.8396 g, 0.011 mole) in toluene (50 ml), was refluxed for 1 hr 45 min. The product was obtained as a pale yellow solid, yield: 4.51 g (90%), m.p. 130 - 133 °C, ^{1}H NMR data: Table 5.

2,2,2-Trichloroethyl-3-[N-(2,2,2-trichloroethyloxycarbonyl)-D-α-phenylglycyl-amido]-4-(2'-benzothiazolyldithio)-2-azetidinon-1-yl isopropenyl acetate. [20j, R=$C_6H_5CH(NHCOOCH_2CCl_3)CONH$, R'=$CH_2CCl_3$]

To a solution of 2,2,2-trichloroethyl-3-[N-(2,2,2-trichloroethyloxy-carbonyl)-D-α-phenylglycylamido]penicillanate-1β-oxide **14g**, (18.0 g, 0.0268 mole) in toluene, 2-mercaptobenzothiazole (4.93 g, 0.0295 mole) was added and the mixture heated to reflux for 4 h. The reaction mixture was concentrated to half the volume and hexane was added. The separated solid was filtered, dissolved in CH_2Cl_2, concentrated and dried to give a yellow foam, yield: 20g (75%), m. p. 73 - 75 °C, ^1H NMR data: Table 5.

2,2,2-Trichloroethyl-3-[D-2-phenyl-2-[(4-ethyl-2,3-dioxo-piperzin-1-yl)carbonyl-amino]acetamido)-4-(2'-benzothiazolyldithio)-2-azetidinon-1-yl isopropenyl acetate, [20k, R=$C_6H_5CH(NHCO$-N N-$C_2H_5)CONH$, R'=CH_2CCl_3].

In a manner similar to that described above, the title compound was prepared from 2,2;2-trichloroethyl-3-[D-2-phenyl-2-[(4-ethyl-2,3-dioxo-piperzin-1-yl)carbonylamino]acetamido]penicillanate-1β-oxide **14h**, (8.0 g, 0.012 mole) in toluene (150 ml) and 2-mercaptobenzothiazole (2.207 g, 0.0132 mole). The product was a yellow foam, yield: 8.58 g (77%), m. p. 85 - 88 °C, ^1H NMR data: Table 5.

C. *2β-(HALOMETHYL)PENAMS* ***18,***

Significant IR signals are 1770 - 1780 cm^{-1} (β-lactam C=O), 1750 - 1760 cm^{-1} (ester C=O), and 1685 - 1695 cm^{-1} (amide C=O).

Benzhydryl-6β-(phenoxyacetamido)-2β-(chloromethyl)penicillanate, [18a, R=$C_6H_5OCH_2CONH$, R'=$CH(C_6H_5)_2$, X=Cl].

To a solution of the *unsym*-azetidinone disulfide **20f** (0.681 g, 1 mmol) in CH_2Cl_2 (20 ml), cupric chloride (0.1407 g, 1.1 mmol) was added and the

mixture stirred at room temperature for 4 h. The reaction mixture was filtered through a short bed of Celite and the filtrate washed sequentially with aqueous $NaHCO_3$, distilled water and brine and dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated to give a yellow foam, yield: 0.412 g (75%), m.p. 50 - 52 °C, ^1H NMR data: 1.3(3H, s, C_2-C<u>H</u>$_3$); 3.44, 3.48(2H, ABq, J=12 Hz, C_2-C<u>H</u>$_2$); 4.54, 4.58(2H, ABq, J=15 Hz, OC<u>H</u>$_2$); 5.04(1H, s, C_3-<u>H</u>); 5.66(2H, m, C_5-<u>H</u>, C_6-<u>H</u>); 6.90(1H, s, C<u>H</u>(C_6H_5)$_2$); 6.92(1H, d, J=9 Hz, N<u>H</u>); 7.36[15H, m, C_6<u>H</u>$_5$, (C_6<u>H</u>$_5$)$_2$].

In a manner similar to that described above the following compounds were prepared.

2,2,2-Trichloroethyl-6β-(phenoxyacetamido)-2β-(chloromethyl)penicillanate, [18b, R=$C_6H_5OCH_2CONH$, R'=CH_2CCl_3, X=Cl].

The *Unsym*-azetidinone disulfide **20b** (10.33 g, 0.02 mole) and cupric chloride (2.95 g, 0.022 mole) in CH_2Cl_2 (100 ml) gave a pale yellow solid, yield: 7.65 g (59.10%), m. p. 48 - 50 °C, ^1H NMR data: Table 13.

Benzhydryl-6β-(phenylacetamido)-2β-(chloromethyl)penicillanate, [18c, R=$C_6H_5CH_2CONH$, R'=CH(C_6H_5)$_2$, X=Cl].

The *Unsym*-azetidinone disulfide **20i** (6.65 g, 0.01 mole) and cupric chloride (1.48 g, 0.011 mole) in CH_2Cl_2 (120 ml) gave an off-white foam, yield: 4.27 g (80%), m.p. 58 - 60 °C, ^1H NMR data: 1.3(3H, s, C_2-C<u>H</u>$_3$); 3.3(2H, s, C_2-C<u>H</u>$_2$); 3.63(2H, s, C_6H_5C<u>H</u>$_2$); 5.0(1H, s, C_3-<u>H</u>); 5.63(2H, m, C_5-<u>H</u>, C_6-<u>H</u>); 6.56(1H, d, J=9 Hz, N<u>H</u>); 6.93(1H, s, C<u>H</u>(C_6H_5)$_2$); 7.33(15H, m, (C_6<u>H</u>$_5$)$_2$, C_6<u>H</u>$_5$).

2,2,2-Trichloroethyl-6β-(phenylacetamido)-2β-(chloromethyl)penicillanate, [18d, R=$C_6H_5CH_2CONH$, R'=CH_2CCl_3, X=Cl].

The *Unsym*-azetidinone disulfide (10 g, 0.020 mole) and cupric chloride (2.95 g, 0.022 mole) in CH_2Cl_2 (150 ml) gave an off-white foam, yield: 7.5 g (75%), m. p. 111 - 113 °C, ^1H NMR data: Table 12.

p-Nitrobenzyl-6,6-dihydro-2β-(chloromethyl)penicillanate, [18e, R=H, R'=$CH_2C_6H_4$-p-NO_2, X=Cl].

The *Unsym*-azetidinone disulfide (5.013 g, 0.010 mole) and cupric chloride (1.48 g, 0.011 mole) in CH_2Cl_2 (80 ml) gave an off-white foam, yield: 1.85 g (50%), m. p. 65 - 68 °C, ^1H NMR data: 1.53(3H, s, C_2-C<u>H</u>$_3$); 3.16(1H, dd, J=2 Hz, 18 Hz, C_6-<u>H</u>); 3.66(1H, dd, J=4.5 Hz, 18 Hz, C_6-<u>H</u>); 3.6(2H, s, C_2-C<u>H</u>$_2$); 5.16(1H, s, C_3-<u>H</u>); 5.36(2H, s, C<u>H</u>$_2C_6H_4NO_2$); 3.46(1H, dd, J=2 Hz, 4.5 Hz, C_5-<u>H</u>); 7.66, 8.33(4H, d, C_6<u>H</u>$_4$).

Benzhydryl-6,6-dihydro-2β-(chloromethyl)penicillanate, [18f, R=H, R'=$CH(C_6H_5)_2$, X=Cl].

The *Unsym*-azetidinone disulfide (5.32 g, 0.010 mole) and cupric chloride (1.48 g, 0.011 mole) in CH_2Cl_2 (80 ml) gave an off-white foam, yield: 2.73 g (68%), 54 - 58 °C, ^1H NMR data: 1.3(3H, s, C_2-C<u>H</u>$_3$); 3.04(1H, dd, J=2 Hz, 12 Hz, C_6-<u>H</u>); 3.56(1H, dd, J=4.5 Hz, 12 Hz, C_6-<u>H</u>); 3.6(2H, s, C_2-C<u>H</u>$_2$); 5.13(1H, s, C_3-<u>H</u>); 5.4(1H, dd, J=2 Hz, 4.5 Hz, C_5-<u>H</u>): 6.96(1H, s, C<u>H</u>$(C_6H_5)_2$); 7.3(10H, m, $(C_6$<u>H</u>$_5)_2$).

2,2,2-Trichloroethyl-3-[(N-2,2,2,-trichloroethyloxycarbonyl)-D-α-phenylglycyl-amido]-2β-(chloromethyl)penicillanate [18g, R=$C_6H_5CH(NHCOOCH_2CCl_3)CONH$, R'=$CH_2CCl_3$, X=Cl].

The *Unsym*-azetidinone disulfide **20j**, (21.5 g, 0.026 mole) in $CHCl_3$ (350 ml) and cupric chloride (3.873 g, 0.0288 mole) gave a pale yellow foam, yield:

17 g (94%), m. p. 93 - 96 °C, ^{1}H NMR data: 1.66(3H, s, C_2-C$\underline{H}_3$); 3.3, 3.46(2H, ABq, J=12 Hz, C_2-C$\underline{H}_2$); 4.78(2H, s, C_3-COOC$\underline{H}_2$CCl$_3$); 4.86(3H, s, C_3-$\underline{H}$, COOC$\underline{H}_2$CCl$_3$); 5.6(3H, m, C_5-$\underline{H}$, C_6-$\underline{H}$, C_6H_5C$\underline{H}$NH); 6.66(1H, d, J=7.5 Hz, C_6-N$\underline{H}$); 7.86(1H, d, J=10.5 Hz, C_6H_5CHN$\underline{H}$); 7.5(5H, m, $C_6\underline{H}_5$).

2,2,2-Trichloroethyl-3-{D-2-phenyl-2-[(4-ethyl-2,3-dioxo-piperazin-1-yl)-carbonylamino]acetamido}-2β-(chloromethyl)penicillanate [18h, R=C_6H_5CH(NHCO-N N-C_2H_5)CONH, R'=CH_2CCl_3, X=Cl].

The *Unsym*-azetidinone disulfide **20k**, (8.13 g, 0.010 mole) in $CHCl_3$ (150) and cupric chloride (1.47 g, 0.011 mole) gave a yellow foam, yield: 6.63 g, (81.5%), m. p. 85 - 88 °C, ^{1}H NMR data: 1.22(3H, t, J=7.51 Hz, N-CH_2C$\underline{H}_3$); 1.52(3H, s, C_2-C$\underline{H}_3$); 3.26, 3.48(2H, ABq, J=12.02 Hz, C_2-C$\underline{H}_2$); 3.56(2H, q, J=7.51 Hz, N-C$\underline{H}_2$CH$_3$); 3.6, 3.8, 4.8, 5.1(4H, NC$\underline{H}_2$C$\underline{H}_2$N); 4.72, 4.76(2H, ABq, J=12.12 Hz, C$\underline{H}_2$CCl$_3$); 5.04(1H, s, C_3-$\underline{H}$); 5.58(3H, m, C_5-$\underline{H}$, C_6-$\underline{H}$, C_6H_5C$\underline{H}$NH); 7.36(5H, m, $C_6\underline{H}_5$); 7.68(1H, d, J=9.5 Hz, N$\underline{H}$); 10.02(1H, d, J=7.4 Hz, C_6H_5CHN$\underline{H}$).

Benzhydryl-6β-(phenoxyacetamido)-2β-(bromomethyl)penicillanate, [18i, R=$C_6H_5OCH_2CONH$, R'=$CH(C_6H_5)_2$, X=Br].

To a solution of *unsym*-azetidinone disulfide **20f**, (6.81 g, 0.010 mole) in CH_2Cl_2 (80 ml), cupric bromide (3.35 g, 0.015 mole) was added and the mixture stirred at 0 - 5 °C for 4 h. The reaction mixture was filtered through a short bed of Celite and the filtrate washed sequentially with aqueous $NaHCO_3$ solution, distilled water and brine. The organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated to give a pale yellow foam, yield: 5.05 g (85%), m.p. 52 - 55 °C, ^{1}H NMR data: 1.36(3H, s, C_2-C$\underline{H}_3$); 3.40, 3.46(2H, ABq, J=12 Hz, C_2-C$\underline{H}_2$); 4.54(2H, s, OC$\underline{H}_2$); 5.14(1H, s, C_3-$\underline{H}$); 5.64(2H, m, C_5-$\underline{H}$, C_6-$\underline{H}$); 6.93(1H, s, C$\underline{H}$(C_6H_5)$_2$); 7.3(15H, m, ($C_6\underline{H}_5$)$_2$, C_6H_5).

2,2,2-Trichloroethyl-6β-(phenoxyacetamido)-2β-(bromomethyl)penicillanate, [18j, R=$C_6H_5OCH_2CONH$, R'=CH_2CCl_3, X=Br].

In a manner to that described above for **18i**, the title compound was prepared with *unsym*-azetidinone disulfide **20b** (6.465 g, 0.010 mole) and cupric bromide (3.35 g, 0.015 mole) in CH_2Cl_2 (80 ml). The product was obtained as a pale yellow foam, yield: 4.46 g (75%), m. p. 59 - 62 °C, ^1H NMR data: Table 13.

2,2,2-Trichloroethyl-6β-(phenylacetamido)-2β-(bromomethyl)penicillanate, [18k, R=$C_6H_5CH_2CONH$, R'=CH_2CCl_3, X=Br].

To a solution of *unsym*-azetidinone disulfide **20e** (1.8915 g, 3 mmol) in CH_2Cl_2 (30 ml), acetamide (0.15 g) and 1M bromine in CCl_4 (2.93 ml, 3 mmol) were added and the mixture stirred for 50 min in an ice-bath. The separated solid was removed by filtration. The filtrate was washed with aqueous $NaHCO_3$ solution, dried over anhydrous sodium sulfate, filtered and the filtrate was concentrated. Ether was added to the residue and the separated solid filtered and dried to give a pale yellow solid, yield: 1.07 g(60%), m. p. 86 - 89 °C, ^1H NMR data: Table 12.

***p*-Nitrobenzyl-6,6-dihydro-2β-(bromomethyl)penicillanate, [18l, R=H, R'=$CH_2C_6H_4$-*p*-NO_2, X=Br].**

To a cooled solution of *unsym*-azetidinone disulfide **20g**, (10.027 g, 0.02 mole) in CH_2Cl_2 (120 ml), cupric bromide (6.7 g, 0.03 mole) was added and the mixture stirred at 0 °C for 4 h. The reaction mixture was filtered through a short bed of Celite and the filtrate washed sequentially with aqueous $NaHCO_3$ solution, distilled water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated and dried to give a pale yellow solid, yield: 5.67 g (69%), m. p. 84 - 86 °C, ^1H NMR data: 1.56(3H, s,

C_2-C<u>H</u>$_3$); 3:16(1H, dd, J=2 Hz, 16 Hz, C_6-<u>H</u>); 3.63(3H, m, C_6-<u>H</u>, C_2-C<u>H</u>$_2$); 5.16(1H, s, C_3-<u>H</u>); 5.26(2H, s, C<u>H</u>$_2$C$_6$H$_4$NO$_2$); 7.63, 8.3(4H, d, C$_6$<u>H</u>$_4$).

D. *FLUORINATED CEPHAMS*

Significant IR signals are 1775 - 1795 cm^{-1} (β-lactam C=O), 1750 - 1760 cm^{-1} (ester C=O).

Reaction of Benzhydryl-3-phenoxyacetamido-4-(2'-benzothiazolyldithio)-2-azetidinon-1-yl isopropenyl acetate 20f, with Silver Fluoride.

To a solution of the *unsym*-azetidinone disulfide **20f**, (1.7025 g, 2.5 mmol) in CH_2Cl_2 (50 ml) in a flask, wrapped with aluminum foil, AgF (0.635 g, 5 mmol) was added and the mixture stirred at room temperature for 5 d. The reaction mixture was filtered through Celite, and the clear, dark reddish brown filtrate was concentrated and dried to give 1:0762 g of the crude product. The TLC and ^1H NMR of the crude product showed a complex mixture, which was difficult to purify by usual chromatographic techniques.

Oxidation of the Above Crude Reaction Mixture with *m*-Chloroperbenzoic Acid.

To a cooled solution of the crude product (1.0762 g) in CH_2Cl_2 (15 ml) was added dropwise *m*-chloroperbenzoic acid (0.345 g, 2 mmol) in CH_2Cl_2 (15 ml) and the mixture stirred at the same temperature for 1.5 h. The reaction mixture was filtered and the filtrate washed sequentially with saturated $NaHCO_3$ solution, distilled water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated to give a yellow foam (750 mg), which was purified by gradient flash chromatography using ethyl acetate/hexane as eluants, to give **36**, (X=F, 160 mg, 21%) and **26**, (180 mg, 24%). The physical data are summarized in Table 10 and Table 11.

Benzhydryl-7β-(phenoxyacetamido)-3β-fluoro-3α-methylcepham, [22, R=$C_6H_5OCH_2CONH$, R'=$CH(C_6H_5)_2$, X=F].

To a solution of benzhydryl-6β-(phenoxyacetamido)-2[illegible]mo-methyl)penicillanate **18i**, (X=Br; 0.534 g, 1 mmol) in distilled CH_2Cl_2 (20 ml), in a flask wrapped with aluminum foil, AgF was added (0.254 g, 2 mmol), and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated and the residue taken up in ethyl acetate, decolorized by stirring with adsorbent charcoal, filtered through Celite, and the filtrate concentrated to give a white foam. Crystallization of the foam from ethyl acetate-hexane gave **22**, (X=F) as a white solid, yield: 0.507 g (95%). The spectral data and physical constants are listed in Table 10 and Table 11.

Benzhydryl-7β-(phenoxyacetamido)-3β-fluoro-3α-methylcepham-1β-oxide, [36, R=$C_6H_5OCH_2CONH$, R'=$CH(C_6H_5)_2$,]

To a cooled solution of benzhydryl-7β-(phenoxyacetamido)-3β-fluoro-3α-methylcepham **22** (X=F; 0.534 g, 1 mmol) in CH_2Cl_2 (15 ml), meta-chloroperbenzoic acid (0.19 g, 1.1 mmol) in CH_2Cl_2 (10 ml) was added portionwise and the mixture stirred at the same temperature for 1.5 h. The reaction mixture was filtered and the filtrate washed sequentially with saturated $NaHCO_3$ solution, distilled water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated and dried. The crude product was purified by gradient flash chromatography using ethyl acetate/hexane as eluant. The physical data on the pure product, a white powder, yield: 0.44 g (80%), is given in Table 10 and Table 11.

Benzhydryl-7β-(phenoxyacetamido)-3β-fluoro-3α-methylcepham-1,1-dioxide [40; R=$C_6H_5OCH_2CONH$, R'=$CH(C_6H_5)_2$]

To a cooled solution of benzhydryl-7β-(phenoxyacetamido)-3β-fluoro-3α-methylcepham **22**, (X=F; 0.35 g, 0.65 mmol), in glacial acetic acid (6.54 ml)

and water (0.85 ml) was added portionwise $KMnO_4$ (0.237 g, 1.3 mmol) over a period of 1 h. The reaction mixture was stirred at room temperature for 3.5 h and a 30% solution of H_2O_2 added dropwise until decolorization was complete. The resulting solution was poured into ice-water (15 ml) and the resulting solid collected by filtration and taken up in CH_2Cl_2. The solution was washed sequentially with saturated $NaHCO_3$ solution, distilled water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated to a white foam **40**, which weighed 250 mg (60% yield), physical data: Table 10 and Table 11.

E. *2β-(HALOMETHYL)PENAM-1β-OXIDES* **41.**

Significant IR signals are 1780 - 1790 cm^{-1} (β-lactam C=O), 1760 - 1770 cm^{-1} (ester C=O), and 1685 - 1695 cm^{-1} (amide C=O).

Benzhydryl-6β-(phenoxyacetamido)-2β-(chloromethyl)penicillanate-1β-oxide [41a, $R=C_6H_5OCH_2CONH$, $R'=CH(C_6H_5)_2$, X=Cl].

To a cooled solution of benzhydryl-6β-(phenoxyacetamido)-2β-(chloromethyl)penicillanate **18a**, (2.2020 g, 4 mmol), in CH_2Cl_2 (15 ml), *m*-chloroperbenzoic acid (0.759 g, 4.4 mmol) in CH_2Cl_2 (15 ml) was added dropwise and the mixture stirred at the same temperature for 1.5 h. The reaction mixture was filtered and the filtrate washed sequentially with saturated $NaHCO_3$ solution, distilled water and brine. The organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated and dried. The crude product thus obtained was purified by gradient silica-gel column chromatography using ethyl acetate/hexane as eluant to give a white powder, yield: 1.677 g (74%), m.p. 88 - 91 °C, ^1H NMR data: 1.0(3H, s, C_2-$C\underline{H}_3$); 3.94, 4.28(2H, ABq, J=12 Hz, C_2-$C\underline{H}_2$); 4.54(2H, s, $OC\underline{H}_2$); 4.76(1H, s, C_3-$\underline{H}$):

5.0(1H, d, J=6 Hz, C_5-H); 6.18(1H, dd, J=12 Hz, 6 Hz, C_6-H); 7.06[1H, s, CH(C_6H_5)$_2$]; 7.36(15H, m, CH(C_6H_5)$_2$, C_6H_5); 8.24(1H, d, J=12 Hz, NH).

In a manner similar to that described to that above, the following compounds were prepared.

2,2,2-Trichloroethyl-6β-(phenoxyacetamido)-2β-(chloromethyl)penicillanate-1β-oxide [41b, R=$C_6H_5OCH_2CONH$, R'=CH_2CCl_3, X=Cl].

The title compound was prepared from 2,2,2-trichloroethyl-6β-(phenoxyacetamido)-2β-(chloromethyl)penicillanate **18b**, (2.58 g, 5 mmol), and *m*-chloroperbenzoic acid (0.9488 g, 5.5 mmol) in CH_2Cl_2 (50 ml). The product was obtained as a white powder, yield: 1.73 g (65%), m.p. 82 - 85 °C, ^{1}H NMR data: Table 13.

2,2,2-Trichloroethyl-6β-(phenylacetamido)-2β-(chloromethyl)penicillanate-1β-oxide [41c, R=$C_6H_5CH_2CONH$, R'=CH_2CCl_3, X=Cl].

The title compound was prepared from 2,2,2-trichloroethyl-6β-(phenylacetamido)-2β-(chloromethyl)penicillanate **18d**, (2.5 g, 5 mmol), and *m*-chloroperbenzoic acid (0.9488 g, 5.5 mmol) in CH_2Cl_2 (50 ml). The product was obtained as a white powder, yield: 1.625 g (63%), 80 - 84 °C, ^{1}H NMR data: Table 12.

2,2,2-Trichloroethyl-6β-(phenoxyacetamido)-2β-(bromomethyl)penicillanate-1β-oxide [41d, R=$C_6H_5OCH_2CONH$, R'=CH_2CCl_3, X=Br].

The title compound was prepared from 2,2,2-trichloroethyl-6β-(phenoxyacetamido)-2β-(bromomethyl)penicillanate **18g**, (2.7975 g, 5 mmol) and *m*-chloroperbenzoic acid (0.9488 g, 5.5 mmol) in CH_2Cl_2 (50 ml). The product was obtained as a white powder, yield: 1.727 g (60%), m. p. 76 - 79 °C, ^{1}H NMR data: Table 13.

Benzhydryl-6β-(phenoxyacetamido)-2β-(bromomethyl)penicillanate-1β-oxide [41e, R=$C_6H_5OCH_2CONH$, R'=$CH(C_6H_5)_2$, X=Br].

The title compound was prepared with benzhydryl-6β-(phenoxyacetamido)-2β-(bromomethyl)penicillanate **18i**, (0.5949 g, 1 mmol), and *m*-chloroperbenzoic acid (0.2588 g, 1.5 mmol) in CH_2Cl_2 (50 ml). The product was obtained as a white powder, yield: 0.403 g (66%), m.p. 78 - 80 °C, ^{1}H NMR data: 1.68(3H, s, C_2-C<u>H</u>$_3$); 3.60, 3.6(2H, ABq, J=12.75 Hz, C_2-C<u>H</u>$_2$); 4.58(2H, s, OC<u>H</u>$_2$); 4.88(1H, s, C_3-<u>H</u>); 4.98(1H, d, J=3.82 Hz, C_5-<u>H</u>); 5.78(1H, dd, J=8.92 Hz, 3.28 Hz, C_6-<u>H</u>); 6.98[1H, s, C<u>H</u>$(C_6H_5)_2$]; 7.36(15H, m, $CH(C_6$<u>H</u>$_5)_2$, C_6<u>H</u>$_5$); 7.7(1H, d, J=8.92 Hz, N<u>H</u>).

2,2,2-Trichloroethyl-6β-(phenylacetamido)-2β-(bromomethyl)penicillanate-1β-oxide [41f, R=$C_6H_5CH_2CONH$, R'=CH_2CCl_3, X=Br].

The title compound was prepared with 2,2,2-trichloroethyl-6β-(phenylacetamido)-2β-(bromomethyl)penicillanate **18h**, (1.8 g, 3.3 mmol), and *m*-chloroperbenzoic acid (0.85 g, 5 mmol) in CH_2Cl_2 (50 ml). The product was obtained as a white powder, yield: 1.575 g (85%), m. p. 80 - 83 °C, ^{1}H NMR data: Table 12.

F. *SODIUM SALTS OF 2β-(HALOMETHYL)PENAMS* **42** *AND THEIR SULFOXIDES* **43**.

Significant IR signals are 1750 - 1760 cm^{-1} (β-lactam C=O), 1665 - 1675 cm^{-1} (amide C=O) and 1610 - 1620 cm^{-1} (COO$^-$).

Sodium 6β-(phenoxyacetamido)-2β-(chloromethyl)penicillanate [42a, R=$C_6H_5OCH_2CONH$, X=Cl].

To a cooled (0 - -5 °C) solution of 2,2,2-trichloroethyl-6β-(phenoxyacetamido)-2β-(chloromethyl)penicillanate **18b**, (0.516 g, 1 mmol), in DMF (2.5 ml), glacial acetic acid (0.75 ml) and zinc dust (0.58 g, 8.9 mmol) were added and the mixture stirred under cooling for 3 h. The reaction mixture was filtered, the filtrate taken up in ethyl acetate-water mixture (10 ml each). The organic layer was separated, washed with 5% HCl and then thoroughly with distilled water; dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated and dried. The residue, 6β-(phenoxyacetamido)-2β-(chloromethyl)penicillanic acid was dissolved in ethyl acetate (10 ml) and the solution cooled in an ice-bath and a solution of sodium 2-ethylhexanoate was added until the solution reached a pH of pH 7. The separated solid was filtered, washed with ether and dried to give a white powder, yield: 0.203 g (50%), m. p. 116 - 119 °C, ^{1}H NMR data: Table 13.

In a manner similar to that described above, the following compounds were prepared.

Sodium 6β-(phenylacetamido)-2β-(chloromethyl)penicillanate, [42b, R=$C_6H_5CH_2CONH$, X=Cl]

The title compound was prepared form 2,2,2-trichloroethyl-6β-(phenylacetamido)-2β-(chloromethyl)penicillanate **18d**, (0.5 g, 1 mmol), in DMF (2.5 ml), glacial acetic acid (0.75 ml) and zinc dust (0.5818 g, 8.9 mmol). The product was obtained as a white solid, yield: 0.254 g (65%), m. p. 146 - 149 °C, ^{1}H NMR data: Table 12.

Sodium 6β-(phenoxyacetamido)-2β-(bromomethyl)penicillanate, [42c, R=$C_6H_5OCH_2CONH$, X=Br].

The title compound was obtained from 2,2,2-trichloroethyl-6β-(phenoxyacetamido)-2β-(bromomethyl)penicillanate **18j**, (0.5595 g, 1 mmol), in DMF(2.5 ml), glacial acetic acid (0.75 ml) and zinc dust (0.5818 g, 8.9 mmol). The product was obtained as a white powder, yield: 0.225 g (50%), m. p. 135 - 138 °C, ^1H NMR data: Table 13.

Sodium 6β-(phenylacetamido)-2β-(bromomethyl)penicillanate, [42d, R=$C_6H_5CH_2CONH$, X=Br]

The title compound was prepared from 2,2,2-trichloroethyl-6β-(phenylacetamido)-2β-(bromomethyl)penicillanate **18k**, (0.5435 g, 1 mmol) in DMF (2.5 ml), glacial acetic acid (0.75 ml) and zinc dust (0.5818 g, 8.9 mmol). The product was obtained as a white powder, yield: 0.283 g (65%), m. p. 115 - 118 °C, ^1H NMR data: Table 12.

Sodium 6β-(phenoxyacetamido)-2β-(chloromethyl)penicillanate-1β-oxide, [43a, R=$C_6H_5OCH_2CONH$, X=Cl].

The title compound was prepared from 2,2,2-trichloroethyl-6β-(phenoxyacetamido)-2β-(chloromethyl)penicillanate-1β-oxide **41b**, (0.532 g, 1 mmol) in DMF (2.5 ml), glacial acetic acid (0.75 ml) and zinc dust (0.5818 g, 8.9 mmol). The product was obtained as a white powder Yield: 0.275 g (65%), m. p. 110 - 115 °C, ^1H NMR data: Table 13.

Sodium 6β-(phenylacetamido)-2β-(chloromethyl)penicillanate-1β-oxide, [43b, R=$C_6H_5CH_2CONH$, X=Cl].

The title compound was prepared from 2,2,2-trichloroethyl-6β-(phenylacetamido)-2β-(chloromethyl)penicillanate-1β-oxide **41c** (0.516 g, 1 mmol) in DMF (2.5 ml), glacial acetic acid (0.75 ml) and zinc dust (0.5818 g, 8.9

mmol). The product was obtained as a white powder, yield: 0.252 g (62%), m. p. 100 - 103 °C, ^{1}H NMR data: Table 12.

Sodium 6β-(phenoxyacetamido)-2β-(bromomethyl)penicillanate-1β-oxide, [43c, R=$C_6H_5OCH_2CONH$, X=Br].

The title compound was prepared from 2,2,2-trichloroethyl-6β-(phenoxyacetamido)-2β-(bromomethyl)penicillanate-1β-oxide **41d**, (0.5755 g, 1 mmol), in DMF (2.5 ml), glacial acetic acid (0.75 ml) and zinc dust (0.5818 g, 8.9 mmol). The product was obtained as a white powder, yield: 0.313 g (67%), m. p. 115 - 118 °C, ^{1}H NMR data: Table 13.

Sodium 6β-(phenylacetamido)-2β-(bromomethyl)penicillanate-1β-oxide, [43d, R=$C_6H_5CH_2CONH$, X=Br].

The title compound was prepared from 2,2,2-trichloroethyl-6β-(phenylacetamido)-2β-(bromomethyl)penicillanate-1β-oxide **41f**, (0.5755 g, 1 mmol) in DMF (2.5 ml), glacial acetic acid (0.75 ml) and zinc dust (0.5818 g, 8.9 mmol). The product was obtained as a white powder, yield: 0.307 g (68%), m. p. 127 - 130 °C, ^{1}H NMR data: Table 12.

G. *ESTERS OF 2β-(SUBSTITUTED METHYL)PENAMS* **44**

Significant IR signals are 1780 - 1790 cm^{-1} (β-lactam C=O), 1755 - 1770 cm^{-1} (ester C=O), and 1665 - 1695 cm^{-1} (amide C=O).

2,2,2-Trichloroethyl-6β-(phenylacetamido)-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)-thiomethyl]penicillanate, [44a, R=$C_6H_5CH_2CONH$, R'=CH_2CCl_3, R"=5-methyl-1,3,4-thiadiazole].

To a suspension of 2-mercapto-5-methyl-1,3,4-thiadiazole (1.056 g, 8 mmol) and $NaHCO_3$ (0.672 g, 8 mmol), in phosphate buffer, (pH 6.4, 60 ml), a solution of 2,2,2-trichloroethyl-6β-(phenylacetamido)-2β-(chloromethyl)-

penicillanate **18d**, (2 g, 4 mmol) in acetone (160 ml) was added and the mixture heated to reflux for 2 h. The reaction mixture was then stirred at room temperature for 24 h. Acetone was removed by evaporation and the residue extracted with ethyl acetate. The combined organic extracts were washed with distilled water and brine, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated and dried and the residue purified by gradient silica-gel chromatography using ethyl acetate/hexane as eluants. The product was obtained as a white foam, yield: 0.860 g (36%), m.p. 70 - 72 °C, ^1H NMR data: Table 14.

In a manner similar to that described above, the following compounds were prepared.

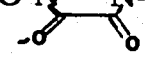
2,2,2-Trichloroethyl-6β-(phenoxyacetamido)-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl)]penicillanate [44b, R=$C_6H_5OCH_2CONH$, R'=CH_2CCl_3, R"=5-methyl-1,3,4-thiadiazole].

The title compound was prepared from 2,2,2-trichloroethyl-6β-(phenoxyacetamido)-2β-(chloromethyl)penicillanate **18b**, (2.064 g, 4 mmol), in acetone (160 ml) and 2-mercapto-5-methyl-1,3,4-thiadiazole (1.056 g, 8 mmol), $NaHCO_3$ (0.672 g, 8 mmol) and phosphate buffer, (pH 6.4, 60 ml). The product was obtained as a white foam, yield: 0.930 g (38%), m. p. 66 - 68 °C, ^1H NMR data: Table 14.

2,2,2-Trichloroethyl-3-[(N-2,2,2-trichloroethyloxycarbonyl)-D-α-phenylglycyl-amido]-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl]penicillanate [44c, R=$C_6H_5CH(NHCOOCH_2CCl_3)CONH$, R'=$CH_2CCl_3$, R"=5-methyl-1,3,4-thiadiazole].

The title compound was prepared from 2,2,2-trichloroethyl-3-[(N-2,2,2-trichloroethyloxycarbonyl)-D-α-phenylglycylamido]-2β-(chloromethyl)-penicillanate (6.9 g, 10 mmol) in acetone (105 ml) and 2-mercapto-5-methyl-

1,3,4-thiadiazole (2.64 g, 20 mmol), $NaHCO_3$ (1.68 g, 20 mmol) and phosphate buffer, (pH 6.4, 35 ml). The product was obtained as a white foam, yield: 1.965 g (25%), m. p. 89 - 92 °C, ^1H NMR data: Table 15.

2,2,2-Trichloroethyl-3-{D-2-phenyl-[(4-ethyl-2,3-dioxo-piperazin-1-yl)carbonyl-amino]acetamido}-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl]-penicillanate [44d, R=C_6H_5CH(NHCO-  -C_2H_5)CONH, R'=CH_2CCl_3, R"=5-methyl-1,3,4-thiadiazole].

The title compound was prepared from 2,2,2-trichloroethyl-3-{D-2-phenyl-[(4-ethyl-2,3-dioxo-piperazin-1-yl)carbonylamino]acetamido}-2β-(chloromethyl)penicillanate (2.7 g, 5 mmol) in acetone (50 ml) and 2-mercapto-5-methyl-1,3,4-thiadiazole (1.32 g, 10 mmol), $NaHCO_3$ (0.84 g, 10 mmol) and phosphate buffer, (pH 6.4, 20 ml). The product was obtained as a white foam, yield: 0.955 g (30%), m. p. 125 - 128 °C, ^1H NMR data: Table 16.

2,2,2-Trichloroethyl-6β-(phenylacetamido)-2β-[(1-methyl-1,2,3,4-tetrazol-5-yl)-thiomethyl]-penicillanate, [44e, R=$C_6H_5CH_2CONH$, R'=CH_2CCl_3, R"=1-methyl-1,2,3,4-tetrazole]

To a suspension of 5-mercapto-1-methyl-1,2,3,4-tetrazole (0.96 g, 8.28 mmol) and $NaHCO_3$ (0.695 g, 8.28 mmol) in phosphate buffer, (pH 6.4, 60 ml), a solution of 2,2,2-trichloroethyl-6β-(phenylacetamido)-2β-(chloromethyl)-penicillanate **18d**, (2.16 g, 4.32 mmol) in acetone (110 ml), was added and the mixture heated to reflux for 2 h. The reaction mixture was then stirred at room temperature for 24 h. The acetone was removed by evaporation and the residue extracted with ethyl acetate. The combined organic extracts were washed with distilled water and brine; dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated and purified by gradient silica-gel chromatography using ethyl acetate/hexane as eluants. The product was

obtained as a white foam, yield: 0.975 g (39%), m. p. 67 - 70 °C, ^{1}H NMR data: Table 14.

In a manner similar to that described above, the following compounds were prepared.

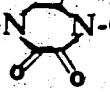
2,2,2-Trichloroethyl-6β-(phenoxyacetamido)-2β-[(1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl]penicillanate, [44f, R=$C_6H_5OCH_2CONH$, R'=CH_2CCl_3, R"=1-methyl-1,2,3,4-tetrazole].

The title compound was prepared from 2,2,2-Trichloroethyl-6β-(phenoxyacetamido)-2β-(chloromethyl)penicillanate **18b**, (2.0644 g, 4 mmol), in acetone (160 ml), and 5-mercapto-1-methyl-1,2,3,4-tetrazole (1.056 g, 8 mmol), $NaHCO_3$ (0.672 g, 8 mmol) and phosphate buffer, (pH 6.4, 60 ml). The product was obtained as a white foam, yield: 1 g (42%), m.p. 68 - 70 °C, ^{1}H NMR data: Table 14.

2,2,2-Trichloroethyl-3-[(N-2,2,2-trichloroethyloxycarbonyl)-D-α-phenylglycyl-amido]-2β-[(1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl]penicillanate [44g, R=$C_6H_5CH(NHCOOCH_2CCl_3)CONH$, R'=$CH_2CCl_3$, R"=1-methyl-1,2,3,4-tetrazole].

The title compound was prepared from 2,2,2-trichloroethyl-3-[(N-2,2,2-trichloroethyloxycarbonyl)-D-α-phenylglyclyamido]-2β-(chloromethyl)-penicillanate (5.14 g, 7.4 mmol), in acetone (80 ml), and 5-mercapto-1-methyl-1,2,3,4-tetrazole (1.72 g, 14.8 mmol), $NaHCO_3$ (1.25 g, 14.8 mmol) and phosphate buffer, (pH 6.4, 35 ml). The product was obtained as a white foam, yield: 2. 4g (41.8%), m. p. 88 - 90 °C, ^{1}H NMR data: Table 15.

2,2,2-Trichloroethyl-3-{D-2-phenyl-[(4-ethyl-2,3-dioxo-piperazin-1-yl)carbonyl-amino]acetamido}-2β-[(1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl]penicillanate [44h, R=C_6H_5CH(NHCO-N N-C_2H_5)CONH, R'=CH_2CCl_3, R"=1-methyl-1,2,3,4-tetrazole].



The title compound was prepared from 2,2,2-trichloroethyl-3-{D-2-phenyl-[(4-ethyl-2,3-dioxo-piperazin-1-yl)carbonylamino]acetamido}-2β-(chloromethyl)penicillanate (2.7 g, 5 mmol), in acetone (50 ml) and 5-mercapto-1-methyl-1,2,3,4-tetrazole (1.15 g, 10 mmol), $NaHCO_3$ (0.84 g, 10 mmol) and phosphate buffer, (pH 6.4, 20 ml). The product was obtained as a white foam, yield: 1.1 g (35%), m. p. 133 - 135 °C, ^1H NMR data: Table 16.

H. *SODIUM SALTS OF 2β-(SUBSTITUTED METHYL)PENAMS* 45.

Significant IR signals are 1750 - 1765 cm^{-1} (β-lactam C=O), 1665 - 1675 cm^{-1} (amide C=O) and 1615 - 1625 cm^{-1} (COO^-).

Sodium 6β-(phenylacetamido)-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl] penicillanate [45a, R=$C_6H_5CH_2CONH$, R"=5-methyl-1,3,4-thiadiazole].

To an ice-cold solution of 2,2,2-trichloroethyl-6β-(phenylacetamido)-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl]penicillanate **44a**, (1.1 g, 1.8 mmol), in DMF (8 ml), zinc dust (1.047 g, 16.04 mmol) and acetic acid (1.5 ml) were added and the mixture stirred under cooling for 2 h. The reaction mixture was filtered and the filtrate extracted with ethyl acetate. The combined organic extracts were washed sequentially thrice with 5% HCl, twice with distilled water and brine; dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated. To an ice-cold solution of the acid (0.63 g, 80% yield) in ethyl acetate (15 ml) and water (15 ml), $NaHCO_3$ (0.107 g, 90%), was added and the mixture stirred for 15 - 20 min. The aqueous layer was separated and freeze

dried. The sodium salt was obtained as a white powder, yield: 0.78 g (90%), m. p. 85 - 88 °C, ^{1}H NMR data: Table 14

In a manner similar to that described above, the following compounds were prepared.

Sodium 6β-(phenoxyacetamido)-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl]penicillanate [45b, R=$C_6H_5OCH_2CONH$, R"=5-methyl-1,3,4-thiadiazole]

The title compound was prepared from 2,2,2-trichloroethyl-6β-(phenoxyacetamido)-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl]-penicillanate **44b**, (1.22 g, 2 mmol), in DMF (8 ml), and zinc dust (1.16 g, 17.8 mmol) and acetic acid (1.5 ml). The sodium salt was obtained as a white solid, yield: 0.73g (70%), m.p. 120 - 122 °C, ^{1}H NMR [illegible] 14.

Sodium 2β-[(5-methyl-1,3,4-thiadiazol-2-yl)t[illegible]mpicillin [45c, R=$C_6H_5CH(NH_2)CONH$, R"=5-methyl-[illegible]diazole].

The title compound was obtained from 2,2,2-trichloroethyl-3-[(N-2,2,2-trichloroethyloxycarbonyl)-D-α-phenylglycylamido]-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl]penicillanate **44c**, (1.53 g, 2 mmol), in DMF (8 ml) and zinc dust (1.16 g, 17.8 mmol) and glacial acetic acid (1.5 ml). The sodium salt was obtained as a white solid, yield: 0.738 g (77%), m. p. 185 - 188 °C, ^{1}H NMR data: Table 15.

Sodium 2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl]piperacillin, [45d, R=$C_6H_5CH(NHCO$-N⟨ ⟩N-$C_2H_5)CONH$, R"=5-methyl-1,3,4-thiadiazole].

The title compound was prepared from 2,2,2-trichloroethyl-3-{D-2-phenyl-2-[(4-ethyl-2,3-dioxo-piperazin-1-yl)carbonylamino]acetamido}-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl]penicillanate **44d**, (1.51 g, 2 mmol), in DMF (8 ml) and zinc dust (1.16 g, 17.8 mmol) and glacial acetic acid (1.5 ml).

The sodium salt was obtained as a white solid, yield: 0.843 g (63%), m.p. 160 - 162 °C, ^{1}H NMR data: Table 16.

Sodium 6β-(phenylacetamido)-2β-[(1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl]-penicillanate [45e, R=$C_6H_5CH_2CONH$, R"=1-methyl-1,2,3,4- tetrazole].

The title compound was prepared from 2,2,2-trichloroethyl-6β-(phenylacetamido)-2β-[(1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl]penicillanate **44e**, (1.0 g, 1.8 mmol) in DMF (8 ml), zinc dust (1.04 g, 16 mmol) and acetic acid (1.5 ml). The sodium salt was obtained as a white solid, yield: 0.676 g (80%), m. p. 140 - 143 °C, ^{1}H NMR data: Table 14.

Sodium 6β-(phenoxyacetamido)-2β-[(1-methyl-1,2,3,4-tetrazol-5-yl)thio-methyl]penicillanate [45f, R=$C_6H_5OCH_2CONH$, R"=1-methyl-1,2,3,4-tetrazole].

The title compound was prepared from 2,2,2-trichloroethyl-6β-(phenoxyacetamido)-2β-[(1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl]-penicillanate **44f**, (1.19 g, 2 mmol), in DMF (8 ml) and zinc dust (1.16 g, 17.8 mmol) and acetic acid (1.5 ml). The sodium salt was obtained as a white solid, yield: 0.8262 g (85%), m. p. 151 - 154 °C, ^{1}H NMR data: Table 14.

Sodium 2β-[(1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl]ampicillin [45g, R=$C_6H_5CH(NH_2)CONH$, R"=1-methyl-1,2,,3,4-tetrazole].

The title compound was prepared from 2,2,2-trichloroethyl-3-[(N-2,2,2-trichloroethyloxycarbonyl)-D-α-phenylglycylamido]-2β-[(1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl]penicillanate **44g**, (1.54 g, 2 mmol), in DMF (8ml) and zinc dust (1.16 g, 17.8 mmol) and acetic acid (1.5 ml). The sodium salt was obtained as a white solid, yield: 0.666g (72%), m. p. 210 - 212 °C, ^{1}H NMR data: Table 15.

Sodium 2β-[(1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl]]piperacillin, [45h, R=C_6H_5CH(NHCO--C_2H_5)CONH, R"=1-methyl-1,2,3,4-tetrazole]

The title compound was prepared from 2,2,2-trichloroethyl-3-{D-2-phenyl-2-[(4-ethyl-2,3-dioxo-piperazin-1-yl)carbonylamino]acetamido}-2β-[(1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl]-penicillanate **44h**, (1.48 g, 2 mmol), in DMF (8 ml), and zinc dust (1.16 g, 17.8 mmol) and acetic acid (1.5 ml). The sodium salt was obtained as a white solid, yield: 0.705 g (56%), m.p. 148 - 150 °C, ^{1}H NMR data: Table 16.

I. *2β-(HALOMETHYL)PENAM-1,1-DIOXIDES, 19.*

Significant IR signals are 1800 - 1810 cm^{-1} (β-lactam C=O) and 1755 - 1765 cm^{-1} (ester C=O).

p-Nitrobenzyl-6,6-dihydro-2β-(chloromethyl)penicillanate-1,1-dioxide, [19a, R=H, R'=$CH_2C_6H_4$-*p*-NO_2, X=Cl].

To an ice-cold solution of *p*-nitrobenzyl-6,6-dihydro-2β-(chloromethyl)-penicillanate **18e** (3.705 g, 10 mmol) in glacial acetic acid (70 ml) and water (9.3 ml) was added portionwise, $KMnO_4$ (3.476 g, 22 mmol), over a period of 1 h. The reaction mixture was stirred at room temperature for 3.5 h. A 30% solution of H_2O_2 was added dropwise until decolorization was complete. The resulting solution was poured into ice-water (150 ml). The solid collected by filtration was dissolved in CH_2Cl_2 and washed sequentially with aqueous $NaHCO_3$, distilled water and brine. The organic layer was dried over anhydrous Na_2SO_4 and filtered and the filtrate was concentrated and dried. The product was obtained as a white solid, yield: 3.34 g (83%), m.p. 80 - 82 °C, ^{1}H NMR data: Table 17.

p-Nitrobenzyl-6,6-dihydro-2β-(bromomethyl)penicillanate-1,1-dioxide, [R=H, R'=$CH_2C_6H_4$-*p*-NO_2, X=Br].

In a manner similar to that described above, the title compound was prepared from *p*-nitrobenzyl-6,6-dihydro-2β-(bromomethyl)penicillanate 18l, (4.329 g, 10 mmol) in glacial acetic acid (82.2 ml), water (10.6 ml) and $KMnO_4$ (3.476 g, 22 mmol). The product was obtained as a white solid, yield: 3.99 g, (86%), m. p. 73 - 76 °C, ^1H NMR data: Table 17.

Sodium 6,6-dihydro-2β-(chloromethyl)penicillanate-1,1-dioxide, [19a, R=H, R'=Na, X=Cl].

To a solution of *p*-nitrobenzyl-6,6-dihydro-2β-(chloromethyl)-penicillanate-1,1-dioxide (1.0 g, 2.5 mmol) in THF (35 ml) and water (25 ml), Pd-C catalyst (10%, 1.0 g) was added and the mixture hydrogenated at 50 p.s.i. for 3 h. The reaction mixture was filtered through a short bed of Celite. To the filtrate $NaHCO_3$ (0.093 g, 90%), was added and the solution evaporated to remove THF. The aqueous portion was washed twice with ether and the separated aqueous portion was freeze dried. The sodium salt was obtained as a white solid, yield: 0.2533g (35%), m.p. 125 - 130 °C, ^1H NMR data: Table 17.

Sodium 6,6-dihydro-2β-(bromomethyl)penicillanate-1,1-dioxide [19a R=H, R'=Na, X=Br].

In a manner similar to that described above the title compound was prepared from *p*-nitrobenzyl-6,6-dihydro-2β-(bromomethyl)penicillanate (1.0 g) in THF (35 ml) and water (25 ml), and Pd-C (10%) catalyst. The sodium salt was obtained as a white solid, yield: 0.2719 g (35%), m.p. 190 - 192 °C, ^1H NMR data: Table 17.

J. *6,6-DIHYDRO-2β-[(5-METHYL-1,3,4-THIADIAZOL-2-YL)THIOMETHYL]-PENAM AND ITS OXIDATION PRODUCTS*

Significant IR signals are 1780 - 1805 cm^{-1} (β-lactam C=O) and 1755 - 1765 cm^{-1} (ester C=O).

Benzhydryl-6,6-dihydro-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl]-penicillanate, [47, R=H, R'=$CH(C_6H_5)_2$].

To a suspension of 2-mercapto-5-methyl-1,3,4-thiadiazole, (2.64 g, 20 mmol), and $NaHCO_3$ (1.68 g, 20 mmol) in phosphate buffer, (pH 6.4, 16.9 ml), a solution of benzhydryl-6,6-dihydro-2β-(chloromethyl)penicillanate **18f**, (4.015 g, 10 mmol) in acetone (50.6 ml) was added and the mixture heated to reflux for 2.5 h. The reaction mixture was then stirred at room temperature for 24 h. The acetone was [redacted] the residue extracted twice with ethyl acetate. The combined organic extracts were washed thrice with distilled water; dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated and dried to give a yellow foam. The crude product 3.53 g (70%), was purified by gradient silica-gel column chromatography using ethyl acetate/hexane as eluants. The product was obtained as an off-white powder, yield: 1.74 g (35%), m.p: 56 - 59 °C, ^1H NMR data: Table 18, ^{13}C NMR data: Table 19

Benzhydryl-6,6-dihydro-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl]-penicillanate-1β-oxide [48, R=H, R'=$CH(C_6H_5)_2$].

To a solution of benzhydryl-6,6-dihydro-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl]penicillanate **47**, (1.91 g, 3.8 mmol) in CH_2Cl_2 (30 ml), a 30% solution of H_2O_2 (1.0448 g, 9.2 mmol) and glacial acetic acid (1.82 g, 30 mmol) were added and the mixture stirred at room temperature for 24 h. The reaction mixture was washed sequentially with aqueous $NaHCO_3$ solution,

distilled water and brine; dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated and the crude product 1.68g (85%), was purified by gradient silica-gel column chromatography using ethyl acetate/hexane as eluants. The product was obtained as a white foam, yield: 0.985 g (50%), m.p. 83 - 85 °C, ^1H NMR data: Table 18, ^{13}C NMR data: Table 19.

Benzhydryl-6,6-dihydro-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfinylmethyl]-penicillanate 1,1-dioxide [49, R=H, R'=$CH(C_6H_5)_2$].

To a solution of benzhydryl-6,6-dihydro-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl]penicillanate-1β-oxide 48, (1.8 g, 3.5 mmol) in CH_2Cl_2 (15 ml), a solution of *m*-chloroperbenzoic acid (1.3283 g, 7.7 mmol) in CH_2Cl_2 (15 ml) was added over a period of 30 min. The reaction mixture was stirred at room temperature for 6 h. It was then washed sequentially with aqueous $NaHCO_3$, distilled water and brine; dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated and dried to a foam. The crude product, 1.623 g (85%) thus obtained was purified by gradient silica-gel column chromatography using ethyl acetate/hexane. The product was obtained as a white foam, yield: 0.956 g (50%), m.p. 67 - 70 °C, ^1H NMR data: Table 18, ^{13}C NMR data: Table 19.

Benzhydryl-6,6-dihydro-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl]-penicillanate-1,1-dioxide [50, R=H, R'=$CH(C_6H_5)_2$].

To a solution of benzhydryl-6,6-dihydro-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfenylmethyl]penicillanate-1,1-dioxide 49, (0.545 g, 1 mmol), in CH_2Cl_2 (10 ml) P_2S_5 (0.4889 g, 1.1 mmol) and pyridine (0.3164 g, 4 mmol) were added and the mixture stirred at room temperature for 24 h. Then the reaction mixture was poured into water (10 ml) and the resulting mixture extracted with CH_2Cl_2. The combined organic extracts were washed sequentially with distilled water (4 times), aqueous $NaHCO_3$ solution and brine; dried over

anhydrous Na_2SO_4 and filtered. The filtrate was concentrated and dried to give a yellow foam; (0.2116 g, 40%) which was purified by preparative silica-gel chromatography using ethyl acetate/toluene (2:3) as solvent system. A band visible under UV light was cut out, extracted with ethyl acetate and the ethyl acetate solution concentrated and dried. The product was obtained as a white foam, yield: 0.132g (25%), m.p. 90 - 93 °C, ^1H NMR data: Table 18, ^{13}C NMR data: Tabl

Benzhydryl-6,6-dihydro-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfonylmethyl]-penicillanate-1,1-dioxide, [51, R=H, R'=$CH(C_6H5)_2$].

To a cooled solution of benzhydryl-6,6-dihydro-2β-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl]penicillanate-1β-oxide 48, (0.8 .6 mmol), in glacial acetic acid (14.96 ml) and water (1.82 ml) $KMnO_4$ (0.7585 g, 4.7 mmol) was added portionwise over a period of 1 h. The reaction mixture was stirred at room temperature for 3.5 h. A 30% solution of H_2O_2 was added dropwise until decolorization was complete. The resulting solution was poured into ice-water (25 ml), and the solid that separated was collected by filtration and dissolved in CH_2Cl_2. This solution was washed sequentially with aqueous $NaHCO_3$ solution, distilled water and brine; dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated and dried. The product was obtained as a white foam, yield: 0. 62 g (77%), m.p. 111 - 113 °C, ^1H NMR data: Table 18, ^{13}C NMR data: Table 19.

K. *Biological section*

Preparation of Media

The Mueller Hinton Agar was suspended in purified water, mixed throughly and heated to boiling with frequent agitation to completely dissolve the powder. Suitable volume of media was then poured into flasks.

Sterilization

The media was sterilized by autoclaving at 121 °C for 15 min.

Preparation of Antibiotic Solution

A stock solution of antibiotic was prepared by dissolving 50 mg of antibiotic in 5 ml of sterile distilled water, (10,000 µg/ml), and filtered through a 0.22 µ millipore filter.

The working solutions of antibiotic were prepared by the serial dilution method as follows. Two series of 13 sterile test tubes were set upon a rack and labelled 2 -14. 1.5 ml sterile distilled water was aseptically transferred to each of the test tubes. 1.5 ml of stock antibiotic solution was added aseptically to the test tube 2, (5000µg/ml). With a fresh pipette, the contents of the tube 2 were mixed and 1.5 ml was transferred to tube 3; the two fold dilution was completed by repeating the preceding step, using a fresh pipette after each transfer. Thus two series of working antibiotic solutions ranging in concentration from 10,000µg/ml to 1.22 µg/ml were prepared and the experiment carried out in duplicate.

Preparation of Antimicrobial Plates

Sterile Mueller Hinton Agar in a flask was melted, cooled to 45 - 50 °C, and held at that range in a water bath. 14 sterile round petri dishes were labelled 1 to 14. 19 ml of melted agar was transferred to each plate. 1 ml of

stock antibiotic solution was added to plate 1 and mixed. Similarly 1 ml of antibiotic solution in tubes 2 to 14 was transferred to plates 2 to 14 and mixed. The plates were allowed to solidify. Thus plates with antibiotic concentration ranging from 500µg/ml to 0.06µg/ml were prepared.

Preparation of Inoculum

All ATCC organisms were standard and other organisms were clinical isolates obtained from the University of Alberta Hospital, Microbiology Department.

A loopful (0.001 ml) of each organism was inoculated to a tube containing 4 ml of trypticase soya broth and incubated for 24 h at 37 °C.

The overnight broth culture was diluted in sterile distilled water to a turbidity equivalent to a $BaSO_4$ standard prepared by adding 0.5 ml of 0.048 M $BaCl_2$ (11.7 g of $BaCl_2$. $2H_2O$ per liter) to 99.5 ml of 1% (v/v) H_2SO_4 (0.25N).

The final inoculum was made by diluting the above standard suspension to yield a bacterial CFU of 10^6/ml. This was achieved by diluting the Gram -ve cultures to 100 times (0.1 ml + 0.9 ml broth) and Gram +ve cultures to 10 times (0.01ml + 0.99 ml broth)[203].

Inoculation Of Plates

A suitable template was made to identify the type of organism at a particular spot. The loop calibrated to deliver 0.001 ml was sterilized and the test cultures were spot inoculated on to the plates.

Incubation

The plates were left undisturbed for 30 min and then incubated for 18 h at 37 °C.

Reading the Plates

After 18 h, each plate was placed on the template and the concentration at which the organism failed to grow (one colony or hazy growth was neglected) was noted as the MIC^{203}. Results Tables: 20 - 23.

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