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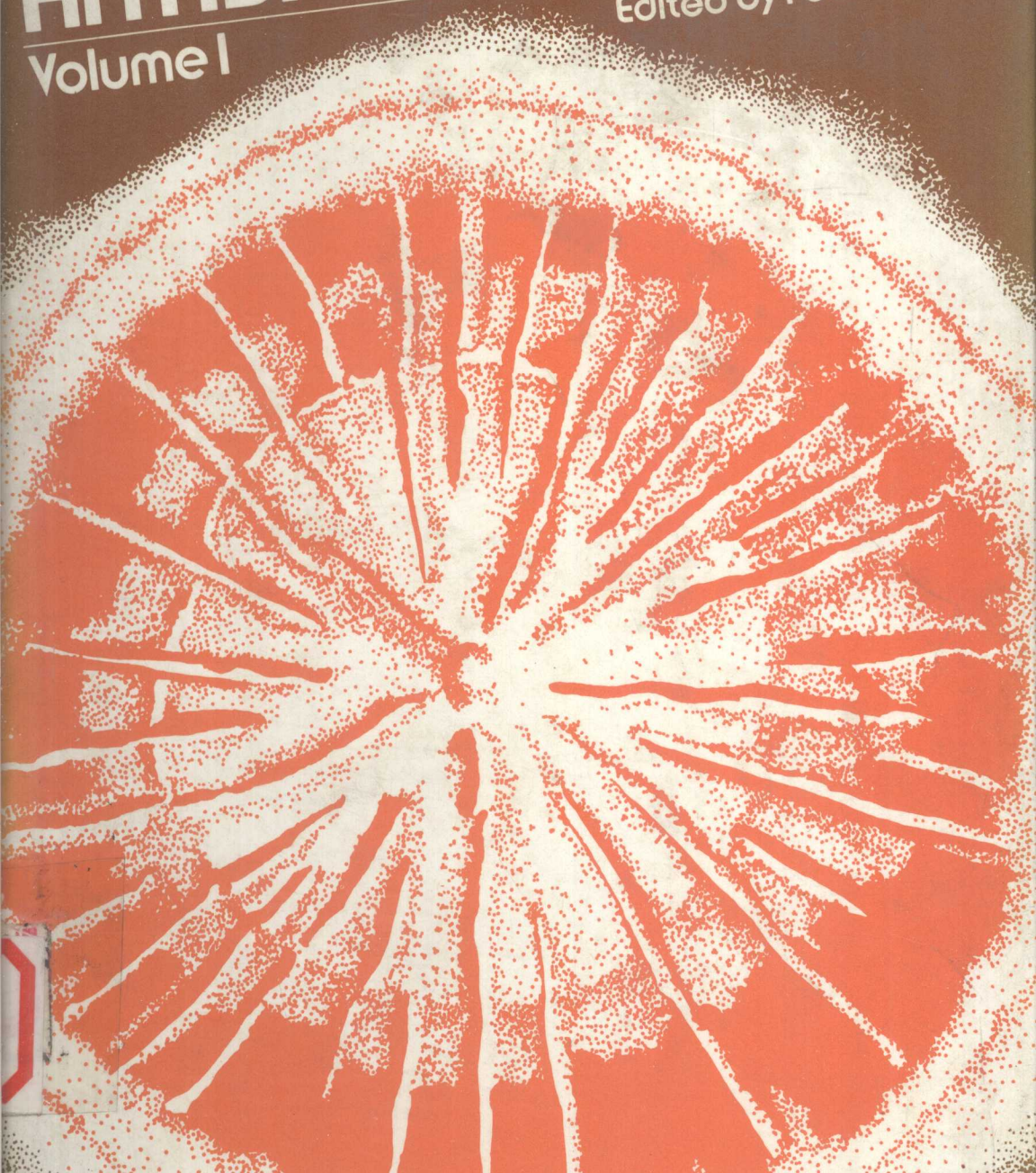
TOPICS IN ANTIBIOTIC CHEMISTRY

Edited by Peter Sammes

Volume I



Topics in Antibiotic Chemistry

Volume 1

AMINOGLYCOSIDES and ANSAMYCINS

Edited by

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EDITOR'S PREFACE

Today the announcement of the discovery of a new antibiotic and the elucidation of its structure causes little stir in the scientific community because modern methods of screening and isolation, accompanied by the new techniques available in chemistry, make such new discoveries commonplace. Complex antibiotics with molecular weights well in excess of 1000 can often be obtained and then characterised in a matter of months rather than years, and techniques such as computerised X-ray crystallographic analysis are routinely applied to define precise stereochemical details. By contrast, our understanding of how such antibiotics work is at its dawning and the mechanisms by which antibiotics operate are hazily emerging. Rapid strides are being made in our knowledge of structure-activity relationships and the precise roles played by antibiotics in their interference with normal biochemical processes.

Relatively few people realise that man's war against diseases caused by pathogenic agents is a continuing battle. Many of the antibiotics of yesterday are no longer effective against these invaders, since they have learnt how to defend themselves against these older weapons using a variety of resistance mechanisms. In order to overcome these resistant species we need to develop new antibiotics and to understand how these resistant species arise. Such studies involve a variety of scientific disciplines amongst which chemistry is playing an increasingly important part.

Topics in Antibiotic Chemistry is aimed at keeping all interested workers abreast of this progress by providing up-to-date articles, written by experts who are actively engaged in such research. Although chemical aspects of the subject will be emphasises, contributors have been briefed not to limit themselves to these and, as a result of this broadening of coverage, it is expected that a much wider range of readers will find herein material of interest. In this foundation volume the authors have tried to deal with the mode of action of the antibiotics, detoxification and resistance mechanisms, and the effect of structural modifications on the activity of these drugs.

Editor's Preface

In Part A workers from Pfizer Limited (U.K.), led by Dr. David Cox, summarise the chemistry of the broad range of aminoglycoside antibiotics. Members of this family are important agents against gram negative pathogens. The article is aimed at both the novice wishing to find an authoritative introduction into the area, and at the specialist who needs a comprehensive and recent survey of this field.

In Part B Professor Mario Brufani, by training an X-ray crystallographer, reviews the ansamycin group of antibiotics, his emphasis being given to the commercially more important group of rifamycin derivatives. His article provides some original suggestions as to the essential features of these compounds which are required for activity against bacteria.

It is planned that this volume should be the forerunner to a sequence of volumes in the field. *Topics in Antibiotic Chemistry* is envisaged to cover not only chemical aspects of the subject but also associated areas of interest, such as modern methods for screening, isolation, and production of this very important group of drugs. The Editor and Publisher will very much welcome critical comments and suggestions from its readers, including suggestions for future articles.

The appearance of this book has depended a great deal on the encouragement and help from others. May I, as Editor, take this chance to record my thanks to all of these, especially my secretary, Mrs. Rose Hills, to Mrs. Valerie Gingell, who typed the manuscript, and Mr. James Gillison, who provided much of the art-work.

Department of Chemistry
The City University
London

1st January, 1977.

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PART A

THE AMINOGLYCOSIDES

by

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PFIZER CENTRAL RESEARCH, PFIZER LIMITED, SANDWICH, KENT

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1. INTRODUCTION

Streptomycin, was the first member of an entirely new group of antibiotics to be discovered called the aminoglycosides. Reported in 1944[1] streptomycin remains today a first-line drug for the treatment of tuberculosis. Clinical use of streptomycin is reserved almost entirely for the treatment of this disease, where it is normally used in combination with isoniazid and *p*-aminosalicylic acid. This landmark was swiftly followed by the discovery of neomycin[2] and, later, by other fermentation-derived aminoglycosides, including the kanamycins[3], gentamicin[4], ribostamycin[5,6] and butirosin[7]. Of these, gentamicin[8] has achieved considerable clinical status as a powerful antibiotic for the treatment of serious gram-negative infections, and has provided an important stimulus for the rapid growth of chemical, biological and clinical research activity seen in this field in recent years.

Gentamicin's pre-eminent position in the treatment of this type of infection arises from its broad spectrum of antimicrobial activity compared with other major classes of antibacterial agents (Table 1.1). No other antibiotic, outside the aminoglycosides, provides such comprehensive cover of the pathogens most commonly found in the hospital environment. The relative incidence of such pathogens in acute care hospitals with more than 100 beds in the U.S.A. is illustrated in Table 1.2, whilst the susceptibility of these organisms to gentamicin compared to a select group of other antibiotics is given in Table 1.3.

The early penicillins, exemplified by penicillin G, were predominantly active against gram-positive organisms such as the Staphylococci and Streptococci (Table 1.1) and their extensive use led to an increased incidence of infections caused by penicillin-resistant Staphylococci and gram-negative organisms such as *Escherichia coli*, particularly in the hospital environment (Table 1.2). In the penicillin field this trend led to the development of semi-synthetic derivatives possessing activity against penicillin-resistant Staphylococci (e.g. methicillin) and derivatives with a broader spectrum of activity such as ampicillin and carbenicillin (Table 1.1). In spite of these developments, however, and of the advent of the tetracyclines and cephalosporins, the gram-negative infections, particularly those caused by opportunist pathogens such as *Pseudomonas aeruginosa*, continued to present problems. It is against this type of infection that the aminoglycoside antibiotics have proved invaluable.

Table 1.1 Antibacterial Spectrum of Gentamicin Compared with Other Major Antibiotics and Antibiotic Classes

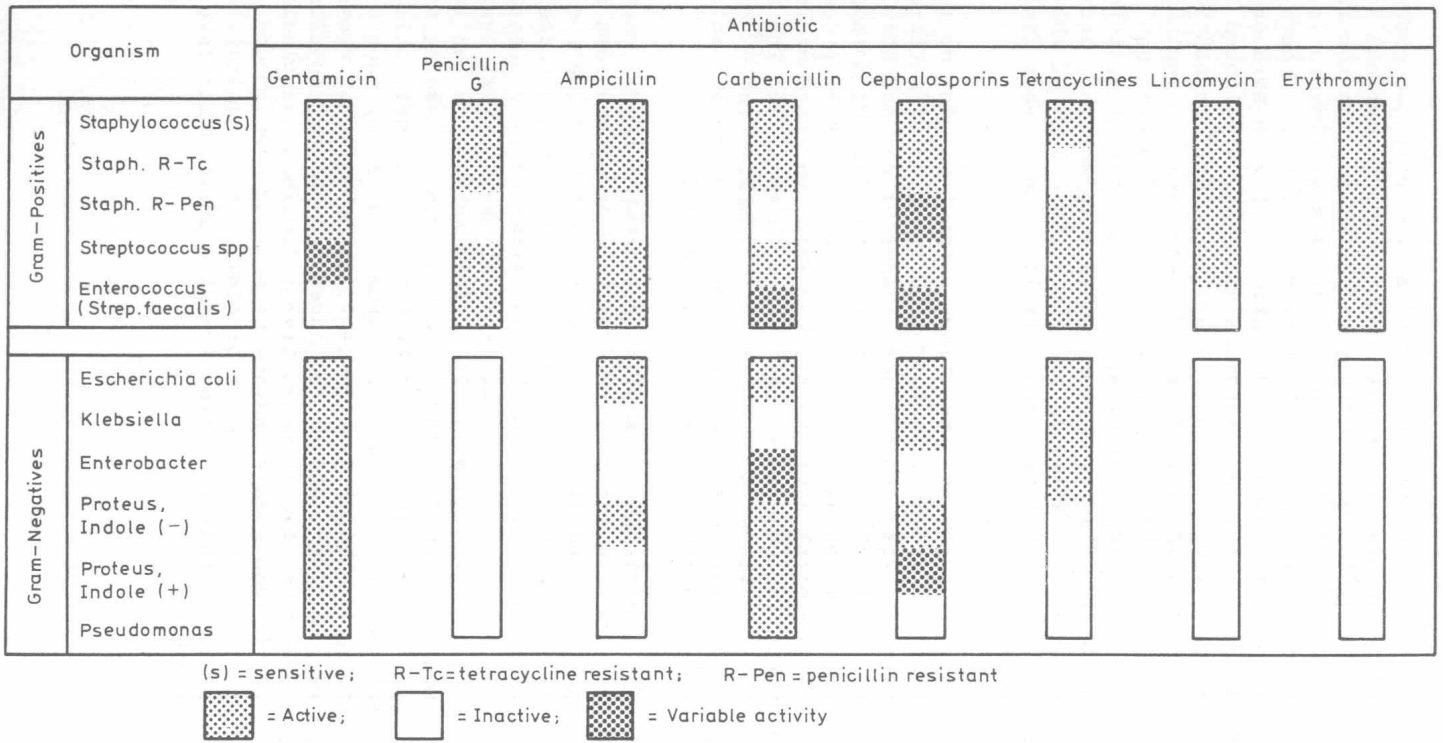

Table 1.2 Acute Care Hospitals Survey

Projected Incidence for All >100 Bed Hospitals in U.S.A.*

	Winter 1974-1975	Rank in Incidence	Percent
E. coli	566,687	1	20.69
Staph. aureus	351,826	2	12.84
Streptococcus (spp)	283,874	3	10.36
Staph. epidermidis	201,697	4	7.36
Klebsiella	196,325	5	7.17
Pseudomonas (spp)	188,881	6	6,90
Enterococcus	170,591	7	6.23
Proteus mirabilis	150,677	8	5.50
Haemophilus (spp)	79,989	9	2,92
Enterobacter (spp)	64,302	10	2.35
Diplococcus pneumonia	56,660	11	2.07
Proteus (indole-positive)	31,012	12	1.13
Serratia	24,278	13	0.89
Citrobacter	23,218	14	0.84
Bacteroides	15,265	15	0.56
All Other Bacteria	333,759		12.19
Total Number of Isolates	2,739,041		100%

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The aminoglycosides act at the ribosomal level and inhibit protein synthesis. Detailed mode of action studies have been reviewed by Tanaka[9]. They are bactericidal in action and their spectrum of antibacterial activity includes the Staphylococci, a wide range of gram-negative bacteria, and the tubercle bacillus. The only notable spectrum deficiency lies in their poor activity against Streptococci, for which the antibiotic of choice is penicillin.

The antibacterial spectrum of the earlier aminoglycoside antibiotics, such as kanamycin, was limited by resistance. In many species of bacteria this resistance is determined by the presence of R factors which direct the synthesis of a new

Table 1.3 Percent Susceptibility of 15 Key Organisms to Selected Antibiotics*

Organisms According to Incidence	Gentamicin	Penicillin G	Ampicillin	Carbenicillin	Cephalothin	Erythromycin	Tetracycline	Clindamycin	Chlor-amphenicol
E. coli	99	X	78	81	82	X	74	25	96
Staph. aureus	99	X	20	57	98	93	87	95	98
Streptococcus	59	93	92	92	96	93	57	90	95
Staph. epidermidis	98	36	41	77	97	75	54	82	93
Klebsiella	99	X	X	X	88	X	85	20	93
Pseudomonas	94	X	X	74	X	X	X	20	X
Enterococcus	58	38	94	63	33	65	23	X	90
Prot. mirabilis	99	21	92	96	92	X	X	24	91
Haemophilus influenza	94	64	89	93	89	79	95	22	99
Enterobacter	96	X	X	79	X	X	88	25	94
Diplococcus	55	96	97	95	98	98	86	95	99
Proteus (indole +ve)	81-97	X	19-34	67-93	17-32	X	9-60	24-33	46-83
Serratia	96	X	X	67	X	X	31	28	83
Citrobacter	98	X	29	51	49	X	79	29	93
Bacteroides	21	28	45	79	39	79	59	91	97

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X = Less than 20% of strains susceptible.

family of enzymes[10]. These enzymes inactivate aminoglycosides by three separate mechanisms: acetylation, adenylylation and phosphorylation. A thorough understanding of the mechanisms of resistance allows a prediction to be made concerning the activity spectrum of structurally related aminoglycosides. This may be illustrated by reference to *Pseudomonas aeruginosa*, many strains of which carry enzymes capable of inactivating kanamycin by phosphorylation of the 3'-hydroxyl group. In contrast gentamicin lacks this functional group and is thus highly active against this clinically important organism. Resistance to aminoglycosides is reviewed in detail in chapter 4.

The aminoglycosides are limited to parenteral routes of administration for the treatment of systemic infections. They are not absorbed when given by mouth, but are excreted unchanged in the faeces. This lack of absorption is utilised in certain clinical situations: for example, neomycin or kanamycin may be given orally to achieve sterilisation of the gut prior to bowel surgery in order to minimise the risk of post-operative wound infection. Aminoglycosides have also been applied topically, for instance to prevent infection in cases of severe burn.

Intramuscular injection is the normal mode of administration in cases of systemic infection. Good absorption occurs by this method, to give therapeutically effective blood levels and satisfactory distribution to most tissues. Excretion is *via* the kidney and high levels of unchanged drug are found in the urine. Metabolism does not occur to any significant extent in this class of antibiotics.

The clinical use of aminoglycosides has been reviewed by Guisti[11]. Gentamicin and the more recently introduced aminoglycosides such as tobramycin, dibekacin, and amikacin are of particular value in the treatment of gram-negative septicaemia, as well as in lung and urinary tract infections caused by *Pseudomonas aeruginosa* and other gram-negative pathogens. There are several reports (e.g. ref.[12]) describing a synergistic action between gentamicin and carbenicillin, the Pseudomonas-active pencillin.

To varying degrees, all of the aminoglycosides are potentially toxic (e.g. streptomycin[13]) and controlled administration is of the utmost importance and necessity, particularly in patients with impaired kidney function. Ototoxicity is the most serious toxic manifestation and occurs in the inner ear, where both cochlear (auditory) and vestibular (balance) functions can be affected. Ototoxic damage is irreversible and cumulative, in that subsequent aminoglycoside treatment may lead to progressive functional impairment. Vestibular disturbance may be partially

offset by normal compensatory mechanisms[11] and the symptoms usually disappear on termination of treatment.

Individual aminoglycosides vary in the type of ototoxicity they produce. Gentamicin, for example, is more widely reported as causing vestibular damage than loss of hearing, whilst the reverse is true of kanamycin. In a recent clinical study[14], Noone and co-workers detected no symptomatic ototoxicity in patients undergoing treatment of serious gram-negative sepsis with gentamicin. Audiometry revealed no impairment of auditory function in the patients tested but a detailed investigation of the eigth cranial nerve function revealed an 18% incidence of vestibular function abnormalities, probably related to gentamicin therapy.

The aminoglycoside antibiotics also have the potential to cause nephrotoxicity[15]. This is generally thought to be a less serious problem in that the nephrotoxicity produced is reversible and can be readily detected at an early stage by appropriate examination of urinary enzymes and other urinary parameters. However, a knowledge of the patient's kidney function is most important, since pre-existing renal impairment may lead to decreased urinary excretion of the antibiotic with a concomitant rise in the antibiotic blood level to toxic levels with an increased potential for ototoxicity. It is for such reasons that blood level monitoring of aminoglycosides can be of crucial importance, particularly in patients with underlying kidney disease. Rapid and reliable measurement of blood levels of aminoglycosides can be made by conventional bioassay or by radio-labelling techniques which employ specific aminoglycoside inactivating enzymes using radio-labelled acetyl coenzyme A or ATP as appropriate[16].

Despite the potential toxicity hazards associated with aminoglycoside therapy, gentamicin has been in clinical use now for more than ten years and, under controlled hospital conditions, can be used with a high degree of confidence[8]. The average therapeutically effective blood levels are approximately 5μg/ml for gentamicin. Provided that the dosage is such that levels in the blood do not regularly exceed approximately 12μg/ml the incidence of toxicity is minimised. For patients with renal impairment, nomograms have been devised[17] which relate recommended dosage to the patient's kidney function.

The aminoglycosides as a class also possess neuromuscular blocking activity[18,19,20] although this seldom presents problems at therapeutic dose levels. Complications may arise, however, if aminoglycosides are used in conjunction with anaesthetics, since this combination may result in respiratory depression[21].

In considering the current status and future trends in aminoglycoside therapy, it seems likely that streptomycin will continue to be important in the therapy of tuberculosis and that the hospital use of gentamicin will continue to grow as understanding of the drug increases and assay procedures become more widely available and utilised. Of the newer agents, tobramycin is more potent than gentamicin against *Pseudomonas aeruginosa*, but is otherwise very similar in its overall biological profile, as also are dibekacin and sisomicin. Amikacin, which is a new semi-synthetic derivative of kanamycin A, differs in showing activity against a high proportion of gentamicin-resistant organisms and is likely to assume increasing importance: the incidence of gentamicin resistance, although low at present, appears to be growing, as testified by reports (e.g. ref.[22]) from individual hospital centres. Although amikacin is intrinsically less toxic than gentamicin it is unlikely to possess a significant safety advantage, because it is also less potent, necessitating higher dose levels.

In summarising this introductory section it can be said that the aminoglycoside antibiotics, particularly as exemplifed by gentamicin, have established an important position in the antibiotic armamentarium of the clinician. Knowledge of the enzymatic modes of aminoglycoside inactivation have encouraged the chemist to develop semi-synthetic aminoglycosides which are less susceptible to inactivation. The first examples of new antibiotics specifically designed in this way are dibekacin (3',4'-dideoxykanamycin B) and amikacin.

Although the aminoglycoside antibiotics, particularly the new ones, are impressive in terms of antibacterial potency and spectrum, there has been a distinct lack of progress with respect to reduced toxicity. Since it is undoubtedly this factor alone which prevents the more widespread use of these agents beyond the controlled hospital environment, it is apparent that an improved potency/toxicity ratio represents a research goal of fundamental importance. It is to be hoped that advances will be made in this direction during the coming decade as the development of semi-synthetic aminoglycosides gathers momentum and as our understanding of the effect of structural modification on antibacterial activity and toxicity increases.

This topic concentrates on structural relationships amongst this rapidly increasing group of antibiotics, on the type of known resistance mechanisms operating against the aminoglycosides, and on structure-activity correlations. These aspects are followed by a survey of the more important chemical transformations on these systems and on recent synthetic studies leading to these antibiotics.

2. STRUCTURAL INTER-RELATIONSHIPS

Prototypes of each of the major structural classes of aminoglycoside antibiotics have been derived naturally from the fermentation liquors of various moulds or, less frequently, bacteria. Micro-organisms from the genus *Streptomyces* have been the most productive and aminoglycosides from this source have included streptomycin, neomycin, kanamycin and spectinomycin. The gentamicins* and sisomicin* were obtained from organisms belonging to the genus *Micromonospora*, whilst butirosin is rather unusual in being derived from a bacterial source, namely a strain of *Bacillus circulans*. This section of the topic deals with the structural interrelationships between the major classes of naturally derived aminoglycosides and includes the most important semi-synthetic derivatives.

2.1 Streptomycin

The ineffectiveness of penicillin against gram-negative bacteria prompted Waksman and his co-workers to search for new antibiotics with a broader spectrum of activity. From 1939 many thousands of micro-organisms were screened, particularly from soil samples, and in 1943 a strain of *Streptomyces griseus* was isolated which was subsequently shown to produce streptomycin[1], the first aminoglycoside antibiotic.

The structures of streptomycin and related aminoglycosides are depicted in Figure 2.1. and, except for dihydro-and dihydrodeoxy-streptomycin, are fermentation derived compounds. It is of interest to note that N-demethylstreptomycin was produced[30] from *S. griseus* grown in the presence of DL-ethionine, an inhibitor of L-methionine-mediated methylation.

The streptomycin group of antibiotics is characterised by the presence of an L-glucosamine moiety and streptose[23] or its oxidised or reduced form, which is a unique branched-chain sugar first synthesised in 1965 by Dyer and co-workers[24]. The aglycone is the aminocyclitol streptidine[25], or a modified form thereof as in bluensomycin[26]. The glycosidic linkages have been shown to be of the α-L configuration by n.m.r. studies[27], and the absolute configuration has been elucidated by application of Reeve's copper complexing method to various degradation products of streptomycin and dihydrostreptomycin[28]. An X-ray crystallographic analysis of streptomycin oxime selenate has confirmed the assigned structure and nature of the glycoside linkages[29].

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*It has been recommended that the suffix "mycin" should be reserved for antibiotics derived from the genus *Streptomyces*.