DERIVATIVES OF DIHYDROSTREPTOMYCIN

T H E S I S

submitted to

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by

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SUMMARY

The work already carried out on the chemical modificat: of naturally occurring antibiotics is reviewed. The reasons for carrying out these modifications are discussed. Antibiotics which have been modified chemically in some way include the penicillins, the cephalosporins, the tetracyclines, griseofulvin and the aminoglycoside antibiotics, including streptomycin itself. The value of these modifications varies from compound to compound, the most successful derivatives which have been prepared, to date, being the derivatives of penicillin. Compounds have been prepared which are mo active, which are active orally and which are active against penicilli producing micro-organisms. Derivatives of other antibiotics have, generally, been less successful, although valuable information about structure-action relationships has been obtained. The derivatives of dihydrostreptomycin which are considered in this thesis are glycosides of dihydrostreptobiosamine, a disaccharide composed of dihydrostreptose linked glycosidically with N-methyl - L glucosamine.

The methods available for glycoside synthesis, the Koenigs-Knorr, the Helferich and the Fischer syntheses, are reviewed. A modification of the Fischer synthesis was used for the preparation o the benzyl glycoside of dihydrostreptobiosamine, using methyl dihydrostreptobiosaminide as starting material. No clear mechanism for this reaction has so far been postulated and therefore a series of reactions was carried out in order to elucidate the mechanism.

α - and β- methyl dihydrostreptobiosaminides were prepared and separated in the form of their acetates which were then used to prepare benzyl penta-acetyldihydrostreptobiosaminide by transglycosidation. The fact that the α- anomer was formed in both cases indicates that, every time, it is the more stable isomer which is formed. Direct benzyl alcoholysis of dihydrostreptomycin also gave α- benzyl penta-acetyldihydrostreptobiosaminide, confirming t theory.

Methanolysis of α- benzyl-acetyldihydrostrepto-biosaminide gave only the α- anomer of methyl penta-acetyldihydro-streptobiosaminide.

There is some indication that the furanose ring of dihydrostreptose facilitates the reaction, in analogy with the Fischer glycoside synthesis.

A C K N O W L E D G M E N T S

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C O N T E N T S

INTRODUCTION

The Chemical Modification of Naturally Occurring Antibiotics

The current era of antibiotic therapy began in 1929 with the now celebrated observation of Fleming and his coining the term penicillin to designate the then unidentified antibacterial metabolic product of the mould Penicillium notatum.[1] Since that time an ever-increasing number of antibiotics have been discovered and used to considerable effect in the treatment of bacterial and fungal infections. Running parallel with the discovery of new naturally occurring antibiotics has been the chemical modification of antibiotics already known and used clinically, whose structure has already been elucidated. The aim of these modifications is two-fold, one, the investigation of structure-action relationships and secondly the more immediate search for improved antibiotics, either more active, less toxic or having a different antibacterial spectrum. These studies have, by necessity, been carried out only on compounds whose structures are already known, hence comparatively few of the hundreds of antibiotics available have been so investigated. These, however, form the bulk of antibacterial compounds used routinely in clinical practice to-day.

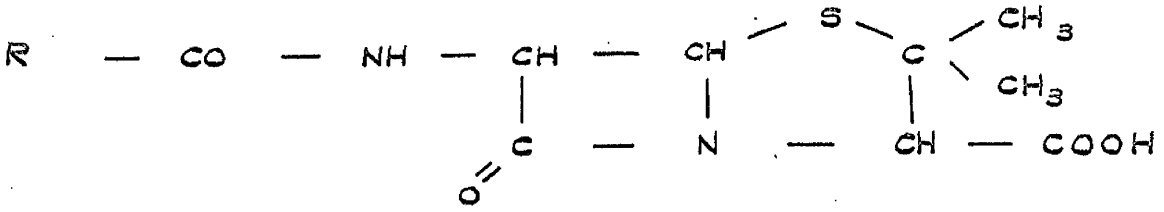
As will be seen in succeeding pages, in the large majority of cases, chemical modification has had an adverse, or at least no beneficial, effect on the antimicrobial effect of the drugs. In some selected instances, however, notably the penicillins and cephalosporins, modification of the antibiotic by chemical means has given compounds which have considerably increased the value of the compound clinically.

The successes achieved with these drugs, and the need for new antimicrobial agents, should encourage further research on the chemical modification of existing antibiotics.

The Penicillins

The first penicillins, discovered by Sir Alexander Fleming in 1929,[1] were originally produced from the mould Penicillium notatum by surface culture. Further investigations [2] showed that the process of submerged culture, using P. chrysogenum gave much higher yields at considerably less cost, and by 1946, most of the difficulties for producing penicillin on a commercial scale had been solved.

Examination of the penicillin produced showed that the antibiotic was composed of several related compounds, designated G, X, F and K,[3] all having the same general structural formula (I).



WHERE R = $C_6H_5CH_2-$ G

$HO-C_6H_4-CH_2-$ X

$CH_3CH_2CH=CHCH_2-$ F

$CH_3(CH_2)_5CH_2-$ K

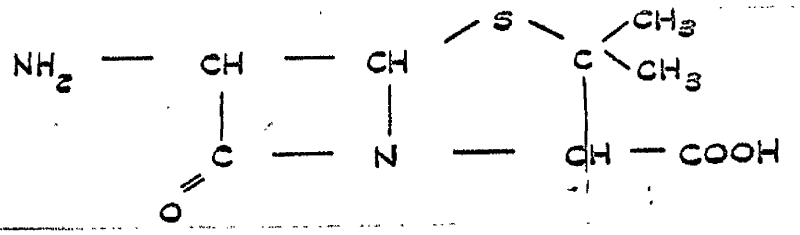
(I)

A little later it was shown that the yield of any particular penicillin could be increased by the addition of a particular chemical to the culture medium. These side-chain precursors, for example phenylacetic acid, resulted in a virtually 100% yield of the penicillin desired, in this case penicillin G, benzylpenicillin.

The first modifications to the penicillin molecule were effected by the addition of various different side-chain precursors to the culture medium. However, the range of penicillins which could be produced in this way was strictly limited because only certain chemical structures generally monobasic carboxylic acids with non-polar groupings

(i.e. R. CH_2 COOH), are acceptable to the mould for incorporation as penicillin side-chains.

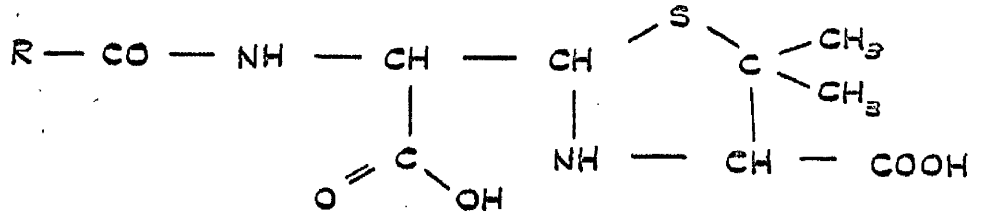
Further modifications of the penicillin molecule were made possible by the isolation of the penicillin nucleus, 6 - amino penicillanic acid (6-APA) (II).



(II)

6-APA can be isolated in several ways. In 1950 it was announced that the penicillin nucleus could be obtained by the action of an amidase on benzylpenicillin.[4] The amidase involved was formed by a strain of P. chrysogenum, and catalysed the hydrolysis of the phenylacetyl side-chain. Since then, at least two types of amidases have been shown [5,6] to exist in a variety of micro-organisms. One, occurring in certain fungi and actinomycetes hydrolyses phenoxymethylpenicillin, n- amylpenicillin and n- heptylpenicillin more rapidly than it does benzylpenicillin; another, occurring in bacteria of the genera Escherichia and Alcaligenes hydrolyses benzylpenicillin more rapidly than phenoxymethylpenicillin.

Tackling the problem from a different angle, Sheehan reported [7] in 1959 that 6-APA had been obtained by total synthesis, the culmination of an extensive series of investigations. Penicilloic acid (III) had been



(III)

synthesised during war-time work on penicillin, but attempts to reconstruct the β - lactam ring had been frustrated by the ease with

which the alternative, oxazolone, ring had formed. The solution of the problem was found in the use of side-chains, for example, triphenylmethyl, from which oxazolone formation could not occur, together with a new ring closing agent dicyclohexylcarbodiimide.

However, since the total synthesis of 6-APA involved a number of stages of fairly low yield, it could not be used in the commercial production of large quantities of the compound.

Also in 1960, Doyle and his co-workers [8] isolated 6-APA from penicillin fermentations which were carried out in the complete absence of any side-chain precursors. It had been observed for some time that there were discrepancies between the results of microbiological and chemical assays, and it had been thought that this discrepancy could be explained by the presence in the broth of a penicillin-like material without anti-bacterial properties. This was shown to be 6-APA by removing the natural penicillins from the fermentation liquor by solvent extraction at low pH and treating the remaining solution with an excess of phenylacetyl chloride in the presence of a weak base, such as sodium bicarbonate. This resulted in the formation of an antibiotic substance which was readily destroyed by penicillinase and which behaved similarly to benzylpenicillin when chromatographed. Chemical analysis also indicated the structure of 6-APA.

6-APA was found to have antibacterial properties of a much lower order than benzylpenicillin and the spectrum of activity is of a different type. It is destroyed by penicillinase but at a much slower rate than benzylpenicillin.

The value of 6-APA lies in the fact that the 6- amino group is unsubstituted and hence amenable to side-chain addition. An almost unlimited variation in side-chains is therefore possible, leaving the way open for the production of penicillins suitable for a variety of purposes.

Although natural penicillins continue to be widely used and are highly effective against many infections, they nonetheless have a number of drawbacks. These can be summarised thus: [9]

(1) Repeated injections of penicillin are necessary to control acute infection.

(2) Large doses are necessary.

(3) Oral dosage forms are preferable to injections which can be painful.

(4) The alarming increase of resistance of staphylococcal infection to the action of natural penicillins.

(5) The increasing incidence of allergic reaction to intremuscular penicillin.

Possibly the most pressing of these failings is the widespread appearance of resistant staphylococci. Resistance in organisms has been produced experimentally but clinically only staphylococcal infections present any problem, and this has been increased by the fact that such staphylococci show a tendency to become resistant to other antibiotics also. The resistance of these organisms has been found to be due to the production by the organisms of penicillinase,[10] an enzyne which destroys penicillin by catalysing the hydrolytic opening of the β-lactam ring of penicillin with the formation of inactive penicilloic acid. The syntheses of penicillinase has been shown, in some bacteria, to be inducible, occurring at a greatly increased rate when the cells come into contact with penicillin.

In order, therefore, to prepare penicillins which were active orally, resistant to penicillinase and which showed no cross-resistance with the natural penicillins, the following methods were adopted in the production of new penicillins from 6-APA.

(1) reaction with an acid chloride in semi-aqueous or anhydrous media;

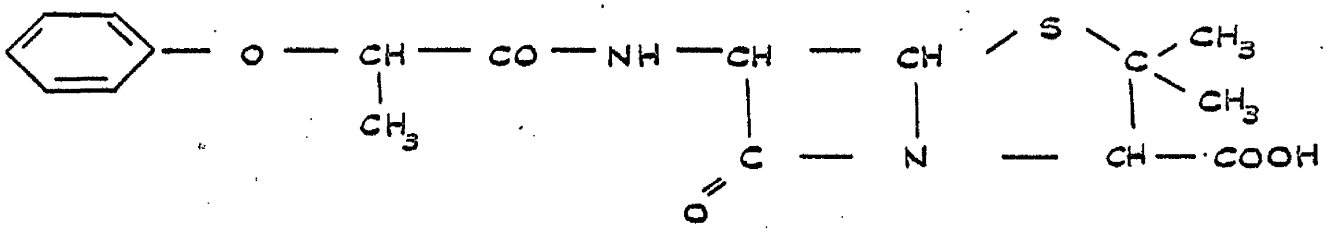
(2) reaction with an acid anhydride or mixed anhydride in semi-aqueous media;

(3) reaction with an acid in the presence of a coupling agent, such as N,N' - dicyclohexylcarbodiimide;

(4) reaction with an activated derivative of an acid in the presence of an amidase enzyme.

Of the numerous penicillins synthesised, the first to be introduced

clinically was 6-(α- phenoxypropianamido -) penicillanic acid or phenoxyethylpenicillin [11] (IV).

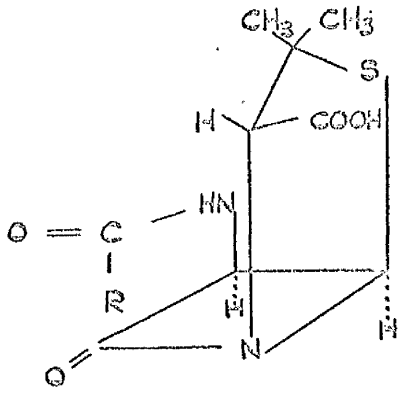


(IV)

The main advantage which phenethicillin has over benzylpenicillin is that it has a high resistance to decomposition by acids and is therefore suitable for oral administration. This is also the case with phenoxymethylpenicillin originally produced directly by fermentation although its stability to acid was not realised until later.

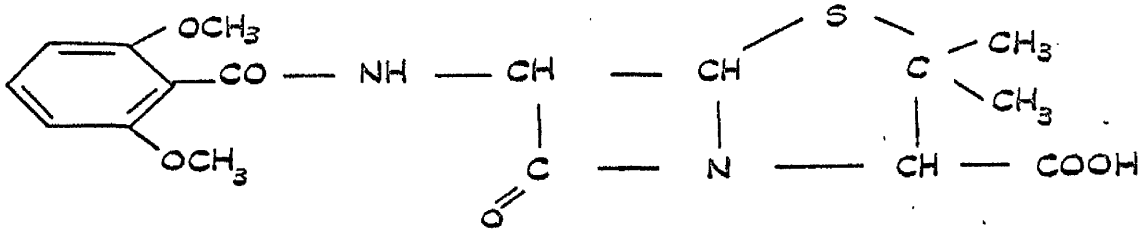
Attention was then turned towards the production of a penicillin which would resist inactivation by staphylococcal penicillinase. One class of penicillins possessing this valuable property was found[12] by acylating 6-APA with aromatic or heteroaromatic carboxylic acid chlorides substituted in both ortho positions when the ring structure of the side-chain was 6-membered, almost any pair of ortho substituents conferred marked stability towards penicillinase, but when it was 5-membered, one or both of the substituents had to be relatively bulky, for example, phenyl or substituted phenyl. Such findings illustrate the primary importance of steric effects in determining stability towards penicillinase. In this respect, the chemical constitution of the side-chain is of much less significance, although it has a profound influence on antibacterial activity and on stability to acids.

Examination of three dimensional structure of penicillin (V) shows[13] that the side-chain can indeed assume a position close to the β - lactam ring so that a bulky side-chain will sterically hinder the lactam carbonyl group.



(V)

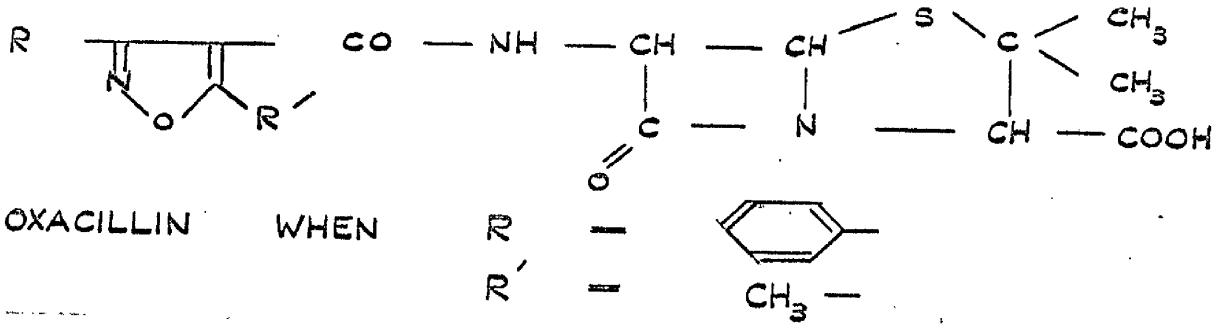
The first compound to be synthesised as a result of this work [12] was 2,6 - dimethoxyphenylpenicillin (VI), (Methicillin).



(VI)

It is very effective in staphylococcal infections but it is unstable to acid and poorly absorbed when given orally and hence must be administered by intramuscular injection. [14] Since the bactericidal activity of methicillin is slower and less complete than that of benzylpenicillin against strains sensitive to both drugs, in this respect methicillin has no advantage over benzylpenicillin. The great value of methicillin lies in its ability to resist penicillinase, even with large innocula.

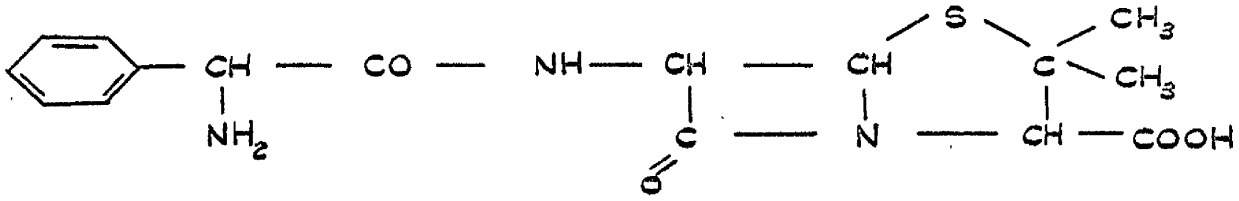
Later, [12] a series of 3,5 - disubstituted 4 - iso - oxazolyl penicillins (VII) were prepared, many of which, especially those with one of the ortho substituents a phenyl or substituted phenyl and the other methyl or other alkyl, exhibit high antibacterial activity both in vivo and in vitro.



(VII)

Since they are also stable at low pH values, they are therefore suitable for oral administration, and are well absorbed.

One disadvantage shared by all penicillins described so far is their lack of activity against gram-negative organisms. This disadvantage has been overcome by the introduction of ∝ - amino-phenylpenicillin (VIII) (Ampicillin).



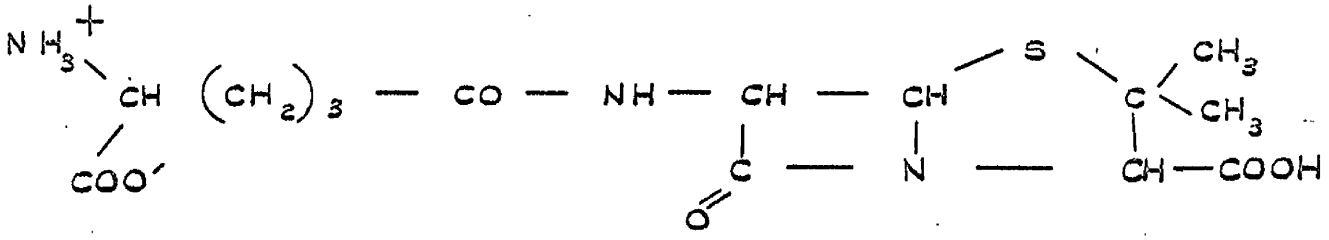
(VIII)

Ampicillin possesses bactericidal activity against a wide range of organisms.[15] In its intensity of bactericidal action it resembles benzylpenicillin, but its range, in terms of therapeutically obtainable concentrations, is much wider. The range is comparable, in fact, to that of the 'broad spectrum' antibiotics such as the tetracyclines and chloramphenicol, which are essentially bacteriostatic and have a lower intrinsic activity. However, many gram-negative organisms are

penicillinase producers and since ampicillin, unlike methicillin, is destroyed by penicillinase, there will be some gram-negative organisms which are not susceptible.

The Cephalosporins

The cephalosporins were discovered initially by Brotzu in 1948, isolated from a fungus, Cephalosporium, found in the sea near a sewage outfall off Sardinia.[17] Brought to the notice of British workers, the compound cephalosporin - N was isolated, and its structure elucidated. Cephalosporin - N was shown[18] to be a penicillin derivative, (D - 4 - amino - 4 carboxybutyl) penicillin (IX).

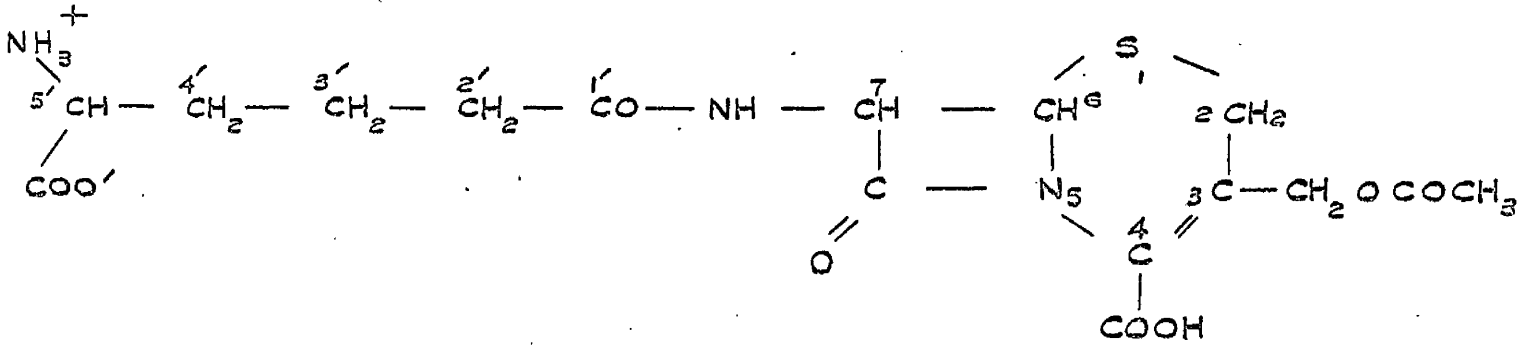


(IX)

The polar groups in the side-chain of cephalosporin - N give it extremely hydrophilic properties with the result that it has a range of antibacterial properties quite different from the conventional penicillins. It has less than 1% of the activity of benzylpenicillin against Staphylococcus aureus but is considerably more active against Salmonella typhi and other gram-negative organisms. Acylation of the amino group, rendering the side-chain non-polar, increases the activity against Staphylococcus while reducing activity against Salmonella. Hence radical changes in biological properties can be brought about by certain changes in the side-chain of the molecule, as has already been demonstrated with other derivatives of penicillanic acid.

Cephalosporin - N was found to be rapidly destroyed by penicillinase but it was noticed[19] that partially purified samples of the antibiotic were contaminated by a compound which possessed some activity but which was unaffected by penicillinase. This compound, designated cephalosporin-C,

was shown [20,21] to contain a fused β-lactam-dihydrothiazine ring system (X) in place of the fused β-lactam - thiazolidine ring system of the penicillins.

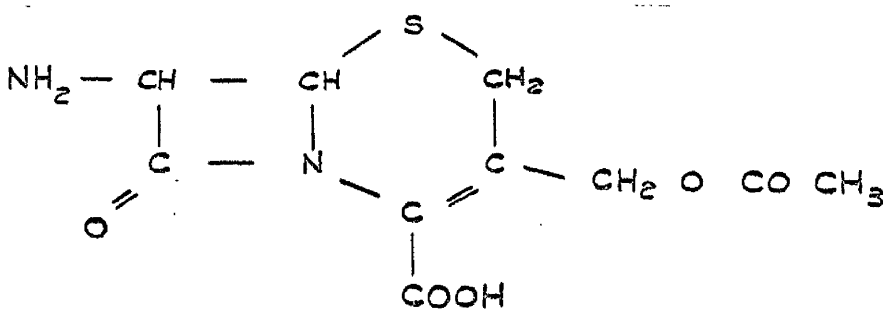


(X)

The sodium salt of cephalosporin - C was found to be even less toxic to mice than sodium benzylpenicillin. Its mode of action is similar to that of the penicillins, bringing about the lysis of staphylococci and appearing to inhibit the synthesis of the staphylococcal cell walls.[22] It is a powerful inducer of penicillinase by Staph.aureus and B.cereus but its rate of hydrolysis by penicillinase is about 5,000 times smaller than that of benzylpenicillin.

Cephalosporin - C is therefore as active in vitro against penicillinase-producing strains of Staph.aureus as against strains that do not produce penicillinase. However, it has a low activity, about 0.1% of that of benzylpenicillin and it seemed worthwhile to explore the possibility of obtaining a derivative of cephalosporin - C that retained the resistance of the parent compound to penicillinase, but showed a much higher activity against staphylococci. Since benzylpenicillin was more than 100 times as active as cephalosporin - N against penicillin sensitive strains of Staph.aureus, it was thought probable [19] that a very large increase would result from an exchange of the α - aminoadipyl side-chain in cephalosporin - C for a side-chain derivative from phenylacetic acid. The side-chain was successfully removed [23] from cephalosporin-C

by mild acid hydrolysis without affecting the rest of the molecule, and although the process gave a low yield, it enabled the nucleus of cephalosporin - C, 7- aminocephalosporanic acid (7-ACA) (XI) to be isolated in a pure form.



(XI)

Acetylation of 7-ACA with phenylacetyl chloride yielded a derivative several hundred times as active as cephalosporin - C and about one fifth as active as benzylpenicillin. However, unlike benzylpenicillin, the derivative was highly active in vitro against strains of staphylococcus that produced penicillinase.

The yield of 7-ACA from acid- or base- catalysed hydrolysis had been limited by the lability of the molecule under these conditions. A mild selective cleavage of α - aminoadipylamide was thought [22] to result from a reaction in which the intramolecular interaction of the amide with the C- 5' centre in cephalosporin - C could occur. De-amination of cephalosporin - C and cleavage of the amide occurs under mild conditions, since treatment with excess nitrous acid in aqueous acetic acid solution gives 2 moles nitrogen per mole cephalosporin - C. The second mole of nitrogen presumably arises from reaction of the reagent with 7-ACA. With anhydrous acetic acid and nitrosyl chloride 7-ACA was isolated in 7% yield, the yield increasing to 40% when carried out in formic acid, which is a better solvent for cephalosporin - C and has a greater volatility. By this

method, practical quantities of 7-ACA, for the preparation of acylamido - cephalosporanic acids, can be produced.

A large range of N - acyl derivatives of 7-ACA have since been prepared [24] by reaction of 7-ACA with the appropriate acid, acid chloride or mixed anhydride and isolated as the sodium or potassium salt or as the free acid.

Comparison of the benzylpenicillin bio-assay of the cephalosporins and their penicillin congeners [25] revealed some interesting correlations. Such comparisons are valid since the concentration/activity curves of the cephalosporins and benzylpenicillin are parallel.

The activities of the cephalosporins roughly paralleled those of the corresponding penicillin, except that the cephalosporins were uniformly less active. The side-chain structural requirements for high activity against a test organism for both cephalosporins and penicillin are similar. Certain exceptions to this generalisation do occur but study of a larger number of cephalosporins indicates that the trend is quite consistent. The most active group of compounds were arylmercaptoacetyl and alkylmercaptoacetyl derivatives, and the next potent assemblage was composed largely of aryloxyacetyl, arylacetyl and heterocyclic acetyl amides. Uniformly poor antibiotics resulted when 7-ACA was acylated with aroyl and carbonic acids.

Modification of the more active compounds by branching at the α - carbon consistently lowered activity and double branching reduced activity still further. Similar effects have been observed in the penicillin series.

Mixed effects resulted from ring substitution in the aromatic side-chains. No enhancement of activity resulted from aromatic substitution in the arylmercaptoacetyl cephalosporins; however, considerable increases in the potencies of the aryloxyacetyl and arylacetyl cephalosporins were the consequences of appropriate substitution. An m - chloro substituent doubled the activity of both the phenoxyacetyl - and phenylacetyl - cephalosporins, although it had no effect on the phenylmercaptoacetyl - cephalosporins. The preceding may be contrasted with the effect of an

o - methoxy group which reduced the activity of all three groups of compounds.

Enzymatic cleavage of the O - acetyl function gave antibiotics which were, at best, only half as active as their precursors. Cyclisation of the deacetyl - cephalosporin to its lactone increased the potency to the level of the acetyl compound.

It is thought that the low activity of cephalosporin - C derivatives *in vivo* is due to the fact that in man the acetyl group is metabolised off.

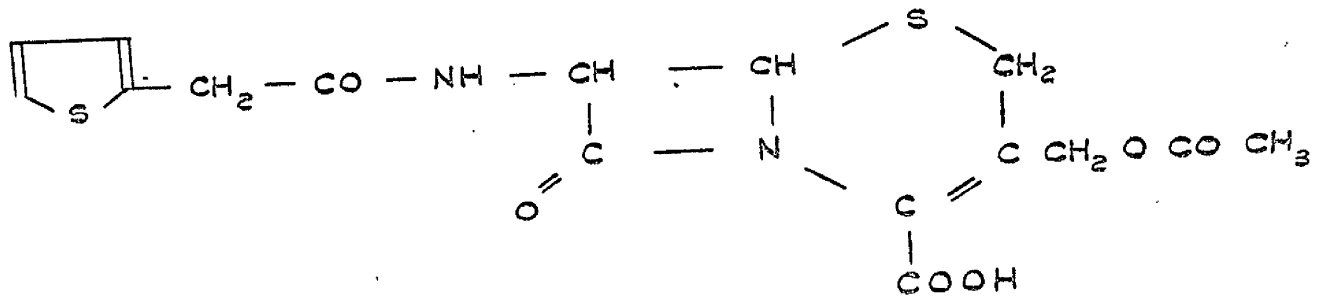
It was found that in neutral aqueous solution, cephalosporin - C reacted with pyridine to form a new compound with antibacterial activity. The acetyl group of cephalosporin - C is in an allylic position and is hence susceptible to nucleophilic substitution with bases such as pyridine and it is thought that the formation of the new compound involved displacement of the acetyl group in cephalosporin - C by a tertiary base, followed by conversion to the quaternary derivative.

The higher activity of this group of compounds is attributed to the fact that the basic group is not metabolised in the same way as the acetyl group.

Although their activities toward penicillin - sensitive staphylococci varied considerably, the cephalosporins were consistently effective against penicillin-resistant strains of these micro-organisms, except for those cephalosporins with very low activities.

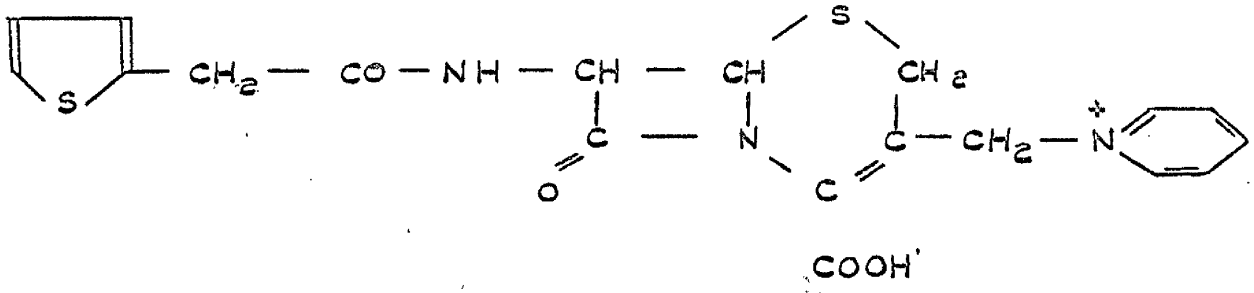
Susceptibility of gram-negative organisms to cephalosporins was limited to a relatively small number of compounds. The thiophene - 2 - acetyl side-chain was the most outstanding of an initial series of cephalosporins. Other acid radicals related to the thiophene - 2 - acetyl showed gram-negative activity. A number of halo-substituted low molecular weight aliphatic acids likewise imparted gram-negative activity to their cephalosporin conjugates. The deacetyl derivative of 7 - (thiophene - 2 - acetamido -) cephalosporanic acid possessed less gram-negative activity than did the parent compound. Apparently, presence of the acetyl function is necessary for significant potency against these bacteria.

Extended clinical trials have been carried out on 7 - (thiophene - 2 - acetamido) - cephalosporanic acid, the sodium salt of which has been given the generic name cephalothin. (XII). It was found to inhibit the growth of Proteus mirabilis, Escheriohia coli and Klebsiella but to be inactive against Pseudomonas. No toxic side effects were noted.



(XII)

A cephalosporin derivative which has recently [26] been made available commercially as Ceporin, is cephalodrine, 7 - ((2 - thienyl) - acetamido) - 3 - (1 - pyridylmethyl) - 3 - cephem - 4 - carboxylic acid betaine. (XIII).



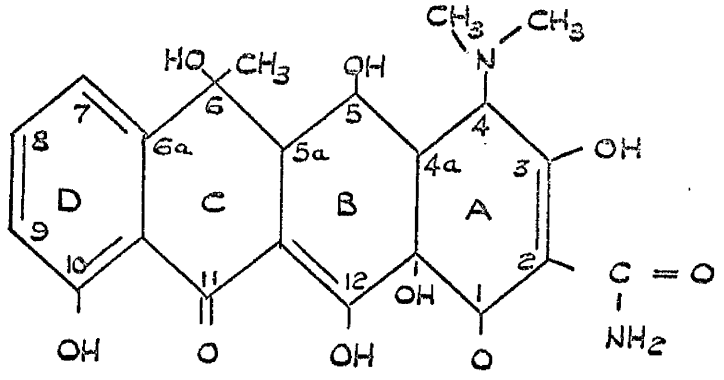
(XIII)

It is a broad spectrum antibiotic of low toxicity, active against Staph. aureus (including penicillin-resistant strains), E. coli and Salmonella spp. It has been shown to be more active against Staph.aureus than ampicillin, tetracycline, chloramphenicol or methicillin.

The Tetracyclines

The history of the tetracycline family of antibiotics began in 1947 with a discovery of chlortetracycline,[27] a potent, broad-spectrum antibiotic effective against a wider range of pathogenic micro-organisms than any agent in use up to that time. A few years later, the second member of the series, oxytetracycline, was discovered [28] and introduced to the medical profession. In 1953, the 'parent' compound of the series, tetracycline, was discovered [29,30] and rapidly became the tetracycline of choice. This latter compound may be prepared either by fermentation or by catalytic dehalogenation of chlortetracycline. In 1957, a new strain of Streptomyces aureofaciens was found [31] which produced a new class of tetracyclines, those lacking a methyl group at the 6 - position. One of this latter group, 6 - demethylchlortetracycline is the most recent member of the series to be produced in quantity and used widely in the practice of medicine.

The chemical structures of these compounds were, of course, not known during the first years of their use in medicine, but were the subject of intensive investigation during the early 1950's. The first structure of the series to be elucidated was that of oxytetracycline (XIV), [32] followed [33] later in 1952 by that of chlortetracycline (XV).



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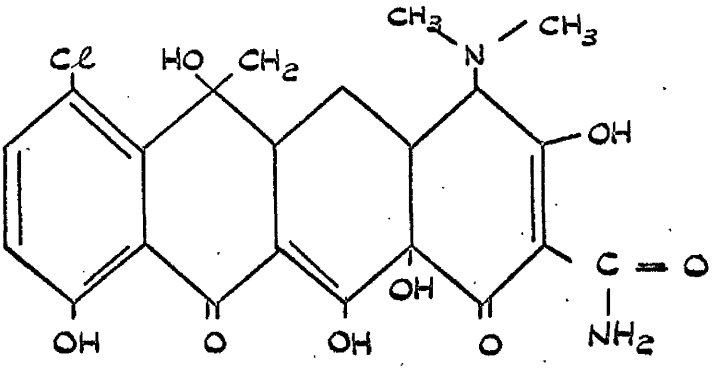
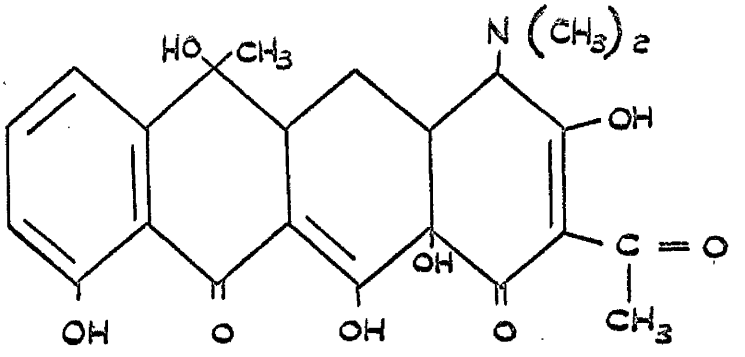


(XV)

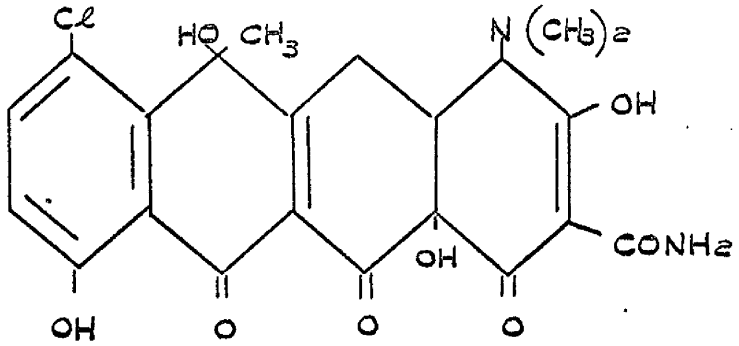
Based on this foundation, the structures of the other members of the series followed rapidly.

The number of naturally occurring or biosynthetically produced tetracyclines is not large, the five compounds already mentioned (chlortetracycline, oxytetracycline, tetracycline, 6 - demethylchlortetracycline and 6 - demethyltetracycline) being the most important ones. 7 - bromotetracycline, a highly active compound, can also be produced by supplying bromide ion and excluding chloride ion in the fermentation. All the above compounds are highly potent antibacterial agents. Studies of the metabolism of mutant strains of streptomyces have at times produced compounds related structurally to the tetracyclines but having little or no antibacterial activity, for example,[34] 2 - acetyl - 2 - decarboxamidotetracycline (XVI) having



(XVI)

a methyl ketone in place of the carboxamide group or 5a(11a) - dehydrochlortetracycline (XVII) containing one double bond in addition to the normal unsaturation of the tetracyclines.[35]



(XVII)

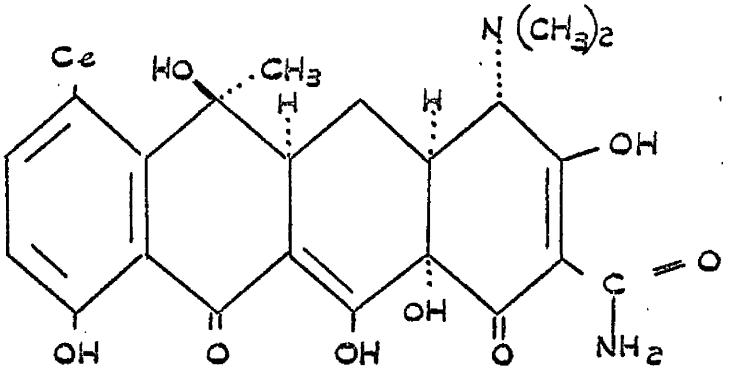
In addition, there are a number of more distantly related compounds produced by different streptomyces. These quinone pigments differ from one another by the number and position of oxygen functions and by the sugars which are attached via glycosidic linkages. Members of this group have been isolated by a number of different laboratories and in some cases the names are overlapping. Although these antibiotics show activity as antibacterial agents in vitro and, in some cases, antiviral action has been claimed, the lack of further published biological testing or clinical data tends to indicate that for one reason or another they are not clinically useful compounds.

Compared with the biosynthetically produced tetracyclines, the chemically modified derivatives are rather more numerous. Large numbers of compounds have been prepared from the naturally occurring compounds, both for the purpose of studying structure-action relationships and, more important, also to find compounds which have higher activity or have activity against a different spectrum of micro-organisms.

The derivatives which have been prepared can best be discussed in groups according to the modifications which have been carried out.

(1) Modification to the Stereochemistry.

The stereochemistry of the tetracyclines was finally elucidated by at least two x-ray crystallographic studies, [36,37] although partially determined by chemical means. Tetracycline has five asymmetric centres and at least some information is available as to the configurations required for biological activity at three of these centres (XVIII).

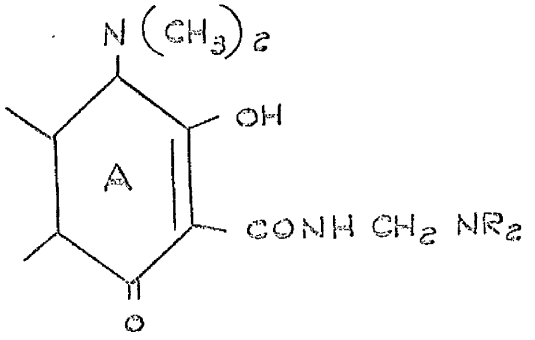


(XVIII)

Position 4 can be epimerised [38] with great ease and solutions of the antibiotic at intermediate pH ranges eventually reach a stable equilibrium mixture containing approximately 50% of each epimer. The 4 - epitetracyclines have a low activity and even this is probably due to reconversion to a normal tetracycline during the assay. Although position 4 is the only asymmetric position that can be epimerised directly catalytic reduction of 5a (11a) - dehydrochlortetracycline, a product of one of the mutant strains of Streptomyces, produces [39] a mixture of tetracycline and 5a - epitetracycline. The latter compound is nearly devoid of activity. All of the biosynthetically produced tetracyclines bear a hydroxyl group at position 6 which can be removed by hydrogenolysis in acidic conditions, using a palladium-charcoal catalyst.[40] It has been shown [41] that this hydrogenolysis inverts the 6-methyl group, thus producing 6 - deoxy - 6 - epitetracycline. Although there is a quantitative difference between the normal and epi form of 6 - deoxytetracycline, both are potent antibacterial substances.

(2) Modifications to Rings A and B.

The carboxamide group in the 2 - position has been subjected to several chemical changes, one being the simple dehydration to a nitrile,[42] which is completely inactive. Treatment of the tetracyclines with formaldehyde and a primary or secondary amine gives [43] aminomethyl derivatives (XIX). These compounds usually have the property of increased solubility in water over a broad pH range. However, such a chemical link is very liable to hydrolysis and it is probably this ready hydrolysis back to the parent compound which accounts for the biological activity of these compounds.



(XIX)

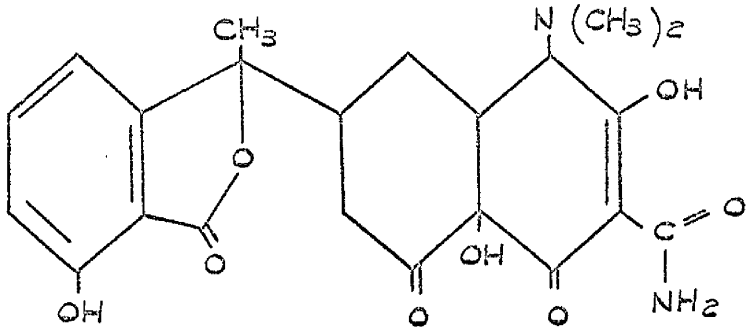
The dimethylamino group at position 4 can be epimerised as already described, it can also be quaternised [44] by treatment with methyl iodide, or it can be replaced by hydrogen by a zinc/acetic acid reduction of the methiodide. Each of these changes results in either greatly diminished or complete loss of activity.

The hydroxyl group at position 12a, which breaks up the conjugation of the 5 /3 - dicarbonyl groups thus preventing one continuous chromophore, is a group necessary for biological activity. Elimination of this group may be accomplished either by reduction with zinc and ammonium hydroxide [45] or by catalytic hydrogenation of the 12-a formyloxy derivative.[46] These intermediate 12-a acyloxy esters have full biological activity, but this again is thought to be due to the extreme ease with which hydrolysis of the ester groups takes place. Also derived from the 12-a acyloxy compounds are the 4-a (12-a) anhydro derivatives, prepared by pyrolysis.[47] These compounds possess minimal antibacterial properties.

Position 11a can be substituted by halogenation [48,49] which breaks up the β - diketone conjugation of the BCD chromophore. Very little has been published on the biological activity of these compounds but it is known that certain of them are readily reconverted into the starting material under very mild reducing conditions, and this may only be another example of activity due to regeneration of the parent antibiotic under biological conditions.

(3) Modifications to Ring C.

Some of the earliest studied chemical modifications of the tetracyclines involve the 6 - hydroxyl group of ring C. Strong acids induce a dehydration to the anhydrotetracyclines [33] involving this hydroxyl group and the 5a hydrogen, thus creating a naphthalene ring system. On the other hand, in alkaline solution, the 6 - hydroxyl group participates with the 11 - ketone cleaving the 11 to 11a bond, and forming a new lactone ring, giving a type of compound called an isotetracycline (XX).



(XX)

Both these modifications cause a severe loss of activity. Several other modifications of the 6 - position have been accomplished, however, with retention of biological activity. The 6 - hydroxyl group can be replaced by hydrogen, as described earlier, and, as already mentioned, if a methyl group is present its configuration is inverted. In addition, the 6 - deoxy - 6 - methylenetetracyclines, prepared [50] *via* the 11a - halo - 6,12 - hemiketals, have been added to the list of variations possible at this position. Also, a number of new compounds have been prepared [41] by

adding various thio compounds, such as benzyl mercaptan or thiophenol, to the double bond of the methylene derivative. All these modifications give highly active antibacterial agents.

(4) Modifications to Ring D.

Although a few biosynthetically produced tetracyclines have a halogen substituent on ring D, most of the compounds so substituted have been prepared by chemical modification. Substitution in the aromatic ring D usually requires the presence of strong acids, conditions which induce dehydration of the naturally produced 6 - hydroxy compounds. Thus it was the discovery that the 6 - hydroxyl group could be removed by hydrogenolysis and the resulting 6 - deoxy compounds retained the antibacterial properties of the parent antibiotics that stimulated the preparation of a number of 7 - and 9 - substituted derivatives. For example, 6 - deoxy - 6 - demethyltetracyclines can be nitrated in concentrated sulphuric acid to yield a mixture of isomeric 7 - nitro and 9 - nitro compounds each of which can be reduced by standard procedures to the corresponding amino - derivatives. The amino-tetracyclines can be converted to diazonium compounds by treatment with nitrous acid [52] and the diazonium group can be replaced by a number of different groups. In addition to nitration to introduce substituents, halogens may also be inserted [51,52] but in this case only 7 - substitution occurs. Disubstituted derivatives are also well-known, having either the same or different substituents in the 7 and 9 positions. [51] Thus a large variety of compounds of this type have become known and their antibacterial properties measured.

Although these different derivatives differ quantitatively from one another in biological activity, all of them fall into the active category, with the exception of those having a nitro group in the 9 - position. The nitro group in this position is thought to hydrogen bond with the ortho phenolic groups thus changing the hydrogen bonding and enolisation of the BCD chromophore, and causing a loss of most of the antibacterial activity. On the other hand, 7 - nitro - 6 - demethyl - 6 - deoxy tetracyclines has the highest in vitro activity of any of the derivatives so far reported, being about seven times as potent as tetracycline. This

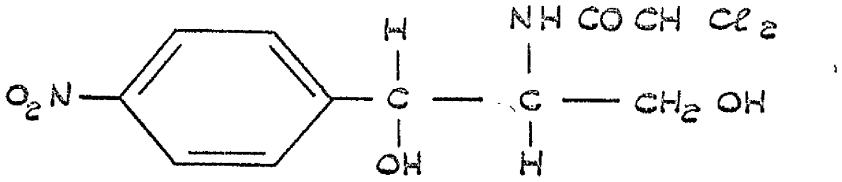
ratio is not repeated on *in vivo* studies, however.

Thus by modification of the tetracycline molecule, it can be shown that substituents at positions 5, 6, 7 and 9 can be altered with retention of activity. Other substituents on which changes can be made, for example, the 12-a formyloxy or some of the 11-a halo compounds probably retain activity because of their ready conversion back to the parent antibiotic.

Chloramphenicol

Chloramphenicol was originally isolated [53] in 1947 from the culture filtrate of an actinomycete found in the soil in Caracas, Venezuela, later designated Streptomyces venezuelae, and was also almost simultaneously isolated [54] from the same organism in Illinois.

Its structure was established, [55] in 1949, and confirmed by synthesis, [56] as D(-) threo - p - nitrophenyl - 2 - dichloro - acetamido - 1, 3 - propanediol (XXI).



(XXI)

Chloramphenicol has an unusually broad range of antibacterial action, low toxicity, great stability and effective absorption from the intestinal tract. [57] It was found to be of real value in the treatment of rickettsial infections and in typhoid fever.

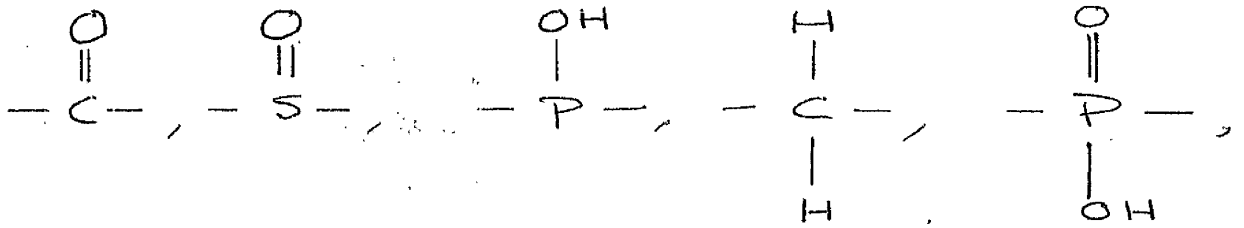
During investigations on the mode of action of chloramphenicol a large number of derivatives were prepared in order to elucidate the essential structural requirements for the anti-bacterial activity of the drug.

For the purpose of discussion, the molecule has been considered as three parts (a) the propanediol moiety, (b) the dichloroacetamide side-chain and (c) the p - nitrophenyl group.

(a) The propanediol moiety.

X-ray crystallography has shown [58] that in its crystalline form the two hydroxyl groups of chloramphenicol approach each other closely, with the formation of a strong hydrogen bond, hence forming a 6-membered ring. While this probably does not occur in solution, some chloramphenicol

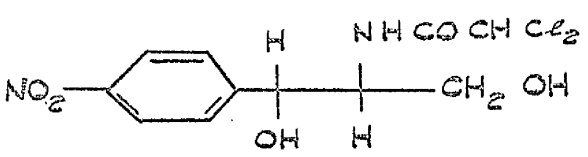
derivatives have been synthesised which possess stable six-membered ring structures closed by: [59,60,61]



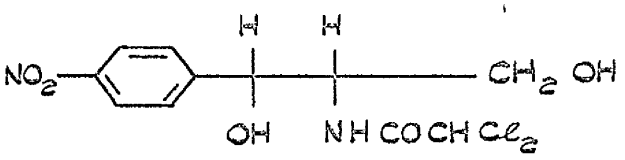
The cyclic carbonate, sulphite and phosphite are active antibiotics, while the cyclic phosphate and the 1,3 - dioxane derivatives are devoid of activity. It is suggested, however, that any activity is due to the restoration of the original chloramphenicol molecule in the system.

Structural changes that affect the character of the hydroxyls as free functional groups, for example acetylation or replacement of hydrogen, or alter the length of the propane residue, [60,62,63] tend to reduce activity.

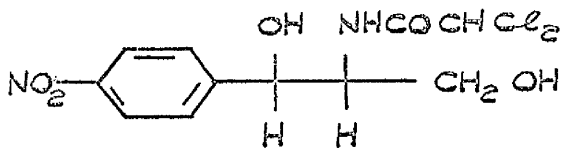
In chloramphenicol, the steric configuration of the substituents attached to the propane chain is of key importance for activity. Two asymmetric carbon atoms give rise to two pairs of stereoisomers all of which have been synthesised (XXII). [64]



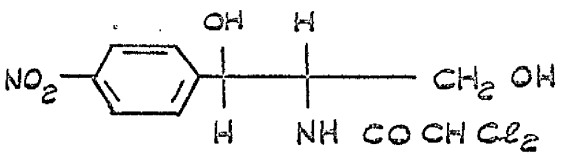
D (−) THREO
(CHLORAMPHENICOL)



L (+) ERYTHRO



D (−) ERYTHRO



L (+) THREO

(XXII)

The D(-) threo isomer, chloramphenicol, and the L(+) erythro isomer, slightly bacteriostatic to a number of organisms, have the hydroxyl group attached to carbon C-1 in an identical position relative to the plane of the asymmetric carbon atoms. In contrast, the other pair of stereoisomers are largely devoid of activity. There is, however, a fiftyfold difference between the activity of the D(-) threo compound and the L(+) erythro compound, indicating the importance of the stereochemistry at carbon C-2.

(b) The dichloroacetamide side-chain.

More than one hundred chemical variations of the acetamide side-chain have been reported.[65] Although data on the antibiotic properties of these derivatives is scanty, it appears that the principal factors that determine biological activity are the molar volume and the electronegativity of the acyl substituent.

It has been pointed out[62] that the structural requirements for antibiotic action are particularly rigid for the acyl side-chain and that the size of the dichloroacetyl cationic head is of critical importance. The preparation of compounds in which the terminal electronegative substituents have been maintained but in which the size of the acyl residue has been altered appreciably indicates that an increase in the molar volume of the head of the side-chain leads to the loss of antibiotic activity.

Despite rather wide variations, it appears there is also a rough correlation between the electronegativities of the acyl substituents and the antibacterial activities of the substituted chloramphenicol derivatives, provided that the molar volumes of the substituents are kept within narrow limits. It seems that a decrease in electronegativity tends to parallel a decrease in antibiotic activity.

The presence of the halogenacyl side-chain as such is one of the prerequisites for antibiotic action in the chloramphenicol series, hydrolysis of the amide bond yields a base whose activity is less than one fiftieth of that of chloramphenicol.[55] The amide linkage itself also appears to have a functional significance. Substitution of a methyl

group for the amide hydrogen atom or for the hydrogen atom attached to carbon C-2 of the propane moiety leads to a loss of activity.[60] It has been suggested[60] that this would hinder sterically an essential juxtaposition between chloramphenicol and its site of action.

(c) The p - nitrophenyl group.

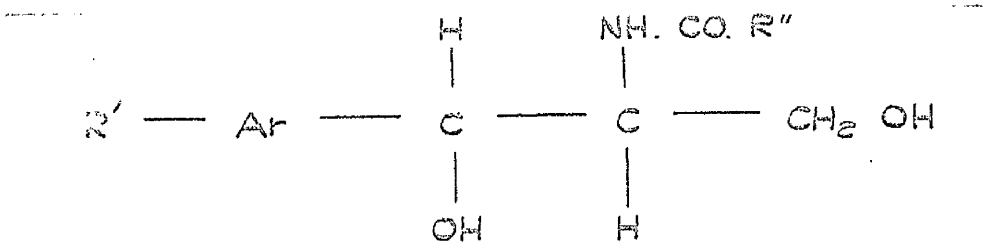
The appearance of the nitro group in biological substances is quite unusual. Consequently the aromatic nitro group was thought[66] to be of key importance for the antibiotic action of chloramphenicol until it became known that variations of the para substituent yield compounds with a wide range of antibiotic activity. Examination of these compounds together with the relative electronegativities of their para substituents shows a direct relationship between activity and the electronegativity of the substituent. For example, the nitro group with a relative electronegativity of 37.6 has a biological activity of 100% while the methyl group having an electronegativity of 4.24 gives a chloramphenicol derivative with a negligible biological activity.

This effect is thought to be due to increased resonance in the ring caused by substituents of higher electronegativity. This hypothesis is consistent with the observation that para substituents of less electronegativity but known ability to take part in p - quinoid type of structures, which contribute to the actual resonance of aromatic molecules, can give rise to chloramphenicol derivatives of extremely high biological activity. For example, a number of biphenyl derivatives show very high activity. Conversely para substitution by the more electronegative phenoxy or phenylmercapto groups, in which conjugation through both rings is much less important results in derivatives of considerably lower antibiotic activity.[67]

There is also incomplete knowledge concerning the influence that changes in position of the electronegative substituent might have on the activity of chloramphenicol. The meta nitroisomer has been reported to be devoid of activity, while for the ortho isomer,[68] no activity data has been published.

The information discussed above has shown that a number of structural features of the chloramphenicol molecule have specific

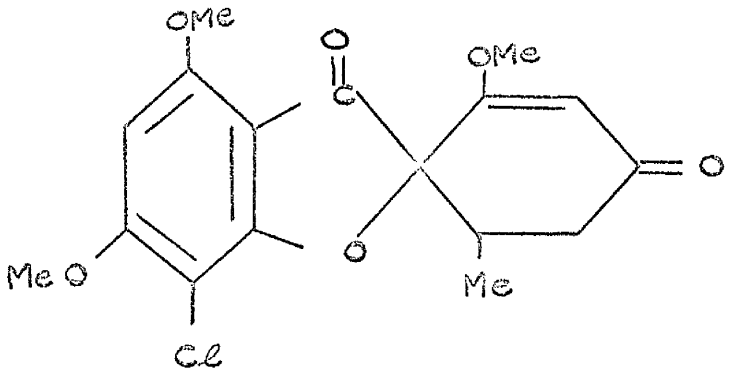
influence on its antibacterial action, as a result of this analysis, a generalised structure (XXIII) has been formulated [69] which embodies the structural features essential for the antibiotic action of compounds of chloramphenicol type, and which indicates the points at which specific kinds of chemical variations will cause predictable modifications of the antibacterial activity. It should be noted, however, that no chemical derivative has been noted which surpasses significantly the antibacterial activity of the natural drug itself.



(XXIII)

Griseofulvin

Griseofulvin is a fermentation product of three species of Penicillium : P.griseofulvum Dierck X, P.janczewski and P. patulum. It was discovered [70] by Oxford, Raistrick & Simonart in 1939, but its antifungal properties were unnoticed until the late 1940's, when they were described by Brian and his co-workers.[71] They noticed that in low concentrations, griseofulvin produced excessive branching with considerable distortion of the hyphal elements of a number of fungal species including Botrytis allis. The structure of griseofulvin was then investigated and shown [72,73] to be a coumaranone derivative, (2S,6'R) 7 - chloro - 4,6,2' - trimethoxy - 6'- methyl - gris - 2' -ene - 3,4'dione (XXIV).



(XXIV)

It was found that other varieties of Penicillium, namely, P.raistrickii [74] and P.nigricans [75] were also capable of producing griseofulvin. No-one attempted to use the drug clinically until it was found, in 1955, that griseofulvin in vitro inhibited the growth of many pathogenic dermatophytes, and it was even later that Gentles,[76] after infecting guinea pigs with Microsporum canis and Trychophyton mentagrophytes, demonstrated the in vivo effect of griseofulvin when given orally. He administered the drug in doses of 60mg./Kg. and

noted that in the treated animals only the tips of the hairs fluorised under filtered ultra-violet light and that on microscopic examination the dermatophyte could be found only in the portion of the hair shaft that fluoresced.

Since then griseofulvin has been widely used in the oral treatment of superficial dermatomycoses due to species of Trichophyton and Microsporum and also due to Epidermaphyton floccosum but has little effect in deep mycoses and has no antibacterial action.[77]

Dechlorgriseofulvin occurs[78] to some extent in normal media but can be produced in larger quantities in chloride-deficient media. Bromogriseofulvin has been isolated[79] from a mould grown in a medium containing bromide instead of chloride. Both are less active than the parent compound.

A wide range of griseofulvin derivatives have been prepared in an attempt to find a more active antifungal agent. The modifications which have been carried out include 4,6 & 2' alkoxy derivatives and various halogen derivatives.[80-84]

The 2' - n - propyl and n - butyl homologues cause helical waving of the fungal hyphae at concentrations less than one twentieth of the minimal effective concentration of griseofulvin. However, neither is as good as griseofulvin when administered systemically to plants for anti-fungal protection.

Over 300 analogues of griseofulvin have been evaluated[85] by in vitro tests against a representative selection of dermatophytes and plant pathogenic fungi. None of the analogues was found to be active against bacteria or yeasts. Enhancement of activity relative to that of griseofulvin was usually specific to one or a few species, except in the case of 2' -alkoxy - 3' benzyl griseofulvin analogues, of which the most active member 2' - ethoxy - 3' benzyl griseofulvin had greater activity than griseofulvin against all but one of the test fungi. Further increase in the length of the alkoxy side-chain first made more specific then increased anti-fungal activity and then reduced activity against all test fungi.

Increased activity against the plant pathogens predominated over enhanced action against dermatophytes.

Combination of the more active substituents at the 3' position with the more active groups at the 2' position showed a potentiated response in several analogues: some of the analogues of this type exhibited exceptionally high specific activity in the hyphal-curling test on B.allii, the 2' - butoxy - 3' - bromo, 2' - propoxy - 3' - iodo and 2' - propoxy - 3' - benzyl analogues being more than 500 times as active as griseofulvin.

Most replacements at any other position in the griseofulvin molecule diminished anti-fungal activity relative to that of griseofulvin, but a few of the 4 - or 6 - alkoxy analogues of griseofulvin gave improved performance against some species in vitro, although the anti-fungal spectrum was limited. Demethylation at either the 4, 6 or 2' positions greatly reduce the anti-fungal action of the analogue.

It was noted that there was a tendency for the 2' - alkoxy derivatives to accumulate in plant roots, so that only 1% of the dose reached the shoots.[86] It has been suggested that the low water solubility coupled with a high oil/water partition coefficient might diminish movement in the transpiration stream and lead to accumulation in the lipid of plant roots ie. the physical properties contribute considerably to the activity of griseofulvin analogues.

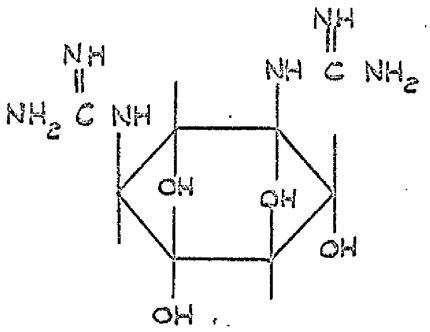
Stereochemical considerations also have a decisive influence over activity;[89] of the four stereoisomers possible, only griseofulvin itself is active and racemic griseofulvin has only half the activity of (d,d) griseofulvin.

The Streptomycin Group

This group of related antibiotics comprises streptomycin, mannosidostreptomycin, hydroxystreptomycin and bluensomycin. Other antibiotics which are related structurally include the neomycins and the kanamycins.

Streptomycin was isolated by Waksman [88] in 1944 from Streptomyces griseus, as the result of an extensive search for an antibiotic active against gram-negative bacteria. Following its isolation, detailed chemical investigations into the structure of streptomycin were carried out in several laboratories.

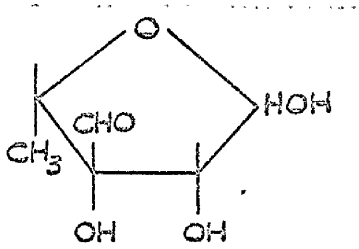
Acid hydrolysis was found [89] to split the antibiotic into two fragments, designated streptidine and streptobiosamine. Streptidine was shown [90] to be 1,3 - diguanido - scyllo - inositol (XXV)



(XXV)

Streptobiosamine was shown to be a disaccharide,

composed of N - methyl - L - glucosamine, linked glycosidically to streptose, 3 - C - formyl - L - lyxomethylose (XXVI).



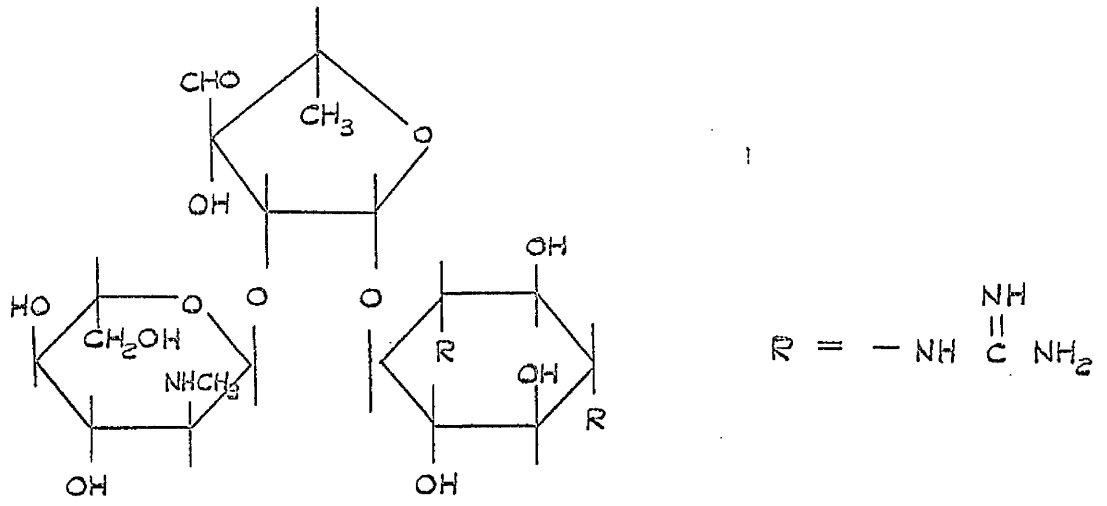
(XXVI).

N - methyl - L - glucosamine and streptose are linked through C - 2 of streptose. Streptidine is linked to streptobiosamine through C - 1 of streptose and C - 4 or C - 6 of streptidine.

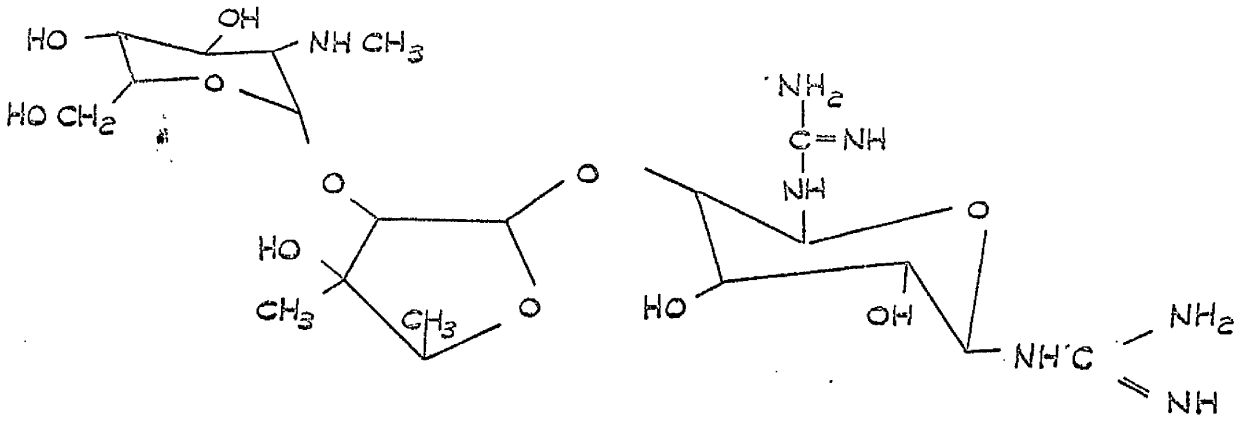
Wolfrom and his co-workers [93] utilising benzoylated derivatives of streptomycin, streptidine and methyl dihydrostreptobiosaminide for optical rotation experiments suggests that the streptidine - streptose link is β- L - and the hexosamine - streptose link is α- L -.

The absolute configuration of streptidine has been established more recently, [94] hence the absolute configuration of the whole molecule is known, except for the streptose ring.

The structural formula (XXVII) and absolute conformation of streptomycin (XXVIII) is shown below.



(XXVII)



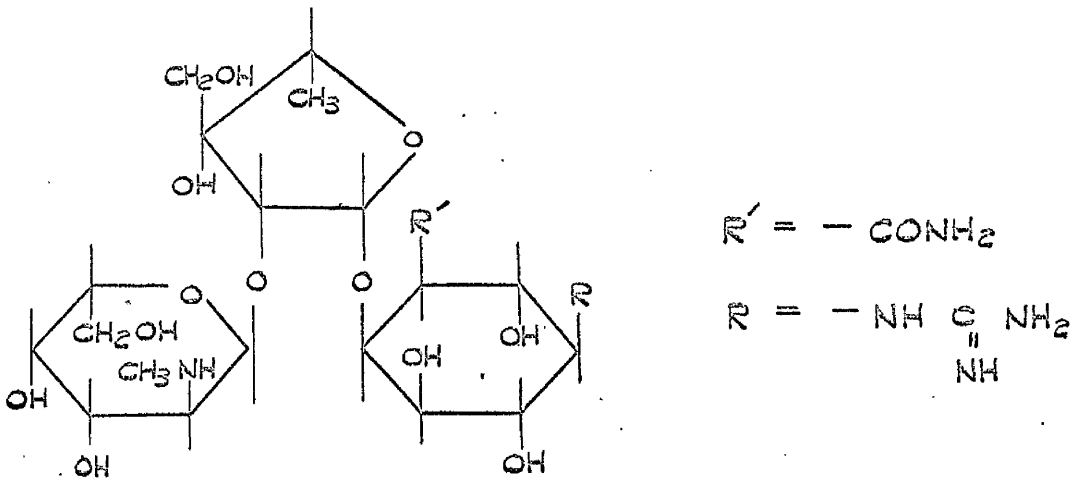
(XXVIII)

Mannosidostreptomycin, which has about one-fifth of the activity of streptomycin, has been isolated [95] from culture filtrates during the production of streptomycin. Its structure is identical except for an additional mannose residue, linked glycosidically to C-4 of N - methyl - L - glucosamine.

Hydroxystreptomycin, isolated [96] from culture filtrates of Streptomyces griseo-carnus, differs from streptomycin in having an additional hydroxyl group, attached to the streptose moiety. It is not as active as streptomycin but appears to be more stable to acid.

Bluensomycin, from Streptomyces bluensis, was isolated [97] in 1962, and shown to be very closely related to streptomycin. Acid

hydrolysis yields [98] dihydrostreptobiosamine and bluensidine, - 1 - deoxy - 1 - guanido - 3-O carbamoyl - scyllo - inositol. Glebomycin, isolated [99] from a new Streptomyces species in Japan, appears from preliminary investigations to be identical [97] with bluensomycin (XXIX).



(XXIX)

Bluensomycin is cross resistant with and less active than streptomycin.

The synthetic approach towards new members of this group of antibiotics has achieved only limited success. Biological activity is retained only if the antibiotic is subjected to a minor modification such as the formation of dihydrostreptomycin from streptomycin.

A number of salts of streptomycin have been prepared and claimed to be less toxic than the parent compound. These include pantothenates, i.e. salts of (D(+) - N - (α , γ - dihydroxy - β ,β- dimethyl butyryl) -β- alanine; methionates, [100] and N - methanesulphonates (- CH_2SO_2 Na) and N - methanesulphonates (-CH_3SO_3Na). [101]

Alteration of the streptidine portion of streptomycin leads to a decrease or complete loss in activity. This is demonstrated in bluensomycin and also in alkaline degradation products, [102,103]

for example, streptures dihydrostreptobiosaminide and streptamine dihydrostreptobiosaminide.

The most successful derivative of streptomycin is dihydrostreptomycin, prepared by catalytic hydrogenation [104] of streptomycin, thus reducing the carbonyl function on streptose to a primary alcohol group. It is highly active against Mycobacterium tuberculosis but has the disadvantage of causing deafness in a fairly large number of cases even after comparatively small doses. Clinically, dihydrostreptomycin is often used as a 50% mixture with streptomycin.

The remaining derivatives of streptomycin which have been prepared show alterations in the streptobiosamine residue. Comrie, Mital, and Stenlake [102] synthesised a series of streptamine and streptidine glycosides using the acetylated derivatives of streptidine or streptamine with one free hydroxyl group and the glycosyl halide. None of the compounds synthesised showed any appreciable activity against Mycobacterium tuberculosis.

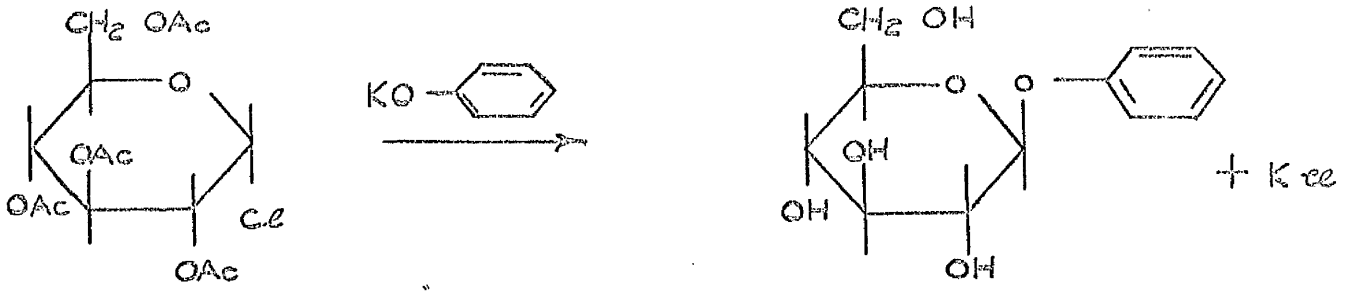
DISCUSSION

The original aim of this course of research was to prepare glycosides of dihydrostreptose in order to study further the structure-action relationships of the streptomycin antibiotics. Although dihydrostreptose has now been isolated,[105] the difficulties encountered in preparing reasonable quantities of this sugar have so far prevented the study of these derivatives. As an interim approach, therefore, the preparation of glycosides of dihydrostreptobiosamine was investigated. Methyl dihydrostreptobiosaminide, which is readily available, was used as starting material. Various methods are available for the synthesis of glycosides and a brief review of these methods is given below.

Methods of Glycoside Synthesis

The synthesis of many of the simplest glycosides dates back to the nineteenth century, and often no modification of the original preparation is to be found in the literature. There is no doubt, however, that in many instances the synthesis of a given compound could easily be simplified and improved. Several new general methods, or modified procedures, for glycoside synthesis have been introduced in the last thirty years.

In the first reported synthesis of a glycoside,[106] phenyl β- D - glucopyranoside, potassium phenoxide was condensed with tetra - O - acetyl -α- D - glucopyranosyl chloride, made by the treatment of D - glucose with acetyl chloride. Under the conditions of the reaction, the acetyl groups were removed and unacetylated glycoside was produced (XXX).



(XXX)

This method, with modern refinements, is of value in the preparation of phenyl glycosides but cannot be used for glycosides of alcohols, or for disaccharide syntheses.

Polyacetylated sugars are widely used [107] as intermediates in glycoside synthesis, being employed in both the Koenigs-Knorr and Helferich syntheses. Acetic anhydride is used exclusively for their preparation, and a catalyst is essential, the choice of catalyst often determining the predominant anomer obtained. The principal catalysts used in acetylation are anhydrous sodium acetate, zinc chloride, pyridine, perchloric acid, and concentrated sulphuric acid.

Sodium acetate gives predominantly the β - anomer, the acid catalysts the α - form, while pyridine results in a mixture of the α - and β - acetates, which can often be separated by fractional recrystallisation.

In the Koenigs-Knorr syntheses, [108] the fully acetylated sugar is treated with the hydrogen halide, usually the bromide, although there are some instances in which the chloride is preferred, in acetic acid, giving the O - acetylated glycosyl halide.

However, in the method described by Bárczai-Martos and Körösy for the preparation of bromides, [109] acetylation and bromination are carried out successively without isolation of the fully acetylated sugar.

The Koenigs-Knorr reaction, in which O - acetyl - glycosyl halides are condensed with alcohols or phenols, in the presence of a heavy metal or organic base, has been extensively reviewed elsewhere, [110,111]

Although Koenigs and Knorr isolated [108] a small yield of methyl β - D - glucopyranoside from a solution of tetra - O - acetyl - α - D - glucopyranosyl bromide in methanol that had stood at room temperature for several days it is customary to add an acid receptor to speed up the reaction and to prevent deacetylation of the product. Silver, in the form of the oxide or a salt, was the first acid acceptor to be employed, and it is still the one in most common use. Unless the aglycon is a simple alcohol, it is usual to dissolve the reactants in a solvent, which is often an organic base, to act as an additional acid acceptor. Walden inversion at C-1 is almost invariably the rule when the reaction is carried out in the presence of silver ion. [112] In special circumstances, however, both anomeric glycosides may be obtained. Walden

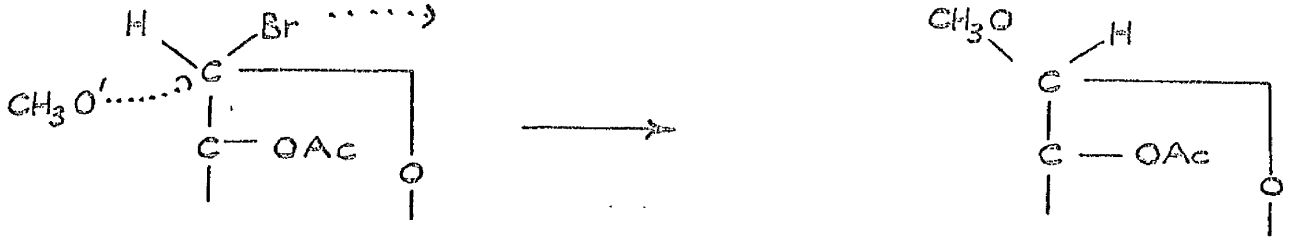
inversion at C-1 is also the rule when alkali is employed as the condensing agent.[113] An organic base (nearly always quinoline) may be used in the same way, but in the absence of silver, a mixture of the α - and β - glycoside acetates results.

In their original experiments,[108] Koenigs and Knorr used silver carbonate or concentrated aqueous silver nitrate to remove the hydrogen halide produced in the condensation; silver oxide was subsequently found to be equally effective. The exclusion of water during this reaction is of prime importance and calcium chloride or calcium sulphate has been used as an internal dessicant, to remove water produced by the reaction of the halogen halide and silver oxide.

While Walden inversion at C-1 is the rule in the presence of silver compounds, other factors may modify the reaction and so affect the final result.[114]

When, as in tetra - O - acetyl - α - D - glucopyranosyl bromide, the halogen and the neighbouring C-2 acetoxy group are cis, the halogen is replaced with inversion by a negative group from the environment, methoxyl in this case.

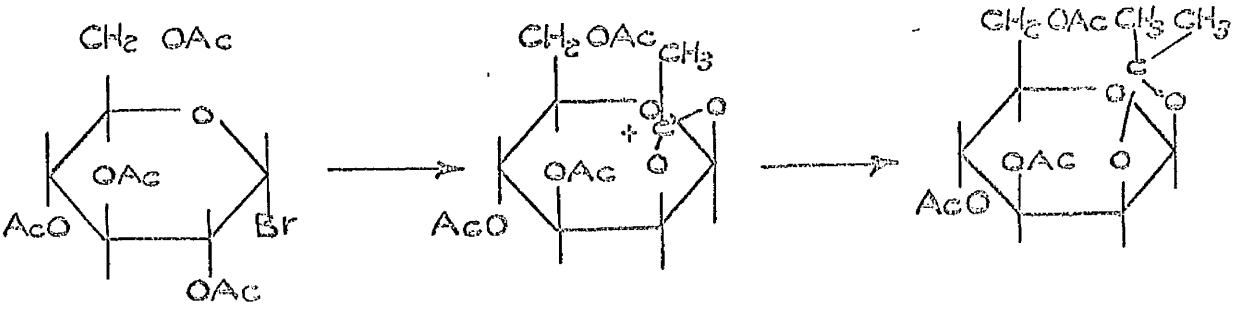
Recent work indicates this is an S_n1 substitution.[115]



(XXXI)

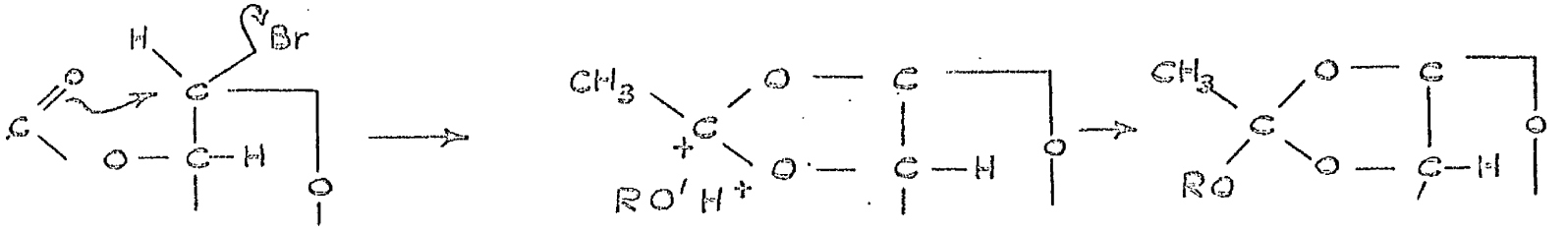
Where silver oxide or carbonate is used, the equilibrium is driven to the right by removal of the bromide ion. An organic base catalyst, for example quinoline, removes the hydrogen ion.

Reaction of the corresponding α- D mannopyranosyl bromide (a trans halide) results in a much more complex situation.[114] (XXXII).



(XXXII)

As the halogen departs, the nucleophilic oxygen of the neighbouring acetyl group attacks the opposite face of C-1 to give an orthoester carbonium ion which is electron deficient and under the Koenigs-Knorr basic conditions reacts with the solvent, in this case methanol, to give stable orthoesters which are diastereoisomers, for which full stereo structures have now been assigned on the evidence of nuclear magnetic resonance spectra



(XXXIII)

A competing reaction is the replacement of halogen without participation of the 2 - acetoxy group to give the alkyl glycoside with inversion but this is a minor product as the rate controlling step, the dissociation of the halogen, is speeded up by the neighbouring group effect. Subsequent methanolysis of the orthoacetate gives a mixture of the anomeric glycosides.

Helferich & Wedemeyer found [116] that a wide miscellany of metallic oxides and salts and other compounds, including albumin, are efficient 'acid acceptors' in the condensation of tetra - O - acetyl α - D glucopyranosyl bromide with methanol at room temperature to give methyl tetra - O - acetyl - β - D - glucopyranoside.

The Koenigs-Knorr synthesis is useful for the preparation of phenyl and alkyl glycosides as well as for disaccharide synthesis. A limitation of the reaction, besides the orthoester formation described above, is the difficulty of forming α - linkages. Most of the glycosyl halides are stable in the α - form as predicted on conformational grounds, hence on Walden inversion, β - glycosides are obtained. The reaction is generally used for pyranoside formation but ethyl β - galactofuranoside has been reported as being formed by this method. [117]

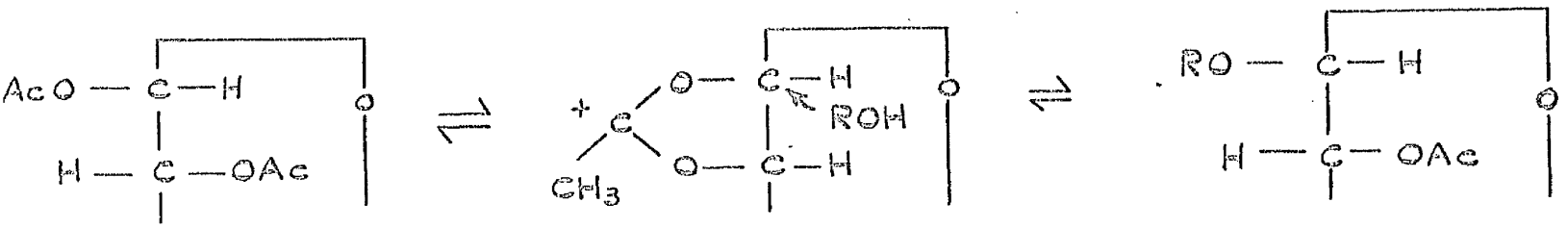
When O - benzoyl glycoside bromides are used instead of O - acetyl-glycosyl bromides, Walden inversion occurs during the reaction in the presence of the silver ion. It has, however, been shown [118] that the O - benzoylglycosyl bromides react rapidly with simple alcohols at room temperature in the absence of any acid acceptor and without debenzoylation of the product. Under these conditions, the configuration of the product is determined by steric hindrance. As a general rule, in the absence of an acid acceptor, all the benzoylated glycopyranosyl halides which have a benzoyloxy group at C-2 trans to the halogen react with methanol without net Walden inversion while those halides having a cis relationship between the groups on C-1 and C-2 react with inversion at C-1. Thus, since the aglycon always takes up a trans position with respect to the benzoyl group at C-2, this reaction yields β - D - glucosides, β - D - ribosides and β - D - xylosides, but α - D mannosides and α - D - arabinosides.

In the method of glycoside preparation developed by Helferich, [119] phenols are condensed with fully acetylated sugars in the presence of a catalyst. The use of this reaction has been confined almost entirely to the synthesis of phenolic glycosides but there is no reason why the reaction should not be carried out using alcohols instead of phenols. So far as is known, benzoylated sugars have not been employed in the Helferich reaction.

Catalysts used in this reaction are either anhydrous zinc chloride or p - toluenesulphonic acid; the former favours the formation of the α and the latter the β anomer. Improved yields result [120] from the removal, under reduced pressure, of the acetic acid produced in the reaction.

A few catalysts other than zinc chloride and p - toluenesulphonic acid have been successfully employed in the Helferich reaction, [107] for example, phosphoroyl chloride, sulphuric acid and anhydrous stannic chloride, all of which yield the β - anomer. Boron trifluoride also gives the β - glycoside.

A mechanism discussed [121] for the reaction suggests that the sugar acetate dissociates to give carbonium and acetate ions, followed by reaction of the carbonium ion with the phenol. (XXXV).



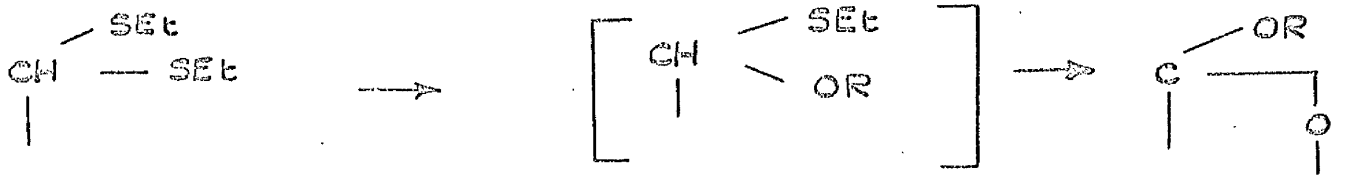
(XXXV)

Tri - O - acetyl - 1,2 - anhydro α - D - glucopyranose, (Brigl's anhydride) has been used for forming α - or β - D - glucopyranosides depending on the conditions used. Brigl [122] originally used it for the preparation of methyl β - D - glucopyranoside 3, 4, 6 - triacetate by evaporating a solution of the anhydride in methanol to dryness. Lemieux [123] has used the same anhydride in the synthesis of naturally occurring disaccharides, sucrose, maltose and trehalose by treating the anhydride with the appropriate O - acetyl sugar, for example, 1,3,4,6 - tetra - O - acetyl - D - fructose in the case of sucrose. Mechanisms of reaction have been postulated but there is no clear route.

The preparation of glycosides from dithioacetals [124] has the advantage of being particularly suitable for the synthesis of furanosides.

By varying temperature, quantity and nature of catalyst, usually mercuric compounds, α or β furanosides or pyranosides may be obtained.

It was considered [125] that during alkylglycoside formation from the acyclic mercaptal, a mixed acetal with one alkoxy and one thioethyl group is an intermediate. (XXXVI).



(XXXVI)

It has been shown [126] that this is the case in some examples but not in others. D-glucose diethyl mercaptal with methanol and mercuric chloride gave ethyl α- thio D - glucofuranoside. The S - ethyl - O - methyl monothioacetal gave methyl β- glucofuranoside indicating that the mixed acetal is not an intermediate in this reaction. The mixed acetal of D - galactose with mercuric chloride in ethanol gave ethyl β- D - galactofuranoside. D - galactose diethyl mercaptal gave the same product, indicating that the mixed acetal is in this case an intermediate.

When a sugar is alkylated with one equivalent of dimethyl sulphate in alkaline conditions, the glycosidic hydroxyl group is preferentially alkylated.[127] D - mannose treated in this way gave a mixture of α- and β - methyl D - mannopyranosides, substances not readily obtained by the Koenigs-Knorr reaction due to orthoester formation. Direct alkylation may also be carried out by using methyl iddide with silver oxide.[128] These methods are, however, rarely used in alkyl glycoside formation.

One of the oldest and simplest methods of glycoside synthesis is the Fischer reaction, in which the sugar is condensed directly with an alcohol in the presence of an acidic catalyst. Fischer [129] carried out the reaction in a sealed tube but refluxing with alcoholic hydrogen chloride has been found to be satisfactory. The catalyst most commonly used is hydrogen chloride, generally 0.5 - 2% w/v but an elegant improvement in

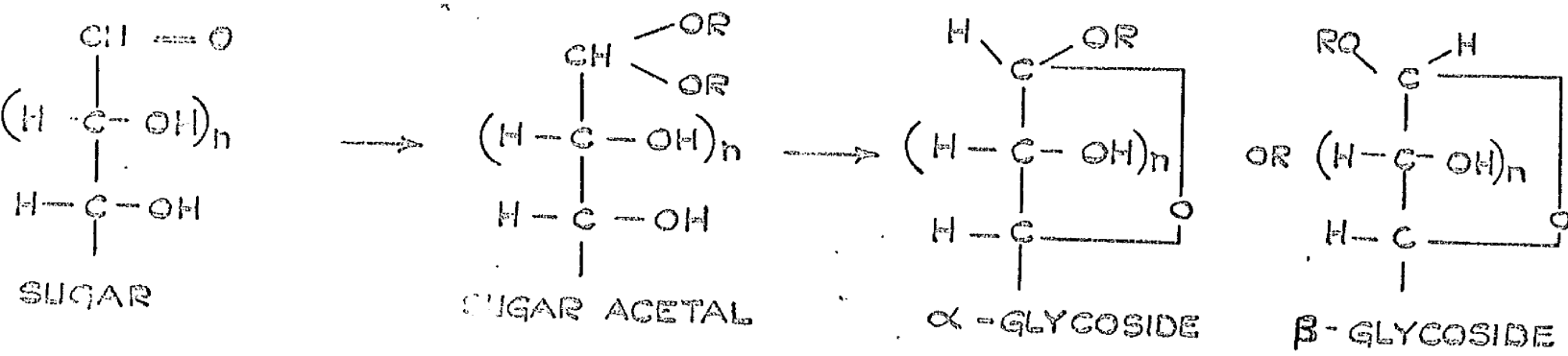
technique was made by Cadotte, Smith and Spriersterbach, [130] introducing cation - exchange resins as catalysts. The resin has the advantage of being filtered off at the end of the reaction.

This method is suitable for the synthesis of glycosides of lower aliphatic alcohols. It is not generally suitable for the preparation of glycosides of disaccharides since they are generally cleaved by alcoholysis. Amino-sugars are, however, an exception since the glucosaminide link is stable to hydrolysis.

In the Fischer reaction, one or other, or both, anomers may be separated from the reaction mixture, some sugars such as D - mannose, give essentially only one anomer. There is no way of altering the $\alpha : \beta$ ratio in the final equilibrium mixture and mixtures of anomeric glycosides are not as a rule easy to separate. It may thus be nearly impossible to separate the anomer required, even if it is formed in significant amounts. Newer methods of separation, however, including cellulose [131] and ion - exchange [132] column chromatography, silicate earth chromatography [133] and gas-liquid chromatography, using methylated derivatives, [134] have lead to an increased interest in the reaction, especially since it is a useful method of furanoside synthesis.

Newer methods of analysis have also enabled investigations to be made more satisfactorily into the mechanism of reaction. Previous studies had relied on hydrolytic, rather than synthetic, methods.

Campbell and Link, [135] investigating the hydrolysis of D - galactose dimethyl acetal with methanolic hydrogen chloride used polarimetric evidence to show that furanoside formation takes place first and subsequently the more stable pyranosides are formed. They, therefore, suggested the following reactions for glycoside synthesis. (XXXVII).



(XXXVII)

Levène, Raymond and Dillon, [136] studied in detail the changes taking place during methyl glycoside formation of a number of common sugars. The composition of the reaction mixture was determined by analysis for reducing sugars before and after hydrolysis under strongly acid conditions, when both pyranosides and furanosides are hydrolysed and under weakly acid conditions when only furanosides are hydrolysed.

In every case they found that furanosides were formed early in the reaction but their quantity decreased with time. Pyranoside synthesis, on the other hand, increased progressively with time.

Mowery and Ferrante, [133] re-investigating the glycoside formation of D - galactose using modern chromatographic procedures for analysis obtained results which substantially agreed with those of Levène. It was also shown that β -isomers are formed first, changing later to α -isomers, the change being accelerated at higher temperatures or hydrogen chloride concentration. The change from furanoside to pyranoside was shown to take place simultaneously with furanoside formation of D - mannose and D - arabinose.

Brown, and Baskowski [137] attempted to rationalise the difference in behaviour of furanose and pyranose sugars in terms of the I - strain concept, a general stereochemical theory proposed for cyclic carbon systems. I - strain is defined [137,138] as that change in internal strain accompanying the change in the co-ordination number of the ring atom participating in the reaction. For small rings (3 - 4 members) the internal strain arises primarily from distortion of the normal bond angles. In 5- and 6- and larger rings the strain is attributed primarily to repulsion terms arising from unfavourable conformations. It is suspected that comparatively small differences in internal strain can have large effects on rates and equilibrium of reactions of these compounds. For example, any enlargement of the ring carbon angles by nucleophilic substitution in cyclohexane will cause conformational change, increase non-bonded H - H repulsions, decrease the symmetry and increase the internal strain.

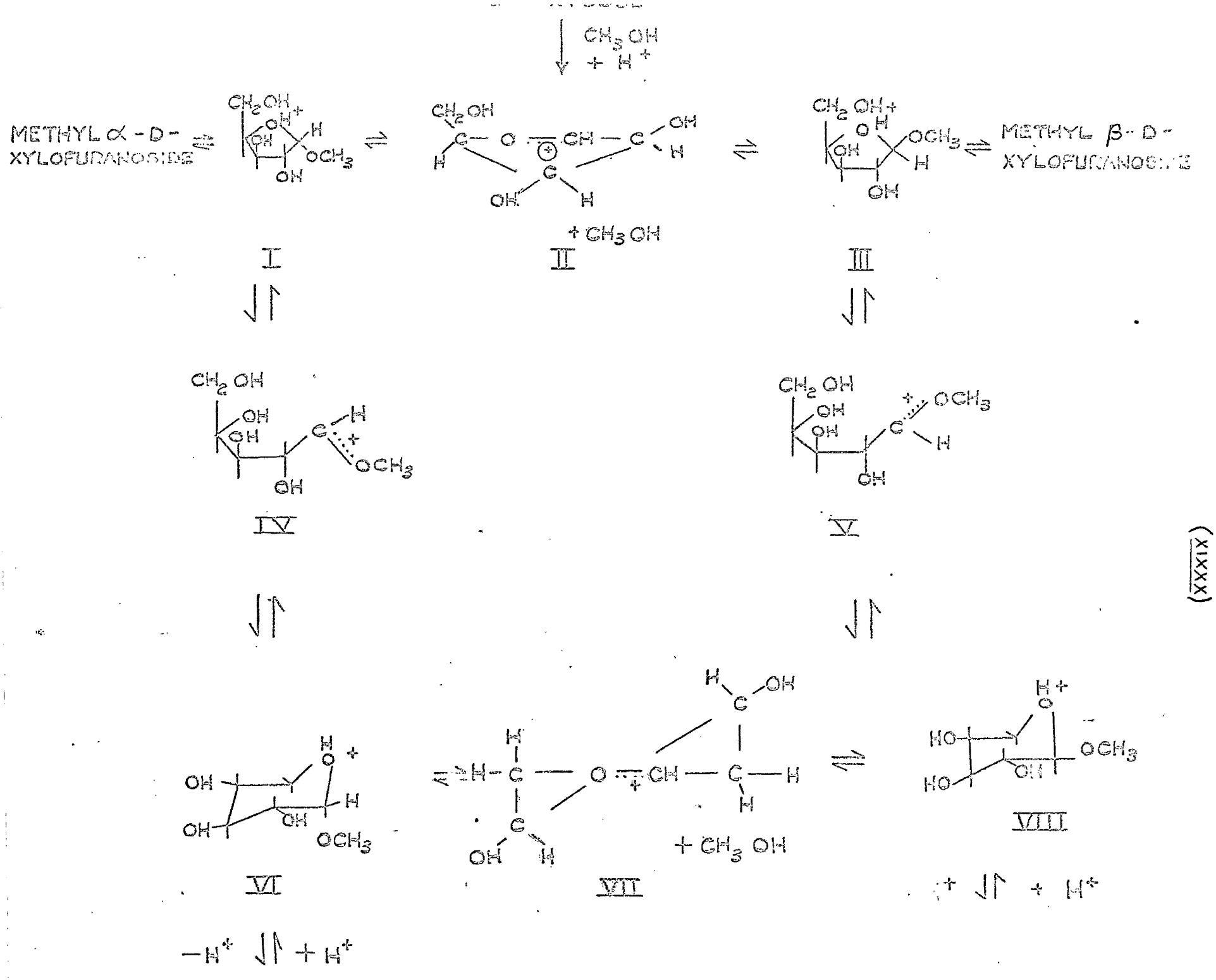
It was suggested that the I - strain offers an explanation for the marked difference in behaviour of furanose and pyranose forms of the

sugars. Under ordinary conditions, the sugars exist in solution as the hemiacetals in an equilibrium mixture of ring structures with the pyranose form greatly predominating. However, treatment of the mixture with methanol and hydrogen chloride results in the preferential formation of methyl furanosides.

It has previously been pointed out that solvolysis of 1 - methyl - 1 - chlorocyclopentane [139] proceeds at a rate some 100-fold greater than that of the corresponding cyclohexane derivative. The greater reactivity of the 5- ring derivative can be accounted for in terms of the I - strain concept. In the cyclohexane derivative, C- bond angles 109.5° confer greater stability and so less strain and less reactivity. Presumably the same factors operate to render the 5-membered furanose form far more reactive than the pyranose form. Therefore in the initial stages of the reaction the furanose derivative will react preferentially. Bishop and Cooper [140,141] made a detailed study of the kinetics of the methanolysis of D - xylose, D - arabinose, D - lyxose and D - ribose. The reactions, under controlled conditions, were followed by gas-liquid chromatography of the fully methylated, or acetylated, derivatives. Rate curves were established for each of the four anomers of each sugar and the results obtained indicated that the following sequence of reactions occurs during methyl glycoside formation.

(1) sugar ⟶ furanosides
(2) anomerisation of furanosides
(3) furanosides ⟶ pyranosides
(4) anomerisation of pyranosides

The relative rates of these four reactions in order of decreasing velocity are 2, 1, 3, 4. Reaction (3) was thought to proceed with retention of configuration at the anomeric centre. Capon, Loveday and Overend, [142] however, held the view that ring expansion occurred with inversion at the anomeric centre, quoting evidence from the study of α - and β- glucosides. Bishop and Cooper postulated the following mechanism for the formation of methyl D-xylosides. (XXXIX).


CH3OH + H+
METHYL α-D-XYLOFURANOSIDE
+ CH3OH
METHYL β-D-XYLOFURANOSIDE
I
II
III
IV
V
VI
VII
VIII
−H+ + H+
+ H+
METHYL α-D-XYLOPYRANOSIDE
METHYL β-D-XYLOPYRANOSIDE

(XXXIX)

The differencesin the observed rates of the anomerisation reaction were explained by the postulation of cyclic carbonium ion intermediates II and VII. The higher rate of reaction (2) (see page 10) may thus be explained by the ease of formation of ion II from I and III. The ring atoms C-4, ring O, C-1 and C-2 of the methyl D - xylofuranosides I and III would be coplanar and practically no conformational change would be involved in the formation of II. Conversely, the production of ion VII in the half chair conformation from the stable chair forms VI and VIII requires considerable change in conformation and the energy demands make the reaction the slowest of the four.

It was thus conclusive that glycoside compositions at equilibrium could be interpreted in terms of stabilities of each of the four glycosides, as influenced by steric and ionic effects.

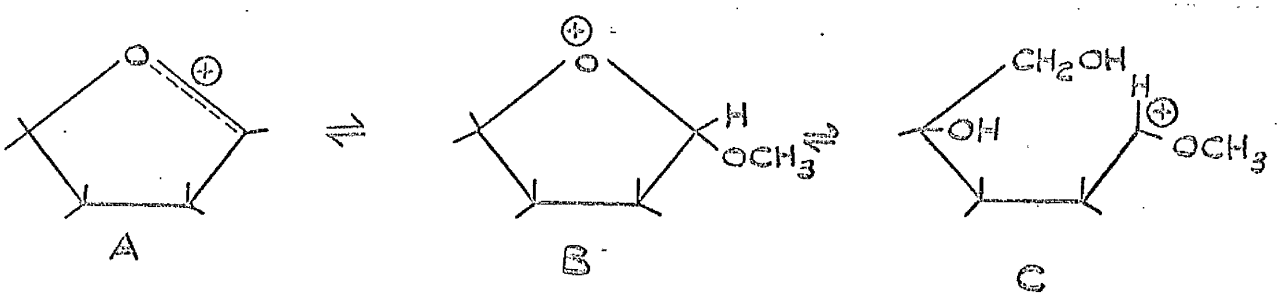
Nuclear magnetic resonance studies [143] on D - ribofuranose in nucleotides had shown that a specific conformation could be assigned to furanoid rings.

Bishop and Cooper thus adopted a system of conformational analysis for the furanosides. This was based [144] on the fact that the strain inherent in a 5-membered ring, cyclopentane or furanoid, can be relieved by a slight puckering brought about by movement of one or two atoms out of the plane of the ring. For cyclopentane, in which all atoms are equivalent, this results in two possible conformations (a) the C_s conformation in which a single atom is displaced from the plane of the other four, and (b) the C_2 conformation in which two atoms are displaced, one above and the other below the plane of the remaining three atoms. In the furanoid sugars a more definitive designation of conformation is required because of the specific stereochemistry of the carbon atoms in the ring. One atom out of plane gives the E (envelope) form while with two atoms out of plane, the T (twist) form exists. The atoms out of plane are indicated by subscripts or superscripts to show displacement below or above the plane of the ring. Carbon atoms are given by numbers, and the ring oxygen by O.

In the furanoside ring, the effective interactions are those between the eclipsed groups on adjacent carbon atoms and the most favoured

conformations will allow maximum staggering. Methyl ∝- D - arabinofuranoside has all of its large substituents in a trans orientation and the strain on the ring can therefore be relieved by the maximum staggering afforded by a T_2^3 or T_3^2 conformation. In methyl β - D - arabinofuranoside there is an eclipsed interaction between the C-1 and C-2 substituents which should force them away from one another, giving an E_2 conformation. On present evidence it was considered impossible to decide unequivocally between alternative conformations for a single furanoside.

Suggested possible intermediates for furanoside anomerisation (I) and for furanoside to pyranoside conversion (II), both arising from the protonated furanoside (III) are shown below, (XXXVIII).



(XXXVIII)

The non-bonded interactions between large eclipsed groups in the furanosides and protonated furanoside, C, will be relieved by opening of the ring, or in A if it has a puckered form with C-3 above or below the plane of the other atoms. Dissociation at C-1 in A removes any interaction between C-1 and C-2 substituents and displacement of C-3 in an E_3 or E^3 conformation relieves interactions between substituents on C-3, C-2 and C-1. Hence the relative orders of reactivity of the pentoses should be the same for reactions (2) and (3) (see page 10) and should depend on the strength and number of eclipsed interactions. The same argument would hold if reactions(2) and (3) proceeded through a common intermediate which decomposes at different rates into furanoside and pyranoside. Experimental data confirmed this theory. Table (I) gives the preferred conformations and eclipsed interactions of methyl

furanosides. It can be seen that the lyxosides, with the highest number of unfavourable interactions should have the highest reactivity, with regard to anomerisation or solvolysis; on the other hand the α - arabinofuranosides with no eclipsed interactions should be the least reactive, and in fact are the slowest reacting of the pentofuranosides.

The differences in rates of pyranoside anomerisation was also explained, using a similar theory.

Table I

Methyl D - pentofuranoside		Conformation	Interactions	Total
Arabinoside	α	T_2^3 , T_3^2	-	0
	β	E_2	C-1···C-2	1
Riboside	α	E^3	C-2···C-3	1
	β	E^2	C-1···C-2, C-2···C-3	2
Xyloside	α	T_3^2	C-3···C-4	1
	β	T_3^2 , E_3	C-3···C-4	1
Lyxoside	α	T_2^3 , E_3	C-2···C-3, C-3···C-4	2
	β	T_2^3	C-1··· C-2, C-2···C-3 C-3···C-4	3

Glycosides of Dihydrostreptobiosamine

Methanolysis of dihydrostreptomycin yields two isomeric methyl glycosides of dihydrostreptobiosamine. These glycosides have proved difficult to separate but acetylation of the anomeric mixture yields α - and β - methyl penta-acetyldihydrostreptobiosaminides which are easily separated because of a difference in solubility in refluxing ether. This was first demonstrated [92] during the elucidation of the structure of streptomycin and has been confirmed in this work. The acetylated methyl glycosides have been shown to be 90% α and 10% β and this is assumed to reflect the composition of the unacetylated anomeric mixture.

McGilveray, [110] using methyl dihydrostreptobiosaminide hydrochloride as starting material, has prepared, by solvolysis, a number of analogues of methyl dihydrostreptobiosaminide, viz: the phenyl, benzyl, 2 - bromethyl and cyclohexyl derivatives. These were obtained as the hydrochlorides, amorphous, hygroscopic solids, which were difficult to handle, and with the exception of the benzyl derivative, difficult to obtain in the form of the base. Rotational evidence, however, indicated that they occurred mainly, if not wholly, as one anomer.

The present experiments were carried out with several aims in mind, firstly to confirm that only one isomer is obtained from the solvolysis reaction, secondly to elucidate the stereochemistry of the product, and thirdly to investigate the mechanism of the solvolysis reaction, i.e. to determine whether or not the reaction proceeds by inversion, and the extent to which the stereochemistry of the product is determined by conformational considerations.

The formation of an anomeric mixture of methyl dihydrostreptobiosaminides, consisting (presumably) mainly of the α - anomer, from dihydrostreptomycin, in which the furanoside link is β -, suggests that the reaction might conceivably proceed by inversion. Differences in conformational stability of the two anomers may not be so high as to preclude subsequent equilibrium, even although the α - anomer, which would be the initial product in a reaction by inversion, permits fewer unfavourable interactions and hence, according to Bishop and Cooper, should be the more stable of the two anomers.

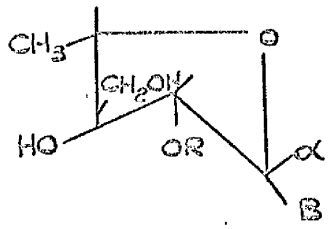
The examples of solvolysis found in the literature, mainly concerning pyranosides, give no clear indication of the mechanism involved in the reaction. Purves and Hudson [145] prepared β-benzyl D - fructopyranoside from α - methyl D - fructofuranoside and from α - benzyl D - fructofuranoside. The same workers also prepared β - methyl D - fructopyranoside from β - benzyl D - fructopyranoside. Pigman and Laffre [147] prepared α - n - butyl D - glucopyranoside from α - methyl D - glucopyranoside. Vernon and his colleagues [146] found that the methanolysis of α - and β - phenyl D - glucopyranosides occurred with predominant inversion.

The present experiments show that the benzyl alcoholysis of anomeric methyl dihydrostreptobiosaminides gives only one product, showing only one spot on thin-layer chromatography on alumina. This product is thought to be the α - anomer, from rotational evidence, in comparison with the rotations of other streptomycin derivatives. (Table II). It is of interest to note that although the rotational values of the α - anomers are all of the same order, while

Table II Derivative	$[\alpha]_D^{\circ}$
Streptomycin	- 78
Dihydrostreptomycin	- 94.5
Dodeca - acetyl dihydrostreptomycin	- 67
α- and β-methyl dihydrostreptobiosaminides HCl	- 125
α-methyl penta-acetyldihydrostreptobiosaminide	- 120
β-methyl penta-acetyldihydrostreptobiosaminide	- 34
Strepturea β - L - dihydrostreptobiosaminide	- 90.1
Benzyl dihydrostreptobiosaminide HCl	- 110
Benzyl penta-acetyldihydrostreptobiosaminide	- 138
2-Bromethyl dihydrostreptobiosaminide HCl	- 100
Phenyl dihydrostreptobiosaminide HCl	- 131

acetylation of the methyl glycosides gave a small decrease in rotation, acetylation of the benzyl anomer gave an increase in rotation. This is possibly due to a slight difference in conformation of the different glycosides.

The homogeneity of the product was confirmed by complete acetylation which again gave only one product, showing one spot on thin-layer chromatography. Rotational evidence again indicated the α - isomer. The product was completely insoluble in refluxing ether, showing a parallel with methyl α - penta-acetyldihydrostreptobiosaminide, and no ether-soluble material could be obtained. The α - anomer would be expected, on conformational grounds, existing in either the T_2^3 or T_3^2 conformation and showing unfavourable interactions at C-2 & C-3, and C-3 & C-4, to be the more stable isomer, whereas the β - anomer in the more probable E^2 form, has three unfavourable interactions. (XXXX)



(XXXX)

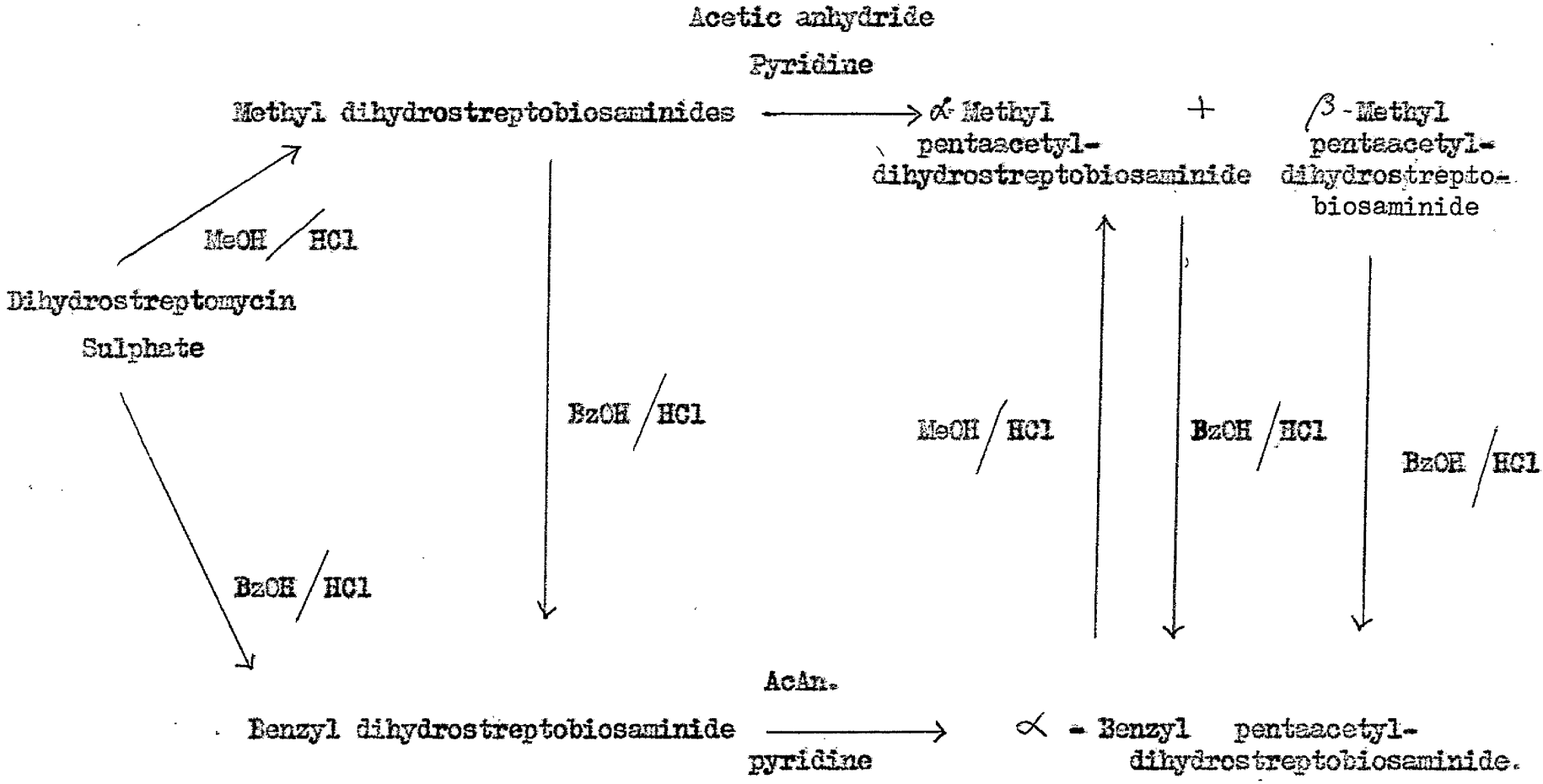
The same product, as indicated by infra-red absorption and rotational evidence, uncontaminated by the corresponding β - anomer was obtained by benzyl alcoholysis of pure α - methyl penta-acetyldihydrostreptobiosaminide and also of β - methyl penta-acetyldihydrostreptobiosaminide. Since only one anomer is consistently formed, there is no clear evidence therefore that the reaction proceeds by inversion and it can only be said that, irrespective of the reaction mechanism, the more stable isomer, on conformational grounds, is formed. On this basis, and on rotational evidence, this anomer may reasonably be concluded to be the α - anomer. This conclusion is supported by the fact that only α - methyl penta-acetyldihydrostreptobiosaminide could be isolated from the methanolysis of the benzyl penta-acetyldihydro-streptobiosaminide. No trace of the β - anomer could be found, although the yield of the α - anomer did not indicate that β - anomer formation was impossible. The β - anomer would be the expected, initial product if the transglycosidation proceeded by inversion.

Similarly the benzyl alcoholysis of dihydrostreptomycin itself gave the same anomer of benzyl penta-acetyldihydrostreptobiosaminide, as indicated by infra-red absorption, melting-point and rotational evidence, although once again the yield obtained did not rule out completely the possibility of the formation of the other anomer.

It should be noted that the benzyl group, because of its size, is much more important with regard to the conformational stability of the molecule than the methyl group, hence equilibration between the two anomers of the benzyl glycoside is less likely.

It is of interest to note that streptomycin, as it occurs naturally, is a β - lyxofuranoside, the more unstable of the configurations. Anomerisation of the glycosidic link to α - would give useful information to the structure-action relationships of streptomycin. All attempts, using acid-catalysts in non-aqueous media have so far been unsuccessful, resulting in either unchanged or hydrolysed reactant.[102]

Scheme of Reactions



Materials

Dihydrostreptomycin sulphate was supplied by Glaxo Laboratories from a non-sterile, freeze-dried batch.

Pyridine was dried by refluxing over potassium hydroxide and distilling.

Acetic anhydride was redistilled, the early and late runnings being discarded.

α - and β - Methyl dihydrostreptobiosaminides.

(Preparation after Fried and Wintersteiner.)

Dihydrostreptomycin sulphate (10g.) which had been dried for two hours in a high vacuum pistol over phosphorus pentoxide at 100° was dissolved in methanolic hydrogen chloride (N, 200ml.) and the solution kept at room temperature for 24 hours. Dry ether (400ml.) was then added, precipitating streptidine hydrochloride (5.4g.) which was filtered off as a white hygroscopic solid. The methanol-ether filtrate was concentrated in vacuo to approximately 25ml., any solid removed by centrifugation, and poured into dry ether (250ml.) and left at 0-5° for two hours. The oily precipitate was separated by decantation, washed with dry ether (2 X 250ml.), and dissolved in methanol (10ml.). The solution was passed down a De-Acidite FF (OH' form) column which was washed with methanol (500ml.). The eluate was evaporated to dryness in vacuo to a pale cream amorphous solid, a mixture of the anomeric methyl dihydrostreptobiosaminides, (4.5g.,91.2%), $[\alpha]_D^{20}$ -125°.

Found, N, 3.45%; $C_{21}H_{42}N_4O_9$ requires N, 3.6%

α - and β - Methyl penta - acetyldihydrostreptobiosaminides.

Methyl dihydrostreptobiosaminides (4.5g.) dried in vacuo over phosphorus pentoxide at 60° was dissolved in pyridine (10ml.). Acetic anhydride (15ml.) was added and the solution left at room temperature overnight. Ice-cold water (50ml.) was then added and the solution evaporated to dryness in vacuo. The product was dissolved in chloroform

(25ml.), the solution washed with water (20ml.), dilute sulphuric acid (20ml.), and water (20ml.). The chloroform was distilled off under vacuum to give a creamy amorphous solid, (2.1g.) a mixture of α - and β - methyl penta-acetyldihydrostreptobiosaminides.

α - Methyl penta-acetyldihydrostreptobiosaminide.

The mixture of α - and β - methyl dihydrostreptobiosaminides (2.1g.) was boiled with dry ether (200ml.) for two minutes, and the ether decanted off. The ether-soluble material was crystallised from ethanol-ether, giving α-methyl penta-acetyldihydrostreptobiosaminides (1.5g.) which on recrystallisation from ethanol gave white shining needles, mp. 192°, $[\alpha]_D^{20}$ - 117°.

Infra-red spectrum showed ν_{max} at 3300 (NH,OH stretch, 2900 (C-H stretch), 1600 (NH bend), 310 (α - glycoside) $cm.^{-1}$

Found, N, 2.6%; calculated for $C_{24}H_{37}NO_3$, N,2.5%.

β - Methyl penta-acetyldihydrostreptobiosaminide.

The ether-soluble material from the above reaction was isolated by evaporating the ether, and washing the residue with petroleum ether. The material was recrystallised from dry ethanol giving prisms of β - methyl penta-acetyldihydrostreptobiosaminide (100mg.) mp. 156-157°, $[\alpha]_D^{20}$ - 34° (c., 1% in chloroform).

Benzyl dihydrostreptobiosaminide.

Methyl dihydrostreptobiosaminide (1g.), dried in vacuo at 60° for two hours, was dissolved in benzyl alcoholic hydrogen chloride (N, 50ml.) and the solution left at room temperature for 24 hours. The solution was then poured into dry ether (250ml.) and kept at 0-5° for two to three hours. The ether was then decanted off and the precipitate dissolved in a minimum of ethanol and washed down a column of De-Acidite FF (OH' form) with methanol (500ml.). The eluate was evaporated to dryness in vacuo at 60° giving benzyl dihydrostreptobiosaminide (550mg.) as a white, amorphous solid, $[\alpha]_D^{20}$ - 125 (c. 1% in methanol).

Found, N, 3.3%, calculated for $C_{20}H_{21}NO_9$ 3.5%

α- Benzyl penta-acetyldihydrostreptobiosaminide

Benzyl dihydrostreptobiosaminide (1g.), dried in vacuo at 60° was dissolved in pyridine (10ml.). Acetic anhydride (10ml.) was added and the solution left overnight at room temperature. Ice-cold water (20ml.) was then added and the solution evaporated to dryness in vacuo. The product was dissolved in chloroform (20ml.) and the solution washed with water, dilute sulphuric acid, and water. The chloroform was distilled off in vacuo and the residue crystallised from ethanol-ether. Recrystallisation from ethanol gave needles of α- benzyl penta-acetyldihydrostreptobiosaminide (700 mg.), mp.141 - 143°, $[\alpha]_D^{20}$ - 138°.

Infra-red spectrum gave $\nu_{max.}$ at 330 (N-H, O-H stretch), 2800 (C-H stretch), 1550 (C-O stretch), 1520 (N-H bend), 1480 and 1230 (aromatic), 1130 (benzyl ether), 820 (glycoside), 745 and 690 (aromatic) cm.$^{-1}$.

The product was completely insoluble in ether; no ether soluble material was isolated.

Found, C, 57.2%, H, 6.6%, N, 2.2%

C, 57.9%; H, 6.4%; N, 2.2%

C H NO requires
30 41 3

Benzyl alcoholysis of dihydrostreptomycin sulphate.

Dihydrostreptomycin sulphate (2g.) dried in vacuo at 60° for two hours was stirred into benzyl alcoholic hydrogen chloride (2N, 250 ml.) at 40°. The mixture was stirred continuously for 96 hours at 40°. At the end of this time, the undissolved streptidine hydrochloride was filtered off. The filtrate was reduced in volume (10-15ml.) under high vacuum and dry ether added. The resultant white precipitate was triturated with dry ether, dissolved in a minimum of methanol and passed down a column of De-Acidite FF (OH' form). The washings (250ml.) were evaporated in vacuo at 60° to give benzyl dihydrostreptobiosaminide, as a colourless amorphous solid (800mg.). The base was dissolved in pyridine (10ml.) and acetic anhydride added and the solution left overnight at room temperature. The solution was worked

up as before giving crystals of α- benzyl penta-acetyldihydrostreptobiosaminide (630mg.) m.p. 141-142°, undepressed on admixture with authentic material, $[\alpha]_D^{20}$ - 138° (c., 1% in chloroform). Infra-red absorption identical with that of authentic material.
Found: N, 2.2%; $C_{30}H_{41}NO_3$ requires N, 2.2%.

Benzyl alcoholysis of α- methyl penta-acetyldihydrostreptobiosaminide.

Benzyl alcoholysis of α- methyl penta-acetyldihydrostreptobiosaminide.

α- methyl penta-acetyldihydrostreptobiosaminide (500mg.) was dissolved in benzyl alcoholic hydrogen chloride (2N, 5ml.) and the solution left at room temperature for 24 hours. Pyridine (10ml.) and acetic anhydride (10ml.) were then added and the solution left overnight. Excess pyridine and acetic anhydride were then evaporated off in vacuo. The resulting solution was chromatographed on neutral alumina. Elution with ether gave benzyl acetate and elution with chloroform gave α- benzyl penta-acetyldihydrostreptobiosaminide which was recrystallised from ethanol, giving colourless needles (200mg.), m.p. and mixed m.p. 141-142°, $[\alpha]_D^{20}$ - 138°. Infra-red absorption identical with that of authentic material.

Benzyl alcoholysis of β- methyl penta-acetyldihydrostreptobiosaminide.

β- methyl penta-acetyldihydrostreptobiosaminide (85mg.) treated as above gave crystals of α- benzyl penta-acetyldihydrostreptobiosaminide (25mg.), m.p. and mixed m.p. 141-142°, $[\alpha]_D^{20}$ - 138°, Infra-red absorption identical with that of authentic material.

Methanolysis of ∝- benzyl penta-acetyldihydrostreptobiosaminide.

∝ - benzyl penta-acetyldihydrostreptobiosaminide (500mg.) was dissolved in methanolic hydrogen chloride (N, 25ml.) and the solution left overnight at room temperature. The solvents were then removed under vacuum, pyridine (5ml.) and acetic anhydride (7.5ml.) added, and the solution left overnight at room temperature. Removal of the solvents under vacuum gave ∝ - methyl penta-acetyldihydrostreptobiosaminide (320mg.) which on recrystallisation from ethanol gave colourless needles, mp. and mixed mp. 141-142°, $[\alpha]_D^{20}$ - 117°, infra-red absorption identical with that of authentic material.

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SUMMARY

SUMMARY

The work already carried out on the chemical modification of naturally occurring antibiotics is reviewed. The reasons for carrying out these modifications are discussed. Antibiotics which have been modified chemically in some way include the penicillins, the cephalosporins, the tetracyclines, griseofulvin and the aminoglycoside antibiotics, including streptomycin itself. The value of these modifications varies from compound to compound, the most successful derivatives which have been prepared, to date, being the derivatives of penicillin. Compounds have been prepared which are more active, which are active orally and which are active against penicillinase-producing micro-organisms. Derivatives of other antibiotics have, generally, been less successful, although valuable information about structure-action relationships has been obtained. The derivatives of dihydrostreptomycin which are considered in this thesis are glycosides of dihydrostreptobiosamine, a disaccharide composed of dihydrostreptose linked glycosidically with N - methyl - L glucosamine.

The methods available for glycoside synthesis, the Koenigs-Knorr, the Helferich and the Fischer syntheses, are reviewed. A modification of the Fischer synthesis was used for the preparation of the benzyl glycoside of dihydrostreptobiosamine, using methyl dihydrostreptobiosaminide as starting material. No clear mechanism for this reaction has so far been postulated to elucidate the mechanism.

α - and β - methyl dihydrostreptobiosaminides were prepared and separated in the form of their acetates which were then used to prepare benzyl penta-acetyldihydrostreptobiosaminide by transglycosidation. The fact that the α - anomer was formed in both cases indicates that, every time, it is the more stable isomer which is formed. Direct benzyl alcoholysis of dihydrostreptomycin also gave α - benzyl penta-acetyldihydrostreptobiosaminide, confirming this theory.

Methanolysis of α- benzyl-acetyldihydrostreptobiosaminide gave only the α - anomer of methyl penta-acetyldihydrostreptobiosaminide.

There is some indication that the furanose ring of dihydrostreptose facilitates the reaction, in analogy with the Fischer glycoside synthesis.