

COMPARATIVE CHEMISTRY OF SOME AMINOGLYCOSIDE ANTIBIOTICS

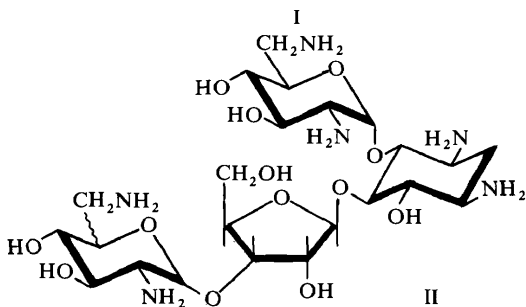
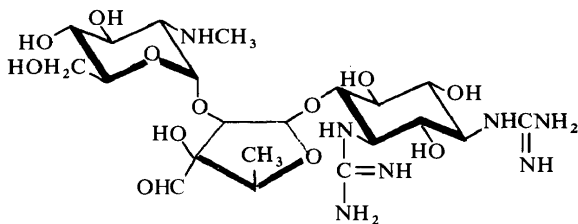
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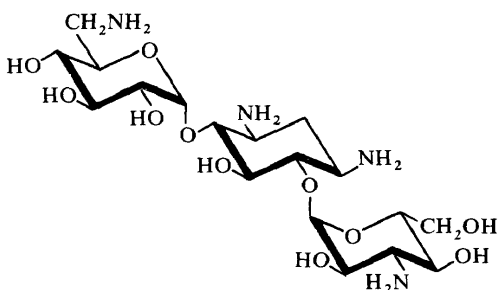
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ABSTRACT

In the field of antibiotics, the term 'aminoglycoside' is restricted in its use to those compounds in which the aglycone is a cyclitol or an aminocyclitol; the sugar moieties usually contain at least one amino-group. The three clinically important groups are exemplified by streptomycin (I), neomycin (II) and kanamycin A (III). This paper will be concerned largely with the kanamycin group and particularly with the gentamicin C components (IV). Some discussion of structure determination will be included as well as some approaches to the synthesis of new aminoglycosides.

In its most general sense, the term 'aminoglycoside' refers to any carbohydrate containing an amino-function and linked via the C-1 oxygen to a second moiety—the aglycone. In the field of antibiotics, however, the term is restricted in its use to those compounds in which the aglycone is a cyclitol or an aminocyclitol. The clinically important aminoglycosides fall into three 'families' exemplified by streptomycin (I), neomycin (II) and kanamycin A (III). In streptomycin the aglycone is streptomine; in the other two cases





III

the related aminocyclitol 2-deoxystreptamine is present. The sugar linkages to 2-deoxystreptamine are noteworthy in that they are disposed to the 4,6-positions in kanamycin and the 4,5-positions in neomycin.

In general the aminoglycosides are broad-spectrum antibiotics of major application in the treatment of Gram-negative infections. In particular, the high potency of some of the pseudotrisaccharides^{1,2} of the kanamycin group versus *Pseudomonas* organisms has been responsible for their relatively widespread use in both topical and life-threatening systemic infections; e.g. a cumulative *in vitro* tube dilution study³ of Garamycin® is shown in Table 1. It can be seen that at 10 µg/ml—a therapeutically attainable blood

Table 1. Garamycin® sulphate. Cumulative *in vitro* tube dilution studies 1963–1970

	No. Strains	Percent inhibited	
		5 µg/ml	10 µg/ml
<i>E. coli</i>	2124	83	92
<i>Klebsiella-Enterobacter</i>	1938	90	97
<i>Proteus</i> spp.	1487	57	85
<i>Providencia</i>	20	70	100
<i>P. aeruginosa</i>	2027	82	96
<i>Salmonella</i> spp.	345	75	93
<i>Serratia marcescens</i>	175	93	100
<i>Neisseria gonorrhoeae</i>	113	92	100
<i>S. aureus</i>	1640	96	97

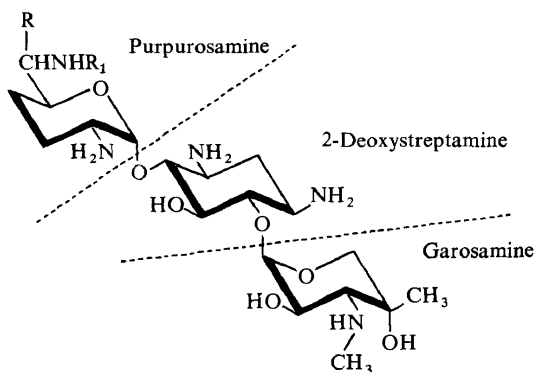
level—96 per cent of the 2027 strains of *Pseudomonas aeruginosa* screened have proved sensitive to the drug. That the compounds have not found wider general application is due largely to two factors; (a) they are not orally absorbed in man and (b) the risk of toxic side effects, especially renal toxicity and eighth-nerve damage, is too high to warrant use in non-critical situations. Some of the more recently available kanamycin-like antibiotics do possess very favourable therapeutic ratios and these will be discussed later.

The work carried out by the author and his associates at the Schering Corporation has been concerned largely with the gentamicins^{1,4}, a group of antibiotics closely related to the kanamycins. The ensuing discussion will be limited to the kanamycin family of aminoglycosides and in particular to the gentamicins.

The gentamicin complex—a mixture of a number of aminoglycoside

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antibiotics—is produced in submerged fermentations by the organisms *Micromonospora purpurea* (NRRL 2953) and *Micromonospora echinospora* (NRRL 2985)⁴. It can be readily isolated using the well-established general techniques of aminoglycoside isolation⁵. Removal of inorganic salts and separation of the antibiotics into two sub-complexes—the gentamicin A/B complex and the gentamicin C complex—is accomplished by chromatography on Dowex 1. The gentamicin C complex comprises some 75 per cent of the total complex and contains three closely-related aminoglycosides designated gentamicins C₁, C₂ and C_{1a}^{6, 7}; the structures (IVa, b, c) of these



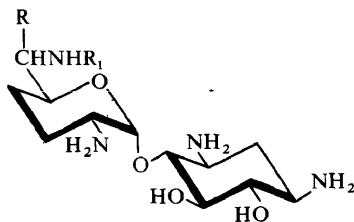
- IV (a) Gentamicin C₁, R = R₁ = CH₃
 (b) Gentamicin C₂, R = CH₃; R₁ = H
 (c) Gentamicin C_{1a} R = R₁ = H

compounds have been determined^{8, 9}. The nomenclature of the sugar units is shown in (IV); the common sugar unit has been named garosamine and the dissimilar 2,6-diamino sugars have been named purpurosamine A, B and C corresponding to gentamicins C₁, C₂ and C_{1a} respectively. It should also be noted that one literature report¹⁰ has referred to 'gentamicin D' instead of gentamicin C_{1a}. The former term is incorrect and the generally accepted nomenclature—gentamicin C_{1a} will be used throughout this and subsequent publications.

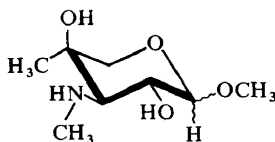
The gentamicin C components were separated by column chromatography on silica gel using the lower phase of the system chloroform–isopropanol–ammonia (1:2:1, developer), (1:1:1, eluent). This task was performed using two 5' × 4" glass columns (designed and built by 'Glenco,' Houston, Texas), linked in series and having all-Teflon® end plates and connections. The columns were wet-packed in the developing solvent system and were run at a constant flow rate of approximately 40–80 ml/min using an all-Teflon® metering pump. In this way approximately 750 g of gentamicin could be separated into its components. The structure studies will not be presented here in detail. It is, however, instructive to examine examples of some of the procedures used, especially where they relate to aminoglycoside structure determination in general.

Methanolysis of the C gentamicins^{8, 9} in methanolic hydrochloric acid gave the gentamines (V) and an anomeric mixture of methyl garosaminide

(VI). Cleavage of the purpurosamine sugar does not occur under these conditions; protonation of the 2-amino group prevents hydrolytic cleavage of the glycosidic linkage¹¹. The reaction is thus diagnostic for the presence of a



V



VI

2-amino-substituted sugar. The gentamines and methyl garosaminide were separated as free bases by a chromatographic procedure analogous to the gentamicin separation method. Methyl garosaminide was a syrupy mixture of anomers separable by fractional crystallization of the *N*-acetyl-derivative and subsequent deacetylation with hydrazine. Analytical data and mass spectrometry established the molecular formula to be $C_8H_{17}NO_4$. Periodate oxidation studies showed that methyl garosaminide consumed two equivalents of periodate; the *N*-acetyl-derivative consumed none, a result in agreement with the structural formulation. N.m.r. analysis of the anomerically pure species⁸ agreed unequivocally with the structure assignment and, further, confirmed the conformation of the molecule in solution. Application of Reeves' cuprammonium procedure¹² to methyl garosaminide showed a change in molecular rotation of -748° , a result most compatible with the *L*-arabino stereochemistry although not completely excluding the *D*-xylo configuration. Examination of the behaviour of the model compounds (VII) and (VIII) toward benzaldehyde showed that (VII) reacted exothermically in ethanol to give the epimeric oxazolidines (IX). Compound (VIII) did not react even on heating with neat benzaldehyde. Methyl garosaminide reacted with benzaldehyde exothermically and stereospecifically to give the crystalline oxazolidine (X); the n.m.r. spectrum of this compound (Figure 1) indicated little change in conformation, in agreement with the postulated *cis*-condensation of the oxazolidine ring.

The gentamines (V) were characterized by micro-analysis and mass spectrometry of their crystalline tetra-*N*-acetyl derivatives. Degradation by mercaptolysis using ethanethiol and aqueous hydrochloric acid afforded a quantitative yield of *N,N'*-diacetyl-2-deoxystreptamine, readily separable from the crystalline, chloroform soluble purpurosamine derivatives (XIa, b, c). The mass spectra of these compounds were characteristic for derivatives of

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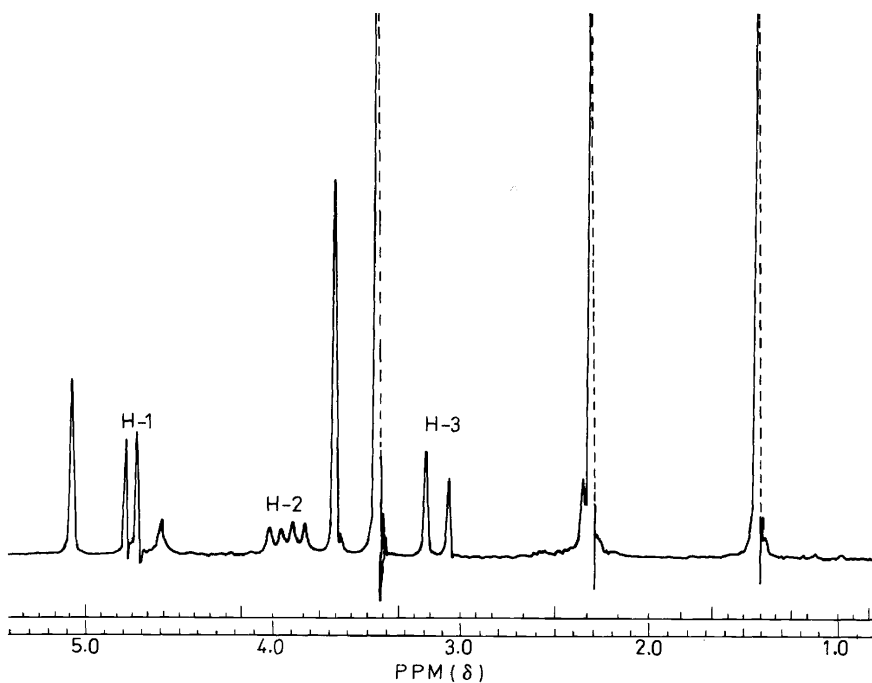
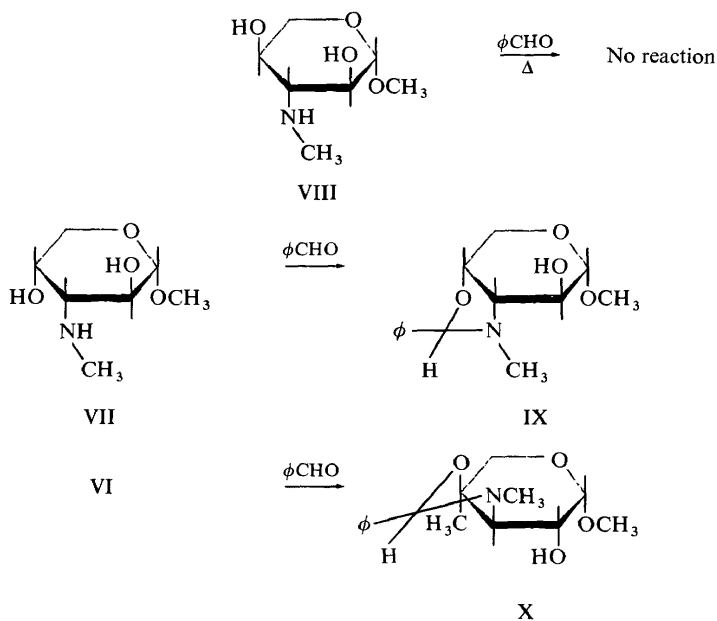
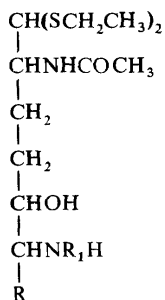


Figure 1. ¹H n.m.r. spectrum (60 MHz, CDCl₃) of methyl 3,4-*N*-*O*-benzylidene-3-deoxy-4-*C*-methyl-3-methylamino-β-*L*-arabinopyranoside

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XI(a) Purpurosamine A derivative $\text{R} = \text{R}_1 = \text{CH}_3$

(b) Purpurosamine B derivative $\text{R} = \text{CH}_3$
 $\text{R}_1 = \text{COCH}_3$

(c) Purpurosamine C derivative $\text{R} = \text{H}$
 $\text{R}_1 = \text{COCH}_3$

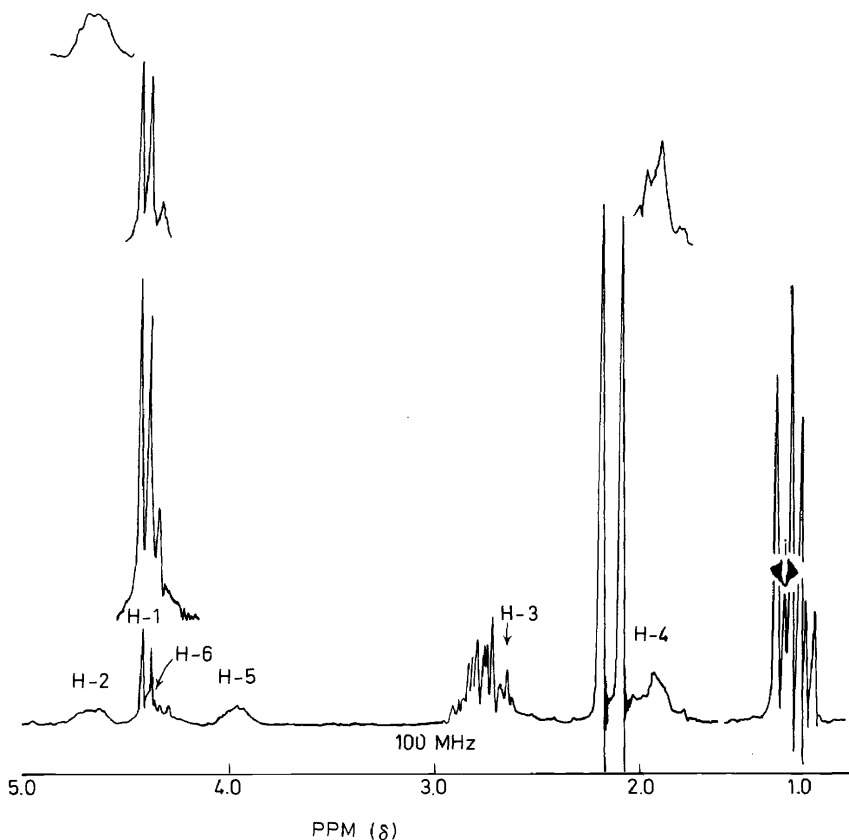
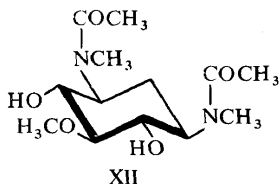


Figure 2. ^1H n.m.r. spectrum ($\text{C}_5\text{D}_5\text{N}$, 100 MHz) of *N,N'*-diacetylpurpurosamine B diethyl-dithioacetal

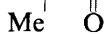
2-*N*-acetyl sugar thioacetals¹³. Confirmation of the structural assignments was obtained by analysis of the n.m.r. spectra (e.g. the purpurosamine B derivative, Figure 2) in which critical assignments were verified by double irradiation studies.

The stereochemistry of 2-deoxystreptamine in the gentamines has been investigated by Dr R. D. Guthrie *et al.* at the University of Sussex, Brighton, England. The circular dichroism of the tetra-*N*-acetyl gentamines in cuprammonium solution showed a positive maximum at 570 m μ and a negative minimum at 280 m μ compatible with a K chelate^{9,14} and thus a negative dihedral angle between the free hydroxyl groups of the 2-deoxystreptamine moiety, confirming the stereochemistry shown in (V).

The point of attachment of garosamine to 2-deoxystreptamine was ascertained by methylation of the gentamicin per-*N*-acetyl derivatives using sodium hydride and methyl iodide in dimethylformamide followed by acid hydrolysis to give *N,N'*-diacetyl-*N,N'*-5-*O*-trimethyl-2-deoxystreptamine (XII), confirming the attachment of garosamine to C-6 of the 2-deoxystreptamine unit. The structure of (XII) was indicated by its lack of optical

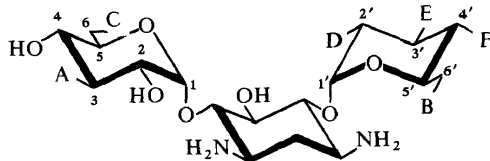


activity. However, the n.m.r. spectra in *d*₆-DMSO was complex due to restricted rotation about the —N—C— bond. That this was the case was



shown by re-running the spectrum at an elevated temperature whereupon it simplified to that expected for the symmetrical (XII). In terms of stereochemistry and disposition of amino-groups the gentamicins are closely analogous to the kanamycins¹⁵. A considerable body of literature has now been assembled on the kanamycins and the gentamicins as well as some information on the related nebramycin antibiotics². An analysis of structure-activity and to some degree structure-toxicity relationships is now possible. Table 2 illustrates the variation of antibacterial activity with position of amino-groups, the stereochemistry remaining constant. The compound (XIIIa) containing no amino-groups is inactive. Introduction of amino-groups at the 6,6'-positions (XIIIb) resulted in measurable activity, but only when the kanamycin C configuration (XIIIc) is reached, with amino-groups in the 3 and 2' positions, does activity become good. Introduction of an additional amino-group in the 3'-position of kanamycin A results in low activity. Optimal activity appears to be reached for kanamycin B (XIIIe) and tobramycin (XIIIh), both of which contain a 2',6'-diaminosugar and a 3-amino-sugar. The latter configuration is also common to the C gentamicins (IV) all of which possess high broad-spectrum antibiotic activity. The generalization can be made that in considering synthesis of new aminoglycosides the five amino-group configuration of the known highly active compounds will

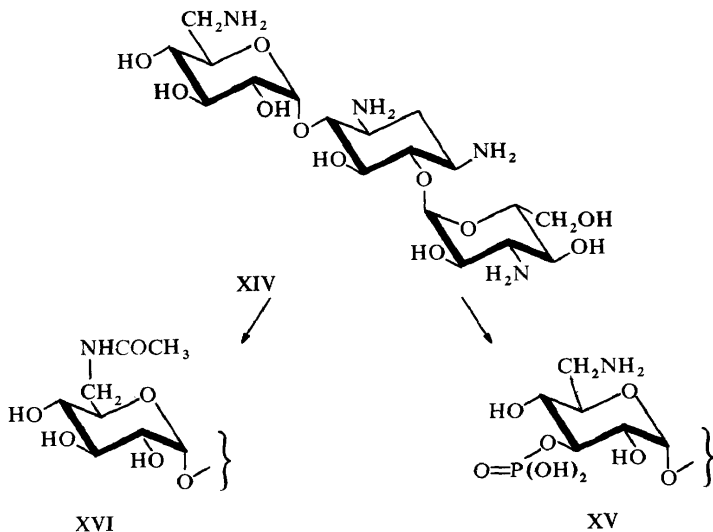
Table 2. Effect of positions of amino groups on activity of the kanamycin-like antibiotics



	A	B	C	D	E	F	Activity	Source
XIII a	OH	OH	OH	OH	OH	OH	none	(Lemieux, 1970)
b	OH	NH ₂	NH ₂	OH	OH	OH	fair	(Lemieux, 1970)
c	NH ₂	OH	OH	NH ₂	OH	OH	good	(Kanamycin C)
d	NH ₂	NH ₂	OH	OH	OH	OH	high	(Kanamycin A)
e	NH ₂	NH ₂	OH	OH	NH ₂	OH	low	(S. Inoue, 1965)
f	NH ₂	NH ₂	OH	NH ₂	OH	OH	very high	(Kanamycin B)
g	NH ₂	NH ₂	NH ₂	OH	OH	OH	good	(S. Inoue, 1965)
h	NH ₂	NH ₂	OH	NH ₂	H	OH	very high	(Tobramycin, Lilly)

probably be necessary for optimal activity. With regard to stereochemistry, little data have thus far been accumulated.

The structure-toxicity relationships in the kanamycin series are somewhat less available. In general, the compounds containing only four amino-groups (kanamycin A, kanamycin C) are considerably less toxic than those containing five amino-groups^{1, 16} (kanamycin B, tobramycin, gentamicin C). However, they are also very much less active and the therapeutic ratios for e.g. gentamicin C are much more favourable than those of e.g. kanamycins A and C. On this point hinges the much greater clinical versatility of the gentamicins. Published data² suggest that tobramycin may be less ototoxic than gentamicin although the experimental conditions used by the Eli Lilly group were not in any way comparable to those used by Waitz *et al.*¹⁷ for the gentamicins.



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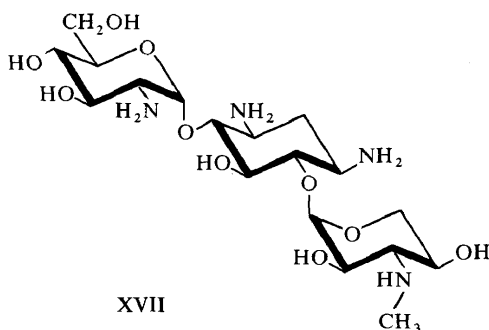
An ever increasing problem in aminoglycoside therapy is likely to be posed by the incidence of resistance mediated by R factors¹⁸. Thus far two such R factors are known to affect the kanamycin group and can be exemplified by reference to kanamycin A (XIV). One factor biologically inactivates the molecule by phosphorylation of the vacant 3-hydroxyl-group¹⁹ giving (XV). The other factor results in acetylation of the 6'-amino-group (XVI)²⁰ again with biological inactivation. The *in vitro* action of these two R factors in the kanamycin series has been extensively examined by Dr Julian Davies and his associates at the University of Wisconsin. Their results are summarized in Table 3. In general in the kanamycin series compounds having

Table 3. Effects of the known R factors on the kanamycin antibiotics

Antibiotic	Phosphorylated	Inactivated	Acetylated	Inactivated
Kanamycin A	Yes	Yes	Yes	Yes
Gentamicin A	Yes	Yes	No	No
Gentamicin C ₁	No	No	No	No
Gentamicin C ₂	No	No	Slight	No
Gentamicin C _{1a}	No	No	Yes	No
Tobramycin	No	No	Yes	Yes

a free 3-OH group are phosphorylated and those having a 6-amino-group are acetylated, in both cases with resulting biological inactivation. The gentamicin C components, however, differ. No phosphorylation occurs because of the absence of a 3-OH group (this is also true of tobramycin). Acetylation occurs completely only in the case of gentamicin C_{1a}. Gentamicin C₂ acetylates poorly and C₁ not at all. Presumably the latter two compounds offer steric hindrance to the acetylating enzyme. Interestingly, acetylation of gentamicin C_{1a} does not completely destroy its biological activity and it remains a moderately active antibiotic. It is evident that these unique features of the C gentamicins offer a significant clinical advantage over the other members of the kanamycin family.

Our studies on the gentamicins have included not only those of established clinical importance but also many of the co-produced compounds contained in the gentamicin A/B complex²¹. Some of these compounds have been purified and their structures investigated; one of them, gentamicin A (XVII),



was initially studied by Dr Carl Schaffner and his associates at Rutgers University and a gross structure was established²². Stereochemistry of the 3-deoxy-3-methylamino fragment (XVIII) was established by the Schering group by total synthesis and was reported at the 158th National Meeting of the American Chemical Society in September 1969²³. The same synthesis was reported by the Rutgers group in early 1970 in a paper submitted on 6 September 1969¹⁰. Figure 3 summarizes the work performed at Schering including the synthesis of the *arabino*-compound (XIX) mentioned earlier in relation to the establishment of the stereochemistry of garosamine. The crystalline and stable ketoepoxide (XX) is interesting in that it represents a new type of sugar intermediate.

The goal of our extensive investigations of structure-activity relationships in the gentamicin series was to develop knowledge that, combined with the available literature, would enable us to embark on a programme of semi-synthetic and, perhaps, synthetic aminoglycosides designed to minimize

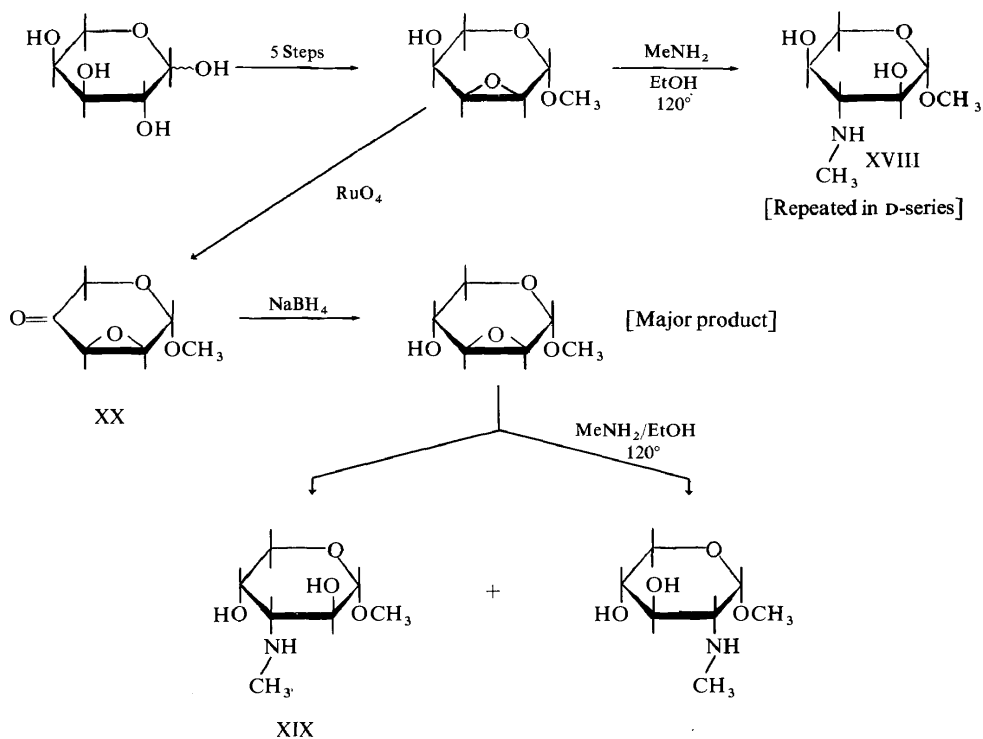


Figure 3

side effects while maintaining desirable biological activity. The key to synthesis in the kanamycin series lies in the efficiency with which a monosaccharide can be linked via an α -glycosidic bond to 2-deoxystreptamine. The classical method of glycoside synthesis—the Koenigs-Knorr reaction—does not fulfil the criterion of efficiency and furthermore usually results in

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the formation of largely β -anomer. The use of the Koenigs-Knorr reaction has been exploited by the Japanese school in the synthesis of kanamycin analogues²⁴⁻²⁸. Even in favourable cases, however, the reactions do not approach commercial significance.

Recently R. U. Lemieux^{29,30} described a new synthesis of α -glycosides detailed in Figure 4. The readily-available glucals (XXI) are converted to

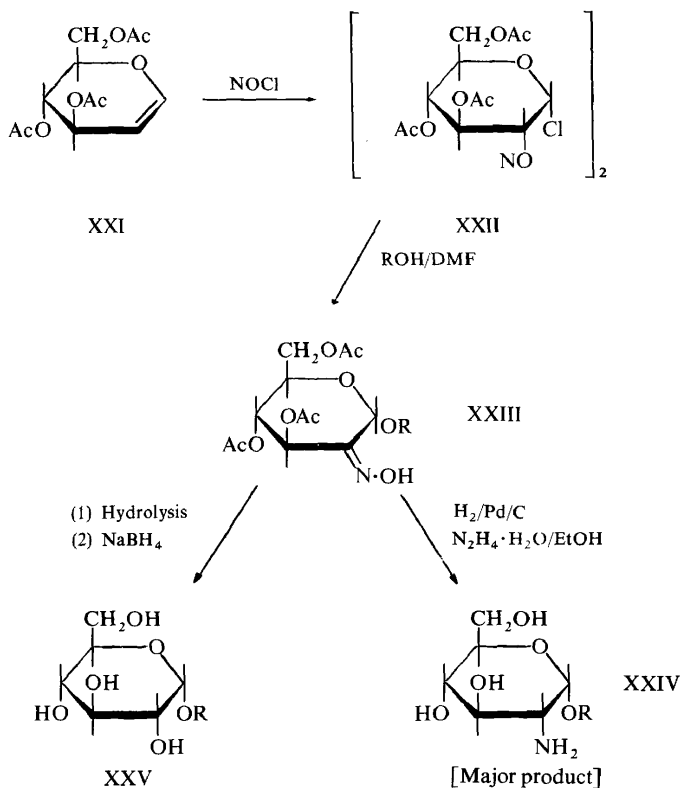


Figure 4

nitrosochlorodimers (XXII) that will react readily with alcohols^{30,31} (including very hindered alcohols) in dichloromethane or dimethylformamide to give the 2-oximino- α -glycoside (XXIII) in high yield. The product can be converted to the 2-aminosugar (XXIV) by hydrogenation or to the 2-hydroxy-compound (XXV) via borohydride reduction of the ketose. Lemieux has demonstrated³⁰ the applicability of the method to the synthesis of kanamycin-like antibiotics from 2-deoxystreptamine in yields approaching commercial interest. Total synthesis of kanamycin itself by the Lemieux procedure awaits the development of synthetic methods for the as yet undescribed 3-amino- or potentially 3-aminoglucals.

It seems likely that in the future we can look forward to semi-synthetic gentamicins into which considerable variation has been incorporated. It

may well be that this group of antibiotics stands now in the position occupied by penicillin at the time of isolation of 6-APA. In any event the era of second generation aminoglycosides is now with us and if sufficient separation of toxicity and activity can be attained, they may well become the broad spectrum antibiotics of choice. Available evidence suggests that gentamicin is already making inroads into the markets formerly dominated by β -lactams and tetracycline. The increasing problem of resistance to the latter compounds and the excellent resistance-profile of gentamicin and some of the related aminoglycosides are further telling arguments for the active development of these compounds as therapeutic agents.

ACKNOWLEDGEMENTS

I wish to extend my grateful thanks to Dr Marvin J. Weinstein of the Schering Corporation for his generosity in making available to me recently acquired biological results. I would also like to take this opportunity to thank the many other dedicated members of the gentamicin team with whom I am fortunate to have been associated.

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