SEMISYNTHETIC AMINOGLYCOSIDE ANTIBIOTICS. II. SYNTHESIS OF ANALOGUES OF PAROMOMYCIN MODIFIED IN THE GLUCOSAMINE MOIETY[1)]

Sir:

The accomplishment of an efficient method for obtaining the previously unknown 1,3,2‴,6‴-tetra-N-acetylparomomycin (**1**) and the subsequent preparation of the pseudotrisaccharide 5-O-[3-O-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-L-ribo-furanosyl]-2-deoxystreptamine(**5**), as described in the preceding paper[1)], by nitrous acid deamination, suggested a route for providing a useful intermediate for the synthesis of novel aminoglycoside antibiotics related to paromomycin and neomycin and modified in the glucosamine moiety. In order to investigate the structure-activity requirements for the glycoside unit linked to the C-4 hydroxy group of 2-deoxystreptamine and to obtain new analogues, we have undertaken the preparation of a selectively protected derivative of the pseudotrisaccharide **5** derived from paromomycin and lacking the glucosamine moiety.

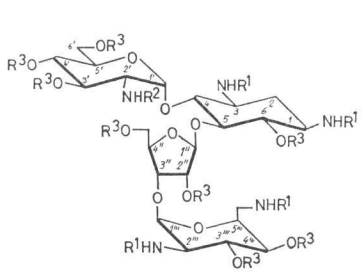
Reaction of 1,3,2‴,6‴-tetra-N-acetylparomomycin (**1**)[1)] with benzyloxycarbonyl chloride gave the corresponding 2′-N-benzyloxycarbonyl derivative (**2**) (m.p. 198~200°C; $[\alpha]_D$ +57°, MeOH), that was O-acetylated to give the fully acylated compound **3** (m.p. 269~271°C; $[\alpha]_D$ +56°, MeOH).

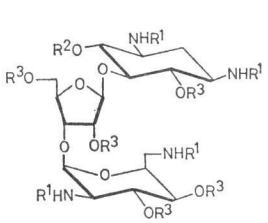
Hydrogenolysis of the benzyloxycarbonyl group in acidic methanol on 10% palladium-carbon afforded 1,3,2‴,6‴-tetra-N-acetyl-octa-O-acetyl-paromomycin (**4**) as the hydrochloride (m.p. 200~205°C; $[\alpha]_D$ +62°, MeOH).

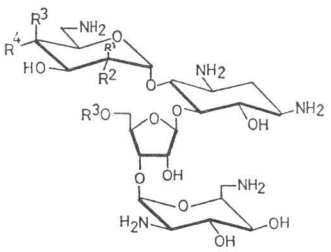
Nitrous acid deamination of **4** gave a mixture containing two main products which were separated by column chromatography on silica gel. The less polar component was identified as 5-(acetoxymethyl)-2-furaldehyde after comparison with an authentic sample prepared by deamination of 1,3,4,6-tetra-O-acetyl-2-amino-2-deoxy-α-D-glucopyranose hydrochloride[2)]. The major product (m.p. 157~160°C; $[\alpha]_D$ +17°, $CHCl_3$), obtained in an overall yield of 50%, was identified as 6-O-acetyl-1,3-di-N-acetyl-5-O-[2,5-di-O-acetyl-3-O-(2,6-diacetamido-3,4-di-O-acetyl-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribo-furanosyl]-2-deoxystreptamine.

Since no analogues of paromomycin have been found to contain the 6-amino-6-deoxy-α-D-glycopyranosyl unit linked to the 4-position of the 2-deoxystreptamine ring, it seemed worthwhile to prepare such analogues from compound **6**. For the glycosidation of compound **6**, we applied the LEMIEUX-NAGABHUSHAN reaction which has been reported to give α-glycosides[3)] and which had been successfully used for the synthesis of kanamycin analogues[4)].

As an example of the general procedure, the synthesis of **9** is reported.







1 : R^1=Ac , R^2 = R^3 = H

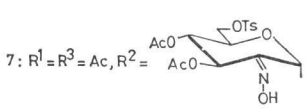
2 : R^1=Ac , R^2 = Cbz , R^3 = H

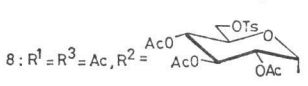
3 : R^1=R^3=Ac , R^2 = Cbz

4 : R^1 = R^3 = Ac , R^2 = H

5 : R^1 = R^2 = R^3 = H

6 : R^1 = R^3 = Ac , R^2 = H





9 : R^1 = R^3 = H , R^2 = R^4 = OH

10 : R^1 = R^4 = OH , R^2 = R^3 = H

11 : R^1 = R^4 = H , R^2 = R^3 = OH

Table 1. The minimum inhibitory concentration of paromomycin and analogs

	Inactivating* enzyme	Paromomycin	9	10	11
Staphylococcus aureus FDA 209P		3.1	6.2	50	50
Escherichia coli B		6.2	12.5	50	100
Escherichia coli K-12-R112	APH (3′) I	250	62.5	250	250
Escherichia coli K-12-W677	ANT (2″)	250	125	250	250
Proteus sp-1		500	125	250	250

* For abbreviation of the inactivating enzymes, see S. MITSUHASHI; L. ROSIVAL and V. KRCMERY: Drug inactivating enzymes and antibiotic resistance. p. 115, Springer-Verlag, Berlin, 1975.

The dimer of 3,4-di-O-acetyl-2-deoxy-2-nitroso-6-O-tosyl-α-D-glucopyranosyl chloride[5] was condensed with compound **6** in dimethylformamide at 20°C for 50 hours to give, with a 44% yield, the 4-O-(3,4-di-O-acetyl-2-oximino-6-O-tosyl-α-D-arabino-hexopyranosyl)-N-O-acetylated pseudotrisaccharide (**7**) (m.p. 152~153°C; $[\alpha]_D$ +18°, $CHCl_3$). Deoximation with levulinic acid followed by sodium borohydride reduction[6] of the intermediate 2′-ketone and O-acetylation gave, with a 30% yield, the 4-O-(2,3,4-tri-O-acetyl-6-O-tosyl-α-D-glucopyranosyl)-N-O-acetylated pseudotrisaccharide (**8**) (m.p. 171~172°C) as the main product. Treatment of the latter with sodium azide, followed by catalytic hydrogenation over 10% palladium-carbon and deprotection with hot aqueous sodium hydroxide[7] gave a crude mixture that was chromatographed on a cation-exchange resin column. The main product, namely 4-O-(6-amino-6-deoxy-α-D-glucopyranosyl)-5-O-[3-O-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-2-deoxystreptamine (**9**) (m.p. 190°C dec.; $[\alpha]_D$ +46°, H_2O) was obtained in an overall yield of 6%. The structure assigned to **9** was confirmed by ^1H and ^{13}C N.M.R. spectrometry and by chemical degradation. Hydrolysis with dilute acid gave 4-O-(6-amino-6-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine (m.p. 190°C dec.; $[\alpha]_D$ +90°, H_2O) and neobiosamine, isolated as the hydrochlorides and identified by direct comparison with authentic samples obtained respectively by hydrolysis of kanamycin A[8] and of paromomycin[9].

A second biologically active compound was obtained as a by-product (**10**) (m.p. 173~175°C; $[\alpha]_D$ +29°, H_2O) in a 2% overall yield and was identified on the basis of its H N.M.R. spectrum as the 2′-manno-analog of **9**, resulting from the non-stereoselective sodium borohydride reduction of the 2′-ketone[10].

In order to investigate the effect of inversion of stereochemistry at the 4′-position on biological activity, the galacto-analog of **9** was also synthesized by the above-described procedure. Starting from the dimer of 3,4-di-O-acetyl-2-deoxy-2-nitroso-6-O-tosyl-α-D-galactopyranosyl chloride (m.p. 113°C; $[\alpha]_D$ +99°, $CHCl_3$) and compound **6**, the galacto-analog (**11**) (m.p. 180~182°C; $[\alpha]_D$ +40°, H_2O) was obtained in a 4% overall yield*.

Among these novel semisynthetic aminoglycoside antibiotics having different 6-amino-6-deoxy-glycopyranosyl units α-linked to the 4-position of the pseudotrisaccharide **5**, the gluco-analog **9** shows similar or slightly reduced potency when compared with paromomycin against sensitive organisms, and a two-fold increased activity against some resistant strains of gram-negative bacteria, while the manno- and galacto-analogs show a weak antibacterial activity, as shown in Table 1.

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* All new compounds gave correct microanalyses and exhibited N.M.R. spectral characteristics that were in accord with their structures.

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