Communications to the editor

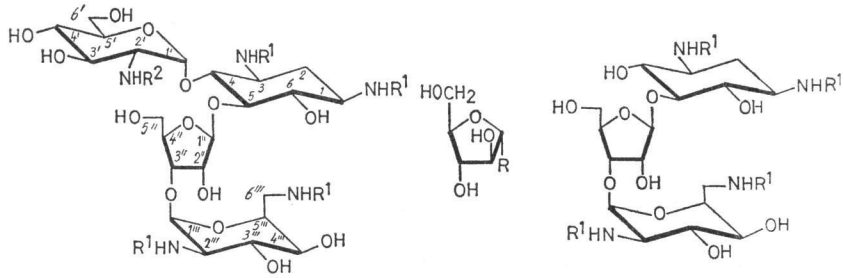
SEMISYNTHETIC AMINOGLYCOSIDE ANTIBIOTICS. I. NEW REACTIONS OF PAROMOMYCIN AND SYNTHESIS OF ITS 2′-N-ETHYLDERIVATIVE[1]

Sir:

The aminoglycoside antibiotics are useful chemotherapeutic agents because of their activity against gram-negative bacteria not readily susceptible to other antibiotics. Their clinical use has led to a growing number of resistant bacterial strains, whose resistance usually results from enzymatic inactivation of the antibiotic. In the case of paromomycin (**1**), such inactivation includes acetylation of one of the amine groups attached to carbons 2′ and 3, and phosphorylation of the hydroxyl groups attached to carbons 3′ and 5″[2]. The purpose of our investigation in this field was the synthesis of novel derivatives and analogues modified at the sites of bacterial inactivation with the specific aim of broadening their antibacterial activity.

We now report the synthesis of 2′-N-ethylparomomycin, which shows a broader antibacterial spectrum of activity when compared with paromomycin. We have found that addition of an excess of acetic anhydride to an aqueous methanolic solution containing equimolar amounts of paromomycin free base and of hydrochloric acid gives 1, 3, 2‴, 6‴-tetra-N-acetylparomomycin (**2**) (m.p. 200°C dec.; $[\alpha]_D$ +60.5°, MeOH) as the major product in 57% yield after chromatography. The selectivity observed in the acetylation reaction is probably due to differences in basicity of the amino groups of paromomycin as well as different rates of reaction due to steric factors.

The structure of **2** was established by chemical degradation. Nitrous acid deamination gave 2,5-anhydro-D-mannose (**3**)[3] and a tetra-N-acetylpseudotrisaccharide (**5**) (m.p; 180~190°C dec.; $[\alpha]_D$ −18.5°, MeOH) which were separated by column chromatography. It is known that the deamination of glycosides of 2-amino-2-deoxypyranoses, in which the amine function is equatorial, results in ring contraction and concomitant cleavage of the glycosidic linkage[3]. Compound **3**, obtained as a syrup, was then reduced with buffered sodium borohydride[4] to give crystalline 2,5-anhydro-D-mannitol (**4**) (m.p. 98~99°C; $[\alpha]_D$ +56°, H_2O)[5]. Compounds **3** and **4** were identified by direct comparison with authentic samples obtained by the deamination-reduction sequence on methyl 2-amino-2-deoxy-α-D-glucopyranoside as previously reported[4]. Treatment of **5** with hot aqueous sodium hydroxide[6] gave a pseudotrisaccharide (**6**), isolated as the amorphous sulphate (m.p.200~210°C dec.; $[\alpha]_D$ +5.5°, H_2O), that shows very weak antibacterial activity. Compound **6** was then hydrolysed with 0.4 N methanolic hydrogen chloride to give 2-deoxystreptamine and a mixture of α- and β-methyl neobiosaminides as the hydrochlorides, identified by direct comparison with authentic samples obtained by hydrolysis



1 : $R^1 = R^2 = H$ 3 : R = CHO 5 : R^1 = Ac

2 : R^1 = Ac, R^2 = H 4 : $R = CH_2OH$ 6 : R^1 = H

7 : R^1 = H, R^2 = Et

Table 1. The minimum inhibitory concentrations (mcg/ml) of paromomycin (**1**) and 2′-N-ethyl-paromomycin (**7**)

Strain	Inactivating enzyme*	**1**	**7**
Staphylococcus aureus 209P		3.1	3.1
Escherichia coli B		6.2	25
Escherichia coli K12-R112	APH (3′)-I	250	125
Escherichia coli K12-W677	ANT (2″)	250	125
Salmonella abortus equi		25	25

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

of paromomycin[7]. The structure assigned to compound **6**, namely, 5-O-[3-O-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-2-deoxystreptamine, was also confirmed by its ^1H and ^{13}C N.M.R. spectra. Pseudotrisaccharide **6** has been recently found as a minor component in commercial samples of neomycin[8] and it has also been obtained by sequential oxidative and β-elimination degradation of neomycin B and paromomycin[9].

1,3,2‴,6‴-Tetra-N-acetylparomomycin (**2**) is a useful intermediate for the synthesis of a number of novel N-2′-alkyl derivatives by reductive N-alkylation followed by de-N-acetylation. As an example of general procedure the synthesis of **7** is reported. A cooled aqueous solution of **2** at pH 8.5, in the presence of an excess of acetaldehyde was treated with sodium borohydride. To the reaction mixture sodium hydroxide was added and refluxed for six hours. After cooling the solution was extracted with 10% benzaldehyde in *n*-butanol; the organic phase was then extracted with 0.1 N aqueous sulphuric acid to give 2′-N-ethylparomomycin as crude sulphate. Chromatography on carbon-diatomaceous earth column, using water as eluent, gave, in a 46% yield, pure 2′-N-ethylparomomycin (**7**) sulphate (m.p. 285°C dec.; $[\alpha]_D$ +36°, H_2O), whose structure was also confirmed by ^1H and ^{13}C N.M.R. spectra.* 2′-N-Ethylparomomycin shows similar potency, when compared with paromomycin, against sensitive organisms and a two fold increased activity against some resistant strains of gram-negative bacteria, as shown in Table 1.

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

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