Communications to the editor

TRANSGLYCOSYLATION OF NEAMINE

Sir:

The aminoglycoside antibiotics are well recognized for their potent antimicrobial activities and broad antimicrobial spectra. Among those of clinical importance at the present time are gentamicin, kanamycin, neomycin, paromomycin and streptomycin.

The clinical usefulness of these antibiotics appears to be limited by the emergence of resistant bacterial cultures which enzymatically inactivate these antibiotics[1] as well as their lack of significant oral absorption. We have investigated the preparation of glycosides of a number of aminoglycoside antibiotics in a search for derivatives with resistance to the enzyme inactivation and with oral absorption properties.

Neamine (neomycin A, a degradation product of neomycins B and C) was converted by PAN's transglycosidation reaction[2] using a reaction mixture containing 1 g neamine (free base), 4 g maltose, 1 g Clarase^R, and sufficient McILVAINE's buffer (pH 3.0) to give a total volume of 40 ml. The solution was incubated at 30°C for 4 days.

At the end of the incubation period, the reaction mixture was analyzed by paper ionophoresis (pH 1.8, 50 volts/cm, 20 min., Whatman No. 1 paper)[3], descending paper chromatography using 2-butanone - *iso*-propanol - 6.5 N NH_4OH (8 : 2 : 3)[4], gas liquid chromatography (0.75 % OV-1 on gaschrome Q, 100~120 mesh, 2 meters×3 mm column)[5], and thin-layer chromatography on silica gel with $CHCl_3$ - CH_3OH - conc. NH_4OH (1 : 3 : 2) as developing solvent*. The neamine and derivatives were detected on the chromatograms and ionopherograms by spraying with ninhydrin reagent and by bioautographs using *Staphylococcus aureus* FDA 209. Under the best conditions approximately 15 % of the neamine was converted to a slower moving compound in the chromatographic systems.

This transformation product was isolated from the reaction mixture by ion-exchange chromatography using CG-50 (NH_4^+ form) with NH_4OH as eluting solution. The isolated material had the following characteristics:

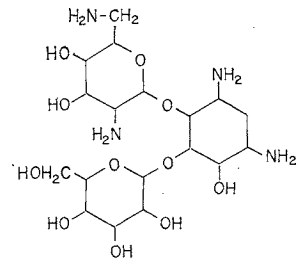
m.p. 170°C (with decomposition)

$[\alpha]_D^{29}$ +129° (concentration, 0.0625 in H_2O)

C/N ratio, 4.62 (theory for a neamine glucoside, 4.50)

After hydrolysis with 1 N H_2SO_4 at 100°C for 1 hour, the glycosylated neamine was decomposed and neamine and a sugar moiety were detected in the chromatograms of the reaction mixture. Since periodate oxidation followed by hydrolysis with 48 % HBr did not result in release of deoxystreptamine and neosamine C (or a decomposition product), we concluded that the glucose was joined to the C-5 of the deoxystreptamine or the C-4′ of the neosamine C. Further study in which the glycosyl-neamine N-acetate was oxidized with periodate and the hydrolyzed with HBr yielded deoxystreptamine (detected by thin-layer chromatography). The proposed structure is shown in Fig. 1.

Fig. 1.



5-Glucosyl-neamine

In vitro studies of 5-glucosyl-neamine are summarized in Table 1. It is evident that it is more active against the organisms tested than neamine. However, it did not inhibit an *Escherichia coli* culture resistant to neomycin B which inactivates neomycin B and kanamycin by acetylation. The 5-glucosyl-neamine did not show significant

* Private communication from Dr. W. M. STARK, Eli Lilly Research Laboratories.

Table 1. Antimicrobial activity of neamine and 5-glucosyl-neamine

Test organism	Minimal inhibitory concn., mcg/ml*	
	Neamine	5-Glucosyl-neamine
Bacillus subtilis Marburg	2	0.5
Sarcina lutea	>32	8
Staphylococcus aureus 209	2	1
Escherichia coli B	4	2
Klebsiella species A10	4	2

* As determined by 2-fold agar dilution test.

therapeutic activity in mice infected with *Streptococcus pyogenes* C203 under conditions where neomycin B was found active.

Other aminoglycoside antibiotics which were found to be acceptors in the transglycosylation system include gentamicin (C complex), kanamycin, and paromomycins.

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