PREPARATION OF TWO NEW AMINOGLYCOSIDE ANTIBIOTICS

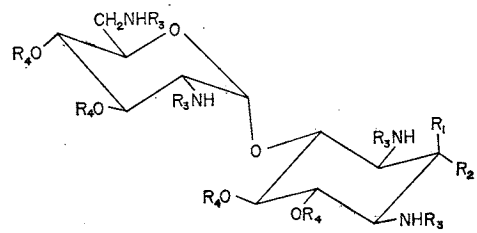
Sir:

A remarkable feature of the antibiotic neomycin[1] is the retention of considerable antibiotic activity by neamine (I, Fig. 1), a portion amounting to approximately one-half of the neomycin molecule. Indeed, the original name for neamine was neomycin A and, when first isolated, it was regarded as a component of the antibiotic complex[2]. Although the activity of neamine is always less than that of neomycin B it approximates that of neomycin C against many test organisms[3].

In view of the antibiotic activity of neamine noted above it was deemed of interest to ascertain the activity of those portions of the hybrimycins (Fig. 1) corresponding to the neamine portion of neomycin. We now report the selective hydrolysis of hybrimycins A and B to yield these two new antibiotics, for which we propose the names hybrimycins A3 (II) and B3 (III), respectively.

Hybrimycin A and B complexes were hydrolyzed separately in 0.5 N HCl for 5 hours at 85°C, the mixtures were evaporated to dryness, and hybrimycins A3 and B3, respectively, were isolated from the residues by preparative paper chromatography in the solvent system methanol – concentrated ammonium hydroxide (4 : 1).

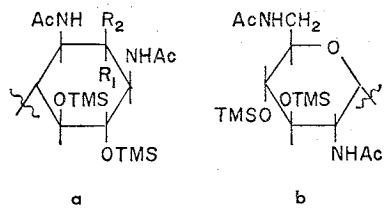
Fig. 1. Antibiotics and derivatives described.



	R_1	R_2	R_3	R_4
I (Neamine):	H	OH	H	H
II (Hybrimycin A3):	OH	H	H	H
III (Hybrimycin B3):	H	H	CH_3CO	H
IV:	H	OH	CH_3CO	H
V:	OH	H	CH_3CO	H
VI:	H	H	CH_3CO	$Si(CH_3)_3$
VII:	H	$OSi(CH_3)_3$	CH_3CO	$Si(CH_3)_3$
VIII:	$OSi(CH_3)_3$	H	CH_3CO	$Si(CH_3)_3$
IX:				

We recently reported the isolation of a mutant of *Streptomyces fradiae* incapable of synthesizing neomycin[4] in the absence of added 2-deoxystreptamine (1,3-diamino-1,2,3-trideoxy-*scyllo*-inositol), the aminocyclitol subunit of the antibiotic. This mutant was also shown[4] to convert two other aminocyclitols, streptamine (1,3-diamino-1,3-dideoxy-*scyllo*-inositol, a portion of streptomycin) and 2-epistreptamine (1,3-diamino-1,3-dideoxy-*myo*-inositol, part of the antibiotic spectinomycin) into four new antibiotics: hybrimycins A1 and A2 from streptamine and hybrimycins B1 and B2 from 2-epistreptamine.

The structures of the new antibiotics were established by methods employed earlier[4]. Comparison of the mass spectra of tetra-N-acetyl-tetra-O-trimethylsilyl-hybrimycins A3 (VIII) and B3 (IX) with that of the corresponding derivative of neamine (VII)[4,5] showed that numerous peaks including the molecular ion M (m/e 866 *vs* 788) were shifted 88 m. u. higher, corresponding to an additional trimethylsilyloxy group. Since the peak corresponding to the aminocyclitol moiety (**a**) was shifted (m/e 461 *vs* 373) while that arising from the aminosugar moiety (**b**, m/e 389) was not, the additional functional group must be located on the aminocyclitol moiety



a b

m/e 461 (R_1 or R_2=OTMS) m/e 389
m/e 373 (R_1=R_2=H)

Table 1. Properties of N-acetyl derivatives

Antibiotic derivatized	$[\alpha]_D^{22}$ (c 0.01, in H_2O)	Rf*
Neamine	76.2°	0.32
Hybrimycin A3	65.2°	0.23
Hybrimycin B3	83.5°	0.27

* Rf values for paper chromatography in 1-butanol-pyridine-water (6 : 4 : 3).

Table 2. Antibacterial activity of hybrimycins A3 and B3.

Test organism*	MIC (μg/ml)**		
	Neamine	Hybrimycin A3	Hybrimycin B3
Staphylococcus aureus OSU 284	6.4	50	200
Staphylococcus aureus ATCC 151	3.2	50	200
Staphylococcus hemolyticus UC 152	25.0	100	200
Escherichia coli ATCC 26	50	200	>200
Klebsiella pneumoniae ATCC 10031	6.4	100	>200
Salmonella schottmuelleri ATCC 9149	50	200	>200
Bacillus subtilus UC 564	3.0	25	50

* OSU, Ohio State University ; UC, The Upjohn Co. ; ATCC, American Type Culture Collection.

** The antibiotic preparations were dissolved in brain-heart infusion broth and twofold decrements were made from 200 μg/ml. An 18-hour culture of each test organism was diluted 1 : 2000 and 1 drop of the diluted suspension was added to 1 ml of broth with the antibiotic. The test system was estimated to contain 10^5 organisms per ml. All tubes were incubated at 37℃ for 24 hours. The minimum inhibitory concentration (MIC) is the lowest concentration of antibiotic which prevented growth.

of hybrimycins A3 and B3. The nmr spectra of hybrimycins A3 and B3 show no absorption by methylene protons (ABX_2 multiplet near δ 2.0), characteristic of deoxystreptamine derivatives[6], conclusively locating the additional hydroxyl group on the carbon between the amino groups of the aminocyclitol moiety. The ORD plain curves in the region 240 to 600 mμ, as well as the specific rotation (see Table 1), for tetra-N-acetylneamine (IV) lie approximately equidistant between those for tetra-N-acetylhybrimycins A3 (V) and B3 (VI), in accord with the expected rotatory effects of opposite absolute configurations at C-2 of the aminocyclitol moiety. The greater mobility of tetra-N-acetylhybrimycin B3 *vs* tetra-N-acetylhybrimycin A3 in paper chromatography is consistent with an axial hydroxyl group in the former and an equatorial hydroxyl in the latter[7]. Thus, the hybrimycin A3 and B3 structures are established as in Fig. 1.

The antibacterial properties of hybrimycins A3 and B3 are compared with those of neamine in Table 2. Hybrimycin A3 is somewhat less active than neamine and hybrimycin B3 is less active still. The relative activities mirror those of hybrimycins A (A1 plus A2) and B (B1 plus B2) *vs* neomycin (B plus C)[4]. These data indicate that the methylene group in the deoxystreptamine moiety of neamine is not essential for its antibacterial activity, and that the stereochemistry of substitution by a hydroxyl group has an influence on the antibacterial activity of the modified antibiotic. These conclusions are essentially in agreement with the structure-activity relationships drawn by MASUKAWA and TANAKA[8] from the codon misreading activity of other aminoglycoside antibiotics.

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