SYNTHESES OF 3′- AND 4′-O-METHYLENEAMINE

Sir:

Recently we have synthesized 3′-deoxykanamycin A[1], 3′,4′-dideoxykanamycin B[2] and 3′,4′-dideoxyneamine[3], and it has been found that these 3′-deoxy and 3′,4′-dideoxy variants are remarkably active against resistant bacteria. The syntheses were designed to take advantage of the mechanism of resistance as elucidated by H. UMEZAWA *et al.*[4,5,6] They found that resistant bacteria produce enzymes which inactivate aminoglycosidic antibiotics such as kanamycins, paromomycins and neomycins by blocking a specific hydroxyl group. Kanamycins were found to be inactivated by enzymic phosphorylation of their 3′-hydroxyl groups. Inactivation of neamine was found to be due to the phosphorylation of the 3-hydroxyl group of the 2, 6-diamino-2, 6-dideoxy-D-glucose moiety.

On the other hand, we were interested in blocking the 3′-hydroxyl group with a small alkyl group and so synthesized 3′-O-methylkanamycin A[1], which was almost without antibacterial activity. Since removal of both 3′- and 4′-hydroxyl groups from kanamycin B and neamine gave compounds active against resistant bacteria, we were interested in the effect on antibiotic activity of blocking the 3′- and 4′-hydroxyl groups of neamine with a methyl group. This paper describes the syntheses of 3′- and 4′-O-methylneamines and their antibiotic activity.

To a solution of 5,6-O-cyclohexylidene-tetra-N-methoxycarbonylneamine[3] (**1**) in acetone, 30 % aqueous sodium hydroxide and dimethyl sulfate were added; after 1 hour, the starting material disappeared and a mixture of mono-O-methyl derivatives [**2** and **4**; Rf 0.26 on TLC with silica gel and chloroform – 2-propanol, 15 : 1)] appeared as major products accompanied by minor amounts of the di-O-methyl derivative (Rf 0.5). Fortunately, the methylation was virtually free from N-methylation. The mixture of mono-O-methyl derivatives (yield ~50 %) was acetylated and chromatographed on silica gel with benzene – ethyl acetate (2 : 5) containing 0.25 % triethylamine to afford the 4′-O-acetyl-3′-O-methyl and 3′-O-acetyl-4′-O-methyl derivatives (**3** and **5**) in yields of 42 % and 52 %, respectively. Compound **3**: $[\alpha]_D^{20}$ +46° (*c* 1, methanol), Rf 0.26 (TLC with silica gel and benzene – ethyl acetate, 1 : 4); NMR (in $CDCl_3$) τ 8.2~8.8 (~11H, broadened signal, cyclohexylidene protons and H-2_{ax}), 7.88 (3H, s, OAc), 6.55 (3H, s, OCH_3), 6.31 (12H, s, $NHCOOC\underline{H}_3$), 5.12 (1H, t, *J* 9.5 Hz, H-4′), 4.80 (1H, d, *J* 3.5 Hz, H-1′). Compound **4**: $[\alpha]_D^{20}$ +44° (*c* 1, methanol), Rf 0.39 (with the same system as above); NMR (in $CDCl_3$) τ 8.2~8.8 (~11H), 7.92 (3H, s, OAc), 6.53 (3H, s, OCH_3); 6.35 (3H), 6.32 (6H) and 6.28 (3H) (singlets, $NHCOOC\underline{H}_3$), 4.83 (in $CDCl_3$-D_2O, 1H, t, *J*~10 Hz, H-3′), 4.80 (in $CDCl_3$-D_2O, 1H, d, *J*~3.5 Hz, H-1′).

Deacetylation and demethoxycarbonylation of **3** with barium hydroxide in refluxing aqueous methanol followed by decyclohexylidenation with 1 N hydrochloric acid and purification by resin column chromatography (Amberlite CG-50, eluted with 0.15 N ammonia) gave 3′-O-methylneamine (**6**) in a yield of 76 %, $[\alpha]_D^{20}$ +122° (*c* 1, water), $\Delta[M]_{TACu}^{436}$ +670°; NMR (in D_2O) τ 8.80 (1H, q, *J*~12 Hz, H-2_{ax}), 8.02 (1H doublet of triplets, *J* 4,4 and 13 Hz, H-2_{eq}), 6.40 (3H, s, OCH_3), 4.71 (1H, d, *J* 3.5 Hz, H-1′).

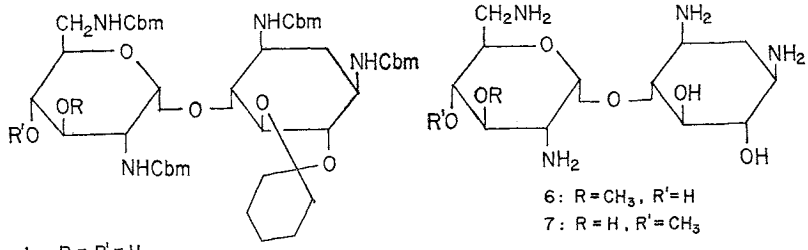
Analogously, **5** gave 4′-O-methylneamine (**7**) in a yield of 83 %, $[\alpha]_D^{20}$ +126° (*c* 1, water), $\Delta[M]_{TACu}^{436}$ −210°; NMR (in D_2O) τ 8.82 (1H, q, *J*~12 Hz, H-2_{ax}), 8.05 (1H doublet of triplets, *J* 4.4 and 13 Hz, H-2_{eq}), 6.46 (3H, s, OCH_3), 4.74 (1H, d, *J* 3.5 Hz, H-1′).

The positions of the O-methyl groups in **6** and **7** were determined by their $\Delta[M]_{TACu}$ values[7], as described above. In the case of

Table 1. Antibacterial spectra

Test organisms*	Minimal inhibitory concentration (mcg/ml)		
	3′-O-Methyl-neamine	4′-O-Methyl-neamine	Neamine
Staphylococcus aureus FDA 209P	500	100	2
Salmonella typhosa T63	250	50	8
Escherichia coli K-12	1,000	100	1
〃 K-12 ML1630	1,000	>1,000	>1,000

* Nutrient bouillon, 37℃, 18 hours.



1: R = R' = H
2: R = CH_3, R' = H
3: R = CH_3, R' = Ac
4: R = H, R' = CH_3
5: R = Ac, R' = CH_3

6: R = CH_3, R' = H
7: R = H, R' = CH_3

7, tetrammine copper (II) sulfate (TACu) can form copper complexes at both 1-NH_2 and 6-OH, and at 2'-NH_2 and 3'-OH, resulting in a small $\Delta[M]_{TACu}$ value by the intramolecular compensation of the Δ[M] contributions of the two complexes opposite in sign and approximately equal in magnitude; in the case of **6**, TACu can form a complex only at 1-NH_2 and 6-OH.

The antibacterial activity of 3'-O-methylneamine (**6**), 4'-O-methylneamine (**7**) and neamine is shown in Table 1. These results show that blocking of a 3'-hydroxyl group with a methyl group causes a marked decrease in antibacterial activity of the parent antibiotic neamine except for the slight activity against resistant *Escherichia coli* 1630. A similar tendency was also seen in the case of 3'-O-methylkanamycin.[1] Blocking of the 4'-hydroxyl group with methyl also causes a marked decrease in the antibacterial activity of neamine. However, it should be noted that blocking of the 4'-hydroxyl group does not bring about activity against *E. coli* 1630 carrying R factor and that the activity decrease of 4'-O-methylneamine is less than that of 3'-O-methylneamine.

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