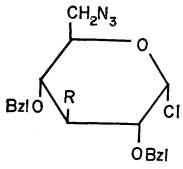
SYNTHESIS OF 3′-DEOXYKANAMYCIN EFFECTIVE AGAINST KANAMYCIN-RESISTANT *ESCHERICHIA COLI* AND *PSEUDOMONAS AERUGINOSA*

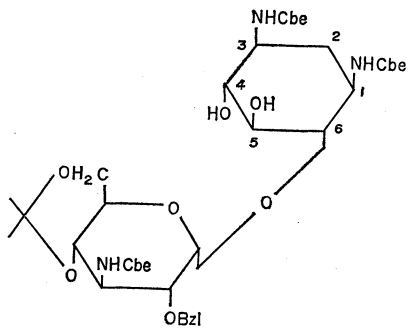
Sir:

Treatment of resistant bacterial infections is the most important subject of chemotherapeutic studies. H. UMEZAWA *et al.*[1,2,3] have clarified the mechanism of resistance of *E. coli* K12 carrying R factor and resistant *Pseudomonas*: these organisms produce an enzyme transfering phosphate from ATP to 3-hydroxyl group of 6-amino-6-deoxy-D-glucose, 2,6-diamino-2,6-dideoxy-D-glucose or D-glucosamine moiety of deoxystreptamine-containing antibiotics and as the result of the phosphorylation, the antibiotics such as kanamycins, paromomycins and neomycins are inactivated. This mechanism of inactivation suggested us that blocking or removal of the hydroxyl group which is phosphorylated will give new compounds effective against the resistant organisms. Thus, the authors attempted, for the first approach, to prepare 3′-O-methylkanamycin (I) by a synthetic method. It was prepared by condensation of newly prepared 6-azido-2,4-di-O-benzyl-6-deoxy-3-O-methyl-α-D-glucopyranosyl chloride (II) with 6-O-(2-O-benzyl-3-deoxy-3-ethoxycarbonylamido-4,6-O-isopropylidene-α-D-glucopyranosyl)-N,N′-diethoxycarbonyl-2-deoxystreptamine (III) followed by reduction of the azido to an amino group and removal of the three kinds of protecting groups. The structures of the condensation product and 3′-O-methylkanamycin derived from it were proved by physical methods and elemental analysis although they were shown to be a mixture of 4-O-α-D- and 4-O-β-D-glycosyldeoxystreptamine isomers in the ratio of approximately 1:1. The condensation product: m.p. 226°C, $[\alpha]_D^{15}$ +92.6° (*c* 0.4, $CHCl_3$); Found: C 59.23, H 6.78, N 8.11. Calcd. for $C_{52}H_{70}N_6O_{17}$: C 59.42, H 6.71, N 8.00 %; IR: 2100 cm^{-1} (N_3); NMR (in dimethylsulfoxide-d_6): τ 8.94 (3H t, $CH_2C\underline{H}_3$), 8.86 (6H t, $CH_2C\underline{H}_3$), 8.76 (3H s, Isop.), 8.63 (3H s, Isop.), 2.72, 2.73, 2.76 (each 5H s, Phenyl). 3′-O-Methylkanamycin: m.p. 167~169°C, $[\alpha]_D^{15}$ +106° (*c* 0.5, H_2O); $Rf_{kanamycin}$ 2.2 (ppc with *n*-butanol - pyridine - water - acetic acid 6:4:3:1); Found: C 46.20, H 7.47, N 11.00. Calcd. for $C_{19}H_{38}N_4O_{11}$: C 45.78, H 7.68, N 11.24 %; NMR (in D_2O); τ 6.37 (3H s, OCH_3), 4.95 (1H d, J~3 Hz, H-1 of 3-amino-3-deoxy-α-D-glucoside moiety), 4.63 (~0.5 H d, J~3 Hz, H-1 of 3-O-methyl-α-D-glycoside moiety attached to C-4 of 2-deoxystreptamine). The prepared 3′-O-methylkanamycin, that is, a mixture of 4-O-(6-amino-6-deoxy-3-O-methyl-α-D- and β-D-glucopyranosyl)-6-O-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamines in the ratio of approximately 1:1, however, showed no antibacterial activity except a slight activity against *Bacillus subtilis*.

This result suggests that the approach of 3′-O-methylkanamycin to bacterial ribosomes would be inhibited by the presence

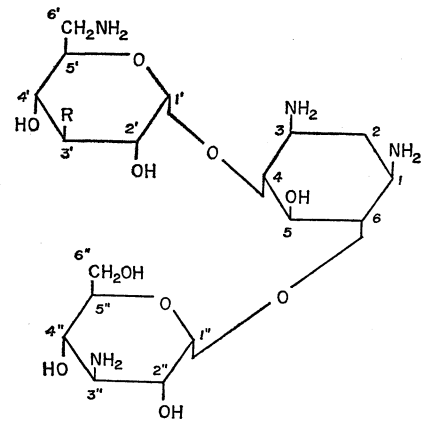


II R: OCH_3
V R: H



III

Bzl: $CH_2C_6H_5$ Cbe: $CO_2C_2H_5$



Kanamycin	R:	OH
3′-O-Methylkanamycin (I)	R:	OCH_3
3′-Deoxykanamycin (IV)	R:	H

Table 1. Antibacterial spectra of 3′-deoxykanamycin and kanamycin

Test organisms*	Minimal inhibitory concentration (mcg/ml)	
	3′-Deoxy-kanamycin	Kanamycin
Staphylococcus aureus FDA 209P	1.56	1.56
Escherichia coli NIHJ	3.12	1.56
〃 K-12 CS-2	3.12	1.56
〃 K-12 ML 1629	3.12	>50
〃 K-12 ML 1630	3.12	>50
〃 K-12 ML 1410	3.12	0.78
Salmonella typhosa T-63	0.78	0.39
Pseudomonas aeruginosa A3	3.12	50
〃 No. 11	12.5	>50
〃 No. 12	12.5	50
〃 No. 39	12.5	>50
〃 No. 45	1.56	50
〃 No. 67	12.5	>50
Proteus rettgeri GN 311	12.5	6.25
〃 GN 466	6.25	3.12

* Nutrient agar, 37℃, 18 hours.

of the bulky methoxyl group. Therefore, the synthesis of 3′-deoxykanamycin was attempted. The synthesized 3′-deoxykanamycin (**IV**), as described next, showed antibacterial activity of the similar strength as kanamycin and moreover showed the activity against *E. coli* 1629, 1630 carrying R factor and *P. aeruginosa* as shown in Table 1. It is thought that this principle could be applied to prepare the analogues of other aminoglycosidic antibiotics active against resistant strains.

The preparation of 3′-deoxykanamycin is similar to that of 3′-O-methylkanamycin, that is, newly prepared 6-azido-2,4-di-O-benzyl-3,6-dideoxy-α-D-glucopyranosyl chloride (**V**) was condensed with **III** in anhydrous benzene-dioxane (3 : 1) at reflux for 15 hours in the presence of mercuric cyanide-Drierite and the main condensation product, 4-O-(6-azido-2,4-di-O-benzyl-3,6-dideoxy-α-D-glucopyranosyl)-6-O-(2-O-benzyl-3-deoxy-3-ethoxycarbonylamido-4, 6-O-isopropylidene-α-D-glucopyranosyl)-N, N′-diethoxycarbonyl-2-deoxystreptamine was isolated by column chromatography in a yield of ~25 %, m.p. 263~264°C, $[\alpha]_D^{20}$ +97° (*c* 0.9, pyridine); Found: C 59.79, H 6.76, N 7.91. Calcd. for $C_{51}H_{68}N_6O_{16}$: C 59.98, H 6.71, N 8.23 %; IR: 2100 cm^{-1} (N_3); NMR (in pyridine-d_5): τ 8.90 (3H t, $CH_2C\underline{H}_3$), 8.87 (3H t, $CH_2C\underline{H}_3$), 8.85 (3H t, $CH_2C\underline{H}_3$), 8.57 (3H s, Isop.), 8.54 (3H s, Isop.), 2.4~2.7 (15H m, Phenyl). The condensation product was then deacetonated with 80 % acetic acid and the azido group was reduced with RANEY nickel and hydrogen to an amino group. The resulting product, after acylation of the amino group with carboethoxy chloride, was debenzylated with palladium black and hydrogen and then de-N-acylated with barium hydroxide. Purification of the final product with column chromatography of Amberlite CG50 (NH_4^+ form) and 0~0.3 N ammonia yielded 3′-deoxykanamycin (**IV**), $[\alpha]_D^{20}$ +146° (*c* 0.2, H_2O); $Rf_{kanamycin}$ 2.2 (ppc with *n*-butanol - pyridine - water - acetic acid 6 : 4 : 3 : 1). Found: C 46.10, H 7.70. Calcd. for $C_{18}H_{36}N_4O_{10}$: C 46.14, H 7.75 %. Hydrolysis with 6 N hydrochloric acid and periodate oxydation of **IV** confirmed the structure of **IV** as expected.

SUMIO UMEZAWA
TSUTOMU TSUCHIYA
RYUJIRO MUTO
YOSHIO NISHIMURA

Dept. of Applied Chemistry,
Faculty of Engineering,
Keio University,
Koganei-shi, Tokyo, Japan

HAMAO UMEZAWA

Institute of Microbial Chemistry,
Shinagawa-ku, Tokyo, Japan

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