Communications to the editor

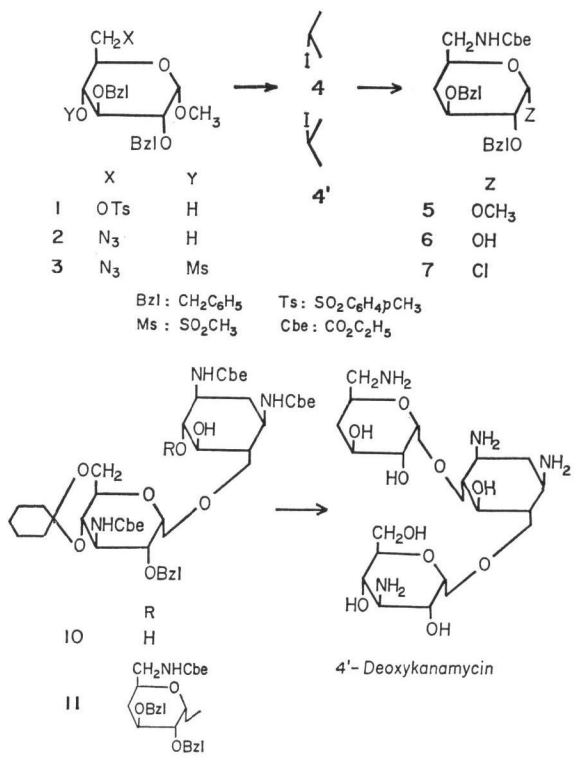
SYNTHESIS OF 4′-DEOXYKANAMYCIN AND ITS RESISTANCE TO KANAMYCIN PHOSPHOTRANSFERASE II

Sir:

As reported in a previous paper[1], resistance to kanamycin, neomycin and paromomycin of resistant bacteria is mainly due to phosphotransferases which phosphorylate the 3′-hydroxyl group of these antibiotics. However, there remained the possibility that the 3′-phosphorylated products might be produced by migration of the phosphate group from the 4′- to the 3′-hydroxyl group, the former being the initial phosphorylating position. Therefore, we synthesized 4′-deoxykanamycin and studied the phosphorylation of this derivative by kanamycin-neomycin phosphotransferases I[2] and II[3].

In order to make an unequivocal preparation of 4′-deoxykanamycin*, this deoxy derivative was synthesized by coupling a protected derivative of 6-O-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine (3AD) with a protected glycosyl chloride of 6-amino-4, 6-dideoxy-D-*xylo*-hexose.

Methyl 2, 3-di-O-benzyl-α-D-glucopyranoside[4] was selectively tosylated to give the 6-O-tosyl derivative **1** in 72% yield, $[\alpha]_D^{18}$+16.4° (*c* 1.9, $CHCl_3$). [Calcd. for $C_{28}H_{32}O_8S$: C, 63.62; H, 6.10; S, 6.07. Found: C, 63.65; H, 6.18; S, 6.23.] Treatment of **1** with sodium azide in DMF gave the 6-azido derivative **2** in 86% yield, $[\alpha]_D^{18}$+20° (*c* 2.0, $CHCl_3$), ir 2110 cm^{-1} (N_3). [Calcd. for $C_{21}H_{25}N_3O_5$: C, 63.14; H, 6.31; N, 10.52. Found: C, 62.68; H, 6.20; N, 10.79.] Mesylation of **2** and treatment of its product **3** with sodium iodide in DMF gave, after column-chromatographic separation, two products, which were proved, by nmr analysis, to be the 4-deoxy-4-iodogluco isomer (**4**, 43% from **2**) and the galacto isomer (**4′**, 30% from **2**). **4**: $[\alpha]_D^{20}$− 21° (*c* 4.6, $CHCl_3$). [Calcd. for $C_{21}H_{24}IN_3O_4$: C, 49.52; H, 4.75; I, 24.92; N, 8.25. Found: C, 49.76; H, 4.79; I, 24.72; N, 8.28.] **4′**: $[\alpha]_D^{20}$+84° (*c* 0.6, $CHCl_3$). [Found: C, 49.83; H, 4.78; I, 24.72; N, 8.50.] Treatment of **4** or **4′** with Raney nickel and hydrogen resulted in replacement of the iodine atom with hydrogen and reduction of the azido to an amino group. Treatment of the crude single product thus obtained with ethoxycarbonyl chloride gave methyl 2, 3-di-O-benzyl-4, 6-dideoxy-6-ethoxycarbonylamino-α-D-*xylo*-hexopyranoside (**5**) in a yield of approximately 60% from either **4** or **4′**, $[\alpha]_D^{18}$+56° (*c* 1, $CHCl_3$). [Calcd. for $C_{24}H_{31}NO_6$: C, 67.11; H, 7.28; N, 3.26. Found: C, 67.31; H, 7.11, N, 3.36.] Hydrolysis of **5** with 2N hydrochloric acid-acetic acid (2:1) at 80°C for 7 hours gave the free sugar derivative **6** as needles (from *n*-hexane) in 50% yield, mp 79~80°C, $[\alpha]_D^{20}$+31° (*c* 1.7, $CHCl_3$). [Calcd. for $C_{23}H_{29}NO_6$: C, 66.49; H, 7.04; N, 3.37. Found: C, 66.55; H, 7.18; N, 3.49.]



* The preparation of 4′-deoxykanamycin from kanamycin was presented at the 192nd Meeting of the Japan Antibiotics Research Association, March 22, 1974.

Treatment of **6** with thionyl chloride gave the corresponding α-D-glycosyl chloride **7** as a syrup in yield of 85%, $[\alpha]_D^{18}+58°$ (*c* 1.0, $CHCl_3$), nmr: τ 3.90 (1H d, *J* 3.5 Hz, H-1). [Calcd. for $C_{23}H_{28}NO_5Cl$: C, 63.66; H, 6.50; N, 3.23; Cl, 8.17. Found: C, 63.80; H, 6.48; N, 3.39; Cl, 8.40].

The 3AD derivative was prepared as follows: The tri-N-ethoxycarbonyl derivative[5] of 3AD was treated with 1, 1-dimethoxycyclohexane in a similar manner to that previously reported[6], to give the 4, 5;4′, 6′-di-O-cyclohexylidene derivative **8** in 83% yield, $[\alpha]_D^{24}+65°$ (*c* 1.4, $CHCl_3$). [Calcd. for $C_{33}H_{53}N_3O_{13}$: C, 56.64; H, 7.63; N, 6.01. Found: C, 56.84; H, 7.82; N, 5.84.] Benzylation of **8** with benzyl bromide-barium oxide-barium hydroxide in DMF gave the 2′-O-benzyl derivative **9** in 63% yield, $[\alpha]_D^{24}+61.3°$ (*c* 0.8, pyridine). [Calcd. for $C_{40}H_{59}N_3O_{13}$: C, 60.82; H, 7.53; N, 5.32. Found: C, 61.04; H, 7.94; N, 5.22.] Decyclohexylidenation of **9** with 60% acetic acid followed by selective cyclohexylidenation in a similar manner to that previously reported[6] gave the 4′, 6′-O-cyclohexylidene derivative **10** in 40% yield from **9**, $[\alpha]_D^{18}+52°$ (*c* 0.7, pyridine); $\Delta[M]_{436(CuAm)}^{18}+2040°$ (*c* 0.2 in water and CuAm[7]). [Calcd. for $C_{34}H_{51}N_3O_{13}$: C, 57.53; H, 7.24; N. 5.92. Found: C, 57.28; H, 7.19; N, 5.94.]

Condensation of **7** and **10** was carried out in a similar manner to that reported[8] and the column-chromatographic separation of the condensation products gave the 4-O-α-D-glycosyl derivative **11** in 23% yield from **10**, $[\alpha]_D^{18}+60°$ (*c* 1.0, $CHCl_3$). [Calcd. for $C_{57}H_{78}N_4O_{18}$: C, 61.83; H, 7.10; N, 5.06. Found: C, 61.64; H, 6.81; N, 5.04.] Removal of cyclohexylidene groups with 50% acetic acid followed by removal

Table 1. Antibacterial spectra of 4′-deoxykanamycin and kanamycin

Test organisms*	Minimal inhibitory concentration (mcg/ml)	
	4′-Deoxykanamycin	Kanamycin
Staphylococcus aureus FDA 209P	0.78	0.78
Sarcina lutea PCI 1001	25	6.25
Bacillus subtilis NRRL B-558	<0.2	<0.2
Klebsiella pneumoniae PCI 602	0.78	0.78
Salmonella typhosa T-63	0.78	0.39
Escherichia coli NIHJ	1.56	1.56
〃 K-12	1.56	1.56
〃 〃 ML 1629	>100	>100
〃 〃 ML 1630	>100	>100
〃 〃 ML 1410	6.25	1.56
〃 〃 〃 R 81	>100	>100
〃 〃 LA 290 R 55	100	100
〃 〃 〃 R 56	25	12.5
〃 〃 W 677	0.78	1.56
〃 〃 JR 66/W 677	>100	>100
Pseudomonas aeruginosa A3	1.56	50
〃 No. 12	6.25	25
〃 GN 315	>100	>100
〃 TI 13	6.25	>100
〃 99	25	>100
Proteus rettgeri GN 311	6.25	6.25
〃 GN 466	6.25	3.12
Mycobacterium smegmatis ATCC 607**	1.56	0.78

* Agar dilution streak method (nutrient agar, 37°C, 18 hours).
** 48 hours.

of the benzyl groups with palladium black and hydrogen gave tetra-N-ethoxycarbonyl derivative **12** in 81 % yield from **11**, $[\alpha]_D^{18}+90°$ (*c* 0.6, H_2O). [Calcd. for $C_{30}H_{52}N_4O_{18}\cdot H_2O$: C, 46.50; H, 7.03; N, 7.23. Found: C, 46.87; H, 6.99; N, 6.95.] Treatment of **12** with 1 N barium hydroxide followed by purification of the crude product on a column of CM Sephadex C-25 employing 0~0.03 N ammonia gave 4′-deoxykanamycin, $[\alpha]_D^{18}+129°$ (*c* 0.5, H_2O), nmr: τ 7.5~9.2 (4H broad signals, deoxyprotons at C-2 and C-4′), 4.55 and 4.88 (each 1H d, *J* 3.5 Hz, anomeric protons). [Calcd. for $C_{18}H_{36}N_4O_{10}\cdot 2/3H_2O$: C, 44.99; H, 7.83; N, 11.66. Found: C, 44.85; H, 7.58; N, 11.58.]

The synthetic 4′-deoxykanamycin showed antibacterial activity as strong as that of the parent antibiotic, kanamycin. *Escherichia coli* K12 ML1629 which produced the phosphotransferase I[2] was resistant to this 4′-deoxy derivative as well as to kanamycin, but this derivative exhibited remarkable activity against several strains of *Pseudomonas aeruginosa* (Table 1). This suggested that *P. aeruginosa* might inactivate kanamycin in a somewhat different way from those previously known. Therefore, we studied the types of phosphotransferases in *Pseudomonas* strains, and as will be reported in another paper[9], we found that there are *Pseudomonas* strains which produce kanamycin-neomycin phosphotranferase Type-I, Type-II or both of them. Based on this observation, we examined the phosphorylation of the 4′-deoxy derivative by Type-I and Type-II enzymes.

Kanamycin-neomycin phosphotransferases I and II were prepared from *E. coli* K12 R11-2 and *E. coli* JR66/W677 and purified by affinity chromatography as reported previously[2,3]. Phosphorylation of 4′-deoxykanamycin or kanamycin was carried out at 37°C in a reaction mixture (1.0 ml) which contained 0.05 μmole of an antibiotic, 4 μmoles of adenosine triphosphate, 10 μmoles of magnesium acetate, 60 μmoles of potassium chloride, 10 μmoles of 1, 4-dithiothreitol, 0.1 ml of 1 M tris-hydrochloric acid buffer (pH 7.8) and 0.1 ml of kanamycin-neomycin phosphotransferase I or II. After a 1-hour reaction, the residual antibiotic activity was determined by the disc-plate method using *Bacillus subtilis* PCI 219 as the test organism. Kanamycin was phosphorylated by kanamycin-neomycin phosphotransferase I more rapidly that 4′-deoxykanamycin. Moreover, 4′-deoxykanamycin hardly undergoes the reaction of kanamycin-neomycin phosphotransferase II.

These results indicate that the 4′-hydroxyl group plays some role in the reaction of kanamycin-neomycin phosphotransferase I and is involved in the binding to the phosphotransferase II, and that the resistant strains producing the latter enzyme are sensitive to the 4′-deoxy derivative.

Sumio Umezawa
Yoshio Nishimura
Yoshiaki Hata
Tsutomu Tsuchiya

Department of Applied Chemistry,
Faculty of Engineering, Keio University
Hiyoshi, Yokohama, Japan

Morimasa Yagisawa
Hamao Umezawa

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

(Received April 24, 1974)

References

1) Umezawa, H.: Mechanism of inactivation of aminoglycosidic antibiotics by enzymes of resistant organisms of clinical origin. progress in Antimicrobial and Anticancer Chemotherapy Vol. 2, pp. 567~571, 1970, University of Tokyo Press: Related references are cited therein.

2) 2) Umezawa, H.; H. Yamamoto, M. Yagisawa, S. Kondo, T. Takeuchi & Y. A. Chabbert: Kanamycin phosphotransferase I: Mechanism of cross resistance between kanamycin and lividomycin. J. Antibiotics 26: 407~411, 1973

3) Yagisawa, M.; H. Yamamoto, H. Naganawa, S. Kondo, T. Takeuchi & H. Umezawa: A new enzyme in *Escherichia coli* carrying R-factor phosphorylating 3′-hydroxyl of butirosin A, kanamycin, neamine and ribostamycin. J. Antibiotics 25: 748~750, 1972

4) Bell, D. & J. Lorber: Use of the benzyl radical in syntheses of methylated sugars. I. 4 : 6-Dimethyl glucose. J. Chem. Soc. 1940: 453~455, 1940

5) UMEZAWA, H.; T. TSUCHIYA, R. MUTO & S. UMEZAWA: The synthesis of 3′-O-methylkanamycin. Bull. Chem. Soc. Japan 45: 2842~2847, 1972
6) IKEDA, D.; T. SUZUKI, T. TSUCHIYA, S. UMEZAWA & H. UMEZAWA: Syntheses of 3′, 4′-dideoxy and 3′, 4′, 5″-trideoxyribostamycin. Bull. Chem. Soc. Japan 46: 3210~3213, 1973
7) UMEZAWA, S.; T. TSUCHIYA & K. TATSUTA: Configurational studies of aminosugar glycosides and aminocyclitols by a copper complex method. Bull. Chem. Soc. Japan 39; 1235~1243, 1966
8) For example, see NISHIMURA, Y.: T. TSUCHIYA & S. UMEZAWA: Synthesis of 4-O- and 6-O-(4-amino-4-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine. Bull. Chem. Soc. Japan 46: 1263-1265, 1973
9) UMEZAWA, H.: Types of kanamycin-neomycin phosphotransferases in *Pseudomonas aeruginosa*, in preparation