SYNTHESES OF 6′-N-METHYLKANAMYCIN AND 3′, 4′-DIDEOXY-6′-N-METHYLKANAMYCIN B ACTIVE AGAINST RESISTANT STRAINS HAVING 6′-N-ACETYLATING ENZYMES

Sir:

3′,4′-Dideoxykanamycin B[1] (DKB) is active against kanamycin-resistant strains which phosphorylate the 3′-hydroxyl group of kanamycin (KM). However, DKB was inactivated by another kind of kanamycin-resistant strains. One of them is *Escherichia coli* K-12 R-5, which inactivates KM by 6′-N-acetylation.[2] More recently[3] *Pseudomonas aeruginosa* GN 315 was found to be strongly resistant to KM, kanamycin B, DKB and other related antibiotics by the same mechanism.

Modification of the 6′-amino groups of KM and DKB was, therefore, undertaken in order to make the derivatives active against the resistant strains. Here we describe the 6′-N-methylation of KM and DKB.

The initial step of the synthesis, selective 6′-N-carbobenzyloxylation was successfully effected with benzyl *p*-nitrophenyl carbonate.[4] This reagent (1.24 g) was added to a solution of KM (2 g) in aqueous dioxane (1 : 2, 80 ml) and the solution was allowed to stand at 0~5°C overnight. On paper chromatography (ppc) with 1-butanol – pyridine – water – acetic acid (6 : 4 : 3 : 1) (Solvent A), the solution showed a major spot at $Rf_{kanamycin}$ 10 accompanied by several minor spots. Evaporation of the solution gave a solid, which was treated with water. Evaporation of the aqueous layer gave a solid (3.0 g), which was charged on a column of Amberlite IRC 50 (40 ml). After the column had been washed with water, it was developed with 0.025 N ammonia. The fraction containing $Rf_{kanamycin}$ 10 was evaporated to give a solid (**1**, 1.5 g, 59 %). Recrystallization from aqueous ethanol (1 : 30) afforded 6′-N-benzyloxycarbonylkanamycin (**1**), mp 219~220°C, $[\alpha]_D^{18}$ +108° (*c* 1.4, water); ir (KBr) 1705 (amide I), 1590 (NH_2) and 1525 (amide II). [Found: C 50.26, H 6.64, N 8.83. Calcd. for $C_{26}H_{42}N_4O_{13}$: C 50.48, H 6.84, N 9.06]. The selective formation of a single isomer was demonstrated by the fact that, on repeated recrystallization, no change of the melting point was observed and that crude **2** obtained by the succeeding procedure gave a ppc pattern identical with that of pure **2**.

N-Methylation was performed as follows: To a suspension of **1** (1 g) in THF (40 ml), $LiAlH_4$ (0.61 g) was added and the suspension was refluxed for 20 hours. A paper-chromatogram (Solvent A) of an aliquot of the suspension, after treatment with water, showed a single spot ($Rf_{kanamycin}$ 1). On paper-electrophoresis (3,500 V) with a solution of formic acid – acetic acid – water (1 : 3 : 36), it also gave a single spot ($Rf_{kanamycin}$ 1). The suspension was poured into a large amount of water, and, after removal of insoluble matter by centrifugation, the aqueous layer was evaporated to give a solid (1.4 g, crude **2**). To an aqueous solution of the solid, anisaldehyde (1.3 g) was added, and the solution was stirred for 1 hour. The resulting precipitate was separated, washed with petroleum ether and dissolved in chloroform. The solution was, after washing with water, treated with 0.2 N hydrochloric acid. The acidic aqueous solution was neutralized with Dowex 1×2 (OH^- form) to pH 4 and concentrated. To the concentrate, acetone was added to give a solid (0.5 g). The solid was chromatographed successively with columns of Amberlite IRA

Chart 1.

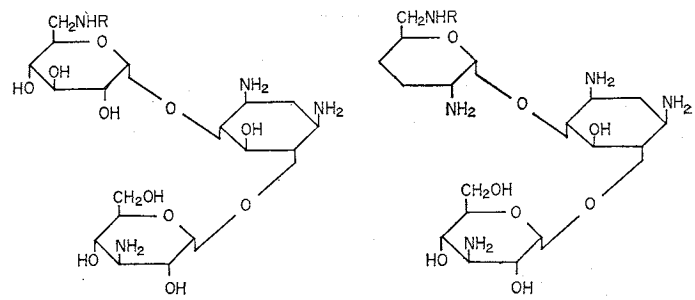

Table 1. Antibacterial spectra of 6′-N-methylkanamycin (**2**) and 3′, 4′-dideoxy-6′-N-methylkanamycin B (**4**). Antibacterial spectra of KM and DKB were also shown.

Test organisms*	Minimal inhibitory concentration (mcg/ml)			
	2	KM	**4**	DKB
Staphylococcus aureus FDA 209P	1.56	1.56	0.78	0.78
Escherichia coli K-12	0.78	0.78	1.56	1.56
〃 ML 1629	>100	>100	3.12	1.56
〃 ML 1410	3.12	1.56	3.12	1.56
〃 〃 R 81	>100	>100	3.12	1.56
〃 R 5	1.56	25	1.56	25
〃 LA 290 R 55	12.5	50	25	>100
〃 〃 R 56	3.12	12.5	6.25	25
〃 〃 R 64	3.12	12.5	6.25	25
Escherichia coli W 677	0.78	0.78	1.56	1.56
〃 JR 66/W 677	>100	>100	25	100
Pseudomonas aeruginosa A3	>100	50	3.12	3.12
〃 No. 12	>100	25	6.25	3.12
〃 TI 13	>100	>100	6.25	3.12
〃 GN 315	>100	>100	12.5	>100
〃 99	>100	>100	25	12.5
Mycobacterium smegmatis ATCC 607**	1.56	0.78	1.56	<0.39

* Nutrient agar, 37℃, 18 hours. ** Nutrient agar, 37℃, 48 hours.

900 (OH^- form) and CM Sephadex C-25 (NH_4^+ form) with water and 0~0.1 N ammonia, respectively, to give solid of 6′-N-methylkanamycin (**2**, 210 mg, 26 %); $[\alpha]_D^{18}$ +136° (*c* 1, water), nmr (in D_2O) τ 8.75 (1H q, H-2_{ax}), 7.97 (1H double triplets, H-2_{ex}), 7.58 (3H s, NCH_3), 4.90 (1H d, ~4 Hz, H-1″), 4.63 (1H d, ~3 Hz, H-1′). [Found: C 44.83, H 7.79, N 11.05. Calcd. for $C_{19}H_{38}N_4O_{11}\cdot{}^1/_2H_2O$: C 44.96, H 7.74, N 11.04]. On treatment of **2** with 6 N hydrochloric acid at 90°C for 30 minutes, the hydrolyzate showed, on ppc with Solvent A, a ninhydrin-positive spot of $Rf_{6\text{-amino-6-deoxy-D-glucose}}$ 1.3 (Rf_{DST} 2.2), which indicated the presence of 6-deoxy-6-methylamino-D-glucose in the N-methylated KM, in addition to the spots corresponding to 2-deoxystreptamine (DST), 3-amino-3-deoxy-D-glucose (3AG, Rf_{DST} 2.9) and the starting material (Rf_{DST} 0.29).

3′, 4′-Dideoxy-6′-N-methylkanamycin B was similarly prepared starting from DKB. 3′,4′-Dideoxykanamycin B was treated with benzyl *p*-nitrophenyl carbonate as described above to afford 6′-N-benzyloxycarbonyl-3′, 4′-dideoxykanamycin B (**3**) in a 50 % yield, mp 130~131°C, $[\alpha]_D^{18}$ +110° (*c* 1, water). [Found: C 53.48, H 7.50, N 11.73. Calcd. for $C_{26}H_{43}N_5O_{10}$: C 53.32, H 7.40, N 11.96]. Compound **3** was treated with $LiAlH_4$ as described above and 3′,4′-dideoxy-6′-N-methylkanamycin B (**4**) was obtained in 32 % yield; $[\alpha]_D^{18}$ +124° (*c* 1, water). nmr (in D_2O): τ 9.0~7.8 (6H m), 7.65 (3H s, NCH_3), 4.93 (1H d, *J*~4 Hz, H-1″), 4.83 (1H d, *J*~4 Hz, H-1′). [Found: C 47.44, H 8.56, N 14.37. Calcd. for $C_{19}H_{39}N_5O_8\cdot H_2O$: C 47.19, H 8.55, N 14.48]. On hydrolysis of **4** with 6 N hydrochloric acid, the hydrolyzate gave a spot of $Rf_{6\text{-amino-6-deoxy-D-glucose}}$ 1.1, which suggested the presence of 2-amino-2, 3, 4, 6-tetradeoxy-6-methylamino-D-glucose in the N-methylated DKB, in addition to the spots corresponding to DST, 3AG and the starting material (Rf_{DST} 0.35).

Synthetic 6′-N-methylkanamycin (**2**) and 3′, 4′-dideoxy-6′-N-methylkanamycin B (**4**) showed strong antibacterial activity at the level of the parent substances KM and DKB, respectively, against most of bacteria tested. Moreover, **2** showed activity against *E. coli* K-12 R-5 and LA 290 R-56 and R-64, and **4** showed activity against *P. aeruginosa* GN 315 at a concentration of 12.5 mcg/ml, as well as to the above strains (Table 1). The fact that **2** is not active against *P. aeruginosa* GN 315 suggests that the strain may produce a 3′-O-phosphorylating enzyme in addition to the N′-acetylating enzyme.

The above results show that methylation

of the special amino group which is to be acetylated by drug-resistant bacteria successfully provides a mean for the preparation of compounds active against the resistant organisms.

HAMAO UMEZAWA

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

YOSHIO NISHIMURA
TSUTOMU TSUCHIYA
SUMIO UMEZAWA

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

(Received October 16, 1972)

References

1) UMEZAWA, H.; S. UMEZAWA, T. TSUCHIYA & Y. OKAZAKI : 3′,4′-Dideoxykanamycin B active against kanamycin-resistant *Escherichia coli* and *Pseudomonas aeruginosa*. J. Antibiotics 24 : 485～487, 1971
2) UMEZAWA, H.; M. OKANISHI, R. UTAHARA, K. MAEDA & S. KONDO : Isolation and structure of kanamycin inactivated by a cell free system of kanamycin-resistant *E. coli*. J. Antibiotics 20 : 136～141, 1967
3) YAGISAWA, M.; H. NAGANAWA, S. KONDO, T. TAKEUCHI & H. UMEZAWA : 6′-N-Acetylation of 3′, 4′-dideoxykanamycin B by an enzyme in a resistant strain of *Pseudomonas aeruginosa*. J. Antibiotics 25 : 495～496, 1972
4) KHOSLA, M.C.; F.M. BUMPUS & R.R. SMEBY : Benzyl *p*-nitrophenylcarbonate. Indian J. Chem. 5 : 279, 1967