AMINOGLYCOSIDE ANTIBIOTICS. X CHEMICAL CONVERSION OF KANAMYCIN B TO KANAMYCIN C AND 6′-DEOXY-KANAMYCIN C

SOICHIRO TODA, SUSUMU NAKAGAWA and TAKAYUKI NAITO

Bristol-Banyu Research Institute, Ltd.
Meguro, Tokyo, Japan

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Kanamycin C was produced as a minor component in the fermentation broth of *Streptomyces kanamyceticus*[1] and its total synthesis was reported by UMEZAWA *et al*[2]. In connection with our aminoglycoside modification program, there was a need to obtain a sizable amount of kanamycin C. Since it was found laborious to isolate a pure sample of kanamycin C from the kanamycin fermentation, we attempted to prepare kanamycin C from a sample of commercially available kanamycin B. This paper reports the chemical conversion of kanamycin B to kanamycin C and its 6′-deoxy derivative.

The 6′-amino group of kanamycin B (**1**) was protected with a carbobenzoxy (Cbz) group by the activated ester method[3,4] to give 6′-N-Cbz-kanamycin B (**2**). The remaining free amino groups of **2** were acetylated with acetic anhydride in methanol to afford the tetra-N-acetyl derivative **3** in 99% yield, mp >300°C;

Anal. Calc'd for $C_{34}H_{51}N_5O_{16}\cdot\frac{3}{2}H_2O$: C 50.24, H 6.70, N 8.62.
Found: C 50.34, H 6.58, N 8.57.

Catalytic hydrogenation of **3** in the presence of 10% palladium on charcoal gave quantitatively the 6′-amino compound **4**. IR (KBr): $\nu_{c=0}$ 1650 cm^{-1}; NMR (D_2O, δ ppm): 2.00 (12H), 5.06 (1H, d, J=4 Hz), 5.36 (1H, d, J=4 Hz). Deamination of **4** in dil. H_2SO_4 with $NaNO_2$ followed by isolation on Amberlite IR-120 and IRA-410 columns yielded tetra-N-acetylkanamycin C (**5**) in 96% yield. Crystallization from MeOH-H_2O gave colorless needles, mp >300°C; IR (KBr): 1640, 1530, 1430, 1370, 1310, 1015 cm^{-1}.

Anal. Calc'd for $C_{26}H_{44}N_4O_{15}\cdot H_2O$: C 46.56, H 6.91, N 8.35.
Found: C 46.53, H 7.13, N 8.49.

Compound **5** was hydrolyzed by heating under reflux with aq. $Ba(OH)_2$ for 7 hours. The hydrolysate was neutralized with $(NH_4)_2CO_3$ and filtered to remove the resulting precipitate ($BaCO_3$). Chromatography of the filtrate on a CG-50 column (NH_4^+) gave kanamycin C in 43% yield, mp 195~198°C (dec.); $[\alpha]_D^{25.3}$ +118° (*c* 1.0, H_2O); NMR (D_2O, δ ppm): 5.08 (1H, d, J=4 Hz), 5.33 (1H, d, J=4 Hz). TLC (S-110*, Rf 0.60) was same as that of the authentic sample of kanamycin C.

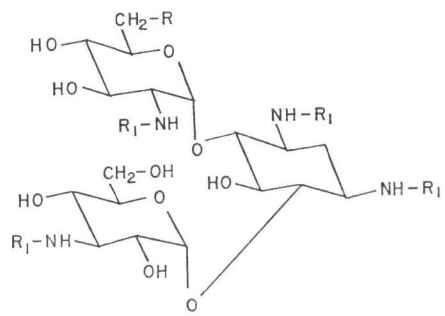
Anal. Calc'd for $C_{18}H_{36}N_4O_{11}\cdot\frac{1}{2}H_2CO_3\frac{3}{2}H_2O$:
C 40.97, H 7.44, N 10.33.
Found: C 41.25, H 7.65, N 10.05.

Deamination of **4** in 48% HBr with $NaNO_2$ in the cold gave **5** in 19% yield along with the 6-bromo derivative (**7**) in 53% yield, which were separated by silica-gel chromatography. **7**: mp 234~238°C. IR (KBr): $\nu_{c=0}$ 1640 cm^{-1};

Anal. Calc'd for $C_{26}H_{43}BrN_4O_{14}\cdot\frac{3}{2}H_2O$: C 42.21, H 6.24, N 7.45, Br 10.76.
Found: C 42.38, H 6.64, N 7.46, Br 10.82.

Hydrogenolysis of **7** with 10% palladium on charcoal and triethylamine gave tetra-N-acetyl-6′-deoxykanamycin C (**8**), which showed a doublet at δ 1.14 ppm (J=6 Hz) due to the 6′-methyl group in the NMR spectrum in D_2O. The

Chart 1.



1	R = NH_2	R_1 = H	(Kanamycin B)
2	R = NHCbz	R_1 = H	
3	R = NHCbz	R_1 = Ac	
4	R = NH_2	R_1 = Ac	
5	R = OH	R_1 = Ac	
6	R = OH	R_1 = H	(Kanamycin C)
7	R = Br	R_1 = Ac	
8	R = H	R_1 = Ac	
9	R = H	R_1 = H	(6′-Deoxykanamycin C)

* silica-gel plate, $CHCl_3$ - MeOH - 28% NH_4OH - H_2O (1: 4: 2: 1)

Table 1. Antibacterial activity of synthetic kanamycin C (**6**) and 6′-deoxykanamycin (**9**)

Test organism	MIC (mcg/ml)		
	Synthetic kanamycin C (**6**)	6′-Deoxy-kanamycin C (**9**)	Kanamycin C
Escherichia coli NIHJ	3.1	>100	6.3
〃 〃 K-12	3.1	>100	6.3
〃 〃 K-12 NR79/W677*	3.1	>100	3.1
〃 〃 K-12 JR35/C600**	>100	>100	>100
Klebsiella pneumoniae D11	0.4	12.5	0.4
Serratia marcescens A20019	3.1	50	3.1
Pseudomonas aeruginosa D-15	>100	>100	>100
Proteus vulgaris A9436	0.8	25	0.8
Proteus mirabilis A9554	6.3	25	12.5
Proteus morganii A9553	3.1	>100	1.6
Streptococcus aureus Smith	0.8	12.5	0.8
Mycobacterium smegmatis ATCC 607	6.3	>100	6.3

* aminoglycoside-6′-acetyltransferase producing strain.
** aminoglycoside-3′-phosphotransferase I producing strain.

acetyl groups of **8** were removed by heating with aq. $Ba(OH)_2$ to yield 6′-deoxykanamycin C (**9**), mp. 177~181°C (dec.); TLC (S-110): Rf 0.70; NMR (D_2O, δ ppm): 1.25 (3H, d, J=6 Hz), 5.05 (1H, d, J=4 Hz), 5.27 (1H, d, J=4 Hz).

Anal. Calc'd for $C_{18}H_{36}N_4O_{10}\cdot\frac{1}{2}H_2CO_3\frac{1}{2}H_2O$:
C 43.69, H 7.53, N 11.02.
Found: C 43.61, H 7.49, N 10.82.

The antibacterial activity of **6** (synthetic kanamycin C) and **9** (6′-deoxykanamycin C) is shown in Table 1. The minimum inhibitory concentrations were determined by the two-fold agar dilution method on MUELLER-HINTON agar plates using the Steers' multi-inoculating apparatus. An authentic sample of kanamycin C, tested comparatively as a reference compound, showed the same antibacterial spectrum and activity as those of synthetic kanamycin C, while the 6′-deoxy derivative showed only very weak activity against most of the microorganisms tested.

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