THE TOTAL SYNTHESIS OF KANAMYCIN A

Sir:

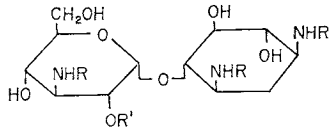
In the previous paper[1], we have reported the total synthesis of kanamycin C. We now wish to report the synthesis of kanamycin A[2], one of the kanamycin congeners, which is composed of 6-O-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine (3AD) and 6-amino-6-deoxy-D-glucose. Since we have previously synthesized 3AD[3] (I), the combined achievements constitute the total synthesis of kanamycin A.

Tri-N-carbobenzoxy-3AD (II), m.p. 282°C (decomp.), $[\alpha]_D^{18}$ +54° (c 0.67, DMF), was treated with 2,2-dimethoxypropane and *p*-toluenesulfonic acid in N,N-dimethylformamide (DMF) at 110°C to give the diisopropylidene derivative (III) in a quantitative yield; m.p. 234°C, $[\alpha]_D^{18}$ +53° (c 0.67, DMF). Benzylation of III with benzyl bromide in the presence of barium oxide and barium hydroxide in DMF gave IV in a 75 % yield; m.p. 258°C, $[\alpha]_D^{18}$ +43° (c 0.67, DMF). Deacetonation of IV by treatment with 80 % acetic acid gave quantitatively 6-O-(2-O-benzyl-3-carbobenzoxyamino-3-deoxy-α-D-glucopyranosyl)-N,N'-dicarbobenzoxy-2-deoxystreptamine (V); m.p. 288°C (decomp.), $[\alpha]_D^{18}$ +40° (c 0.67, DMF). Partial acetonation of V with 2,2-dimethoxypropane and *p*-toluenesulfonic acid in DMF at about 5°C gave monobenzyl-monoisopropylidene derivative (VI) in a 64 % yield; m.p. 240°C, $[\alpha]_D^{18}$ +40° (c 0.67, DMF).

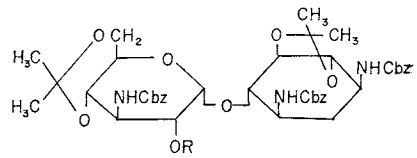
Anal. Found: C 64.43, H 6.09, N 4.76
Calcd. for $C_{46}H_{53}N_3O_{13}$:
C 64.55, H 6.24, N 4.91 %

Methyl 6-acetamido-6-deoxy-α-D-glucopyranoside[4] was benzylated with benzyl chloride in the presence of sodium hydride to afford the benzylated derivative, which was converted to 2,3,4-tri-O-benzyl-6-N-benzylacetamido-6-deoxy-α-D-glucopyranosyl chloride*, m.p. 91~92°C (decomp.), $[\alpha]_D^{23}$ +70° (c 1.0, $CHCl_3$), by successive acetolysis and chlorination.

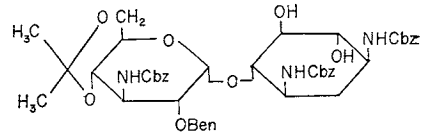
The condensation of VI with the benzylated glycosyl chloride was conducted as follows: To a mixture of VI (1.65 g), mercuric cyanide (0.68 g) and Drierite (5.4 g) in dioxane (10 ml), an anhydrous solution of the glycosyl chloride (2.21 g) in benzene (30 ml) was added with stirring, and the mixture was then vigorously stirred at 100°C for 10 hours to give the condensation product. The product was treated with acetic acid to remove the isopropylidene group, hydrogenatd in a mixture of dioxane – water – conc. hydrochloric acid (20 : 4 : 1) over palladium black with occasional addition of water to remove the O-benzyl and N-carbobenzoxy groups, de-N-acetylated with



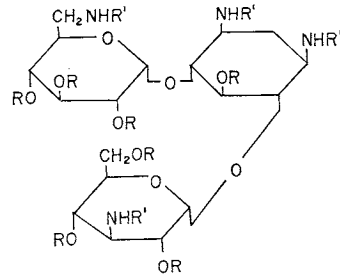
I : R, R'=H (3AD)
II : R=$COOCH_2C_6H_5$, R'=H
V : R=$COOCH_2C_6H_5$, R'=$CH_2C_6H_5$

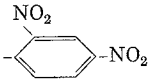


III : R=H
IV : R=$CH_2C_6H_5$



VI



VII : R=$COCH_3$, R'= 
VIII : R, R'=H (Kanamycin A)

* This compound was reported by S. UMEZAWA *et al.* in the abstracts of papers, 20th Annual Meeting of the Chemical Society of Japan, Tokyo, March 31, 1967, Vol. III, p. 596.

barium hydroxide, and again hydrogenated to remove the N-benzyl group. The ninhydrin-positive product was dinitrophenylated with 2,4-dinitrofluorobenzene in aqueous ethanol in the presence of sodium bicarbonate and then O-acetylated with acetic anhydride and anhydrous sodium acetate. The resulting product, which showed about six spots with Rf-values of 0.54, 0.34, 0.30, 0.27 (main), 0.25 and 0.15 on a thin-layer chromatogram with solvent system (A): toluene-MEK (3:1), was chromatographed on a silica-gel column (49 ×270 mm) with the same solvent. The substance having an Rf-value of 0.27 was isolated and recrystallized from toluene-MEK affording yellow crystals of **VII**; yield 281 mg (10.1 % over-yield from **VI**); m. p. 210~213°C (decomp.), $[\alpha]_D^{18}$ + 50° (c 1.0, acetone). IR spectrum (KBr): 3340, 1625, 1600, 1550, 1525, 1340, 835, 745 (NH-DNP), 1765, 1370, 1220 (OAc) cm^{-1}.

Anal. Found: C 46.71, H 4.22, N 11.58
Calcd. for $C_{56}H_{58}N_{12}O_{34}$:
C 46.61, H 4.05, N 11.65 %

On the other hand, kanamycin A[2] was dinitrophenylated and acetylated to give hepta-O-acetyl-tetra-N-(2,4-dinitrophenyl)-kanamycin A; m. p. 210~213°C (decomp.), $[\alpha]_D^{18}$ +52° (c 1.0, acetone).

Anal. Found: C 46.74, H 4.10, N 11.69
Calcd. for $C_{56}H_{58}N_{12}O_{34}$:
C 46.61, H 4.05, N 11.65 %

On thin-layer chromatography with solvent system (A), the synthetic product **VII** and the above-mentioned derivative of natural kanamycin A showed identical mobilities. Their infrared spectra were superimposable. Hydrolysis of **VII** with methanolic ammonia followed by treatment with an excess of Dowex 1X2 (OH^-) resin gave a crude free base, which was purified by chromatography on a column of Dowex 1X2 (OH^-) resin using water and recrystallized from aqueous methanol-ethanol to give a crystalline free base of **VIII**; $[\alpha]_D^{18}$ +149° (c 0.87, water).

Anal. Found: C 44.47, H 7.69, N 11.27
Calcd. for $C_{18}H_{36}N_4O_{11}$:
C 44.62, H 7.49, N 11.56 %

The natural kanamycin A showed $[\alpha]_D^{18}$ +151° (c 1.0, water). On descending paper chromatography by ninhydrin coloration using a solvent system: *n*-butanol-pyridine-water-acetic acid (6:4:3:1), the Rf-value of the synthetic product **VIII** agreed with that of the natural kanamycin A. Infrared spectra of **VIII** and the natural kanamycin A were identical. The antibiotic spectra and minimal inhibitory concentrations (MIC) of the synthetic product **VIII** against test organisms were in agreement with those of the natural kanamycin A as shown in Table 1.

Table 1. Minimum inhibitory concentration of synthetic (VIII) and natural kanamycin A as determined by the dilution method in bouillon

Test organisms	mcg/ml	
	VIII	Kanamycin A
Bacillus subtilis PCI 219	0.2	0.2
Mycobacterium tuberculosis 607	1.0	1.0
Escherichia coli	1.9	1.9
Staphylococcus aureus 209P	1.9	1.9

The details of the present work will be published in Bull. Chem. Soc. Japan.

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