SYNTHESIS OF 3'-EPI-4'-DEOXYKANAMYCIN B THROUGH 3'-EPI-3',4'-ANHYDRO INTERMEDIATE.

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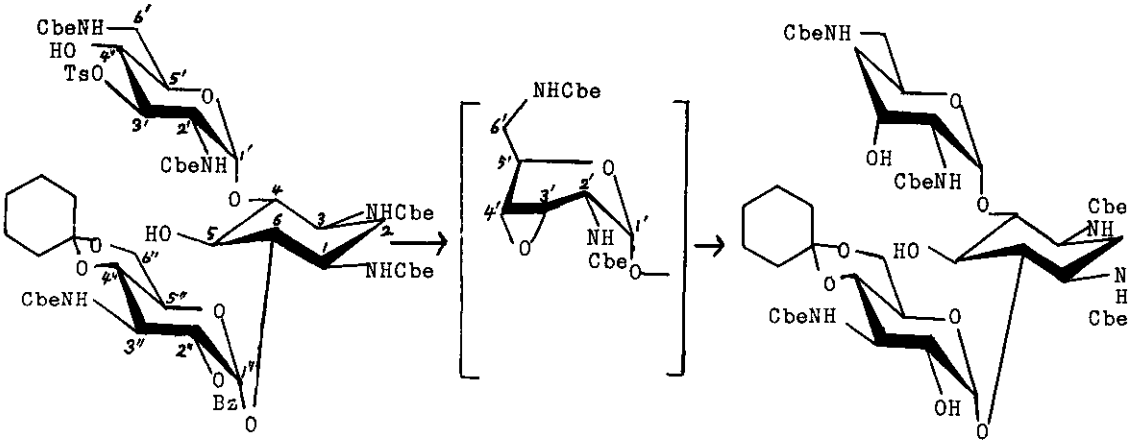
Abstract —— 3'-Epi-4'-deoxykanamycin B was synthesized from a protected derivative of 3'-O-tosylkanamycin B by the reductive ring opening of the assumed intermediate epoxide with sodium borohydride, and the structure of the objective compound was confirmed by PMR, molecular rotation value of its copper complex, and periodate oxidation of its hydrolysate.

During an attempt for the synthesis of 3'-deoxykanamycin B,[1] we found that the reaction of penta-N-ethoxycarbonyl-2"-O-benzoyl-3'-O-tosyl-4",6"-cyclohexylidene-kanamycin B (1)[2] with sodium borohydride did not yield the direct detosyloxylation product,[3] but gave a 3'-epi-4'-deoxy compound. Thus we prepared the titled substance in order to examine the effect of 3'-OH configuration on the antimicrobial activity against drug resistant organisms.

Sodium borohydride was added to the agitated suspension of 1 in diglyme and the mixture was kept stirring for two hours at 65°C to give penta-N-ethoxycarbonyl-3'-epi-4'-deoxy-4",6"-cyclohexylidenekanamycin B (3) in 87.3% yield [not completely purified; Rf 0.44($CHCl_3$, Me_2CO, MeOH. 40:8:3, silica gel, TLC), 0.41(EtOAc, EtOH. 20:1, silica gel, TLC)], which was deprotected by successive treatment with acetic acid and barium hydroxide, and the resulting product was purified by developing on a column of Amberlite CG50(NH_4^+) to give 3'-epi-4'-deoxykanamycin B (4a) in a considerable yield(37.4% from 1): 356mg of 4a was rechromatographed on a column of silica gel (n-BuOH, EtOH, $CHCl_3$, 17%NH_4OH. 4:5:2:5) to give 150mg; m.p. 167°C(dec.), $[\alpha]_D$ +114.8° (c 1.0, H_2O), Anal. Calcd. for $C_{18}H_{37}N_5O_9 \cdot H_2O$: C 44.52%, H 8.09%, N 14.47%, Found: C 44.02%, H 7.83%, N 14.37%, δ (D_2O) 1.0~2.2 (4H, $C_{2ax.}$-H, $C_{2eq.}$-H, $C_{4'ax.}$-H, $C_{4'eq.}$-H), 5.05 (1H, d, J 3.7 Hz, $C_{1''}$-H), 5.25 (1H, d, J 4.4 Hz, $C_{1'}$-H), 2.7~4.4 (16H), Rf 0.21(n-BuOH, EtOH, $CHCl_3$, 17%NH_4OH. 4:5:2:5, silica gel, TLC).

Since 4a is the main product of this reaction, and among minor components 3'-deoxykanamycin B was not detected at all, the possibility of direct detosyloxylation was excluded, and the reaction was supposed to proceed through formation of 3',4'-anhydro intermediate (2), followed by the attack on C4' position by the reducing agent.



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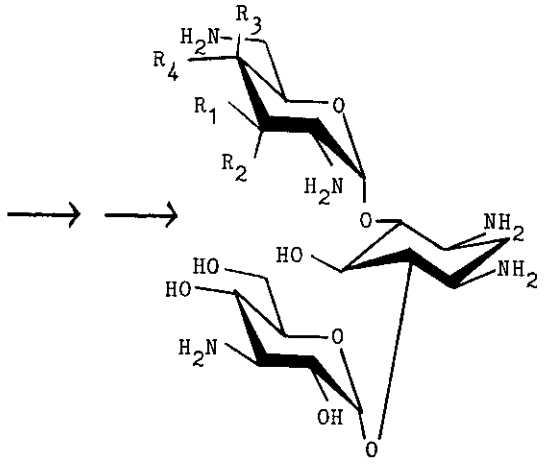

	R_1	R_2	R_3	R_4
4a	H	OH	H	H
4b	H	H	H	OH
4c	OH	H	H	H
4d	H	H	H	H
4e	OH	H	H	OH

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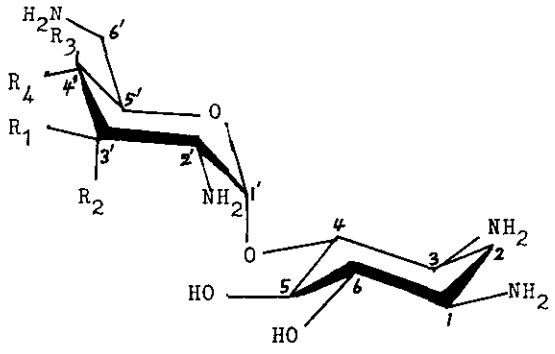
Very few examples are known of reductive epoxide ring opening reaction using sodium borohydride,[4] in contrast to the established method using lithium aluminum hydride.[5] In the present work, lithium aluminum hydride was not tried since ethoxycarbonyl protecting group was considered to be sensitive to the re-

agent.

According to the accepted mechanism of epoxide ring formation,[6] structure of the intermediate should be 2, in consequence, only the structure 4a or 4b is possible for the product, and as 4b was not detected, the product is inevitably 4a. This conclusion was confirmed by the periodate oxidation of the acid hydrolysate of 4a. When 4a was hydrolyzed by 5 N HCl at 100°C for 50 minutes, it gave 3-aminoglucose, 2-deoxystreptamine, and 3'-epi-4'-deoxyneamine (5a), after column chromatographic separation.

Three moles per mole of periodate was consumed on periodate oxidation of 5a by the method of Rammler and Rabinowitz [7] as the structure 5a suggested, while the structure 5b require only two moles per mole of periodate.

PMR *spectrum of 5a taken in deuterium oxide* with 100 MHz aparatus showed four protons at δ 1.0~2.2 which were assigned to C_2-H, and $C_{4'}$-H respectively. Irradiation of $C_{1'}$-H (δ 5.2) changed δ 2.94 triplet to doublet, the coupling constant of which was 3.8 Hz. As $C_{2'}$-H is axial, $C_{3'}$-H should be equatorial when considered this J value. Consequently $C_{3'}$-OH must be axially oriented in the molecule, conforming to the structure 5a. When $C_{2'}$-H was irradiated no difference in PMR spectrum was observed in δ1.0~2.2 region suggesting the absence of methylene protones on the neighbouring $C_{3'}$. The splitting pattern of the two protons at δ 2.2~1.6 was simplified on irradiation at $C_{3'}$-H, suggesting the presence of methylene protons at $C_{4'}$. These data also conform with the structure 5a.



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	R_1	R_2	R_3	R_4
5a	H	OH	H	H
5b	H	H	H	OH

Additional proof was given by the tetraammine copper complex method (Umezawa's TACu method)[8]. Molecular rotation values of the copper complexes of 4a, 3'-deoxykanamycin B (4b), and kanamycin B (4e) were compared with each other.

The presence of equatorial OH of 4e gives the levorotatory effect when compared with the molecular rotation values of 4e and 4b. As 4b is dextrorotatory, 4a with its axial OH, is expected to be more dextrorotatory than 4b, according to the theory.[8, 9] A larger dextrorotatory value of + 1850° was observed with 4a (See Table 1).

Table 1. Molecular Rotation Value of TACu-Complex

Substance	C2'-NH_2	C3'-OH	$\Delta[M]_{436(TACu)}$ [8]
4a	eq.	ax.	+ 1850
4b	eq.	none	+ 950 (Synthetic)[3]
			+ 900 (Natural)[3]
4e	eq.	eq.	- 450 (Natural)[3]
			- 483 (Natural)
4d	eq.	none	+ 800 (Synthetic)[3]

The compound 4a, which is one of the isomers of 4b and 4'-deoxykanamycin B (4c),[10] is structurally related to 3',4'-dideoxykanamycin B (4d).[11] It is well established that the absence of OH group on C3' in kanamycin B series is advantageous for inhibition of microorganisms carrying 3'-phosphotransferase I.[12] When 4a was tested on the microorganisms carrying the same enzyme, it showed, though a little weaker, a similar antibiotic activity to 4d suggesting the correlation between C3'-OH configuration and the action of 3'-phosphotransferase I (See Table 2).

Table 2. Minimum Inhibitory Concentration of Antibiotics (μg ml)

Microorganism	4a	4c	4d	4e
E. coli K12 ML1629	3.12	50	0.78	>100
ML1410 R81	12.5	100	0.78	>100
J5R11-2	3.12	25	0.39	—

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