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Modifiction of Aminocyclitol Antibiotics. 7. Preparation of 5-Epikanamycin B

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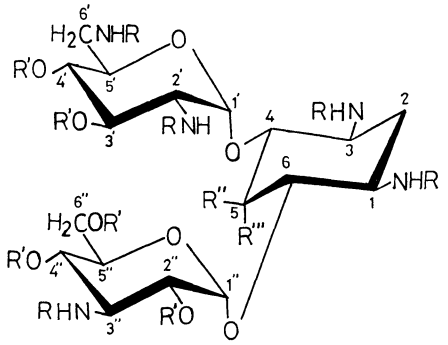
Synopsis. A configuration of the OH group on C-5 of 2-deoxystreptamine moiety of kanamycin B has been epimerized. 5-Epikanamycin B thus obtained was tested against several microorganisms. The structure of the antibiotic was elucidated by ^{1}H and ^{13}C NMR spectrometry.

In connection with the preceding paper of this series,[1] we have attempted to prepare 5-epikanamycin B (**4**), since 5-epineamine[2] showed an improved activity against a resistant strain of bacteria, compared to the parent neamine.

When 3′,4′,2″,4″,6″-penta-*O*-benzoyl-1,3,2′,6′,3″-pentakis-*N*-(ethoxycarbonyl)kanamycin B[1] (**1**) was treated with methanesulfonyl chloride in pyridine, a 5-*O*-mesyl derivative (**2**) was obtained. Nucleophilic substitution of **2** with an acetate ion in DMF, followed by purification afforded 5-*O*-acetyl-3′,4′,2″,4″,6″-penta-*O*-benzoyl-1,3,2′,6′,3″-pentakis-*N*-(ethoxycarbonyl)5-epikanamycin B (**3**). Hydrolysis of **3** in barium hydroxide solution, followed by purification on a CG-50(NH_4^+) resin column gave **4**, which was converted to a penta-*N*-acetyl derivative (**5**).

The ^{1}H NMR spectrum of **3** revealed acetoxyl methyl protons at δ 2.20 as a singlet, indicating an existence of an axial acetoxyl group, since the corresponding 5-*O*-acetylkanamycin B derivative revealed an equatorial acetoxyl methyl signal at δ 2.07.[3]

The structure of **4** was confirmed by ^{13}C NMR spectrometry. The spectrum of **4** was determined at pD above 11, showing 18 carbon signals which are assigned in accordance with the data for kanamycin B.[1,4] When the spectrum of **4** is compared with that of kanamycin B, it is obvious that the epimerization causes no appreciable shift of the signals of carbons in the aminosugar moieties, except that of C-1′, *i.e.*, the signal of C-1′ shifts to a higher field by 4.5 ppm, and analogous shifts have been described in the cases of 5-deoxykanamycin B[1] and 5-deoxyneamine.[7] While, the signals of carbons in the 2-deoxystreptamine moiety are shifted more or less to a higher field, except



1 R=C_2H_5OCO, R′=C_6H_5CO, R″=OH, R‴=H
2 R=C_2H_5OCO, R′=C_6H_5CO, R″=OSO_2CH_3, R‴=H
3 R=C_2H_5OCO, R′=C_6H_5CO, R″=H, R‴=CH_3COO
4 R=R′=R″=H, R‴=OH
5 R=CH_3CO, R′=R″=H, R‴=OH

Table 2. Antimicrobial activity of 5-epikanamycin B (**4**) and kanamycin B

Test organisms	Compound **4**	Kanamycin B
	MIC (mcg/ml)	
Staphyloccocus aureus ATCC 6538P	1.56	0.39
Staphyloccoccus epidermidis ATCC 12228	0.77	0.2
Diplococcus pneumoniae Type 3	0.39	0.1
Bacillus subtilis ATCC 6633	0.39	0.1
Escherichia coli NIH JC-2	6.25	1.56
Klebsiella pneumoniae 602	3.12	0.78
Pseudomonas aeruginosa IAM 1007	100	12.5
Proteus vulgaris OX-19	0.78	0.2
Salmonella pvratyphi A 1015	1.56	0.39
Salmonella paratyphi B	3.12	1.56
Shigella flexneri 2a·SH-74-1	12.5	3.12

Table 1. The ^{13}C NMR chemical shifts[a] of 5-epikanamycin B and kanamycin B

	5-Epikanamycin B (**4**)		Kanamycin B[1]	
	pD 11	pD 1	pD 11	pD 1
C-1	48.0	48.2	50.5	50.5
C-2	36.6	28.8	36.5	28.6
C-3	47.3	47.6	50.3	49.3
C-4	79.9	73.8[c]	87.4	77.5
C-5	68.4	65.7	75.2[d]	75.1
C-6	86.1	81.0	88.6	84.5
C-1′	96.0	91.3	100.5	96.2
C-2′	55.2	54.1	56.3	54.4
C-3′	74.8	69.5	74.9[d]	69.0
C-4′	72.9[b]	71.5	73.3[e]	71.8
C-5′	73.9	69.9	74.7	70.1
C-6′	42.6	41.0	42.8	41.2
C-1″	101.9	100.8	101.4	101.4
C-2″	72.4[b]	68.8	73.0[c]	69.0
C-3″	55.8	55.7	56.5	55.8
C-4″	71.1	66.4	71.3	66.3
C-5″	73.5	73.7[c]	74.0	73.7
C-6″	62.1	61.3	62.0	60.8

a) In parts per million downfield from tetramethylsilane.
b—e) The signals may be reversed.

that of C-2. That is, the signal of C-5 shifts by 6.8 ppm, and this is coincident with the fact that a shift of the signal of C-3 was observed between β-D-gluco and β-D-allopyranoses.[5] The signals of C-4 and 6 shift by 7.5 and 2.5 ppm, owing to a shielding effect of an adjacent axial OH group on C-5. Also, the signals of C-1 and 3 shift by 2.5 and 3.0 ppm, respectively. An analogous shielding effct of an axial OH group was described in a literature.[5,6]

When ^{13}C NMR spectrum of **4** was determined at pD 1 to find out a *N*-protonation effect,[4,8] a β-shielding effect is observed as was described in the case of kanamycin B.[1] Therefore, the inversion of the configuration has been confirmed.

Antimicrobial activities of **4** were determined against several microorganisms. The MIC (minimum inhibition concentration) values are listed in Table 2. No improvement of the activity was achieved by the epimerization of the OH group on C-5 in kanamycin B.

Experimental

General Methods. The same method was used as described in the preceding paper.[1]

3′,4′,2″,4″,6″-Penta-O-benzoyl-1,3,2′,6′,3″-pentakis-N-(ethoxycarbonyl)-5-O-mesylkanamycin B (2). To a stirred solution of 3′,4′,2″,4″,6″-penta-*O*-benzoyl-1,3,2′,6′,3″-pentakis-*N*-(ethoxycarbonyl)kanamycin B[1] (**1**, 311 mg) in pyridine (3 ml), mesyl chloride (0.5 ml) was added. After 160 h, the solution was quenched into ice–water (50 ml), and precipitates were collected by filtration. The precipitates were dissolved in $CHCl_3$, and the solution was passed through a short alumina column and evaporated. The residue was dissolved in benzene, and hexane was added to the solution to give 232 mg (71%) of **2** as amorphous powder, mp 121—126 °C, $[\alpha]_D^{21}$ +76.8° (*c* 0.5, chloroform), R_f 0.37 on TLC in 7 : 1 (v/v) benzene–methanol ^1H NMR: δ 3.32 (s, 3, SO_2CH_3).

Found: C, 57.80; H, 5.54; N, 4.90; S, 1.92%. Calcd for $C_{69}H_{79}N_5O_{27}S$: C, 57.45; H, 5.52; N, 4.86; S, 2.22%.

5-O-Acetyl-3′,4′,2″,4″,5″-penta-O-benzoyl-1,3,2′,6′,3″-pentakis-N-(ethoxycarbonyl)-5-epikanamycin B (3). A mixture of **2** (1.0 g) and sodium acetate (622 mg) in DMF (16 ml) was heated at 100 °C with agitation. After 68 h, the mixture was poured into ice–water (80 ml), and precipitates were collected by filtration. The precipitates were dissolved in chloroform, and the solution was washed with water, dried over Na_2SO_4 and evaporated. The residue was purified on a silica gel column with 20 : 1 (v/v) benzene–methanol. Fractions homogeneous on TLC (R_f 0.43) in 7 : 1 (v/v) benzene–methanol were combined and concentrated. The residue was dissolved in benzene and hexane was added to the solution to give 571 mg (54%) of **3**, mp 132—138 °C, $[\alpha]_D^{21}$ +100.6° (*c* 1.03, chloroform). ^1H NMR: δ 2.20 (s, 3, OAc).

Found: C, 59.45; H, 5.59; N, 4.70%. Calcd for $C_{70}H_{79}N_5O_{26}$: C, 59.78; H, 5.66; N, 4.98%.

5-Epikanamycin B (4). To a stirred solution of $Ba(OH)_2 \cdot 8H_2O$ (4 g) in water (14 ml), a solution of **3** (518 mg) in methanol (6 ml) was added. The mixture was heated at 100 °C for 15 h, and CO_2 was bubbled into the mixture. The precipitates were filtered off and the filtrate was concentrated. The residue was purified on a column of Amberlite CG-50 (NH_4^+) resin as described in the preceding paper[1] to give 20 mg (11%) of **4**, mp 172—178 °C (dec), $[\alpha]_D^{21}$ +159° (*c* 0.6, water).

A part of **4** (11 mg) was acetylated with acetic anhydride in methanol to give 15 mg (93%) of the penta-*N*-acetyl derivative (**5**), mp 217—223 °C (dec).

Found: C, 45.69; H, 6.88; N, 9.36%. Calcd for $C_{28}H_{47}N_5O_{15} \cdot 2H_2O$: C, 46.08; H, 7.04; N, 9.59%.

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