SYNTHESES AND PROPERTIES OF KANAMYCIN C DERIVATIVES ACTIVE AGAINST RESISTANT BACTERIA

Sir:

The enzymatic mechanism of resistance to aminoglycoside antibiotics has been explored by UMEZAWA.[1,2] Many clinically isolated strains of aminoglycosides-resistant bacteria produce enzymes which phosphorylate, adenylylate or acetylate specific positions of aminoglycoside antibiotics. Kanamycin and kanamycin B are inactivated by aminoglycoside 3′-phosphotransferases, 2″-nucleotidyltransferase or 6′-acetyltransferases. Furthermore, it has been shown that the 3′-phosphotransferase-producing bacteria are inhibited by 3′-deoxy derivatives of these antibiotics and 2″-nucleotidyltransferase-producing bacteria by the 1-N-[(*S*)-4-amino-2-hydroxybutyryl] derivatives. However, the 6′-modification of these antibiotics has not yet produced derivatives which inhibit all 6′-acetyltransferase-producing strains.[3] Kanamycin C isolated from a culture filtrate of *Streptomyces kanamyceticus* as a minor component has the 6′-hydroxyl in place of the 6′-amino group in kanamycin B, and is not inactivated by 6′-acetyltransferases. In this communication, we report chemical conversion of kanamycin B and its deoxy derivatives into kanamycin C and its deoxy derivatives, and synthesis of their 1-N-[(*S*)-4-amino-2-hydroxybutyryl] derivatives.

Kanamycin B was converted into kanamycin C (**I**) in good yield by the following route. The free amino groups of 6′-N-BOC*-kanamycin B monohydrate[4] were acetylated with acetic anhydride in methanol at room temperature for 5 hours and the BOC group was removed in 90% trifluoroacetic acid at room temperature for 45 minutes. The 1,3,2′,3″-tetra-N-acetylkanamycin B trifluoroacetate thus obtained was treated with sodium nitrite in 33% aqueous acetic acid for 1 hour at ice temperature and then for 2 hours at room temperature. The reaction mixture was concentrated to dryness and the residue was dissolved in 2 N NaOH. After refluxing the alkaline solution for 12.5 hours, **I** in the solution was adsorbed on a column of Amberlite CG-50 (70% NH_4^+) resin and eluted with 0.5 N ammonia. Rechromatography on a Amberlite CG-50 (NH_4^+) column eluted with 0.05 N, 0.1 N and then 0.2 N ammonia gave pure **I** in 49% yield.

The deoxy derivatives, 3′-deoxykanamycin C (**II**) and 3′,4′-dideoxykanamycin C (**III**) were synthesized from 6′-N-BOC-3′-deoxykanamycin B** and 6′-N-BOC-3′,4′-dideoxykanamycin B[6] by the method described above in 40% and 24% yields, respectively.

The 1-N-acyl derivatives with (*S*)-4-amino-2-hydroxybutyric acid were synthesized from partially protonated forms of **I**, **II** and **III** without any protection of amino groups. In an aqueous solution at pH 6.5±0.2 adjusted with 1 N HCl, **I**, **II** or **III** was acylated with 1.5 equivalents of the N-hydroxysuccinimide ester of N-BOC-(*S*)-4-amino-2-hydroxybutyric acid[6] in dimethylformamide at room temperature for 6 hours and then the BOC group was removed in 90% trifluoroacetic acid at room temperature for 1 hour to afford 1-N-[(*S*)-4-amino-2-hydroxybutyryl]-kanamycin C (IV), -3′-deoxykanamycin C (**V**) or -3′,4′-dideoxykanamycin C (**VI**) in 8.0%, 8.5% or 8.3% yield, respectively. These derivatives were purified by column chromatography on Amberlite CG-50 (NH_4^+) resin eluted with 0.2 N and 0.5 N ammonia, and on silica gel (Mallinckrodt, CC-7) developed with a mixture of chloroform, methanol and 17% ammonia (1: 4: 2 in volume). With the resin chromatography, unreacted **I**, **II** or **III** was recovered in 40%, 34% or 45% yield, respectively. From the silica gel chromatography, a positional isomer having weak biological activity, 3-N-[(*S*)-4-amino-2-hydroxybutyryl]-kanamycin C(**VII**), -3′-deoxykanamycin C (**VIII**) or -3′,4′-dideoxykanamycin C (**IX**) was separated in 11.0%, 6.1% or 7.4% yield, respectively.

The properties of all derivatives described above are summarized in Table 1. The structures of the acyl derivatives were confirmed by PMR and acid hydrolysis[6]. On the carbon-13 FOURIER-transform NMR spectra of **I**, **IV**, **V**, **VI** and **VII**, the chemical shifts were assigned as shown in Table 2.[7]

The minimum inhibitory concentration of **I** and its five derivatives are shown in Table 3.

* *tert*-Butyloxycarbonyl

** 6′-N-BOC-3′-deoxykanamycin B (mp 142~154°C (dec.), $[\alpha]_D^{22}$ +111° (*c* 1, water), $C_{23}H_{45}N_5O_{11}\cdot H_2O$) was prepared from 3′-deoxykanamycin B[5] by the method reported in a previous paper.[4]

Table 1. The properties of kanamycin C derivatives

Derivative	mp (dec.)	$[\alpha]_D$ in H_2O	Molecular formula*	Rf on TLC**	
				A	B
II	180~220°C	+110° at 26°C	$C_{18}H_{36}N_4O_{10}\cdot\frac{1}{2}H_2O$	0.38	0.59
III	200~220°C	+118° at 26°C	$C_{18}H_{36}N_4O_9\cdot\frac{1}{2}H_2O$	0.49	0.66
IV	167~180°C	+91° at 26°C	$C_{22}H_{43}N_5O_{13}\cdot H_2O$	0.18	0.19
V	151~160°C	+83° at 27°C	$C_{22}H_{43}N_5O_{12}\cdot H_2O$	0.22	0.24
VI	142~158°C	+76° at 28°C	$C_{22}H_{43}N_5O_{11}\cdot H_2O$	0.31	0.30
VII	148~153°C	+87° at 24°C	$C_{22}H_{43}N_5O_{13}\cdot H_2O$	0.21	0.28
VIII	136~144°C	+80° at 24°C	$C_{22}H_{43}N_5O_{12}\cdot H_2O$	0.27	0.36
IX	135~147°C	+78° at 24°C	$C_{22}H_{43}N_5O_{11}\cdot H_2O$	0.34	0.44

* Satisfactory results of elemental analyses were obtained for all compounds.

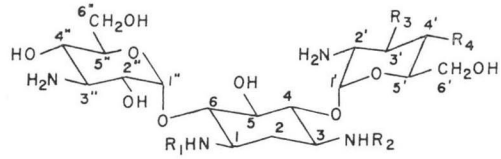
** Thin-layer chromatography on Silica gel G (Merck, Art 5721) using solvent A; butanol - ethanol - chloroform - 28% ammonia (4: 5: 2: 8 in volume) (kanamycin C: Rf 0.31) and solvent B; chloroform - methanol - 28% ammonia - water (1: 4: 2: 1 in volume) (kanamycin C: Rf 0.52).

Table 2. Carbon-13 chemical shifts

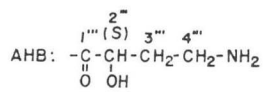
Carbon	Chemical shift (δ)				
	I	IV	V	VI	VII
1	51.3	49.9	50.0	50.0	51.1
2	36.2	33.7	33.6	33.1	34.2
3	50.3	50.5	50.4*	50.4*	49.0
4	87.9	87.4	87.2	87.1	81.2
5	75.2	75.7	75.8	75.8	76.0
6	88.5	81.1	81.5	81.5	88.1
1′	101.3	101.4	100.5	101.0	100.8*
2′	56.2	56.2	50.0*	50.6*	55.9
3′	74.5	74.5	35.7	26.0	74.4
4′	70.7	70.5	65.5	26.5	70.5
5′	73.8	73.7	74.3	70.9	73.3
6′	61.6	61.6	61.7	64.9	61.6
1″	100.7	99.2	99.2	99.2	100.2*
2″	72.6	72.4	72.4	72.3	72.6
3″	55.1	55.0	55.0	54.9	55.1
4″	70.0	70.0	70.1	70.0	70.0
5″	72.9	73.0	72.9	72.9	72.9
6″	61.2	61.2	61.2	61.2	61.1
1‴		176.4	176.4	176.2	176.4
2‴		70.7	70.6	70.6	70.5
3‴		35.2	35.3	35.2	35.0
4‴		38.0	37.9	37.9	37.6

δ: ppm from TMS in D_2O using dioxane (δ= 67.4 ppm) as the internal reference.

* Assignments within any vertical column may be reversed.



	R_1	R_2	R_3	R_4
I:	H	H	OH	OH
II:	H	H	H	OH
III:	H	H	H	H
IV:	AHB	H	OH	OH
V:	AHB	H	H	OH
VI:	AHB	H	H	H
VII:	H	AHB	OH	OH
VIII:	H	AHB	H	OH
IX:	H	AHB	H	H



Among these derivatives, **V** is most active against all bacterial strains tested. This derivative showed 73% and 25% of the bacteriostatic activity of amikacin by the cylinder plate method using *Bacillus subtilis* PCI 219 and *Escherichia coli* JR66/W677, respectively, as the test organisms. These derivatives had low toxicity; when administered intravenously, mice tolerated a single dose of 400 mg/kg of **II**, **IV** or **V**, but the same dose of **III** or **VI** caused death.

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Table 3. The antimicrobial spectra of kanamycin C derivatives

Organisms	Minimum inhibitory concentrations (μg/ml)					
	I	II	III	IV	V	VI
Staphylococcus aureus FDA 209P	6.25	3.13	12.5	6.25	6.25	12.5
Escherichia coli NIHJ	6.25	12.5	25	6.25	6.25	12.5
Escherichia coli K-12	12.5	12.5	50	12.5	6.25	12.5
Escherichia coli K-12 R5[a]	6.25	12.5	25	6.25	6.25	12.5
Escherichia coli K-12 ML1629[b]	>100	12.5	25	6.25	6.25	6.25
Escherichia coli K-12 ML1630[b]	>100	12.5	25	12.5	12.5	12.5
Escherichia coli K-12 ML1410	25	12.5	25	25	6.25	25
Escherichia coli K-12 ML1410 R81[b]	>100	25	100	12.5	12.5	25
Escherichia coli LA290 R55[d]	>100	>100	>100	12.5	12.5	12.5
Escherichia coli LA290 R56	50	100	>100	6.25	6.25	6.25
Escherichia coli LA290 R64	50	50	>100	12.5	3.13	12.5
Escherichia coli W677	12.5	6.25	12.5	12.5	6.25	12.5
Escherichia coli JR66/W677[c,d]	>100	>100	>100	25	12.5	25
Klebsiella pneumoniae PCI 602	6.25	6.25	50	3.13	3.13	6.25
Klebsiella pneumoniae 22#3038[c,d]	>100	>100	>100	12.5	6.25	25
Pseudomonas aeruginosa A3	>100	12.5	>100	12.5	6.25	12.5
Pseudomonas No. 12	>100	100	>100	50	50	>100
Pseudomonas aeruginosa H9[c]	>100	100	>100	>100	100	>100
Pseudomonas aeruginosa TI-13[b]	>100	50	>100	50	50	>100
Pseudomonas aeruginosa GN315[a]	>100	50	>100	50	50	>100
Pseudomonas aeruginosa 99[e]	>100	100	>100	100	100	>100
Pseudomonas aeruginosa H11	>100	50	>100	100	50	>100

Resistance mechanisms: a) 6′-acetyltransferase, b) 3′-phosphotransferase I, c) 3′-phosphotransferase II, d) 2″-nucleotidyltransferase, e) 3-acetyltransferase.

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