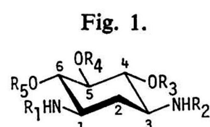
SYNTHESIS OF 1-*N*-(2-AMINOETHANESULFONYL)-KANAMYCINS AND RELATED AMINOGLYCOSIDE ANTIBIOTICS

Sir:

In this paper, we wish to report the synthesis of a new series of aminoglycoside derivatives. These include 1-*N*-(2-aminoethanesulfonyl) derivatives[1] of ribostamycin (I), kanamycin (II), kanamycin B (III), and dibekacin (IV). These derivatives are strongly active against Gram-positive and Gram-negative bacteria including resistant strains as shown in Table 1.

Compounds I, II, III and IV were synthesized by the following general procedure: each antibiotic was dissolved in water and *N,N*-dimethylformamide and triethylamine was added to the solution under stirring at 0~5°C. *N*-Trifluoroacetyl-2-aminoethanesulfonyl chloride[2] in cold *N,N*-dimethylformamide was then added dropwise to the mixture under vigorous stirring at 0~5°C within 5~10 minutes. The mixture was stirred at 0~5°C for 60 to 70 minutes and at room temperature overnight. Water was then added to the reaction mixture. The resultant aqueous solution was treated with concd. NH_4-OH and warmed at 70°C for 1~2 hours (amounts

Fig. 1.



	R_1	R_2	R_3	R_4	R_5
I	AES	H	26DAG	RIB	H
II	AES	H	6AG	H	3AG
III	AES	H	26DAG	H	3AG
IV	AES	H	2634G	H	3AG
V	AES	H	H	H	H
VI	AHB	H	6AG	H	3AG
VII	AHB	H	H	H	H
VIII	AESAHB	H	H	H	H
IX	AHB	AES	H	H	H
X	H	AES	H	H	H
XI	H	H	H	H	H

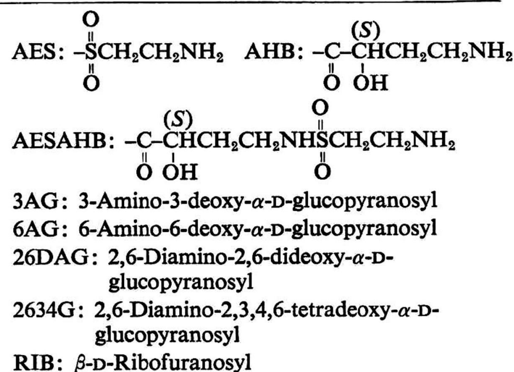


Table 1. Antibacterial spectra of I, II, III, and IV.

Test organisms	MIC (μg/ml)				
	I	II	III	IV	Kanamycin B
Staphylococcus aureus 209 P	6.25	6.25	<0.39	3.12	0.39
Escherichia coli NIHJ	3.12	6.25	1.56	12.5	0.78
E. coli K-12	1.56	3.12	1.56	12.5	0.78
E. coli K-12 ML-1410	1.56	—	1.56	—	0.78
E. coli K-12 ML-1629	25	6.25	3.12	12.5	>100
E. coli K-12 ML-1630	3.12	6.25	3.12	50	>100
E. coli K-12 ML-1410 R 81	3.12	6.25	3.12	25	>100
E. coli K-12 LA 290 R 55	3.12	6.25	3.12	50	12.5
E. coli K-12 LA 290 R 56	3.12	3.12	1.56	12.5	3.13
E. coli K-12 LA 290 R 64	1.56	1.56	1.56	12.5	3.13
E. coli JR 66/W 677	>100	6.25	6.25	50	>100
E. coli K-12 J 5 R 11-2	1.56	3.12	1.56	6.25	—
Klebsiella pneumoniae 22 #3038	>100	12.5	12.5	50	>100
Mycobacterium smegmatis 607	6.25	25	6.25	12.5	0.78
Pseudomonas aeruginosa A 3	12.5	3.12	1.56	6.25	50
P. aeruginosa No. 12	100	12.5	12.5	25	12.5
P. aeruginosa H 9	>100	25	25	>100	—
P. aeruginosa TI-13	>100	25	25	>100	100
P. aeruginosa 99	>100	25	25	>100	>100
P. aeruginosa H 11	>100	25	12.5	>100	—

37°C, nutrient agar.

Table 2. Physico-chemical properties of I, II, III, and IV.

Properties	I[a]	II[a]	III[a]	IV[b]
Mp (dec.) (°C)	157~218	180~242	150~250	130~210
$[\alpha]_D^{23}$	+31°[c]	+154°[c]	+117°[c]	+95°[d]
	(*c* 0.58, H_2O)	(*c* 0.57, H_2O)	(*c* 0.35, H_2O)	(*c* 0.3, H_2O)
ORD	Plain pos.[c]	Plain pos.[c]	Plain pos.[c]	Plain pos.[d]
NMR[e] δ (in D_2O)	5.91 (*J*=1~2)	5.57 (*J*=4)	5.76 (*J*=3~4)	5.75 (*J*=3~4)
	6.16 (*J*=4)	5.93 (*J*=4)	6.00 (*J*=3~4)	6.01 (*J*=3~4)
TLC Rf ($R_{std.}$)[f]	0.25 (1.21)[g]	0.22 (1.18)[g]	0.24 (1.10)[g]	0.42 (1.08)[g]
	0.15 (1.24)[h]	0.17 (1.29)[h]	0.13 (1.18)[i]	0.28 (1.13)[i]

[a] Elemental analysis of I, II, and III are in good agreement with the calculated value.

[b] EI Mass spectroscopy of penta-*N*-salicylidene IV shows M^+ (*m/z* 1,078).

[c] Instrument: ORD/UV-5 (Japan Spectroscopic Co., Ltd.).

[d] Instrument: J-20 (Japan Spectroscopic Co., Ltd.).

[e] Varian T60 A: Anomeric proton shift value in ppm.

Although our δ values fall outside the range found for 1-*N*-aminoacylkanamycin-type aminoglycosides, we consider it possible that compounds I, II, III and IV having $-SO_2NH-$ group which acts rather as an acid than a neutral amide, show somewhat deviated δ values as above. Because both conformation and electronic environment of anomeric protons may be different from those of the ordinary 1-*N*-aminoacyl compounds either by bulkiness of aminoethanesulfonyl group or by intramolecular electrostatic field effect between $-SO_2N^-$– and neighboring $-NH_3^+$.

[f] Standard for I: ribostamycin, for II: kanamycin, for III: kanamycin B, for IV: dibekacin.

[g] BuOH - MeOH - $CHCl_3$ - concd. NH_4OH (4: 5: 2: 5)/silica gel (Merck, Art 5721).

[h] BuOH - EtOH - $CHCl_3$ - 17% NH_4OH (4: 5: 2: 5)/silica gel (Merck, Art 5721).

[i] EtOH - $CHCl_3$ - concd. NH_4OH (4: 1: 2)/silica gel (Merck, Art 5721).

of each antibiotic, water, *N,N*-dimethylformamide, triethylamine, *N*-trifluoroacetyl-2-aminoethanesulfonyl chloride/*N,N*-dimethylformamide, water and concd. NH_4OH for the synthesis of I, II, III and IV were as follows: 2,900 mg, 8 ml, 8 ml, 1.4 ml, 3,300 mg/11 ml, 80 ml, 12 ml; 3,000 mg, 8 ml, 8 ml, 1.4 ml, 3,300 mg/11 ml, 80 ml, 12 ml; 1,347 mg, 4 ml, 3 ml, 0.488 ml, 1,504 mg/5 ml, 40 ml, 6 ml; 1,268 mg, 4 ml, 3 ml, 0.47 ml, 1,502 mg/5 ml, 40 ml, 6 ml).

This solution was chromatographed on a column of Dowex 1-X2 (OH^-) and the column eluted with dilute acetic acid. The eluate was rechromatographed on a column of Amberlite CG-50 (NH_4^+) being eluted with dilute NH_4OH (amounts of Dowex 1-X2 (OH^-), acetic acid concentration, and amounts of Amberlite CG-50 (NH_4^+) were as follows: 150 ml, 0.1%, 100 ml; 100 ml, 0.2%, 100 ml; 100 ml, 0.2%, 20 ml; 100 ml, 0.2%, 20 ml).

Each crude substance thus prepared was further purified by another column chromatography (columns used/developing solvent used, material obtained, weight and yield were as follows: Amberlite CG-50 (NH_4^+) 20 ml/dilute NH_4OH, Dowex 1-X2 (OH^-) 10 ml/0.05% acetic acid, I, 74.16 mg, 2.08%; Wakogel C-200 50 g/ BuOH - EtOH - $CHCl_3$ - 17% NH_4OH (4: 5: 2: 5), Amberlite CG-50 (NH_4^+) 20 ml/dilute NH_4OH, Dowex 1-X2 (OH^-) 20 ml/0.05% acetic acid, II, 136.9 mg, 3.7%; Amberlite CG-50 (NH_4^+) 10 ml/ dilute NH_4OH, Dowex 1-X2 (OH^-) 10.5 ml/ 0.05% acetic acid, Wakogel C-200 21 g/EtOH - $CHCl_3$ - concd. NH_4OH (4: 1: 2), III, 42.73 mg, 2.59%; Wakogel C-200 25 g/EtOH - $CHCl_3$ - concd. NH_4OH (4: 1: 1), IV, 23.84 mg, 2.06%).

The column chromatography was monitored by bioassay using a paper disc method with *Escherichia coli* and *Pseudomonas aeruginosa* on agar plates and only active fractions showing single spots on TLC were collected. Physico-chemical properties of I, II, III and IV are summarized in Table 2.

It is evident that I, II, III and IV thus obtained need structural verification considering the synthetic method described above. ^{13}C NMR spectroscopy failed to definitely identify C-1 as the site of substitution although the signals for the C-1 and C-6 carbons of III differed considerably from those of the corresponding carbons in kanamycin B whereas those of the C-3 and C-4 carbons did not (Table 3). Although the original method

Table 3. ^{13}C Chemical shifts of 1-*N*-(2-aminoethanesulfonyl)kanamycin B (III) and kanamycin B.

Chemical shifts (ppm)			Assignment
III	Kanamycin B		
	Observed	Literature[4]	
54.1*	51.2	50.2	C-1
36.4	36.3	35.4	C-2
49.7	50.2	49.2	C-3
86.4**	87.3	86.4	C-4
76.0***	74.4	74.2	C-5
84.0**	88.8	87.7	C-6
101.0	101.1	100.1	C-1′
56.1	56.2	55.3	C-2′
73.2***	74.2	73.4	C-3′
72.2	72.3	71.2	C-4′
73.5***	73.5	72.8	C-5′
42.3	42.4	41.6	C-6′
100.2	100.7	99.5	C-1″
72.8	72.6	71.7	C-2″
55.1*	55.1	54.2	C-3″
70.1	70.2	69.1	C-4″
74.3***	73.0	71.9	C-5″
61.3	61.2	60.2	C-6″
55.8			C-1‴
36.6			C-2‴
67.4 (dioxane)	67.4 (dioxane)	66.3 (dioxane)	

Observed chemical shifts were taken by Varian XL-100A at pH *ca.* 10 (D_2O+dioxane).
*, **, ***: Assignment may be interchangeable.

of positional structural determination[3] could not be applied in this case because of the resistance of the 2-aminoethanesulfonyl side chain to acid hydrolysis, the structural determination of I, II, III and IV was accomplished by alternative degradation studies as follows.

I, II, III and IV and their *N*-acetates were treated with 6 N HCl at 110°C for 3 hours and the reaction mixtures were neutralized with sodium hydrogen carbonate, charged on columns of Amberlite CG-50 (NH_4^+, pH 8.7) and eluted with concd. NH_4OH - H_2O (1: 150 to 1: 100) to give compound V which was considered to be 1-*N*-(2-aminoethanesulfonyl)-2-deoxystreptamine. Samples of V derived from I, II, III and IV showed the same Rf value on TLC on silica gel as *N*-(2-aminoethanesulfonyl)-2-deoxystreptamine prepared from 2-deoxystreptamine (amounts of V obtained, starting material and its amounts were as follows: 6.95 mg, I, 44.0 mg; 5.17 mg, II, 32.56 mg; 24.54 mg, III, 128.0 mg; 16.32 mg, IV, 50.45 mg). For comparison 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl]-2-deoxystrept-

Table 4. Physico-chemical properties of 1-*N*-(2-aminoethanesulfonyl)-2-deoxystreptamine (V) and 3-*N*-(2-aminoethanesulfonyl)-2-deoxystreptamine (X).

		V				X
	Origin	I	II	III	IV	VI
ORD	$[\alpha]_{400}$	−46°	−35°	−40°	−39°	+50°
	Curve	Plain negative				Plain positive
TLC R_{DSA}		1.26*				1.26**
		1.14**				1.14**
^1H NMR		Identical				Identical
^{13}C NMR		33.36				33.41
		35.14				35.12
		50.64				50.62
		51.20				51.19
		54.12				54.13
		73.27				73.28
		74.90				74.90
		76.03				76.04
		67.39 (dioxane)				67.39 (dioxane)

ORD data of V from I, II and IV were taken by Model J-20; V from III and X from VI by Model ORD/UV-5.

^1H NMR: Varian T-60, ^{13}C NMR: Varian FT-80A.

TLC systems: * BuOH - MeOH - $CHCl_3$ - concd. NH_4OH (4: 5: 2: 5)/silica gel; ** EtOH - $CHCl_3$ - concd. NH_4OH (4: 1: 2)/silica gel.

DSA: Z-Deoxystreptamine.

Fig. 2. Preparation of 3-*N*-(2-aminoethanesulfonyl)-2-deoxystreptamine.

VII
1. TEA, TFATCL/DMF
2. concd. NH_4OH
3. Amberlite CG-50 (NH_4^+)

VIII and IX
1. 6 N HCl, 110°C
2. Amberlite CG-50 (NH_4^+)

X   XI

TFATCL: *N*-Trifluoroacetyl-2-aminoethanesulfonyl chloride

amine (VII) was prepared by periodate oxidation of amikacin (VI) followed by HCl hydrolysis[3].

Compound VII (329 mg) was dissolved in aqueous *N,N*-dimethylformamide (DMF) and to the solution was added triethylamine (TEA). *N*-Trifluoroacetyl-2-aminoethanesulfonyl chloride (93 mg) in cold *N,N*-dimethylformamide was then added dropwise and the solution stirred at room temperature overnight. The reaction mixture was diluted with water and the residual reagent was decomposed by addition of concd. NH_4OH with warming. The resultant mixture was concentrated to dryness and redissolved in water. The aqueous solution was charged on a column of Amberlite CG-50 (NH_4^+, pH 8, 60 ml). After the column was washed with water and concd. NH_4OH - H_2O (1: 150), a mixture of compounds VIII and IX were eluted with concd. NH_4OH - H_2O (1: 75) to give a solid (TLC R_{DSA}: 0.95 and 1.07 (BuOH - MeOH - $CHCl_3$ - concd. NH_4OH (4: 5: 2: 5)/silica gel); 0.8 and 1.07 (EtOH - $CHCl_3$ - concd. NH_4OH (4: 1: 2)/silica gel)). Further elution with concd. NH_4OH - H_2O (1: 30) resulted in the recovery of the starting material (VII) (31 mg).

The mixture of VIII and IX was hydrolyzed by 6 N HCl at 110°C for 4.25 hours. The hydrolysate was neutralized to pH 7 with $NaHCO_3$, diluted with water and charged on a column of Amberlite CG-50 (NH_4^+, pH 8, 20 ml). After the column was washed with water, a mixture of X and XI was eluted with concd. NH_4OH - H_2O (1: 100). This was re-chromatographed on the same column (20 ml) and X (20.1 mg) and XI (61.3 mg) were isolated by successive elution with concd. NH_4OH - H_2O (1: 200) and (1: 100).

From the experimental procedure and physico-chemical properties, X was unambiguously determined to be 3-*N*-(2-aminoethanesulfonyl)-2-deoxystreptamine. As shown in Table 4, the physico-chemical properties of V were identical with those of X with respect to TLC R_{DSA} values, ^1H NMR and ^{13}C NMR spectra, however the ORD data was reversed indicating that V and X are antipodal compounds. Thus the structure of V was shown to be 1-*N*-(2-aminoethanesulfonyl)-2-deoxystreptamine. Accordingly the site of substitution in I, II, III and IV was proved to be the 1-*N* position.

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