SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 6′-N-ALKYL DERIVATIVES OF 1-N-[(*S*)-4-AMINO-2-HYDROXYBUTYRYL]-KANAMYCIN

Sir:

KAWAGUCHI *et al*[1] reported that a semi-synthetic antibiotic, 1-N-[(*S*)-4-amino-2-hydroxybutyryl]-kanamycin (amikacin, BB-K8) was effective against kanamycin-sensitive and -resistant bacteria. However, amikacin was enzymatically inactivated by 6′-N-acetylation[2]. UMEZAWA *et al*[3] synthesized 6′-N-methyl derivatives of kanamycin and 3′,4′-dideoxykanamycin B, which were active against kanamycin-resistant strains producing 6′-N-acetyltransferase. In this communication, we report the synthesis of 1-N-[(*S*)-4-amino-2-hydroxybutyryl]-6′-N-methylkanamycin (**I**) and 1-*N*-[(*S*)-4 amino-2-hydroxybutyryl]-6′-*N*-ethylkanamycin (**II**) which are hardly affected by a 6′-N-acetyltransferase.

6′-N-*tert*-Butyloxycarbonylkanamycin[4] (1.754 g, 3 mmoles) in a mixture of water (12.5 ml) and dimethoxyethane (12.5 ml) was acylated with N-hydroxysuccinimide ester (1.156 g, 3.3 mmoles) of (*S*)-4-benzyloxycarbonylamino-2-hydroxybutyric acid[5] in dimethoxyethane (25 ml) at room temperature for 24 hours. Subsequently, free amino groups of the acylated product were protected by benzyloxycarbonylation. To the solution of the acylated product and sodium bicarbonate (1g, 11.9 mmoles) in a mixture of water (12.5 ml) and acetone (12.5 ml), benzyloxycarbonyl chloride (1.68 g, 9.9 mmoles) was added under ice-cooling. After stirring for 18 hours, a colorless precipitate (3 g) was obtained. The 6′-N-*tert*-butyloxycarbonyl group of the product was removed in 90 % trifluoroacetic acid (75 ml) at room temperature for 1 hour. After evaporation of the reaction mixture and washing with ether (50 ml), the colorless crude powder (2.87 g) containing 3,3″-di-N-benzyloxycarbonyl-1-N-[(*S*)-4-benzyloxycarbonylamino-2-hydroxybutyryl]-kanamycin (**III**) was obtained.

The crude powder without purification was used for 6′-N-alkylation. To the solution of the crude powder (575 mg) in methanol (8ml) and 1 N NaOH (1 ml), 37 % aqueous formaldehyde (0.25ml) and then sodium borohydride (222mg) were added. After standing overnight at room temperature, the reaction mixture was concentrated to dryness and the residue was washed with water (7 ml) to afford a colorless powder (675 mg). The N-benzyloxycarbonyl groups were removed by catalytic hydrogenation with 5 % palladium on carbon (300 mg) in a mixture of acetic acid (5 ml), methanol (4ml) and water (1ml) under atmospheric pressure for 4.5 hours. After removal of catalyst by filtration, evaporation of the reaction mixture gave a colorless powder which was charged on a column of Amberlite CG50 (NH_4^+ form, 30ml). After washing the column with water (200 ml) and 0.3 N ammonia (150 ml), **I** was eluted with 0.5 N ammonia. The fractions containing **I** were detected by activity against *Bacillus subtilis* PCI219 and *Escherichia coli* JR66/W677, and by thin-layer chromatography (Rf 0.13) on Silica gel G (Merck, Art. 5721) using chloroform-methanol-28 % ammonia-water (1:4:2:1 in volume). The purified **I** was obtained as a colorless carbonate in 14 % yield from 6′-N-*tert*-butyloxycarbonylkanamycin, mp 163 ~ 166°C (dec.), $[\alpha]_D^{22}+78°$ (*c* 1, water).

Anal. Calcd. for $C_{23}H_{45}N_5O_{13}\cdot H_2CO_3$:
C43.56, H7.16, N10.59
Found: C43.13, H7.17, N10.13

II was synthesized by a similar method. The crude powder (1.13 g) containing **III** was ethylated with 90 % aqueous acetaldehyde (0.74 ml) and sodium borohydride (444 mg) in a mixture of methanol (16ml) and 2 N NaOH (1.8 ml). The N-benzyloxycarbonyl groups of the ethylated product (804mg) were removed by catalytic hydrogenation and then

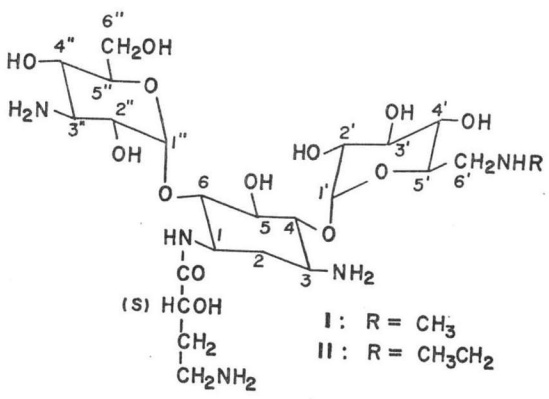

Table 1. The antimicrobial spectra of 1-N-[(*S*)-4-amino-2-hydroxybutyryl]-6′-N-methylkanamycin (**I**) and -6′-N-ethylkanamycin (**II**)

Test organisms	Minimum inhibitory concentrations (mcg/ml)	
	I	**II**
Staphylococcus aureus FDA 209P	0.78	1.56
S. aureus Smith	<0.20	0.39
S. aureus Terajima	<0.20	<0.20
Sarcina lutea PCI 1001	3.13	6.25
Bacillus anthracis	<0.20	<0.20
B. subtilis PCI 219	<0.20	<0.20
B. subtilis NRRL B-558	<0.20	0.78
B. cereus ATCC 10702	0.78	3.13
Corynebacterium bovis 1810	3.13	6.25
Mycobacterium smegmatis ATCC 607	0.39	3.13
Shigella dysenteriae JS 11910	3.13	6.25
S. flexneri 4b JS 11811	3.13	12.5
S. sonnei JS 11746	3.13	6.25
Salmonella typhosa T-63	0.78	1.56
S. enteritidis 1891	0.78	3.13
Proteus vulgaris OX 19	0.78	1.56
Klebsiella pneumoniae PCI 602	0.78	1.56
K. pneumoniae 22#3038	6.25	25
Escherichia coli NIHJ	1.56	1.56
E. coli K-12	0.78	1.56
E. coli K-12 R5	1.56	1.56
E. coli K-12 ML1629	1.56	3.13
E. coli K-12 ML1630	3.13	6.25
E. coli K-12 ML1410	1.56	3.13
E. coli K-12 ML1410 R81	3.13	12.5
E. coli LA290 R55	1.56	3.13
E. coli LA290 R56	0.78	1.56
E. coli LA290 R64	0.78	1.56
E. coli W667	1.56	1.56
E. coli JR66/W677	6.25	25
Pseudomonas aeruginosa A3	6.25	25
P. aeruginosa No. 12	25	25
P. aeruginosa TI-13	25	50
P. aeruginosa GN315	25	100
P. aeruginosa 99	25	100

II was purified by column chromatography on Amberlite CG50 (NH_4^+ form) eluted with 0.5 N ammonia. On thin-layer chromatography as described above, **II** showed Rf 0.20. The purified **II** was obtained as a colorless carbonate in 12% yield from 6′-N-*tert*-butyloxycarbonylkanamycin, mp 184~188°C (dec.), $[\alpha]_D^{25}$ +80° (*c* 1, water).

Anal. Calcd. for $C_{24}H_{47}N_5O_{13}\cdot H_2CO_3$:
C44.44, H7.31, N10.37
Found: C44.43, H6.97, N 9.81

The structures of **I** and **II** were confirmed by pmr spectra and acid hydrolysis. In the mass spectra of tetra-N-acetyl derivatives of **I** and **II**, M^+ peaks were not observed, but the following fragment peaks were shown; **I**: *m/e* 204 (from 3-acetamido-3-deoxyglucose moiety), 218 (from N-acetyl-6-deoxy-6-methylaminoglucose moiety) and 358 (from 1-N-[(*S*)-4-acetamido-2-hydroxybutyryl]-3-N-acetyl-2-deoxystreptamine moiety), **II**: *m/e* 204, 232 (from N-acetyl-6-deoxy-6-ethylaminoglucose moiety) and 358.

The antimicrobial spectra of **I** and **II** are given in Table 1, showing that **I** and **II** are more active than kanamycin against 6′-N-acetyltransferase-producing strains, *Escherichia coli* K-12 R5 and *Pseudomonas aeruginosa* GN 315. When the activities were assayed by the cup plate method using *Bacillus subtilis* PCI 219 and *Escherichia coli* JR66/W677 as test organisms, **I** showed 90% and 72% of the activity of BB-K8, respectively, and **II** showed 49% and 36%, respectively. The results of acetylation studies with a 6′-acetyltransferase obtained from *Pseudomonas aeruginosa* GN315 are reported in the next paper[6].

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