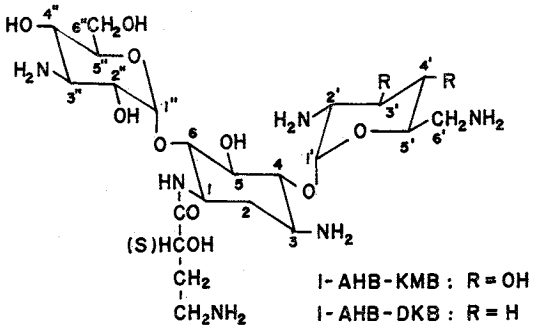
SYNTHESES OF 1-N-{(S)-4-AMINO-2-HYDROXYBUTYRYL}-KANAMYCIN B AND -3′, 4′-DIDEOXYKANAMYCIN B ACTIVE AGAINST KANAMYCIN-RESISTANT BACTERIA

Sir:

Recently, KAWAGUCHI *et al.*[1] reported the synthesis of BB-K8 having (S)-4-amino-2-hydroxybutyric acid (AHBA) at the 1-amino group of kanamycin, and found to be active against resistant bacteria.

In the course of the chemical derivation of kanamycins based on our studies on mechanisms of resistance to aminoglycosidic antibiotics, 1-N-{(S)-4-amino-2-hydroxybutyryl}-kananamycin B (1-AHB-KMB) and -3′, 4′-dideoxykanamycin B(1-AHB-DKB), which are not affected by kanamycin-phosphotransferases I[2,3] and II[4], and kanamycin-nucleotidyltransferase[5~8] have been synthesized.



We studied the selective protection of the five amino groups in kanamycin B and 3′, 4′-dideoxykanamycin B, and found that with benzyloxycarbonyl chloride or *tert*-butyloxycarbonyl azide the 2′-amino group is preferentially acylated after acylation of the 6′-amino group. Therefore, the two title compounds were synthesized by acylation of di-N-protected kanamycin B and 3′, 4′-dideoxykanamycin B[9,10] with AHBA using the active ester method.

6′-N-Benzyloxycarbonylkanamycin B (**I**) was selectively prepared in 37% yield from kanamycin B by reaction with an equimolar amount of benzyloxycarbonyl chloride followed by column chromatography on Amberlite CG 50 (NH_4^+), m.p. 213~220°C (dec.), $[\alpha]_D^{22}$+106° (*c* 1.0, water).

Anal. Calcd. for $C_{26}H_{43}N_5O_{12}$:
C 50.56, H 7.02, N 11.34
Found:
C 49.89, H 6.98, N 10.92

Mono-N-*tert*-butyloxycarbonylation of **I** (1.0 g, 1.62 mmole) with *tert*-butyloxycarbonyl azide (256 mg, 1.78 mmole) in a mixture of water, pyridine and triethylamine (10:10:1 in volume) at room temperature for 21 hours afforded 1.27 g of a yellowish powder (**II**). Thin-layer chromatography showed that **II** was a mixture of **I** and 6′-N-benzyloxycarbonyl-2′-N-*tert*-butyloxycarbonylkanamycin B (**III**) containing a trace of positional isomers of **III**. The **III** was purified by silicic acid chromatography and the structure was confirmed by acid hydrolysis after N-ethoxycarbonylation.

The **II** without purification in water (10 ml) and dimethoxyethane (10 ml) was acylated with 623 mg (1.78 mmole) of N-hydroxysuccinimide ester (**IV**) of (S)-4-benzyloxycarbonylamino-2-hydroxybutyric acid in dimethoxyethane (20 ml) at room temperature for 24 hours. The **IV** was prepared from L-2, 4-diaminobutyric acid by selective protection of the 4-amino group, deamination of the 2-amino group[11] and esterification. The N-protective groups of the acylated product were removed by acidic catalytic hydrogenation with 5% palladium on carbon (640 mg) in 90% trifluoroacetic acid (13 ml) and methanol (9 ml) under atmospheric pressure for 5 hours. After removing the catalyst by filtration, evaporation of the solution gave a colorless powder, which was charged on a column of Amberlite CG 50 in NH_4^+ form (80 ml). After washing the column with water and 0.3 N ammonia, 1-AHB-KMB was eluted with 0.5 N ammonia. The fractions containing 1-AHB-KMB were detected by activity against *Escherichia coli* JR 66/W 677 and by thin-layer chromatography (Rf 0.07) on Silica gel G (E. Merck) using *n*-butanol-ethanol-chloroform-17% ammonia (4:5:2:5 in volume). The 1-AHB-KMB was separated from 3-, 2′- and 3″-acylated derivatives and a small amount of diacylated derivatives by resin chromatography on Amberlite CG 50(NH_4^+). The purified 1-AHB-KMB (176 mg) was obtained as a colorless powder as the dicarbonate in 15.3% yield from **I**, m.p. 181~183°C (dec.), $[\alpha]_D^{20}$+85° (*c* 1.0, water).

Anal. Calcd. for $C_{22}H_{44}N_6O_{12}\cdot 2H_2CO_3$: C 40.67, H 6.83, N 11.86.
Found: C 41.31, H 6.28, N 11.64.

The IR spectrum shows amide bands at 1650 and 1575 cm^{-1}. The PMR spectrum in D_2O using tetramethylsilane as an external reference ($\delta=0$) shows signals at δ *ca.* 2.5 (*m*, 3-H_2 of AHBA), δ 3.58(*t*, 4-H_2 of AHBA), δ 4.70 (*q*, 2-H of AHBA), δ 5.52(*d*, 1''-H) and δ 5.67 ppm (*d*, 1'-H).

The 1-AHB-DKB was synthesized by a similar method. The 6'-N-*tert*-butyloxycarbonyl-3', 4'-dideoxykanamycin B (V) was prepared in 49% yield from 3', 4'-dideoxykanamycin B by reaction with an equimolar amount of *tert*-butyloxycarbonyl azide in a mixture of water, pyridine and triethylamine (10:10:1 in volume) at room temperature for

Table 1. The antimicrobial spectra of kanamycin B, 1-AHB-KMB and 1-AHB-DKB

Test organisms	Minimum inhibitory concentrations (mcg/ml)		
	Kanamycin B	1-AHB-KMB	1-AHB-DKB
Staphylococcus aureus FDA 209P	0.39	0.78	0.78
Staphylococcus aureus Smith	<0.20	<0.20	<0.20
Staphylococcus aureus Terajima	<0.20	0.20	<0.20
Sarcina lutea PCI 1001	1.56	3.13	1.56
Bacillus anthracis	<0.20	<0.20	<0.20
Bacillus subtilis PCI 219	<0.20	<0.20	<0.20
Bacillus subtilis NRRL B-558	<0.20	<0.20	<0.20
Bacillus cereus ATCC 10702	0.78	1.56	1.56
Corynebacterium bovis 1810	1.56	0.39	0.39
Mycobacterium smegmatis ATCC 607	0.78	0.78	<0.20
Shigella dysenteriae JS 11910	3.13	3.13	6.25
Shigella flexneri 4b JS 11811	3.13	3.13	6.25
Shigella sonnei JS 11746	1.56	1.56	3.13
Salmonella typhosa T-63	0.20	0.39	<0.20
Salmonella enteritidis 1891	1.56	3.13	1.56
Proteus vulgaris OX 19	0.78	1.56	0.39
Klebsiella pneumoniae PCI 602	0.78	0.39	0.78
Klebsiella pneumoniae 22#3038	>100	6.25	1.56
Escherichia coli NIHJ	0.78	0.78	0.78
Escherichia coli K-12	0.78	0.78	0.78
Escherichia coli K-12 R5	1.56	0.78	0.78
Escherichia coli K-12 ML1629	>100	1.56	0.78
Escherichia coli K-12 ML 1630	>100	1.56	0.78
Escherichia coli K-12 ML 1410	0.78	3.13	0.78
Escherichia coli K-12 ML1410 R81	>100	1.56	1.56
Escherichia coli LA290 R55	12.5	1.56	0.78
Escherichia coli LA290 R56	3.13	0.39	0.39
Escherichia coli LA290 R64	3.13	0.39	0.78
Escherichia coli W677	0.39	0.39	0.39
Escherichia coli JR66/W677	>100	3.13	1.56
Pseudomonas aeruginosa A3	50	6.25	3.13
Pseudomonas aeruginosa No. 12	12.5	6.25	1.56
Pseudomonas aeruginosa TI-13	100	6.25	3.13
Pseudomonas aeruginosa GN315	>100	50	25
Pseudomonas aeruginosa 99	>100	25	12.5

18 hours, followed by column chromatography on Amberlite CG 50 (NH_4^+) to remove other derivatives and 36% of unreacted 3′, 4′-dideoxykanamycin B; m.p. 136~140°C (dec.), $[\alpha]_D^{26}$+112° (*c* 1.0, water).

Anal. Calcd. for $C_{23}H_{45}N_5O_{10}\cdot H_2O$:
C 48.50, H 8.31, N 12.29.
Found: C 48.56, H 8.11, N 12.14.

The reaction of **V** (1.1 g, 2.0 mmole) with *tert*-butyloxycarbonyl azide (320 mg, 2.2 mmole) at the same conditions afforded 1.41 g of a yellowish powder. The powder without purification in water (20 ml) and dimethoxyethane (10 ml) was acylated with N-hydroxysuccinimide ester (696 mg, 2.2 mmole) of (S)-4-*tert*-butyloxycarbonylamino-2-hydroxybutyric acid in dimethoxyethane (10 ml) at room temperature for 20 hours. The *tert*-butyloxycarbonyl groups were removed in 90% trifluoroacetic acid (10 ml) at room temperature for 40 minutes. The reaction mixture was concentrated to dryness and charged on a column of Amberlite CG 50 in NH_4^+ form (50 ml). After washing the column with water and 0.5N ammonia, 1-AHB-DKB was eluted with 0.75N ammonia. The fractions containing 1-AHB-DKB were detected by activity and thin-layer chromatography (Rf 0.17) as described above. The purified 1-AHB-DKB (162 mg) was obtained as a colorless dicarbonate in a 12% yield from **V**, m.p. 178°C (dec.), $[\alpha]_D^{24}$+87° (*c* 0.77, water).

Anal. Calcd. for $C_{22}H_{44}N_6O_{10}\cdot 2H_2CO_3$:
C 42.60, H 7.15, N 12.42.
Found: C 42.91, H 6.95, N 12.17.

The IR spectrum shows amide bands at 1650 and 1575 cm^{-1}. The PMR spectrum shows signals at δ *ca.* 2.5 (*m*, 3-H_2 of AHBA), δ 3.60(*t*, 4-H_2 of AHBA), δ 4.72 (*q*, 2-H of AHBA), δ 5.54(*d*, 1″-H) and δ 5.67(*d*, 1′-H).

The penta-N-ethoxycarbonyl derivatives of 1-AHB-KMB and 1-AHB-DKB were prepared by the usual SHOTTEN-BAUMANN procedure and hydrolyzed in 6N hydrochloric acid at 100°C for 30 minutes. From the hydrolyzates, mono-N-ethoxycarbonyl-2-deoxystreptamine was isolated by resin chromatography of Amberlite CG 50 as a colorless powder, m.p. 199~201°C, $[\alpha]_D^{22}$+18° (*c* 0.22, water).

Anal. Calcd. for $C_9H_{18}N_2O_5\cdot 1/2H_2O$:
C 44.43, H 7.87, N 11.52.
Found: C 44.68, H 7.35, N 11.35.

By application of the TACu method,[12] the structure of the compound was determined to be 3-N-ethoxycarbonyl-2-deoxystreptamine. From these results, the structures of 1-AHB-KMB and 1-AHB-DKB were completely confirmed.

The antimicrobial spectra of kanamycin B, 1-AHB-KMB and 1-AHB-DKB are shown in Table 1, showing that 1-AHB-KMB and 1-AHB-DKB have strong activity against kanamycin-resistant bacteria producing kanamycin-phosphotransferase I (*E. coli* K-12 ML1629 and K-12 ML1410 R81), kanamycin-phosphotransferase II (*E. coli* JR66/W677) and kanamycin-nucleotidyltransferase (*E. coli* JR66/W677 and LA290 R55)

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