SYNTHESIS OF 1-N-ACYL DERIVATIVES OF 3′, 4′-DIDEOXY-6′-N-METHYL-KANAMYCIN B AND THEIR ANTIBACTERIAL ACTIVITIES

Sir:

As previously reported[1], 3′, 4′-dideoxy-kanamycin B (DKB) is active against kanamycin-resistant strains which phosphorylate the 3′-hydroxyl group of kanamycins. However, DKB is enzymatically inactivated by 6′-N-acetylation[2] and 2″-nucleotidylation.[3~5] As reported in other papers,[6,7] 1-N-(ω-amino-α-hydroxyacyl) derivatives of DKB are active against resistant strains producing 3′-phosphotransferases[8~10] and 2″-nucleotidyltransferase.[3~5] In this communication, we report the syntheses of 1-N-(DL-isoseryl), 1-N-(L-isoseryl), 1-N-[(*S*)-4-amino-2-hydroxybutyryl] and 1-N-[(*S*)-5-amino-2-hydroxy-*n*-valeryl] derivatives of 3′, 4′-dideoxy-6′-N-methylkanamycin B[11] (abbreviated as DL-IS-MDKB, IS-MDKB, AHB-MDKB and AHV-MDKB, respectively) which inhibit resistant strains producing 6′-N-acetyltransferase. These derivatives are not affected by the other enzymes inactivating deoxystreptamine-containing antibiotics.

3′, 4′-Dideoxy-6′-N-methylkanamycin B (MDKB) (500 mg, 1.08 mmoles) prepared by the method of UMEZAWA *et al.*[11] was partially acylated with BOC* azide (465 mg, 3.24 m moles) in a mixture (55 ml) of water, pyridine and triethylamine (3:5:5 in volume) at room temperature for 28.5 hours. The evaporation of the reaction mixture afforded a yellowish powder (727 mg) which was a mixture of the mono-N- and di-N-BOC derivatives containing a trace of the tri-N-BOC derivative. Column chromatography on Amberlite CG-50 (NH_4^+ form) showed that the powder contained more than 20 % of the 2′, 6′-di-N-BOC derivative.** Since complete purification was difficult, the powder without purification was used for 1-N-acylation with an equimolar amount of the N-hydroxysuccinimide ester of N-BOC-DL-isoserine,[7] N-BOC-L-isoserine,[7] N-BOC-(*S*)-4-amino-2-hydroxybutyric acid[6,12] or N-BOC-(*S*)-5-amino-2-hydroxy-*n*-valeric acid*** in a mixture of water and dimethoxyethane at room temperature for 18~24 hours by a method similar to that described in a

Table 1. Properties of 1-N-acyl derivatives of 3′,4′-dideoxy-6′-N-methylkanamycin B.

Derivative	mp (dec)	$[\alpha]_D$ in H_2O	Molecular formula[a]	Rf on TLC[b]	MS of penta-N-acetyl deriv.[c] (*m/e*)	Activity (%)[d]	
						B. sub.	*E. coli*
DL-IS-MDKB	165~169°	+96° at 24°	$C_{22}H_{44}N_6O_{10}\cdot H_2CO_3$	0.51	204, 227, 344	62	105
IS-MDKB	162~166°	+80° at 24°	$C_{22}H_{44}N_6O_{10}\cdot H_2CO_3$	0.51	204, 227, 344	49	94
AHB-MDKB	158~161°	+71° at 25°	$C_{23}H_{46}N_6O_{10}\cdot H_2CO_3$	0.38	204, 227, 358	187	114
AHV-MDKB	152~155°	+79° at 24°	$C_{24}H_{48}N_6O_{10}\cdot H_2CO_3$	0.39	204, 227, 372	129	92

a Satisfactory elemental analyses were obtained for all compounds.

b Thin-layer chromatography on Silica gel G (Merck, Art. 5721) developed with butanol - ethanol - chloroform - 28 % ammonia (4:5:2:8 in volume), and detected by ninhydrin reaction.

c Penta-N-acetyl derivatives were prepared with acetic anhydride in methanol. Fragment peak at *m/e* 204 corresponds to the 3-amino-3-deoxy-α-D-glucose moiety; at *m/e* 227 to 2, 6-diamino-2, 3, 4, 6-tetradeoxy-6-N-methyl-α-D-*erythro*-hexopyranose moiety: at *m/e* 344, 358 and 372 to 1-N-(isoseryl)-, 1-N-(4-amino-2-hydroxybutyryl)- and 1-N-(5-amino-2-hydroxy-*n*-valeryl)-2-deoxystreptamine moieties, respectively.

d The activities were compared by the cup plate method. Assay standard: 1-N-[(*S*)-4-amino-2-hydroxybutyryl]-3′, 4′-dideoxykanamycin B[6] (100 %). Test organisms: *Bacillus subtilis* PCI 219 and *Escherichia coli* K-12.

* BOC: *tert*-butyloxycarbonyl group.

** The 2′, 6′-di-N-BOC-3′, 4′-dideoxy-6′-N-methylkanamycin B showed Rf 0.60 by thin-layer chromatography on Silica gel G using butanol - ethanol - chloroform - 17% ammonia (4:5:2:3 in volume). The structure was confirmed by the pmr spectrum and the mass spectrum of the tri-N-acetyl derivative.

*** N-BOC-(*S*)-5-amino-2-hydroxy-*n*-valeric acid was prepared by preferential deamination of L-ornithine monohydrochloride by the method of OHSHIRO *et al.*[13] followed by N-protection with *tert*-butyl S-4, 6-dimethylpyrimid-2-ylthiocarbonate.[14]

Table 2. The antimicrobial spectra of 3′, 4′-dideoxy-6′-N-methylkanamycin B (MDKB) and its 1-N-acyl derivatives.

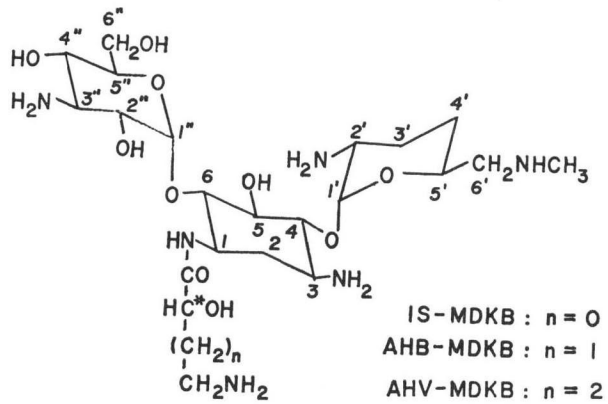
Test organisms	Minimum inhibitory concentrations (mcg/ml)				
	DL-IS-MDKB	IS-MDKB	AHB-MDKB	AHV-MDKB	MDKB
Staphylococcus aureus FDA 209P	0.78	<0.20	0.78	0.39	<0.20
S. aureus Smith	<0.20	<0.20	<0.20	<0.20	<0.20
S. aureus Terajima	<0.20	<0.20	<0.20	<0.20	<0.20
Sarcina lutea PCI 1001	6.25	3.13	3.13	12.5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

previous paper.[6] The N-BOC groups of the acylated product were removed in 90% trifluoroacetic acid at room temperature for 1 hour. The reaction mixture was concentrated to dryness, dissolved in water and charged on a column of Amberlite CG-50 (NH_4^+ form). After washing the column with five resin-volumes each of water and 0.3 N ammonia, DL-IS-MDKB or IS-MDKB was eluted with 0.5 N ammonia, and AHB-MDKB or AHV-MDKB was eluted with 0.75 N ammonia. The eluate was cut into one-tenth resin volume fractions. These in fractions were tested by thin-layer chromatography (Rf values are shown in Table 1) and the activity against *Bacillus subtilis* PCI 219 and *Escherichia*

coli JR66/W677 was examined. Further purification of the products obtained from the active fractions was accomplished by column chromatography on silicic acid (Mallinckrodt, CC-7) developed with methanol - chloroform - 17 % ammonia (4:1:2 in volume). The purified products DL-IS-MDKB, IS-MDKB, AHB-DKB and AHV-DKB were obtained as colorless carbonates in 4 %, 7 %, 3 % and 3 % yield, respectively, from MDKB.

The properties of the four derivatives described above are summarized in Table 1. The structures were confirmed by the pmr spectra, the mass spectra of the penta-N-acetyl derivatives (Table 1) and the acid hydrolysis. The method of synthesis described above is known to give 1-N-acyl derivatives in preference to the 3-N-acyl derivatives which have only weak activity.[12]

The antimicrobial spectra of these four derivatives are shown in Table 2. The antibacterial activity assayed by the cup plate method using *Bacillus subtilis* PCI 219 and *Escherichia coli* K-12 as test organisms is shown in Table 1. These 1-N-acyl derivatives of 3′,4′-dideoxy-6′-N-methylkanamycin B are broadly active against kanamycin-resistant strains producing 3′-phosphotransferases I and II, 2″-nucleotidyltransferase, and 6′-N-acetyltransferase (*Escherichia coli* K-12 R5 and *Pseudomonas aeruginosa* GN315). The AHB-MDKB compound was especially effective against kanamycin-resistant strains.



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