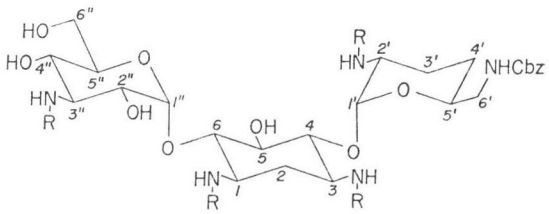
SYNTHESES AND PROPERTIES OF THE 6′-C-ALKYL DERIVATIVES OF 3′,4′-DIDEOXYKANAMYCIN B

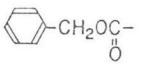
Sir:

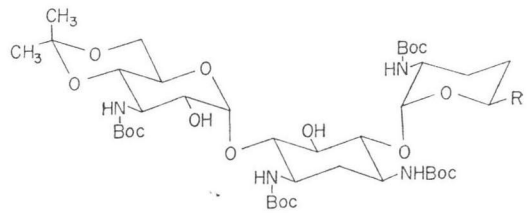
The chemical modification of kanamycins to give derivatives active against resistant strains which form 3′-O-phosphotransferases has been successful[1,2]. However, the 6′-N-methylation of aminoglycoside antibiotics has been not enough to inhibit the reaction of all 6′-N-acetyltransferases, and 6′-N-ethyl and 6′-deamino derivatives have one fourth or lower activity than the parent antibiotics[3,4]. In this communication, we wish to report syntheses and properties of 6′-C-alkyl derivatives of 3′,4′-dideoxykanamycin B. Both 6′(*S*)- and 6′(*R*)-C-alkyl derivatives are strongly active against sensitive and resistant strains. The former is more active than the latter in inhibiting some resistant strains producing 6′-N-acetyltransferase.

By the method reported in a previous paper[5], 6′-C-alkyl derivatives of 3′,4′-dideoxykanamycin B were synthesized through a 5′-deaminomethyl-5′-C-formyl derivative. The free amino groups of 6′-N-benzyloxycarbonyl-3′,4′-dideoxykanamycin B[6] (**1**) were protected with *tert*-butoxycarbonyl group by reaction with *tert*-butyl S-4,6-dimethylpyrimid-2-ylthiocarbonate in aqueous dioxane at room temperature for 22 hours in 99% yield. Treatment of the 6′-N-benzyloxycarbonyl-1,3,2′,3″-tetra-N-*tert*-butoxycarbonyl derivative (**2**) with 2,2-dimethoxypropane in anhydrous N,N-dimethylformamide in the presence of *p*-toluenesulfonic acid at 60°C for 1 hour followed by silicic acid column chromatography (chloroform - methanol, 100: 1) gave 6′-N-benzyloxycarbonyl-1,3,2′,3″-tetra-N-*tert*-butoxycarbonyl-4″,6″-O-isopropylidene-3′,4′-dideoxykanamycin B (**3**) in 79% yield. The N-benzyloxycarbonyl group in **3** was removed by catalytic hydrogenation with 5% palladium-barium carbonate in a mixture of ethanol and methanol under atmospheric pressure for 6 hours to afford the 1,3,2′,3″-tetra-N-*tert*-butoxycarbonyl-4″,6″-O-isopropylidene derivative (**4**) in 78% yield. Oxidation of a primary amino group in **4** with ninhydrin and sodium hydrogencarbonate in a heterogeneous mixture of chloroform and water at room temperature for 42.5 hours followed by silicic acid column chromatography (dichloromethane - ethanol, 40: 1) afforded 1,3,2′,3″-tetra-N-*tert*-butoxycarbonyl-5′-deaminomethyl-5′-C-formyl-4″,6″-O-isopropylidene-3′,4′-dideoxykanamycin B (**5**) in 59% yield, mp 205~207°C (decomp.), PMR(dioxane-d_8): δ 9.57 (s, CHO).

Treatment of **5** in dichloromethane with an excess of ethereal diazomethane (0.5 M) at room temperature for 18 hours followed by silicic acid column chromatography (choloroform - methyl ethyl ketone, 2: 1) gave the 5′-C-ethanoyl derivative (**6**) in 81% yield, mp 216~218°C (decomp.), $[\alpha]_D^{22}$ +64° (*c* 0.3, methanol), PMR (chloroform-d): δ 2.21 (s, $COCH_3$). Reductive amination of **6** in anhydrous methanol with ammonium acetate and sodium cyanoborohydride followed by silicic acid column chromatography (chloroform - methanol - 17% aqueous ammonia, 80: 10: 1) afforded two diastereomers, the 6′(*S*)-



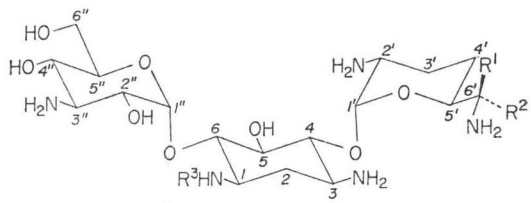
1 R=H
2 R=Boc
Cbz = 
Boc=$(CH_3)_3COC(=O)$-



3 R=-CH_2NHCbz
4 R=-CH_2NH_2
5 R=-CHO
6 R=-$COCH_3$
7 R=-$COCH_2CH_3$
8 R=-$CH(NH_2)CH_3$ (S)
9 R=-$CH(NH_2)CH_3$ (R)
10 R=-$CH(NH_2)CH_2CH_3$ (S)
11 R=-$CH(NH_2)CH_2CH_3$ (R)

C-methyl derivative (**8**) and 6′(*R*)-C-methyl derivative (**9**) in each 31% yield. The N-*tert*-butoxycarbonyl groups and O-isopropylidene group in **8** were removed in 90% trifluoroacetic acid at room temperature for 45 minutes to afford 6′(*S*)-C-methyl-3′,4′-dideoxykanamycin B (**12**) as a monocarbonate, which was purified by column chromatography on Amberlite CG-50 (NH_4^+) resin and eluted with 0.3 N ammonia in a quantitative yield. The compound **9** was also converted into a monocarbonate of 6′(*R*)-C-methyl-3′,4′-dideoxykanamycin B (**13**).

The treatment of **5** with diazoethane (70% yield), reductive amination of the 5′-C-propanoyl derivative (**7**) with ammonium acetate and sodium cyanoborohydride, separation of two diastereomers (**10** and **11**) by silicic acid column chromatography (41% and 32% yield, respec-



12 $R^1, R^3=H, R^2=-CH_3$
13 $R^1=-CH_3, R^2, R^3=H$
14 $R^1, R^3=H, R^2=-CH_2CH_3$
15 $R^1=-CH_2CH_3, R^2, R^3=H$
16 $R^1=H, R^2=-CH_3, R^3=AHB$
17 $R^1=-CH_3, R^2=H, R^3=AHB$

AHB = $-CO\overset{1'''}{C}H(OH)\overset{2'''}{C}H_2\overset{3'''}{C}H_2\overset{4'''}{C}H_2NH_2$ (S)

Table 1. Properties of 6′-C-alkyl derivatives of 3′,4′-dideoxykanamycin B.

Compound	mp (decomp.)	$[\alpha]_D$ in H_2O	Molecular formula	MS *m/e*	Rf on TLC*
12	169~173°C	+ 93° at 22°	$C_{19}H_{39}N_5O_8 \cdot H_2CO_3$	466 $(M+1)^+$	0.48
13	162~167°C	+103° at 22°	$C_{19}H_{39}N_5O_8 \cdot H_2CO_3$	466 $(M+1)^+$	0.43
14	145~152°C	+111° at 22°	$C_{20}H_{41}N_5O_8 \cdot H_2CO_3$	479 M^+	0.53
15	149~155°C	+117° at 22°	$C_{20}H_{41}N_5O_8 \cdot H_2CO_3$	479 M^+	0.51
16	156~164°C	+ 70° at 25°	$C_{23}H_{46}N_6O_{10} \cdot 2H_2CO_3$		0.42
17	156~166°C	+ 76° at 25°	$C_{23}H_{46}N_6O_{10} \cdot 1\frac{1}{2}H_2CO_3$		0.38

* TLC: on cellulose (Avicel) plates using butanol - ethanol - chloroform - 17% aq. ammonia (4: 5: 2: 5, v/v).

Table 2. The carbon 13 chemical shifts.

Carbon	Chemical shift (ppm)						Carbon	Chemical shift (ppm)					
	12 pD 4.2	**13** pD 4.3	**14** pD 4.6	**15** pD 4.2	**16** pD 4.6	**17** pD 4.5		**12** pD 4.2	**13** pD 4.3	**14** pD 4.6	**15** pD 4.2	**16** pD 4.6	**17** pD 4.5
1	50.6*	50.0*	50.5*	50.6*	49.7*	49.7*	7′	15.2	13.1	22.7	22.1	15.2	13.6
2	28.9	28.4	29.3	29.5	32.0**	31.6	8′			9.7	10.3		
3	49.5*	48.9*	49.5*	49.4*	49.7*	49.6	1″	101.3	100.8	101.3	101.3	98.8	98.8
4	77.7	77.6	78.5	79.1	79.4	79.3	2″	68.9	68.4	68.9	68.9	68.9	68.8
5	75.2	74.6	75.2	75.1	75.8	75.7	3″	55.7	55.2	55.7	55.7	55.9	55.9
6	84.6	84.1	84.7	84.9	81.2	81.1	4″	66.3	65.7	66.2	66.3	66.5	66.5
1′	95.5	95.6	95.7	96.5	95.7	96.2	5″	73.7	73.1	73.6	73.6	72.9	72.9
2′	49.6*	49.2*	49.7*	49.6*	49.7*	49.7*	6″	60.7	60.2	60.7	60.7	60.7	60.6
3′	21.1	20.9	21.1	21.4	21.3	21.5	1‴					176.2	176.2
4′	26.1	22.7	26.2	22.6	26.1	23.2	2‴					70.4	70.4
5′	70.9	69.2	69.5	68.9	70.8	69.7	3‴					31.6**	31.6
6′	51.9*	50.0*	57.2	56.2	51.9*	50.5*	4‴					37.8	37.8

The ^{13}C FT NMR spectra were taken with a Varian XL-100 spectrometer in D_2O. Dioxane (67.4 ppm) was used as the internal reference. Similar values with asterisks within each column may be interchanged.

Table 3. Minimum inhibitory concentrations (μg/ml).

Test organism	Inactivating enzyme	12	13	14	15	16	17	DKB*
Staph. aureus FDA 209P		0.78	0.78	1.56	1.56	1.56	0.78	0.78
Staph. aureus Smith		<0.20	<0.20	0.39	<0.20	<0.20	<0.20	<0.20
Staph. aureus Ap01	ANT(4′)	0.78	0.78	1.56	3.13	1.56	0.78	0.78
Staph. epidermidis 109	ANT(4′)	0.78	0.78	1.56	1.56	1.56	0.78	0.78
Micrococcus flavus FDA16		12.5	12.5	50	100	1.56	1.56	12.5
Sarcina lutea PCI1001		12.5	100	50	50	1.56	3.13	25
B. anthracis		<0.20	<0.20	<0.20	0.39	<0.20	<0.20	<0.20
B. subtilis PCI219		<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
B. subtilis NRRL B-558		<0.20	<0.20	0.39	0.39	<0.20	<0.20	<0.20
B. cereus ATCC10702		1.56	1.56	3.13	3.13	1.56	1.56	1.56
Corynebact. bovis 1810		12.5	25	50	50	3.13	3.13	25
Mycob. smegmatis ATCC607		0.39	1.56	1.56	3.13	0.78	0.78	0.78
E. coli NIHJ		1.56	1.56	3.13	6.25	3.13	1.56	1.56
E. coli K-12		1.56	6.25	3.13	3.13	1.56	0.78	1.56
E. coli K-12 R5	AAC(6′)	6.25	100	12.5	25	6.25	100	>100
E. coli K-12 R388		0.78	0.78	3.13	3.13	1.56	0.78	1.56
E. coli K-12 J5R11-2	APH(3′)-I	1.56	1.56	3.13	3.13	1.56	0.78	0.78
E. coli K-12 ML1629	APH(3′)-I	1.56	3.13	3.13	3.13	3.13	3.13	3.13
E. coli K-12 ML1630		1.56	1.56	6.25	6.25	3.13	3.13	1.56
E. coli K-12 ML1410		1.56	1.56	6.25	3.13	12.5	6.25	0.78
E. coli K-12 ML1410 R81	APH(3′)-I	3.13	1.56	6.25	3.13	1.56	1.56	1.56
E. coli K-12 LA290 R55	ANT(2″)	>100	>100	>100	>100	3.13	3.13	>100
E. coli K-12 LA290 R56		25	50	100	25	1.56	1.56	25
E. coli K-12 LA290 R64		25	12.5	50	12.5	1.56	3.13	12.5
E. coli W677		1.56	1.56	3.13	1.56	1.56	3.13	0.78
E. coli JR66/W677	APH(3′)-II ANT(2″)	100	>100	>100	100	6.25	6.25	100
E. coli K-12 C600 R135	AAC(3)	1.56	1.56	6.25	1.56	1.56	0.78	1.56
E. coli JR225	AAC(3)	>100	>100	>100	>100	1.56	1.56	>100
Kl. pneumoniae PCI602		3.13	1.56	3.13	3.13	1.56	1.56	3.13
Sh. dysenteriae JS11910		6.25	3.13	12.5	12.5	6.25	6.25	3.13
Sh. flexneri 4b JS11811		6.25	3.13	12.5	6.25	6.25	6.25	6.25
Sh. sonnei JS11746		12.5	6.25	12.5	12.5	6.25	6.25	3.13
Salm. typhi T-63		0.39	0.39	1.56	1.56	0.78	0.39	0.78
Salm. enteritidis 1891		3.13	1.56	6.25	3.13	1.56	1.56	3.13
Proteus vulgaris OX19		0.78	0.78	1.56	1.56	0.78	0.78	0.39
Proteus rettgeri GN311		12.5	6.25	12.5	12.5	100	50	6.25
Proteus rettgeri GN466		6.25	6.25	12.5	12.5	12.5	12.5	6.25
Serratia marcescens		50	25	25	12.5	50	25	50
Serratia sp. SOU		100	50	>100	12.5	100	50	>100
Providencia sp. Pv16	AAC(2′)	100	>100	100	100	12.5	50	>100
Providencia sp. 2991	AAC(2′)	100	>100	>100	>100	25	50	>100

(to be continued)

Table 3. (continued)

Test organism	Inactivating enzyme	12	13	14	15	16	17	DKB*
Ps. aeruginosa A3		3.13	3.13	12.5	6.25	6.25	3.13	12.5
Ps. aeruginosa No. 12		100	50	100	50	50	25	12.5
Ps. aeruginosa H9	APH(3′)-II	50	25	50	50	25	25	6.25
Ps. aeruginosa H11		50	25	100	100	>100	100	12.5
Ps. aeruginosa TI-13	APH(3)′-I	25	25	50	50	25	25	6.25
Ps. aeruginosa GN315	AAC(6′)	25	100	50	50	50	50	>100
Ps. aeruginosa 99	AAC(3)	50	25	100	50	100	50	6.25
Ps. aeruginosa B-13	APH(3′)-I, -II	50	25			100	50	12.5
Ps. aeruginosa 21-75	APH(3′)-III	>100	>100	>100	>100	100	100	>100
Ps. aeruginosa PST1	AAC(3)	>100	>100	>100	>100	100	50	>100
Ps. aeruginosa ROS134/PU21	AAC(3)	>100	>100	>100	>100	100	50	>100
Ps. aeruginosa K-Ps102	Permeability	50	25	100	50	50	25	6.25
Ps. maltophilia GN907	Permeability	>100	>100	100	>100	100	>100	>100

* DKB is the abbreviation of 3′,4′-dideoxykanamycin B.

tively), removal of the protecting groups in **10** and **11**, followed by resin chromatography on Amberlite CG-50 (NH_4^+) gave 6′(*S*)-C-ethyl-3′,4′-dideoxykanamycin B (**14**) and 6′ (*R*)-C-ethyl-3′,4′-dideoxykanamycin B (**15**) as monocarbonates.

The 1-N-[(*S*)-4-amino-2-hydroxybutyryl] derivatives, **16** and **17** were prepared by 1-N-acylation of the 3,2′,6′-tri-N-protected derivatives of **12** and **13** with the N-hydroxysuccinimide ester of (*S*)-4-benzyloxycarbonylamino-2-hydroxybutyric acid followed by removal of the N-protecting groups.

The properties of the six compounds described above are shown in Table 1. The chemical shifts of carbon-13 FOURIER-transform NMR spectra of these compounds were assigned as shown in Table 2. Absolute structures at C-6′ in **12** and **13** were confirmed by optical rotations and PMR spectra of di-N-acetyl diethyldithioacetals of purpurosamine B[7,8] and 6-*epi*-purpurosamine B[9] which we derived from **13** and **12**, respectively. The stereochemistry at C-6′ in **14** and **15** was also confirmed by the comparison of their optical rotations (Table 1), Rf values on TLC (Table 1) and carbon-13 chemical shifts of C-4′ (Table 2).

As shown in Table 3, the minimum inhibitory concentrations of six compounds (**12**, **13**, **14**, **15**, **16** and **17**) were tested. These compounds showed similar activity to 3′,4′-dideoxykanamycin B except for the activity against resistant strains producing 6′-N-acetyltransferase. It is an especially interesting finding that 6′(*S*)-C-alkyl derivatives are much more active than 6′(*R*)-alkyl derivatives against a 6′-acetyltransferase-producing resistant strain (*Escherichia coli* K-12 R5).

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References

1) UMEZAWA, H.: Biochemical mechanism of resistance to aminoglycosidic antibiotics. *in* Advances in Carbohydrate Chemistry and Biochemistry. Vol. 30, *ed.* by R. S. TIPSON & D. HORTON, pp. 183~225, Academic Press, New York, 1974

2) UMEZAWA, H.: Biochemical mechanism of resistance to aminoglycosidic antibiotics. *in* Drug Action and Drug Resistance in Bacteria. 2. Aminoglycoside antibiotics. *ed.* by S. MITSUHASHI, pp. 211~248, University of Tokyo Press, Tokyo, 1975

3) YAGISAWA, M.; S. KONDO, T. TAKEUCHI & H. UMEZAWA: Aminoglycoside 6′-N-acetyltransferase of *Pseudomonas aeruginosa*. Structural

requirements of substrate. J. Antibiotics 28: 486~489, 1975

4) KONDO, S.; T. MIYASAKA, K. YOSHIDA, K. IINUMA & H. UMEZAWA: Syntheses and properties of kanamycin C derivatives active against resistant bacteria. J. Antibiotics 30: 1150~1152, 1977

5) IKEDA, D.; T. MIYASAKA, K. YOSHIDA, K. IINUMA, S. KONDO & H. UMEZAWA: The chemical conversion of gentamine C_{1a} into gentamine C_2 and its 6'-epimer. J. Antibiotics 32: 1357~1359, 1979

6) UMEZAWA, H.; Y. NISHIMURA, T. TSUCHIYA & S. UMEZAWA: Syntheses of 6'-N-methylkanamycin and 3',4'-dideoxy-6'-N-methylkanamycin B active against resistant strains having 6'-N-acetylating enzymes. J. Antibiotics 25: 743~745, 1972

7) DANIELS, P. J. L.: The elucidation of the structures of gentamicin and sisomicin and the current status of clinical resistance to these antibiotics. *in* Drug Action and Drug Resistance in Bacteria. 2. Aminoglycoside Antibiotics. *ed.* by S. MITSUHASHI, pp. 77~111, University of Tokyo Press, Tokyo, 1975

8) COOPER, D. J.; M. D. YUDIS, H. M. MARIGLIANO & T. TRAUBEL: The purpurosamines, a new class of naturally occuring 2,6-diaminomonosaccharides. J. Chem. Soc. (C) 1971: 2876~2879, 1971

9) EGAN, R. S.; R. S. STANASZEK, M. CIROVIC, S. L. MUELLER, J. TADANIER, J. R. MARTIN, P. COLLUM, A. W. GOLDSTEIN, R. L. DEVAULT, A. C. SINCLAIR, E. E. FAGER & L. A. MITSCHER: Fortimicins A and B, new aminoglycoside antibiotics. III. Structural identification. J. Antibiotics 30: 552~563, 1977