SYNTHESES OF ISOSERYL DERIVATIVES OF KANAMYCINS AND THEIR ANTIBACTERIAL ACTIVITIES

Sir:

KAWAGUCHI *et al.*[1] reported the synthesis of BB-K8 with (S)-4-amino-2-hydroxybutyryl group at the 1-amino group of kanamycin and its activity against resistant bacteria. In the course of our studies on the chemical derivation of kanamycins based on biochemical mechanisms of resistance to aminoglycosidic antibiotics, we synthesized 1-N-[(S)-4-amino-2-hydroxybutyryl]-kanamycin B and -3′, 4′-dideoxykanamycin B[2], and 1, 2′-di-N-[(S)-4-amino-2-hydroxybutyryl]-3′,4′-dideoxykanamycin B[3] which were active against kanamycin-resistant bacteria producing kanamycin phosphotransferases I[4,5] and II[6], and kanamycin nucleotidyl transferase[7~10].

In this communication, the syntheses and antibacterial activities of several derivatives of kanamycin, kanamycin B and 3′, 4′-dideoxykanamycin B acylated at the 1-amino group with isoserine instead of 4-amino-2-hydroxybutyric acid are reported.

It was found that 1-N-(DL-isoseryl)-kanamycin (**I**) and 1-N-(D-isoseryl)-kanamycin (**II**) are about as active as 1-N-(L-isoseryl)-kanamycin (**III**) in inhibiting growth of kanamycin-sensitive and -resistant bacteria, while 1-N-[(R)-4-amino-2-hydroxybutyryl]-kanamycin has about one-fourth activity of BB-K8 with S-configuration as reported by NAITO *et al.*[11] HASKELL *et al.*[12] has reported butirosin analogs introducing configurational isomers of isoserine as amino acid moieties.

I was synthesized from 6′-N-BOC*-kanamycin by acylation with BOC-DL-isoserine. The 6′-N-BOC-kanamycin was prepared in a 45 % yield from kanamycin by reaction with an equimolar amount of BOC-azide in a mixture of water, pyridine and triethylamine (10 : 10 : 1 in volume) at room temperature for 20 hours, and purified by column chromatography on Amberlite CG 50 (NH_4^+) to remove the other derivatives and 37 % of unreacted kanamycin. The 6′-N-BOC-kanamycin ($C_{23}H_{44}N_4O_{13}\cdot H_2O$) shows mp 202~203°C (dec) and $[\alpha]_D^{27}+112°$ (*c* 1, water).

The 6′-N-BOC-kanamycin (635 mg, 1.0 mmole) in a mixture of water (10 ml) and dimethoxyethane (5 ml) was acylated with the N-hydroxysuccinimide ester[13] (303 mg, 1.0 mmole) of BOC-DL-isoserine** in dimethoxyethane (5 ml) at room temperature for 21 hours. The BOC groups were removed in 90 % trifluoroacetic acid (5 ml) at room temperature for 45 minutes. The reaction mixture was concentrated to dryness and charged on a column of Amberlite CG 50 (NH_4^+, 25 ml). After washing the column with water (125 ml), **I** was eluted with 0.25 N ammonia. **I** in fractions (5 ml each) was detected by activity

Table 1. The properties of isoseryl derivatives of kanamycins

Derivatives	mp (dec)	$(\alpha)_D$ in H_2O	Molecular formula[a]	Rf on TLC[b]	
				A	B
I (1-IS-KM)	174~177°	+89° at 25°	$C_{21}H_{41}N_5O_{13}\cdot 2H_2CO_3$	0.09	0.29
II (1-D-IS-KM)	184~188°	+82° at 24°	$C_{21}H_{41}N_5O_{13}\cdot 2H_2CO_3$	0.09	0.29
III (1-L-IS-KM)	184~187°	+74° at 24°	$C_{21}H_{41}N_5O_{13}\cdot 2H_2CO_3$	0.09	0.29
IV (1-IS-KMB)	179~184°	+86° at 26°	$C_{21}H_{42}N_6O_{12}\cdot 2H_2CO_3$	0.11	0.33
V (1-IS-DKB)	174~175°	+82° at 26°	$C_{21}H_{42}N_6O_{10}\cdot 2H_2CO_3$	0.21	0.45
VI (1, 2′-IS-DKB)	175~178°	+96° at 27°	$C_{24}H_{47}N_7O_{12}\cdot H_2CO_3$	0.11	0.27

[a] Satisfactory results of elemental analyses were obtained for all compounds.

[b] Thin-layer chromatography on Silica gel G (E. Merck) using solvent A; butanol-ethanol-chloroform-17 %ammonia (4 : 5 : 2 : 5 in volume) and solvent B; chloroform-methanol-28 %ammonia-water (1 : 4 : 2 : 1 in volume).

* Abbreviated for *tert*-butyloxycarbonyl group.

** Three configurational isomers of BOC-isoserine were prepared from DL-, D- and L-isoserine by reaction with *tert*-butyl S-4, 6-dimethylpyrimid-2-ylthiocarbonate[14]. These isoserines were kindly supplied by Pharmaceutical Product Development Laboratories, Meiji Seika Kaisha, Ltd.

Table 2. The antimicrobial spectra of isoseryl derivatives of kanamycins

Test organisms	Minimum inhibitory concentrations (mcg/ml)					
	1-IS-KM (I)	1-D-IS-KM (II)	1-L-IS-KM (III)	1-IS-KMB (IV)	1-IS-DKB (V)	1, 2′-IS-DKB (VI)
Staphylococcus aureus FDA 209P	0.78	0.78	1.56	1.56	0.78	1.56
S. aureus SMITH	0.20		0.20	<0.20	<0.20	
S. aureus TERAJIMA	<0.20		0.20	<0.20	<0.20	
Sarcina lutea PCI 1001	3.13		6.25	6.25	1.56	
Bacillus anthracis	<0.20		<0.20	<0.20	<0.20	
B. subtilis PCI 219	<0.20		<0.20	<0.20	<0.20	
B. subtilis NRRL B-558	<0.20		0.20	<0.20	<0.20	
B. cereus ATCC 10702	0.78		0.78	1.56	1.56	
Corynebacterium bovis 1810	1.56		1.56	1.56	0.78	
Mycobacterium smegmatis ATCC 607	0.39	0.39	0.20	0.78	<0.20	1.56
Shigella dysenteriae JS 11910	3.13		3.13	6.25	3.13	
S. flexneri 4b JS 11811	3.13		6.25	3.13	3.13	
S. sonnei JS 11746	1.56		1.56	6.25	3.13	
Salmonella typhosa T-63	0.78		0.39	0.78	0.78	
S. enteritidis 1891	0.78		1.56	0.78	0.39	
Proteus vulgaris OX 19	0.39		0.20	0.78	0.20	
Klebsiella pneumoniae PCI 602	0.39	0.39	0.39	0.78	0.39	3.13
K. pneumoniae 22#3038	1.56	1.56	1.56	6.25	1.56	12.5
Escherichia coli NIHJ	1.56	1.56	1.56	3.13	1.56	6.25
E. coli K-12	0.78	0.78	0.78	1.56	1.56	6.25
E. coli K-12 R5	25	25	25	6.25	6.25	50
E. coli K-12 ML1629	1.56	0.78	1.56	3.13	1.56	6.25
E. coli K-12 ML1630	1.56	0.78	1.56	3.13	1.56	6.25
E. coli K-12 ML1410	1.56	1.56	1.56	1.56	1.56	6.25
E. coli K-12 ML1410 R81	1.56	1.56	1.56	3.13	1.56	6.25
E. coli LA290 R55	1.56	0.78	1.56	1.56	1.56	6.25
E. coli LA290 R56	0.78	0.39	0.78	0.78	0.39	6.25
E. coli LA290 R64	0.78	0.78	0.78	0.78	0.39	6.25
E. coli W677	0.78	0.39	0.78	0.78	0.78	3.13
E. coli JR66/W677	3.13	1.56	3.13	12.5	3.13	12.5
Pseudomonas aeruginosa A3	3.13	1.56	3.13	6.25	3.13	6.25
P. aeruginosa No. 12	0.78	3.13	3.13	6.25	1.56	25
P. aeruginosa TI-13	3.13	3.13	3.13	6.25	3.13	12.5
P. aeruginosa GN315	>100	>100	>100	50	12.5	>100
P. aeruginosa 99	6.25	6.25	6.25	6.25	3.13	50

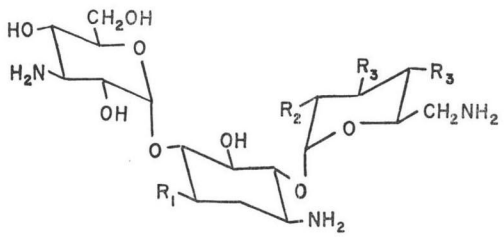
against *Escherichia coli* JR66/W677, a producer of kanamycin phosphotransferase II and kanamycin nucleotidyl transferase, and by thin-layer chromatography (Rf values shown in Table 1). Further purification of the crude powder (190 mg) obtained from the active fractions was accomplished by column chromatography on silicic acid (Mallinckrodt, 12 g) developed with methanol - chloroform - 17% ammonia (4 : 1 : 2 in volume). The purified **I** (97 mg) was obtained as a colorless dicarbonate in 14% yield from 6′-N-BOC-kanamycin.

The derivatives, **II** and **III** were synthesized from 6′-N-BOC-kanamycin by acylation with BOC-D-isoserine** and BOC-L-isoserine**, respectively, according to the method described above.

The 1-N-(DL-isoseryl)-kanamycin B (**IV**) was synthesized from 6′-N-BOC-kanamycin B in 12 % yield by a method similar to that described previously[2]. The 6′-N-BOC-kanamycin B was prepared from kanamycin B in 40 % yield by reaction with an equimolar amount of *tert*-butyl S-4, 6-dimethylpyrimid-2-ylthiocarbonate[14] (purchased from Kokusan Chemical Works, Tokyo) in a mixture of water and dioxane at room temperature for 18 hours, and purified by Amberlite CG 50 (NH_4^+) chromatography, mp 168~172°C(dec), $[\alpha]_D^{27}$ +110° (*c* 1, water), $C_{23}H_{45}N_5O_{12}\cdot 2H_2O$.

The derivatives, 1-N-(DL-isoseryl)- and 1, 2′-di-N-(DL-isoseryl)-3′, 4′-dideoxykanamycin B (**V** and **VI**) were also synthesized from 6′-N-BOC-3′, 4′-dideoxykanamycin B[2] by similar methods described previously[2,3], in 13 % and 12 % yield, respectively.

The properties of all isoseryl derivatives of kanamycins described above are summarized in Table 1. The structures of these derivatives were confirmed by the pmr spectra, paperchromatography of acid hydrolyzate after N-ethoxycarbonylation, and rotation of mono-N-ethoxycarbonyl-2-deoxystreptamine[2]. The antimicrobial spectra of these six derivatives are shown in Table 2. It is noteworthy that the three isomers of 1-N-isoseryl kanamycins have similar strong activities against kanamycin-sensitive and -resistant bacteria. Each isomer showed about 80 % of the activity of BB-K8*, when the activity was assayed by the cup plate method using *Bacillus subtilis* PCI 219 and *Escherichia coli* JR66/W677 as test organisms.



I (1-IS-KM):
(RS)
R_1=NH-CO-CHOH-CH_2NH_2, R_2, R_3=OH
II (1-D-IS-KM):
(R)
R_1=NH-CO-CHOH-CH_2NH_2, R_2, R_3=OH
III (1-L-IS-KM):
(S)
R_1=NH-CO-CHOH-CH_2NH_2, R_2, R_3=OH
IV (1-IS-KMB):
(RS)
R_1=NH-CO-CHOH-CH_2NH_2, R_2=NH_2, R_3=OH
V (1-IS-DKB):
(RS)
R_1=NH-CO-CHOH-CH_2NH_2, R_2=NH_2, R_3=H
VI (1, 2′-IS-DKB):
(RS)
R_1, R_2=NH-CO-CHOH-CH_2NH_2, R_3=H

SHINICHI KONDO
KATSUHARU IINUMA
MASA HAMADA
KENJI MAEDA
HAMAO UMEZAWA

Institute of Microbial Chemistry
Kamiosaki, Shinagawa-ku, Tokyo, Japan

(Received November 9, 1973)

References

1) KAWAGUCHI, H.; T. NAITO, S. NAKAGAWA & K. FUJISAWA: BB-K8, a new semisynthetic aminoglycoside antibiotic. J. Antibiotics 25: 695~708, 1972
2) KONDO, S.; K. IINUMA, H. YAMAMOTO, K. MAEDA & H. UMEZAWA: Syntheses of 1-N-[(S)-4-amino-2-hydroxybutyryl]-kanamycin B and -3′, 4′-dideoxykanamycin B active against kanamycin-resistant bacteria. J. Antibiotics 26: 412~415, 1973
3) KONDO, S.; K. IINUMA, H. YAMAMOTO, Y. IKEDA, K. MAEDA & H. UMEZAWA: Syntheses of (S)-4-amino-2-hydroxybutyryl derivatives of 3′, 4′-dideoxykanamycin B and their antibacterial activities. J. Antibiotics 26: 705~707, 1973
4) UMEZAWA, H.; M. OKANISHI, S. KONDO, K. HAMANA, R. UTAHARA, K. MAEDA & S. MITUHASHI: Phosphorylative inactivation of aminoglycosidic antibiotics by *Escherichia coli* carrying R factor. Science 157: 1559~1561, 1967
5) UMEZAWA, H.; H. YAMAMOTO, M. YAGISAWA, S. KONDO, T. TAKEUCHI & Y. A. CHABBERT: Kanamycin phosphotransferase I: Mechanism of cross resistance between kanamycin and lividomycin. J. Antibiotics 26: 407~411, 1973
6) YAGISAWA, M.; H. YAMAMOTO, H. NAGANAWA, S. KONDO, T. TAKEUCHI & H. UMEZAWA: A new enzyme in *Escherichia coli* carrying R-factor phosphorylating 3′-hydroxyl of butirosin A, kanamycin, neamine and ribostamycin. J. Antibiotics 25: 748~

* BB-K8 was kindly supplied by Dr. H. KAWAGUCHI, Bristol-Banyu Research Institute, Ltd.

750, 1972

7) YAGISAWA, M.; H. NAGANAWA, S. KONDO, M. HAMADA, T. TAKEUCHI & H. UMEZAWA: Adenylyldideoxykanamycin B, a product of the inactivation of dideoxykanamycin B by *Escherichia coli* carrying R factor. J. Antibiotics 24: 911~912, 1971

8) NAGANAWA, H.; M. YAGISAWA, S. KONDO, T. TAKEUCHI & H. UMEZAWA: The structure determination of an enzymatic inactivation product of 3′, 4′-dideoxykanamycin B. J. Antibiotics 24: 913~914, 1971

9) YAGISAWA, M.; H. NAGANAWA, S. KONDO, T. TAKEUCHI & H. UMEZAWA: Inactivation of 3′, 4′-dideoxykanamycin B by an enzyme solution of resistant *E. coli* and isolation of 3′, 4′-dideoxykanamycin B 2″-guanylate and 2″-inosinate. J. Antibiotics 25: 492~494, 1972

10) YAGISAWA, M.; H. NAGANAWA, S. KONDO, M. HAMADA, T. TAKEUCHI & H. UMEZAWA: Studies on kanamycin nucleotidyltransferase. The 21st meeting of Japan Society of Chemotherapy at Sapporo, June 1973

11) NAITO, T.; S. NAKAGAWA, Y. ABE, S. TODA, K. FUJISAWA, T. MIYAKI, H. KOSHIYAMA, H. OHKUMA & H. KAWAGUCHI: Aminoglycoside antibiotics. II. Configurational and positional isomers of BB-K8. J. Antibiotics 26: 297~301, 1973

12) HASKELL, T. H.; R. RODEBAUGH, N. PLESSAS, D. WATSON & R. D. WESTLAND: The preparation and biological activity of novel amino acid analogs of butirosin. Carbohydrate Research 28: 263~280, 1973

13) ANDERSON, G. W.; J. E. ZIMMERMAN & F. M. CALLAHAN: The use of ester of N-hydroxysuccinimide in peptide synthesis. J. Amer. Chem. Soc. 86: 1839~1842, 1964

14) NAGASAWA, T.; K. KUROIWA, K. NARITA & Y. ISOWA: New agents for *t*-butyloxycarbonylation and *p*-methoxybenzyloxycarbonylation of amino acids. Bull. Chem. Soc. Jap. 46: 1269~1272, 1973