SYNTHESIS OF TETRA-N-PHENYLALKYLKANAMYCINS AND THEIR ANTIMICROBIAL ACTIVITIES

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Sixteen N, N′, N″, N‴-tetraphenylalkylkanamycins (hereafter simply written as tetra-N-phenylalkylkanamycins) were prepared, and their antimicrobial activities *in vitro* were determined. They were less active against most strains tested than kanamycin. But one of them, tetra-N-4-chlorobenzylkanamycin (XI) was slightly more active against *Ps. aeruginosa* than kanamycin. It was suggested that their activities relate with their lipophilicity. Seven of kanamycin derivatives were found active against kanamycin-resistant *Escherichia coli* and *Staphylococcus aureus* as well as sensitive strains.

N-Substituted kanamycin derivatives so far reported may be classified into two groups according to their antimicrobial activities *in vitro*. The N-acylkanamycins[1,2,3] exhibit weak or no antimicrobial activity. The N-methanesulfonates[4], N-substituted-methanesulfonates[5], N-methanesulfinate[6] of kanamycin and SCHIFF's bases of kanamycin[3] exhibit strong activity *in vitro*. The compounds of the former group are stable under physiological conditions, while the latter are rather unstable, liberate the substituents gradually and then exhibit activity[5]. UMEZAWA *et al.*[7] reported that kanamycin-6′-acetamide was produced by the reaction of an enzyme which was obtained from *E. coli* CS-2 (R-5) carrying R factor resistant to kanamycin. This inactivated compound belongs to the first group above.

In this paper, the synthesis and antimicrobial activities of tetra-N-phenylalkylkanamycins are reported. Although these derivatives are considered to be stable, some of these are active *in vitro* against Gram-positive, Gram-negative bacteria and mycobacteria. Therefore, these derivatives belong to neither of the above two groups. Moreover, it is interesting that these compounds are active against kanamycin-resistant *E. coli* and *S. aureus*.

Synthesis and Isolation of Tetra-N-phenylalkylkanamycins

The tetra-N-phenylalkylkanamycins described in Table 1 were synthesized by reduction with sodium borohydride of the SCHIFF's base derived from the appropriate aldehyde and kanamycin A.

$$\text{KM-(NH}_2)_4 + 4\,\text{R-CHO} \longrightarrow \text{KM-(N=CH-R)}_4 \xrightarrow[\text{NaBH}_4]{} \text{KM-(NH-CH}_2\text{-R)}_4$$

KM-(NH$_2$)$_4$: kanamycin A

Kanamycin A base and a small excess of adequate aldehyde were mixed and stirred in aqueous methanol and ethanol to afford a clear solution of the SCHIFF's

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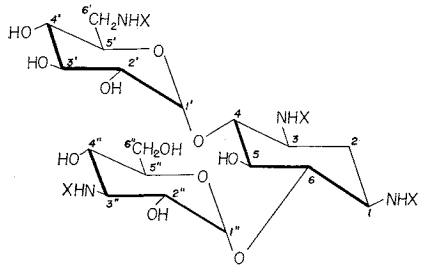
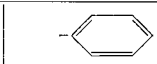
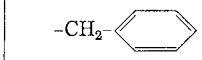
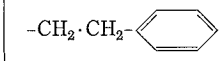
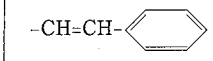
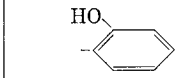
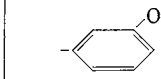
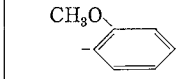
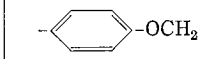
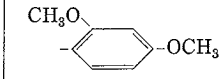
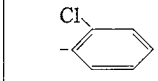
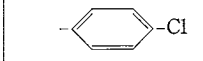
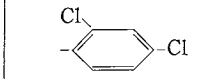
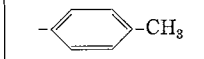
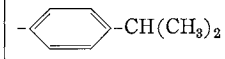
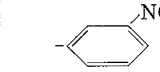
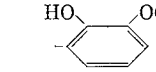
Table 1. Structure of tetra-N-phenylalkylkanamycins

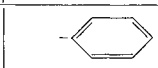
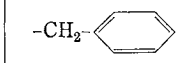
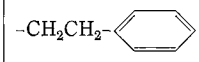
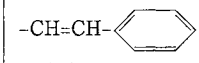
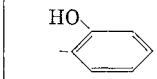
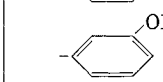
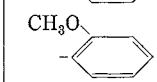
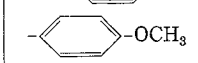
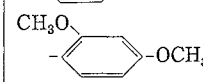
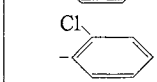
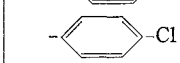
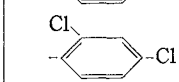
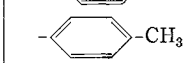
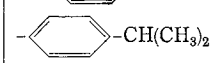
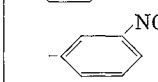
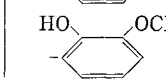


X : H Kanamycin A
$-CH_2-R$ Tetra-N-phenylalkylkanamycin

Compounds	R	KM-(-NH-CH_2-R)$_4$
I		Tetra-N-benzylkanamycin
II		Tetra-N-phenylethylkanamycin
III		Tetra-N-phenylpropylkanamycin
IV		Tetra-N-cinnamylkanamycin
V		Tetra-N-2-hydroxybenzylkanamycin·Na_2
VI		Tetra-N-3-hydroxybenzylkanamycin
VII		Tetra-N-2-methoxybenzylkanamycin
VIII		Tetra-N-4-methoxybenzylkanamycin
IX		Tetra-N-2,4-dimethoxybenzylkanamycin
X		Tetra-N-2-chlorobenzylkanamycin
XI		Tetra-N-4-chlorobenzylkanamycin
XII		Tetra-N-2,4-dichlorobenzylkanamycin
XIII		Tetra-N-4-methylbenzylkanamycin
XIV		Tetra-N-4-isopropylbenzylkanamycin
XV		Tetra-N-3-nitrobenzylkanamycin
XVI		Tetra-N-2-hydroxy-3-methoxybenzylkanamycin·Na_2

base. The SCHIFF's base was then reduced by adding excess sodium borohydride to the reaction mixture affording the tetra-N-phenylalkylkanamycin as the main product. The resulting tetra-N-phenylalkylkanamycin was extracted with chloroform or precipitated from the alkaline reaction mixture by the addition of water. The crude powder obtained from the chloroform extract or the precipitate was purified

Table 2. Properties aud analytical data of tetra-N-phenylalkylkanamycins, KM-(NH-CH_2-R)$_4$

Compounds	R	Formulae	MW	Elemental analysis (%)							
				C		H		N		Cl	
				Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found
I		$C_{46}H_{60}N_4O_{11}$	845.0	65.38	64.91	7.16	7.29	6.63	6.69	—	—
II		$C_{50}H_{68}N_4O_{11}$	901.1	66.64	66.31	7.61	7.49	6.22	5.73	—	—
III		$C_{54}H_{76}N_4O_{11}$	957.2	67.76	67.05	8.00	7.92	5.85	5.67	—	—
IV		$C_{54}H_{68}N_4O_{11}$	949.1	68.33	68.00	7.22	7.34	5.91	5.92	—	—
V		$C_{46}H_{58}N_4O_{15}Na_2$	952.9	57.97	57.34	6.14	6.77	5.88	5.66	—	—
VI		$C_{46}H_{60}N_4O_{15}$	909.0	60.78	60.21	6.65	6.97	6.16	6.16	—	—
VII		$C_{50}H_{68}N_4O_{15}$	965.1	62.22	61.52	7.10	7.15	5.81	5.74	—	—
VIII		$C_{50}H_{68}N_4O_{15}$	965.1	62.22	61.66	7.10	7.10	5.81	5.99	—	—
IX		$C_{54}H_{76}N_4O_{19}$	1085.2	59.76	59.23	7.06	7.18	5.16	5.43	—	—
X		$C_{46}H_{56}N_4O_{11}Cl_4$	982.8	56.22	55.57	5.74	5.85	5.70	5.96	14.43	13.95
XI		$C_{46}H_{56}N_4O_{11}Cl_4$	982.8	56.22	55.57	5.74	5.85	5.70	6.22	14.43	13.71
XII		$C_{46}H_{52}N_4O_{11}Cl_8$	1120.6	49.30	48.79	4.68	4.79	5.00	5.65	25.31	24.59
XIII		$C_{50}H_{68}N_4O_{11}$	901.1	66.64	66.25	7.61	7.83	6.22	6.47	—	—
XIV		$C_{58}H_{84}N_4O_{11}$	1013.3	68.74	68.78	8.36	8.33	5.53	5.74	—	—
XV		$C_{46}H_{56}N_8O_{19}$	1025.0	53.90	53.16	5.51	5.59	10.93	10.66	—	—
XVI		$C_{50}H_{66}N_4O_{19}Na_2$	1073.0	55.96	55.57	6.20	5.56	5.22	5.17	—	—
Kanamycin·H_2SO_4·H_2O		$C_{18}H_{40}N_4O_{16}S$	600.6	36.00	—	6.71	—	9.33	—	(S) 5.34	—

*: in water

to give one spot on the thin-layer chromatograms (Table 4) by reprecipitation and/or recrystallization. The crude tetra-N-cinnamylkanamycin (IV) was purified by counter-current distribution as shown in Fig. 1.

Ultraviolet and infrared absorption spectra indicate successful N-phenylalkylation as shown in Table 3. VAN SLYKE nitrogen determination[8] data (Table 2) show the absence of primary amine and elemental analysis data (Table 2) coincide with the theoretical values for the tetra-N-phenylalkylkanamycins. Accordingly the compounds isolated must have the structure, KM-(NH-CH_2-R)$_4$ as shown in Table 1. The Rf

Van Slyke N(%) Found	m.p. (°C) (Decomp.)	$[\alpha]_D^t$ in dioxane, (c=) t
0.35	(above 188)	+75° (2.0) 23
0.50	120~125 (above 207)	+64° (2.0) 23
0.63	114~124 (above 210)	+62° (2.0) 23
0.07	101~103 (178~220)	+66° (1.0) 22
0.25	(above 189)	+75° (2.0) 23
0.00	115~146 (186~192)	+70° (1.0) 23
0.13	118~125 (above 180)	+71° (2.0) 23
0.00	(189~202)	+71° (1.0) 23
0.34	99~102 (192~213)	+59°(0.85) 23
0.00	105~114 (180~190)	+63° (2.0) 21
0.24	101~105 (186~236)	+63° (1.0) 23
0.18	103~109 (above 192)	+53° (2.0) 21
0.00	102~109 (180~189)	+74° (2.0) 21
0.00	98~105 (180~191)	+66° (2.0) 21
0.52	113~122 (173~181)	+66° (1.0) 20
0.58	(175~180)	+65° (1.0) 19
9.55	(above 268)	+121°(1.0) 23*

Table 3. Ultraviolet and infrared absorption spectra of tetra-N-phenylalkylkanamycins

Compounds	Ultraviolet absorption λ_{max} : mμ (ε)	Solvent	Infrared absorption δ'CH in aromatic ring (950~650 cm^{-1}) KBr
I	248(780), 253(830), 258(880), 264(650)	MeOH	740, 695
II	248(1,170), 253(1,200), 259(1,200), 264(970), 268(760)	MeOH	745, 698
III	248(620), 254(770), 259(860), 262(860), 264(680), 268(670)	MeOH	745, 698
IV	252(71,200), 285(6,640), 293(4,750)	MeOH	745, 692
V	215(25,700), 276(9,100)	0.1 N HCl-dioxane	754
	239(33,700), 293(13,900)	0.1 N NaOH-dioxane	
VI	217(22,200), 276(7,300)	0.1 N HCl-dioxane	864, 788, 694
	240(32,700), 293(10,900)	0.1 N NaOH-dioxane	
VII	220(shoulder), 273(8,100), 277(7,800)	MeOH	751
VIII	226(43,700), 275(5,600), 281(shoulder)	MeOH	812
IX	228(42,200), 278(10,600), 282(9,800)	MeOH	930, 830
X	258(850), 266(890), 273(620)	MeOH	750
XI	221(39,900), 254(890), 261(1,000), 268(1,100), 276(780)	MeOH	803
XII	258(1,600), 266(1,600), 273(1,800), 281(1,400)	MeOH	865, 818
XIII	260(shoulder), 265(1,100) 273(850)	MeOH	804
XIV	251, 257(shoulder), 263(1,200), 272(860)	MeOH	817
XV	262(27,500)	MeOH	910, 802, 690
XVI	220(26,600), 282(10,700)	0.1 N HCl-dioxane	770, 730
	244(28,100), 294(16,100)	0.1 N NaOH-dioxane	

values of tetra-N-phenylalkylkanamycins on thin-layer chromatography and paper chromatography are shown in Tables 4 and 5 respectively.

Tetra-N-phenylalkylkanamycins thus obtained are scarcely soluble in alkaline water, slightly soluble in acidic and neutral water, soluble in alcohols, dimethylsulfoxide, dimethylformamide, dioxane, and chloroform, slightly soluble in benzene, ethyl acetate, ether and acetone, and insoluble in *n*-hexane and petroleum ether. Thus, these derivatives are rather lipophilic in contrast to kanamycin.

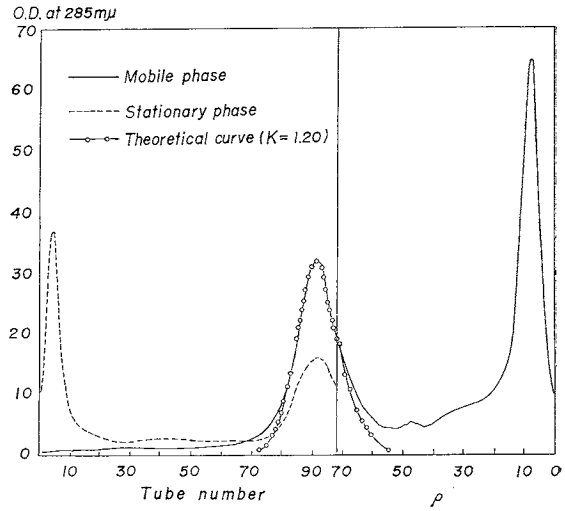
Table 4. Thin-layer chromatography of tetra-N-phenylalkylkanamycins

Compounds	Rf	Rf_{IV}*
I	0.83	1.03
II	0.82	1.02
III	0.81	1.01
IV	0.80	1.00
crude powder	0.80, 0.67	1.00, 0.838
of IV**	0.38, 0.19	0.475, 0.238
V	0.85	1.05
VI	0.85	1.05
VII	0.84	1.04
VIII	0.78	0.972
IX	0.77	0.961
X	0.86	1.07
XI	0.82	1.02
XII	0.84	1.04
XIII	0.85	1.06
XIV	0.83	1.03
XV	0.80	0.995
XVI	0.65	0.809
Kanamycin A	0.03	0.043

Plate : Silica gel G
Detection : 10 % H_2SO_4 spray and heating.
System : Methanol-water-conc. NH_4-OH (20 : 4 : 1) freshly prepared.
* Relative Rf value when the value of IV is taken as 1.00.
** All tetra-N-phenylalkylkanamycins were differentiated from corresponding mono-, di-, and tri-N-phenylalkylkanamycins, showing smaller Rf values.

Fig. 1. Counter-current distribution of tetra-N-cinnamyl-kanamycin.

Tube number 1~98 : mobile and stationary phase remaining in tubes.
ρ0~70 : mobile phase withdrawn from the last tube.
Solvent system : *n*-butanol - *n*-hexane - 0.1 N hydrochloric acid (2 : 1 : 3 v/v)
Each volume of upper and lower layer : 10 ml
Sample (300 mg) was charged equally into the initial 5 tubes.
Temperature : 21~23°C



Antimicrobial Activity

The antimicrobial activities of tetra-N-phenylalkylkanamycins, determined by the agar-streak method on nutrient agar plates (Gram-positive and Gram-negative bacteria) or glycerine-nutrient agar plates (*Mycobacteria*), are shown in Table 6. All the derivatives tested are less active than kanamycin, against all the tested strains except *Pseudomonas aeruginosa* and *Sartina lutea*. But some differences in the extent of the dimunition in activity are observed among the derivatives and strains.

In tetra-N-phenylethylkanamycin (II), tetra-N-phenylpropylkanamycin (III), tetra-N-cinnamylkanamycin (IV), tetra-N-2-chlorobenzylkanamycin (X), tetra-N-4-chlorobenzylkanamycin (XI) and tetra-N-4-methylbenzylkanamycin (XIII), the dimunition in activity against all the tested strains is small. In particular, tetra-N-4-chlorobenzylkanamycin (XI) is a little more active against *P. aeruginosa* than kanamycin. In tetra-N-3-hydroxybenzylkanamycin (VI), tetra-N-2,4-dichlorobenzylkanamycin (XII), tetra-N-4-isopropylbenzylkanamycin (XIV), tetra-N-3-nitrobenzylkanamycin (XV), tetra-N-2,4-dimethoxybenzylkanamycin (IX) and tetra-N-2-hydroxy-3-methoxybenzylkanamycin (XVI), the dimunition in activity against all the tested strains is large. In others, tetra-N-benzylkanamycin (I), tetra-N-2-hydroxybenzylkanamycin (V), tetra-N-2-methoxybenzylkanamycin (VII) and tetra-N-4-methoxy-

Table 5. Paper chromatography of tetra-N-phenylalkylkanamycins

Compounds	a		b		c		d		e	
	Rf	Rf_{IV}	Rf	Rf_{IV}	Rf	Rf_{IV}	Rf	Rf_{IV}	Rf	Rf_{IV}
I	0.858	0.966	0.845	0.845	0.760	0.777	0.175	0.802	0.545	0.812
II	0.889	1.00	1.00	1.00	0.964	0.986	0.286	1.31	0.710	1.05
III	0.910	1.03	1.00	1.00	0.974	0.996	0.252	1.16	0.761	1.13
IV	0.889	1.00	1.00	1.00	0.976	1.00	0.218	1.00	0.671	1.00
V	0.815	0.918	0.695	0.695	0.646	0.662	0.030	0.138	0.355	0.529
VI	0.737	0.830	0.423	0.423	0.544	0.556	0.038	0.174	0.020	0.030
VII	0.868	0.977	0.859	0.859	0.704	0.720	0.107	0.491	0.360	0.537
VIII	0.843	0.949	0.648	0.648	0.687	0.703	0.068	0.491	0.139	0.207
IX	0.868	0.976	0.916	0.916	0.832	0.852	0.077	0.353	0.296	0.441
X	0.864	0.972	1.00	1.00	0.769	0.786	0.727	3.34	0.707	1.05
XI	0.884	0.995	1.00	1.00	0.966	0.988	0.489	2.24	0.688	1.02
XII	0.902	1.01	1.00	1.00	0.962	0.986	0.987	4.53	0.705	1.05
XIII	0.900	1.01	0.920	0.920	0.954	0.975	0.316	1.45	0.725	1.08
XIV	0.942	1.06	1.00	1.00	0.974	0.995	0.496	2.27	0.801	1.19
XV	0.779	0.876	0.636	0.636	0.704	0.720	0.084	0.385	0.055	0.082
XVI	0.674	0.758	0.316	0.316	0.588	0.602	0.084	0.385	0.105	0.156
Kanamycin A	0.079	0.089	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Rf_{IV} : Relative Rf value when the value of IV is taken as 1.00.
Solvent systems : (a) ethanol - conc. NH_4OH - water (8 : 1 : 1 v/v)
(b) *n*-butanol - conc. NH_4OH - water (8 : 1 : 1 v/v)
(c) *n*-butanol - acetic acid - water (4 : 1 : 4 upper layer)
(d) *n*-butanol - pH 7.0, M/15 phosphate buffer (3 : 1 upper layer)
Filter paper was treated with the buffer.
(e) *n*-octanol - water - conc. NH_4OH (5 : 4 : 1 upper layer)
Development : ascending method using Toyo-roshi No. 51 at room temperature.
Detection : autobiography using *Bac. subtilis* PCI-219 and ultraviolet absorption.

benzylkanamycin (VIII), the dimunition in activity is small against *Mycobacteria*, but large against other strains tested.

The decrease in activity seems to correlate with Rf values of paper chromatography described in Table 5. The derivatives showing a small decrease in activity have relatively large Rf values except for the derivatives with molecular weights above 1,000 (Fig. 2). Therefore, it is suggested that the extent of dimunition in activity is related to the lipophilicity of the derivatives.

The antimicrobial activities of several tetra-N-phenylalkylkanamycins such as II, III, IV, X, XI, XIII and VII against kanamycin-resistant *E. coli* and *Staph. aureus* were determined by the agar-streak method on nutrient agar plate as shown in Table 7. A strain of *E. coli* K-12, CS-2

Fig. 2. Relations among antimicrobial activities, Rf_{IV} and molecular weight.
* ▲ expresses a concentration at more than 100 mcg/ml.
** Solvent system : (e) shown in Table 5.

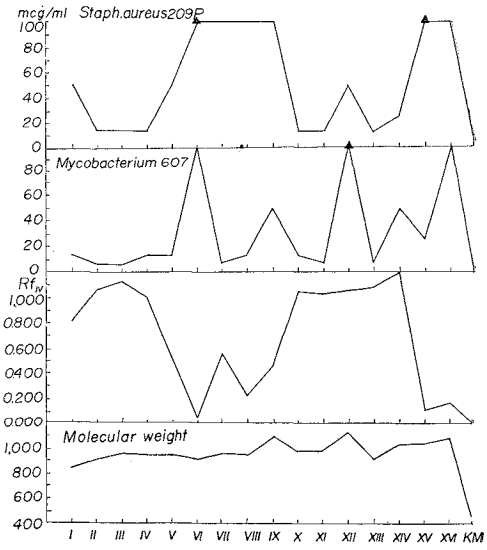


Table 6. Antibacterial spectra of tetra-N-phenylalkylkanamycins (MIC : mcg/ml)

Test organisms	Compounds							
	I	II	III	IV	V	VI	VII	VIII
Staphylococcus aureus 209P	50	12.5	12.5	12.5	50	>100	100	100
Bacillus subtilis PCI 219	50	12.5	12.5	6.25	6.25	>100	6.25	25
Sarcina lutea ATCC 9341	100	50	25	25	100	>100	100	>100
Escherichia coli	100	50	25	25	>100	>100	>100	>100
Salmonella enteritidis 1891	50	100	100	50	100	>100	>100	>100
Pseudomonas aeruginosa A_3	—	>100	>100	>100	—	—	—	—
Pseudomonas aeruginosa No. 4	—	>100	>100	>100	—	—	—	—
Pseudomonas aeruginosa No. 12	—	>100	>100	100	—	—	—	—
Pseudomonas aeruginosa No. 44	—	>100	>100	>100	—	—	—	—
Pseudomonas aeruginosa No. 48	—	50	100	100	—	—	—	—
Mycobacterium 607	12.5	6.25	6.25	12.5	12.5	100	6.25	12.5
Mycobacterium phlei	12.5	12.5	12.5	12.5	12.5	>100	6.25	12.5

Table 7. Antimicrobial activities of tetra-N-phenylalkylkanamycins against drug-resistant *E. coli* and *Staph. aureus* (MIC : mcg/ml)

Test organisms	Compounds							
	II	III	IV	VII	X	XI	XIII	KM
*E. coli**	50	25	25	>100	100	25	25	6.25
E. coli K-12, CS-2[9]	100	50	100	>100	100	25	25	3.12
E. coli K-12, CS-2 (R-5)[9]	100	50	50	>100	50	25	25	50
E. coli K-12, ML-1629[10,11]	100	50	50	>100	100	25	50	>1,000
Staph. aureus 209P*	25	6.25	6.25	50	12.5	6.25	12.5	3.12
Staph. aureus 3-B-22**	6.25	6.25	6.25	50	12.5	6.25	12.5	>1,000
Staph. aureus 3-B-29**	12.5	12.5	6.25	100	12.5	6.25	12.5	>1.000
Staph. aureus 3-B-44**	12.5	6.25	6.25	50	12.5	6.25	6.25	>1,000
Staph. aureus 3-B-75**	6.25	6.25	6.25	50	12.5	6.25	6.25	>1,000
Staph. aureus 3-B-78**	6.25	12.5	6.25	50	12.5	6.25	12.5	>1,000
Staph. aureus 3-B-102**	12.5	12.5	12.5	100	50	6.25	12.5	>1,000
Staph. aureus 3-B-116**	12.5	6.25	6.25	50	25	3.12	12.5	500
Staph. aureus 3-B-135**	12.5	6.25	12.5	100	12.5	6.25	12.5	1,000

* sensitive to kanamycin. KM : kanamycin monosulfate monohydrate

** isolated from patients (in the Hospital of Tokyo University)

(R-5) was shown to inactivate kanamycin[9,10] and UMEZAWA *et al.*[7] found that the inactivated form is kanamycin-6′-acetamide. Another strain of *E. coli* K-12, ML-1629[10] was proved to inactivate kanamycin by transforming it into kanamycin-3′-O-phosphate[11]. Both strains of *E. coli* have R-factor capable of transferring drug-resistance to chloramphenicol, tetracycline, streptomycin, kanamycin and sulfa-drugs. The tested kanamycin-resistant *Staph. aureus* strains were isolated from patients in the Hospital of Tokyo University.

The derivatives tested against these resistant strains have activities practically equal to those against sensitive strains. OKAMOTO *et al.*[9] reported that the inactivation mechanism in *E. coli* having R-factor play an important part in drug-resistance mechanisms. Therefore, the derivatives tested are considered to exhibit activity without inactivation by the organisms, probably because the derivatives have substituents bulky enough to prevent inactivating enzymes from forming a complex with

IX	X	XI	XII	XIII	XIV	XV	XVI	KM
100	12.5	12.5	25	12.5	25	100	>100	3.12
25	12.5	6.25	25	6.25	6.25	50	100	<1.56
100	100	25	>100	50	50	>100	>100	25
>100	50	25	>100	100	>100	>100	>100	12.5
>100	50	25	>100	50	>100	100	>100	12.5
—	>100	100	—	100	—	—	—	>100
—	>100	50	—	100	—	—	—	>100
—	>100	50	—	100	—	—	—	>100
—	>100	50	—	100	—	—	—	>100
—	>100	25	—	100	—	—	—	50
50	12.5	6.25	>100	6.25	50	25	100	3.12
25	12.5	12.5	>100	12.5	100	50	50	3.12

the kanamycin molecule. Similarly the bulky substituents must have a "hindrance effect" on cell wall permeability and complex formation with the site of action, that is, ribosome-mRNA complex. The diminution in activity against sensitive strains due to N-substitution can be explained by the "hindrance effect" mentioned above.

Experimental

Melting point are not corrected. Ultraviolet absorption spectra (Table 3) were taken with a Hitachi recording spectrometer EPS-2U. Infrared absorption spectra (Table 3) were taken in KBr tablet with a Hitachi infrared spectrophotometer EPI-S2. Primary amine nitrogen determination (Table 2) by VAN SLYKE method[11] was carried out by deaminating the derivatives (0.025 m mole) in a mixture of 30 % sodium nitrite (15 ml) and glacial acetic acid (25 ml) for 5 minutes. Elemental analysis data, optical rotation and melting points are shown in Table 2.

Tetra-N-benzylkanamycin (I): To a solution containing 300 mg (0.620 m mole) of kanamycin A base, 400 mg (3.76 m mole) of benzaldehyde, 3 ml of methanol and 0.3 ml of distilled water, 150 mg of sodium borohydride was slowly added. The reaction mixture was allowed to stand at room temperature for 1 hour and poured into 50 ml of water. Resulting precipitate was filtered and washed with water to obtain 728 mg of the crude powder. The crude powder was reprecipitated from dioxane-water to give 387 mg of pure amorphous powder I.

Tetra-N-phenylethylkanamycin (II): To a mixture of 600 mg (1.24 m mole) of kanamycin A base and 816 mg (6.73 m mole) of phenylacetaldehyde, 10 ml of distilled water and 15 ml of ethanol were added. The mixture was stirred under cooling with ice-water for 45 minutes to give a clear solution, to which 700 mg of sodium borohydride was added. The solution was allowed to stand at room temperature for 1 hour, kept in a refrigerator over night, and acidified to pH 2 to decompose the excess sodium borohydride. The reaction mixture was again adjusted to pH 10 with sodium hydroxide, made to 40 ml of total volume with water and extracted with four 20-ml portions of chloroform. The chloroform extract was concentrated to dryness under reduced pressure, giving 1.2 g of crude powder. The crude powder was purified by reprecipitation from benzene-petroleum ether, yielding 950 mg of pure amorphous colorless powder II.

Tetra-N-phenylpropylkanamycin (III): To a mixture of 600 mg (1.24 m mole) of kanamycin A base and 1,000 mg (7.44 m mole) of phenylpropionaldehyde, 6 ml of methanol and 1 ml of distilled water were added. The mixture was stirred under cooling with ice-water for 30 minutes to give a clear solution, to which 450 mg of sodium borohydride was added. The reaction mixture was kept at room temperature for 3 hours. An isolation procedure similar to that used with II yielded 1.09 g of pure colorless amorphous powder III.

Tetra-N-cinnamylkanamycin (IV): To an aqueous solution (4 ml) containing 1.6 g (3.3 m mole) of kanamycin A base, 8 ml of ethanol and 2 g (15.2 m mole) of cinnamaldehyde (trans) were added. The mixture was stirred at room temperature for 1 hour and

32 ml of methanol was added to give a clear solution, to which 750 mg of sodium borohydride was added. The reaction mixture was kept at room temperature for 1 hour. A procedure similar to that used for I gave 2.77 g of crude powder, which showed four spots on the thin-layer chromatogram (Table 4). This powder was purified by a counter-current distribution process. Three hundred mg of the crude powder dissolved in 50 ml of the lower layer of the solvent system (Fig. 1) was charged equally into the initial five tubes of the CRAIG type apparatus having 98 tubes. Then 163 transfers were carried out. The fundamental procedure and upper layer single withdrawal procedure were applied to the initial 93 transfers and next 69 transfers respectively. The peak at Nos. 80~98 and ρ 63~69 in the distribution curve (Fig. 1) contained tetra-N-cinnamylkanamycin, coincided well with the theoretical curve (K=1.20), and gave 118 mg of powder by the isolation procedure used for II. Crystallization from methanol gave 65 mg of needle crystals IV.

Tetra-N-2-hydroxybenzylkanamycin·2Na (V): To a solution containing 300 mg (0.620 m mole) of kanamycin A base, 450 mg (3.68 m mole) of salicylaldehyde, 3 ml of methanol and 0.6 ml of distilled water, 200 mg of sodium borohydride was added. The reaction mixture was treated by the same procedure as I, giving 314 mg of colorless amorphous powder V.

Tetra-N-3-hydroxybenzylkanamycin (VI): To a solution containing 485 mg (1.0 m mole) of kanamycin A base, 634 mg (5.2 m mole) of *m*-hydroxybenzaldehyde, 3 ml of methanol and 0.5 ml of distilled water, 300 mg of sodium borohydride was added. The reaction mixture was allowed to stand at room temperature for 5 hours, adjusted to pH 8.8 and combined with 20 ml of water. Resulting precipitate was filtered, washed with water, and reprecipitated from ethanol to give 370 mg of amorphous powder VI.

Tetra-N-2-methoxybenzylkanamycin (VII): To a solution containing 300 mg (0.620 m mole) of kanamycin A base, 500 mg (3.67 m mole) of *o*-methoxybenzaldehyde, 3 ml of methanol and 0.3 ml of distilled water, 200 mg of sodium borohydride was added. The reaction mixture was kept at room temperature for 1 hour, and treated as described for I, yielding 490 mg of colorless amorphous powder VII.

Tetra-N-4-methoxybenzylkanamycin (VIII): To a solution containing 485 mg (1.0 m mole) of kanamycin A base, 706 mg (5.2 m mole) of *p*-methoxybenzaldehyde, 3 ml of methanol and 0.2 ml of distilled water, 300 mg of sodium borohydride was added. The reaction mixture was treated as described for I, yielding 1.07 g of crude powder. Recrystallization from chloroform-ethanol gave 460 mg of needle crystal VIII.

Tetra-N-2,4-dimethoxybenzylkanamycin (IX): To a solution containing 485 mg (1.0 m mole) of kanamycin A base, 781 mg (5.2 m mole) of 2,4-dimethoxybenzaldehyde, 10 ml of methanol and 2 ml of distilled water, 300 mg of sodium borohydride was added. The reaction mixture was treated as described for I, yielding 890 mg of crude powder. The crude powder was reprecipitated from ethanol-petroleum ether and ethanol successively, yielding 210 mg of pure amorphous powder IX.

Tetra-N-2-chlorobenzylkanamycin (X): Kanamycin A base (485 mg) and 732 mg (5.2 m mole) of *o*-chlorobenzaldehyde were treated by the same procedure as VIII, yielding 970 mg of crude powder. The crude powder was reprecipitated from methanol-water and chloroform-petroleum ether successively, giving 680 mg of pure amorphous powder X.

Tetra-N-4-chlorobenzylkanamycin (XI): Kanamycin A base (485 mg) and 732 mg (5.2 m mole) of *p*-chlorobenzaldehyde was dissolved in a mixture of 50 ml of ethanol and 20 ml of water. To this solution, 500 mg of sodium borohydride was added. The reaction mixture was kept at room temperature over night, concentrated to 10 ml under reduced pressure and combined with 30 ml of water. Resulting precipitate was filtered, washed with water, and reprecipitated from methanol-water, yielding 590 mg of crude powder. Recrystallization from ethanol gave 410 mg of needle crystal XI.

Tetra-N-2,4-dichlorobenzylkanamycin (XII): To a solution containing 485 mg (1.0 m mole) of kanamycin A base, 910 mg (5.2 m mole) of 2,4-dichlorobenzaldehyde, 22 ml of methanol and 1 ml of distilled water, 300 mg of sodium borohydride was added. The reaction

mixture was kept at room temperature for 5 hours and treated as described for I, yielding 1.04 g of crude powder. Recrystallization from ethanol gave 670 mg of needle crystal XII.

Tetra-N-4-methylbenzylkanamycin (XIII): To a solution containing 485 mg (1.0 m mole) of kanamycin A base, 624 mg (5.2 m mole) of *p*-methylbenzaldehyde, 5 ml of methanol and 0.2 ml of distilled water, 300 mg of sodium borohydride was added. The reaction mixture was treated as described for XII, yielding 906 mg of crude powder. The crude powder was reprecipitated from methanol-water and chloroform-petroleum ether successively, giving 660 mg of pure amorphous powder XIII.

Tetra-N-4-isopropylbenzylkanamycin (XIV): Kanamycin A base (485 mg) and 769 mg (5.2 m mole) of *p*-isopropylbenzaldehyde were treated by the same procedure as XIII, yielding 710 mg of crude powder. The crude powder was reprecipitated from methanol-water and chloroform-petroleum ether succesively, giving 440 mg of pure amorphous powder XIV.

Tetra-N-3-nitrobenzylkanamycin (XV): To a solution containing 485 mg (1.0 m mole) of kanamycin A base, 785 mg (5.2 m mole) of *m*-nitrobenzaldehyde, 20 ml of methanol and 0.5 ml of distilled water, 300 mg of sodium borohydride was added. The reaction mixture was treated as described for XII, yielding 860 mg of crude powder. The crude powder was reprecipitated from methanol-water and ethanol successively, giving 350 mg of pale yellow powder XV.

Tetra-N-2-hydroxybenzylkanamycin·2Na (XVI): To a solution containing 485 mg (1.0 m mole) of kanamycin A base, 738 mg (5.2 m mole) of 2-hydroxy-3-methoxylbenzaldehyde, 5 ml of methanol and 1.5 ml of water, 300 mg of sodium borohydride was added. The reaction mixture was treated as described for XII, yielding 1.01 g of crude powder. The crude powder was reprecipitated from methanol-water and chloroform-petroleum ether successively, giving 785 mg of amorphous powder XVI.

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