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241. Shigeharu Inouye*1: Chemical Modification of Kanamycin. III.*2 Syntheses of 6-Amino-6-deoxy-2-manno-kanamycin and 6-Hydrazino-6-deoxy-2-manno-kanamycin.*3,4

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Syntheses of the title compounds starting from 2,6-di-O-*p*-tolylsulfonyl-tetra-N-acetyl-kanamycin *via* 6-azido- and 6-hydrazino-2,3-oxazolinum intermediates. Terminal amination and configurational inversion at C_2 of the 3-amino-glucose moiety were confirmed by nuclear magnetic resonance spectroscopy, molecular rotations and hydrolysis data. The structures of two diamino-derivatives of tetra-N-acetyl-kanamycin obtained as minor products were discussed.

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In the preceding paper,*2 two kinds of kanamycin derivatives, 3′-amino-3′-deoxy-kanamycin and 3′-amino-3′-deoxy-2′-manno-kanamycin were reported. In this paper, some results of synthetic experiments starting from di-O-*p*-tolylsulfonyl-tetra-N-acetyl-kanamycin will be described.

As mentioned in Part I of this series,[1] the reaction of tetra-N-acetyl-kanamycin (I) with 2 moles of *p*-tolylsulfonyl chloride in pyridine afforded a mixture of mono- and di-O-sulfonates, of which mono-O-sulfonate was predominated. When reacted with more amounts of *p*-tolylsulfonyl chloride (3.4 moles), di-O-sulfonates were now obtained as main products, together with mono-O-sulfonates, tri-O-sulfonates and the unreacted material (I). It was noted in this investigation that even though treated with more amounts of the reagent, considerable amounts of I remained unreacted. A similar phenomenon was encountered in the case of polysulfonylation of I with 3.0 moles of methanesulfonyl chloride, where the unreacted I was recovered in addition to penta-O-methanesulfonates.[2]

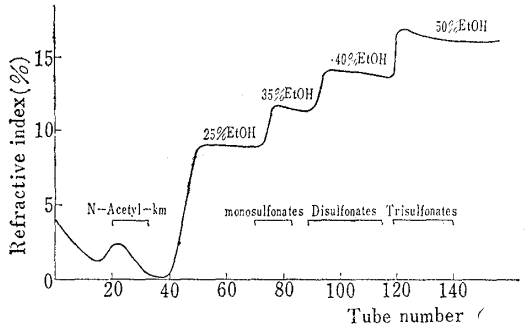

Fig. 1. Resin Chromatographic Separation of a Mixture of O-*p*-Tolylsulfonyl-tetra-N-acetyl-kanamycins.
Column, Dowex 50W×2 (NEt_3^+) 3.3×30 cm. Effluents were collected in 10 ml. fractions.

The separation of the sulfonate mixture was possible by means of the silicic acid chromatography described in the previous paper,[1] but it was more effectively accomplished by the use of the resin chromatography on a column of Dowex 50W×2, developing with water that contained increasing amount of ethanol. As illustrated in Fig. 1, the unreacted N-acetyl-kanamycin (I) was first eluted by water, then mono-O-sulfonates by 25~35% ethanol, di-O-sulfonates by 40% ethanol and finally tri-O-sulfonates by 50%

*1 Morooka, Kohoku-ku, Yokohama-shi (井上重治).
*2 Part II: This Bulletin, in press.
*3 Preliminary report, "Abstracts of papers, 9th Symposium on the Chemistry of Natural Products," Oct. 13, 1965, Osaka, p. 7.
*4 The kanamycin derivatives in which the 3-amino-3-deoxy-α-D-glucopyranose moiety was replaced, respectively, by 3,6-diamino-3,6-dideoxy-α-D-mannopyranose and 3-amino-6-hydrazino-3,6-dideoxy-α-D-mannopyranose. As for the simplified naming of kanamycin derivatives, see reference 1).

1) S. Inouye: J. Antibiotics, Ser. A, **20**, 6 (1967).
2) *Idem*: Unpublished data.

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ethanol. The resin chromatography, as compared with the silicic acid chromatography, had an advantage in that larger quantity of a sulfonate mixture could be separated on a smaller column. The di-O-sulfonates thus obtained were still a mixture of two isomers, as evidenced by the doubled spots in the thin-layer chromatograms shown in Table Ⅵ. Of these, a main product (Ⅱ) having larger Rf value crystallized from aqueous ethanol. Since the complete separation of these isomers by the repeated crystallization resulted in a great loss of Ⅱ, a crude preparation was used as such in the following reactions.

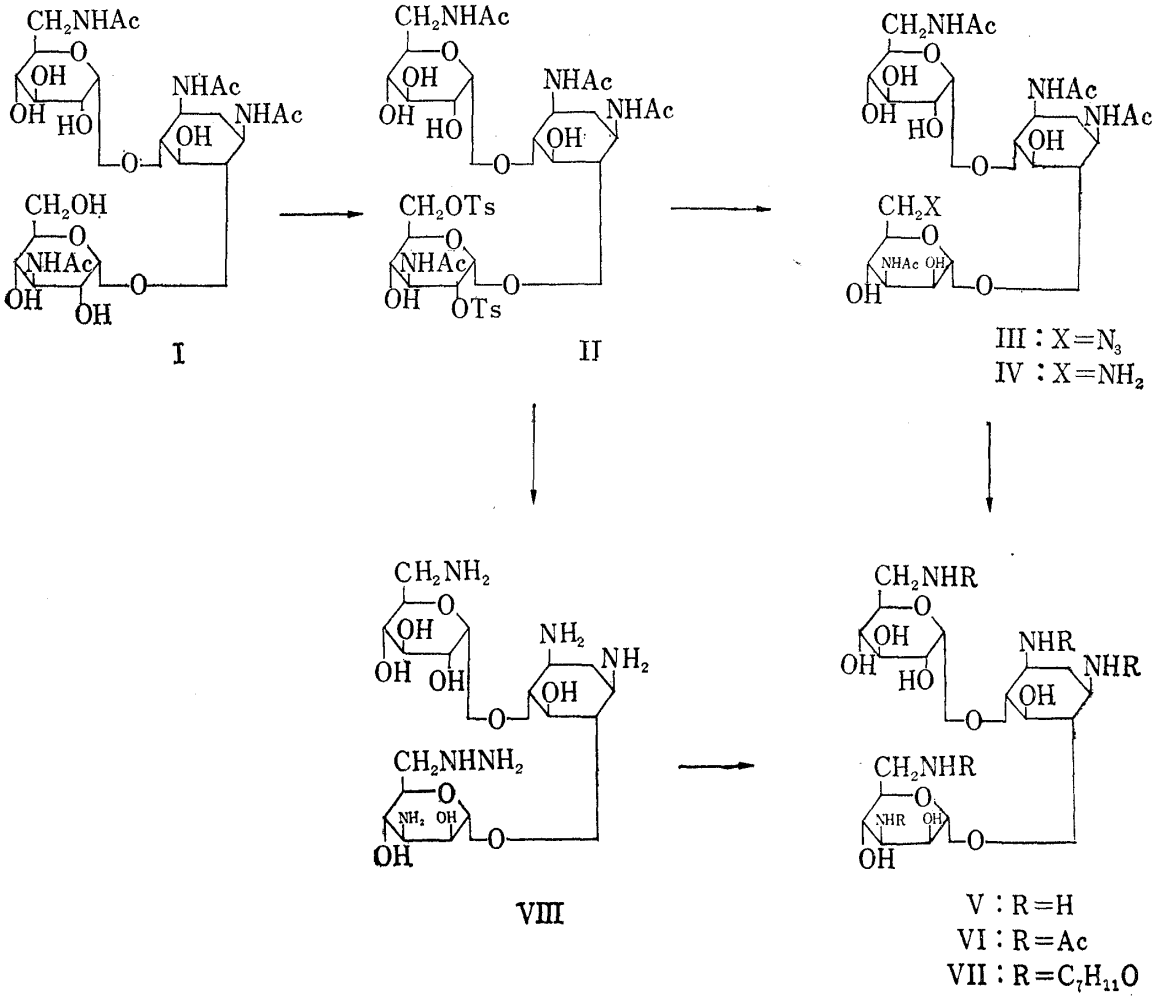

Chart 1.

Treatment of the crude di-O-sulfonate (Ⅱ) with sodium azide in refluxing dimethylformamide followed by the resin chromatography on a column of Dowex 50W×2 developed with water yielded a mixture of azido-derivatives, in which a mono-azido-compound (Ⅲ) was a main product. By the elution with 50% ethanol, mono-azido-mono-O-sulfonates and then mono-azido-di-O-sulfonates were recovered from the resin column. The crude Ⅲ was reduced with Raney nickel to give the amino-derivative which was subjected to

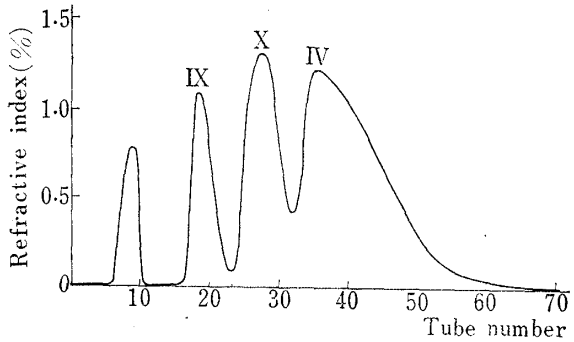

Fig. 2. Resin Chromatographic Separation of a Mixture of Aminated Tetra-N-acetyl-kanamycins

Resin Column, Dowex 1×2 (OH⁻) 100 ml. Aqueous effluents were collected in 6.3 ml. fractions.

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the resin chromatography on a column of Dowex 1×2 (OH^- form). When developed with water, three kinds of amino-derivatives were successively eluted from the column (Fig. 2). By the ninhydrin photometry (Table I), the former two compounds (R_{Km} 0.48 (IX) and 1.05 (X)) were found to be diamino-derivatives, while the lastly eluted main product (R_{Km} 1.55 (IV)) was a monoamino-derivative, which was shown to be 6-amino-6-deoxy-2-manno-tetra-N-acetyl-kanamycin (IV). Selective N-acetylation of IV with acetic anhydride in methanol gave penta-N-acetate (VI).

Treatment of the crude di-O-sulfonate (II) with anhydrous hydrazine at 140° for 40 hours gave a mixture of hydrazino-compounds, from which 6-hydrazino-6-deoxy-2-manno-kanamycin (VIII) was isolated by the repeated resin chromatography on a Dowex 1×2 (OH^- form) column. VIII consumed the iodine reagent owing to the presence of a hydrazino group. The Rf values of VIII in paper and resin chromatographies were summarized in

Table I. "Ninhydrin Color Values"[a] and "Aminomethylfurfural Values"[b] in Amino-derivatives of Tetra-N-acetyl-kanamycin, and Related Compounds

Compound	Ninhydrin color value	Aminomethyl-furfural value
R_{Km} 0.48 Substance (IX)	179	11
R_{Km} 1.05 Substance (X)	185	60
6-Amino-2-manno-tetra-N-acetyl-kanamycin (IV)	92	87
Tetra-N-acetyl-kanamycin (I)	0	90
2′-Acetamido-tetra-N-acetyl-kanamycin	0	10

a) $E^{1\%}_{1cm.}$ at 570 mμ after heating with the ninhydrin reagent[7] at 100° for 20 min.
b) $E^{1\%}_{1cm.}$ at 282 mμ after heating with 50% sulfuric acid for 1.5 hr.

Table II. Rf Values of 6-Amino-6-deoxy-2-manno-kanamycin (V) and Related Compounds in Paper and Resin Chromatographies

Compound	R_{Km}[a]	
	Resin chromat.[7] Dowex 1×2(OH)	Paper chromato.[b] *n*-BuOH-pyr.-AcOH-H_2O (6:4:1:3)
6-Amino-2-manno-tetra-N-acetyl-kanamycin (IV)	1.55	4.8
R_{Km} 0.48 Substance (IX)	0.48	5.6
R_{Km} 1.05 Substance (X)	1.05, 0.81,[c] 0.72[c]	4.5
6-Amino-2-manno-kanamycin (V)	0.72	0.7
6-Hydrazino-2-manno-kanamycin (VIII)	0.64	0.6

a) Relative rate of flow against kanamycin (1.00).
b) Developed descendingly with Tōyō Roshi No. 50 filter paper. Spots were detected by spraying 1% ninhydrin in acetone-pyridine (9:1) or by autobiography.
c) Minor component.

Table II, indicating the differences from those of the parent antibiotic (kanamycin). Polyhydrazino-compounds which were eluted before VIII were difficult to isolate in a pure state. Reduction of the 6-hydrazino-compound (VIII) with Raney nickel at room temperature followed by the chromatographic purification by the use of a Dowex 1×2 (OH) column afforded 6-amino-6-deoxy-2-manno-kanamycin (V) as a homogeneous powder in paper and resin chromatographies (Table II). The compound V gave penta-N-acetate (VI), identical with the sample prepared *via* the azido-intermediate, and penta-N-salicylidene derivative (VII).

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Evidence for the terminal amination on the 3-acetamido-hexose moiety was presented by the nuclear magnetic resonance spectrum of Ⅳ in deuterium oxide. An asymmetric methylene signal (AB part of an ABX system) weakly deshielded by the free amino group newly introduced was recognized at about 7.08 p.p.m., and the peak area of this signal compared well with the combined area of two anomeric proton signals which appeared at 5.06 p.p.m. ($J_{1,2}$=1.5 c.p.s.) and 4.64 p.p.m. ($J_{1,2}$=2.9 c.p.s.). When measured in the form of hydrochloride, the anomeric signal at 5.06 p.p.m. moved to a lower field (5.02 p.p.m.), while the latter remained unchanged (4.64 p.p.m.). Since the down-field shift should be attributable to the electrostatic field effect of an ammonium cation,[3] the anomeric signal with smaller $J_{1,2}$ value could be assigned to the H_1 of the 3-acetamido-6-aminohexose moiety, and hence the signal with larger $J_{1,2}$ to the 6-acetamido-glucose moiety.

The assignment of the α-D-mannopyranose configuration to the diamino-hexose moiety in Ⅳ and Ⅵ was based on the fact that the H_1 signals of α-D-mannopyranose derivatives appeared consistently at higher field with unusually low $J_{1,2}$ values than the

Table Ⅲ. H_1 Chemical Shifts and Apparent Coupling Constants ($J_{1,2}$) of 6-Amino-6-deoxy-2-manno-tetra-N-acetyl-kanamycin (Ⅳ) and Related Compounds in Deuterium Oxide at 60 M.c.p.s.[a]

Compound	H_1 (τ Value)	($J_{1,2}$ c.p.s.)
6-Amino-2-manno-tetra-N-acetyl-kanamycin (Ⅳ) free base	5.06(1.5)	4.64(2.9)
6-Amino-2-manno-tetra-N-acetyl-kanamycin (Ⅳ) HCl	5.02(1.3)	4.64(2.6)
6-Acetamido-2-manno-tetra-N-acetyl-kanamycin (Ⅵ)	5.09(1.7)	4.63(3.0)
6-Amino-tetra-N-acetyl-kanamycin (Ⅺ)[1] free base	4.88(3.9)	4.64(2.8)
6-Amino-tetra-N-acetyl-kanamycin (Ⅺ)[1] HCl	4.84(3.8)	4.65(2.8)
6-Acetamido-tetra-N-acetyl-kanamycin (Ⅻ)[1]	4.90(3.7)	4.62(2.8)
R_{Km} 0.48 Substance (Ⅸ) free base	5.03(2.0)	4.86(3.5)
R_{Km} 0.48 Substance (Ⅸ) HCl	5.00(2.0)	4.83(3.5)
R_{Km} 1.05 Substance (Ⅹ) free base	4.88(3.9)	4.64(2.8)
R_{Km} 1.05 Substance (Ⅹ) HCl	4.84(3.8)	4.65(2.8)

a) Internal standard, sodium 2,2-dimethyl-1,2-silapentane-5-sulfonate.

H_1 of α-D-glucopyranose and α-D-galactopyranose derivatives in the monosaccharide series.[3] Table Ⅲ summarized the H_1 chemical shifts (τ values) and $J_{1,2}$ values in Ⅳ, Ⅵ and their corresponding glucose counterparts (Ⅺ) and (Ⅻ). When these τ values and $J_{1,2}$ values were compared mutually, it was immediately seen that the H_1 signals of the diamino-hexose moieties in Ⅳ and Ⅵ appeared at higher field with low $J_{1,2}$ values than the H_1 of the diamino-glucose moieties in Ⅺ and Ⅻ, suggesting the α-D-mannose configuration. The H_1 signal of the 6-acetamido-glucose moiety, on the other hand, showed little alteration in the four compounds, reflecting the intact component.

The α-D-mannopyranose configuration was further supported by the low molecular rotations ($[M]_D$) of Ⅳ and Ⅴ, compared with those of the glucose derivatives (Ⅺ, XIII) and the parent antibiotic, since the α-D-mannose derivatives including 3,6-diamino- and 3-acetamido-6-amino-sugars all exhibited lower $[M]_D$ than the corresponding glucose and galactose isomers as shown in Table Ⅳ. Paper chromatographic examination of the acid hydrolyzate of Ⅵ showed 2-deoxystreptamine, 6-amino-glucose, 5-aminomethyl-furfural (a degradation product of 6-amino-glucose)[4] and a new amino-sugar, whose Rf value was coincided with that of the authentic sample of 3,6-diamino-3,6-dideoxy-D-mannose[5] (Table Ⅴ). Thus, the structures of 6-amino-6-deoxy-2-manno-kanamycin (Ⅴ)

3) S. Inouye: This Bulletin, **14**, 1210 (1966).
4) H. Ogawa, T. Ito, S. Kondo, S. Inouye: Bull. Agr. Chem. Soc. Japan, **23**, 289 (1959).
5) S. Inouye: This Bulletin, **14**, 902 (1966).

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Table Ⅳ. Molecular Rotations ($[M]_D$) of 6-Amino-6-deoxy-2-manno-kanamycin (Ⅴ) and Related Compounds in Water

Compound	$[M]_D$[a]	Compound	$[M]_D$[a]
6-Amino-2-manno-kanamycin (Ⅴ)	205[b]	Methyl 3,6-Diamino-α-D-glucopyranoside[5]	290
6-Amino-kanamycin (Ⅻ)[1]	300[b]	Methyl 3-Acetamido-6-amino-α-D-mannopyranoside[5]	110
Kanamycin	310[b]	Methyl 3-Acetamido-6-amino-α-D-glucopyranoside[5]	350
6-Amino-2-manno-tetra-N-acetyl-kanamycin (Ⅳ)	235[b]	Methyl α-D-mannopyranoside	150
6-Amino-tetra-N-acetyl-kanamycin (Ⅺ)[1]	365[b]	Methyl α-D-glucopyranoside	310
Tetra-N-acetyl-kanamycin (Ⅰ)	350[b]	Methyl α-D-galactopyranoside	370
Methyl 3,6-Diamino-α-D-mannopyranoside[5]	110		

a) $[\alpha]_D \times$ mol. wt./100. b) $[M]_D \times 1/2$.

Table Ⅴ. Rf Values[a] of Acid Hydrolyzates of 6-Acetamido-6-deoxy-2-manno-tetra-N-acetyl-kanamycin (Ⅵ), the R_{Km} 0.48 Substance (Ⅸ), the R_{Km} 1.05 Substance (Ⅹ), and Tetra-N-acetyl-kanamycin (Ⅰ) in Paper Chromatography[b]

Component amino-sugar	Ninhydrin color	Ⅵ[c]	Ⅵ[d]	Ⅸ	Ⅹ	Ⅰ
2-Deoxystreptamine	black-purple	1.00	1.00	1.00	1.00	1.00
6-Amino-glucose	purple	1.47	1.46		1.46	1.46
3-Amino-glucose	brown					2.20
3,6-Diamino-mannose	purple	1.29	1.29	1.27		
Unknown	purple			1.17	1.09	

a) Relative rate of flow against 2-deoxystreptamine (1.00) runned on the same paper.
b) Developed descendingly with *n*-BuOH-pyridine-AcOH-H_2O (6:4:1:3) for 20~40 hr. Spots were detected by spraying 1% ninhydrin in acetone-pyridine (9:1) or 2.5% aniline hydrogenphthalate in *n*-BuOH saturated with H_2O.
c) Prepared *via* hydrazino-intermediate.
d) Prepared *via* azido-intermediate.

and their derivatives were established, and therefore the di-O-sulfonate (Ⅱ) must be 2,6-di-O-*p*-tolylsulfonyl-tetra-N-acetyl-kanamycin (Ⅱ).

The preferred formation of the 2,6-di-O-sulfonate in stead of 4,6- or 2,4-di-O-sulfonates*5 agreed with the result obtained from dimolar *p*- tolylsulfonylation of methyl 3-acetamido-3-deoxy-α-D-glucopyranoside.[5] It was further interesting to note that sulfonylation of N-acetyl-kanamycin (Ⅰ) occurred predominantly at C_2 of the 3-amino-glucose moiety, rather than at C_2 of the 6-amino-glucose moiety. The less reactivity of the 6-amino-glucose portion may be understandable from the conformation of kanamycin molecule, where the hydroxyl groups on C_2, C_3 and C_4 of the 6-amino-glucose were sterically shielded by the 3-amino-glucose moiety. Inversion of the *p*-tolylsulfonyloxy group on C_2 without replacement by the powerful nucleophilic reagents (azide and hydrazine) was undoubtedly due to the neighboring group participation[6] of the 3-acetamido group that was situated *trans* to the C_2 substituent.

Of the two diamino-derivatives of tetra-N-acetyl-kanamycin obtained as minor products, the R_{Km} 0.48 substance (Ⅸ) was characterized as hexa-N-acetate, which consumed one mole of periodate and did not afford an ultraviolet absorption band

*5 From the 4,6-di-O-sulfonate and 2,4-di-O-sulfonate, the formation of 3,6-diamino-galactose and 3-amino-talose would be expected, respectively.

6) B.R. Barker: "Methods in Carbohydrate Chemistry," Vol. I, Section Ⅺ. Academic Press (1963).

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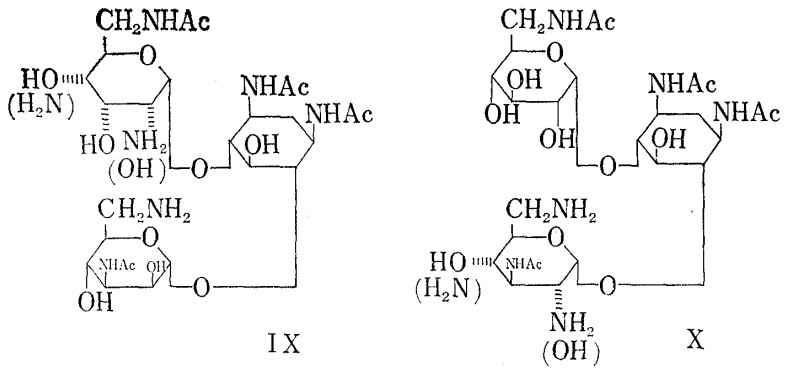

Chart 2.

characteristic for 5-aminomethylfurfural[4] when heated with 50% sulfuric acid, similar to the behavior of 2′-acetamido-2′-deoxy-kanamycin (Table I). Hydrolysis with 3*N* hydrochloric acid for 1.5 hours gave 2-deoxystreptamine and two new reducing amino-sugars in place of 3-amino-glucose and 6-amino-glucose (Table V). Of these, the Rf 1.27 sugar was indistinguishable from 3,6-diamino-3,6-dideoxy-D-mannose in paper chromatography. The 3,6-diamino-mannose moiety was supported by the nuclear magnetic resonance spectrum of IX in Table III, which showed the H_1 signal with small $J_{1,2}$ value (2.0 c.p.s.), typical for α-D-mannose, at 5.03 p.p.m. in the form of free base and at 5.00 p.p.m. in the form of hydrochloride. These results indicated that one of the two amino groups newly introduced must be located on C_6 of the 3-amino-glucose portion with configurational inversion at C_2 and the other on C_2 or C_4 of the 6-amino-glucose portion (the configuration was not determined). Therefore, the compound IX must be derived from the tri-O-sulfonate contaminated to II.

The R_{Km} 1.05 substance (X), though not yet obtained in an analytically pure state (see Table II), gave 5-aminomethylfurfural when heated with 50% sulfuric acid (Table I), and 2-deoxystreptamine, 6-amino-glucose and a new reducing amino-sugar having $R_{deoxystreptamine}$ 1.09 when hydrolyzed with 3*N* hydrochloric acid (Table V). The H_1 signal appeared at 4.64~4.65 p.p.m. in the resonance spectra (Table III) may be assigned to the intact 6-acetamido-glucose moiety. These results suggested that the two amino groups might be not introduced to the 6-amino-glucose portion, but to the 3-amino-glucose moiety, probably on C_6 and C_2 or C_6 and C_4.

The antibacterial activities of V and VIII will be reported later.

Experimental*6

2,6-Di-O-*p*-tolylsulfonyl-tetra-N-acetyl-kanamycin (II)——A suspension of tetra-N-acetyl-kanamycin (I) (20.0 g.) in pyridine (200 ml.) was treated with *p*-tolylsulfonyl chloride (8.0 g.) at 5°. The mixture was stirred at room temperature overnight and then filtered to remove the insoluble materials. Additional *p*-tolylsulfonyl chloride (12 g.) was added to the filtrate at 5° and left overnight. After the addition of a little H_2O (5 ml.), the solution was brought to dryness. The residue was dissolved in EtOH and the crude di-O-sulfonates were precipitated by the addition of ether, followed by reprecipitation from iso-PrOH–ether and finally from hot iso-PrOH. Yield, 19.5 g. This was extracted with H_2O (110 ml.), the remaining insoluble materials were dissolved in 50% EtOH (10 ml.), and the combined extracts were passed through a column (3.3 × 29 cm.) of Dowex 50W × 2 (NEt_3^+) resin. Elution was carried out with H_2O (300 ml.), 25% EtOH (250 ml.), 35% EtOH (200 ml.), 40% EtOH (200 ml.) and 50% EtOH (300 ml.) in that order. The chromatogram was shown in Fig. 1. The unreacted N-acetyl-kanamycin (2.0 g.) was recovered from aqueous effluents mono-O-sulfonates (3.4 g.) from 25~35% EtOH eluates, di-O-sulfonates (7.0 g.) from 40% EtOH eluates and tri-O-sulfonates (1.2 g.) from 50% EtOH eluates, respectively. Rf Values of mono-, di- and tri-O-sulfonates

*6 Melting points were uncorrected. Determination of ultraviolet, infrared and nuclear magnetic resonance spectra and analysis by means of thin-layer, paper and resin chromatographies were carried out as described in the preceding paper.*2

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Table Ⅵ. Rf Values of *p*-Tolylsulfonyl-tetra-N-acetyl-kanamycins on Thin-layer Chromatography[a)]

Plate	Solvent	N-Acetyl-Km	Mono-O-sulfonates	Di-O-sulfonates	Tri-O-sulfonates
Silicic acid	Di-isobutylketone-AcOH-H_2O (8:5:1)		0.33	0.66	0.79
Silicic acid	*n*-BuOH-AcOH-H_2O (4:1:5)	0.10	0.34, 0.39[b)]	0.47, 0.43[b)]	0.65[c)]
Silica gel	*n*-BuOH-pyr.-H_2O (6:4:3)	0.26	0.55	0.69	
Silica gel	*n*-BuOH-AcOH-H_2O (4:2:1)	0.00	0.10	0.38	
Silica gel	*n*-BuOH-AcOH-H_2O (8:2:5)	0.18	0.44, 0.47[b)]	0.53, 0.50[b)]	

a) Spots were detected by spraying 1% anthrone in sulfuric acid.
b) Minor spot. *c*) Tailing.

in thin-layer chromatography were show in Table Ⅵ. A portion (1.97 g.) of the 40% eluates was dissolved in aqueous EtOH, from which was crystallized Ⅱ (0.65 g.) upon standing. Recrystallization from aqueous EtOH gave Ⅱ as needles, m.p. 173~176°, $[\alpha]_D^{24}$+81° (c=0.91, EtOH). UV λ_{max} mμ (ε): 227 (24,400), 265 (1,500), 274 (1,150). IR cm^{-1}: 1,170 (sulfonate ester). *Anal.* Calcd. for $C_{36}H_{56}O_{19}N_4S_2 \cdot 2H_2O$: C, 48.1; H, 6.1; N, 5.6; S, 6.4. Found: C, 47.5; H, 6.0; N, 5.7; S, 6.4. *Anal.* Calcd. for $C_{36}H_{56}O_{19}N_4S_2$: C, 49.2; H, 5.9; N, 5.8; S, 6.7. Found for the sample dried at 100° for 8 hr.: C, 49.3; H, 6.0; N, 5.9; S, 7.0.

6-Amino-6-deoxy-2-manno-tetra-N-acetyl-kanamycin (Ⅳ)——A solution of crude 2,6-di-O-sulfonate (Ⅱ) (14.0 g.) and sodium azide (8.0 g.) and urea (0.5 g.) in dimethylformamide (150 ml.) containing enough H_2O (20 ml.) to give a homogeneous solution, was refluxed for 10 hr. The black solution was evaporated to dryness, the residue was extracted with hot EtOH, and the extracts were again brought to dryness. After washing with ether, the residue (25.5 g.) was dissolved in H_2O (20 ml.) and chromatographed on a column of Dowex 50W×2 (H^+, 140 ml.) resin. Elution with H_2O gave a mixture of azido-derivatives (8.26 g.). A portion of this mixture was twice precipitated from EtOH-iso-PrOH and analyzed. IR cm^{-1}: 2,100 (azide). *Anal.* Calcd. for $C_{26}H_{43}O_{14}N_7$ (monoazide (Ⅲ)): C, 46.1; H, 6.4; N, 14.5. Found: C, 44.5; H, 6.5; N, 13.0.

A mixture of crude azido-compound (Ⅲ) (6.5 g.) and Raney Ni (W-4, 40 ml.) in H_2O (100 ml.) was shaken for 2 hr. at room temperature and left overnight. Fresh catalyst (11 ml.) was added and, after it was further shaken for 2 hr., the catalyst was filtered off and the solution was evaporated to a residue (5.1 g.), which was dissolved in H_2O (15 ml.) and chromatographed on a column of Dowex 1×2 (OH) resin (100 ml.), developing with H_2O. Effluents were collected in 6.3 ml. fractions. The chromatogram was illustrated in Fig. 2. The first ninhydrin-negative fractions was discarded and next ninhydrin-positive, alkaline fractions were gathered in 3 parts: tube nos. 17~23 (Ⅸ), 340 mg., tube nos. 24~31 (Ⅹ), 480 mg., tube nos. 32~65 (Ⅳ), 1.34 g. Ninhydrin photometry and quantitative determination of 5-aminomethylfurfural on the three fractions were shown in Table I, while the Rf values in paper and resin chromatographies were summarized in Table Ⅱ. The monoamino-derivative (Ⅳ) crystallized from the last fraction upon addition of MeOH and recrystallized from the same solvent. Yield, 1.0 g., m.p. 247~252° (decomp.), $[\alpha]_D^{24}$+68° (c=0.91, H_2O). *Anal.* Calcd. for $C_{26}H_{45}O_{14}N_5 \cdot 2H_2O$: C, 45.4; H, 7.2; N, 10.2. Found: C, 45.0; H, 7.4; N, 10.1.

6-Acetamido-6-deoxy-2-manno-tetra-N-acetyl-kanamycin (Ⅵ)——a) To a suspension of the free base of Ⅳ (1.06 g.) in MeOH (15 ml.) was added Ac_2O (0.8 ml.). The resulting clear solution was kept at room temperature overnight and evaporated to a sirup, which crystallized from aqueous EtOH upon standing. Recrystallization from aqueous EtOH gave penta-N-acetate (Ⅵ), 950 mg., m.p. 293~295° (decomp.), $[\alpha]_D^{24}$+70° (c=1.06, H_2O). Hydrolysis of Ⅵ with the boiling 3*N* HCl for 1.5 hr., followed by paper chromatography revealed 2-deoxystreptamine, 6-amino-glucose and 3,6-diamino-mannose (Table Ⅴ). *Anal.* Calcd. for $C_{28}H_{47}O_{15}N_5 \cdot 2H_2O$: C, 46.2; H, 7.0; N, 9.6. Found: C, 45.9; H, 7.2; N, 9.3.

b) A solution of 6-amino-6-deoxy-2-manno-kanamycin (Ⅴ) (50 mg.) prepared *via* the hydrazino-intermediate and Ac_2O (0.2 ml.) in MeOH (10 ml.) was left at room temperature overnight and then evaporated to dryness. The sirup that remained after washing with ether to remove AcOH, was dissolved in a little of H_2O. Crystals of Ⅵ (30 mg.) were obtained from this solution by the addition of EtOH followed by seeding, m.p. 291~294° (decomp.). Acid hydrolysis of this sample gave three amino compounds, whose Rf values were coincided with those of Ⅵ obtained under a). *Anal.* Calcd. for $C_{28}H_{47}O_{15}N_5 \cdot 2H_2O$: C, 46.2; H, 7.0; N, 9.6. Found: C, 45.8; H, 7.1; N, 9.3.

6-Hydrazino-6-deoxy-2-manno-kanamycin (Ⅷ)——A solution of crude di-O-sulfonate (Ⅱ) (6.0 g.) dried at 80° for 24 hr. in anhydrous hydrazine (27 ml.) was heated in a sealed tube at 135° for 50 hr. After removing much of excess hydrazine by evaporation, the residue was dissolved in H_2O (15 ml.) and chromatographed on a column of Dowex 1×2 (OH) resin (100 ml.). Aqueous effluents were collected in 6.3 ml. fractions. Evaporation of tube nos. 18~21 gave the 6-hydrazino-compound (1.5 g.), which was re-chromatographed on a new column of Dowex 1×2 (OH) (100 ml.). The reducing, ninhydrin-positive fractions were pooled and lyophilized to a white powder (0.57 g.). Re-precipitation from MeOH (6 ml.)-EtOH (12 ml.) gave a free

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base of 6-hydrazino-6-deoxy-2-manno-kanamycin (Ⅷ) (56 mg.). *Anal.* Calcd. for $C_{18}H_{38}O_{10}N_6$: N, 16.9. Found: N, 16.6.

Pentahydrochloride of Ⅷ was prepared from the above mother liquor by the addition of *N* HCl (5 ml.) followed by precipitation with EtOH (20 ml.)-acetone (60 ml.). Re-precipitation from the same solvent mixture gave chromatographically homogeneous hydrochloride (260 mg.), m.p. 200~210° (decomp.). Rf Values of Ⅷ in paper and resin chromatographies were shown in Table Ⅱ. *Anal.* Calcd. for $C_{18}H_{38}O_{10}N_6 \cdot 5HCl$: C, 31.8; H, 6.4; N, 12.3; Cl, 26.0. Found : C, 32.3; H, 7.5; N, 12.1; Cl, 25.3.

6-Amino-6-deoxy-2-manno-kanamycin (V)——To a solution of Ⅷ (1.0 g.) in H_2O (20 ml.) was added Raney Ni (6 ml.). The mixture was shaken mechanically for 5 hr. and left overnight. Fresh catalyst (2 ml.) was added, and, after it was shaken for an additional 2 hr., the solution was filtered and lyophilized to a solid (633 mg.). This was dissolved in H_2O (5 ml.) and chromatographed on a column of Dowex 1×2 (OH) resin (55 ml.), the effluents being collected in 3.3 ml. fractions. Evaporation of tube nos. 19~29 gave a white powder (248 mg.). Re-chromatography on a new column of Dowex 1×2 (OH) resin (50 ml.) gave analytically pure 6-amino-6-deoxy-2-manno-kanamycin (V) (110 mg.). $[\alpha]_D^{24}+85°$ (c=1.02, H_2O). The compound V consumed no iodine and gave "ninhydrin color value" ($E_{1cm}^{1\%}$ at 570 mμ) 562, equivalent to that of 2′-amino-2′-deoxy-kanamycin (558) but definitely larger than that of kanamycin (468) by ninhydrin photometry.[7] *Anal.* Calcd. for $C_{18}H_{37}O_{10}N_5$: N, 14.5. Found : N, 14.2. Penta-N-salicylidene derivative (Ⅶ) was prepared according to the usual procedure and analyzed. $[\alpha]_D^{24}+54°$ (c=0.91, MeOH). *Anal.* Calcd. for $C_{53}H_{57}O_{15}N_5$: C, 63.6; H, 5.7; N, 7.0. Found : C, 61.7; H, 5.8; N, 7.0.

Hexa-N-acetate of the R_{km} 0.48 Substance (IX)——A portion (100 mg.) of fractions 17~23 obtained in the resin chromatography mentioned above was treated with Ac_2O (0.2 ml.) in MeOH (5 ml.) for 4 hr. The solution, after addition of a little of H_2O, was evaporated to a sirup, which was washed with ether and crystallized from aqueous EtOH upon standing for 3 days at room temperature. Yield, 35 mg. m.p. 200° (decomp.). $[\alpha]_D^{25}+50°$ (c=1.09, H_2O). *Anal.* Calcd. for $C_{30}H_{50}O_{15}N_6 \cdot 5H_2O$: C, 43.7; H, 7.3; N, 10.2. Found : C, 44.1; H, 7.5; N, 10.1.

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7) S. Inouye, H. Ogawa : J. Chromatog., **13**, 536 (1964).

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