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Chemical Modification of Kanamycin. II.[1] Syntheses of 3′-Amino-3′-deoxykanamycin and 3′-Amino-3′-deoxy-2′-mannokanamycin[2,3]

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Cyclization of the dialdehyde, obtained by periodate oxidation of tetra-N-acetylkanamycin, with nitromethane, followed by reduction and hydrazinolysis gave the titled compounds. Structural assignment of these derivatives was accomplished by NMR spectroscopy and degradation to the respective component sugars. Direct treatment of 3′-nitro-3′-deoxyderivative with hydrazine gave, *via* decomposition of the nitro sugar portion, O-α-D-3-amino-3-deoxyglucopyranosyl(1→6)-2-deoxystreptamine.

In the previous paper,[1] the syntheses of 6-amino-6-deoxykanamycin and 6-hydrazino-6-deoxykanamycin *via* the replacement of the terminal hydroxyl group of the 3-amino-3-deoxy-α-D-glucopyranose moiety by an amino or a hydrazino group were described. This paper was concerned with the chemical modification of the 6-amino-6-deoxy-α-D-glucopyranose moiety by the application of the nitromethane-cyclization reaction[5] to the periodate oxidation product of tetra-N-acetylkanamycin (I) to obtain 3′-amino-3′-deoxyderivatives.

It was already shown[6] that tetra-N-acetylkanamycin (I) consumed two moles of periodate with liberation of one mole of formic acid, indicating the ring cleavage of the 6-aminoglucose portion. The dialdehyde (II) thus obtained was not isolated, but, after removal of much of inorganic materials, condensed directly with nitromethane in the presence of sodium methoxide. The resulting sodium salt of 3′-*aci*-nitro-3′-deoxy-N-acetylkanamycin was neutralized with the cation exchange resin to generate a mixture of 3′-nitro compounds (III). An attempted separation of III by means of the resin chromatography on a column of Dowex 50 W×2 (H form) developed with water was fruitless. The mixture was then hydrogenated

1) Part I: S. Inouye, *J. Antibiotics* (Tokyo), Ser. A, **20**, 6 (1967).
2) Preliminary report, "Abstracts of papers, 9th Symposium on the Chemistry of Natural Products," Oct. 13, 1965, Osaka, p. 7.
3) The kanamycin derivatives in which the 6-amino-6-deoxy-α-D-glucopyranose portion was exchanged, respectively, by 3,6-diamino-3,6-dideoxy-α-D-glucopyranose and by 3,6-diamino-3,6-dideoxy-α-D-mannopyranose. For the abbreviated naming of kanamycin derivatives, see part I of this series[1].
4) Location: *Morooka, Kohoku-ku, Yokohama-shi.*
5) F.W. Lichtenthaler, *Angew. Chem.*, **76**, 84 (1964).
6) H. Ogawa, T. Ito, S. Kondo, and S. Inoue, *Bull. Agr. Chem. Soc. Japan*, **23**, 289 (1959).

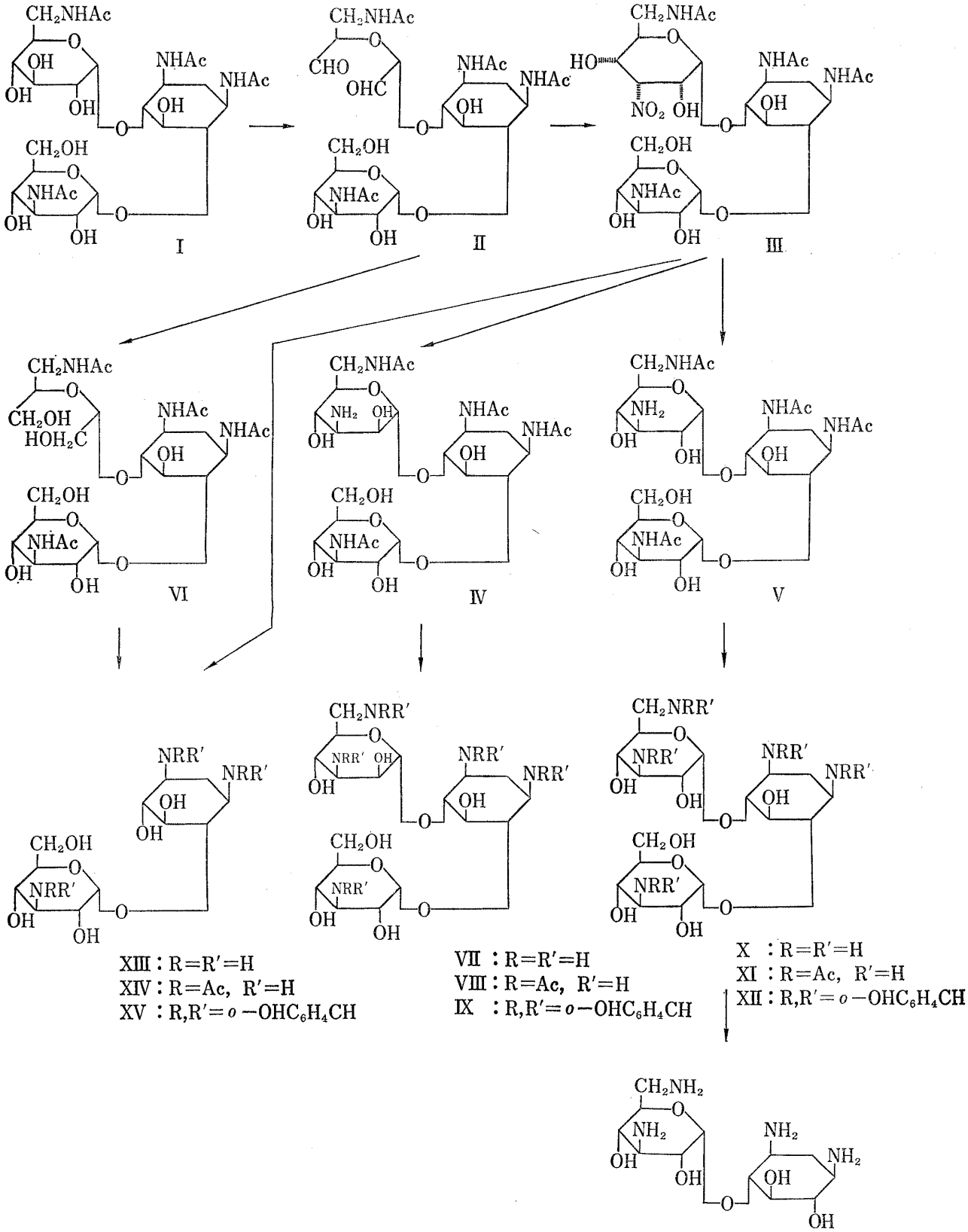

Chart 1

over Raney nickel, and a mixture of 3'-amino compounds produced was separated by the use of the resin chromatography on a column of Dowex 1×2 (OH form) developing with water. A ninhydrin-negative, non-reducing compound was eluted first, to which was assigned tenta-

tively the dialcohol structure (VI), since it gave a partial hydrolysis product XIII by subsequent hydrazinolysis. The second eluate, incompletely separated from the first (VI), was ninhydrin-positive, which contained 3′-amino-3′-deoxy-2′-manno-tetra-N-acetylkanamycin (IV) as described later.

The lastly eluted compound (V), well separated from IV and VI, was also ninhydrin-positive. Treatment of V with hydrazine hydrate at 140° for 60 hours afforded an amino-deoxykanamycin (X) which was characterized by conversion into a penta-N-acetate (XI) and a penta-N-salicylidene Schiff base (XII). The structure of X was determined as follows. The penta-N-acetate (XI) which displayed five methyl signals at 7.96, 7.96, 7.98, 8.00 and 8.05 ppm in the nuclear magnetic resonance (NMR) spectrum in deuterium oxide, consumed no periodate in the acetate buffer of pH 4.7, indicating that the amino group newly introduced was located at C-3 of the 6-aminoglucose moiety. The NMR spectrum of the tetra-N-acetate (V) in the form of free base showed, in addition to four methyl singlets at 7.96, 7.96, 8.00 and 8.03 ppm, a triplet at 7.07 ppm, which was assigned to the C-3 methine proton weakly deshielded by the amino group introduced.[7] The large coupling constants obtained from the analysis of this signal ($J_{2,3}=J_{3,4}=8.2$ cps) indicated *trans*-axial orientations of H-2, H-3 and H-4. It followed from this that a new amino-sugar component in X was 3,6-diaminoglucose in the chair conformation with all the equatorial substituents except C-1. The $J_{1,2}$ values estimated from the line-spacing of the two H-1 signals in the NMR spectra of V and XI (Table I) were also compatible with the magnitude of the equatorial-axial coupling between H-1 and H-2 of α-D-glucopyranose derivatives.[7]

Table I. Chemical Shifts and Apparent Coupling Constants of Anomeric Proton Signals of 3′-Amino-3′-deoxy-tetra-N-acetylkanamycin (V), 3′-Amino-3′-deoxy-2′-manno-tetra-N-acetylkanamycin (IV) and Their Penta-N-acetates (XI and VIII) in Deuterium Oxide

Compound	H-1′ (3,6-Diaminohexose)	H-1 (3-Aminoglucose)
3′-Amino-N-acetylkanamycin (V) free base	4.65(3.1)[a]	4.85(3.5)
3′-Amino-N-acetylkanamycin (V) HCl	4.55(3.5)	4.84(3.5)
3′-Acetamido-N-acetylkanamycin (XI)	4.68(3.5)	4.87(3.8)
3′-Amino-2′-manno-N-acetylkanamycin (IV) free base	4.76(1.8)	4.86(3.5)
3′-Amino-2′-manno-N-acetylkanamycin (IV) HCl	4.69(1.6)	4.85(3.5)
3′-Acetamido-2′-manno-N-acetylkanamycin (VIII)	4.78(1.7)	4.86(3.5)

a) Chemical shift in ppm (τ). Number in parentheses was an apparent coupling constant in cps.

Paper chromatographic examination of the acid hydrolyzate of XI revealed 2-deoxystreptamine, 3-aminoglucose ($R_{\text{deoxystreptamine}}$, 2.25) and a new amino sugar ($R_{\text{deoxystreptamine}}$, 1.20) in stead of 6-aminoglucose. The *Rf* value of the last compound coincided with that of an authentic sample of 3,6-diaminoglucose.[8] In order to further confirm the above results, XI was degraded by refluxing with methanolic hydrogen chloride. Fractionation of the methanolyzate by means of the resin chromatography on a Dowex 1×2 (OH) column yielded 2-deoxystreptamine, methyl 3,6-diamino-3,6-dideoxy-α-D-glucopyranoside characterized as a crystalline N-salicylidene Schiff base and methyl 3-amino-3-deoxy-α-D-glucopyranoside, all of which were identified by comparing their melting points, IR spectra and *Rf* values in paper chromatography with those of authentic samples.[6,8] Thus, the structures of X, VI,

7) S. Inouye, *Chem. Pharm. Bull.* (Tokyo), **14**, 1210 (1966).
8) S. Inouye, *Chem. Pharm. Bull.* (Tokyo), **14**, 902 (1966).

XI and XII must be 3′–amino–3′–deoxykanamycin and its derivatives.

Besides the three components described above, a partial hydrolysis product (XVI) was isolated from the methanolyzate of XI, which afforded a crystalline tetra–N–salicylidene derivative. The structure of XVI was shown to be O–α–D–3,6–diamino–3,6–dideoxyglucopyranosyl–(1→4)–2–deoxystreptamine, since it gave 2–deoxystreptamine and 3,6–diaminoglucose, but no 3–aminoglucose on acid hydrolysis.

A mixture of IV and VI, whose complete separation was difficult as such, was subjected to the hydrazinolysis at 140° for 60 hours. Separation of a mixture of the de–N–acetylated products was again unsuccessful by the resin chromatography using Dowex 1×2 (OH form), but accomplished by the preparative paper chromatography. When developed with the mixed solvent of *n*–butanol–pyridine–acetic acid–water (6:4:1:3), it was separated into two ninhydrin-positive compounds, of which the faster moving compound (XIII) was a monoglycoside affording a tri–N–acetate (XIV) and a tri–N–salicylidene derivative (XV). The compound XIV was shown by comparison of optical rotation, IR and NMR spectra to be identical with an authentic specimen of tri–N–acetyl–O–α–D–3–amino–3–deoxyglucopyranosyl–(1→6)–2–deoxystreptamine.[6] In support of this, acid hydrolysis of XIV gave 2–deoxystreptamine and 3–aminoglucose, identified on paper chromatograms.

The slower moving compound (VII) was an isomer of 3′–aminokanamycin (X), and characterized by conversion into a penta–N–acetate (VIII) and a penta–N–salicylidene derivative (IX). The NMR spectrum of the tetra–N–acetate (IV) in deuterium oxide showed, besides four methyl singlets at 7.95, 7.97, 8.00 and 8.03 ppm, anomeric proton signals at 4.86 ppm ($J_{1,2}$=3.5 cps) and 4.76 ppm ($J_{1,2}$=1.8 cps) (Table I). When measured in the form of hydrochloride, the former with larger J value remained almost unchanged (Δ=0.01 ppm), while the latter having smaller J value showed down–field shift (Δ=0.07 ppm), owing to the electrostatic deshielding effect of the cationic nitrogen atom.[7] It followed from this that the latter signal was due to H–1′ of the 3–amino–6–acetamidohexose moiety, and the former due to H–1 of the 3–acetamidoglucose moiety. The respective anomeric proton signal pairs in V, VIII and XI were assigned similarly as shown in Table I, which indicated that the anomeric protons of the 3,6–diamino sugar portion in IV and VIII resonated at higher field with very low $J_{1,2}$ values than those of the 3,6–diaminoglucose moiety in V and XI. This suggested the mannose configuration to the diamino sugars in IV and VIII, since it was widely recognized that the anomeric protons in α–D–mannose derivatives resonated at higher field with unusually low $J_{1,2}$ values than those of the α–D–glucose derivatives including 3,6–diamino–sugars.[7,9] Acid hydrolysis of VII followed by paper chromatographic examination revealed 2–deoxystreptamine, 3–aminoglucose and a new amino sugar, whose *Rf* value ($R_{\text{deoxystreptamine}}$, 1.30) was indistinguishable from that of an authentic sample of 3,6–diaminomannose.[8] The three components of VI was finally confirmed by methanolysis. From the methanolyzate of VI was isolated methyl 3,6–diamino–3,6–dideoxy–α–D–mannopyranoside as its N–salicylidene derivative, together with 2–deoxystreptamine hydrochloride and methyl 3–amino–3–deoxy–α–D–glucopyranoside. Based on these results, the structures of VII, IV, VIII and IX were shown to be 3′–amino–3′–deoxy–2′–mannokanamycin and its derivatives.

Direct treatment of the mixture of 3′–nitro–N–acetylkanamycin (III) with hydrazine hydrate, without the catalytic reduction in the intermediate step, yielded a partial degradation

9) S. Inouye, *Chem. Pharm. Bull.* (Tokyo), **14**, 1179 (1966).

product of kanamycin (XIII), instead of 3′-aminokanamycin derivative. This result indicated that the 3′-nitro sugar portion was not reduced by hydrazine, but decomposed. The structure of XIII was determined by direct comparison of physical constants of a crystalline tri-N-acetate (XIV) and a tri-N-salicylidene Schiff base (XV) with those of the samples obtained above.

Detailed biological properties of VII and X will be reported later.

Experimental[10]

3′-Nitro-3′-deoxy-tetra-N-acetylkanamycin (III)——To a cooled solution of tetra-N-acetylkanamycin (I) (50 g) in H_2O (250 ml) was added $NaIO_4$ (35 g). The mixture was stirred for 4 hr at room temperature, and the released formic acid was neutralized with solid $NaHCO_3$ (6.5 g). The mixture was then poured into MeOH (250 ml) and the precipitated salts were filtered off. The filtrate was concentrated to a sirup, to which was added a mixture of *iso*-propanol and ether. The precipitate was collected and extracted with MeOH (300 ml), and the methanolic extracts were treated with nitromethane (10 ml) followed with a solution of NaOMe (5 g) in MeOH (100 ml). The mixture was kept at room temperature for 3 hr, and then neutralized with Dowex 50W×8 (H) resin. After removal of the resin, the solution was concentrated to dryness, the residue dissolved in H_2O (100 ml) and chromatographed on a column of Dowex 50W×2 (H) (500 ml). Aqueous effluents were collected by 16 ml fractions. Evaporation of the fractions 23—85 gave a mixture of the 3′-nitro derivatives (III) (36.8 g). UV Spectrum in 0.025N NaOH showed a band at 247 mμ ($E^{1\%}_{1cm}$ 135), typical for an *aci*-nitro chromophore. Repeated chromatography of III by the use of a Dowex 50W×2 resin column resulted no further separation.

3′-Amino-3′-deoxykanamycin (X)——A solution of a mixture of the 3′-nitroderivatives (III) (2.3 g) in H_2O (20 ml) was hydrogenated over Raney Ni (W-4) (10 ml) at the atmospheric pressure for 8 hr. The catalyst was filtered off and the filtrate was evaporated to dryness to give a mixture of the 3′-amino compounds (2.4 g), which showed no more UV band characteristic for the *aci*-nitro group. Resin chromatography of the amino compounds on a column of Dowex 1×2 (OH) (50 ml) with H_2O as an eluant gave three fractions: a) tube nos. 9—14 (684 mg), ninhydrin-negative, $[\alpha]_D^{29}$ +67° (c=0.91, H_2O); b) tube nos. 19—25 (70 mg),

Table II. *Rf* Values[a] of 3′-Amino-3′-deoxykanamycin (X), 3′-Amino-3′-deoxy-2′-mannokanamycin (VII) and Related Compounds in Paper and Resin Chromatographies

Compound	R_{Km}	
	Paper chromato.[b]	Resin chromato.[c]
3′-Aminokanamycin (X)	0.9	1.13
3′-Amino-2′-mannokanamycin (VII)	0.9	0.43
3′-Amino-tetra-N-acetylkanamycin (V)	3.5	
3′-Amino-2′-manno-tetra-N-acetylkanamycin (IV)	2.9	
O-α-D-3-Aminoglucosyl-2-deoxystreptamine (VIII)	2.2	0.43
O-α-D-3,6-Diaminoglucosyl-2-deoxystreptamine (XVI)	1.8	1.13

a) Relative rate of flow against kanamycin (1.00).
b) Developed descendingly with *n*-BuOH-pyridine-AcOH-H_2O (6:4:1:3) for 2—4 days.
c) Developed with H_2O on a column of Dowex 1×2 (OH) resin.

10) Melting points were uncorrected. UV Spectra were taken in MeOH with a Hitachi recording spectrometer EPS-2 and NMR spectra at 60 Mc using a Varian A-60 spectrometer. Sodium 2,2-dimethyl-1,2-silapentane-5-sulfonate was used as an internal standard in deuterium oxide. Paper chromatography was carried out on Tōyō Roshi No. 50 filter paper by the descending method with a solvent of *n*-BuOH-pyridine-AcOH-H_2O (6:4:1:3). Spots were detected by dipping in 0.5% ninhydrin in acetone-pyridine (10:1), or by autobiography. *Rf* Values were indicated by the relative rate of flow against 2-deoxystreptamine or kanamycin. Resin chromatographic analysis was done on a column (2×30 cm) of Dowex 1×2 (OH) resin (200—400 mesh), using H_2O as a developer. Effluents were collected in 3.3 ml fractions and analyzed by the ninhydrin method.[11] Results were shown by the ratio of the elution volume of a sample to that of kanamycin (1.00).

11) S. Inouye and H. Ogawa, *J. Chromatog.*, **13**, 536 (1964).

ninhydrin-positive, $[\alpha]_D^{25}$ +78° (c=0.85, H_2O); c) tube nos. 29—41 (370 mg), ninhydrin-positive, $[\alpha]_D^{27}$ +84° (c=0.91, H_2O).

3′-Amino-3′-deoxy-tetra-N-acetylkanamycin (V) was crystallized from the last fraction (c) and recrystallized from aqueous EtOH. mp 267—268° (decomp.), $[\alpha]_D^{27}$ +90° (c=1.04, 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, 45.2; H, 7.2; N, 9.9.

A solution of V(3.0 g) in 80% hydrazine hydrate (18 ml) was heated in a sealed tube at 140° for 60 hr. An excess of hydrazine was removed by evaporation, the residue dissolved in H_2O (10 ml) and chromatographed on a column of Dowex 1×2 (OH) resin (200—400 mesh, 110 ml). Aqueous effluents were collected by 6.3 ml fractions. The ninhydrin-positive, alkaline fractions (tube nos. 30—70) were pooled and lyophilized to give 3′-amino-3′-deoxykanamycin (X) (1.21 g) as a white powder. $[\alpha]_D^{20}$ +124° (c=1.0, H_2O). The *Rf* values of X in paper and resin chromatographies were shown in Table II.

Selective N-acetylation of X (180 mg) with acetic anhydride in MeOH gave, after recrystallization from aqueous MeOH, a penta-N-acetate (XI) (91 mg). mp >270°. *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, 46.1; H, 7.5; N, 9.5.

The compound X was further characterized as a penta-N-salicylidene derivative (XII), prepared by the usual procedure. mp 185—190°, $[\alpha]_D^{26}$ +123° (c=0.91, MeOH). *Anal.* Calcd. for $C_{53}H_{57}O_{15}N_5$: C, 63.4; H, 5.7; N, 7.0. Found: C, 62.5; H, 5.7; N, 7.1.

Methanolysis of XI——A solution of XI (861 mg) in 500 ml of MeOH saturated with HCl was heated under reflux for 24 hr, and then evaporated to dryness. Extraction of the residue with small amounts of MeOH and EtOH left insoluble materials (568 mg) which consisted mainly of 2-deoxystreptamine and partial hydrolysis products, as indicated by paper chromatography. This was dissolved in H_2O (5 ml), and fractionated by passing through a column of Dowex 1×2 (OH) resin (75 ml) to four main fractions: (a) 127 mg, (b) 28 mg, (c) 65 mg, and (d) 140 mg. Evaporation of the alcoholic extracts (366 mg) and chromatography of the residue on a Dowex 1×2 (OH) column gave two main fractions: (e) 45 mg and (f) 121 mg.

The chief component of the firstly eluted fraction (a) was 2-deoxystreptamine, which crystallized from MeOH and EtOH. Yield, 90 mg. mp 218—219° (decomp.). It was identified by comparison of melting point, IR spectrum and *Rf* values in paper chromatography with those of an authentic sample. Fractions (b) and (e) were combined and treated with a solution of excess salicylaldehyde in EtOH, whereupon crystals of N-salicylidene Schiff base (95 mg) were immediately deposited. After recrystallization from EtOH, it showed mp 239—242°, $[\alpha]_D^{24}$ +160° (c=1.06, MeOH). The UV and IR spectra of this product were superimposable on those of an authentic methyl 3,6-disalicylideneamino-3,6-dideoxy-α-D-glucopyranoside.[8] The melting point was not depressed when mixed with a genuine specimen,. *Anal.* Calcd. for $C_{21}H_{24}O_6N_2$: C, 63.1; H, 6.1; N, 7.0. Found: C, 63.2; H, 6.3; N, 7.1.

A mixture of fractions (c) and (f) was triturated with EtOH to yield crystals of an amino-sugar (76 mg), which was recrystallized from EtOH. mp 164—165°, $[\alpha]_D^{24}$ +150° (c=1.11, H_2O). Admixture of this product with an authentic sample of methyl 3-amino-3-deoxy-α-D-glucopyranoside did not change the melting point. IR spectral comparison of the two compounds also confirmed their identity.

The lastly eluted fraction (d) which contained XVI gave rise to a crystalline N-salicylidene derivative (125 mg) on treatment with an excess of salicylaldehyde in aqueous EtOH. A second recrystallization from MeOH and EtOH afforded an analytical sample. mp 215° (sintered at 173°). $[\alpha]_D^{24}$ +169°(c=1.0, MeOH). Refluxing a solution of XVI (20 mg) in 3N HCl (5 ml) for 3 hr liberated 2-deoxystreptamine and 3,6-diaminoglucose, which were identified by paper chromatography. *Anal.* Calcd. for $C_{40}H_{42}O_{10}N_4$: C, 65.0; H, 5.7; N, 7.6. Found: C, 64.6; H, 6.0; N, 7.5.

3′-Amino-3′-deoxy-2′-mannokanamycin (VII)——The fractions 19—25 obtained by the resin chromatography of the mixture of 3′-amino derivatives mentioned above was twice re-chromatographed on a Dowex 1×2 (OH) resin column and finally precipitated from aqueous MeOH-*iso*-propanol to afford impure 3′-amino-3′-deoxy-2′-manno-tetra-N-acetylkanamycin (IV).

A mixture of IV and VI (2.40 g) was treated with anhydrous hydrazine (9 ml) at 140° for 60 hr, and the de-N-acetylated products were chromatographed on a column of Dowex 1×2 resin (100 ml). Effluents were collected by 6.3 ml fractions and tube nos. 15—19 were pooled and evaporated to give a free base (1.69 g). No signal for an acetyl group in the NMR spectrum. It gave a single peak in the analytical resin chromatography (R_{Km} 0.43), but, two spots in the paper chromatography developed with *n*-BuOH-pyridine-AcOH-H_2O (6:4:1:3). Then, the free base (800 mg) dissolved in H_2O was placed on 3 sheets of Tōyō Roshi No. 526 (40×40 cm) and developed descendingly with the same solvent for 4 days. After drying the sheets, 3′-amino-3′-deoxy-2′-mannokanamycin tetraacetate (VII) (344 mg) was eluted from the R_{Km} 0.9 fraction. $[\alpha]_D^{23}$ +75° (c=0.91, H_2O).

A solution of the tetraacetate (VII) (180 mg) and acetic anhydride (0.4 ml) in MeOH (20 ml) was kept at room temperature overnight and evaporated to a powder which crystallized from aqueous EtOH. mp 255—258° (decomp.), $[\alpha]_D^{25}$ +83° (c=0.89, H_2O). NMR (D_2O), 7.94 (3Ac), 7.99 (Ac), 8.03 (Ac). *Anal.* Calcd. for $C_{28}H_{47}O_{15}N_5 \cdot 4H_2O$: C, 44.0; H, 7.2; N, 9.1. Found: C, 44.1; H, 7.3; N, 8.6.

Penta-N-salicylidene derivative (IX) was prepared and re-precipitated from hot benzene. $[\alpha]_D^{26}$ +101° (c=0.97, MeOH). *Anal.* Calcd. for $C_{53}H_{57}O_{15}N_5$: C, 63.6; H, 5.7; N, 7.0. Found: C, 62.7; H, 5.7; N, 6.9.

Methanolysis of VIII——A solution of a crude VIII (850 mg) in MeOH saturated with HCl (500 ml) was heated under reflux for 19 hr. Concentration of the reaction mixture yielded crystals of 2-deoxystreptamine hydrochloride (230 mg). mp 215—220°. The hydrochloride was characterized by comparison of its IR spectrum and *Rf* values in paper chromatography with those of an authentic sample.[6]

The mother liquor was evaporated to dryness, and the residue was extracted with EtOH. The EtOH insoluble materials (260 mg) gave, after chromatography on a Dowex 1×2 (OH) resin (75 ml), three main fractions: (a) 104 mg (partial hydrolysis products), (b) 19 mg and (c) 37 mg. The EtOH soluble materials (400 mg) were separated in a similar way into two main fractions: (d) 50 mg, and (e) 150 mg. Fractions (b) and (d) were combined and treated with salicylaldehyde to afford a N-salicylidene Schiff base (98 mg), which recrystallized twice from benzene. mp 175—177°, $[\alpha]_D^{24}$ −7° (c=0.854, MeOH). The melting point and IR spectrum of this product were in agreement with those of an authentic sample of methyl 3,6-disalicylidene-amino-3,6-dideoxy-α-D-mannopyranoside crystallized from benzene, but different from those crystallized from EtOH.[8,12] *Anal.* Calcd. for $C_{21}H_{24}O_6N_2$: C, 63.1; H, 6.1; N, 7.0. Found: C, 62.5; H, 6.5; N, 7.3.

Trituration of a mixture of fractions (c) and (e) with EtOH induced crystallization of methyl 3-amino-3-deoxy-α-D-glucopyranoside (135 mg), which, after recrystallization from EtOH, showed mp 165—166°, $[\alpha]_D^{24}$ +154° (c=1.11, H_2O). The identity was confirmed by a mixed melting point determination and IR spectral comparison.

O-α-D-3-Amino-3-deoxyglucopyranosyl-(1→6)-2-deoxystreptamine (XIII)——The R_{Km} 2.2 fraction obtained by the preparative paper chromatography was re-precipitated from EtOH and ether to give XIII (440 mg) as a triacetate. Its chromatographic bahaviors on paper and resin were indistinguishable from those of an authentic sample of XIII prepared by the partial acid hydrolysis of kanamycin[6] (Table II). Identity was further confirmed by conversion into the crystalline tri-N-acetate (XIV), mp 270° (decomp.), $[\alpha]_D^{26}$ +72° (c=0.84, H_2O). Its IR spectrum coincided with that of an authentic sample.[6] NMR (D_2O), 7.94 (Ac), 7.99 (Ac), 8.03 (Ac). 4.84 (H-1, $J_{1,2}$=3.5 cps). Hydrolysis of XIV with 3N HCl at 100° for 1.5 hr yielded 2-deoxystreptamine and 3-aminoglucose identified on paper chromatograms. *Anal.* Calcd. for $C_{18}H_{31}O_{10}N_3 \cdot \frac{1}{2} H_2O$: C, 47.2; H, 7.0; N, 9.2. Found: C, 47.2; H, 6.9; N, 9.1.

Treatment of XIII with an excess of salicylaldehyde in EtOH gave a tri-N-salicylidene Schiff base (XV), which crystallized from benzene. mp 288—292°, $[\alpha]_D^{26}$ −24° (c=0.93, MeOH). *Anal.* Calcd. for $C_{33}H_{37}O_{10}N_3$: C, 62.4; H, 5.9; N, 6.6. Found: C, 62.7; H, 6.4; N, 6.9.

Reaction of 3′-Nitro-3′-deoxy-tetra-N-acetylkanamycin (III) with Hydrazine——A solution of the mixture of 3′-nitro compounds (III) (1.98 g) in anhydrous hydrazine (9 ml) was heated in a sealed tube at 120° for 48 hr. During the reaction, considerable decomposition was observed. An excess of hydrazine was removed by evaporation and the residue was chromatographed on a Dowex 1×2 (OH) column (105 ml). No fraction containing VII or X could be found. Instead, XIII (700 mg) was isolated in 75% yield. It gave a tri-N-acetate (XIV) and a tri-N-salicylidene Schiff base (XV), both of which were shown to be identical with the corresponding derivatives prepared above by comparison of melting points, $[\alpha]_D$, IR spectra, elemental analysis and hydrolysis experiments.

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12) The disagreement was due to the different crystalline forms which arised from tautomerism of N-salicylidene chromophore between the phenolimine and the ketoamine forms.[13] Considering from the relative intensities of the characteristic IR bands for the phenolimine (1580 cm^{-1}) and the ketoamine (1530 cm^{-1}),[13] the Schiff base crystallized from benzene had lower ketoamine content than that crystallized from EtOH. As a matter of fact, both of the crystals showed identical UV and IR spectra in solution.

13) S. Inouye, *Chem. Pharm. Bull.* (Tokyo), **15**, 1540 (1967).