Studies of Antibiotics and Related Substances. XXI. The Synthesis of Deoxy and Chlorodeoxy Derivatives[1] of Kanamycin

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In the course of our investigation into the effects of structural changes in kanamycin on its antibacterial activity, the syntheses of some aminosugar glucosides of deoxystreptamine and related compounds were previously reported.[2] The present report is concerned with the extension of this work.

Since kanamycin[3] contains two aminosugar moieties in the sequence 3-amino-3-deoxy-D-glucose—deoxystreptamine—6-amino-6-deoxy-D-glucose, an interesting type of structural variant would be the exchange of the aminosugars for another sugar by a partial synthesis from the antibiotic. Modifications of the primary alcoholic group in the 3-amino-3-deoxy-D-glucose moiety will be described in this paper.

As has previously been reported,[4] 4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-deoxystreptamine and 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-deoxystreptamine were isolated from the acid-hydrolyzate of kanamycin by controlled hydrolysis; it was thus found that the former showed considerable antibacterial activity, whereas the latter did not show any activity against microorganisms tested. This suggested that (6-amino-6-deoxyglucosyl)-deoxystreptamine moiety may take the leading role in exhibiting the antibacterial activity of kanamycin. Accordingly, the synthetic variation of the 3-amino-3-deoxyglucose part of kanamycin was undertaken.

Kanamycin (I) contains seven alcoholic hydroxyl groups, one of which is primary; the others are all secondary. The difference[5]

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[1] Presented at the 17th Annual Meeting of the Chemical Society of Japan, Tokyo, March, 31, 1964. A brief communication has already been given of the synthesis of deoxykanamycin: T. Tsuchiya, S. Iriyama and S. Umezawa, *J. Antibiotics*, **A16**, 173 (1963).

[2] a) S. Umezawa and Y. Ito, This Bulletin, **34**, 1540 (1961); b) T. Suami, S. Ogawa and S. Umezawa, ibid., **36**, 460 (1963); T. Suami, S. Ogawa, T. Yoshizawa and S. Umezawa, ibid., **37**, 1538 (1964); c) T. Tsuchiya, H. Fujita and S. Umezawa, *J. Antibiotics*, **A17**, 181 (1964); d) S. Umezawa and S. Koto, ibid., **A17**, 186 (1964).

[3] S. Umezawa, Y. Ito and S. Fukatsu, ibid., **A11**, 120, 162 (1958); This Bulletin, **32**, 81 (1959).

[4] S. Umezawa and T. Tsuchiya, *J. Antibiotics*, **A15**, 51 (1962).

[5] See, e. g., R. S. Tipson, "Advances in Carbohydrate Chem.," Vol. 8, Academic Press. Inc., New York, N. Y. (1953), p. 127.

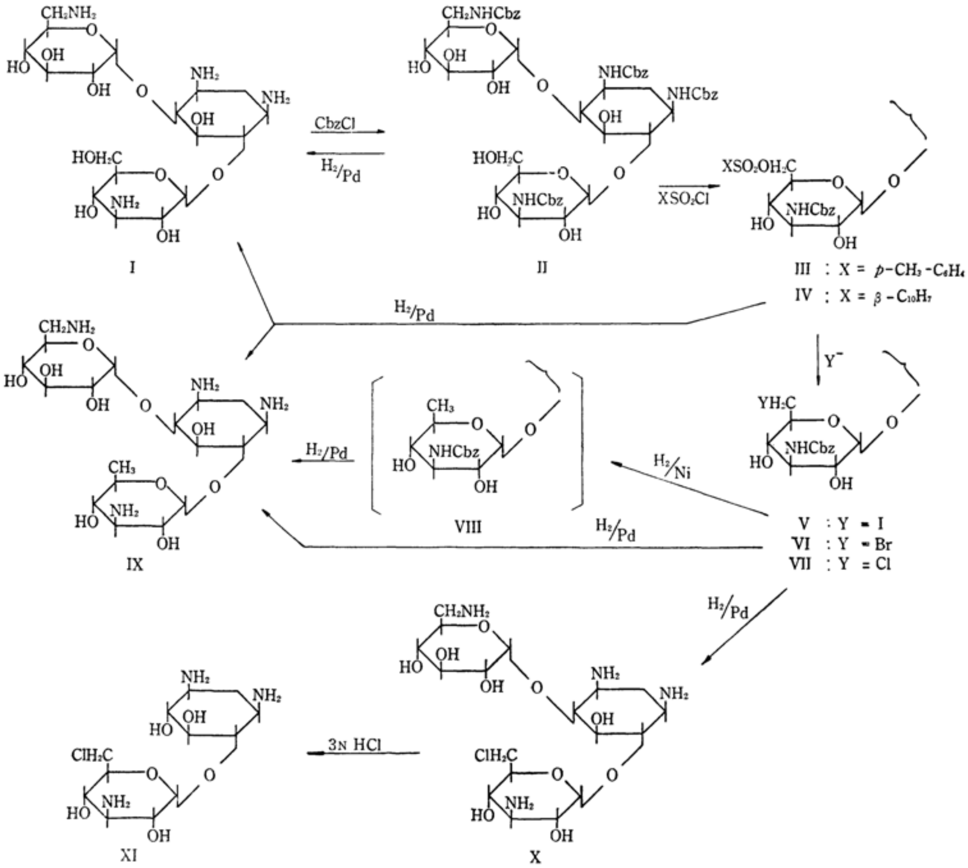

Chart 1

in the ease of the arylsulfonylation of primary and secondary alcoholic groups—the primary hydroxyl can be more easily esterified than the secondary ones—has been used for the present synthesis.

The treatment of an aqueous acetone suspension of kanamycin and sodium carbonate with carbobenzoxy chloride in toluene afforded tetra-*N*-carbobenzoxykanamycin (II). Since difficulty was experienced in avoiding contamination by the formation of an *O*-carbobenzoxylated product, the crude product was treated with dilute ethanolic hydrogen chloride at room temperature to afford an analytically pure sample of II (Chart 1).

The arylsulfonylation of the primary hydroxyl group of II was effected by *p*-toluenesulfonyl chloride, followed by fractional precipitation and silica gel column chromatography; by this method, a mono-*O*-tosylated product (III) was obtained in about a 20% yield. Alternatively, β-naphthalenesulfonyl chloride was used to convert II to a mono-*O*-nasylated product (IV). However, the yield of arylsulfonylation was only slightly improved.

The replacement of the tosyloxy group of III or the nasyloxy group of IV by an iodine atom was readily brought about by heating the ester (III or IV) with sodium iodide in an acetone-dioxane solution, thus affording the deoxyiodo derivative (V) of tetra-*N*-carbobenzoxykanamycin (II) in a 94% yield (from III). On the basis of the extensive studies by Oldham and Rutherford[6] and other workers[7], it can be said that the facility of the replacement reaction indicates that the tosyl group in III was joined to the primary hydroxyl group. The corresponding chlorodeoxy and bromodeoxy derivatives (VII and VI) were also obtained from IV by the use of lithium chloride and bromide in similar reactions. The well-known order of nucleophilic reactivity, $I^- > Br^- > Cl^-$, has been again observed in the fact that sodium iodide reacted faster than lithium bromide, which in turn reacted faster than lithium chloride, to precipitate the corresponding alkaline salt of β-naphthalenesulfonic acid. The

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[6] J. W. H. Oldham and J. K. Rutherford, *J. Am. Chem. Soc.*, **54**, 366 (1932).

[7] W. T. Haskins, R. M. Mann and C. S. Hudson, ibid., **64**, 132 (1942); G. R. Barker and R. W. Goodrich, *J. Chem. Soc.*, **1949**, *Supplm.*, 233; H. Schmid and P. Karrer, *Helv. Chim. Acta*, **32**, 1371 (1949).

crude product of VII, which could not be readily obtained in a pure form, was used for the next step without further purification.

The deoxyiodo derivative (V) was converted, by hydrogenolysis with a Raney nickel catalyst, to the corresponding deoxy derivative (VIII) of tetra-*N*-carbobenzoxykanamycin, and thence, by repeated hydrogenolysis with palladium black in a dioxane - water - acetic acid solution, to deoxykanamycin. Attempts to effect direct hydrogenolysis by the palladium catalyst to deoxykanamycin without the prior use of a Raney nickel catalyst were unsuccessful.

The hydrogenolysis of the bromodeoxy derivative (VI) with palladium black by a similar procedure also gave the deoxykanamycin.

A similar hydrogenolysis of the chlorodeoxy derivative (VII) with a palladium catalyst, followed by column chromatography on Dowex 1 X2 resin, led to chlorodeoxy-kanamycin (X).

The hydrogenolysis of the nasyl derivative (IV) by the palladium catalyst in aqueous dioxane containing a small amount of acetic acid proceeded smoothly, and the formation of the expected kanamycin, accompanied by deoxykanamycin, was confirmed by paper chromatography.

Paper chromatography of the deoxykanamycin (IX) and chlorodeoxy-kanamycin (X) by ninhydrin coloration, using a *t*-amylalcohol - pyridine - water - acetic acid (6 : 4 : 3 : 1) solvent system and Toyo filter paper No. 50, showed R_f 1.3 and 2.2 respectively, the R_f-value of kanamycin being taken as 1.0.

Structural evidence for chlorodeoxy-kanamycin (X) and deoxykanamycin (IX) was further obtained from the results of the controlled hydrolysis of X. (3-Amino-6-chloro-3, 6-dideoxy-D-glucosyl)-deoxystreptamine (XI) has been isolated from the hydrolyzate of X by a paper chromatographic technique.

We[2c)] have previously reported that the R_{f_D}-value*[1] of a di-glucoside of deoxystreptamine is approximately given by the product of the two R_{f_D}-values of the constituent mono-glucosides. The application of the R_{f_D}-values of XI (R_{f_D} 1.33) and 4-*O*-(6-amino-6-deoxyglucosyl)-deoxystreptamine (R_{f_D} 0.39) to the multiplication rule showed a good accordance with the experimental value of R_{f_D} (0.52) of X: $1.33 \times 0.39 = 0.52$.*[2]

Deoxykanamycin (IX) was hydrolyzed by refluxing it in 3 N hydrochloric acid, and the hydrolyzate was submitted to paper chromatography. Detection by ninhydrin-coloration showed a spot of R_f (1.67) relative to the R_f-value of 3-amino-3-deoxy-D-glucose taken as 1.0, in addition to the spots corresponding to deoxystreptamine and 6-amino-6-deoxy-D-glucose. Evidently, the new spot indicated the presence of 3-amino-3, 6-dideoxy-D-glucose in the deoxykanamycin. The paper chromatography of the hydrolyzate obtained by mild hydrolysis of chlorodeoxy-kanamycin showed the spot of (3-amino-6-chloro-3, 6-dideoxy-D-glucosyl)-deoxystreptamine (XI), in addition to the spots of deoxystreptamine, 6-amino-6-deoxy-D-glucose, 4-*O*-(6-amino-6-deoxy-D-glucosyl)-deoxystreptamine[4)] and unchanged chlorodeoxy-kanamycin.

Both deoxykanamycin and chlorodeoxykanamycin showed strongly antibacterial activity, and their antibiotic spectra*[3] were essentially similar to that of kanamycin as shown in Table I. XI had no antibacterial activity against organisms tested.

Experimental

Paper Chromatography and Bioautography.— Paper chromatography was performed by the descending technique, using Toyo filter paper No. 50. The solvent system used was *t*-amylalcohol - pyridine - water - acetic acid (6 : 4 : 3 : 1). Aminosugars and their glucosides were detected by ninhydrin-coloration, using a general procedure. Spots corresponding to the antibacterial components were also detected on the bioautograms by a general procedure[8)] using *Bacillus subtilis* as the test organism.

Tetra-*N*-carbobenzoxykanamycin (II).— A mixture of a kanamycin base (1.5 g. 3.1 mmol.) and sodium carbonate decahydrate (4.1 g.) in 75% aqueous acetone (60 ml.) was cooled to about −5°C, and into the mixture was then vigorously stirred a solution of carbobenzoxy chloride (2.15 g. 12.6 mmol.) in toluene (7 ml.) in portions over a 5 min. period. After the addition was complete, stirring was continued at about 5°C for 3 hr. The reaction mixture became almost clear once, and then the resulting product began to separate. The reaction mixture was placed in a refrigerator overnight. The mixture was poured into a large volume of a mixture of water and ether, and then vigorously agitated. The flocculent product which had been precipitated between two layers was collected and pressed between filter papers to remove the solvents.

The product was added to a solution of ethanol and concentrated hydrochloric acid (10 : 1), and the mixture was stirred at room temperature for 1.5 hr.

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*[1] R_{f_D} is an R_f-value relative to the R_f-value of deoxystreptamine, taken as 1.0.

*[2] In this experiment, by the general procedure described in the beginning of the "Experimental" section and analogous to that described in the report of Ref. 2c) The R_{f_D}-values of (3-amino-3-deoxy-D-glucosyl)-deoxystreptamine and kanamycin were 0.60 and 0.23 respectively. The experimental value for kanamycin also showed a good accordance with the calculated value: $0.60 \times 0.39 = 0.23$.

*[3] The antibiotic spectrum of deoxykanamycin was previously presented in a short communication by T. Tsuchiya, S. Iriyama and S. Umezawa; see Ref. 1.

8) The method first used for the analysis of penicillins: R. R. Goodall and A. A. Levi, *Nature*, **158**, 675 (1946); *Analyst*, **72**, 277 (1947).

The product was filtered and washed with water; yield 3.09 g. (96%), m. p. 259—261°C (decomp.). A sample (1.0 g.) was recrystallized from dioxane-water to give an analytically pure sample (0.76 g.) of the title compound (II); m. p. 259—261°C, $[\alpha]_D^{18}$ +68.2° (*c* 2.038, in dimethyl formamide).

Found: C, 58.89; H, 6.18; N, 5.41. Calcd. for $C_{50}H_{60}O_{19}N_4$: C, 58.81; H, 5.92; N, 5.49%.

It was confirmed by van Slyke determination that the pure sample did not contain the free NH_2 group.

The Hydrogenolysis of II to Kanamycin.—A sample (400 mg.) of II was dissolved in 15 ml. of aqueous dioxane (1 : 1) containing a drop of acetic acid and hydrogenated over palladium black (100 mg.) under atmospheric pressure at 45°C for 13 hr. The reaction mixture was filtered, and the filtrate was evaporated to dryness. An aqueous solution of the residue (270 mg.) was passed through a column of Amberlite IRA-400 resin (OH form) (4 ml.). Ninhydrin-positive, strongly-alkaline fractions were collected and concentrated to a small volume by evaporation. The addition of ethanol to the concentrate caused crystallization; yield 120 mg. The product was proved to be identical with intact kanamycin by elementary analysis and biological assay.

Found: C, 44.63; H, 7.26; N, 11.46; 11.2 (van Slyke). Calcd. for $C_{18}H_{36}O_{11}N_4$: C, 44.62; H, 7.49; N, 11.56%.

Tetra-*N*-carbobenzoxy-mono-*O*-tosylkanamycin (III).—A sample (10.2 g. 10 mmol.) of II was dissolved in dry pyridine (60 ml.) and cooled to −18°C, and then to it was added *p*-toluenesulfonyl chloride (2.10 g.) in one portion. The mixture was vigorously shaken and then allowed to stand at about the same temperature for 4 hr., at 0°C for 20 hr., and additionally at room temperature for 24 hr. After a small volume of water had been added and after it had stood for 1 hr., the reaction mixture was poured into a large volume of ice water. The precipitate was collected by centrifuge and washed with water; yield 10.5 g. The crude product was extracted with three 30-ml. portions of hot ethyl acetate in order to remove the higher tosylated product (1.05 g.; S, 5.28%). The insoluble residue was pressed between filter papers and dried in a desiccator; yield 7.8 g. The product was powdered and extracted with three 300-ml. portions of hot dry acetone to separate the unchanged II, which is almost insoluble in acetone. The resulting acetone extract was filtered and concentrated to a volume of about 65 ml. After the concentrate had been allowed to stand at room temperature for a while, the resulting solid was filtered; yield 1.9 g., S, 1.83%.

A sample (450 mg.) of the product was dissolved in a solvent of *n*-butanol - acetic acid - water (3 : 2 : 1) and was chromatographed through a silica gel column (17×310 mm.) using the same solvent system. After 30 ml. of the eluate had been collected, the desired product emerged in the next 13 ml. This fraction, after evaporation to dryness, weighed 370 mg. (13.3%). Additional material (770 mg.) was obtained by the concentration of the above acetone mother-liquor to 20 ml.; the total yield was 20%; m. p. 200—202°C (decomp.), $[\alpha]_D^{14}$ +67° (*c* 1.96, in dimethyl formamide).

Found: C, 58.29; H, 5.88; S, 2.41. Calcd. for $C_{57}H_{66}O_{21}N_4S$: C, 58.25; H, 5.66; S, 2.73%.

Tetra-*N*-carbobenzoxy-mono-*O*-nasylkanamycin (IV).—A sample (7.0 g.) of II was dissolved in dry pyridine (40 ml.) and cooled to about −18°C, and to it was added β-naphthalenesulfonyl chloride (1.95 g.). The reaction mixture was then submitted to a procedure similar to that described above for III, giving crude IV (8.4 g.). The crude product (720 mg.) was extracted with acetone (35 ml.) by refluxing it for a while, and, after it had cooled to room temperature, the acetone extract was filtered and evaporated to dryness (510 mg.). This was again extracted with ethyl methyl ketone (75 ml.) by refluxing it for a while; the extract was filtered and evaporated to dryness to give a residue (390 mg.) with a sulfur content of 3.03%. The product was further treated with hot ethyl acetate (20 ml.), and the insoluble residue was collected and dried in a desiccator; yield 230 mg. (32%); m. p. 186—187°C (decomp.).

Found: S, 2.69%. Calcd. for $C_{60}H_{66}O_{21}N_4S$: S, 2.53%.

Tetra-*N*-carbobenzoxy-deoxyiodo-kanamycin (V).—A solution of III (1.0 g.) and sodium iodide (0.38 g.) in anhydrous dioxane - acetone (1 : 1) was heated in a sealed tube in a boiling water bath for about 50 min. After the mixture had been heated for 5 min., sodium *p*-toluenesulfonate began to precipitate. After cooling, the reaction mixture was filtered and evaporated to dryness; the residue was washed with water and dried in a desiccator. A mixture of the product and anhydrous acetone was refluxed for a while and then allowed to stand overnight. The precipitate was collected by filtration to give the title compound; yield 900 mg. (94%); m. p. 220—223°C (decomp.).

Found: C, 53.32; H, 5.14; I, 11.30. Calcd. for $C_{50}H_{59}O_{18}N_4I$: C, 53.20; H, 5.26; I, 11.20%.

The infrared spectrum of the product showed the absence of the absorption at 1170 cm^{-1} (ν_s SO_2) which was found in the spectrum of III.

This compound was also prepared from IV and sodium iodide by a procedure similar to that described above. In this reaction, the precipitation of sodium β-naphthalenesulfonate began after the reaction mixture had been heated for 10 min.

Deoxykanamycin (IX).—A sample of V (200 mg.) in 80% aqueous dioxane (6.0 ml.) was refluxed, hydrogen being passed through in the presence of Raney nickel (W-4) (0.2 ml.) for 10 min. After cooling, the mixture was filtered, and the filtrate was repeatedly treated with fresh Raney nickel and hydrogen. The concentration of the filtrate, followed by the addition of water, afforded a crude product of tetra-*N*-carbobenzoxy-deoxykanamycin (VIII); yield 150 mg.

The product was dissolved in a mixed solvent dioxane - water - acetic acid (4 : 1 : 0.04) (10 ml.) and hydrogenated with palladium black (40 mg.) under hydrogen-pressure of 3.0 atm. at 35°C for 1 hr. A small quantity of water was added to the mixture, and again the hydrogenation was continued for about 2 hr. The repetition of this procedure, followed by filtration and evaporation, gave a crude product, which was washed with hot acetone. The resulting solid was dissolved in 95% ethanol, treated with a small quantity of active charcoal, and evaporated to give deoxykanamycin tetraacetate; yield 82 mg., $[\alpha]_D^{15}$ +99.6° (*c* 0.88, in water).

Found: C, 44.24; H, 7.13; N, 8.08. Calcd. for $C_{18}H_{36}O_{10}N_4 \cdot 4CH_3CO_2H$: C, 44.06; H, 7.39; N, 7.91%.

By the general procedure of paper chromatography, the product showed an R_f-value of 1.3 relative to an R_f-value of kanamycin of 1.0.

The acetate (70 mg.) was dissolved in a small quantity of water, placed on the top of a column of Amberlite IRA-400 resin (OH form) (1 ml.), and developed with water. Ninhydrin-positive fractions were collected, filtered, and evaporated to dryness. The residue was dissolved in a small quantity of hot 90% ethanol and filtered; concentration, followed by the addition of ethanol to turbidity, afforded the free base of deoxykanamycin; yield 25 mg.

Found: C, 46.36; H, 7.51; N, 11.81. Calcd. for $C_{18}H_{36}O_{10}N_4$: C, 46.15; H, 7.75; N, 11.96%.

A small quantity of IX was heated with 3 N hydrochloric acid for 2 hr. in a boiling water bath, and the hydrolyzate was submitted to paper chromatography by the general procedure. Detection by ninhydrin coloration showed the spot of 3-amino-3,6-dideoxy-D-glucose (R_f 1.67, relative to an R_f-value of 3-amino-3-deoxy-D-glucose of 1.0), in addition to the spots corresponding to deoxystreptamine and 6-amino-6-deoxy-D-glucose.

Chlorodeoxy-kanamycin (X). — A mixture of a crude sample (400 mg.) of IV and dry lithium chloride in 14 ml. of anhydrous dioxane-acetone (1 : 1) was heated in a sealed tube for 5 hr. in a boiling water bath. No precipitation of lithium β-naphthalenesulfonate occurred until the heating of the mixture had been continued for 2 hr. After cooling, the resulting mixture was filtered and worked up by a procedure similar to that described above for V; the yield of the crude product of VII was 360 mg. (Cl, 2.83%).

A crude sample (1.74 g.) of VII was dissolved in a mixture of dioxane (40 ml.), water (25 ml.) and acetic acid (0.4 ml.) and hydrogenated with palladium black (250 mg.) under a hydrogen-pressure of 3.0 atm. at 35°C for 15 hr. When water (20 ml.) was added, the reaction mixture became turbid; this indicated that the hydrogenation was not completed. Hydrogenation was therefore continued for a further 3 hr. Water (15 ml.) was added to the reaction mixture, and the hydrogenation was again continued for about 10 hr., after which the reaction mixture was filtered and the filtrate was concentrated to about 50 ml. Since the paper chromatography of the concentrate still showed three, strongly ninhydrin-positive spots (R_f 4.3, 8.7 and 14, relative to an R_f-value of X of 1.0) other than X, the concentrate was again treated with a fresh palladium catalyst (250 mg.) and hydrogen for 20 hr. and filtered. The filtrate was concentrated to about 10 ml., placed on a column (27×280 mm.) of Dowex 1 X2 (OH form), and developed with water at the rate of 0.5 ml./min.; the eluates were submitted to paper chromatography and bioautography. After 350 ml. of eluate had been collected, kanamycin emerged in the next 130 ml., and then chlorodeoxykanamycin emerged from 590 ml. to 710 ml. The former fractions (fraction A) were collected, neutralized with hydrochloric acid to pH 3.0, and concentrated, and acetone was added to give kanamycin tetrahydrochloride; yield 90 mg. By a similar procedure, the latter fractions (fraction B) gave a syrup, which was dissolved in a small quantity of methanol and treated with active charcoal, and acetone was added to afford chlorodeoxy-kanamycin tetrahydrochloride; yield 170 mg., $[\alpha]_D^{15}$ +95° (*c* 0.8, in water).

Found: C, 32.31; H, 6.19; N, 8.60; Cl, 26.20. Calcd. for $C_{18}H_{35}O_{10}N_4Cl \cdot 4HCl \cdot H_2O$: C, 32.42; H, 6.20; N, 8.40; Cl, 26.59%.

In another experiment, fraction B was neutralized

TABLE I. MINIMAL INHIBITORY CONCENTRATION OF CHLORODEOXY-KANAMYCIN TETRAACETATE, mcg./ml.

Test organism	Chlorodeoxy-kanamycin tetraacetate	Kanamycin tetraacetate
Staphylococcus aureus 209p	3.12	3.12
Staphylococcus aureus Terashima	1.56	3.12
Streptococcus faecalis ATCC 8043	50	50
Streptococcus haemolyticus Cook	50	50
Sarcina lutea	12.5	25
Diplococcus pneumoniae Type I	1.56	3.12
Escherichia coli var. *communis*	12.5	12.5
Salmonella typhi O-901-W	3.12	3.12
Salmonella typhi T-58	3.12	6.25
Shigella dysenteriae shigae	1.56	3.12
Proteus vulgaris OX 19	25	50
Pseudomonas aeruginosa	100	100
Bacillus subtilis PCI 219	3.12	3.12
Mycobacterium phlei No. 56	1.56	3.12
Mycobacterium 607	1.56	1.56
Mycobacterium tuberculosis H37Rv	3.12	3.12

with acetic acid to pH 4 and worked up by a procedure similar to that used for the above hydrochloride to give chlorodeoxy-kanamycin tetraacetate; $[\alpha]_{\rm D}^{15}$ +90° (*c* 0.9, in water).

Found: C, 39.42; H, 7.66; Cl, 4.54. Calcd. for $C_{18}H_{35}O_{10}N_4Cl \cdot 4CH_3CO_2H \cdot 2H_2O$: C, 40.08; H, 7.11; Cl, 4.55%.

A small quantity of X was heated with 3 N hydrochloric acid in a boiling water bath for 20 min., and the hydrolyzate was submitted to paper chromatography by the general procedure. Detection by ninhydrin coloration showed the spot ($Rf_{\rm D}$ 1.33) of XI, in addition to the spots of deoxystreptamine ($Rf_{\rm D}$ 1.00), 6-amino-6-deoxy-D-glucose ($Rf_{\rm D}$ 1.72), 4-*O*-(6-amino-6-deoxy-D-glucosyl)-deoxystreptamine ($Rf_{\rm D}$ 0.39) and unchanged chlorodeoxy-kanamycin ($Rf_{\rm D}$ 0.52).

6-*O*-(3-Amino-6-chloro-3, 6-dideoxy-*α*-D-glucopyranosyl)-deoxystreptamine Hydrochloride (XI).* — A sample (108 mg.) of chlorodeoxy-kanamycin tetrahydrochloride was dissolved in 3 N hydrochloric acid and heated for 20 min. in a boiling water bath. The reaction was quenched by cooling the mixture with tap water, and then the resulting solution was evaporated to dryness under reduced pressure and the residue dried in a desiccator to give a solid (109 mg.). This was dissolved in a small quantity of water, paper chromatographed on 3 sheets of Toyo filter paper No. 50 (40×40 cm.), and developed with the *t*-amylalcohol - pyridine - water - acetic acid (6 : 4 : 3 : 1) solvent system by the descending method for 40 hr. A zone corresponding to the compound of $Rf_{\rm D}$ 1.33 was clipped from each paper and extracted with water, and the aqueous extract was evaporated to dryness. The residue (28 mg.) dissolved in a small quantity of water was placed on a column (4×110 mm.) of Dowex 1 X2 resin (OH form) and developed with water. After a small amount of deoxystreptamine has been eluted, the desired compound emerged from 1.7 to 3.5 ml. of the eluate. The fraction, which was strongly alkaline and positive in the Beilstein test for halogen, was neutralized with hydrochloric acid to pH 3.0 and evaporated to dryness. The residue was dissolved in a small quantity of methanol, and acetone was added to precipitate the title compound; yield 11 mg.; $[\alpha]_{\rm D}^{20}$ +73° (*c* 0.7, in water).

Found: C, 32.33; H, 6.25. Calcd. for $C_{12}H_{24}O_6N_3Cl \cdot 3HCl$: C, 31.94; H, 6.03%.

The product showed no reducing property.

Tetra-*N*-carbobenzoxy-bromodeoxy-kanamycin (VI) and Hydrogenolysis.—A mixture of a sample (30 mg.) of IV and dried lithium bromide (10 mg.) in 0.5 ml. of anhydrous dioxane - acetone (1 : 1) was heated in a sealed tube for 2 hr. in a boiling water bath. When the mixture was heated for 30 min., lithium *β*-naphthalenesulfonate was precipitated. The resulting mixture was worked up by a procedure similar to that described above for V to give 26 mg. of VI.

Found: Br, 7.7. Calcd. for $C_{50}H_{59}O_{18}N_4Br$: Br, 7.4%.

A small quantity of the above sample was hydrogenated with palladium black by a procedure similar to that used for the hydrogenolysis of VII to afford deoxykanamycin, which was detected by both paper chromatography and bioautography.

Summary

1) Deoxykanamycin has been synthesized in a four-stage sequence beginning with kanamycin. Kanamycin has been converted to tetra-*N*-carbobenzoxykanamycin, and this transformed in turn to tetra-*N*-carbobenzoxy-mono-*O*-tosylkanamycin (or the corresponding nasyl derivative), to tetra-*N*-carbobenzoxy-deoxyiodo-kanamycin, and finally to deoxykanamycin.

2) The above nasyl derivative has been transformed via tetra-*N*-carbobenzoxy-chlorodeoxy-kanamycin to chlorodeoxy-kanamycin.

3) 6-*O*-(3-Amino-6-chloro-3, 6-dideoxy-*α*-D-glucosyl)-deoxystreptamine hydrochloride has been obtained by the controlled acid-hydrolysis of chlorodeoxy-kanamycin.

4) The hydrogenolysis of tetra-*N*-carbobenzoxy-bromodeoxy-kanamycin has given deoxykanamycin.

5) It has been found that deoxykanamycin and chlorodeoxy-kanamycin are strongly antibacterial and that their antibiotic spectra are essentially similar to that of kanamycin.

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* According to Rinehart and Hichens, 3-amino-3-deoxy-D-glucose is linked to the C-6 of deoxystreptamine; see K. L. Rinehart and M. Hichens, *J. Am. Chem. Soc.*, **85**, 1547 (1963).