BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 42 537—541 (1969)

Studies of Aminosugars. XXIII. The Total Synthesis of Kanamycin B[1]

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(Received July 16, 1968)

4-*O*-(3,4-Di-*O*-benzyl-2,6-dicarbobenzoxyamino-2,6-dideoxy-*α*-D-glucopyranosyl)-*N*,*N*′-dicarbobenzoxy-2-deoxystreptamine has been prepared from neamine and condensed with 3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-*α*-D-glucopyranosyl chloride by a modified Koenigs-Knorr reaction to give an *α*,*α*-diglycoside. The identity of the product with a substance of the same structure derived from natural kanamycin B was shown. Removal of the masking groups from the product gave the synthetic kanamycin B, which was identical with natural kanamycin B.

Paper chromatography[2] and ion-exchange chromatography[3] of the kanamycin complex revealed the presence of two minor congeners, kanamycin B and C, in addition to kanamycin A. Kanamycin B was isolated by counter-current distribution of the salicylidene derivatives and by chromatography.[4,5] Kanamycin B is composed of 2-deoxystreptamine, 3-amino-3-deoxy-D-glucose and 2,6-diamino-2,6-dideoxy-D-glucose.[4,6] The structure of kanamycin B was established as *O*-(2,6-diamino-2,6-dideoxy-*α*-D-glucopyranosyl)-(1→4)-*O*-[3-amino-3-deoxy-*α*-D-glucopyranosyl-(1→6)]-1,3-diamino-1,2,3-trideoxy-*myo*-inositol.[6,7] In other words, kanamycin B is an *α*-glycoside of neamine[8] (I) and 3-

1) Part XXXVIII of "Studies on Antibiotics and Related Substances" by Sumio Umezawa. A part of this work has been briefly communicated: S. Umezawa, S. Koto, K. Tatsuta, H. Hineno, Y. Nishimura and T. Tsumura, *J. Antibiotics*, **21**, 424 (1968).

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3) J. W. Rothrock, R. T. Goegelman and F. J. Wolf, *Antibiotics Annual*, **1958/59**, 796.

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8) R. L. Peck, C. E. Hoffhine, Jr., P. Gale and K. Folkers, *ibid.*, **71**, 2950 (1949); R. L. Peck, C. E. Hoffhine, Jr., F. H. Gale and K. Folkers, *ibid.*, **75**, 1018 (1953).

amino-3-deoxy-D-glucose. In this paper, we describe the synthesis of kanamycin B from neamine and 3-amino-3-deoxy-D-glucose. Since we[9] have synthesized neamine (I) from paromamine which was synthesized[10] from glucosamine and 2-deoxystreptamine, the combined achievements constitute the total synthesis of kanamycin B.

Neamine was converted into tetra-*N*-carbobenzoxy-neamine (II). Treatment of II with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid in *N*,*N*-dimethylformamide (DMF) at 110°C followed by chromatography on silica gel gave a mixture of monoisopropylidene derivatives (IIIa and IIIb) in a 41% yield. The mixture was benzylated with benzyl bromide in the presence of barium oxide and barium hydroxide in DMF and followed by chromatography on silica gel to afford 4-*O*-(3,4-di-*O*-benzyl-2,6-dicarbobenzoxyamino-2,6-dideoxy-*α*-D-glucopyranosyl)-*N*,*N*′-dicarbobenzoxy-5,6-*O*-isopropylidene-2-deoxystreptamine (IVa) mp 185°C, $[\alpha]_D^{18}$ +57° (*c* 0.67, DMF) and 4-*O*-(2,6-dicarbobenzoxy-amino-2,6-dideoxy-3,4-*O*-isopropylidene-*α*-D-glucopyranosyl)-5,6-di-*O*-benzyl-*N*,*N*′-dicarbobenzoxy-2-deoxystreptamine (IVb) mp 230—231°C, $[\alpha]_D^{18}$ +33° (*c* 0.67, DMF) in 12% and 37% yield respectively.

Deacetonation of IVa by treatment with aqueous acetic acid gave 4-*O*-(3,4-di-*O*-benzyl-2,6-dicarbobenzoxyamino-2,6-dideoxy-*α*-D-glucopyranosyl)-*N*,*N*′-dicarbobenzoxy-2-deoxystreptamine (V) in an 86% yield, mp 221—222°C, $[\alpha]_D^{18}$ +58° (*c* 0.67, DMF).

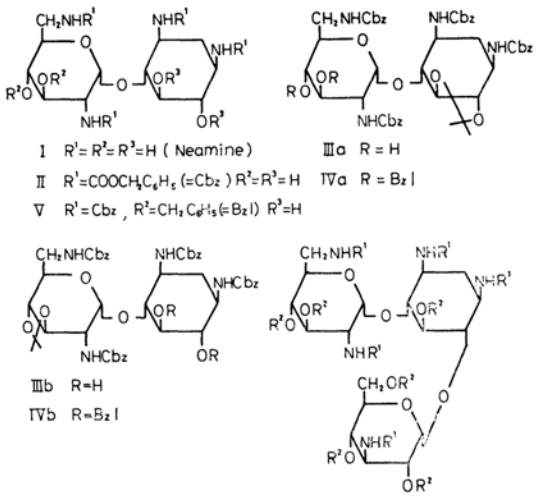
The compound V was condensed with 3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-*α*-D-glucopyranosyl chloride, which was already utilized for the synthesis of kanamycin C,[11] in the presence of mercuric cyanide and Drierite in benzene-dioxane. The condensation product was hydrogenated in a mixture of dioxane–water–concentrated hydrochloric acid over palladium black to remove the *O*-benzyl and *N*-carbobenzoxy groups, and de-*N*-acetylated with hot aqueous barium hydroxide to give a ninhydrin-positive product. Paper chromatography and bioautography of the product showed that the product mainly contained the substance whose R_f-value coincided with that of natural kanamycin B, however, attempts to isolate it were unsuccessful. Then the product was *N*-dinitrophenylated with 2,4-dinitrofluorobenzene in aqueous ethanol in the presence of sodium bicarbonate and then *O*-acetylated with acetic anhydride and sodium acetate. The product, which showed about four spots of R_f-values 0.40, 0.33, 0.13 and 0.08 on a thin layer chromatogram with a solvent system (c): toluene–methyl ethyl ketone (4 : 1), was chromatographed on a silica gel column with the same solvent system. The substance of R_f-value 0.08 was isolated and proved to be synthetic hexa-*O*-acetyl-penta-*N*-(2,4-dinitrophenyl)-kanamycin B (VI) mp 212—213°C (decomp.), $[\alpha]_D^{18}$ +220° (*c* 0.3, acetone), overall yield from V being 5%.

On the other hand, natural kanamycin B was dinitrophenylated and acetylated by the above-mentioned procedure to afford the hexa-*O*-acetyl-penta-*N*-(2,4-dinitrophenyl) derivative of kanamycin B, mp 217—218°C (decomp.), $[\alpha]_D^{18}$ +240° (*c* 0.4, acetone).

The identity of the synthetic VI to the above-mentioned derivative of natural kanamycin B was established by elemental analyses, specific rotations, by their failure to depress the mixed melting point and identical mobilities on thin layer chromatography and infrared spectra.

We have investigated the above-mentioned removal of *O*-benzyl and *N*-carbobenzoxy groups in some detail and found that treatment of the condensation product with sodium in liquid ammonia instead of catalytic hydrogenation with palladium black gave a better result and the overall yield of VI from V was raised to about 15%.

Finally, ammonolysis of the synthetic VI with methanolic ammonia followed by hydrolysis with excess of Dowex 1×2 (OH^-) resin afforded a free base, which was purified by chromatography on a column of the same resin. The free base melted at 178—182°C (decomp.), $[\alpha]_D^{18}$ +130° (*c* 0.5, water). The identity of the free base to the natural kanamycin B [mp 179—181°C (decomp.), $[\alpha]_D^{18}$ +128° (*c* 0.5, water)] was further established by their failure to depress the mixed melting point,



9) K. Tatsuta, E. Kitazawa and S. Umezawa, This Bulletin, **40**, 2371 (1967).

10) S. Umezawa and S. Koto, *ibid.*, **39**, 2014 (1966).

11) S. Umezawa, S. Koto, K. Tatsuta and T. Tsumura, *J. Antibiotics*, **21**, 162 (1968) (brief communication); See the foregoing paper.

identical mobilities on thin layer chromatography and infrared spectra. The antibiotic spectra and minimal inhibitory concentrations[1] of the synthetic kanamycin B against test bacteria were identical with those of the natural kanamycin B.

Experimental

General Procedures. Thin layer chromatography (TLC) was carried out on "Silica-Rider for TLC" (Dai-ichi Pure Chemicals Co.) using three solvent systems: (a) benzene-ethanol (7 : 1), (b) benzene-methyl ethyl ketone (MEK) (4 : 1), and (c) toluene-MEK (4 : 1), as mobile phases. Paper chromatography was performed in the previously described fashion.

Tetra-*N*-carbobenzoxy-neamine (II). To a solution of neamine (I) (15 g) and sodium carbonate decahydrate (39 g) in water (150 m*l*) was added acetone (450 m*l*) under agitation and the mixture was cooled to −5 — −10°C. Carbobenzoxychloride in toluene (30%; 104 g) was added for one hour. After having been vigorously stirred for about 4 hr at −5— −10°C, the reaction mixture was set aside in a refrigerator overnight. The resulting colorless solid was collected by filtration and tightly pressed between filter papers to remove oily matter and moisture. The waxy product was pulverized with 1 N hydrochloric acid (600 m*l*), filtered and dried *in vacuo* to give a colorless powder; yield 43 g. The product was dissolved in a hot mixture of dioxane (850 m*l*) and water (6 m*l*) and insoluble matters were filtered off. When the filtrate was allowed to stand at room temperature overnight, a colorless gelatinous mass precipitated; yield 34 g. The mother liquor deposited a second crop, 1.2 g. Total yield 89%, mp 259°C (decomp.), $[\alpha]_D^{18}$ +44° (*c* 0.67, DMF), IR spectrum (KBr): 1698 and 1538 cm^{-1} (NHCbz).

Found: C, 61.59; H, 5.97; N, 6.64%. Calcd for $C_{44}H_{50}N_4O_{14}$: C, 61.54; H, 5.86; N, 6.52%.

4-*O*-(3,4-Di-*O*-benzyl-2,6-dicarbobenzoxyamino-2,6-dideoxy-α-D-glucopyranosyl)-*N*,*N*′-dicarbobenzoxy-5,6-*O*-isopropylidene-2-deoxystreptamine (IVa) and 4-*O*-(2,6-Dicarbobenzoxyamino-2,6-dideoxy-3,4-*O*-isopropylidene-α-D-glucopyranosyl)-5,6-di-*O*-benzyl-*N*,*N*′-dicarbobenzoxy-2-deoxystreptamine(IVb). A mixture of II (5.2 g), *p*-toluenesulfonic acid monohydrate (0.052 g) and 2,2-dimethoxypropane (1.4 m*l*) in anhydrous DMF (30 m*l*) was heated at 110°C for 4 hr. The resulting reddish-brown solution was cooled and neutralized with about 10 m*l* of Amberlite IRA-400 (OH type) washed with methanol before use. The resin was filtered off and the solution was evaporated. The residue was chromatographed on a silica-gel column (500 g; 47×680 mm) using the solvent system (a) to separate two fractions of R_f-values 0.65 and 0.55 on TLC with the solvent system (a). The fraction of R_f 0.55 appeared between 930 m*l* and 2100 m*l*. The fraction was still a mixture of two kinds of mono-*O*-isopropylidene derivatives (IIIa and IIIb); yield 3.37 g (63%), $[\alpha]_D^{18}$ +42° (*c* 0.67, DMF).

Found: C, 63.03; H, 6.31; N, 6.34. Calcd for $C_{47}H_{54}N_4O_{14}$: C, 62.80; H, 6.05; N, 6.23%.

The mixture (5.28 g) of monoisopropylidene derivatives was dissolved in anhydrous DMF (70 m*l*) and to the solution were added powdery barium oxide (5.04 g) and finely crushed barium hydroxide octahydrate (6.35 g). After being cooled to −10°C, benzyl bromide (5.1 m*l*) was added dropwise into the mixture, and vigorously stirred at 0°C for 4 hr and then at room temperature for an additional 20 hr. The slightly colored mixture was diluted with chloroform (140 m*l*) and the insoluble matters were filtered off. The filtrate was evaporated *in vacuo* and the residue was extracted with chloroform. Concentration of the extract gave an oily substance, which was chromatographed on a column of silica-gel (500 g, 50×695 mm), using the solvent system (b). Each 15 g fraction was examined by TLC. The fractions containing only a substance of an R_f-value 0.50 appeared in tube Nos. 20—29. Evaporation afforded IVa, yield 0.74 g (12%), mp 185°C, $[\alpha]_D^{18}$ +57° (*c* 0.67, DMF), IR spectrum (KBr): 3350, 1700 and 1536 (NHCbz), 1137 and 1060 cm^{-1} (ketal).

Found: C, 68.07; H, 6.32; N, 5.30%. Calcd for $C_{61}H_{66}N_4O_{14}$: C, 67.89; H, 6.16; N, 5.19%.

Fractions (850 m*l*) after tube No. 43 contained the substance of R_f 0.25. Evaporation gave IVb, yield 2.33 g (37%), mp 230—231°C, $[\alpha]_D^{18}$ +33° (*c* 0.67, DMF), IR spectrum (KBr): 3350, 1700 and 1535 (NHCbz), 1157, 1133, 1060 and 1020 cm^{-1} (ketal).

Found: C, 67.43; H, 6.37; N, 5.32%. Calcd for $C_{61}H_{66}N_4O_{14}$: C, 67.89; H, 6.16; N, 5.19%.

4-*O*-(3,4-Di-*O*-benzyl-2,6-dicarbobenzoxyamino-2,6-dideoxy-α-D-glucopyranosyl)-*N*,*N*′-dicarbobenzoxy2-deoxystreptamine (V). A sample (0.74 g) of IVa was dissolved in warm aqueous acetic acid (80%, 25 m*l*), and the solution was kept standing at room temperature overnight. The resulting solid was collected, washed well with water and dried *in vacuo* to give an analytically pure solid of V; yield 0.64 g (90%), mp 221—222°C, $[\alpha]_D^{18}$ +58° (*c* 0.67, DMF), IR spectrum (KBr): 3340, 1699 and 1540 (NHCbz), 1037 cm^{-1} (*O*-benzyl).

Found: C, 66.74; H, 6.41; N, 5.58%. Calcd for $C_{58}H_{62}N_4O_{14}$: C, 67.04; H, 6.01; N, 5.39%.

Proof for the Structure of V. Each sample (10 mg) of V and IVb was dissolved in a mixture of acetic acid (0.6 m*l*) and acetic anhydride (0.1 m*l*) and to the solution was added a mixture of acetic acid and sulfuric acid (5 : 1; 0.07 m*l*) at 0°C. The solutions were stirred at room temperature overnight and then poured onto cracked ices (about 3 g) to separate an oily substance, which was extracted with benzene (6 m*l*). Each extract was washed with aqueous 5% sodium bicarbonate solution and water. After drying over sodium sulfate, each benzene-solution was evaporated and the residue was de-*O*-acetylated by treatment with saturated methanolic ammonia (10 m*l*) in a refrigerator overnight. The mixture was evaporated *in vacuo* and to the residue was added 1 N aqueous barium hydroxide (8 m*l*). After heating at 100°C for 4 hr, each hydrolyzate was neutralized with 3 N sulfuric acid to pH 2 and insoluble matters were removed by centrifuge. Each supernatant was treated with Dowex 1×2 (OH type) to pH 8—9 and the resin was filtered off. Evaporation and drying *in vacuo* afforded a mixture of free bases. Each mixture was examined by paper chromatography with the solvent system of (d) by ninhydrin coloration. 2-Deoxystreptamine was present in the hydrolyzate from V, while IVb did not produce 2-deoxystreptamine.

Synthetic Hexa-*O*-acetyl-penta-*N*(2,4-dinitrophenyl)-kanamycin B (VI). A mixture of V (0.8 g), well pulverized mercuric cyanide (0.31 g) and freshly activated Drierite (3.3 g) in a mixed solvent of benzene (9 ml) and dioxane (3 ml) was heated to reflux with stirring under anhydrous conditions and then cooled to room temperature. To the mixture was added well dried 3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-*α*-D-glucopyranosyl chloride (0.60 g; 1.7 eq.) at once and the mixture was vigorously stirred at reflux temperature for 20 hr. During the course of the reaction the mixture gradually became somewhat thick and brown. After removal of insoluble matters by filtration, the solution was evaporated. The dark brown residue was extracted with ethyl acetate (70 ml) and the extract was washed with five 20 ml portions of 20% aqueous potassium bromide and then three 20 ml portions of water. After being dried over anhydrous sodium sulfate, the ethyl acetate solution was evaporated to give a sirup, 0.75 g. The condensation product was hydrogenated over palladium black (0.3 g) in a mixture (24 ml) of dioxane, water, methanol and concentrated hydrochloric acid (20 : 1 : 2 : 1) at about 40°C under 3.5 atm of hydrogen pressure with occasional additions of small portions of water, whose total volume was 5 ml. After removal of the catalyst, the hydrogenated solution was evaporated to dryness *in vacuo*. The residue was again hydrogenated over fresh palladium black (0.15 g) in the same manner as mentioned above until the total volume of water reached 10 ml. The same hydrogenation procedure was further repeated twice. The total duration of hydrogenation took 150 hr. After removal of the catalyst, the solution was evaporated to give a ninhydrin-positive sirup, 0.55 g, which was hydrolyzed by heating with 1 N barium hydroxide (10 ml) on a boiling water water bath for 2.5 hr. After cooling to room temperature, the mixture was neutralized with 3 N sulfuric acid to pH 2 and the insoluble matter was removed by centrifuge. The solution was neutralized with Dowex 1×2 (OH type) to pH 7—8, and the resin was filtered. Evaporation of the filtrate afforded a sirup of a mixture of free bases, 0.51 g. All attempts to obtain kanamycin B from the product in a pure form have so far been unsuccessful.

The mixture (0.5 g) of free bases was dinitrophenylated with 2,4-dinitrofluorobenzene (1.2 g) in the presence of sodium bicarbonate (0.55 g) in a mixture (60 ml) of water and ethanol (1 : 1) under stirring at room temperature overnight. The resulting mixture was neutralized with 0.1 N hydrochloric acid and dried up *in vacuo* to give a yellowish-orange residue, from which excess 2,4-dinitrofluorobenzene was removed by co-distillation with *n*-butanol. The residue was fully acetylated by heating with acetic anhydride (20 ml) and anhydrous sodium acetate (1.1 g), at 110°C, stirring vigorously for 5 hr. The mixture was dried up *in vacuo* and extracted with acetone. The extract was again evaporated to afford a yellow sirup, 1.5 g, which showed about four spots of R_f-values 0.40, 0.33, 0.13 and 0.08, on TLC, developing with the solvent system (c). The sirup was chromatographed on a column of silica-gel (10 g, 22×55 mm) with the solvent system (c), each fraction of the effluent being 10 g. The substance of R_f-value 0.08 appeared in the fractions of Nos. 12—25, which was evaporated to give yellow needles of VI, 0.06 g (5% overall-yield from V). Recrystallization from the solvent system (c); mp 212—213°C (decomp.), $[\alpha]_D^{18}$ +220° (*c* 0.3, acetone), IR spectrum (KBr): 3350, 3120, 1625, 1600, 1530, 1340, 835 and 745 (NHDNP), 1760 and 1220 cm^{-1} (OAc).

Found: C, 46.16; H, 4.23; N, 12.96%. Calcd for $C_{60}H_{59}N_{15}O_{36}$: C, 46.01; H, 3.80; N, 13.42%.

On TLC the R_f-value of the synthetic VI agreed with that of the hexa-*O*-acetyl-penta-*N*-(2,4-dinitrophenyl) derivative of natural kanamycin B (see below). Infrared spectra of VI and the above derivative were superimposable. The melting point of VI was not depressed by admixture with the above derivative.

We have investigated the above-mentioned removal of *O*-benzyl and *N*-carbobenzoxy groups in some detail. Instead of hydrogenation with palladium black, the condensation product (a sirup 0.7 g) was dissolved in liquid ammonia (60 ml) and sodium (0.3 g) was added under cooling in a solid carbon dioxide - methanol bath (−80——85°C). The solution became blue and the reaction was continued for 2 hr with occasional stirring. To the solution was added ammonium chloride until the blue color disappeared. Evaporation of the solution at room temperature left a colorless residue, which was treated with water to precipitate insoluble matters. The mixture was filtered and the solution was evaporated. The resulting colorless, ninhydrin-positive residue was dissolved in 1 N aqueous barium hydroxide solution (50 ml) and refluxed at 100°C for 2 hr. After cooling to room temperature, the solution was acidified with 3 N sulfuric acid to pH 2 and the barium salt was removed by centrifuge. The solution was filtered and neutralized with Dowex 1×2 (OH type) to pH about 7. After removal of the resin, the solution was evaporated *in vacuo* to give a colorless sirup. Dinitrophenylation and acetylation followed by chromatography with silica gel were carried out by similar procedure as described above. The yield of VI was 0.18 g (15% overall yield from V).

Synthetic Kanamaycin B (VII). A sample (77 mg) of VI was dissolved in methanol (50 ml), saturated with dry ammonia gas at 0°C and the red solution was set aside at room temperature overnight. After evaporation under reduced pressure, the residual yellow sirup was taken up with acetone (5 ml) and treated with excess Dowex 1×2 (*ca.* 2 ml of wet OH type) at room temperature for 12 hr with stirring. After removal of resin, the solution was evaporated *in vacuo* to give a glassy residue (24 mg) of VII, which was purified by chromatography with a small column of Dowex 1×2 (6× 50 mm). Treatment of the product with ethanol containing a small amount of water gave an analytically pure VII; 17 mg (72%), mp 178—182°C (decomp.), $[\alpha]_D^{18}$ +130° (*c* 0.5, water).

Found: C, 44.90; H, 7.34; N, 14.51. Calcd for $C_{18}H_{37}N_5O_{10}$: C, 44.71; H, 7.71; N, 14.49%.

On a descending paper chromatogram the R_f-value of the synthetic VII was in agreement with that of natural kanamycin B, after developing for 72 hr at room temperature. The antibiotics spectra and minimal inhibitory concentrations[1] of synthetic VII against test organisms coincided with those of natural kanamycin B.

Hexa-*O*-acetyl-penta-*N*-(2,4-dinitrophenyl) Derivative of Natural Kanamycin B. Free base of natural kanamycin B (40 mg) was dinitrophenylated with 2,4-dinitrofluorobenzene (59 mg) in the presence

of sodium bicarbonate (26 mg) in a mixture of water (0.5 m*l*) and ethanol (0.5 m*l*) with vigorous agitation at room temperature overnight. The yellow precipitate was collected and washed thoroughly with water and ethanol; yield 77 mg.

The product (75 mg) was then fully *O*-acetylated by heating with acetic anhydride (7.5 m*l*) in the presence of anhydrous sodium acetate (750 mg) at 110°C for 4 hr under stirring. The mixture was dried up to give a residue, which was extracted with an excess of acetone. The extract was evaporated *in vacuo* to give a yellow product; 99.9 mg. The product was purified on a column of silica-gel (10 g; 22×55 mm) with toluene-MEK (2 : 1), the effluent being cut into 1 m*l* each. After the initial effluent of 24 m*l*, hexa-*O*-acetyl-penta-*N*-(2,4-dinitrophenyl) derivative of natural kanamycin B was eluted in tube Nos. 5—24; 77 mg (49.2%), mp 217—218°C (decomp.), $[\alpha]_D^{18}$ +240° (*c* 0.4, acetone), IR spectrum (KBr): 3350, 3120, 1625, 1600, 1530, 1340, 835 and 745 (NHDNP), 1760 and 1225 cm^{-1} (OAc).

Found: C, 46.31; H, 4.21; N, 13.38%. Calcd for $C_{60}H_{59}N_{51}O_{36}$: C, 46.01; H, 3.80; N, 13.42%.

The authors are indebted to the Kawasaki-Factory of Meiji-Seika Co. Ltd., for supplying kanamycin B. Thanks are also due to Professor Hamao Umezawa of Institute of Microbial Chemistry for helpful advice and to Mr. Saburo Nakada for microanalysis.