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Studies of Aminosugars. XXII. The Total Synthesis of Kanamycin A[1]

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6-*O*-(3-Amino-3-deoxy-*α*-D-glucopyranosyl)-2-deoxystreptamine, which was previously synthesized, was masked with carbobenzoxy, isopropylidene and benzyl groups to give 6-*O*-(2-*O*-benzyl-3-carbobenzoxyamino-3-deoxy-4,6-*O*-isopropylidene-*α*-D-glucopyranosyl)-*N*,*N*′-dicarbobenzoxy-2-deoxystreptamine, with which 2,3,4-tri-*O*-benzyl-6-(*N*-benzylacetamido)-6-deoxy-*α*-D-glucopyranosyl chloride was condensed by a modified Koenigs-Knorr reaction, to give an *α*,*α*-diglycoside compound. The identity of the product with a substance of the same structure derived from natural kanamycin A was shown. Removal of the masking groups gave the synthetic kanamycin A, which was identical to the natural specimen.

Kanamycins[2] belong to a group of water-soluble, basic antibiotics that are entirely carbohydrate in nature and show broad-spectrum antibacterial activity. They were discovered by H. Umezawa *et al.* in cultures of *Streptomyces kanamyceticus* n. sp. Okami *et* Umezawa. Kanamycin A is the major component of kanamycin complex produced by the above-mentioned strain. Cleavage products of kanamycin A are 3-amino-3-deoxy-D-glucose (kanosamine), 6-amino-6-deoxy-D-glucose and 2-deoxystreptamine. The former two kinds of aminosugars had not previously been encountered in natural products. (3-Amino-3-deoxy-*α*-D-glucopyranosyl)-2-deoxy-streptamine[3] and (6-amino-6-deoxy-*α*-D-glucopyranosyl)-2-deoxy-streptamine[4] were also isolated from the partial hydrolysis product. The structure of kanamycin A[5-7] (VIII) was established as *O*-(6-amino-6-deoxy-*α*-D-glucopyranosyl)-(1→4)-*O*-[3-amino-3-deoxy-*α*-D-glucopyranosyl-(1→6)]-1,3-diamino-1,2,3-trideoxy-*myo*-inositol. Absolute configuration of kanamycins

1) Part XXXVII of "Studies on Antibiotics and Related Substances" by Sumio Umezawa. This paper was read before the 160th Research Meeting of Japan Antibiotic Research Association, March 22, 1968. A part of this work has been briefly communicated: S. Umezawa, K. Tatsuta and S. Koto, *J. Antibiotics*, **21**, 367 (1968).

2) H. Umezawa, M. Ueda, K. Maeda, K. Yagishita, S. Kondo, Y. Okami, R. Utahara, Y. Osato, K. Nitta and T. Takeuchi, *J. Antibiotics*, **A10**, 181 (1957).

3) K. Maeda, M. Murase, H. Mawatari and H. Umezawa, *ibid.*, **A11**, 163 (1958).

4) S. Umezawa and T. Tsuchiya, *ibid.*, **A15**, 51 (1962).

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

6) M. J. Cron, O. B. Fardig, D. L. Johnson, D. F. Whitehead, I. R. Hooper and R. U. Lemieux, *J. Am. Chem. Soc.*, **80**, 4115 (1958).

7) H. Ogawa, T. Ito, S. Inoue and S. Kondo, *J. Antibiotics*, **A11**, 169 (1958); H. Ogawa, T. Ito, S. Kondo and S. Inoue, *Bull. Agr. Chem. Soc. Japan*, **23**, 289 (1959).

were recently revealed.[8-10]

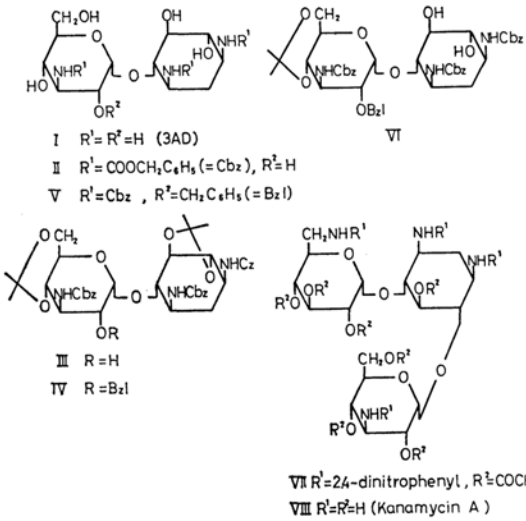
After having completed structural studies on kanamycin A, we turned to contemplation of its synthesis. There appeared to be two possible routes to kanamycin A, one of which was a route *via* a masked derivative of 6-*O*-(3-amino-3-deoxy-*α*-D-glucopyranosyl)-2-deoxystreptamine (I, 3AD) and the other was a route *via* a masked derivative of 4-*O*-(6-amino-6-deoxy-*α*-D-glucopyranosyl)-2-deoxystreptamine (6AD). We chose the former route, because 3AD is more readily obtainable than 6AD from the partially hydrolyzed product of kanamycin A in order to identify with the synthetic specimen. In the present paper, the total synthesis of kanamycin A and related compounds are described.[11]

The initial phase of the synthesis was thus concerned with the preparation of 3AD, which has very recently been reported.[12] The 3AD now had to be suitably masked. Treatment of 3AD with carbobenzoxychloride in aqueous sodium carbonate gave tri-*N*-carbobenzoxy derivative (II) in a 84% yield. Acetonation of II with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid in dimethylformamide (DMF) at 110°C afforded the diisopropylidene derivative (III), mp 234°C, $[\alpha]_D^{18}$ +53° (*c* 0.67, DMF), in a quantitative yield. Benzylation of III with benzyl bromide in the presence of barium oxide and barium hydroxide in DMF afforded the benzyl derivative (IV), mp 258°C, $[\alpha]_D^{18}$ +43° (*c* 0.67, DMF), which was deacetonated by treatment with 80% acetic acid to give 6-*O*-(2-*O*-benzyl-3-carbobenzoxyamino-3-deoxy-*α*-D-glucopyranosyl)-*N*,*N*′-dicarbobenzoxy-2-deoxystreptamine (V), mp 288°C, (decomp.), $[\alpha]_D^{18}$ +40° (*c* 0.67, DMF), quantitatively. Treatment of V with 2,2-dimethoxypropane at 5°C in the presence of *p*-toluenesulfonic acid in DMF successfully gave monoisopropylidene derivative (VI), mp 240°C, $[\alpha]_D^{18}$ +40° (*c* 0.67, DMF), in a 64% yield. It is worth mentioning that the hydroxyl groups at C-4 and C-6 in the 3-amino-3-deoxy-D-glucose moiety were acetonated in preference to the hydroxyl groups in the deoxystreptamine. This reaction is similar to that carried out in the preparation of the corresponding derivative of paromamine in the case of the total synthesis of kanamycin C in the foregoing report.

We could now proceed along the main synthetic pathway. In analogy to the foregoing studies on kanamycin C, the intermediate VI was condensed with 6-(*N*-benzylacetamido)-2,3,4-tri-*O*-benzyl-6-deoxy-*α*-D-glucopyranosyl chloride[13] in the presence of mercuric cyanide and Drierite in dioxane-benzene. The product was treated with aqueous acetic acid to remove the isopropylidene group, hydrogenated over palladium black to remove carbobenzoxy and *O*-benzyl groups, de-*N*-acetylated with barium hydroxide, and again hydrogenated to remove the *N*-benzyl group. The ninhydrin-positive product was dinitrophenylated with 2,4-dinitrofluorobenzene and then *O*-acetylated with acetic anhydride and anhydrous sodium acetate. The product, which showed about six spots with R_f-values of 0.54, 0.34, 0.30, 0.27 (main), 0.25 and 0.15 on a thin-layer chromatogram (TLC) with a solvent system (e): toluene-MEK (3 : 1), was chromatographed on a silica-gel column with the same solvent. The main product (VII) of the R_f-value 0.27 was isolated as yellow needles in an overall-yield 10.1% from VI.

On the other hand, kanamycin A was dinitrophenylated and acetylated to give hepta-*O*-acetyl-tetra-*N*-(2,4-dinitrophenyl)-kanamycin A.

The synthetic yellow needles (VII), mp 210—213°C (decomp.), $[\alpha]_D^{18}$ +50° (*c* 1.0, acetone), were proved to be identical to the above-mentioned derivative of natural kanamycin A by elemental analyses, specific rotations, by their failure to depress the mixed melting point, identical mobilities on TLC and infrared spectra.



8) M. Hichens and K. L. Rinehart, Jr., *J. Am. Chem. Soc.*, **85**, 1547 (1963).

9) S. Tatsuoka, S. Horii, K. L. Rinehart, Jr., and T. Nakabayashi, *J. Antibiotics*, **A17**, 88 (1964).

10) S. Umezawa, K. Tatsuta and T. Tsuchiya, This Bulletin, **39**, 1244 (1966).

11) For brief report of an alternative, independent synthesis of the kanamycin A, *cf.* M. Nakajima, A. Hasegawa, N. Kurihara, H. Shibata, T. Ueno and D. Nishimura, *Tetrahedron Letters*, **1968**, 623.

12) S. Koto, K. Tatsuta, E. Kitazawa and S. Umezawa, *J. Antibiotics*, **21**, 365 (1968); This Bulletin, **41**, 2769 (1968).

13) This compound was reported by S. Umezawa *et al.* at the 20th Annual Meeting of the Chemical Society of Japan, Tokyo, Mar. 31, 1967. (See abstracts of the papers of the Meeting, Vol. III, p. 596). S. Koto, T. Tsumura, Y. Kato and S. Umezawa, This Bulletin, **41**, 2765 (1968).

Before dinitrophenylation of the ninhydrin-positive product, we made efforts to directly separate the synthetic kanamycin A from the crude product by chromatographic technique, but it was unsuccessful. We found that VII could be separated when the crude product was subjected to dinitrophenylation and acetylation.

Hydrolysis of VII with methanolic ammonia followed by treatment with an excess of Dowex 1×2 (OH^-) resin afforded a free base, which was purified by chromatography on a column of Dowex 1×2 (OH^-) resin and recrystallized from aqueous methanol - ethanol to afford the synthetic kanamycin A of $[\alpha]_D^{18}$ $+149°$ (*c* 0.87, water). The natural kanamycin A showed $[\alpha]_D^{18}$ $+151°$ (*c* 1.0, water). The identity of the synthetic kanamycin A with the natural specimen was further established by elemental analyses, identical mobilities on TLC, superimposable infrared spectra and by their failure to depress the mixed melting point. The antibiotic spectra and minimal inhibitory concentrations[1] of the synthetic kanamycin A against test organisms were in agreement with those of the natural kanamycin A.

Experimental

General Procedures. Thin layer chromatography (TLC) and paper chromatography were carried out similarly as described in the foregoing report, using solvent systems: (a) benzene-ethanol (4 : 1), (b) benzene-methyl ethyl ketone (MEK) (4 : 1), (c) benzene-methanol (4 : 1), (d) benzene-ethyl acetate (1 : 2), (e) toluene-MEK (3: 1), and (f) *n*-butanol - pyridine - water - acetic acid (6 : 4 : 3 : 1).

6-*O*-(3-Carbobenzoxyamino-3-deoxy-α-D-glucopyranosyl)-*N*,*N*′-dicarbobenzoxy-2-deoxystreptamine (II). To a solution of 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine (I) (6.0 g) and sodium carbonate (18 g) in a mixture of water (60 m*l*) and acetone (180 m*l*) was added carbobenzoxychloride in toluene (30%, 32 g) under agitation at $-10°C$ during an hour. Having been stirred for about 4 hr, the reaction mixture was set aside in a refrigerator overnight. The resulting solid was shaken with a mixture (600 m*l*) of water and ether (15: 1 v/v), collected and pressed between filter papers. The waxy product was triturated with 1 N hydrochloric acid (300 m*l*), filtered, washed with water and dried. Recrystallization from dioxane (900 m*l*) afforded colorless solid; 9.5 g. The mother liquor gave a second crop; 1.8 g. Total yield 84%; mp 280—282°C (decomp.), $[\alpha]_D^{18}$ $+54°$ (*c* 0.67, DMF), IR spectrum: 3330, 1693 and 1547 cm^{-1} (NHCbz).

Found: C, 59.86; H, 6.25; N, 5.66%. Calcd for $C_{36}H_{43}N_3O_{13}$: C, 59.58; H, 5.97; N, 5.79%.

6-*O*-(3-Carbobenzoxyamino-3-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranosyl)-*N*,*N*′-dicarbobenzoxy-4,5-*O*-isopropylidene-2-deoxystreptamine (III). To a solution of II (4.6 g) in anhydrous DMF (30 m*l*) was added *p*-toluenesulfonic acid monohydrate (0.06 g) and 2,2-dimethoxypropane (7.0 m*l*), and the solution was heated at 110°C for 4 hr with occasional stirring. The mixture was neutralized with Amberlite IRA-400 (OH type; 10 m*l*) washed with methanol before use, filtered and the solution was evaporated *in vacuo* to give a residue, which was solidified by treatment with water; yield 4.9 g (94%). An analytically pure sample was obtained by chromatography on a silica-gel column, using the solvent system (a); mp 233—234°C, $[\alpha]_D^{18}$ $+53°$ (*c* 0.67, DMF), IR spectrum: 3350, 1700 and 1537 (NHCbz), 1175 and 1063 cm^{-1} (ketal).

Found: C, 62.67; H, 6.59; N, 5.35%. Calcd for $C_{42}H_{51}N_3O_{13}$: C, 62.60; H, 6.38; N, 5.21%.

6-*O*-(2-*O*-Benzyl-3-carbobenzoxyamino-3-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranosyl)-*N*,*N*′-dicarbobenzoxy-4,5-*O*-isopropylidene-2-deoxystreptamine (IV). To a solution of III (4.9 g) in anhydrous DMF (80 m*l*) was added pulverized barium oxide (4.9 g) and barium hydroxide octahydrate (5.9 g) with stirring and the mixture was cooled to $-15°C$. Benzyl bromide (3.5 m*l*) was added dropwise to the mixture under agitation, and the reaction temperature was allowed to raise gradually to 0°C during 4 hr. The mixture was further stirred for 20 hr at room temperature and filtered through a thick layer of Celite. The filtrate and washings were combined and evaporated *in vacuo* to give a sirup, which was taken up into chloroform (200 m*l*) to precipitate a gelatinous mass. This product was transferred to the top of a thick layer of Hyflosupercel (60×60 mm) and washed with chloroform (300 m*l*) and then with hot chloroform (300 m*l*) to afford a fraction containing IV, which was evaporated *in vacuo* to give an analytically pure solid of IV; 4.1 g, mp 258°C; $[\alpha]_D^{18}$ $+43°$ (*c* 0.67, DMF), IR spectrum: 3340, 1700 and 1538 (NHCbz), 1177 and 1066 cm^{-1} (ketal).

Found: C, 65.88; H, 6.66; N, 4.51%. Calcd for $C_{49}H_{57}N_3O_{13}$: C, 65.68; H, 6.41; N, 4.69%.

6-*O*-(2-*O*-Benzyl-3-carbobenzoxyamino-3-deoxy-α-D-glucopyranosyl)-*N*,*N*′-dicarbobenzoxy-2-deoxystreptamine (V). A suspension of IV (4.1 g) in 80% aqueous acetic acid (120 m*l*) was heated at 80°C for a while to give a clear solution, which was kept standing at room temperature overnight to separate a colorless precipitate. This was collected, washed with water and dried to afford a colorless powder of V, mp 286—288°C (decomp.), $[\alpha]_D^{18}$ $+40°$ (*c* 0.67, DMF), IR spectrum: 1695 and 1547 (NHCbz), 1052 cm^{-1} (*O*-benzyl).

Found: C, 63.13; H, 6.32; N, 5.02%. Calcd for $C_{43}H_{49}N_3O_{13}$: C, 63.30; H, 6.05; N, 5.15%.

6-*O*-(2-*O*-Benzyl-3-carbobenzoxyamino-3-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranosyl)-*N*,*N*′-dicarbobenzoxy-2-deoxystreptamine (VI). To a solution of V (2.8 g) in DMF (17 m*l*) was added *p*-toluenesulfonic acid monohydrate (0.03 g) and 2,2-dimethoxypropane (1.2 m*l*), and the mixture was allowed to stand at about 5°C for 16 hr. The mixture was neutralized with Amberlite IRA-400 (OH type; 8 m*l*) washed with methanol before use, and evaporated *in vacuo* to give a sirup, which was solidified by trituration with water. The crude product (2.9 g) was extracted with ethyl acetate (340 m*l*) and the extract was evaporated under reduced pressure. The residue was crystallized from ethanol; yield 1.9 g (64%). Examination of the product by TLC using the solvent system (d) indicated that the product was quite pure, mp 239—240°C, $[\alpha]_D^{18}$ $+40°$ (*c* 0.67, DMF), IR spectrum: 3340, 1698 and 1540 (NHCbz), 1172 and 1063 cm^{-1} (ketal).

Found: C, 64.43; H, 6.09; N, 4.76%. Calcd for

$C_{46}H_{53}N_3O_{13}$: C, 64.55; H, 6.24; N, 4.91%.

Proof for the Structure of VI. The monoisopropylidene derivative VI (26 mg) was benzylated with benzyl bromide (0.036 ml) in the presence of barium oxide (47 mg) and barium hydroxide octahydrate (58 mg), similarly as described for the preparation of IV. TLC using the solvent system (b) showed that VI completely disappeared and a new spot appeared. An oily product (20 mg) was dissolved in a mixture of acetic acid (6.0 ml), methyl cellosolve (3.0 ml), water (1.6 ml) and concentrated hydrochloric acid (1.2 ml) and heated at 100°C for 10 hr. The paper chromatography of the hydrolyzate using the solvent mixture (f) and ninhydrin-coloration showed that hydrolysis of the fully benzylated VI gave neither 3-amino-3-deoxy-D-glucose nor 2-deoxystreptamine, while analogous hydrolysis of II (a control test) gave both of them.

Synthetic Hepta-*O*-acetyl-tetra-*N*-(2,4-dinitrophenyl)-kanamycin A (VII). A mixture of VI (1.65 g), finely pulverized mercuric cyanide (0.7 g) and freshly ignited Drierite (5.4 g) in anhydrous dioxane (10 ml) was heated to reflux for 15 min under stirring and then cooled to room temperature. To the mixture was added well dried 2,3,4-tri-*O*-benzyl-6-(*N*-benzylacetamido)-6-deoxy-*α*-D-glucopyranosyl chloride (2.2 g; 1.9 eq.) in anhydrous benzene (30 ml) and the mixture was stirred vigorously under reflux for 10 hr to give a brown viscous mixture. The mixture was passed through a bed of Celite and washed with dioxane (100 ml) and ethyl acetate (100 ml). The filtrate and washings were combined and evaporated. The brown residue (6.4 g) was extracted with ethyl acetate (120 ml) and the extract was washed with three 100 ml portions of 20% aqueous potassium bromide and 100 ml of water. After drying over anhydrous sodium sulfate, the solution was evaporated to give an oily product (5.8 g). To the product was added 80% aqueous acetic acid (40 ml) and the mixture was kept standing for 30 min with occasional stirring. Evaporation under reduced pressure afforded a colorless sirup (5.0 g). This deacetonated product was hydrogenated over palladium black (0.5 g) in a solvent mixture of dioxane (30 ml), water (2 ml) and concentrated hydrochloric acid (3 ml) under 3.5 atm of hydrogen pressure at about 40°C with occasional addition of small portions of water whose total volume was 30 ml. After 24 hr, the hydrogenation was completed and the catalyst was filtered off. The solution was evaporated under reduced pressure below 30°C and made free from hydrogen chloride by co-distillation with *n*-butanol to afford a colorless sirup (1.4 g). The product was heated with 1 N barium hydroxide (60 ml) at 100°C for 2.4 hr to remove the *N*-acetyl group. The hydrolyzate was neutralized with 3 N sulfuric acid to pH 2. Inorganic matter was removed by centrifuging and the supernatant was treated with Dowex 1×2 (OH type) to pH about 9.5. After removal of the resin, the solution was evaporated to give a colorless sirup (0.8 g). Finally, the *N*-benzyl blocking was removed by catalytic hydrogenation. The sirup (0.8 g) in water (10 ml) and concentrated hydrochloric acid (0.5 ml) was shaken over palladium black (0.3 g) under 3.5 atm of hydrogen pressure for 48 hr at about 40°C. After removal of the catalyst, the solution was evaporated under reduced pressure at 30°C and followed by co-distillation with *n*-butanol to afford a glassy product (0.9 g). The product was ninhydrin-positive and the major R_f value indicated that the product consisted of the hydrochloride of synthetic kanamycin A. However, attempts to isolate it were unsuccessful. Then the product was dinitrophenylated with 2,4-dinitrofluorobenzene (1.7 g) in 50% aqueous ethanol (60 ml) in the presence of sodium bicarbonate (0.63 g) at room temperature overnight under stirring, whereupon a gummy product separated. Addition of water (80 ml) to the mixture and further agitation made the gummy product powdery. The yellow powder (1.73 g) was acetylated with acetic anhydride (30 ml) in the presence of freshly fused sodium acetate (1.5 g) at 100°C for 10 hr with stirring. The resulting mixture was evaporated *in vacuo* and the residue was extracted with excess acetone. The extract was evaporated and made free from acetic anhydride by co-distillation with toluene to give a yellow glass (2.2 g), which showed a major spot of R_f 0.27 together with several minor spots on TLC using the solvent system (e). The product was chromatographed on a silica-gel column (200 g; 4.7×26 cm) with the same solvent system, the effluent being cut into 10 ml each. The main product having R_f value 0.27 appeared in fractions of Nos. 65—70. Evaporation gave yellow needles, 0.28 g (overall yield from VI 10%), recrystallization from the same solvent system gave VII; mp 210—213°C (decomp.), $[\alpha]_D^{18}$ +50° (*c* 1.0, acetone), IR spectrum: 3340, 1625, 1600, 1550, 1525, 1340, 835 and 745 (NHDNP), 1765, 1370 and 1220 cm^{-1} (OAc).

Found: C, 46.71; H, 4.22; N, 11.58%. Calcd for $C_{56}H_{58}N_{12}O_{34}$: C, 46.61; H, 4.05; N, 11.65%.

The melting point of VII was not depressed by admixture with the hepta-*O*-acetyl-tetra-*N*-(2,4-dinitrophenyl) derivative of natural kanamycin A described below. On TLC with the solvent system (e), the synthetic VII and the above-mentioned derivative of kanamycin A showed identical mobilities and their IR spectra were superimposable.

Synthetic Kanamycin A (VIII). A sample (50 mg) of VII was dissolved in methanol (8 ml) saturated with ammonia at 0°C and the solution was allowed to stand at room temperature for 4 hr. Concentration of the solution *in vacuo* gave a sirup, which was treated with Dowex 1×2 (OH type; *ca.* 2 ml of wet resin) in acetone (9 ml) at room temperature with stirring overnight. After removal of the resin, the solution was evaporated and followed by purification by passing through a small column of Dowex 1×2 (OH type; *ca.* 1 ml) to give a colorless glass, which was treated with aqueous methanol containing ethanol to afford the crystalline free base of synthetic kanamycin A (VIII); yield 47%, $[\alpha]_D^{18}$ +149° (*c* 0.87, water).

Found: C, 44.47; H, 7.69; N, 11.27%. Calcd for $C_{18}H_{36}N_4O_{11}$: C, 44.62; H, 7.49; N, 11.56%.

On descending paper chromatography by ninhydrin-coloration using the solvent system (f), the R_f value of the synthetic product VIII agreed with that of the natural kanamycin A. Infrared spectra of VIII and the natural specimen were superimposable. The antibiotic spectra and minimal inhibitory concentrations[14] of the synthetic product VIII against test organisms coincided with that of natural kanamycin A.

Natural Hepta-*O*-acetyl-tetra-*N*-(2,4-dinitrophenyl)-kanamycin A. To a solution of natural kanamycin

14) S. Umezawa, K. Tatsuta and S. Kto, *J. Antibiotics*, **21**, 367 (1968).

A (0.5 g) in water (5 m*l*) was added sodium bicarbonate (0.2 g) and 2,4-dinitrofluorobenzene (0.45 g) in ethanol (5 m*l*), and the mixture was agitated for 2 hr at room temperature to give a yellow gummy substance, which was rubbed by glass rod with water (15 m*l*) to give a yellow powdery solid; 0.9 g (75%).

The dinitrophenyl derivative was then fully acetylated with acetic anhydride (20 m*l*) in the presence of anhydrous sodium acetate (2 g) by heating at 100°C under stirring for 3 hr. The resulting mixture was evaporated *in vacuo* and followed by dilution with excess acetone. Insoluble matters were filtered off and the solution was evaporated and made free from acetic anhydride by co-evaporation with toluene to give a yellow glass, 1.95 g. The product was purified by silica-gel column chromatography (150 g; 3.5 × 35 cm) with a solvent mixture of benzene-MEK (2 : 1), the effluent being cut into 10 g each. The fraction Nos. 34—37 were combined and evaporated to afford *N*-dinitrophenyl-*O*-acetyl derivative of natural kanamycin A; 0.6 g (41% from the base). Recrystallization from the same solvent system gave yellow needles; mp 210—213°C (decomp.), $[\alpha]_D^{18}$ +52° (*c* 1.0, acetone), IR spectrum: 3340, 1625, 1600, 1550, 1525, 1340, 835 and 745 (NHDNP), 1765, 1370 and 1220 cm^{-1} (OAc).

Found: C, 46.74; H, 4.10; N, 11.69%. Calcd for $C_{56}H_{58}N_{12}O_{34}$: C, 46.61; H, 4.05; N, 11.65%.

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