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Studies of Aminosugars. XX. The Synthesis of 6-*O*-(3-Amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine[1]

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6-*O*-(3-Amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine (3AD) which is a glycoside component of kanamycin A has been synthesized. Amino and hydroxyl groups of 2-deoxystreptamine were masked with carbobenzoxy and isopropylidene groups to give racemic mono-*O*-isopropylidene-di-*N,N'*-carbobenzoxy-2-deoxystreptamine, with which 3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-glucopyranosyl chloride was condensed by a modified Koenigs-Knorr reaction. Removal of all the masking groups followed by *N*-dinitrophenylation, acetylation and chromatography gave the product identical with an authentic specimen of the penta-*O*-acetyl-tri-*N*-(2,4-dinitrophenyl) derivative of the natural 3AD. The synthetic product was further led to 3AD identical with a natural sample.

As a part of the investigation of the synthesis of antibiotic aminoglycosides, we previously reported the syntheses of paromamine,[2] neamine,[3] 5-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-deoxystreptamine,[4] 4,6-di-*O*-(3-amino-3-deoxy-β-D-glucopyranosyl)-deoxystreptamine,[5] 4,6-di-*O*-(6-amino-6-deoxy-β-D-glucopyranosyl)-deoxystreptamine,[5] diamino-dideoxy derivative of trehalose,[6] trehalosamine,[7] triamino-trideoxy derivatives of maltose,[6] sucrose[6] and raffinose[8] and hexaamino-hexadeoxy derivative of Schardinger α-dextrin.[8]

In the present paper, the synthesis of 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-deoxystreptamine (IV: abbreviated to 3AD) is described. This is a glycoside component of kanamycin A,[9] an useful antibiotic, and was first isolated by Maeda *et al.*[10] from the hydrolyzate of the antibiotic. Synthesis of this glycoside was undertaken as an approach to the synthesis of kanamycin A which has been briefly communicated.[11]

The key intermediates for the proposed synthesis were the totally *O*-benzylated derivative of 3-acetamido-3-deoxy-α-D-glucopyranosyl chloride on the one hand, and the monoisopropylidene derivative of *N,N'*-masked 2-deoxystreptamine on the other hand. A modified Koenigs-Knorr reaction between the two intermediates formed the desired α-glycosidic linkage, though not exclusively.

The preparation of 3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-glucopyranosyl chloride (I) was

1) Part XXXVI of "Studies on Antibiotics and Related Substances," by Sumio Umezawa. Presented before the 160th Meeting of the Japan Antibiotic Reasearch Association, at the Institute of Medical Science, Tokyo, March 22, 1968.

2) S. Umezawa and S. Koto, This Bulletin, **39**, 2014 (1966); *J. Antibiotics*, **A19**, 88 (1966).

3) K. Tatsuta, E. Kitazawa and S. Umezawa, This Bulletin, **40**, 2371 (1967); *J. Antibiotics*, **A20**, 53 (1967).

4) S. Umezawa, T. Tsuchiya and H. Fujita, *J. Antibiotics*, **A19**, 222 (1966).

5) S. Koto, Y. Ito and S. Umezawa, This Bulletin, **38**, 1447 (1965).

6) S. Umezawa, T. Tsuchiya and S. Nakada, *ibid.*, **40**, 395 (1967).

7) S. Umezawa, K. Tatsuta and R. Muto, *J. Antibiotics*, **A20**, 388 (1967).

8) S. Umezawa and K. Tatsuta, This Bulletin, **41**, 464 (1968).

9) 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).

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

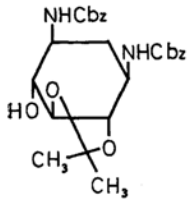
11) S. Umezawa, K. Tatsuta and S. Koto, *ibid.*, **21**, 367 (1968).

previously reported.[12] Treatment of *N,N'*-dicarbobenzoxy-2-deoxystreptamine[5] with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid in *N,N*-dimethylformamide at 110°C gave the monoisopropylidene derivative (II) in racemic form in a quantitative yield.

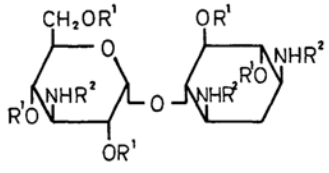
Condensation of I with II in the presence of mercuric cyanide and Drierite in an anhydrous mixture of benzene and dioxane gave a crude product, which was hydrolyzed with 80% acetic acid to remove isopropylidene group and hydrogenated over palladium black to remove benzyl groups. De-*N*-acetylation with barium hydroxide gave a crude ninhydrin-positive product. This was further dinitrophenylated with 2,4-dinitrofluorobenzene and *O*-acetylated. The resulting product showed about five spots including one main (R_f-value 0.3) and four minor spots on a silica-gel thin-layer chromatogram. The product was chromatographed on a silica-gel column and the main fraction was further separated into two fractions (R_f-values 0.30 and 0.28) of nearly equal amounts by preparative thin-layer chromatography. The substance having R_f-value 0.30 was crystallized to give 4,5-di-*O*-acetyl-6-*O*-[2,4,6-tri-*O*-acetyl-3-(2,4-dinitroanilino)-3-deoxy-α-D-glucopyranosyl]-di-*N,N'*-(2,4-dinitrophenyl)-2-deoxystreptamine (III) of mp 195—198°C (decomp.) and $[\alpha]_D$ +24° (*c* 0.9, acetone) in a 12% overall-yield from I.

On the other hand, the natural sample of 3AD was dinitrophenylated and acetylated to give penta-*O*-acetyl-tri-*N*-(2,4-dinitrophenyl) derivative, mp 194—198°C (decomp.) and $[\alpha]_D$ +22° (*c* 1.0, acetone).

The identity of the synthetic derivative III was established by direct comparison with both III and the above-mentioned derivative from natural sample.



II (racemate) Cbz = $COOCH_2C_6H_5$



III $R^1 = COCH_3$ R^2 = 2,4-dinitrophenyl

IV $R^1 = R^2 = H$ (3AD)

V $R^1 = H$, $R^2 = COCH_3$

12) S. Koto, T. Tsumura, Y. Kato and S. Umezawa, This Bulletin, **41**, 2765 (1968).

Hydrolysis of III with methanolic ammonia followed by treatment with Dowex 1*X*2 (OH) resin gave 3AD (IV), whose identity was established by direct comparison with both synthetic IV and natural sample.

Further, the synthetic free aminosugar (IV) was *N*-acetylated with acetic anhydride in methanol to give 6-*O*-(3-acetamido-3-deoxy-α-D-glucopyranosyl)-*N,N'*-diacetyl-2-deoxystreptamine (V), which showed $[M]_{CuAm}$ +1460, by the copper complex method[13] reported from our Laboratory. The observed value agreed with the reported value[14] of the tri-*N*-acetyl derivative of natural 3AD, indicating that IV is the 6-*O*-linked glycoside of 2-deoxystreptamine.

Experimental

General Procedure. Infrared spectra were determined in potassium bromide pellets. Thin layer chromatography (TLC) was performed by "Silica-Rider for TLC" (Dai-ichi Pure Chemicals Co.). Descending paper chromatography was carried out by Toyo filter paper No. 51, using a solvent system of *n*-butanol : pyridine : water : acetic acid = 6 : 4 : 3 : 1, with ninhydrin (0.3% in pyridine) coloration, developing at room temperature for 72 hr.

Racemic Di-*N,N'*-carbobenzoxy-mono-*O*-isopropylidene-2-deoxystreptamine (II). To a solution of 4.3 g of di-*N,N'*-carbobenzoxy-2-deoxystreptamine[5] in freshly distilled *N,N*-dimethylformamide (30 m*l*) was added *p*-toluenesulfonic acid monohydrate (0.06 g) and 2,2-dimethoxypropane (5 m*l*), and the mixture was heated at 110°C for 4 hr to result in a brown solution. After the solution was neutralized with 10 m*l* of Amberlite IRA-400 (OH type) which was washed with methanol before use, the resin was filtered off and the filtrate was evaporated to give a thick sirup, which solidified when treated with water (100 m*l*). Crude product, 4.4 g (94%), was recrystallized from ethyl acetate to give an analytically pure sample of racemic II; yield 4.0 g, 86%, mp 145—146°C; IR spectrum: 3325, 1698 1545 (NHCbz), 1067 cm^{-1} (ketal).

Found: C, 63.63; H, 6.69; N, 5.85. Calcd for $C_{25}H_{30}N_2O_7$: C, 63.82; H, 6.43; N, 5.95%.

4,5-Di-*O*-acetyl-6-*O*-[2,4,6-tri-*O*-acetyl-3-(2,4-dinitroanilino)-3-deoxy-α-D-glucopyranosyl]-di-*N,N'*-(2,4-dinitrophenyl)-2-deoxystreptamine (III). A mixture of II (1.3 g), well dried powdery mercuric cyanide (0.91 g) and Drierite (9.5 g) in a solvent mixture of benzene and dioxane (4 : 1; 40 m*l*) was heated at 110°C for 15 min with stirring and then cooled to room temperature. To this mixture was added dry 3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-glucopyranosyl chloride[11] (1.9 g; 1.3 eq.) and stirred at 110°C for 6 hr and additional 4 hr at room temperature under anhydrous conditions. The mixture was passed through a bed of Celite. The filtrate was combined and evaporated to afford a dark-brown residue, which

13) S. Umezawa, T. Tsuchiya and K. Tatsuta, *ibid.*, **39**, 1235 (1966).

14) S. Umezawa, K. Tatsuta and T. Tsuchiya, *ibid.*, **39**, 1244 (1966).

was extracted with ethyl acetate (40 m*l*). After removal of an insoluble matter, the solution was shaken with four 40 m*l* portions of 20% aqueous potassium bromide and then 20 m*l* portions of distilled water, dried over anhydrous sodium sulfate and evaporated to afford a sirup (2.5 g). This was dissolved in warm 80% aqueous acetic acid (25 m*l*) and the solution was heated at 60°C for 2 hr. Evaporation *in vacuo* gave a sirup residue. The deacetonated product was dissolved in a mixture (15 m*l*) of dioxane, water and concentrated hydrochloric acid (8 : 3 : 1) and hydrogenated over palladium black (0.5 g) at about 30°C for 35 hr under 3.5 atm of hydrogen pressure with occasional addition of water (total volume, 10 m*l*). The mixture was filtered and the solution was evaporated at below 30°C and hydrogen chloride was removed by co-distillation with *n*-butanol to give a residue (0.7 g). The ninhydrin-positive sirup was dissolved in 1 N aqueous barium hydroxide (15 m*l*) and heated on a boiling water bath for 2 hr. The mixture was acidified with 2 N sulfuric acid to pH 2 and filtered. The filtrate was neutralized with 2 m*l* of Dowex 1×2 (OH type) and evaporated *in vacuo* to afford a colorless sirup (0.5 g) of free base. The crude product was dinitrophenylated with 2,4-dinitrofluorobenzene (1.3 m*l*) in the presence of sodium bicarbonate (0.7 g) in 50% aqueous ethanol (10 m*l*) at room temperature under stirring overnight to give a yellow precipitate. To the resulting mixture was added water (10 m*l*) and further stirred to complete precipitation; yellow powder, 1.4 g, was obtained. The product was acetylated with acetic anhydride (11 m*l*) and freshly fused sodium acetate (0.5 g) by heating at 110°C for 12 hr with stirring. Evaporation of excess reagent afforded a brown residue, which was extracted with acetone and followed by evaporation of the extract *in vacuo* to give a yellow glass, 1.25 g. The product showed about five spots with R_f values 0.3 (main), 0.37, 0.45, 0.51 and 0.56 on a silica-gel thin-layer chromatogram with a solvent system of benzene and MEK (3 : 1). The product was chromatographed on a silica-gel column (200 g; 47×290 mm) using a solvent system: benzene and MEK (4 : 1), being cut into 10 g each. The main product having an R_f value 0.3 appeared in fractions of Nos. 77—101 which gave a yellow solid by evaporation *in vacuo*. This fraction was further separated into two fractions of nearly equal amount, which have R_f values 0.30 and 0.28 respectively, by preparative TLC (20×20×0.05 cm) using the above-mentioned solvent system. The substance of R_f value 0.30 was collected and recrystallized from the same solvent system to give yellow needles of III, 340 mg (12% overall-yield from II); mp 195—198°C (decomp.), $[\alpha]_D^{18}$ +24° (*c* 0.9, acetone), IR spectrum: 3340, 3100, 1625, 1595, 1340 and 745 (NHDNP), 1760, 1370 and 1225 cm^{-1} (OAc).

Found: C, 46.50; H, 4.25; N, 12.41. Calcd for $C_{40}H_{41}N_9O_{24}$: C, 46.56; H, 4.01; N, 12.22%.

The melting point of III was not depressed by admixture with the penta-*O*-acetyl-tri-*N*-(2,4-dinitrophenyl) derivative of natural 3AD described below. On TLC with a solvent system, toluene-MEK (3 : 1), the synthetic III and the above-mentioned derivative of natural 3AD showed identical mobilities and their IR spectra were superimposable.

6-*O*-(3-Amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine (IV). A sample (59 mg) of III was dissolved in methanol (15 m*l*) saturated with dry ammonia at 0°C and the red solution was kept standing at room temperature overnight. Evaporation of the reaction mixture *in vacuo* gave a yellow residue, which was treated with Dowex 1×2 (OH type; 10 m*l* of wet resin) in acetone (5 m*l*) under stirring at room temperature overnight. The resin was filtered off and the filtrate was concentrated *in vacuo* to give a glass, 22 mg, which was chromatographed on a column of Dowex 1×2 (OH type; 2 m*l*) with carbon dioxide-free water. Evaporation of the middle ninhydrin positive fraction was followed by trituration with aqueous methanol-ethanol to give an analytically pure sample of III; yield 11 mg (60% from III), $[\alpha]_D^{18}$ +98°C (*c* 0.9, water).

Found: C, 44.24; H, 7.51; N, 12.69. Calcd for $C_{12}H_{25}N_3O_7$: C, 44.57; H, 7.79; N, 13.00%.

On the other hand, the natural 3AD showed $[\alpha]_D^{18}$ +98° (*c* 0.10, water). On descending paper chromatography, the R_f values of the synthetic product IV and the natural 3AD were in agreement. IR spectra of IV and natural 3AD were superimposable.

Penta-*O*-acetyl-tri-*N*-(2,4-dinitrophenyl) Derivative of Natural 6-*O*-(3-Amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine. A mixture of natural 3AD (0.33 g) and sodium bicarbonate (0.4 g) in water (15 m*l*) was stirred for a while, and to the solution was added 2,4-dinitrofluorobenzene (0.62 g) in ethanol (7 m*l*). The mixture was stirred for about 6 hr at room temperature until the yellow gummy product initially formed became powdery; yield 0.74 g (90%). The crude tri-*N*-(2,4-dinitrophenyl) derivative was acetylated with acetic anhydride (10 m*l*) and anhydrous sodium acetate (1.0 g) by heating at 110°C for 10 hr with stirring under anhydrous conditions. Evaporation of the excess reagent gave a residue, which was extracted with acetone. Concentration of the extract gave a yellow glass which was crystallized from acetone by the gradual addition of ethanol. Two recrystallization from benzene-MEK (3 : 1) afforded a chromatographically pure product, fine neeldes, mp 194—198°C (decomp.), $[\alpha]_D^{18}$ +22° (*c* 1.0, acetone), IR spectrum: 3340, 3100, 1625, 1595, 1340 and 745 (NHDNP), 1760, 1370 and 1225 cm^{-1} (OAc).

Found: C, 46.44; H, 4.21; N, 12.36. Calcd for $C_{40}H_{41}N_9O_{24}$: C, 46.56; H, 4.01; N, 12.22%.

6-*O*-(3-Acetamido-3-deoxy-α-D-glucopyranosyl)-*N*,*N*′-diacetyl-2-deoxystreptamine (V). To a suspension of IV (20 mg) in methanol (1.2 m*l*) was added acetic anhydride (0.6 m*l*) at room temperature. The mixture became clear immediately and after 18 hr, the solution became ninhydrin-negative. The solution was evaporated to give a colorless solid, from which acetic anhydride was removed by co-distillation with ethanol and crystallized from acetone to afford the pure acetate V; yield 23 mg (80%), mp 236—239°C (decomp.), $[\alpha]_{589}^{18}$ +74° (*c* 0.91, water), $[\alpha]_{436}^{18}$ +140° (*c* 0.91, water), $[\alpha]_{436}^{18}$ +465° (*c* 0.80, CuAm), $\Delta[M]_{CuAm}$ +1460, IR spectrum: 3400, 1100—1000 (OH), 3290, 1650, 1560 and 1375 cm^{-1} (NHAc).

Found: C, 47.91; H, 6.99; N, 9.08. Calcd for $C_{18}H_{31}N_3O_{10}$: C, 48.10; H, 6.95; N, 9.35%.

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