Studies on Antibiotics and Related Substances. XXII. Syntheses of Di(3-amino-3-deoxy-D-glucosyl)- and Di(6-amino-6-deoxy-D-glucosyl)-deoxystreptamine[1]

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In a previous paper[2] we described the synthesis of di-*O*-(D-glucopyranosyl)-deoxystreptamine, in which two sugars are linked to C-4 and C-6 of deoxystreptamine in a manner analogous to that two amino sugars are linked in kanamycin. In an effort to determine the effect of amino groups on the antibiotic activity of glucosides of this kind, the amino glucosides of the title have been synthesized. The basis of the work of this series[3] arose with the recent discovery of a number of antibiotics which are the glucosides of deoxystreptamine but which differ primarily in the structures of their sugar moieties.

The condensation of *N*, *N'*-dicarbobenzoxy-deoxystreptamine (II)[2] with an excess of 2, 4, 6-tri-*O*-acetyl-3-carbobenzoxyamino-3-deoxy-α-D-glucopyranosyl bromide (I)[4] in nitromethane in the presence of mercuric cyanide gave 4, 6-di-*O*-(2, 4, 6-tri-*O*-acetyl-3-carbobenzoxyamino-3-deoxy-β-D-glucopyranosyl)-*N*, *N'*-dicarbobenzoxy-deoxystreptamine (III) in a 25% yield.

The deacetylation of III with methanolic ammonia gave 4, 6-di-*O*-(3-carbobenzoxyamino-3-deoxy-β-D-glucopyranosyl)-*N*, *N'*-dicarbobenzoxy-deoxystreptamine (IV) in a 68% yield.

The catalytic hydrogenolysis of IV with palladium black gave 4, 6-di-*O*-(3-amino-3-deoxy-β-D-glucopyranosyl)-deoxystreptamine (V) in an 80% yield.

The reaction sequence used in the synthesis of 4, 6-di-*O*-(6-amino-6-deoxy-β-D-glucopyranosyl)-deoxystreptamine (V') was analogous to that of V.

Since the structure of 4, 6-(tetra-*O*-acetyl-β-D-glucopyranosyl)-deoxystreptamine, which corresponds to the hydrogenolysis product of III had been previously[2] confirmed by means of periodate oxidation, the two amino sugar moieties in V and V' were assigned to the 4- and 6-positions in deoxystreptamine.

Futhermore, V' rapidly consumed about four moles of periodate, and the paper chromatography of the hydrolyzate of the oxidation mixture showed the presence of deoxystreptamine and the complete absence of 6-amino-6-deoxy-D-glucose. The survival of deoxystreptamine is indicative of glucosidation at the 4- and 6-positions of deoxystreptamine.

The designation of V and V' as β-anomers was based on their optical rotations and infrared spectra. The optical rotations of V and V' are $[\alpha]_D^{27}$ $-5.5°$ (*c* 1.2, water) and $[\alpha]_D^{15}$ $-0.1°$ (*c* 0.5, water) respectively. Compared with the optical rotation of the kanamycin free

1) Presented in part at the International Congress on Antibiotics, Prague, CSSR, June 15—19, 1964; S. Umezawa, Abstracts of Papers, 134 (1964).

2) S. Umezawa and Y. Ito, This Bulletin, **34**, 1540 (1961).

3) Previous papers of this series: S. Umezawa and T. Tsuchiya, *J. Antibiotics*, **A15**, 51 (1962); T. Suami, S. Ogawa and S. Umezawa, This Bulletin, **36**, 459 (1963); S. Umezawa and T. Tsuchiya, *J. Antibiotics*, **A16**, 173 (1963); T. Tsuchiya, H. Fujita and S. Umezawa, ibid., **A17**, 181 (1964); T. Tsuchiya and S. Umezawa, This Bulletin, **38**, 1181 (1965); Ref. 2.

4) Y. Ito, S. Koto and S. Umezawa, This Bulletin, **35**, 1619 (1962).

base $[\alpha]_D^{13}$ +140° (*c* 1.35, water)[5] with an α-configuration, that of V and V′ are levorotatory, and since the amino sugar moieties in V and V′ have a D-configuration, the assignment of β-configurations to V and V′ appears to be reasonable. The infrared spectra of V showed absorption band at 895 cm^{-1}, indicating a characteristic absorption band for β-glucoside, while the kanamycin free base showed absorption bands at 838 and 823 cm^{-1} corresponding to two α-anomeric configurations.[6]

A similar condensation of *N*, *N*′-dicarbobenzoxy-deoxystreptamine with 3, 4, 6-tri-*O*-acetyl-2-carbobenzoxyamino-2-deoxy-α-D-glucopyranosyl bromide[7] was unsuccessful; only 4, 5-(3, 4, 6-tri-*O*-acetyl-D-glucopyrano)-2-oxazolone[8] was obtained.

Experimental

4, 6-Di-*O*-(2, 4, 6-tri-*O*-acetyl-3-carbobenzoxyamino-3-deoxy-β-D-glucopyranosyl)-*N*, *N*′-dicarbobenzoxy-deoxystreptamine (III).—To a suspension of *N*, *N*′-dicarbobenzoxy-deoxystreptamine (0.38 g., 0.9 mmol.) and 2, 4, 6-tri-*O*-acetyl-3-carbobenzoxyamino-3-deoxy-α-D-glucopyranosyl bromide[4] (1.11 g., 2.2 mmol.) in nitromethane* (20 ml.), powdery mercuric cyanide (0.57 g., 2.3 mmol.) was addded, the suspension was then stirred at 27°C for 24 hr. The resulting mixture was filtered and the filtrate evaporated to dryness at about 40°C under reduced pressure. Extraction with chloroform was followed by repeated washings with water in order to remove mercuric salts. After drying over sodium sulfate, the chloroform solutions was evaporated to dryness under reduced pressure to give a residue (1.05 g.), which was washed with ether to give a crude product (250 mg.) (25%). Recrystallized from absolute ethanol; m. p. 277°C (decomp., sintered at about 277°C), $[\alpha]_D^{15}$ −13.0° (*c* 1.75, dioxane).

Found: C, 58.11; H, 5.75; N, 4.41; Mw, 1150. Calcd. for $C_{62}H_{72}O_{25}N_4$: C, 58.48; H, 5.70; N, 4.40%; Mw, 1273.

4, 6-Di-*O*-(3-carbobenzoxyamino-3-deoxy-β-D-glucopyranosyl)-*N*, *N*′-dicarbobenzoxy-deoxystreptamine (IV). — Three hundred and ten milligrams of III was suspended in 75 ml. of absolute methanol, and to this was added 50 g. of absolute methanol saturated with ammonia. Since, after this mixture had been left overnight, there was a considerable quantity of solid left in the mixture, absolute methanol saturated with ammonia was again added to the mixture. After again having been left overnight at room temperature, a clear solution was obtained; this was filtered, and the filtrate was concentrated under reduced pressure to yield colorless crystals, which were collected and washed with absolute methanol and chloroform in turn; 0.17 g., m. p. 264—265°C (decomp., sintered at about 200°C), $[\alpha]_D^{15}$ +5.0° (*c* 1.22, dimethyl formamide).

Found: C, 58.67; H, 5.60. Calcd. for $C_{50}H_{60}O_{19}N_4$: C, 58.81; H, 5.92%.

4, 6-Di-*O*-(3-amino-3-deoxy-β-D-glucopyranosyl)-deoxystreptamine (V). — One hundred and sixty milligrams of IV was suspended in 12 ml. of a dioxane-water mixture (7:5), and then this mixture was added to a prehydrogenated palladium black (50 mg.) in 1.5 ml. of a dioxane-water mixture (1:1). The mixture was hydrogenated under mechanical shaking at 23—25°C for 1.5 hr. and further at 30—31°C for 4 hr. A further suspension of 20 mg. of prehydrogenated palladium black in dioxane-water was added to the resulting mixture, and hydrogenolysis was continued at 38—42°C for about 7 hr. The resulting mixture was strongly alkaline. After the removal of the catalyst by filtration, the solution was evaporated under reduced pressure to give 60 mg. of an amorphous solid. The product was dissolved in hot methanol, and ethanol was added to yield a crystalline powder of the title compound, $[\alpha]_D^{27}$ −5.5° (*c* 1.2, water).

Found: C, 44.58; H, 7.30; N, 10.95. Calcd. for $C_{18}H_{36}O_{11}N_4$: C, 44.61; H, 7.49; N, 11.57%.

The infrared absorption spectrum of the product in Nujol contained absorptions at 3260 (NH, OH), 1665, 1595 (NH), 1040 (C-O) and 895 cm^{-1} (type 2b of the pyranose ring).

4, 6-Di-*O*-(6-amino-6-deoxy-β-D-glucopyranosyl)-deoxystreptamine (V′). — To a suspension of *N*, *N*′-dicarbobenzoxy-deoxystreptamine (0.42 g., 1.0 mmol.) and 2, 3, 4-tri-*O*-acetyl-6-carbobenzoxyamino-6-deoxy-β-D-glucopyranosyl chloride[9] (1.00 g., 2.2 mmol.) in dry nitromethane (30 ml.), powdery mercuric cyanide (0.56 g., 2.2 mmol.) was added; then the suspension was shaken at about 27°C for 4 days in a dark room. The resulting mixture was filtered, and the filtrate was evaporated under reduced pressure. The residue was extracted with chloroform, and the chloroform extract was washed with water to remove mercuric salts. After it had been dried over sodium sulfate, the chloroform solution was evaporated under reduced pressure. The pale yellow syrup (1.1 g.) obtained was suspended in absolute methanol saturated with ammonia (62 g.). After having been left overnight at room temperature, the mixture was filtered, and the filtrate was evaporated under reduced pressure. The resulting syrup was treated several times with 2-ml. portions of water, and the residue was dried up under reduced pressure to give a colorless syrup (0.480 g.). The residue was dissolved in 10 ml. of 25% aqueous methanol, and to this solution was added a prehydrogenated palladium black (100 mg.). The mixture was hydrogenated under mechanical shaking at room temperatuer for 50 hr. During the hydrogenation, dilute hydrochloric acid was

5) Ph. D. thesis of K. Maeda, Keio University, 1958.
6) M. J. Cron, D. L. Evans, F. M. Palermiti, D. L. Whitehead, I. R. Hooper, Paul Chu and R. U. Lemieux, *J. Am. Chem. Soc.*, **80**, 4741 (1958).
7) L. Zervas and S. Konstas, *Chem. Ber.*, **93**, 435 (1960).
8) S. Konstas, A. I. Photake and L. Zervas, ibid., **92**, 1288 (1959); S. Umezawa, S. Koto and Y. Ito, This Bulletin, **36**, 186 (1963).
* Dry nitromethane obtained by distillation with phosphorus pentoxide.
9) S. Umezawa, S. Koto and Y. Ito, This Bulletin, **36**, 183 (1963).

added several times in order to acidify the reaction mixture.* After the catalyst had been removed, the solution was evaporated under reduced pressure to yield a syrup (324 mg.). Descending-paper chromatography of the product by ninhydrin coloration, using an *n*-butanol - pyridine - water - acetic acid (6 : 4 : 3 : 1) solvent system at room temperature for 19 hr., showed five spots of R_{f6AG} 1.00, 0.65 (deoxystreptamine), 0.31, 0.10 and 0.04.** The spot of R_{f6AG}, 0.10, corresponded to the main product.

The product was mixed with cellulose powder (100—200 mesh) (1.0 g.) and water (3.0 ml.) and dried over phosphorus pentoxide in a desiccator under reduced pressure. It was then placed on a column (11×2.5 cm.) of cellulose powder (15 g.) and developed with the above-metioned solvent system at a rate of 10 ml./hr. The product of R_{f6AG}, 0.10, emerged from 340 to 800 ml. of the eluate. The fractions were then combined and evaporated under reduced pressure at 30—35°C. A solution of the residue (116 mg.) in water (1.0 ml.) free from carbon dioxide was placed on a column (0.5×5 cm.) of Dowex 1X2 (OH form) and developed with water free from carbon dioxide. The ninhydrin-positive fractions were then collected and evaporated under reduced pressure at about 20°C to give a colorless powder of the title compound (65 mg.) (13.5%). Purification for analysis was accomplished by dissolving the product in hot methanol, with the subsequent addition of ethanol; colorless amorphous powder, $[\alpha]_D^{15}$ −0.1° (*c* 0.5, water).

Found: C, 43.90; H, 7.25; N, 11.04. Mw, 480. Calcd. for $C_{18}H_{36}O_{11}N_4$: C, 44.61; H, 7.49; N, 11.57%. Mw, 484.

The Periodate Oxidation of V′.—An accurately-weighed sample (2.42 mg.) of V′ was dissolved in 5.0 ml. of a 0.03 M sodium periodate solution (acetate buffer, pH 4.4). The oxidation was carried out at 2—3°C. in the dark. To each one-ml. aliquot was added a measured excess of 0.05 M sodium arsenite at eight-hour intervals, beginning three hours after the dissolving of the sample, and then these solutions were titrated with a 0.01 M iodine solution. The product consumed 2.1 mol. of periodate at 3 hr. and 3.9 mol. at 24 hr. A parallel experiment using a sample (2.52 mg.) of kanamycin base gave a titration curve similar to that obtained from the product (V′); kanamycin consumed 2.6 mol. of periodate at 3 hr. and 4.0 mol. at 24 hr.

Another weighed sample (2.6 mg.) of V′ was oxidized similarly for 120 hr. After the addition of ethylene glycol (1.0 ml.), the mixture was again allowed to stand over night. To the mixture concentrated hydrochloric acid (5.0 ml.) was then added, and the mixture was heated at 90—95°C for 3 hr. The hydrolyzate was chromatographed on Toyo filter paper No. 51 by the descending method, using *n*-butanol - pyridine - water - acetic acid (6 : 4 : 3 : 1) for 16 hr. Detection by ninhydrin-coloration gave the spot corresponding to deoxystreptamine.

The Paper Chromatography of the Acid Hydrolyzate of V′.—Two 0.5 mg. samples of V′ were dissolved in 1.75 ml. of 2 N and 6 N hydrochloric acid respectively, and then allowed to stand at about 95°C for 6 hr. The hydrolyzates were chromatographed on Toyo filter paper No. 51 by the ascending method, using the above-mentioned solvent system, for 19 hr. Detection by ninhydrin-coloration gave only three spots, corresponding to 6-amino-6-deoxy-D-glucose, deoxystreptamine and unchanged V′.

Bioassay.—Compounds V and V′ did not show any antibacterial activity against *M. Pyogenes var. aureus* 209-p, *E. coli* and *Mycobacterium tuberculosis* 607 in a dilution of 1 : 1000.

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* When the hydrogenation was effected without the addition of hydrochloric acid, the crude product (syrup) showed a remarkable inhibition against *B. subtilis* PCI 219. The antibacterial activity seems to be due to the presence of the complex diorthoester reported by R. U. Lemieux, C. I. C. Medal Lecture, "The Chemical Synthesis of Glucosides," Chemical Institute of Canada (1964).

** R_{f6AG}: R_f values relative to the R_f value of 6-amino-6-deoxy-D-glucopyranose taken as 1.0.