Studies on Antibiotics and Related Substances. XI. Methylated Amino-sugar Moieties from Methylated Kanamycin[1]

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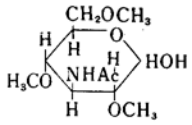
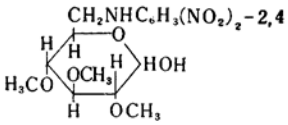
In a previous paper[2] we described the fact that 1,3-diamino-4,6-dihydroxy-5-methoxycyclohexane was isolated from the hydrolyzate of exhaustively methylated *N*-acetyl kanamycin. It was therefore concluded that both of the two aminohexose moieties in kanamycin are directly joined to deoxystreptamine through 4- and 6-hydroxyl groups by glucosidic linkages, the structure of kanamycin[3] being established. Subsequently, attempts have been made to isolate amino-sugar moieties from the same hydrolyzate. The present paper is concerned with the isolation of methylated derivatives of 3-amino-3-deoxy glucose and 6-amino-6-deoxyglucose.

Separation of 1,3-diamino-4,6-dihydroxy-5-methoxycyclohexane from acid-hydrolyzate was accomplished as described in a previous paper[2]. The residue from the mother liquor was chromatographed on a column of cellulose powder with butanol–ethanol–water (4 : 1 : 5). Repetition of the chromatography resulted in the isolation of 3-acetamido-3-deoxy-2,4,6-tri-*O*-methyl-D-glucose (I). The new derivative was converted into a methyl glucoside, which has been identified with the synthetic specimen prepared from D-glucose by the route of Peat and Wiggins[4].

On the other hand, an aqueous solution of



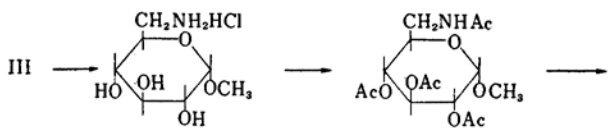
I



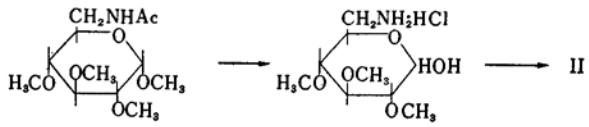
II

the above-mentioned residue from the mother liquor was passed through a column of Amberlite IRC-50 (Na^+ form) to remove *N*-acetylated amino-sugar moieties and developed with dilute hydrochloric acid. The combined residues from the early fractions were subjected to a cellulose powder chromatography to give fractions which showed a single ninhydrin-positive spot and positive Tollens and aniline phthalate reaction. The product was chromatographically identical with the synthetic specimen of 6-amino-6-deoxy-2,3,4-tri-*O*-methyl-D-glucose hydrochloride, the synthesis of which is mentioned below. The product could not be caused to crystallize. However, condensation with 2,4-dinitrofluorobenzene afforded yellow needles of 6-(2,4-dinitrophenylamino)-6-deoxy-2,3,4-tri-*O*-methyl-D-glucose (II).

The synthetic specimen of II has been prepared from 6-amino-6-deoxy-3,5-benzylidene-1,2-isopropylidene-D-glucose[5] (III) by the following scheme.



IV V



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1) Presented before the division of Organic Chemistry of the Annual Meeting of the Chemical Society of Japan at Kyoto, April, 1959 and at Tokyo, April, 1960.

2) S. Umezawa, Y. Ito and S. Fukatsu, This Bulletin, **32**, 81 (1959).

3) Ref. 2, p. 82.

4) S. Peat and L. F. Wiggins, *J. Chem. Soc.*, **1938**, 1810.

5) B. Helferich and R. Mittag, *Ber.*, **71**, 1588 (1938).

In the course of our synthesis of II, an alternative synthesis of methyl 6-amino-6-deoxy-α-D-glucoside hydrochloride (IV) was performed by Cramer et al.[6] The glucoside prepared by our method has been identified with a sample prepared by the alternative method by the mixed melting point method and infrared absorption spectra.

The mixed melting point determination of the natural and synthetic specimens of II was undepressed and the respective infrared spectra were completely superimposable.

Experimental

Isolation of 3-Acetamido-3-deoxy-2, 4, 6-tri-*O*-methyl-D-glucose (I).—The exhaustively methylated tetra-*N*-acetyl-kanamycin was hydrolyzed with 6 N hydrochloric acid and 1, 3-diamino-4, 6-dihydroxy-5-methoxycyclohexane was separated from the hydrolyzate, details of which were described in a previous paper[1]. The mother liquor was evaporated to dryness. Paperchromatographic studies of the residue (brown syrup) showed ninhydrin-positive spots of R_f 0.54 (violet) 0.41 (violet), 0.31 (orange) 0.23 (violet) and 0.08 (brown) using butanol-ethanol-water (4 : 1 : 5). The residue (9.67 g.) was dissolved in the above-mentioned solvent, chromatographed on a column (3×51 cm.) of cellulose powder and developed with the same solvent. One hundred and fourteen fractions were cut into 10 ml. each, evaporated to dryness, weighed and tested for R_f values. The combined fractions of tube Nos. 27—37 (6.89 g., R_f 0.48, 0.41, 0.36) were again chromatographed in the same manner and the combined fractions of tube Nos. 13–30 were evaporated to yield a crystalline residue (600 mg.). Recrystallization from absolute ethanol afforded needles of m. p. 229~230°C (decomp.), $[\alpha]_D^{15}$+107.1° (*c* 0.98, methanol).

Found: C, 49.89; H, 7.90; N, 5.49; OCH_3, 35.20; $COCH_3$, 14.70. Calcd. for $C_{11}H_{21}NO_6$: C, 50.18; H, 8.04; N, 5.32; OCH_3, 35.36; $COCH_3$, 16.35%.

The product feebly reduced Fehling's solution and showed a negative ninhydrin reaction. On hydrolysis by refluxing with 6 N hydrochloric acid for 2 hr., the product gave a hydrolyzate which showed a ninhydrin-positive spot of R_f 0.29 (orange). These results indicated that the product was an *N*-acetylated derivative of methylated hexosamine. By refluxing in excess absolute methanol (40 ml.) in the presence of hydrogen chloride (2%), the product (350 mg.) afforded a methyl glucoside (120 mg.) of m. p. 156~157°C, $[\alpha]_D^{15}$+120.7° (*c* 0.41, chloroform), which had nearly the same m. p. with the methyl 3-acetamido-3-deoxy-2, 4, 6-tri-*O*-methyl-α-D-glucoside reported by Peat and Wiggings[4].

Found: C, 51.87; H, 8.10; N, 5.18; OCH_3, 44.00. Calcd. for $C_{12}H_{23}NO_6$: C, 51.97; H, 8.36; N, 5.05; OCH_3, 44.76%.

The synthetic specimen was therefore prepared from D-glucose by the route of the above authors. The mixed m. p. determination of the natural and synthetic specimen (m. p. 157~158.5°C) was undepressed and the respective infrared spectra were completely superimposable.

Isolation of 6-Amino-6-deoxy-2, 3, 4-tri-*O*-methyl-D-glucose.—The residue (4.89 g.) obtained by evaporation of the mother liquor from 1, 3-diamino-4, 6-dihydroxy-5-methoxycyclohexane was dissolved in water (20 ml.) and passed through a column of Amberlite IRC-50 (Na^+ form, 80 ml.) to remove *N*-acetylated amino-sugar moieties and developed with 0.5 N hydrochloric acid. Thirty-one fractions of eluate were cut into 20 ml. each, evaporated to dryness and tested for R_f values. Fractions of tube Nos. 4-9 showed ninhydrin-positive spots of R_f 0.44~0.46, 0.23~0.24*. The combined residues (4.54 g.) from the fractions were treated with absolute ethanol (30 ml.) to separate off the sodium chloride and the solution was evaporated to dryness to give a syrup (1.60 g.), which was treated with active charcoal in absolute ethanol (20 ml.). To the filtrate was added absolute ether (30 ml.) to precipitate sodium chloride and the filtrate was evaporated. A solution of the resulting syrup (1.39 g.) in methanol (2 ml.) was passed through a column (2×70 cm.) of cellulose powder and developed with *n*-butanol-acetic acid-water (10 : 2 : 5); fifty-seven fractions were cut into 5.0 ml. each. A residue (0.70 g.) obtained from tube Nos. 35-45 showed a single ninhydrin-positive spot of R_f 0.33~0.38 and gave positive Tollens and aniline phthalate reactions. The combined residue (0.60 g.) obtained was mixed with cellulose powder (1.0 g.) in a small quantity of methanol, placed on a column (2×70 cm.) of cellulose powder, washed with *n*-butanol-ligroin-water (19 : 30 : 1) and developed with *n*-butanol-acetic acid-water (10 : 2 : 5); eighty-nine fractions were cut into 5.0 ml. each. Fractions of tube Nos. 43—55 showed a single ninhydrin-positive spot of R_f 0.40 and were chromatographically identical with the synthetic specimen of 6-amino-6-deoxy-2, 3, 4-tri-*O*-methyl-D-glucose hydrochloride.

The combined residues (170 mg.) obtained from the above fractions were dissolved in an aqueous solution (3.0 ml.) of sodium bicarbonate (100 mg.) and to the solutions was added 2, 4-dinitrofluorobenzene (100 mg.) dissolved in absolute ethanol (2.0 ml.); the solution was stirred at room temperature for 1 hr. to give yellow needles. After standing overnight, the needles (180 mg.) were separated and treated with ether to remove a trace of any insoluble part. The ethereal solution was passed through a column of alumina** (10.0 g.) and eluted with ether-petroleum ether and ether in turn. Evaporation of the combined eluates gave a crystalline residue (92 mg.); recrystallization from absolte methanol afforded a pure sample (72 mg.) of 6-(2, 4-dinitrophenylamino)-6-deoxy-2, 3, 4-tri-*O*-methyl-D-glucose (II), m. p. 97~99°C sintered from 92°C), λ_{max}^{EtOH} 261.5 (ε 3833), 348 mμ (ε 8293).

6) F. Cramer, H. Otterbach and H. Springmann, *Chem. Ber.*, **92**, 384 (1959).

* Unless otherwise indicated, the solvent system of *n*-butanol-acetic acid-water (10 : 2 : 5) and Toyo filter paper No. 51 were used.

** Aluminum Oxide, Merck.

Found: C, 46.31; H, 5.20. Calcd. for $C_{15}H_{21}N_3O_9$: C, 46.51; H, 5.46%.

6-Amino-6-deoxy-D-glucose Hydrochloride (IV).—6-Amino-6-deoxy-3,5-benzylidene-1,2-isopropylidene-D-glucose[5] (21.0 g.) was hydrolyzed by refluxing with 75% acetic acid (300 ml.) for 3 hr. and followed by treatment with active charcoal (1.0 g.). The filtrate was evaporated to dryness to give a residue (14.1 g.) which reduced Fehling's solution. To the residue (14.0 g.) which was dried over phosphorus pentoxide in a vacuum desiccator were added silver acetate (10.6 g.), acetic anhydride (11.5 g.) and methanol (141 ml.). The mixture was stirred at room temperature for 3 hr. and refluxed on a water bath for an additional 5 min. The reaction mixture was filtered and washed with hot water; the filtrate was acidified with concentrated hydrochloric acid to Thymol blue to precipitate silver salt. The filtrate was evaporated up to give a crude *N*-acetylated product***, which was dried over phosphorus pentoxide in a vacuum desiccator. The product as converted into a methyl glucoside by refluxing with 300 ml. of methanolic hydrochloride (2%) for 10 hr. The hydrolyzate was neutralized with basic lead carbonate (100 g.), filtered, and the filtrate was evaporated up in vacuo to give a dark syrup, which showed a positive ninhydrin reaction, indicating that de-*N*-acetylation occurred as well as the glucoside formation. The dark product was dissolved in methanol (26 ml.) and the solution was passed through a column of alumina (40 g.), washed with 1 N hydrochloric acid, and developed with methanol; early fractions (170 ml.) were combined and evaporated up to give a brown residue (7.88 g.), An aqueous solution (100 ml.) of the residue was passed through a column of Amberlite IRA-410 (base form, 100 ml.) and the early effluent (77 ml.) was treated with active charcoal (2 g.) and followed by evaporation to dryness to give crude methyl 6-amino-6-deoxy-D-glucoside (3.85 g., overall yield 23%). A solution of the crude product in absolute methanol was acidified with hydrogen chloride and evaporated to dryness. Recrystallization of the resulting solid from absolute methanol-ether afforded colorless crystals (1.5 g.) of methyl 6-amino-6-deoxy-α-D-glucoside hydrochloride, m. p. 200°C. (decomp.).

Methyl 6-Acetamido-6-deoxy-2, 3, 4-tri-*O*-methyl-α-D-glucoside (VI).—A mixture of methyl 6-amino-6-deoxy-α-D-glucoside hydrochloride (3.9 g.), acetic anhydride (15.5 g.) and pyridine (20.7 g.) was stirred at room temperature for 43 hr. The reaction mixture was added to ice water, stirred for 4.5 hr. and then extracted thrice with chloroform (100 ml.). The combined extracts were washed thrice with 35% potassium bisulfate (50 ml.) to remove the pyridine and further washed with a saturated solution (50 ml.) of sodium bicarbonate and water (90 ml.) in turn. The chloroform solution was dried with calcium chloride and evaporated to dryness. To a vigorously stirred solution of the acetylated product (5.90 g.) in methanol (6.0 ml.) was added a solution of dimethyl sulfate (60 ml.) in carbon tetrachloride (24 ml.) and water (4.0 ml.). During this and the subsequent addition of alkali, the temperature of the reaction was kept at about 40°C. A solution of sodium hydroxide (12.5 N, 60 ml.) was added dropwise over a 25 min. period. Again, dimethyl sulfate (20 ml.) was added and 12.5 N sodium hydroxide solution (80 ml.) was added gradually during one-hour period. The reaction mixture was kept at 75°C for 1 hr. and then heated on a boiling water bath for an additional 30 min. The temperature was lowered to about 40°C and the mixture was extracted with two 250-ml. portions of chloroform. After drying with calcium chloride, the combined extract was evaporated to give a crystalline solid (3.80 g. 96.3%). Recrystallization from ethyl acetate gave colorless needles of methyl 6-acetamido-6-deoxy-2,3,4-tri-*O*-methyl-α-D-glucoside, m. p. 121~121.5°C, $[\alpha]_D^{18}$ +250.8° (*c* 1.03, chloroform).

Found: C, 52.13; H, 8.06; N, 5.10; OCH_3, 44.36. Calcd. for $C_{12}H_{23}NO_6$: C, 51.97; H, 8.36; N, 5.05; OCH_3, 44.76%.

6-Amino-6-deoxy-2, 3, 4-tri-*O*-methyl-D-glucose Hydrochloride (VII).—A solution of VI (1.47 g.) in 3 N hydrochoric acid (30 ml.) was refluxed for 6 hr. and treated with active charcoal (100 mg.); the filtrate was evaporated in vacuo. The residue obtained was dissolved in water (30 ml.) and again the solution was evaporated in vacuo. The residue was dried over phosphorus pentoxide in a vacuum desiccator and crystallized from absolute ethanol-absolute ether; colorless needles (1.70 g., 99.6%). Recrystallization from the same solvent gave a pure sample of 6-amino-6-deoxy-2,3,4-tri-*O*-methyl-D-glucose hydrochloride, m. p. 179~180°C (decomp.), $[\alpha]_D^6$ +27.25° → +65.3° (*c* 1.32, water).

Found: C, 42.27; H, 7.56; N, 5.43; OCH_3, 36.37. Calcd. for $C_9H_{20}NO_5Cl$: C, 41.94; H, 7.82; N, 5.43; OCH_3, 36.13%.

6-Deoxy-6-(2, 4-dinitrophenylamino)-2, 3, 4-tri-*O*-methyl-D-glucose.—Condensation of VII with 2,4-dinitrofluorobenzene was accomplished in the same manner as described in the end of the second clause. The crude product (52%) was washed with ether and dissolved in absolute methanol to remove a trace of any insolube part. The residue from the methanol solution was recrystallized from a small quantity of absolute methanol to give an analytically pure sample of m. p. 97~99°C (sintered at about 90°C), $[\alpha]_D^{11}$ +37.7° (*c* 0.61, methanol); λ_{max}^{EtOH} 261.5 (ε 3833), 348 mμ (ε 8393).

Found: C, 46.38; H, 5.24; N, 11.04. Calcd. for $C_{15}H_{21}N_3O_4$: C, 46.51; H, 5.46; N, 10.85%.

Summary

1) 3-Acetamido-3-deoxy-2, 4, 6-tri-*O*-methyl-D-glucose (I) and *N*-(2, 4-dinitrophenyl) derivative (II) of 6-amino-6-deoxy-2, 3, 4-tri-*O*-methyl-D-glucose have been isolated from the hydrolyzate of exhaustively methylated *N*-acetyl kanamycin.

2) II has been synthesized from 6-amino-6-deoxy-3, 5-benzylidene-1, 2-isopropylidene-D-glucose.

*** As direct formation of glucoside from free hexosamine seemed difficult, the glucoside formation was preceded by *N*-acetylation. See T. White, *J. Chem. Soc.*, 1940, 428.

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