Biochemical Studies on Carbohydrates

CCXIX. Total Synthesis of Lactosamine-octaacetate

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Yosizawa¹⁾ synthesized N-acetyllactosamine from lactose in 1950, and Kuhn and Kirschenlohr²⁾ improved the method. The present account deals with a device to totally synthesize the same disaccharide. Since we found that 1,2,3-triacetyl-4,6-benzylidene- α -glucosamine (**I**) is obtainable from N-acetylglucosamine in good yield (1,2,3-Triacetyl-4,6-benzylidene- β -glucosamine (**II**) is yielded much less), it was employed as one of the materials. It was debenzylidenated by heating (20 minutes; 100°C) with 60% acetic acid, and 1,2,3-triacetyl- α -glucosamine (**III**) given was changed into 1,2,3,6-tetraacetyl- α -glucosamine (**V**) by controlled acetylation according to Levene and others³⁾ and condensed with acetobromogalactose in the manner which Reynolds and Evans⁴⁾ applied for preparing gentio-bioseoctaacetate from 1,2,3,4-tetraacetyl-glucose and acetobromoglucose. The product (**VI**) (needles) melted at 224°C and had an $[\alpha]_D$ (chloroform) value of +57.7°. No depression of melting point occurred when it was heated with the octaacetate derived from natural N-acetyllactosamine. And its O-deacetylation product gave a golden yellow spot with R_{lactose} of 1.34 as the N-acetyllactosamine did, when paper-chromatographed with solvent butanol-pyridine-water (5:3:2) and sprayed with the benzidine reagent of Raymonds and Morgan⁵⁾. Thus it was identified with the compound in view, although its condensation was very inefficient, yielding only 4% of theory, probably due to steric hindrance.

EXPERIMENTAL

1,2,3-Triacetyl-4,6-benzylidene- α -glucosamine (**I**) and 1,2,3-triacetyl-4,6-benzylidene- β -glucosamine (**II**). 25 g. of N-acetyl-4,6-benzylidene-glucosamine⁶⁾ was dissolved in a mixture of 100 cc. of dry pyridine and 40 cc. of acetic anhydride and stood in a refrigerator (0––5°C) for 48 hours, the mixture was then poured into 1 l. of iced water, and the white precipitate was washed with water and dried. It was extracted with two 100 cc. portions of chloroform, and the solutions were distilled to dryness and recrystallized from abs. ethanol twice. 25.5 g.

(80% of theory) of fine needles was yielded. F.P. $200.5\text{--}201.5^\circ$ (decomp.). $[\alpha]_D^{25}$ (pyridine) $= -100.9^\circ$ (c 0.7). Fehling positive. Soluble in chloroform, pyridine and methanol; slightly soluble in ethanol; insoluble in ether and water. Found: N 3.4; CH_3CO , 31.7. $\text{C}_{19}\text{H}_{23}\text{O}_8\text{N}$ requires N, 3.6; CH_3CO , 32.8. As compared with the one below regarding optical rotation, this product is judged as the derivative of α -glucosamine.

The chloroform-insoluble residue above was recrystallized twice from hot abs. methanol. About 2 g. (6.3% of theory) of soft long needles was yielded—the derivative of β -glucosamine. F.P. $248\text{--}249^\circ$ (decomp.). $[\alpha]_D^{25}$ (pyridine) $= -70^\circ$ (c 1.0). Found: N, 3.5; CH_3CO , 32.5. Fehling positive. Soluble in pyridine and hot methanol and ethanol; slightly soluble in chloroform and cold methanol and ethanol; insoluble in ether and water.

1,2,3-Triacetyl- α -glucosamine (III). Hydrolysis of I with 60% acetic acid was found preferable to catalytic hydrogenation of it with Pd-black.

a) Catalytic hydrogenation. 7 g. of 1,2,3-triacetyl-4,6-benzylidene- α -glucosamine was taken up in a mixture of 50 cc. of dioxane and 150 cc. of abs.

ethanol, 3.5 g. of Pd-black was added, and the whole mixture was shaken with hydrogen gas at room temperature. When no more hydrogen was absorbed after 24 hours (The hydrogen gas consumed, 840 cc.), it was brought onto a suction funnel, and the filtrate was distilled. The dry still-residue was extracted with two 50 cc. portions of chloroform, and the solutions were washed with three 100 cc. portions of water. The chloroform-insoluble residue was dissolved in the washings of the chloroformic solutions and distilled to dryness. The glassy yellowish residue (1.5 g.) was recrystallized twice from abs. ethanol by addition of ether. 0.6 g. (11.2% of theory) of crystals in the needle form was yielded. F.P. 142°. $[\alpha]_D^{25}$ (abs. ethanol) = +123° (c 1.0). Fehling positive. Soluble in ethanol, water and hot chloroform; insoluble in ether. It gave a spot with $R_{N\text{-acetylglucosamine}}$ 2.8, when paper-chromatographed (descending) with solvent butanol-ethanol-water (5:1:4, upper layer; 20°C). Found: N, 4.8; CH_3CO , 41.7; OH, 11.78. $\text{C}_{12}\text{H}_{19}\text{O}_8\text{N}$ requires N, 4.6; CH_3CO , 42.3; OH, 11.15.

b) Hydrolysis by the aid of 60% acetic acid. 19 g. of 1,2,3-triacetyl-4,6-benzylidene- α -glucosamine was suspended in 200 cc. of 60% acetic acid and refluxed on boiling water, and the clear solution given in 7–10 minutes was heated further on for 20 minutes. When cold, the hydrolysate was washed with two 100 cc. portions of petroleum ether and three 100 cc. portions of chloroform and distilled in vacuo. The syrupy still-residue (light brown; 15 g.) was taken up in about 10 volumes of abs. ethanol, decolorized with charcoal and concentrated to a syrup. It was then dissolved in 50 cc. of abs. ethanol, and after addition of ether to slight cloudiness, placed in a refrigerator. The white crystalline deposit was separated. Yield 12 g. It began to melt at 138° and melted completely at 170°. It gave two spots with $R_{N\text{-acetylglucosamine}}$ values of 2.0 and 2.8 when chromatographed as above, suggesting the di- and tri-acetate. Hence it was dried in an Abderhalden apparatus (P_2O_5 , 56.5°C) and extracted continuously with chloroform in a Soxhlet apparatus for 48 hours. The solution was distilled, and the crystalline still-residue was recrystallized twice from abs. ethanol. 8.7 g. (60% of theory) of needles was given. F.P. 142°. $[\alpha]_D^{25}$ (abs. ethanol) = +125° (c 1.0). The product gave a spot at a position corresponding to the triacetate in a chromatogram developed as above, and the mixed melting point showed no depression.

2,3-Diacetyl-glucosamine (IV). The chloroform insoluble residue above (5 g.) was recrystallized from 50 cc. of a mixture of chloroform and methanol (3:1) three times. 3.6 g. (30.6% of theory) of long needles was obtained. F.P. 173–174°. It mutarotates D-light. $[\alpha]_D^{25}$ (abs. ethanol) = +32.3° (5 min.) $\rightarrow$ +21.0° (45 min.) (c 1.3). Fehling positive. Soluble in water, methanol and hot ethanol; insoluble in chloroform. It showed $R_{N\text{-acetylglucosamine}}$ of 2.0 in a chromatogram developed as above. Found: N, 5.5; CH_3CO , 31.5; OH, 18.8. $\text{C}_{10}\text{H}_{17}\text{O}_7\text{N}$ requires N, 5.3; CH_3CO , 32.7; OH, 19.4.

The same diacetate was given in a yield of 92%, when 1,2,3-triacetyl-4,6-benzylidene- β -glucosamine was treated as above. Namely, here almost all the material lost one acetyl besides the benzylidene.

1,2,3,6-Tetraacetyl- α -glucosamine (V). 5 g. of 1,2,3-triacetyl- α -glucosamine was weighed in the 50 cc. brown flask (a) in Fig. 1, dissolved in 25 cc. of dry pyridine and chilled with ice. The solution was vigorously stirred, and 18.5 cc.

Fig. 1. Apparatus for controlled acetylation. *a*: 50 cc. brown flask. *b*: stirrer, *c*: cork stopper. *d*: 50 cc. burette. *e*: CaCl_2 granules. *f*: ice pieces. *g*: rotatory electric magnet.

of a 10% (w/v) acetic anhydride solution in chloroform was added in drops during 1 hour. The mixture was stirred for another 1 hour under continuous cooling and then stood at room temperature for 12 hours. Followingly, it was poured into 100 cc. of iced water, and after standing for several hours, concentrated to a thin syrup (about 40 cc.). The condensate was distilled with toluene in vacuo repeatedly, until it smelled no more of pyridine. The final dry residue was taken up in 50 cc. of water and extracted with 50 cc. of chloroform 6 times in a separatory funnel. The combined chloroformic solutions were distilled to about 50 cc. under reduced pressure and extracted with an equal volume of water many times (10 times). The watery solutions were united, condensed to a glassy solid. Yield 3.5 g. The product here gave two spots with $R_{N\text{-acetylglucosamine}}$ values of 3.2 and 3.4 coinciding with those of 1,2,3,6-tetraacetyl- α -glucosamine and of pentaacetyl- α -glucosamine in a paper chromatogram developed as above. Therefore, it was dissolved in 15 cc. of hot abs. ethanol and placed in the refrigerator for 5 days. The crystalline deposit (long needles), which was the pentaacetate, was eliminated, ether was added to the mother fluid to faint turbidity, and this mixture was placed in the refrigerator

for 1 week, whereby a small precipitate appeared. The supernatant fluid was decanted and concentrated by vacuum distillation. The hygroscopic still-residue amounted to 2.5 g. (45% of theory). $[\alpha]_D^{25}$ (abs. ethanol) = +87° (c 1.01). Fehling positive. Found: N, 3.7; CH₃CO, 50.5; OH, 4.0. C₁₄H₂₁O₉N requires N, 4.0; CH₃CO, 49.6; OH, 4.9.

α-Lactosamine-octaacetate (VI). A mixture of 2.5 g. of 1,2,3,6-tetraacetyl-*α*-glucosamine, 2 g. of silver oxide, 10 g. of Drierite⁷⁾ and 25 cc. of chloroform was agitated in the 50 cc. brown flask (a) in Fig. 1 for 5 hours, and after addition of 0.4 g. of iodine, 20 cc. of chloroform containing 3.2 g. of acetobromogalactose was further added in drops during 1 hour. The flask was taken off the apparatus, closed with a glass stopper (ground) and shaken for 5 days. The reaction mixture was filtered through charcoal, and the filtrate was washed with water thoroughly and distilled in vacuo to dryness. The still-residue was dissolved in 50 cc. of hot moist ether and placed in the refrigerator. The precipitate was recrystallized in a similar manner. 8 g. of crystals thus given was taken up in 15 cc. of chloroform, petroleum ether was added to permanent cloudiness, and the mixture was also stood in the refrigerator. After 1 week, the crystals in needles were subjected to four repetitions of crystallization. The product weighed 0.18 g. (4% of theory). F.P. 224–225°. $[\alpha]_D^{25}$ (chloroform) = +57.7° (c 1.07). It melted at 223–225° when mixed with the octaacetate derived according to Zilliken *et al.*⁸⁾ from N-acetylactosamine which had been separated from the group mucopolysaccharide of pig stomach mucus after Masamune and Yosizawa.⁹⁾ The product after deacetylation according to Kuhn *et al.*¹⁰⁾ showed a golden yellow spot with R_{lactose} 1.34 in a descending chromatogram by solvent butanol-pyridine-water (5:3:2) (20°C), when sprayed with the benzidine reagent of Raymonds and Morgan.⁵⁾

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