Carbohydrate Research, 35 (1974) 33–37

THE REACTION OF AMMONIA WITH ACETYL DERIVATIVES OF GENTIOBIOSE*†

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(Received December 26th, 1973; accepted for publication January 9th, 1974)

ABSTRACT

The reaction of aqueous ammonia with octa-*O*-acetylgentiobiose gives 1,1-bis-(acetamido)-1-deoxy-6-*O*-β-D-glucopyranosyl-D-glucitol (**1**) and *N*-acetyl-6-*O*-β-D-glucopyranosyl-β-D-glucofuranosylamine (**3**). The structure of the furanoid compound **3** was demonstrated by methylation and hydrolysis techniques, and its anomeric configuration through periodate oxidation and borohydride reduction.

INTRODUCTION

Most of the studies on the ammonolysis of acylated disaccharides have been conducted with substances having a (1→4)-glycosidic linkage[1]; this excluded the possibility of formation of an *N*-acyl-aldobiofuranosylamine through acyl migration. On the other hand, the lack of an acyl group on O-4 eliminated a contribution to the formation of sugars nitrogenated on C-1 that can be important[2].

The acylated disaccharides possessing a (1→6)-glycosidic linkage offer new structural possibilities, as the presence of an acyl group on O-4 and the possibility of cyclization could exert great influence on the favored formation of cyclic, nitrogenated products, among other competitive reactions[3]. This was evidenced in a previous study on ammonolysis of acetyl derivatives of melibiose[4], in which α-melibiose (7.7%), *N*-acetyl-6-*O*-α-D-galactopyranosyl-β-D-glucofuranosylamine (22.5%), and 1,1-bis-(acetamido)-1-deoxy-6-*O*-α-D-galactopyranosyl-D-glucitol (4.7%) were isolated. In this case, the overall yield of nitrogenated sugars (27%) did not differ from that obtained (~27%) from maltose octaacetate[5], despite the fact that this acetate lacked the acetyl group on O-4 presumably contributing highly to the migration pathway.

*Dedicated to Dr. Horace S. Isbell, in honor of his 75th birthday.
†The Reaction of Ammonia with Acylated Disaccharides. XII.
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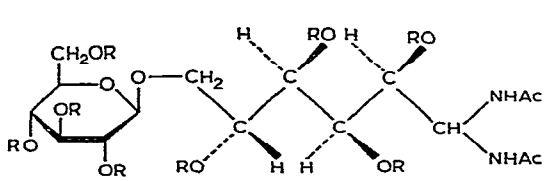
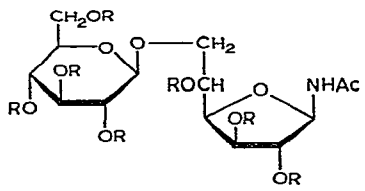
This suggested that the yields depend more on the general configuration of the sugar than on the magnitude of the individual contributing capacity of the acyl groups.

However, in the ammonolysis of octa-*O*-acetylgentiobiose with 25% aqueous ammonia, here reported, 1,1-bis(acetamido)-1-deoxy-6-*O*-*β*-D-glucopyranosyl-D-glucitol (**1**) and *N*-acetyl-6-*O*-*β*-D-glucopyranosyl-*β*-D-glucofuranosylamine (**3**) were obtained in 10 and 29% yields, respectively. The overall yield of nitrogenated sugars (39%) implies a substantial increase over that obtained for melibiose. As the sugar portion involved in the migration reactions is the same in the two disaccharides, the differences can be attributed to more difficult isolation of the melibiose derivatives. As expected, the presence of an acetyl group on O-4 increases the yield of nitrogenated compounds.

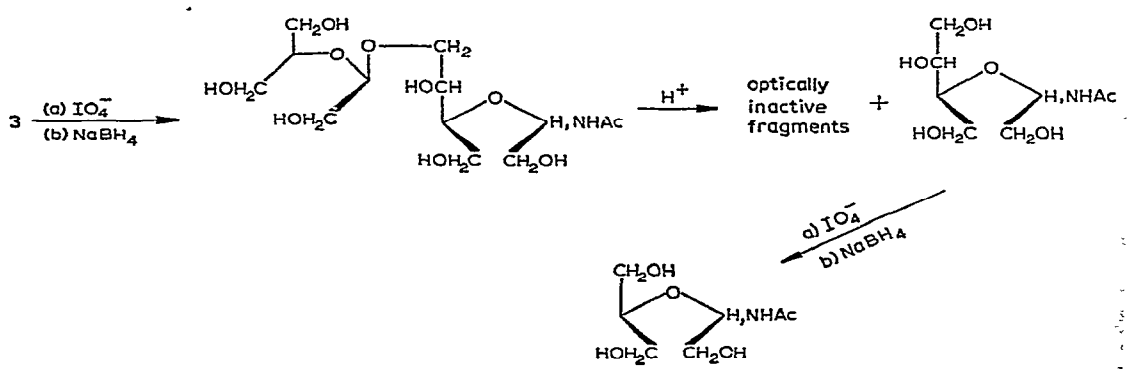


1 R = H
2 R = Ac



3 R = H
4 R = Ac

The furanose structure of **3** was ascertained by methylation, hydrolysis, and isolation of 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,5-tri-*O*-methyl-D-glucose. The anomeric configuration was determined by periodate oxidation and borohydride reduction[4,6] according to Scheme 1. The aldehyde groups produced were reduced with borohydride, the products hydrolyzed at room temperature, and the liberated glycols oxidized with periodate. After further reduction, the optical rotation of the solution was $-7.5°$, which agrees with that for the *β*-anomeric configuration[6].



Scheme I.

EXPERIMENTAL

General procedures. — Melting points are not corrected. Paper chromatography was conducted on Whatman No. 1 paper with the following eluants: (*A*) 10:4:3 (v/v) butyl alcohol–pyridine–water, (*B*) ethyl acetate–acetic acid–water (2:1:2, v/v; top layer), (*C*) butyl alcohol–ethanol–water (5:1:4, v/v; top layer), and (*D*) 5:2:2 (v/v) butyl alcohol–ethanol–water. The spray reagents were (*E*) silver nitrate–sodium methoxide[7] and (*F*) aniline hydrogen phthalate[8]. Evaporations were conducted under diminished pressure, below 60°. Optical rotations were measured at 22°.

Ammonolysis of octa-O-acetylgentiobiose with aqueous ammonia. Isolation of 1,1-bis(acetamido)-1-deoxy-6-O-β-D-glucopyranosyl-D-glucitol (**1**). — Octa-*O*-acetylgentiobiose[9] (13.5 g) was suspended in 25% aqueous ammonia (300 ml) and shaken until dissolution occurred (2.5 h). The solution was kept for 24 h at room temperature, and evaporated to dryness, and the dried, residual syrup was dissolved in methanol (200 ml). The solution was shaken with Zeo-Karb 225 (H^+) resin (20 g) to eliminate basic substances, the suspension was filtered, the filtrate evaporated to dryness, and the residual syrup dried in a vacuum desiccator and then extracted with ethyl acetate (5 × 50 ml) to remove acetamide.

The syrup was chromatographed on a column (55 × 3 cm) of charcoal (Darco G-60)–Celite 535 (5:1); elution was performed successively with increasing concentrations of ethanol in water as follows: 3% (fractions 1–6, 3 liters); 4.5% (fractions 7–15, 4.5 liters), 6% (fractions 16–19, 2 liters), 7% (fractions 20–21; 1 liter); 8% (fractions 22–25, 2 liters), 10% (fractions 26–29, 2 liters), 15% (fractions 30–31; 1 liter) and 95% (fractions 32–35, 2 liters).

By evaporation, and crystallization of the product from methanol, fractions 8–15 gave **1** as needles that, recrystallized from methanol, gave 915 mg (10% yield), m.p. 146–147°, $[\alpha]_D$ −19.6° (*c* 0.2, water).

Anal. Calc. for $C_{16}H_{30}N_2O_{12}.H_2O$: C, 41.73; H, 6.95; N, 6.08. Found: C, 41.87; H, 7.12; N. 5.80.

1,1-Bis(acetamido)-octa-O-acetyl-1-deoxy-6-O-β-D-glucopyranosyl-D-glucitol (**2**). — Compound **1** (100 mg) was dissolved at room temperature in 1:1 acetic anhydride–pyridine (8 ml). After 24 h at room temperature, the solution was evaporated in a vacuum desiccator; 180 mg of crude product was obtained that, by repeated recrystallization from 9:1 benzene–light petroleum, gave **2**, m.p. 107–109°, $[\alpha]_D$ −27.0° (*c* 0.2, chloroform).

Anal. Calc. for $C_{32}H_{46}N_2O_{20}$: C, 49.33; H, 5.96; N, 3.45. Found: C, 49.83; H, 6.06; N, 3.64.

Isolation of N-acetyl-6-O-β-D-glucopyranosyl-β-D-glucofuranosylamine (**3**). — The fractions (from chromatography on the charcoal column) that did not crystallize were collected, and dried to a syrup (4.4 g) that was chromatographed on a column (60 × 5 cm) of cellulose (Whatman), employing system (*A*) as the eluant. Twenty-five fractions (300 ml each) were collected. From fractions 5–9, a syrup (2.2 g, 30% yield)

was obtained that, on paper chromatography with solvents (*A*) and (*B*), and spraying with reagent (*E*), gave only one spot. This syrupy 3 had $[\alpha]_D - 9.3°$ (*c* 0.2, water).

Anal. Calc. for $C_{14}H_{25}NO_{11}$: C, 43.86; H, 6.53; N, 3.65. Found: C, 43.65; H, 6.51; N, 3.25.

From fractions 10–16 was obtained a syrup (2 g) that, on paper chromatography with system (*B*), showed gentiobiose and compound 3, but it was not possible to isolate the components pure.

N-*Acetyl-hepta*-O-*acetyl-6*-O-β-D-*glucopyranosyl*-β-D-*glucofuranosylamine* (**4**). — Compound 3 (100 mg) was dissolved in 1:1 acetic anhydride–pyridine (6 ml); the solution was kept for 24 h at room temperature and then evaporated to dryness in a vacuum desiccator. The residue was crystallized from ethanol, giving needles (80 mg) that, after repeated recrystallization from isopropyl alcohol–light petroleum, had m.p. 161–162°, $[\alpha]_D - 17.2°$ (*c* 1.02, chloroform).

Anal. Calc. for $C_{28}H_{39}NO_{18}$: C, 50.00; H, 6.10; N, 2.31. Found: C, 50.22; H, 6.08; N, 2.41.

Structure of N-*acetyl-6*-O-β-D-*glucopyranosyl*-β-D-*glucofuranosylamine*. — Compound 3 (175 mg) was dissolved in *N*,*N*-dimethylformamide (7 ml), and barium oxide (800 mg) and methyl iodide (1 ml) were added. After being shaken for 12 h at room temperature, the mixture was poured into chloroform (150 ml). The chloroform suspension was filtered, and the filtrate was washed successively with 0.5 M sulfuric acid, a saturated solution of sodium hydrogen carbonate, and water, dried (anhydrous sodium sulfate), and evaporated to dryness, and the residual syrup (191 mg) was hydrolyzed by boiling with 0.5M sulfuric acid (4 ml) during 6 h under reflux. After neutralization of the acid with barium carbonate, the suspension was filtered, and the filtrate was evaporated to dryness. On paper chromatography with solvent (*C*), the residual syrup showed two spots (revealed with reagent *F*). One of them coincided with that for a specimen of pure 2,3,4,6-tetra-*O*-methyl-D-glucose, and the other was an elongated spot of R_G 0.50 (using 2,3,4,6-tetra-*O*-methyl-D-glucose as the reference, methylated sugar).

The mixture was preparatively chromatographed on Whatman 3MM paper by employing solvent (*D*) as the eluant. The methylated sugars were separately extracted with methanol, and purified by successive and repeated dissolutions in water and ethyl ether, until clear, aqueous solutions were obtained. 2,3,4,6-Tetra-*O*-methyl-D-glucose gave $[\alpha]_D + 90.9°$ (*c* 0.8, water); lit.[10] $[\alpha]_D + 84°$ (water).

The other methylated sugar showed $[\alpha]_D - 13.1°$ (*c* 0.7, water), which agrees with the value for 2,3,5-tri-*O*-methyl-D-glucose, lit.[11] $[\alpha]_D - 13.4°$. [For 2,3,4-tri-*O*-methyl-D-glucose, lit.[12] $[\alpha]_D + 60.5°$ (water).]

Anomeric configuration of compound 3. — A solution of compound 3 (29.6 mg, 77 μmoles) in 0.1M sodium periodate (3.5 ml, 350 μmoles) was kept for 24 h at room temperature in the dark. A little ethylene glycol was added to decompose the excess of periodate, and then the solution was made alkaline with sodium hydrogen carbonate (10 mg), and treated with sodium borohydride (10 mg). The solution was acidified (to pH ~1) and was kept for 24 h at room temperature to cleave both moieties of the

degraded 3. Then, a little periodate was added, and the new aldehyde group thus produced was, after basification of the solution with sodium hydrogen carbonate, reduced with borohydride. In this way, the rotatory contributions from C-4 and C-5 were eliminated; that treatment left C-1 unaltered. The optical rotation of the final solution was $-7.5°$, in agreement with the range given[6] for oxidized *N*-acetyl-D-glycosylamines of β-anomeric configuration.

ACKNOWLEDGMENTS

We thank the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina and the Universidad de Buenos Aires for partial financial support.

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