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THE REACTION OF AMMONIA WITH ACYLATED DISACCHARIDES

XI. THE WOHL REACTION WITH OCTA-*O*-ACETYLMELIBIONONITRILE

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ABSTRACT

Octa-*O*-acetylmelibiononitrile (**1**) was prepared from melibiose oxime. The reaction of aqueous ammonia with **1** gave 1,1-bis(acetamido)-1-deoxy-5-*O*-α-D-galactopyranosyl-D-arabinitol (**2**), *N*-acetyl-5-*O*-α-D-galactopyranosyl-α-D-arabinofuranosylamine (**3**), and the anomeric pair of 5-*O*-α-D-galactopyranosyl-D-arabinofuranoses (**4** and **5**). The hepta-*O*-acetyl derivative of **2** was prepared, and the acyclic structure of the nitrogen-containing moiety was established by oxidation with periodate. The α anomeric configuration of **3** was demonstrated by periodate oxidation and subsequent reduction with sodium borohydride and hydrolysis.

INTRODUCTION

The action of ammonia upon acylated nitriles of aldonic acids has been extensively studied in the monosaccharide field[1]. Work previously published on acylated disaccharidic nitriles[2] showed that a similar degradation takes place, with formation of nitrogen-containing sugars as the principal, isolable substances. In this paper, we report on the reaction of octa-*O*-acetylmelibiononitrile with aqueous ammonia.

Zemplén[3] attempted the synthesis of octa-*O*-acetylmelibiononitrile (**1**), but obtained a syrup that contained only 64% of the nitrile. We have modified Zemplén's original technique, and prepared **1** in 66% yield as pure, amorphous material.

The reaction of **1** with 25% aqueous ammonia gave a syrup from which 1,1-bis(acetamido)-1-deoxy-5-*O*-α-D-galactopyranosyl-D-arabinitol (**2**) was isolated by fractional recrystallization; subsequently, by employing cellulose-column chromatography and preparative, paper chromatography, further amounts of **2** were isolated, as well as *N*-acetyl-5-*O*-α-D-galactopyranosyl-α-D-arabinofuranosylamine (**3**), 5-*O*-α-D-galactopyranosyl-α-D-arabinofuranose (**4**), and 5-*O*-α-D-galactopyranosyl-β-D-arabinofuranose (**5**).

Compounds **3**, **4**, and **5** obviously have a furanose structure for the arabinose

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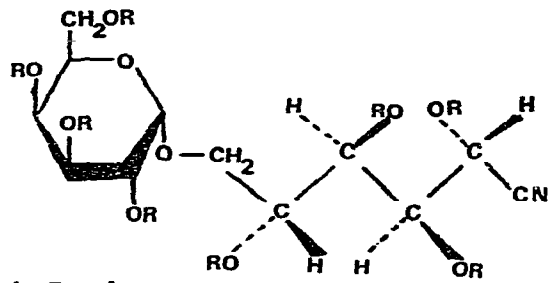
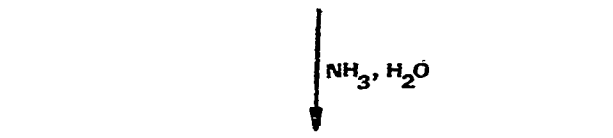
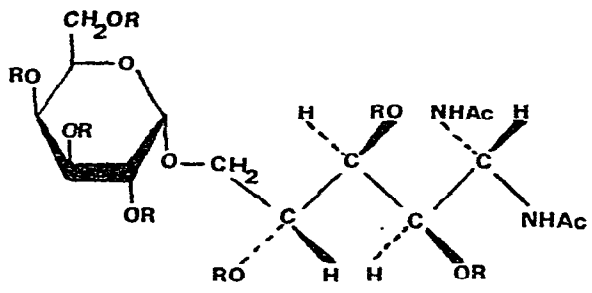


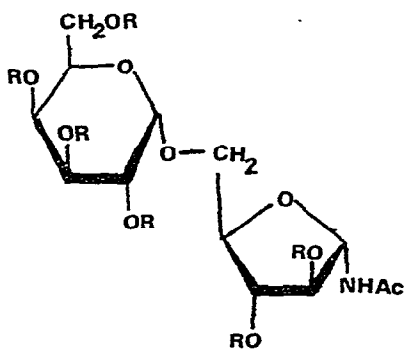
1 R = Ac





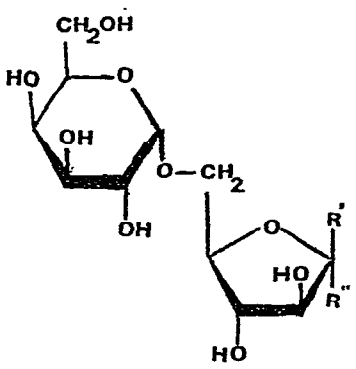
2 R = H

6 R = Ac



3 R = H

7 R = Ac

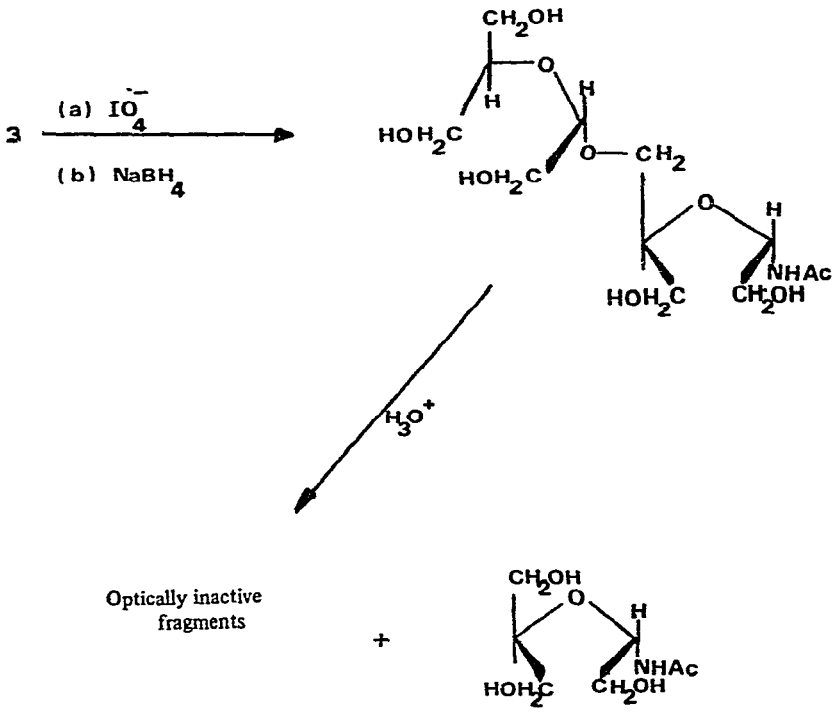


4 R′ = H ; R″ = OH

5 R″ = H ; R′ = OH

portion, as C-5 of this pentose residue is involved in the glycosidic linkage. The anomeric character of the reducing disaccharides **4** and **5** was established on the basis of their rotatory power.

The α anomeric configuration of **3** was assigned experimentally (see Scheme I) by oxidation thereof with periodate, followed by borohydride reduction of the



Scheme I.

aldehyde groups produced and subsequent hydrolysis at room temperature. This treatment eliminates all optically active centers, except C-1 of the original disaccharide. The optical rotation of the resulting solution was +11°; this confirmed the α-anomeric configuration of **3**, because, as demonstrated by Cerezo and Deulofeu[4] for some pairs of anomeric *N*-acetyl-D-glycosylamines, C-1 gives an optical rotation of about +7.1° to +9.5° (in water) for the α anomers.

EXPERIMENTAL

General procedures. — Melting points are not corrected. T.l.c. was performed on Silica Gel G (Merck), with the following mixtures as the mobile phase: (*A*) 49:1 benzene–abs. ethanol; (*B*) 17:3 benzene–abs. ethanol. The spots were detected with iodine vapor, and by the hydroxamic acid test[5].

Paper chromatography was conducted on Whatman No. 1 paper by the descending technique, using D-glucose as the standard, with 5:2:2 (v/v) butyl alcohol–ethanol–water, which was also used in cellulose-column chromatography employing Whatman cellulose CF 11, and in preparative, paper chromatography on 3 MM Whatman paper. Three developments were applied in the preparative, paper chromatography. The reagents used were: (*i*) silver nitrate–sodium methoxide[6] and (*ii*) aniline hydrogen phthalate[7]. The fractions from the cellulose column were evaporated under diminished pressure below 60°, and collected after comparison by paper chromatography. The fractions from the preparative paper were extracted with methanol. Optical rotations were measured at 20°.

Octa-O-acetylmelibiononitrile (**1**). — Sodium (1.54 g) was dissolved in absolute ethanol (60 ml), and the solution was slowly added, with stirring, to a solution, cooled to −5°, of hydroxylamine hydrochloride (7.2 g) in water (5.5 ml). After 0.5 h, the sodium chloride was filtered off, and washed with ethanol (10 ml).

α-Melibiose monohydrate (10 g) was dissolved in warm water (10 ml), and the ethanolic solution of hydroxylamine was slowly added at 60°. After 1 h at 65°, the mixture was concentrated, and melibiose oxime (10 g) was obtained as needles, m.p. 194–195° (dec.), $[\alpha]_D$ +95.9° (water); lit.[3] m.p. 184° (dec.), $[\alpha]_D$ +95° (water).

Anhydrous sodium acetate (10 g) was mixed with melibiose oxime (10 g) and acetic anhydride (60 ml) was added; the mixture was gradually and cautiously warmed on a warm-water bath, with continual shaking, a violent reaction being avoided by inmersion of the flask in ice-water when necessary. When the solid had dissolved, and the reaction had apparently ceased, the solution was kept for 80 min at 100° and then poured into ice-water (300 ml). After 24 h, the liquid was decanted, and the resulting gum was washed by stirring several times with cold water, until a powder was obtained; this was filtered off and washed with water until the washings were neutral. The crude octa-*O*-acetylmelibiononitrile was suspended in warm, light petroleum (b.p. 60–80°), and the gum obtained was cooled with stirring. A solid product (12.6 g, 65.8%) having m.p. 64–66° (softened at 55°), $[\alpha]_D$ +100.6° (*c* 0.8, chloroform) was obtained; t.l.c. (solvent *A*) showed only one spot, R_F 0.64.

Anal. Calc. for $C_{28}H_{37}NO_{18}$: C, 49.75; H, 5.52; N, 2.07. Found: C, 49.43; H, 5.71; N, 1.66.

Ammonolysis of octa-O-acetylmelibiononitrile. 1,1-Bis(acetamido)-1-deoxy-5-O-α-D-galactopyranosyl-D-arabinitol (**2**). — Compound **1** (10 g) was dissolved in 25% aqueous ammonia (250 ml) by shaking for 3 h. After 24 h at room temperature, the solution was evaporated to dryness, and the residual syrup was extracted with hot ethyl acetate (6 × 50 ml) to remove acetamide. The residual syrup was dried in a vacuum desiccator, and was then dissolved in methanol at room temperature. Compound **2** was obtained as needles that, after three recrystallizations from methanol, gave 0.58 g, m.p. 163–165°, $[\alpha]_D$ +78.7° (*c* 0.8, water). Paper chromatography (reagent *i*) gave only one spot, of R_g 0.64. Spraying with reagent (*ii*) did not show any spot, indicating its nonreducing character.

The mother liquors from **2** were evaporated and the resulting syrup (10.9 g) was

chromatographed on a cellulose column (45 × 940 mm); 100 fractions (100 ml each) were collected. Only compound **2** (0.39 g), m.p. 163–165° was isolated.

The fractions (from the column) that did not give crystalline products were collected and evaporated, and the residual syrup was chromatographed on Whatman 3 MM paper, giving 0.18 g of compound 2. The total amount of **2** was 1.16 g (19% yield).

Anal. Calc. for $C_{15}H_{28}N_2O_{11}$: C, 43.68; H, 6.79; N, 6.79. Found: C, 43.25; H, 7.26; N, 6.47.

From the preparative chromatography on 3 MM paper, the following compounds were also obtained.

N-*Acetyl-5*-O-α-D-*galactopyranosyl*-α-D-*arabinofuranosylamine* (**3**). This compound was isolated as a syrup (0.38 g, 7.3%), $[\alpha]_D$ +210.7° (*c* 0.56, water) that, on paper chromatography (reagent *ii*), showed nonreducing character. With reagent (*i*), it showed R_g 0.93.

Anal. Calc. for $C_{13}H_{23}NO_{10}$: C, 44.19; H, 6.51; N, 3.96. Found: C, 44.36; H, 6.27; N, 3.81.

5-O-α-D-*Galactopyranosyl*-α-D-*arabinofuranose* (**4**). Compound **4** was isolated as a syrup showing $[\alpha]_D$ +98° → +82° (*c* 0.91, water; 66 h). Paper chromatography (reagents *i* and *ii*) gave only one reducing spot, R_g 0.44.

Anal. Calc. for $C_{11}H_{20}O_{10}$: C, 42.30; H, 6.41. Found: C, 42.30; H, 6.73.

5-O-α-D-*Galactopyranosyl*-β-D-*arabinofuranose* (**5**). Compound **5** was isolated as a syrup (0.11 g, 2.3%) showing $[\alpha]_D$ +76.2° → +82.2° (*c* 1.01, water, 96 h). Paper chromatography (reagents *i* and *ii*) showed only one reducing spot, R_g 0.18.

Anal. Calc. for $C_{11}H_{20}O_{10}$: C, 42.30; H, 6.41. Found: C, 42.15; H, 6.79.

1,1-Bis(*acetamido*)-*hepta*-O-*acetyl-1-deoxy-5*-O-α-D-*galactopyranosyl*-D-*arabinitol* (**6**). — Compound **2** (60.8 mg) was dissolved in 1:1 pyridine–acetic anhydride (7 ml) by heating for 3 min in a boiling-water bath. The mixture was kept for 24 h at room temperature, and evaporated to dryness in a vacuum desiccator. Recrystallization from methanol gave 103 mg (99%) of **6** as needles, m.p. 193–194°, $[\alpha]_D$ +130.8° (*c* 0.58, chloroform). T.l.c. (solvent *B*) showed one spot, R_F 0.43.

Anal. Calc. for $C_{29}H_{42}N_2O_{18}$: C, 49.29; H, 5.95; N, 3.96. Found: C, 48.98; H, 6.06; N, 3.66.

N-*Acetyl-hexa*-O-*acetyl-5*-O-α-D-*galactopyranosyl*-α-D-*arabinofuranosylamine* (**7**). — Compound **3** (34 mg) was dissolved in 1:1 pyridine–acetic anhydride (1.2 ml) by heating for 5 min in a boiling-water bath. The mixture was kept for 24 h at room temperature, and then evaporated to dryness in a vacuum desiccator. The resulting syrup was purified by preparative t.l.c., with solvent *B* as the eluant. Compound **7** (30.5 mg, 52.4%) was isolated as a syrup giving one spot, R_F 0.37, on t.l.c. (solvent *B*), $[\alpha]_D$ +64.9° (*c* 1.37, chloroform).

Anal. Calc. for $C_{25}H_{35}NO_{16}$: C, 49.59; H, 5.79; N, 2.31. Found: C, 49.31; H, 5.20; N, 2.47.

Oxidation of compound **2**. — A solution of this compound (3.1 mg) in 15mM sodium periodate (3.9 ml) was kept at 5°. Samples (0.1 ml) were taken at intervals, and

diluted with water to 25 ml; the periodate uptake was determined by spectrophotometric methods[8], by using a Beckman DU spectrophotometer at 222.5 nm.

The results were: (time in h, moles of periodate uptake per mole) 0.16 (1.35), 4 (2.53), 24 (3.02), 28 (3.98), 91 (3.98), and 115 (3.98). Formaldehyde was not detected[9].

Anomeric configuration of **3**. — A solution of compound **3** (35.3 mg, 0.1 mmole) in 0.1M sodium periodate (3.5 ml, 0.35 mmole) was kept in the dark for 24 h at room temperature. A few drops of ethylene glycol were added to decompose the excess of periodate, and then the solution was made alkaline with solid sodium hydrogen carbonate (10 mg) and treated with sodium borohydride (10 mg). The solution was kept for 24 h at room temperature, and was then acidified (pH ~1) and kept for 24 h at room temperature to cleave both portions of the degraded **3**. The optical rotation of the final solution was +11°, in agreement with the range given by Cerezo and Deulofeu[4] for oxidized *N*-acetyl-D-glycosylamines of α anomeric configuration.

Hydrolysis of compounds **4** *and* **5**. — Compounds **4** and **5** (10 mg) were hydrolyzed separately by dissolving in 0.5M sulfuric acid (3 ml). The solution was kept for 1 h in a boiling-water bath, and then made neutral with barium carbonate; the suspension was filtered, and the filtrate was concentrated to 0.5 ml. Paper chromatography in several solvent systems gave two reducing spots, identified with D-arabinose and D-galactose standards.

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