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Note

A simple preparation of 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucose and -D-mannose*

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2-Acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucose (**5**, *N*-acetyl-lactosamine) is a disaccharide occurring naturally in milk. It is also one of the disaccharide units frequently found in glycoproteins[1] and glycolipids[2]. Synthesis of **5** has been accomplished by β-D-galactosylation of properly protected 2-acetamido-2-deoxy-D-glucose derivatives[3], or by transformation of 3-*O*-β-D-galactopyranosyl-D-arabinose (**1**), which is now commercially available. Kuhn and Kirschenlohr[4] reported the synthesis of **5** from **1** by the Kiliani reaction (addition of hydrogen cyanide), whereas we described a similar synthesis using the nitromethane addition-reaction[5]. The Kiliani reaction-conditions employed by Kuhn and Kirschenlohr require a large quantity of anhydrous hydrogen cyanide, and are thus undesirable. Although our procedure was developed in order to avoid the use of an excess of hydrogen cyanide, it requires more steps than the Kiliani method.

In view of the growing interest in biochemical and biological reactions involving **5**, it became desirable to improve the synthesis of **5**, for easier preparation and for potential, isotopic labeling. One of the avenues that we have investigated is to conduct the Kiliani reaction with a limited proportion of KCN. While our work was in progress, Walker and Barker[6] reported a synthesis of 2-amino-2-deoxy-D-[1-^{13}C]glucose by a modified Kiliani reaction in which only an approximately equimolar proportion of sodium [^{13}C]cyanide was used, and H^{13}CN was generated *in situ* by addition of glacial acetic acid, thus avoiding the handling of HCN. We report here a simple method of preparing **5** by using a procedure similar to that reported by Walker and Barker[6].

Both the Kiliani reaction and the nitromethane addition create a new chiral carbon atom in the product, so that a pair of 2-epimers is formed. In the present

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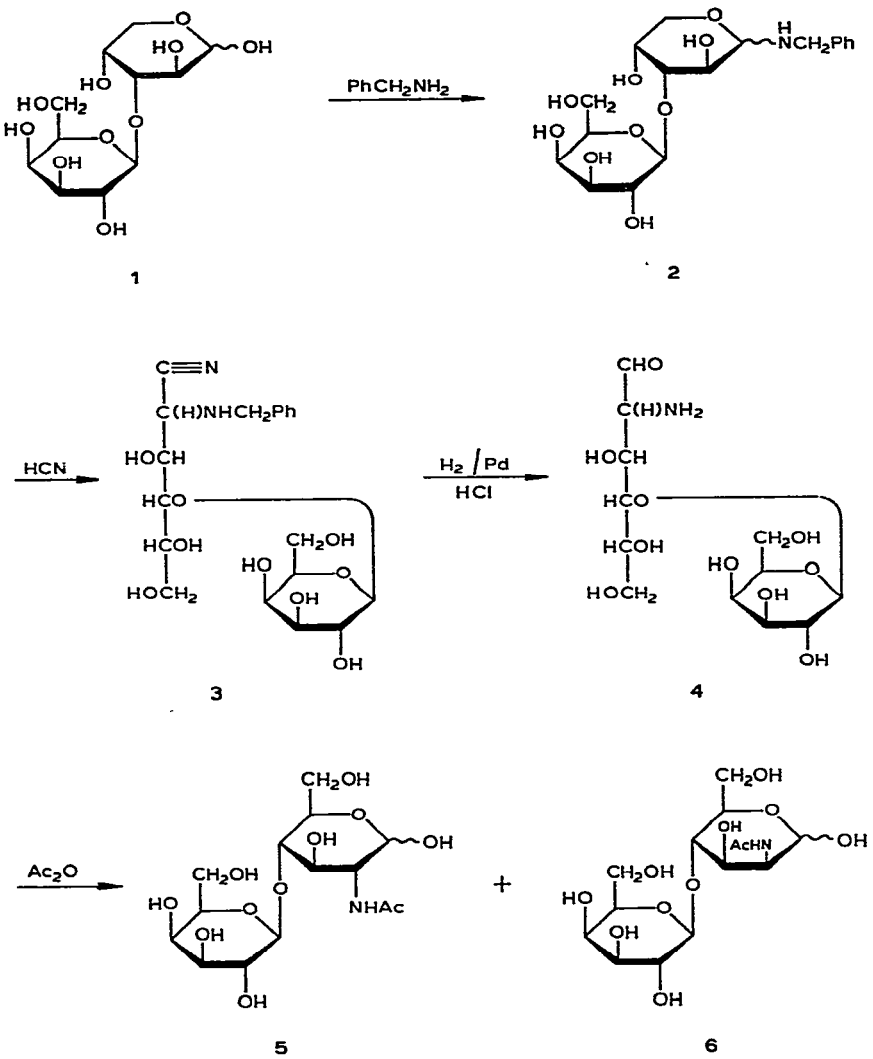

method, the products are 4 to 1 in favor of **5** over its 2-epimer, 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-mannose (**6**), whereas, in the nitromethane addition-reaction[5], **6** is favored 2 to 1 over **5**.

EXPERIMENTAL

General. — The following were obtained as indicated: 3-*O*-β-D-galactopyranosyl-D-arabinose (**1**) from Pfanstiehl Labs, Inc.; 2,4,6-trinitrobenzenesulfonic acid (TNBS) from Sigma Chem. Co.; benzylamine and 10% palladium-on-carbon from J. T. Baker Chem. Co.; Rexyn 201 from Fisher Scientific Co.; and Dowex-1 X-8 and Bio-Gel P-2 from BioRad Labs.

For the determination of the carbohydrate components in column effluents, a modified phenol–sulfuric acid method was used[7]. The amino group was estimated by a modified TNBS method[8], using 6-aminohexanoic acid as the standard. Products were analyzed by means of a neutral-sugar analyzer[9], using a column (0.2 × 28 cm) of Rexyn 201 and 0.27M sodium borate buffer[5], pH 7.8, as the eluant at 56°.

Thin-layer chromatography (t.l.c.) was performed on layers of silica gel G-60 precoated on aluminum sheets (E. Merck), using solvent *A*, 9:4:2 (v/v) ethyl acetate–isopropyl alcohol–water; *B*, 3:2:1 (v/v) ethyl acetate–acetic acid–water; and *C*, 5:5:1:1 (v/v) ethyl acetate–pyridine–acetic acid–water. The carbohydrate components were made visible by spraying the t.l.c. plates with 15% sulfuric acid in 50% ethanol and heating them at 140°. For the detection of amino groups, plates were sprayed with 5% ninhydrin in acetone, and heated briefly.

N-*Benzyl-3*-O-(*β*-D-*galactopyranosyl*)-D-*arabinosylamine* (**2**). — A suspension of finely pulverized 3-*O*-*β*-D-galactopyranosyl-D-arabinose (**1**; 12.6 g, 40 mmol) in dry ethanol (100 mL) and benzylamine (7 mL) was heated under reflux for ~1.75 h (until an almost clear solution resulted). The opalescent solution was filtered while warm, and the filtrate was kept at room temperature to allow crystallization of **2**, which was filtered off, and washed well with dry ethanol; yield, 14.95 g (37 mmol), m.p. 117–118° (lit.[4] m.p. 125–126°). On concentration of the mother liquor, a further 0.2 g (0.5 mmol) of **2** was obtained; total yield, 94%.

2-Benzylamino-2-deoxy-4-O-β-D-galactopyranosyl-D-glucononitrile and -D-mannononitrile. — A suspension of pulverized **2** (4 g, 9.9 mmol) in dry ethanol (65 mL) was magnetically stirred and heated in a three-necked, round-bottomed flask equipped with a reflux condenser and a dropping funnel. When **2** began to dissolve, pulverized KCN (0.68 g, 10.4 mmol) was added, and heating was continued until complete dissolution of KCN occurred. The mixture was then quickly cooled to room temperature in a cold-water bath. Glacial acetic (0.69 mL, 12 mmol) in dry ethanol (5 mL) was added dropwise, and the mixture was kept for 15 min. T.l.c. in solvent *A* then showed essentially a single, u.v.-absorbing (and charring) spot, R_F 0.62, indicating that the cyanide addition had proceeded rapidly to completion. (Compound **2** is non-u.v.-absorbing, and has R_F 0.14 in solvent *A*.) To the mixture was added ether (300 mL), and the precipitate (**3**) was washed with ether and dried in a desiccator, yielding 4.7 g of a solid.

2-Amino-2-deoxy-4-O-β-D-galactopyranosyl-D-glucose and -D-mannose (**4**). — The solid **3** precipitated by ether in the previous step was dissolved in M HCl (30 mL). To this solution was added 10% Pd-on-carbon (6 g), and the mixture was hydrogenated in a Brown hydrogenator for 17 to 20 h, at which time the nitrile was no longer present (t.l.c. in solvent *B*). The mixture contained one major (R_F 0.14) and one minor (R_F 0.27) carbohydrate component. The major component is apparently composed of two amino-containing disaccharide isomers; the minor component matched **1** in R_F in solvent *B*. Dowex-1 X-8 (HCO_3^-) resin (10 g) was added to the mixture and, after foaming had subsided, the suspension was filtered through Celite. The solid on the filter was washed three times with 50% ethanol (20 mL),

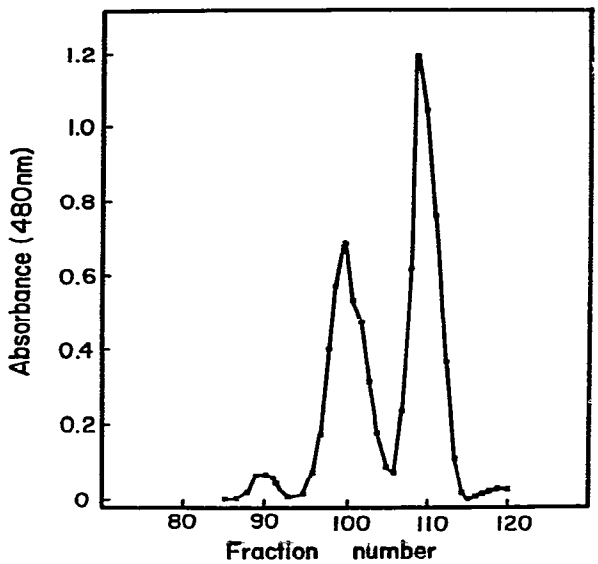

Fig. 1. Fractionation, on a column of Bio-Gel P-2, of the reaction mixture containing **5** and **6**. [Fractions (20-mL) were collected, and a 10-μL aliquot from each fraction was assayed by the phenol–sulfuric acid method.]

and the filtrates were combined and evaporated to a syrup (**4**) that was dissolved in water (30 mL). Analysis for the primary amino group by the TNBS method showed the yield of amino-containing compounds to be 80% overall.

2-Acetamido-2-deoxy-4-O-β-D-galactopyranosyl-D-glucopyranose (**5**) *and 2-acetamido-2-deoxy-4-O-β-D-galactopyranosyl-D-mannopyranose* (**6**). — Potassium hydrogencarbonate (4 g, 40 mmol) was dissolved in the aqueous solution (30 mL) of **4** obtained in the previous step, and a solution of acetic anhydride (2 mL, 20 mmol) in acetone (15 mL) was added. After 0.5 h at room temperature, the mixture contained a negligible amount of primary amino groups (as determined by the TNBS method). The mixture was evaporated briefly to remove acetone, adjusted to pH 6 with 60% acetic acid, and concentrated to ≈30 mL. The mixture was purified in two batches on a column (5 × 160 cm) of Bio-Gel P-2, using 0.1M acetic acid as the eluant; the elution profile is shown in Fig. 1. The following fractions were combined, and evaporated: fractions 96–100 (mainly **5**), fractions 101–105 (**5** and **6**), and fractions 107–112 (**1**). The middle fractions (101–105), containing both **5** and **6**, were re-chromatographed on the column of Bio-Gel P-2, to separate **5** from **6**. From these evaporated fractions, **5** and **6** were obtained as crystals (from water–ethanol), and **1** was obtained as a white powder; m.p. of **5**, 168–169° (lit.[4] m.p. 168–170°); of **6**, 230° (lit.[10] 237°). The yield, from 9.9 mmol of **2**, was 3.22 mmol of **5**, 0.8 mmol of **6**, and 1.06 mmol of **1**; the overall yield of **5** and **6** from **2** was 41%, and of **1**, 38.5%.

Products were identified by comparison with the standard compounds, using (*i*) t.l.c. in solvent *A* or *B*, (*ii*) a sugar analyzer, and (*iii*) analysis of the acid hydrolyzate. In the sugar analyzer, the elution time is characteristic of a particular compound.

This value is 1.4 × the void volume for **5**, 2.4 × the void volume for **6**, and 3.1 × the void volume for **1**. For acid hydrolysis, samples were heated in 2M trifluoroacetic acid for 2.5 h at 100°, and the solutions evaporated to remove the acid, and then analyzed by t.l.c. in solvent *C*. The R_F values are: D-galactose, 0.41; D-arabinose, 0.56; 2-amino-2-deoxy-D-glucose, 0.12; and 2-amino-2-deoxy-D-mannose, 0.16. In each of the aforementioned, analytical methods, the three products were identified as the compounds designated.

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