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SYNTHESIS OF *N*-ACETYLLACTOSAMINE CONTAINING A D-[6-^{3}H]GALACTOPYRANOSYL GROUP

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ABSTRACT

A simple and convenient method for the introduction of radiolabel onto C-6′ of *N*-acetyllactosamine is described. 1-*N*-Benzyl-3-*O*-β-D-galactopyranosyl-D-arabinosylamine (**1**) was synthesized from 3-*O*-β-D-galactopyranosyl-D-arabinose as described by Lee and Lee. Compound **1** was oxidized with D-galactose oxidase, and the product reduced with KB^3H_4 to introduce the label at C-6′. After dilution with unlabeled material, the *N*-benzyl-3-*O*-β-D-[6-^{3}H]galactopyranosyl-D-arabinosylamine was converted into 2-(benzylamino)-2-deoxy-4-*O*-D-[6-^{3}H]galactopyranosyl-D-gluconononitrile, which was subjected to simultaneous hydrogenolysis of the benzylamino and nitrile groups. *N*-Acetylation of the amino group as described by Alais and Veyrières afforded the crystalline title compound in 63% yield.

INTRODUCTION

The disaccharide 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucose (*N*-acetyllactosamine) is an integral portion of the nonreducing termini of many complex oligosaccharides[1–5]. During the course of the assembly of complex oligosaccharides, this disaccharide unit serves as an acceptor for the glycosyltransferases responsible for the biosynthesis of α-(2→3)- and α-(2→6)-sialyl linkages[6,7], of α-(1→2)-L-fucosyl linkages[8], and of α-(1→3)-D-galactosyl linkages[9]. The recent trend toward obtaining structural information regarding oligosaccharide biosynthesis through the use of radioisotopically labeled carbohydrate residues[9–12] has prompted interest in the synthesis of ^{3}H-labeled *N*-acetyllactosamine. This synthesis was accomplished through a combination of enzymic and chemical reactions.

RESULTS AND DISCUSSION

The series of chemical and enzymic reactions described herein provides a simple and convenient method for the introduction of a tritium radiolabel into the D-galactosyl group of *N*-acetyllactosamine. Specificity studies with D-galactose

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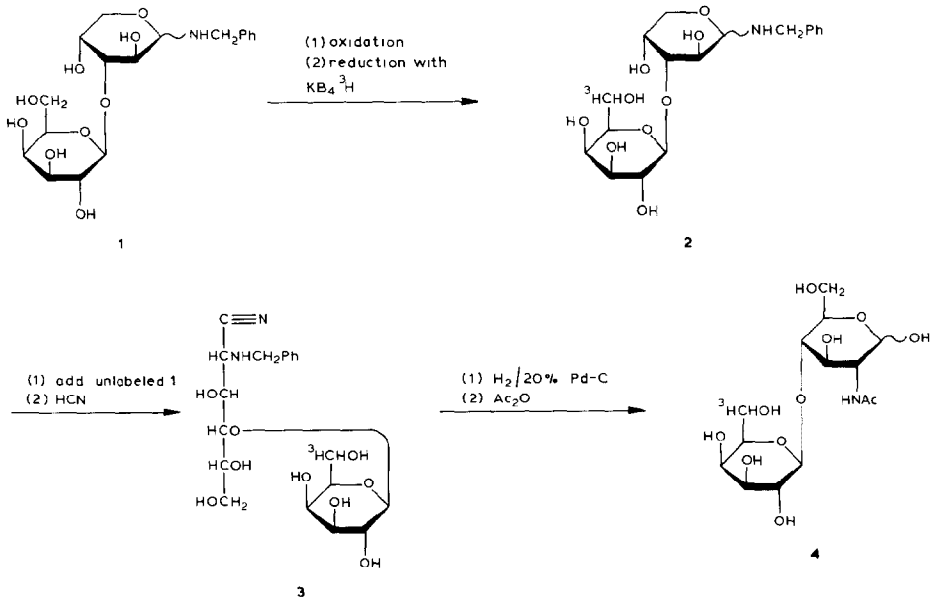
oxidase[13,14] had demonstrated that *N*-acetyllactosamine itself is a relatively poor substrate for the enzyme. In addition, treatment of the so-oxidized *N*-acetyllactosamine with borohydride would reduce not only the aldehydo group introduced on C-5′ of the (non-reducing) D-galactosyl group, but also the (reducing) terminal D-glucose residue, thus yielding a product other than the original disaccharide.

Lee and Lee[15] described a simplified, chemical synthesis of *N*-acetyllactosamine that involves three intermediate compounds. We have discovered that both the starting compound for this synthesis, 3-*O*-β-D-galactopyranosyl-D-arabinose, and its *N*-benzylglycosylamine derivative (**1**) could serve as substrates for D-galactose oxidase. In a continuous, spectrophotometric assay, performed as described by Rao and Mendicino[16], 2mM final concentrations of both 3-*O*-β-D-galactopyranosyl-D-arabinose and its *N*-benzylamino derivative (**1**) reacted with D-galactose oxidase, and yielded rates ~13% of that determined for 2mM free D-galactose.

The benzylamino derivative **1** was chosen for subsequent experiments, as the benzylamino group on C-1 prevented any reduction thereat. Treatment of **1** with D-galactose oxidase introduced a borohydride-reducible group exclusively at C-6 of the D-galactosyl group, and treatment of the oxidation product with KB^3H_4 generated **2**, the original compound, but now specifically labeled at C-6′. After dilution with "cold" **1**, the synthesis of the desired disaccharide **4** was completed, *via* **3**, to afford, in 63% yield, crystalline, labeled *N*-acetyllactosamine (**4**) having a specific activity of 1.38×10^5 c.p.m. μmol^{-1}. For the experiments described herein, the radiolabeled material was diluted with unlabeled carrier before subsequent synthetic steps. The specific activity of the final product may thus be lowered by dilu-



tion with unlabeled material (or be increased by use of a KB^3H_4 preparation of higher specific activity).

EXPERIMENTAL

General methods. — D-Galactose oxidase (EC 1.1.3.9) and catalase (EC 1.11.1.6) were purchased from Sigma Chemical Co. KB^3H_4 (46.25 GBq/mmol) was a product of New England Nuclear, and 2,5-diphenyloxazole was obtained from Research Products International Corp. The following were obtained from the sources indicated: 3-*O*-β-D-galactopyranosyl-D-arabinose from Pfanstiehl Labs., Inc., and palladium hydroxide-on-carbon (Pearlman's catalyst) and benzylamine from Aldrich Chemical Co. Plastic-backed Polygram silica gel G plates (250 μm thick), from Brinkmann Instruments, Inc., were used in detection of the radiolabeled *N*-benzyl-3-*O*-β-D-galactopyranosyl-D-arabinosylamine. T.l.c. was performed on precoated plates of silica gel G-60 (Brinkmann Instruments, Inc.). Unless otherwise stated, the compounds were detected with a spray containing 5% each of ammonium molybdate, phosphoric acid, and sulfuric acid, followed by heating the plates for 10 min at 140°.

N-Benzyl-3-*O*-β-D-galactopyranosyl-D-arabinosylamine (**1**) was synthesized as described by Lee and Lee[15].

1-N-*Benzyl-3*-O-β-D-[*6-^{3}H*]*galactopyranosyl*-D-*arabinosylamine* (**2**). — The enzymic oxidation was conducted in a total volume of 5 mL; this contained **1** (100 mg, 0.25 μmol), D-galactose oxidase (5 mg), catalase (0.5 mg), and toluene (0.05 mL) in 0.1M phosphate buffer, pH 7.0. After incubation for 24 h at 25°, the pH of the mixture was brought to 10 by the addition of M NaOH, and KB^3H_4 (5 μmol, 2.313 GBq) was added. After incubation for 30 min at 40°, unlabeled $NaBH_4$ (25 μmol, dissolved in 0.1M NaOH) was added, and reduction was continued for an ad-

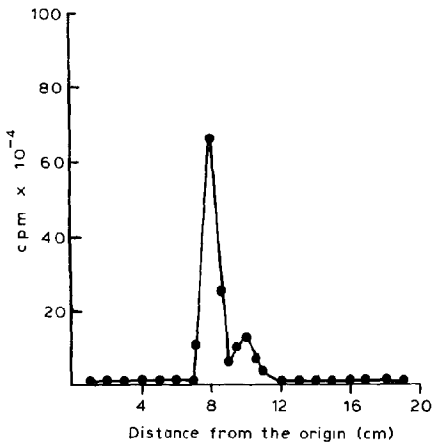


Fig. 1. Thin-layer chromatography of 1-*N*-benzyl-3-*O*-β-D-[6-^{3}H]galactopyranosyl-D-arabinosylamine (**2**).

ditional 30 min. The excess of borohydride was decomposed by acidification with 0.5M H_2SO_4. When the pH reached 5.5, the mixture was incubated for 16 h at 0°, transferred to a round-bottomed flask, and evaporated to dryness. An aliquot of a solution of the residue in ethanol was spotted on a silica-gel plate marked in 0.5-cm segments, 1 cm wide. Standards of *N*-benzyl-3-*O*-β-D-galactopyranosyl-D-arabinosylamine and 3-*O*-β-D-galactopyranosyl-D-arabinose were applied on both sides of the 1-cm segment that contained the radiolabeled compound.

After the plate had been developed with 3:3:2 ethyl acetate–2-propanol–water, the radiolabeled compound was located by cutting the chromatogram into 0.5-cm segments, and counting (see Fig. 1) in a scintillation fluid containing 4.0 g of 2,5-diphenyloxazole/L of toluene.

*2-Benzylamino-2-deoxy-4-*O*-β-*D*-[6-^{3}H]galactopyranosyl-*D*-glucononitrile* (**3**). — "Cold" **1** (0.4 g) was added to the ^{3}H-labeled compound (**2**), unseparated from salts, and the mixture was treated with sodium cyanide and glacial acetic acid as described by Alais and Veyrières[17]. The mixture was stirred for 6 h; undissolved starting-material was then filtered off, and the filtrate was stored overnight in the cold. Addition of ethanol–ether gave a hygroscopic solid (0.7 g); t.l.c. in 3:3:2 (v/v) ethyl acetate–2-propanol–water showed the product (R_F 0.56) to contain a minor impurity (R_F 0.25).

*2-Acetamido-2-deoxy-4-*O*-β-*D*-[6-^{3}H]galactopyranosyl-*D*-glucopyranose* (**4**). — To a solution of the crude nitrile **3** in 0.5M hydrochloric acid (18 mL) was added palladium hydroxide-on-carbon (0.3 g), and the mixture was hydrogenated for 12 h at room temperature and atmospheric pressure; the catalyst was filtered off through Celite, and washed with water. The filtrate and washings were combined, the pH adjusted to 6 by addition of solid sodium hydrogencarbonate, and the mixture was processed and the product *N*-acetylated as described by Alais and Veyrières[17]. The crystalline product (1.38×10^5 c.p.m./μmol), obtained in 63% yield, was homogeneous by t.l.c. in 2:2:1 2-propanol–acetone–lactic acid, the plate being sprayed with a reagent consisting of aniline (4 mL), diphenylamine (4 g), acetone (200 mL), and 85% H_3PO_4 (30 mL).

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