SYNTHESIS OF N-ACETYL-LACTOSAMINE CONTAINING A D-[6-3H]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-D-galactopyranosyl-D-arabinosylamine (1) was synthesized from 3-O-D-galactopyranosyl-D-arabinose as described by Lee and Lee. Compound 1 was oxidized with D-galactose oxidase, and the product reduced with KB₃H₄ to introduce the label at C-6'. After dilution with unlabeled material, the N-benzyl-3-O-[6-3H]galactopyranosyl-D-arabinosylamine was converted into 2-(benzylamino)-2-deoxy-4-O-D-[6-3H]galactopyranosyl-D-glucononitrile, 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. 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, of α-(1→2)-L-fucosyl linkages, and of α-(1→3)-D-galactosyl linkages. The recent trend toward obtaining structural information regarding oligosaccharide biosynthesis through the use of radioisotopically labeled carbohydrate residues has prompted interest in the synthesis of 3H-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
oxidase\textsuperscript{13,14} had demonstrated that N-acetylactosamine itself is a relatively poor substrate for the enzyme. In addition, treatment of the so-oxidized N-acetylactosamine 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\textsuperscript{15} described a simplified, chemical synthesis of N-acetylactosamine that involves three intermediate compounds. We have discovered that both the starting compound for this synthesis, 3-O-\textbeta-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\textsuperscript{16}, 2mM final concentrations of both 3-O-\textbeta-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\textsuperscript{3}H\textsubscript{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-acetylactosamine (4) having a specific activity of \(1.38 \times 10^5\) c.p.m. \(\mu\)mol\textsuperscript{-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$_3$H$_4$ 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$_3$H$_4$ (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-[6-³H]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$_3$H$_4$ (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-[6-³H]galactopyranosyl-D-arabinosylamine (2).
ditional 30 min. The excess of borohydride was decomposed by acidification with 0.5M H₂SO₄. 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-[6-³H]galactopyranosyl-D-glucononitrile (3). — "Cold" 1 (0.4 g) was added to the ³H-labeled compound (2), unseparated from salts, and the mixture was treated with sodium cyanide and glacial acetic acid as described by Alais and Veyrières. 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 (Rₚ 0.56) to contain a minor impurity (Rₚ 0.25).

2-Acetamido-2-deoxy-4-O-β-D-[6-³H]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 hydrogen carbonate, and the mixture was processed and the product N-acetylated as described by Alais and Veyrières. The crystalline product (1.38 x 10⁵ 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% H₃PO₄ (30 mL).

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REFERENCES

6'-3H-LABELLED N-ACETYLACTOSAMINE