THE SYNTHESIS OF 4-*O*-β-D-GALACTOSYL-D-GLUCOSE (LACTOSE)[1]

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ABSTRACT

4-*O*-β-D-Galactosyl-D-glucose (lactose) has been synthesized using a modification of the Koenigs–Knorr reaction.

INTRODUCTION

4-*O*-β-D-Galactosyl-D-glucose (lactose) occurs in all mammalian milk to the extent of about 5%. It has also been reported in the long-styled pollen of forsythia flowers. It was prepared in 1942 by Haskins, Hann, and Hudson (1) from epilactose (4-*O*-β-D-galactosyl-D-mannose), but has not previously been synthesized directly.

One of the most useful methods of disaccharide synthesis is that due to Koenigs and Knorr (2). They prepared tetra-*O*-acetyl-α-D-glucopyranosyl-bromide and found that this, in the presence of silver carbonate or dry pyridine, reacted with methanol to form methyl-β-D-glucopyranoside. The reaction—essentially the elimination of hydrogen halide between a glycosyl halide and a hydroxyl group—was adapted for disaccharide synthesis by Helferich (3) and by Hudson (4). They allowed the acetobrom sugar to react with a suitably substituted sugar derivative in a dry inert medium in the presence of silver carbonate or silver oxide. With modern refinements—use of "Drierite" as drying agent and small amounts of iodine as catalyst—good yields can be obtained in the case of $1 \rightarrow 6$ linked hexose disaccharides, where a primary hydroxyl group is involved. However, up to the present only small yields have been obtained in the case of disaccharides where a secondary hydroxyl group is involved. This is due to the lesser reactivity of the secondary hydroxyl group and to steric hindrance by the alcohol component due to the presence of the hemiacetal ring and the substituent groups. It was thought that this second factor might be lessened—and thus greater yields obtained—by the use of an open-chain sugar derivative as the alcohol reactant. Here the accessibility of the hydroxyl group is much greater than in the corresponding ring compound.

In the present work 2,3-5,6-di-*O*-isopropylidene-D-glucose diethyl acetal was condensed with acetobrom-D-galactose under standard Koenigs–Knorr conditions (5). The reaction mixture was boiled with sodium hydroxide solution to remove acetyl groups from the product and to destroy excess acetobrom-galactose, cooled, and deionized. Hydrolysis of isopropylidene and acetal groups was carried out with hot dilute acetic acid. The resulting mixture of mono- and di-saccharides was separated by charcoal- and paper-chromatography. Lactose was obtained crystalline in 35% yield, which is well in excess of the yields usually obtained from Koenigs–Knorr syntheses where a secondary hydroxyl group is involved. Two other disaccharide fractions were obtained in small yield. These have not been completely identified but presumably result from isomers of 2,3-5,6-di-*O*-isopropylidene-D-glucose diethyl acetal. These are inevitably present in small amount as a result of the method of preparation of this compound (via a mercaptal mixture (8) consisting mainly of the 2,3-5,6-di-*O*-isopropylidene derivative).

EXPERIMENTAL

Optical rotations were measured in water at 21°±2° C. The following solvent systems (v/v) were used to separate sugars on paper chromatograms: (A) ethyl acetate: acetic acid: formic acid: water, 18:3:1:4; (B) *n*-butanol:ethanol: water, 3:1:1. Sugars were

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detected with the *p*-anisidine hydrochloride spray (6). Solutions were concentrated under reduced pressure at 40° C.

D-*Glucose Diethyl Thioacetal* (*Ref. 7*)

D-Glucose (60 g), ice-cold ethanethiol (60 ml), and ice-cold concentrated hydrochloric acid (60 ml) were shaken together in a stoppered flask, and left at room temperature. After 3 hours the material had formed a crystalline mass. Methanol (*ca.* 300 ml) was added and sufficient lead carbonate to neutralize the solution, which was then filtered and the precipitate washed with boiling methanol. The crude product crystallized from the filtrate on cooling and was recrystallized from methanol. Yield: 62 g (65%), m.p. 119–120° C.

Di-O-isopropylidene-D-*glucose Diethyl Thioacetal* (*Ref. 8*)

Glucose diethyl thioacetal (36 g) was added to a mixture of dry acetone (350 ml) and concentrated sulphuric acid (8.5 g) and the resulting solution was left at room temperature for 72 hours. The solution was neutralized with a solution of barium hydroxide in methanol, and filtered. The filtrate was evaporated to a pale yellow mobile syrup. Yield: 35 g.

Di-O-isopropylidene-D-*glucose Diethyl Acetal*

The method used was similar to that employed by Wolfrom *et al.* (9) in the preparation of D-galactose diethyl acetal pentaacetate. Di-*O*-isopropylidene-D-glucose diethyl thioacetal (40 g), mercuric oxide (80 g), and lead carbonate (20 g) were added to dry ethanol (400 ml) and the solution heated to 70–80° C while it was being stirred. A solution of mercuric chloride (176 g) in dry ethanol (300 ml) was then added and the solution stirred for 5 hours at 70–80° C with occasional addition of mercuric oxide. The solution was filtered hot and the precipitate washed with hot ethanol. The filtrate was evaporated to a small volume and then diluted with chloroform (400 ml). This chloroform solution was extracted with water until chloride-free and dried over Drierite. Filtration and subsequent evaporation yielded a syrup (30 g), which was purified by distillation, a fraction (22 g) being collected with b.p. 130–140° C at 0.05 mm.

Condensation of Di-O-isopropylidene-D-*glucose Diethyl Acetal with Acetobromo*-D-*galactose*

Di-*O*-isopropylidene-D-glucose diethyl acetal (15 g, 0.0449 mole) was dissolved in dry, alcohol-free chloroform (200 ml) and to the solution were added Drierite (20 g), silver oxide (20 g), and glass beads (35 g). The solution was shaken in the dark. After 4 hours acetobrom-D-galactose (46 g, 0.112 mole) in dry, alcohol-free chloroform (150 ml) and iodine (1 g) were added and the shaking continued. After 42 hours a test for ionizable bromine was negative. The solution was filtered, evaporated to a syrup, and dissolved in a solution of sodium hydroxide (20 g) in methanol (400 ml). This was heated under reflux for 3.5 hours, cooled, and de-cationized on Amberlite IR-120 resin in the cold. The acidic eluate was heated at 65° C for 1 hour in order to hydrolyze isopropylidene and acetal groups, and then de-ionized by passage through a column of Duolite A-4 resin. Concentration of the eluate yielded a syrup (20 g) which was shown by paper chromatography to consist, in addition to glucose and galactose, of a main fraction with R_{gal} 0.45 (solvent A) and 0.28 (solvent B) and two much smaller spots running slightly in front of, and behind, this spot.

A portion of the syrup (8.1 g) was fractionated on a column of Darco charcoal (4×5 cm) (10). Elution with water removed all the monosaccharide and in the final stages a faint trace of the slower-moving components: subsequent elution with ethanol/water (5:95) removed the bulk of the slower-moving components (2.9 g). This latter syrup

was dissolved in a little moist methanol, nucleated with authentic lactose, and the solvent allowed to evaporate at room temperature. Part of the material crystallized, 1.8 g being obtained. It was recrystallized from moist methanol and was found chromatographically to be a single substance indistinguishable from lactose. Hydrolysis yielded galactose and glucose in equal amounts. It had m.p. 201–202° C undepressed by admixture with an authentic specimen of lactose monohydrate, and $[\alpha]_D$ +86.3 (5 min) → +58.1° (16 hr). The infrared spectra of the material and of authentic lactose monohydrate were identical. Calculated for $C_{12}H_{22}O_{11}.H_2O$: C, 40.00%; H, 6.67%. Found: C, 40.19%; H, 6.82%.

Part of the syrup which failed to crystallize (0.6 g) was separated on filter paper (Whatman No. 3 MM). A further quantity of lactose (0.20 g) was obtained in addition to two other fractions: I (51.2 mg) and II (49.9 mg). Fraction I had R_{gal} 0.33 (solvent A) and 0.15 (solvent B) while II had R_{gal} 0.47 (solvent A) and 0.32 (solvent B). Both fractions yielded galactose and glucose in equal amounts on hydrolysis.

Periodate Oxidation of Fractions I and II

Portions of fractions I and II were oxidized with aqueous sodium metaperiodate solution using standard procedures. The periodate uptake was estimated by the method of Neumüller and Vasseur (11) and the formic acid liberation by that of Andrews *et al.* (12).

The molar periodate consumptions per mole of disaccharide were as follows: fraction I, 3.90 (after 25 min), 4.44 (1 hr), 4.75 (3.5 hr), and 4.83 (22 hr); fraction II, 2.35 (0.5 hr), 2.84 (1 hr), 3.08 (4 hr), and 3.56 (22 hr). The molar productions of formic acid were: fraction I, 3.77 (after 40 min), 4.29 (1.5 hr), 4.92 (4 hr), and 5.34 (22.5 hr); fraction II, 1.01 (40 min), 1.25 (1.5 hr), 1.41 (4 hr), and 2.12 (22.5 hr). Extrapolation of the flat part of the curve to zero time gave values of: fraction I, periodate uptake = 4.78 moles per mole of sugar, formic acid production = 4.41 moles per mole; fraction II, periodate uptake = 2.95 moles per mole sugar, formic acid production = 1.17 moles per mole.

The figures for fraction I are difficult to interpret. The large amount of formic acid could not have come from any normal type of sugar and it may be that this fraction is a mixture of disaccharide and some other material. The figures for fraction II—where the slope of the flat part of the periodate and formic acid curves indicates that over-oxidation was taking place—are those to be expected of a 1 → 3 linked hexopyranose disaccharide. Crystalline derivatives of fractions I and II have not yet been obtained.

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