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The Synthesis and Characterisation of 4-α,6-β-Bis-D-Glucopyranosido-D-Glucose *

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The chemical synthesis of 4-α,6-β-bis-D-glucopyranoside-D-glucose has been accomplished. Chemical and enzymic evidence in support of its structure is described. The trisaccharide hendeca-acetate failed to anomerise when treated with titanium tetrachloride.

Although branched polysaccharides have been known for many years, the isolation and proper characterisation of a branched oligosaccharide has not been reported as a product of hydrolysis from such polymers. (The term branched oligosaccharide will be used to refer to saccharides in which at least two sugar residues are glycosidically linked to a third sugar residue). The chemical [1-3] and enzymic [4] syntheses of several branched trisaccharides have been reported. Klemer [1] reported the synthesis of [4-α, 6-β-bis-D-glucopyranosido-D-glucose (I) and in a later communication [5] determined the velocity constants for the hydrolysis of the glycosidic bonds in this substance.

Some years ago one of us [6] (B.L.) tried to synthesise the same trisaccharide; the synthesis was however not accomplished, due to inadequate fractionation of the final product. The present paper describes the synthesis of the trisaccharide by the route previously reported, confirming the specific optical rotation reported by Klemer and adduces chemical and enzymic evidence in support of its structure.

Essentially, the synthesis involves a Koenings-Knorr condensation of tetra-*O*-acetyl-α-D-glucopyranosyl bromide and 1,2,3,2',2',4',6'-β-maltose heptaacetate [6]. The product, after deacetylation was resolved by charcoal column chromatography [7]. The yield of the amorphous trisaccharide was 8.4 %. It had $[\alpha]_D^{20}$ + 83° in water; Klemer [1] reports + 84°.

The following experimental evidence is presented in support of its representation as I:

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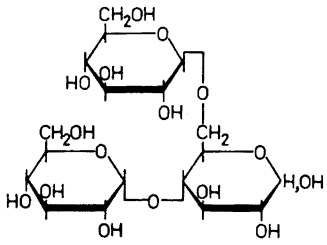
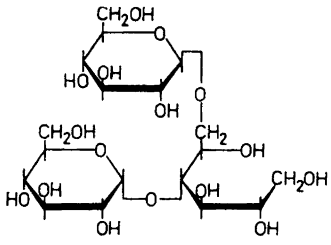


I



II

(*a*) Partial hydrolysis gives rise to glucose, maltose, gentiobiose and unchanged I.
(*b*) Reduction followed by partial hydrolysis affords glucose as the only saccharide reducing to the *p*-anisidine spray reagent.
(*c*) Emulsin cleaves I to give D-glucose and maltose.
(*d*) Paper electrophoresis in borate buffer of pH 10 (M_G 0.37) indicates the presence of a 1,4- or 1,2-linked residue on the reducing moiety.
(*e*) Reduction of I affords II. Periodate oxidation of II resulted in the consumption of 5.9 moles of periodate with the liberation of 3.1 and 0.95 moles of formic acid and formaldehyde, respectively. (Theory requires 6, 3, and 1 moles).
(*f*) Reduction of periodate oxidised II with borohydride and hydrolysis gives glycerol and erythritol in the relative proportions of 2:1.

The only structure which is in agreement with these facts is I. The conversion of gentiobiose octaacetate to an isomaltose derivative on treatment with titanium tetrachloride [8] suggested that I might undergo a similar transformation to 4-*α*,6-*α*-bis-D-glucosido-D-glucose. This branched trisaccharide would be expected as a product of partial hydrolysis from amylopectin and glycogen. I, however, failed to undergo this anomerisation, possibly because the glucosyl substituent in the 4-*O*-position of the reducing residue of gentiobiose prevents the formation of the reactive intermediate, through which a successful anomerisation must pass.

A further attempt to obtain the *α*-anomer was made using quinoline [9] as the acid acceptor in the condensation of tetra-*O*-acetyl-*α*-D-glucopyranosyl bromide and the maltose heptaacetate.

EXPERIMENTAL

All evaporations were conducted *in vacuo*, bath temp. 35—40°. Paper chromatography was conducted on Whatman No. 1 and preparative separations on Whatman 3 MM.

Chromatographic solvents:

1. Ethyl acetate-pyridine-water (10:4:3)
2. Ethyl acetate-acetic acid-water (3:1:3)
3. Dry benzene (on dimethyl sulphoxide impregnated paper [10])

Chromatographic spray reagents:

1. Acetonic silver nitrate and ethanolic sodium hydroxide.
2. *p*-Anisidine hydrogen chloride.

Melting points are corrected.
Optical rotations determined in water (saccharides) or chloroform (acetates), $c = 1$.

4-α,6-β-Bis-D-glucosido-D-glucose

To a solution of 1,2,3,2′,3′,4′,6′-β-maltose heptaacetate [6] (7.3 g, m.p. 147—149°, $[\alpha]_D^{20}$ +64°) in dry, ethanol-free chloroform (35 ml) was added silver oxide (5.0 g) and Drierite (5.0 g) and the mixture was shaken in the absence of light for 1 h at room temperature. A solution of tetra-*O*-acetyl-α-D-glucopyranosyl bromide (4.9 g) and iodine (0.5 g) in dry, ethanol-free chloroform (30 ml) was added in 4 equal portions over 1 h and the mixture shaken mechanically for 72 h. The reaction mixture was filtered through a layer of Celite, washed with dilute sodium thiosulphate solution and water and dried over sodium sulphate. Concentration of the chloroform solution yielded a yellow syrup which crystallised spontaneously and from which unchanged maltose heptaacetate (0.85 g) was recovered. (Chromatography of the remaining material in solvent 3, spray reagent 1, revealed the presence of maltose heptaacetate and two slower components, having $R_{\text{Maltose heptaacetate}}$ 0.65 and 0.34.)

The acetylated mixture was deacetylated by the method of Zemplén, deionised (Amberlite IR 120 and Dowex 3) and concentrated, to give a clear, colourless syrup (5.1 g). Paper chromatographic analysis in solvent 1 gave glucose, maltose and a trisaccharide (R_{Gl} 0.41) with traces of a slower component (R_{Gl} 0.24).

The syrup was fractionated on a charcoal-Celite column (4 × 43 cm) which was eluted first with water, then with a linear gradient of aqueous ethanol (10 l, 0 — 20 %). 40 ml fractions were collected. D-Glucose (1.8 g), m.p. 148—149°, $[\alpha]_D^{20}$ +52° (final) and maltose (2.6 g), m.p. 163—166°, $[\alpha]_D^{20}$ +126° (final) were obtained and characterised, maltose also as the β-octaacetate, m.p. 159—160°, $[\alpha]_D^{20}$ +61°.

The major portion of the trisaccharide (I) was eluted in fractions 158—176. Concentration followed by extraction with 70 % methanol gave I (0.40 g), $[\alpha]_D^{20}$ +82°, contaminated with a trace of maltose.

Higher fractions, including 50 % ethanol eluant, contained I, contaminated with some saccharides having lower R_F-values. The combined material (0.49 g) was refractionated on a smaller column, yielding further amounts of pure I. The total yield of I was 0.49 g.

Structural studies on I

On electrophoresis in borate buffer (pH 10) maltose, gentiobiose and I had the M_G-values 0.36, 0.71, and 0.37, respectively.

I (2 mg) was hydrolysed in 0.33 N sulphuric acid (0.5 ml) at 100° for 0.5 h. Neutralisation followed by chromatography in solvent 2 gave glucose, maltose (R_{Gl} 0.46), gentiobiose (R_{Gl} 0.36) and unchanged I (R_{Gl} 0.19).

I (20 mg) in water (5 ml) was treated with emulsin at pH 5.5 and 25° for 7 days. A chromatographic investigation of the digest showed the presence of glucose, maltose and unchanged I. The components were separated on paper and glucose and maltose isolated and characterised as the crystalline compounds.

I (33 mg) in water (10 ml) was treated with sodium borohydride (20 mg) After standing overnight at room temperature the solution was deionised and boric acid removed by distillation with methanol. Purification on paper gave the trisaccharide alcohol (II) as a syryp (24 mg), $[\alpha]_D^{20}$ +53°. II and its acetate failed to crystallise. Partial acid hydrolysis of II (same conditions as above) gave glucose as the only reducing sugar, using spray reagent 2. Spray reagent 1 also revealed the presence of II and of disaccharide alcohols.

II (19.0 mg) was dissolved in 0.06 N sodium metaperiodate (10 ml) and stored in the dark for one week. After that period periodate consumption (5.9 moles), liberation of formic acid (3.1 moles), and of formaldehyde [11] (0.95 moles) were determined on 1 ml samples of the solution. The remaining solution was neutralised with barium carbonate, filtered and treated with sodium borohydride (0.030 g). After standing overnight the solution was deionised, concentrated and boric acid removed by distillation with methanol. The residue was dissolved in N sulphuric acid (1 ml) and kept at 70° for one hour. Neutralisation (barium carbonate), filtration, concentration and extraction with methanol gave

a syrup consisting solely of glycerol and erythritol (paper chromatography, solvents 1 and 2). The components were separated on paper and the glycerol:erythritol ratio determined to be 2.1:1 by the method of Lambert and Neish [11].

Attempted synthesis of 4-α,6-α-bis-D-glucopyranosido-D-glucose.

(*a*) I (0.38 g) was acetylated with acetic anhydride and pyridine. The resulting anomeric mixture of hendecaacetates (0.75 g) was dissolved in dry, ethanol-free chloroform (10 ml). A solution of titanium tetrachloride (0.7 ml) in the same solvent (10 ml) was added whereupon a canary-yellow precipitate formed. The mixture was refluxed on a glycerol bath (70°) for 5 h, cooled and worked up according to Lindberg [8], yielding the saccharide acetates as a colourless syrup (0.73 g). The mixture was deacetylated by the method of Zemplén. Chromatography (solvent 1, spray 1) revealed the presence of glucose, maltose and gentiobiose in small amounts, together with the major component, a trisaccharide with the same R_F as I. The latter was purified on paper (solvent 2), yielding the original trisaccharide I (0.25 g), $[\alpha]_D^{20}$ +83°. Treatment as above with emulsin gave glucose, maltose and unchanged I.

(*b*) A mixture of 1,2,3,2′,3′,4′,6′-β-maltose heptaacetate (2.0 g) and tetra-*O*-acetyl-α-D-glucopyranosyl bromide (1.3 g) was dissolved in quinoline, to which had been added Drierite (1.0 g). The mixture was shaken in the absence of light for 24 h and then kept at 50° for 3 h. After cooling chloroform (40 ml) was added and the chloroform solution washed successively with water, dilute sulphuric acid, dilute sodium hydrogen carbonate, water, and dried over sodium sulphate. Concentration of the chloroform solution gave a light red syrup which was deacetylated as above. Chromatographic analysis revealed the presence of glucose, maltose, trisaccharide I and traces of other saccharides. Charcoal column chromatography, followed by fractionation on paper, gave I (65 mg), $[\alpha]_D^{20}$ +79°.

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