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A Chemically-Synthesised Galactoglucan *

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The addition polymerisation of a mixture of 1,6-anhydro-β-D-glucopyranose and 1,6-anhydro-β-D-galactopyranose has given rise to a synthetic galactoglucan. A high molecular weight fraction was selected for study. The results indicate a highly branched structure with a high proportion of α- and 1,6-glycosidic linkages.

The acid-catalysed condensation of simple sugars to afford synthetic polysaccharides has been of increasing interest in recent years [1–5]. An alternative approach to such polysaccharides involves addition polymerisation of anhydro sugars.

Pictet [6] initiated such studies as early as 1918. Recently Carvalho and Schuerch [7] improved the conditions for the polymerisation of 1,6-anhydro-β-D-glucopyranose and investigated the nature of the product formed. The polymerisation of other anhydro sugars was also reported by Schuerch and coworkers.[8,9]

This paper describes the copolymerisation of 1,6-anhydro-β-D-glucopyranose and 1,6-anhydro-β-D-galactopyranose to yield a synthetic heteropolymer. A mixture of these sugar derivatives, in equal proportions, containing monochloroacetic acid as catalyst, was heated in a sealed tube to 106°. The material, a brittle glass, was completely soluble in water and was subjected to fractionation by addition of ethanol to its aqueous solution. After two fractionations a fraction, corresponding to 10 % of the monomers, was obtained, which showed $[\alpha]_D^{20}$ +95° and was completely immobile on paper chromatograms. After dialysis against distilled water 92 % of the material was recovered, and used in subsequent studies. It was found to consist solely of D-glucose (52 %) and D-galactose (48 %) residues and to have a $\overline{DP}_n$ of about 90, as determined osmometrically.

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Table 1. Substances, isolated after periodate oxidation, borohydride reduction and hydrolysis of the synthetic polysaccharide.

Substance	Molar %		
	Quantitative determination	By weight	Calculated [a]
Glycerol	58.6	53.7	55
Erythritol	16.0	16.5	20
D-Glucose	12.9	11.7	25
D-Galactose	10.2	10.3	
Arabinose	2.3	7.0[b]	—

[a]) From consumption of periodate and liberation of formic acid.
[b]) Also containing small amounts of xylose.

Treatment of the synthetic polysaccharide with sodium metaperiodate resulted in the consumption of 1.3 moles of periodate and the liberation of 0.55 moles of formic acid per hexose residue. This is equivalent to a content of 55 % 1 → 6-like bonds, 20 % 1 → 4-like bonds and 25 % 1 → 3-like bonds in the polysaccharide, assuming only pyranosidic residues.

The periodate-oxidised polysaccharide was reduced with sodium borohydride and hydrolysed to afford glycerol, erythritol, D-glucose, D-galactose and small amounts of what appeared to be arabinose and xylose. These products (with the exception of the last two) were identified as the crystalline substances or converted to crystalline derivatives. The amount of each was determined analytically as well as by isolation. These values are collected in Table 1. It can be seen that there is reasonably good agreement between the values noted and the results of the periodate oxidation. The presence of threitol in the "erythritol fraction" was considered a possibility, but paper electrophoresis in sulphonated phenyl boronic [10] acid at pH 6.5 failed to reveal its presence.

Partial hydrolysis gave a mixture of oligosaccharides, one of which was obtained crystalline and proved to be melibiose (6-*O*-*α*-D-galactopyranosyl-D-glucose.

It is apparent from the foregoing that a galactoglucan has been prepared chemically. The fact that the material contains approximately equal amounts of D-glucose and D-galactose residues together with the isolation of melibiose indicates a true heteropolymer as opposed to a mixture of glucan and galactan.

The rotation of the synthetic polymer ($[\alpha]_D^{20}$ + 95° in water) is similar to those of synhtetic glucose polymers [1,7] and indicates the presence of a relatively high content of *α*-glycosidic linkages.

The liberation of 0.55 moles of formic acid on periodate oxidation together with the high proportion of glyceritol isolated from the polyalcohol indicates a highly branched structure or a high content of 1 → 6-linked residues or both possibilities. The latter alternative is considered most probable.

The failure to detect any glycerinaldehyde in the hydrolysate of the polyalcohol suggests the lack of D-glucose or D-galactose residues linked solely in the C-2 position or in the C-2 and C-6 positions. On the other hand, the yields of unoxidised D-glucose (12 %) and D-galactose (10 %) indicates the presence or 1,3-linkages or residues which are multiply linked, *e.g.* containing 1,2- and 1,4-linkages.

Erythritol arises from those D-glucose residues which are linked 1,4 or 1,4 and 1,6; the appearent absence of threitol suggests the lack of D-galactose residues, linked in an analogous manner. The presence of arabinose and xylose cannot be accounted for. They could arise from suitably protected furanosidic residues (cleavage between C-5 and C-6).

EXPERIMENTAL

All melting points corrected.
Paper chromatograms were run on Whatman 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) 80 % Aqueous acetone.
Chromatographic spray reagents:
1) Acetonic silver nitrate and ethanolic sodium hydroxide.
2) *p*-Anisidine hydrogen chloride.
3) Periodate-benzidine.

Paper electrophoresis was conducted in borate buffer (pH 10), germanate buffer[11] (pH 10.4) and sulphonated phenylboronic acid[10] (pH 6.5).

All optical rotations were determined in water, *c*, 1.0.

Copolymerisation of 1,6-anhydro-D-glucopyranose and 1,6-anhydro-D-galactopyranose. An intimate mixture of 1,6-anhydro-D-glucopyranose (6.70 g), 1,6-anhydro-D-galactopyranose (6.70 g) and monochloroacetic acid (0.670 g) in a Pyrex tube was dried *in vacuo* at 25° for 0.5 h, sealed and heated at 106° for 16 h. The reaction product, a dark, amber-coloured solid, which fissured upon cooling, was dissolved in water (50 ml) containing sodium hydrogen carbonate (0.65 g). The dark brown solution was filtered, the volume was adjusted to 200 ml and ethanol (930 ml) was added slowly with stirring. The syrup which formed was collected by centrifugation and washed with 85 % ethanol. On treatment of the syrup with anhydrous ethanol a brittle powder was obtained which was washed several times with ethanol and ether and dried *in vacuo* at 40°. The product (9.0 g), $[\alpha]_D^{20}$ +94°, on paper chromatography in solvent 1 showed the absence of low molecular weight substances.

Fractionation of the synthetic polysaccharide. The polysaccharide (8.0 g) was dissolved in water (160 ml) and ethanol added dropwise while stirring mechanically. At intervals the precipitate, a syrup, was collected by centrifugation and worked up as before. The following fractions were obtained:

Fraction 1. 65.5 % ethanol. Yield 2.3 g, $[\alpha]_D^{20}$ +95°.

Fraction 2. 71.5 % ethanol. Yield 1.8 g, $[\alpha]_D^{20}$ +92°.

Fraction 3. 76 % ethanol. Yield 1.5 g, $[\alpha]_D^{20}$ +90°.

Fraction 1 was subfractionated by precipitation of its aqueous solution (50 ml) with ethanol.

Fraction 1A 65.5 % ethanol. Yield 1.33 g.
Fraction 1B 71.5 % ethanol. Yield 0.57 g.
Fraction 1C 76 % ethanol. Yield 0.21 g.

Fraction 1A was dialysed against distilled water. A recovery of 1.22 g, $[\alpha]_D^{20}$ +95°, resulted. $\overline{DP}_n$ of fraction 1A, using a Zimm and Myerson [12] osmometer and »Ultrafein allerfeinst" membrans (Membranfiltergesellschaft, Göttingen) was determined in water to be about 90.

Acid hydrolysis of fraction 1A in 1 N sulphuric acid at 100° for 10 h yielded only D-glucose (52 %) and D-galactose (48 %), both sugars being isolated in a crystalline state and characterised.

Periodate oxidation. Icecold 0.061 N sodium periodate solution was added to a sample of fraction 1A (0.350 g, 4.65 % water) to a volume of 200 ml and the mixture kept at 2°. A portion of the periodate solution served as blank. At intervals, aliquots were withdrawn for determination of periodate consumption and formic acid liberation. After 8 days the values became constant: periodate consumption, 1.3 moles per mole hexose residue; formic acid liberation, 0.55 moles per mole hexose residue.

Reduction and hydrolysis of the periodate oxidised polysaccharide. The reaction mixture was neutralised (barium carbonate), centrifuged and the solution poured into a solution of borohydride (1.0 g) in water (25 ml). After standing overnight, the solution was neutralised (acetic acid), deionised (Amberlite IR 120, Dowex 3) and boric acid removed by distillation with methanol.

The product was hydrolysed by treatment with 0.6 N sulphuric acid (25 ml) at 100° for 12 h, neutralized (barium carbonate), filtered, deionised and concentrated to give a yellow syrup (0.2 g). Paper chromatography (solvent 2, spray 1 + 2) showed the presence of glyceritol (R_{Gl} 2.42) erythritol (R_{Gl} 1.93) glucose, galactose (R_{Gl} 0.95), arabinose (R_{Gl} 1.36) and xylose (R_{Gl} 1.51). The last substance was indistinguishable from xylose also on chromatography in solvent 1 and electrophoresis in germanate buffer.

Part of the syrup (0.020 g) was fractionated on thick filter paper, the components eluted with water and determined quantitatively, the polyols according to Lambert and Neish [13], the sugars according to Saeman *et al.*[14]. The results are presented in Table 1.

The remainder of the syrup (0.16 g) was fractionated on filter paper and the various components purified by extraction with methanol and concentrated to give the amounts, shown in Table 1. D-Glucose, m.p. 144—145°, $[\alpha]_D^{20}$ +57° (final), D-galactose, m.p. 165—166°, $[\alpha]_D^{20}$ +80° (final) and erythritol, m.p. 119—120°, crystallised and were indistinguishable from authentic samples. Glycerol was converted to its tri-*O*benzoyl derivative, m.p. and mixed m.p. 71—72°. The mother liquors from the erythritol, when subjected to electrophoresis in borate and sulphonated phenyl boronic acid appeared to contain no threitol.

Partial hydrolysis. A portion of the synthetic polysaccharide (0.75 g) was dissolved in 0.5 N sulphuric acid (100 ml) and kept on a boiling water bath for 3.5 h. The solution was neutralised (barium carbonate), filtered and concentrated to a syrup which upon chromatographic analysis in solvent 1 showed the presence of glucose, galactose and a number of oligosaccharides, all of which having lower R_F-values than that of maltose. The syrup was placed on a charcoal-Celite column (3.5 × 23.5 cm) which was eluted, first with water (2 l), then with a linear gradient of aqueous ethanol (5 l, 0 → 25 %). Fractions (40 ml) were collected and the elution followed by determination of the optical rotation and by chromatography in solvent 3.

Fractions 119—125 were combined and concentrated. Chromatography in solvent 1 showed the presence of two components with R_{Gl} values 0.70 and 0.62, respectively. Separation on paper (solvent 2) gave melibiose (12 mg), R_{Gl} 0.70, which crystallised as the dihydrate upon seeding with authentic material, m.p. and mixed m.p. 81—83°, $[\alpha]_D^{20}$ +125° (final).

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