Carbohydrate Research, 161 (1987) c1–c3
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Preliminary communication

Inversion of the 2-hydroxyl groups of D-glucosyl units in (1→3)-β-D-glucan

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(Received September 9th, 1986; accepted for publication in revised form, December 26th, 1986)

In the chemical synthesis of polysaccharides[1,2], much effort has been directed towards control of the linkage type and configuration, because the identity of the constituent monosaccharides is fixed by the starting material. There is another way[3,4] in which to obtain an artificial polysaccharide having a regular structure; it involves retaining the glycosidic linkage and configuration of a natural polysaccharide, and converting its monosaccharide units into specified, new monosaccharide units. In previous papers[5,6], we reported that (1→3)-β-D-glucan was isopropylidenated at the 4- and 6-hydroxyl groups of the D-glucosyl units, suggesting that some chemical modification at the 2-hydroxyl groups might be possible. Therefore, we attempted an inversion of the 2-hydroxyl groups of the isopropylidenated polymer, to yield a (1→3)-β-D-glucomannan or -mannan or both. This paper concerns the characterization of the polysaccharide obtained from (1→3)-β-D-glucan by an SN2 reaction at the 2-hydroxyl groups.

Linear (1→3)-β-D-glucan (mol. wt. 45,000), prepared from pachyman by periodate oxidation followed by Smith degradation, was isopropylidenated with 2,2-dimethoxypropane in dimethyl sulfoxide (Me_2SO) as mentioned in previous papers[5,6], in which some properties of a per-*O*-isopropylidenated D-glucan (Ip-glucan) had been noted.

To a solution of Ip-glucan (4.0 g) in dry pyridine (200 mL), cooled to −20°, was slowly added methanesulfonyl chloride (40 mL) while stirring and maintaining this temperature. After standing for 24 h at 4°, the solution was diluted with water, and dialyzed against running water. The mesyl derivative of Ip-glucan was deposited during the dialysis, and was collected by centrifugation and lyophilized, yield 5.2 g; $[\alpha]_D$ −51.4° (*c* 0.6, $CHCl_3$). A small quantity of the derivative was subjected to n.m.r. analysis after confirmation of the absence of hydroxyl absorption in its i.r. spectrum. The ^{1}H-n.m.r. spectrum was recorded at 90 MHz with a Hitachi R-22 spectrometer for a solution in chloroform-*d*, with tetramethylsilane as the internal standard. The ratio between isopropylidene (δ 1.3–1.5) and mesyl (δ 3.1–3.2) group protons in the derivative was 2:1, indicating the presence of one isopropylidene and one mesyl group per D-glucose unit in the polysaccharide.

To a solution of the mesyl derivative (0.5 g) dissolved in dry *N,N*-dimethyl-

0008-6215/87/$ 03.50

TABLE I

SOME PROPERTIES OF THE POLYSACCHARIDES DERIVED FROM (1→3)-β-D-GLUCAN

SN2 reaction		*Mannose:glucose*	$\overline{M}_w$	$[\alpha]_D$ (*c 0.3, Me_2SO*) (*degrees*)	*Yield (%)*
Temp. (°C)	*Time (h)*				
100	8	0.20:1.00	15,000	−55.3	85
120	8	0.27:1.00	15,000	−63.1	71
140	8	0.49:1.00	10,000	−56.9	25

formamide (2 mL) was added potassium acetate (0.25 g), and the mixture was stirred for 8 h at the temperature shown in Table I, diluted with water, and dialyzed against running water. A precipitate that was deposited during the dialysis was collected by centrifugation, and lyophilized. The i.r. spectrum of the product showed the presence of hydroxyl (~3450), acetyl (~1740), mesyl (~1340, ~1190), and isopropylidene (~840 cm^{-1}) groups. Although SN2 displacement was thus not complete under the conditions of the reaction, an attempt was made to regenerate a free polysaccharide. The ratio of acetyl to mesyl group in the product was estimated to be 0.25–0.33:1.00 from their proton ratio in the n.m.r. spectrum.

The product was dissolved in 1,4-dioxane (1 mL) and the solution treated with 28% sodium methoxide in methanol (0.1 mL) for 24 h at room temperature, to remove acetyl and mesyl groups. After de-ionization with Amberlite IR-120 (H^+) resin, the solvent was evaporated, and the residue treated with 25% acetic acid (1 mL) for 48 h at room temperature to remove the isopropylidene groups. The acid was neutralized with aqueous sodium hydroxide and the solution dialyzed against running water. The polysaccharide was recovered from the dialyzed solution by lyophilization, its i.r. spectrum did not show absorption bands due to acetyl, isopropylidene, and mesyl groups.

The polysaccharide (2 mg) was hydrolyzed with 0.5M sulfuric acid (1 mL) for 12 h at 100°, the acid neutralized with barium carbonate, and the neutral hydrolyzate evaporated to dryness. The sugars thus obtained were converted into their alditol acetates, and these were analyzed by g.l.c. with a Shimadzu GC-7A apparatus equipped with a glass column (0.3 × 200 cm) packed with 3% of Silar 10C on Uniport B (60–100 mesh) and programmed from 190 to 240° at 4°/min, with a gas-flow rate of 60 mL of nitrogen per min. Two peaks, corresponding to glucitol and mannitol, were detected. The ratio of glucitol to mannitol was determined from the peak-areas, shown in Table I. The ratio is in good agreement with the ratio of acetyl group to mesyl group in the product after nucleophilic substitution, except for the experiment at 140°. The relatively large difference between these ratios in the case of the product at 140° might be caused by partial cleavage of the acetyl group during the displacement reaction[7].

Another portion of the polysaccharide (2 mg) was dissolved in 0.5M sodium

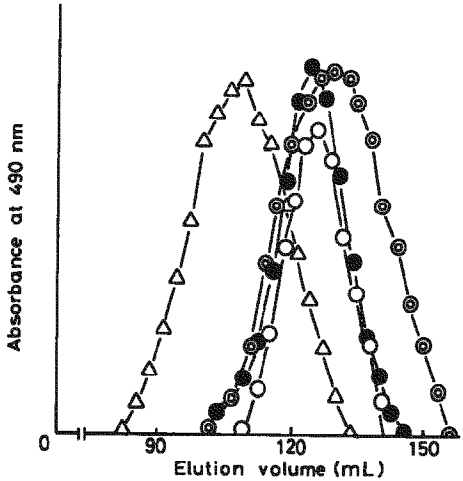

Fig. 1. Gel filtration of the polysaccharides. Pachymaran (—△—), polysaccharide prepared at 140° (—◎—), at 120° (—●—), and at 100° (—○—) for 8 h by the SN2 reaction. Column: Sepharose CL-6B (1.5 × 95 cm); solvent, 0.2M sodium hydroxide; flow rate, 0.25 mL/min.

hydroxide (0.2 mL), and the solution applied to a column (1.5 × 95 cm) of Sepharose CL-6B. The column was equilibrated, and eluted, with 0.2M sodium hydroxide (0.25 mL/min), and the effluent was collected in 1.5-mL fractions. The carbohydrate content of each fraction was determined by the phenol–sulfuric acid method[8]. The column was calibrated with the following dextrans: T-70 (mol. wt. 70,000), T-40 (43,500), T-20 (20,000), and T-10 (10,500), which are products of Pharmacia Fine Chemicals. From the elution volume (see Fig. 1), the molecular weight was determined as shown in Table I. From these results, it could be inferred that (1→3)-β-D-glucomannans having different mannose:glucose ratios may be obtained by choice of the temperature of SN2 displacement, although the reaction is invariably accompanied by a lowering of the molecular weight of the free polysaccharide.

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