Carbohydrate Research, 197 (1990) 181–186
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

INVERSION OF THE 2-HYDROXYL GROUPS OF D-GLUCOSYL UNITS IN (1→3)-β-D-GLUCAN

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(Received March 15th, 1989; accepted for publication, June 28th, 1989)

ABSTRACT

Per-*O*-isopropylidenated (1→3)-β-D-glucan (Ip-glucan) was oxidized with dimethyl sulfoxide–acetic anhydride. Borohydride reduction, followed by *O*-de-isopropylidenation of the products gave free polysaccharides containing D-mannose and D-glucose in ratios that were determined. The 2-keto-β-D-hexosyl residue in the oxidized product of Ip-glucan was found, by mass-chromatographic analysis of the deuteride-reduction product, to be reduced preferentially to the mannose configuration.

INTRODUCTION

In the chemical synthesis of polysaccharides, much effort[1–5] has been directed towards control of the linkage type and the configuration, because the identity of the constituent monosaccharides is fixed by the starting material. There is another way[6,7] 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 a previous paper[8], we reported an inversion of the 2-hydroxyl groups of D-glucosyl units in (1→3)-β-D-glucan by an SN2 reaction. However, the ratio of D-mannose to D-glucose in the resultant polysaccharide was 0.49:1.00 at the most. In continuation of the study, we now describe an inversion of 2-hydroxyl groups of D-glucosyl units in (1→3)-β-D-glucan by an alternative method, namely, oxidation of the 2-hydroxyl groups of per-*O*-isopropylidenated (1→3)-β-D-glucan, followed by reduction, and characterization of the resultant polysaccharide.

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0008-6215/90/$ 03.50

RESULTS AND DISCUSSION

(1→3)-β-D-Glucan was first isopropylidenated at the 4- and 6-hydroxyl groups of the D-glucosyl units, and then the 2-hydroxyl groups were oxidized with a solution of acetic anhydride in dimethyl sulfoxide. In order to learn the relation between the degree of conversion (d.c.) and the oxidation time and temperature of Ip-glucan, an aliquot of the oxidation mixture was taken out from time to time and submitted to study.

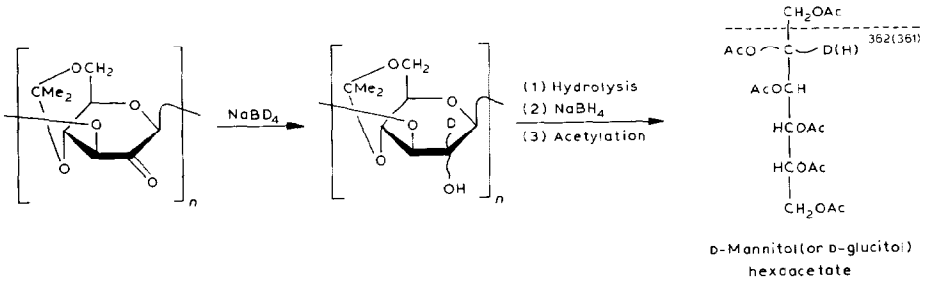
The oxidized product, isolated by precipitation with methanol, showed in its i.r. spectrum not only carbonyl absorption (at 1745 cm^{-1}) but also hydroxyl absorption (3400 cm^{-1}), indicating that some of the 2-hydroxyl groups in Ip-glucan had survived the oxidation. Although the oxidation of the 2-hydroxyl groups was thus not complete under the conditions of the reaction, an attempt was made to regenerate a hexose residue by reduction of the carbonyl groups. Reduction of the carbonyl groups was effected with sodium borohydride, in a mixture of tetrahydrofuran and water, for 14 h at room temperature. As the reduction product showed an absorption band at 850 cm^{-1} due to isopropylidene group, it was treated with 25% acetic acid to regenerate the free polysaccharide.

On acid hydrolysis, the resultant polysaccharide gave glucose and mannose, which were identified by g.l.c. after conversion into their alditol acetates. The ratio of mannose to glucose was determined from the peak-areas, from which the d.c. was calculated. The results are summarized in Table I. The maximum value of d.c. by the present method was 60%, which corresponds to D-mannose:D-glucose of 3:2 (oxidation for 180 min at 40°). The D-glucose content thus estimated might include D-glucose derived from 2-keto-D-hexose residues by the reduction with sodium borohydride. Therefore, a part of the oxidation (180 min at 40°) product was reduced with sodium borodeuteride, by which the 2-keto-D-hexose residues were converted into C-2-deuterated hexose residues. Because the alditol acetates

TABLE I

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

Oxidation conditions		*Mannose:glucose*	M_w
Temp (°C)	*Time (min)*		
60	20	1.11:1.00	24 000
	40	1.17:1.00	
	60	1.16:1.00	
	120	1.16:1.00	16 000
40	30	0.24:1.00	29 000
	60	0.32:1.00	28 000
	120	0.75:1.00	27 000
	180	1.50:1.00	25 000
	240	1.20:1.00	22 000
	300	1.20:1.00	21 000



derived from C-2-deuterated hexose give a fragment ion of *m/z* 362 instead of 361 on mass-spectral analysis (see Figure), the D-glucosyl residues regenerated from 2-keto-D-hexosyl residues by the reduction will be distinguishable from the D-glucosyl residues which survived the oxidation. The ratio of C-2-deuterated D-mannose to C-2-deuterated D-glucose, calculated from the peak-areas on the chromatogram monitored at *m/z* 362 in mass–chromatography, was 16.8:1.0. The result indicates that ~94% of the 2-keto-D-hexosyl residues was reduced to D-mannosyl residues. Bredereck[9] had reported an analogous result for the borohydride reduction of carbonyl groups at C-2 in a dimethyl sulfoxide–acetic anhydride-oxidized 6-*O*-trityl-cellulose, so the result indicates that 3-*O*-substituted, as well as 4-*O*-substituted, 2-keto-β-D-hexosyl residues can be preferentially reduced with sodium borohydride to the D-*manno* configuration.

The mol. wt. of the polysaccharide was determined by the method of gel filtration (see Table I). The mol. wt. decreases with increasing reaction time and temperature. However, the free polysaccharide having the maximum extent of d.c. that is formed by the present method not only contains more mannose residues but also has a mol. wt. larger than that of the polysaccharide prepared by the SN2 reaction. Therefore, the present method is distinctly superior to the SN2 method for inversion of the 2-hydroxyl groups of D-glucosyl units in (1→3)-β-D-glucan.

The maximum extent of d.c. could not be over 60%. This might be a consequence of protection by inter-residue hemiacetal formation between oxidized and neighboring non-oxidized residues.

The polysaccharide (D-mannose:D-glucose = 3:2) was subjected to controlled degradation in order to learn the distribution of D-mannosyl residues in the polysaccharide chain. A mixture of oligosaccharides recovered from an acetolysis product (the degree of hydrolysis as determined for the *O*-deacetylated product was 40%) was fractionated on a column of BioGel P-2. L.c. and p.c. analysis of the fraction corresponding to disaccharides (F2), and of F2 exhaustively digested by β-D-glucosidase, showed that the fraction consisted of laminarabiose (G-G), epilaminarabiose (G-M), *O*-β-D-mannopyranosyl-(1→3)-D-glucose (M-G) and *O*-β-D-mannopyranosyl-(1→3)-D-mannose (M-M) in the ratios of 1:4:2:1. The ratio of the last two disaccharides (2:1) could indicate the presence of isolated mannose units as well as of two consecutive mannose units in the polysaccharide chain. However, considering the ratio of these disaccharides and the 60% d.c., occurrence of

the trisaccharide units composed solely of D-mannosyl (or D-glucosyl) residues could not be so frequent in the polysaccharide.

EXPERIMENTAL

General methods. — Total sugars were estimated by the phenol–sulfuric acid method. Reducing sugars were determined by the Somogyi-Nelson method. Specific rotations were determined at 20° with a Union PM-201 polarimeter. Infrared spectra were recorded with a JASCO IRA-1 infrared spectrometer. Gas–liquid chromatography was performed in 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, Gasukuro Kogyo Co., Ltd.) and programmed from 190 to 240° at 4°/min, with a gas-flow rate of 60 mL of nitrogen per min. Mass–chromatography was carried out by g.l.c.–m.s. conducted with a JEOL Model JMS-D300 apparatus equipped with a Shimadzu fused-silica capillary column of OV-1701 (0.33 mm × 25 m). The temperature of the column was programmed at 32°/min from 60 to 230°, with a flow-rate of helium of 1 mL/min, the mass spectra and peak areas at *m/z* 361 and 362 being recorded at an ionizing potential of 20 eV. Liquid chromatography at 7.8 MPa was performed in a JASCO BIP-I apparatus equipped with a Shodex RI SE-11 differential refractometer as the detector. The stainless-steel column (0.46 × 25 cm) was packed with LiChrosorb-NH_2 (Merck) and operated with a flow-rate of 1.5 mL of 7:3 acetonitrile–water per min. Paper chromatography was carried out by the multiple ascending method on Toyo No. 50 filter paper. The solvent used was 6:4:3 (v/v) 1-butanol–pyridine–water.

Per-O-isopropylidenation of (1→3)-β-D-glucan. — Linear (1→3)-β-D-glucan {mol. wt. 45 000, $[\alpha]_D$ +4.3° (*c* 0.5, 0.5M NaOH)}, prepared from pachyman by periodate oxidation followed by Smith degradation, was isopropylidenated with 2,2-dimethoxypropane in dimethyl sulfoxide as mentioned in previous papers[10,11], in which some properties of a per-*O*-isopropylidenated D-glucan(Ip-glucan) had been noted.

Oxidation of Ip-glucan. — Ip-glucan (1 part) was dissolved in a mixture of dimethyl sulfoxide (40 parts) and acetic anhydride (20 parts). The solution was stirred for the specified time and at the specified temperature, as shown in Table I. The oxidized product was precipitated by pouring into methanol (500 parts), filtered off, washed exhaustively with methanol, and dried under diminished pressure.

Reduction of the oxidized Ip-glucan. — The oxidized Ip-glucan (1 part) was dissolved in a mixture of tetrahydrofuran (150 parts) and water (14 parts). To this solution was added sodium borohydride (1.2 parts), and the mixture was stirred for 14 h at room temperature. For the determination of the ratio of C-2-deuterated hexoses, sodium borodeuteride was used. The excess of borohydride was decomposed by addition of 25% acetic acid, and the solution was dialyzed against running water, and then lyophilized. The residue was dissolved in 25% acetic acid,

and the solution was kept for 24 h at room temperature to remove the isopropylidene groups. The resulting polysaccharide was isolated from the dialyzate by lyophilization; yield 0.6 g from 1 g of Ip-glucan, its i.r. spectrum did not show absorption bands due to isopropylidene and carbonyl groups.

Analysis of constituent monosaccharides of the polysaccharide. — 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. Two peaks, corresponding to glucitol and mannitol, were detected. The ratio was determined from the peak-areas, as shown in Table I. After similar treatment, the C-2-deuterated polysaccharide was submitted to g.l.c. analysis for determination of the ratio of mannose:glucose, and to mass-chromatography monitored at *m/z* 362 for determination of the ratio of C-2-deuterated mannose:glucose.

Determination of molecular weight. — The polysaccharide (2 mg) was dissolved in 0.5M sodium hydroxide (0.2 mL), and the solution was 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. 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, the molecular weight was determined as shown in Table I.

Acetolysis of the polysaccharide. — The polysaccharide {D-mannose:D-glucose 3:2, $[\alpha]_D$ −89.3° (*c* 0.3, Me_2SO), 1 g} was acetylated with acetic anhydride–pyridine in the usual way. The resultant acetate was dissolved in acetic anhydride (16 mL) and the solution kept briefly at 5°. To this solution was added 7 mL of a cold mixture of acetic anhydride (6 parts) and sulfuric acid (1 part), and the whole was kept briefly at 5°. Thereafter, the reaction mixture was kept for 5 days at room temperature, and then poured into ice–water. The derived acetates were isolated by extraction with chloroform in the usual way; the degree of depolymerization as determined for the *O*-deacetylated product was 40.3%. The product was *O*-deacetylated with 0.02M sodium methoxide at 5° and the resultant mixture of free oligosaccharides isolated in the usual way; yield 0.6 g.

Fractionation and identification of disaccharides. — The oligosaccharides were first fractionated on a column (2.5 × 90 cm) of BioGel P-2 at 50°. The effluent was collected in 4-mL fractions. The carbohydrate content of each fraction was determined by the phenol–sulfuric acid method. The fractions corresponding to disaccharide (F2) were combined, and submitted to l.c. analysis. Three peaks (A, B, and C) were detected, in the ratios of 1:6:1.

Oligosaccharide A, corresponding to peak A, was isolated by preparative l.c. This sugar gave only D-glucose on acid hydrolysis, and was chromatographically identical with an authentic specimen of laminarabiose.

Oligosaccharide B, corresponding to peak B, gave two spots (B1 and B2) on p.c. analysis. Therefore each sugar was isolated by preparative l.c. and p.c.

Oligosaccharide B1, on acid hydrolysis, gave D-glucose and D-mannose in the ratio of 1:1. Reduction with sodium borohydride followed by acid hydrolysis gave only D-glucose as reducing sugar; $[\alpha]_D$ +7.9° (*c* 0.5, H_2O).

Oligosaccharide B2, on acid hydrolysis, gave D-glucose and D-mannose in the ratio of 1:1. Reduction with sodium borohydride, followed by acid hydrolysis, gave only D-mannose as reducing sugar; $[\alpha]_D$ +20.7° (*c* 0.3, H_2O).

Oligosaccharide C, corresponding to peak C, was isolated by preparative l.c. This sugar gave only D-mannose on acid hydrolysis. An attempt to determine a specific rotation of the sugar was not successful because of the low yield.

The ratio of the disaccharides in F2. — A part of the F2 fraction was dissolved in 0.2M acetate buffer (pH 5.0) and incubated with β-D-glucosidase (Tokyo Kasei Co., Ltd.), under toluene, for 72 h at 37°. The mixture was heated for 10 min at 100° in order to inactivate the enzyme. Insoluble materials were filtered off, and the filtrate was treated with Amberlite IR-120 (H^+) and IRA-410 (OH^-) ion-exchange resins, and subjected to l.c. analysis. Two peaks, corresponding to peak B (B2) and C, were detected in the ratio of 2:1. Because the ratios of A:B:C are 1:6:1, the result indicates that the ratios of disaccharides A, B1, B2, and C in F2 are 1:4:2:1.

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