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Preliminary communication

Acetonation of linear (1→3)-β-D-glucan

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In a previous paper[1], we reported that acetonation of laminara-triose and -tetraose with 2,2-dimethoxypropane in *N,N*-dimethylformamide gave compounds in which the 4- and 6-hydroxyl groups of the internal glucopyranosyl residue(s) were isopropylidenated. In continuation of the study, we now describe the result of acetonation of linear (1→3)-β-D-glucan.

(1→3)-β-D-Glucan (LAP) was prepared from alkali-soluble polysaccharide from the sclerotia of *Grifora umbellata*[2] by periodate oxidation followed by Smith degradation. The polysaccharide thus obtained gave only D-glucose on both acid hydrolysis and enzymic hydrolysis by exo-(1→3)-β-D-glucanase[2], and had an average molecular weight of 56,200, as revealed by gel filtration (see Fig. 1).

LAP (1 g), $[\alpha]_D$ −9° (*c* 0.4, dimethyl sulfoxide), was dissolved in dry dimethyl sulfoxide (25 mL). To this solution were added *p*-toluenesulfonic acid (50 mg) and 2,2-dimethoxypropane (5 mL), and the mixture was stirred for 6 h at 50°, and diluted with

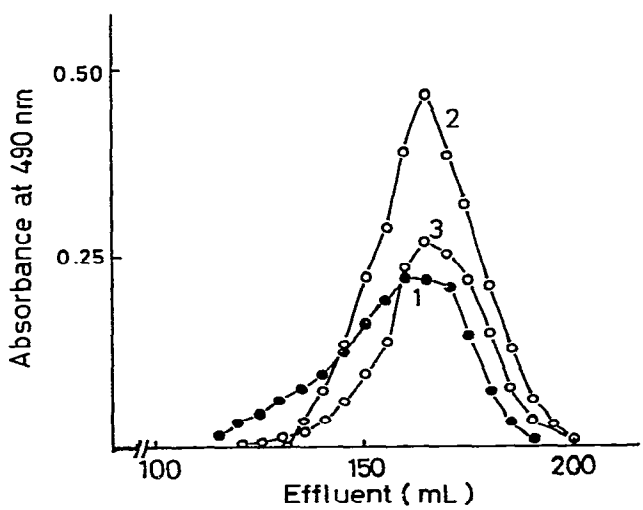

Fig. 1. Gel filtration of linear (1→3)-β-D-glucan LAP (1), acetic acid-treated LAP (2), and LAP isopropylidenated and then *O*-de-isopropylidenated (3).

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water; the acid was neutralized with aqueous sodium carbonate, and the solution was dialyzed against running water for 2 days. A precipitate deposited during the dialysis was collected by centrifugation and lyophilized; yield, 0.73 g; $[\alpha]_D$ −29° (*c* 0.4, dimethyl sulfoxide). In order to learn the degree of substitution by isopropylidene groups, from time to time an aliquot of the isopropylidenated polymer was acetylated with acetic anhydride and pyridine in the usual way, and the acetate was subjected to n.m.r. analysis. The spectra were recorded at 90 MHz, with a Hitachi R-22 spectrometer for solutions in chloroform-*d*, tetramethylsilane being used as the internal standard. The proton ratio between isopropylidene (δ 1.3–1.5) and acetyl (δ 2.0–2.2) groups in the derivative was 2:1 after several tests of the progress of the isopropylidenation.

A portion (5 mg) of the per-*O*-isopropylidenated polymer (**1**) was methylated by the Hakomori procedure[3]. The i.r. spectrum of the methylated product showed an absorption band near 850 cm^{-1}, characteristic of the isopropylidene group, but no band in the region of hydroxyl absorption. The methylated, isopropylidenated polymer was hydrolyzed, first with 90% formic acid for 2 h at 100° and then with 0.25M sulfuric acid for 12 h at 100°. After neutralization of the sulfuric acid with barium carbonate, the sugars in the hydrolyzate were reduced with sodium borohydride, the alditols acetylated with acetic anhydride–pyridine in the usual way, and the acetates analyzed by g.l.c.–m.s. conducted with a Hitachi Model M-52 apparatus equipped with a glass column (0.3 × 100 cm) packed with 2% of OV-1 on Chromosorb W (80–100 mesh) at 200°, the mass spectra being recorded at an ionizing potential of 20 eV.

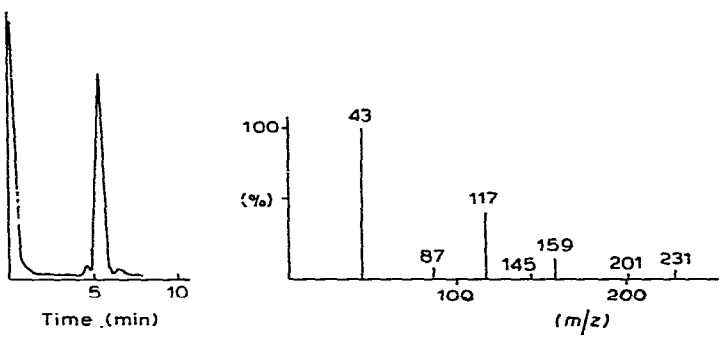
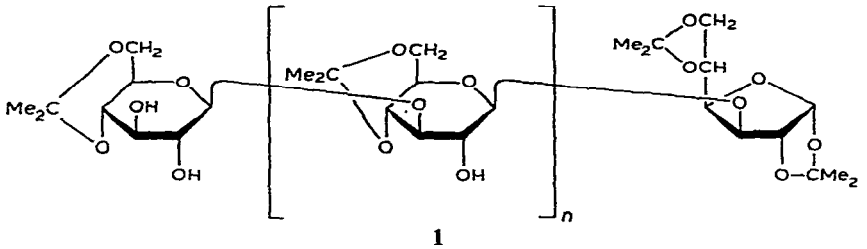


Fig. 2. Gas–liquid chromatogram and mass spectrum of 1,3,4,5,6-penta-*O*-acetyl-2-*O*-methyl-D-glucitol derived from methylated, isopropylidenated (1→3)-β-D-glucan.

As shown in Fig. 2, 1,3,4,5,6-penta-*O*-acetyl-2-*O*-methyl-D-glucitol was the main product; its proportion relative to other products was >90%, calculated from the peak areas on the chromatogram, indicating that the 6- and 4-hydroxyl groups of each D-glucopyranosyl residue in the polysaccharide were substituted by an isopropylidene group. It is, therefore, reasonable to presume, from the results of acetonation of laminara-oligosaccharides[1,4], that one isopropylidene group is not shared by two D-glucosyl residues, but is attached to a single D-glucosyl residue at the 4- and 6-hydroxyl



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groups, and that the potentially reducing end may be a 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose residue, as shown in **1**.

A suspension of the isopropylidenated polysaccharide (20 mg) in 25% aqueous acetic acid (2 mL) was heated for 24 h at 70°, and the acid was neutralized with aqueous sodium hydroxide. The suspension was dialyzed, and the dialyzate lyophilized. The i.r. spectrum of the residual material had no absorption band near 850 cm^{-1}, characteristic of the isopropylidene group, showing that *O*-de-isopropylidenation was complete.

Part (5 mg) of the *O*-de-isopropylidenated material was dissolved in 0.2M sodium hydroxide (0.5 mL), and the solution was applied to a column (1.5 × 90 cm) of Sepharose CL-2B. The column was equilibrated, and eluted, with 0.2M sodium hydroxide, and the effluent was collected in 1-mL fractions. The carbohydrate content of each fraction was determined by the phenol–sulfuric acid method. Native (1→3)-β-D-glucan, and that treated with 25% acetic acid as already mentioned, were also subjected to gel filtration. As shown in Fig. 1, there was almost no degradation of the polysaccharide during the processes of isopropylidenation and *O*-de-isopropylidenation.

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