Polysaccharides of Wood-destroying Fungi *Polyporus squamosus* (HUDS.) FR. and *Phellinus igniarius* (L.) QUEL.

A. KARDOŠOVÁ, K. BABOR, J. ROSÍK and J. KUBALA

Institute of Chemistry, Slovak Academy of Sciences, Bratislava 9

Received February 21, 1969

Extraction of the fruit bodies of *Polyporus squamosus* and *Phellinus igniarius* with water gave a mixture of polysaccharides, fractionation of which afforded a fucomannogalactan and a glucan. Their structural features were established by hydrolysis, methylation analysis and periodate oxidation.

In our previous paper [1], we have mentioned reports dealing with structural investigation on polysaccharides from different species of wood-destroying fungi and described the polysaccharides isolated from fruit bodies of wood-destroying fungus *Fomes fomentarius* (L.) FR.

This communication reports the isolation of, and the structural studies on the polysaccharides isolated from the fruit bodies of *Polyporus squamosus* and *Phellinus igniarius*.

Experimental

Melting points were determined on a Kofler block, optical rotations were measured on an authomatic polarimeter Bendix-Ericson, type 143 A at 22°C. Evaporations were carried out at reduced pressure at temperatures not exceeding 40°C.

Paper chromatography was carried out on Whatman No. 1 and Whatman No. 3 papers with the following solvent system (v/v): ethyl acetate—pyridine—water (8:2:1). Compounds were detected with aniline hydrogen phthalate [2]. Chromatograms were quantitatively evaluated with Lange Chromatometer 3. The R_G values of monosaccharides and the R_{MeG} values of methylated monosaccharides refer to rates of movements relative to that of D-glucose and 2,3,4,6-tetra-*O*-methyl-D-glucose, resp. TLC of methylated saccharides was performed on Kieselgel G (10—40 μm) using benzene—acetone (1 : 1) as solvent. Gas chromatography of methylated methyl glycosides was performed on column of Chromosorb coated with 10% by weight of Carbowax 6000 (operating temperatures 164 and 170°C). Retention times (R_t) are quoted relative to methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside.

Electrophoresis was carried out on Electrophoresegerät 35 (Zeiss, Jena) in borate buffer pH 9.2 and 11, resp. The polysaccharide concentration was 1%.

The sedimentation constant was determined on an ultracentrifuge MOM G 110, diffusion coefficient on a Kern LK 30 device, and the partial specific volume was determined pycnometrically. From these values, molecular weight was calculated.

The periodate uptake and the formed formic acid were determined by thiosulfate method [3] with amperometric titration [4].

Preparation of the parent material

Fruit bodies of *Polyporus squamosus* (grown on a nut-tree) and that of *Phellinus igniarius* (grown on a willow) were stored in acetone immediately after harvesting. After being desintegrated in a Turmix blendor, refluxed with acetone, and air-dried, the material (120 g) was extracted by stirring 2 hours with distilled water (4 l) at 75°C. Part of the desintegrated and air-dried material of *P. squamosus* was extracted by stirring 2 hours with 0.15 M phosphate buffer (3 liters) of a pH 6.3 at 75°C. Extracted polysaccharides were recovered from the concentrated solutions by precipitation with acidified ethanol (10 ml of concd. hydrochloric acid to 1 liter of ethanol) in the ratio of 1 : 3. Yield of the water-extracted polysaccharide was 2 g (1.6%) and that of phosphate buffer-extracted polysaccharide was 1.8 g (1.8%).

Fractionation of the polysaccharide mixture

The mixture of polysaccharides was fractionated on Dowex 1X8 ion-exchanger in acetate form by elution with distilled water and acetic acid as in ref. [1].

Hydrolysis of saccharides

a) Partial hydrolysis of the heteropolysaccharide

Dowex 50 W (H^+-form) ion-exchanger (10 g) was added to a solution containing 200 mg of polysaccharide in 25 ml of distilled water. Hydrolysis product was worked up as in our previous work [1].

b) Partial hydrolysis of the glucan

Polysaccharide (1 g) was heated with 20 ml of 1 N sulfuric acid 1 hour at 100°C. The hydrolyzate was neutralized and evaporated to a syrup. Hydrolysis products were separated by paper chromatography.

c) Total hydrolysis

Polysaccharides and oligosaccharides, resp. were hydrolyzed with 1 N sulfuric acid. Solutions were worked up and hydrolysis products identified as in [1].

Methylation analysis

Polysaccharides were methylated successively with dimethyl sulfate and sodium hydroxide, and methyl iodide and silver oxide. Oligosaccharides were methylated with methyl iodide and silver oxide in *N*,*N*-dimethylformamide. Methanolysis was carried out with 5% methanolic hydrogen chloride and hydrolysis with 70% sulfuric acid. The whole procedure was as in [1]. Hydrolysis products were determined by both paper and TL chromatography and methanolysis products by gas chromatography.

Periodate oxidation

Polysaccharides (0.2% solutions) were oxidized with 0.025 M sodium metaperiodate and worked up as in [1].

Results

Polyporus squamosus

Both water-extracted polysaccharides and that extracted with phosphate buffer were heterogeneous and on hydrolysis gave galactose, mannose, fucose, and glucose in the same molar proportions. Therefore the combined polysaccharides were further investigated.

Fractionation of the polysaccharide mixture on Dowex 1X8 ion-exchanger afforded two fractions. The water-eluted fraction was a heteropolysaccharide composed of galactose, mannose, and fucose and the fraction eluted with 4 N acetic acid was a glucan.

The heteropolysaccharide was homogeneous when examined by boundary electrophoresis and sedimentation in ultracentrifuge, soluble in water, had $M_{s,D} = 47{,}600$, DP = 297, $[\alpha]_D = +174°$ ($c = 0.4$ in water). The molar ratio of D-galactose : D-mannose : L-fucose was 12 : 2 : 1.

Partial hydrolysis with Dowex 50 W ion-exchanger released 6-*O*-α-D-galactopyranosyl-D-galactose together with L-fucose and D-mannose. The disaccharide was characterized as follows:

6-*O*-α-D-Galactopyranosyl-D-galactose — $R_{Gal} = 0.50$, $[\alpha]_D = +141°$ ($c = 1$ in water) (ref. [5] reports $[\alpha]_D = +141°$), on hydrolysis gave D-galactose only. Hydrolysis of the methylated disaccharide afforded 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3,4-tri-*O*-methyl-D-galactose determined by paper chromatography by comparison with authentic samples. The value of optical rotation of the disaccharide indicated α-glycosidic linkage.

A sample of the polysaccharide consumed 1.13 moles of periodate and released 0.54 moles of formic acid per average saccharide unit. Hydrolysis of the periodate-oxidized polysaccharide, followed by paper chromatographic determination of the hydrolyzate, indicated that D-galactose was the only reducing sugar present.

A sample of the polysaccharide was fully methylated and had $[\alpha]_D = +101°$ ($c = 0.4$ in chloroform). Hydrolysis products of the methylated polysaccharide were identified by paper and TL chromatography: 2,3,4,6-tetra-*O*-methyl-D-galactose — $R_{MeG} = 0.90$; 2,3,4,6-tetra-*O*-methyl-D-mannose — $R_{MeG} = 0.96$; 2,4,6-tri-*O*-methyl-D-galactose — $R_{MeG} = 0.73$; 2,3,4-tri-*O*-methyl-D-galactose — $R_{MeG} = 0.66$; possibly 2,6-di-*O*-methyl-D-galactose (traces) — $R_{MeG} = 0.44$, and 2,4-di-*O*-methyl-D-galactose — $R_{MeG} = 0.37$. Gas chromatography of the methanolysis product of the methylated polysaccharide showed the presence of following methyl glycosides: methyl 2,3,4-tri-*O*-methyl-L-fucoside — $R_t = 0.46$; methyl 2,3,4,6-tetra-*O*-methyl-D-mannoside — $R_t = 1.00$; methyl 2,3,4,6-tetra-*O*-methyl-D-galactoside — $R_t = 1.16$; methyl 2,4,6-tri-*O*-methyl-D-galactoside — $R_t = 2.42$ and 3.07; methyl 2,3,4-tri-*O*-methyl-D-galactoside — $R_t = 3.57$ and 5.71, and methyl 2,4-di-*O*-methyl-D-galactoside — $R_t = 15.35$.

The glucan was not homogeneous at sedimentation in ultracentrifuge and boundary electrophoresis, difficultly soluble in water, soluble in alkali and had $[\alpha]_D = +60°$ ($c = 0.4$ in 1 N sodium hydroxide). On acid hydrolysis gave D-glucose only. Partial acid hydrolysis followed by preparative paper chromatography afforded four oligosaccharide fractions: glucobiose — $R_G = 0.50$, $[\alpha]_D = +38°$ ($c = 1$ in water); glucotriose — $R_G = 0.30$, $[\alpha]_D = +6°$ ($c = 1$ in water); glucotetraose — $R_G = 0.08$.

$[\alpha]_D = -18°$ ($c = 1$ in water), and glucopentaose — $R_G = 0.03$, which formed a homologous series (Fig. 1). From the values of optical rotations of these oligosaccharides it was assumed that β-glycosidic linkages were present.

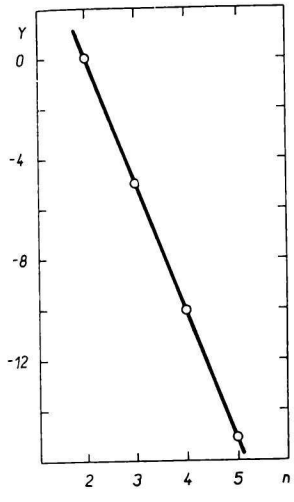


Fig. 1. Homologous series of oligosaccharides. $Y = \log \frac{R_G}{1 - R_G} . 10$; n = number of D-glucose units.

Gas chromatography of the methanolysis products of methylated oligosaccharides showed the presence of methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-glucose, and 2,4,6-tri-*O*-methyl-D-glucose.

The methylated polysaccharide had $[\alpha]_D = +59°$ ($c = 1$ in chloroform) and showed no hydroxyl absorption in the infrared. After hydrolysis of the methylated product, the following methylated sugars were identified by paper chromatography: 2,3,4,6-tetra-*O*-methyl-D-glucose — $R_{MeG} = 1.00$; 2,3,4-tri-*O*-methyl-D-glucose — $R_{MeG} = 0.85$; 2,4,6-tri-*O*-methyl-D-glucose — $R_{MeG} = 0.76$, and an unidentified (probably) dimethylglucose — $R_{MeG} = 0.52$ (traces). Gas chromatography of the methanolysis product from the methylated polysaccharide showed the presence of methyl glycosides of the above mentioned methylated sugars ($R_t = 0.69$ and 1.00; 1.95 and 2.75; 2.41 and 3.49; 5.19 and 7.25).

The polysaccharide consumed 0.92 moles of periodate and released 0.18 moles of formic acid per glucose unit. Some of the D-glucose units were periodate-resistent.

Phellinus igniarius

Fractionation of the water-extracted polysaccharide mixture on Dowex 1X8 ion-exchanger in acetate form afforded two fractions: a water-eluted fraction *A* and a (1→4)-glucan eluted with 4 N acetic acid.

Fraction A was heterogeneous when examined by boundary electrophoresis and sedimentation in ultracentrifuge. On acid hydrolysis furnished D-galactose, D-mannose, L-fucose, D-glucose, and an unidentified saccharide of the $R_G = 1.71$. After recurring fractionation, the ratio of all sugars present was unchanged only the D-

glucose amount decreased. That indicated that this fraction was a mixture of a heteropolysaccharide and a glucan which we failed to separate. The heteropolysaccharide was composed of D-galactose, D-mannose, L-fucose, and of an unidentified compound.

This fraction was soluble in water and had $[\alpha]_D = +65°$ ($c = 0.4$ in water). Gas chromatography of the methanolysis product of the methylated fraction showed the presence of methyl glycosides of 2,3,4-tri-*O*-methyl-L-fucose — $R_t = 0.47$; 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-mannose — $R_t =$ $= 0.69$ and 1.00 (the peaks were overlapped); 2,3,4,6-tetra-*O*-methyl-D-galactose — $R_t = 1.17$; 2,3,4-tri-*O*-methyl-D-galactose — $R_t = 5.96$ and 2,3,6-tri-*O*-methyl-D-glucose — $R_t = 2.69$ and 3.62. Besides, 2,4-di-*O*-methyl-D-galactose — R_{MeG} was identified by paper chromatography.

The (1→4)-glucan was partly soluble in water and had $[\alpha]_D = +99°$ ($c = 1$ in 1 N sodium hydroxide). The polysaccharide was homogeneous at sedimentation in ultracentrifuge and boundary electrophoresis and gave D-glucose as the only hydrolysis product. The methylated polysaccharide had $[\alpha]_D = +110°$ ($c = 1$ in chloroform). 2,3,4,6-Tetra-*O*-methyl-D-glucose — $R_{MeG} = 1.00$ and 2,3,6-tri-*O*-methyl-D-glucose — $R_{MeG} = 0.86$, and their methyl glycosides $R_t = 0.69$ and 1.00 and $R_t = 2.69$ and 3.60 were determined by both paper and gas chromatography.

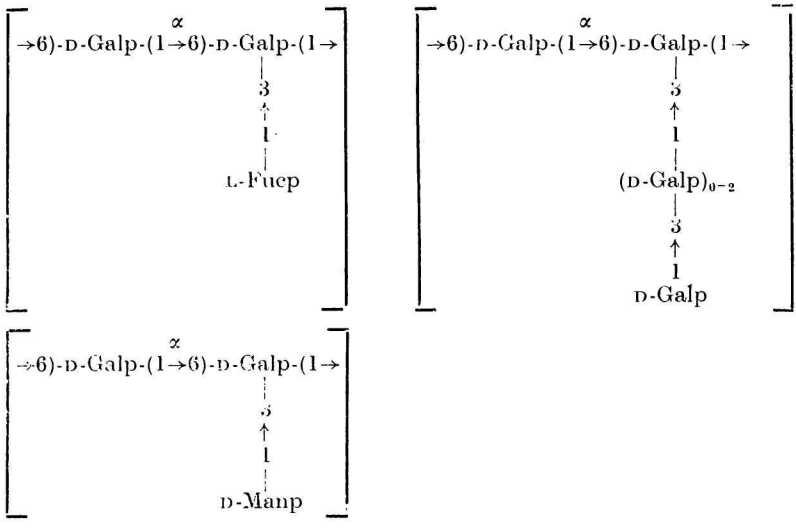
The polysaccharide consumed 0.97 moles of periodate and released 0.14 moles of formic acid per glucose unit.

Discussion

Fucomannogalactan from P. squamosus

It is apparent from the methylation analysis that the heterogalactan is branched. The backbone is formed by D-galactopyranose units linked together with (1→6)-glycosidic bonds. It is clearly shown by the presence of 6-*O*-α-D-galactopyranosyl-D-galactose in the hydrolysis product. From the observed high positive values of optical rotations of the polysaccharide and oligosaccharide it was concluded that the glycosidic linkages were in the α-form. The side chains composed mainly of D-galactose units are attached to the D-galactose units of the main chain in the 3-position. It is indicated by the identification of 2,4-di-*O*-methyl-D-galactose. Identification of 2,4,6-tri-*O*-methyl-D-galactose in hydrolysis product of the methylated polysaccharide indicated that D-galactose units were linked each to other in side chains with (1→3)-linkages. 2,3,4,6-Tetra-*O*-methyl-D-galactose demonstrated that D-galactose was also present as non-reducing end group in side chains. D-Mannose and L-fucose were present exclusively as terminal, non-reducing residues of side chains. It was demonstrated by the identification of 2,3,4,6-tetra-*O*-methyl-D-mannose and 2,3,4-tri-*O*-methyl-L-fucose in hydrolysis and methanolysis products of the methylated polysaccharide, resp.

On the basis of methylation analysis, molar ratios of monosaccharides, and the obtained values of periodate consumption (1.13 moles) and of produced formic acid (0.43 moles), the following structural sequences are believed to form the structure of the polysaccharide:



The theoretical values of periodate consumption (1.13 moles) and of produced formic acid (0.56 moles) calculated for such sequences are in reasonably good agreement with that of experimentally obtained.

However, it is not experimentally established, whether the D-galactose units linked each to other in side chains with (1→3)-linkages bear D-mannose or L-fucose as end-groups. We have preferred this formulation of the sequences with respect to side chains in similar polysaccharides.

Such structural units of the main chain as described above were found in the backbone of polysaccharides isolated from *Polyporus pinicola* and *Armillaria mellea* [5, 6].

Glucan from P. squamosus

The methylation analysis and the prepared oligosaccharides showed the presence of (1→3)- and (1→6)-glycosidic linkages. As all of the oligosaccharide fractions including that of disaccharide were mixed and contained (1→3)- and (1→6)-linkages (laminaribiose and gentiobiose have the same chromatographic movement in the system used), the heterogeneity of the glucan could be explained as follows:

Either it is a polysaccharide of a certain structure with dispersion of its molecular weight, or it is a mixture of two polysaccharides of different structure. In the first case it would be a glucan similar to dextran but instead of it with β(1→3)- and β(1→6)-bonds. In the second case it would be a mixture of β(1→3)-glucan (so-called callose, frequently appearing in higher fungi and some plants) and of a β(1→6)-glucan.

Polysaccharides from P. igniarius

It is apparent from the methylation analysis that the heteropolysaccharide is branched. The backbone is composed of D-galactose units linked together with

(1→6)-glycosidic bonds and the side chains are formed by D-mannose and L-fucose units. This heteropolysaccharide is similar to the mannofucogalactan from *Fomes fomentarius*.

D-Glucose units in the glucan are linked together with (1→4)-bonds as it is confirmed by the results of methylation analysis and periodate oxidation. However, it is not possible to determine the configuration of the glycosidic linkages from the observed value of optical rotation.

References

1. Kardošová A., Babor K., Rosík J., Kubala J., *Chem. Zvesti* ***23***, 454 (1969).
2. Partridge S. M., *Nature* ***164***, 443 (1949).
3. Sowa W., Blackwood A. C., Adams G. A., *Can. J. Chem.* ***41***, 2314 (1963).
4. Babor K., Kaláč V., Tihlárik K., *Chem. Zvesti* ***18***, 913 (1964).
5. Fraser R. N., Karácsonyi Š., Lindberg B., *Acta Chem. Scand.* ***21***, 1783 (1967).
6. Fraser R. N., Lindberg B., *Carbohyd. Res.* ***4***, 12 (1967).

Translated by A. Kardošová