Note

Structure and molecular size of pachyman*

G. C. HOFFMANN, B. W. SIMSON, AND T. E. TIMELL

Department of Forest Chemistry, State University of New York, College of Forestry, Syracuse, N. Y. 13210 (U. S. A.)

(Received May 28th, 1971)

Pachyman is the major constituent of the cell wall of *Poria cocos* Wolf, a fungus that grows on tree roots and is commercially garnered, especially in Japan. It consists of (1→3)-linked β-D-glucopyranose residues[1]. Recently, the claim has been made[2] that a few, internal (1→6)-linkages are also present, and that the native polysaccharide has a degree of polymerization of 255 and may contain four branch points. In connection with a structural analysis of a similar polysaccharide from compression wood of *Larix laricina*[3], more information was needed concerning the molecular properties and degree of branching of pachyman.

Pachyman was obtained in a yield of 90% by direct extraction of the cell-wall material of *Poria cocos* with methyl sulfoxide. The polysaccharide, which had $[\alpha]_D$ −13.0° in this solvent and +21.5° in 10% aqueous sodium hydroxide, gave, on partial hydrolysis with acid, only glucose and a series of polymer-homologous oligosaccharides from laminarabiose to laminaraheptaose. No uronic acids could be detected.

Direct methylation of the fungus polysaccharide afforded an *O*-methylglucan that contained 977 glucose residues per molecule. Methanolysis of this ether, followed by acetylation[4] and examination of the acetates by gas–liquid chromatography (g.l.c.), indicated the presence of residues of di-*O*-methylglucoses, 2,4,6-tri-*O*-methylglucose, and 2,3,4,6-tetra-*O*-methylglucose in the ratios of 5:223:1, corresponding to 4.2 non-reducing end-groups and, accordingly, 3.2 branches, per average macromolecule. When the purified polysaccharide was reduced with sodium borohydride, and the product methylated, the *O*-methyl derivative contained 233 glucose residues per molecule, and had ratios of di- to tri- to tetra-*O*-methylglucose residues of 2:77:1, indicative of 2.0 branches per average *O*-methylated, reduced glucan molecule. Obviously, the pachyman had in this case been degraded during the methylation, despite the absence of the original, reducing end-groups.

A portion of the methyl glucosides obtained in the first experiment was separated by chromatography on silica gel, giving tetra-, tri-, and, in two fractions, di-*O*-

*Dedicated to Dr. Nelson K. Richtmyer in honor of his 70th birthday.

methylglucosides. Each of the last two fractions was acetylated[4], and the product analyzed by g.l.c. The third fraction was found to contain methyl 2,6-di-*O*-methyl-α,β-glucopyranoside, methyl 4,6-di-*O*-methyl-α,β-glucopyranoside, and methyl 2,4-di-*O*-methyl-β-glucopyranoside in the ratios of 1.0:4.3:0.4. The first two methyl α,β-glucosides also occurred in the fourth fraction, together with methyl 2,4-di-*O*-methyl-α-glucopyranoside, in the ratios of 1.0:2.8:53. In addition to these glucosides, a small proportion of methyl 2,3-di-*O*-methyl-α,β-glucopyranoside was also present.

The large excess of di-*O*-methylglucose as compared to tetra-*O*-methylglucose residues shows that de-*O*-methylation had occurred during the methanolysis. The branch points cannot, therefore, be identified with certainty. The preponderance of the 4,6-derivative suggests that branching occurs at C-2. The presence of branch points at C-6[2] only could not be confirmed. No evidence was obtained for the presence of 2,3,4-tri-*O*-methylglucose residues in the *O*-methylglucan, as would be expected were internal, (1 → 6)-linkages present[2]. However, the occurrence of a few 2,3-di-*O*-methylglucose residues indicates that, if residues linked through C-6 exist, they are branched through C-4.

For determination of the molecular weight of the purified pachyman, its nitric ester was subjected to osmotic-pressure measurements. The number-average degree of polymerization obtained was 690, a value considerably higher than that reported[2]. The nitrate had an intrinsic viscosity in acetone of 3.6 dl/g. The corresponding value for a cellulose nitrate of the same size and degree of substitution (d.s.) would have been 7.0 dl/g. The helical conformation of a β-D-(1 → 3)-linked glucan would be expected to give a lower viscosity than the linear cellulose, but part of the difference could also be due to the presence of branches in the pachyman. Reduced pachyman, dissolved in dilute alkali, was subjected to gel-permeation chromatography with Sephadex G-150. The elution curve, shown in Fig. 1, indicates a symmetrical and rather narrow molecular-weight distribution.

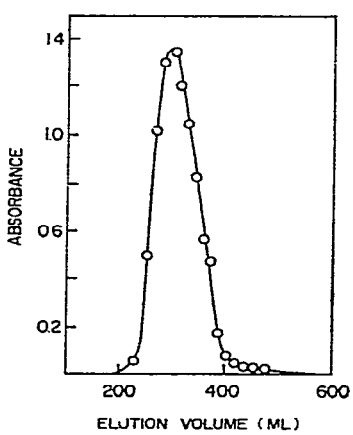

Fig. 1. Diagram of the elution of pachyman from Sephadex G-150.

In summary, 90% of the cell wall of *Poria cocos* is a polysaccharide consisting on the average of about 700 (1→3)-linked β-D-glucopyranose residues containing 3–6 branch points, possibly at C-2. A few, (1→6)-linked, internal residues might also be present; if they are, they appear to be branched at C-4. The relatively high viscosity of the glucan suggests that the branches are short in comparison with the main chain. The molecular-weight distribution is narrow.

EXPERIMENTAL

Isolation of pachyman. — Cell-wall material of *Poria cocos* Wolf (obtained from the Mikuni Co., Ltd., Dosho-machi 2-25, Higashiku, Osaka, Japan) was extracted with methyl sulfoxide for 24 h. The viscous solution was poured into 10 times its volume of water acidified with acetic acid, and the precipitate was collected by centrifugation, washed successively with methanol and petroleum ether, and dried; yield 90%, $[\alpha]_D$ −13° (*c* 1.0, methyl sulfoxide), +21.5° (*c* 1.0, 10% sodium hydroxide).

Methylation analysis. —Crude pachyman (5.1 g) was successively methylated with methyl sulfate–sodium hydroxide, and *N*,*N*-dimethylformamide–methyl iodide–silver oxide, to give an almost fully substituted (by i.r. spectroscopy) *O*-methylglucan (3.2 g, 51%). After methanolysis, a portion of the glucoside mixture was partially resolved by g.l.c., by using a column of 10% of diethylene glycol succinate on Chromosorb W. The peaks obtained indicated the presence of 2,4,6-tri-*O*-methylglucose and 2,3,4,6-tetra-*O*-methylglucose residues in the ratio of 223:1. Another portion of the glucoside mixture was acetylated[4] and the acetate resolved in a similar way by g.l.c., giving ratios of di- to tri- to tetra-*O*-methylglucose residues of 5:223:1.

A third portion of the methanolyzate was resolved by column chromatography on silicic acid (100 mesh) with 3:1 (v/v) chloroform–ethanol as the eluant. The flow rate was 0.25 ml.min^{-1}, and elution of sugars was monitored by t.l.c. Fractions 1 and 2 consisted of methyl tetra- and tri-*O*-methylglucosides, respectively. Fractions 3 and 4 contained mixtures of methyl di-*O*-methylglucosides that were acetylated[4] and the acetates resolved by g.l.c., giving methyl 2,3-, 2,4-, 2,6-, and 4,6-di-*O*-methylglucoside acetates that were identified by comparison with authentic specimens.

Molecular properties. — Purified pachyman was nitrated with a nondegrading mixture[5] of phosphorus pentaoxide, phosphoric acid, and nitric acid (5:13:32) for 1 h at 0°, to give a nitrate having a d.s. of 2.65, in a yield of 97%. Osmotic-pressure measurements were conducted on the nitrate in acetone at 15° with a Mechrolab High Speed Model 503 osmometer. The molecular weight obtained was 194,000, corresponding to a degree of polymerization of 690. The molecular weight of each of the two methylated glucans in toluene was determined at 37°. Measurements of the viscosity in acetone were performed with a Craig–Henderson viscometer[6].

For gel-permeation studies, the purified pachyman was dissolved in 0.1M sodium hydroxide (0.5%), and the solution was added to the top of a column containing Sephadex G-150. The amount of glucan in each fraction (15 ml) was determined spectrophotometrically[7].

ACKNOWLEDGMENTS

The authors express their gratitude to Drs. G. O. Aspinall and B. Lindberg for the gift of sugar samples. This investigation was supported by a grant under the McIntire–Stennis Program, United States Department of Agriculture, Cooperative State Research Service, for which grateful acknowledgment is hereby made.

REFERENCES

1 S. A. WARSI AND W. J. WHELAN, *Chem. Ind.* (London), (1957) 1573.
2 H. SAITO, A. MISAKI, AND T. HARADA, *Agr. Biol. Chem.* (Tokyo), 32 (1963) 1261.
3 G. C. HOFFMANN AND T. E. TIMELL, *Wood Sci. Technol.*, 4 (1970) 159.
4 J. K. N. JONES AND H. G. JONES, *Can. J. Chem.*, 47 (1969) 3269.
5 W. J. ALEXANDER AND R. L. MITCHELL, *Anal. Chem.*, 21 (1949) 1497.
6 A. W. CRAIG AND D. A. HENDERSON, *J. Polym. Sci.*, 19 (1956) 215.
7 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350.