Carbohydrate Research, 26 (1973) 421–425

A HEMICELLULOSIC β-D-GLUCAN FROM MAIZE STEM

A. J. BUCHALA AND H. MEIER

Institut de Biologie végétale et de Phytochimie, Université de Fribourg, CH-1700 Fribourg (Switzerland)

(Received July 19th, 1972; accepted for publication, July 24th, 1972)

ABSTRACT

A glucan of d.p. ~127 has been isolated from the hemicellulosic material of the stem tissues of the mature maize plant, *Zea mays* L. Methylation analysis and periodate-oxidation studies showed that the glucan has (1→3)- and (1→4)-linked D-glucopyranosyl residues in the molar ratio 1.0 to 2.0.

INTRODUCTION

A hemicellulosic β-D-glucan containing (1→3)- and (1→4)-linked D-glucose residues in the molar ratio 1.00 to 1.65 was isolated by Fraser and Wilkie[1] from the young-leaf tissues of the oat plant. Similar glucans were isolated from leaf and stem tissues of oat plants at various stages of maturity[2] and from fully mature barley, rye, and wheat stem-tissues[3]. Glucans have also been reported in oat coleoptiles[4] and maize stalks[5], and are known to be present in the leaf and stem tissues of bamboo[6]. With a view to examining the metabolism of hemicelluloses in the growing maize plant, a pure β-D-glucan has been isolated and subjected to structural studies.

RESULTS AND DISCUSSION

The total hemicellulose, obtained from the chlorite holocellulose by successive treatments with 5 and 24% aqueous potassium hydroxide, contained arabinose, galactose, glucose, and xylose residues (ratio 10:3:5:43), acidic sugars, and a trace of rhamnose. A variety of fractionation methods was examined, but the method finally used to isolate the glucan was based on that of Gramera and Whistler[5]. Graded ethanol precipitation of the water-soluble hemicellulosic material gave fractions enriched in glucan. Repeated washing[5] of such fractions with water gave only very small quantities of pure glucan. Most of the contaminating xylan could be removed by complexing with Fehling's solution, and only traces of xylan remained after washing the material, which did not form an insoluble complex, with water. The glucan isolated accounted for 2.5% of the total hemicellulose; *i.e.* for 30% of the D-glucose residues present in the starting material.

The polysaccharide gave no colour with iodine and was not attacked by alpha- or beta-amylase. The presence of β-D-linked residues was indicated by the low specific rotation (−5.0°) and by the presence of an i.r. peak at 895 cm^{-1}. The i.r. spectrum was very similar to those of the hemicellulosic β-D-glucans isolated from oat tissues[2], and to those of an endospermic β-D-glucan from oat tissue and an α-cellulose from oat stem.

The glucan was methylated by the methods of Haworth[7] and Hakomori[8] to yield a product (~82%) that displayed no i.r. peak attributable to hydroxyl groups. Hydrolysis of the methylated glucan by the formic acid–sulphuric acid method[9] gave 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose, and 2,4,6-tri-*O*-methyl-D-glucose. G.l.c. of the derived alditol acetates and of the methyl glycosides showed that these sugars were present in the ratio of 1:86:40. The absence of any di-*O*-methyl derivatives indicates that the glucan was unbranched, and therefore the d.p. for the methylated glucan was ~127. A value of 110 was obtained for the d.p. of the methylated glucan by vapour-phase osmometry.

The ratio of (1→3) to (1→4) linkages was also determined by periodate oxidation. Hydrolysis of the reduced oxopolysaccharide gave glucose, erythritol, and glycerol in the molar ratio 1.0:2.0:trace. The methylation analysis and the periodate-oxidation values are in good agreement.

Partial, acid hydrolysis of the D-glucan released oligosaccharides containing both β-(1→3) and β-(1→4) linkages, and others with only β-(1→3) or β-(1→4) linkages. The following oligosaccharides were tentatively identified: cellobiose, cellotriose, cellotetraose, laminaribiose, 4-*O*-β-laminaribiosyl-D-glucose and 3-*O*-β-cellobiosyl-D-glucose. Enzymic hydrolysis, using the enzyme mixture from *Cytophaga* which is known to contain β-1,3-glucanase activity[10], gave a similar mixture of oligosaccharides and others of higher d.p. which were not investigated; 4-*O*-β-laminaribiosyl-D-glucose was not detected. The lack of material prevented a detailed examination of the linkage sequences, but it is evident that up to four contiguous β-D-(1→4)-linked residues occur. There was no evidence of contiguous β-D-(1→3) linkages.

The maize β-D-glucan is structurally similar to that found in young oat leaf-tissues[1] and to those found in the endospermic tissues of various cereals[11]. It is of interest to note that the value for the ratio of β-(1→3) to β-(1→4) linkages for all of the β-D-glucan in the maize total hemicellulose was 1.0:2.4, whereas the corresponding value for the isolated glucan was 1.0:2.0. There is evidence to suggest the presence of enzymes in the tissues of various cereals which could degrade such β-D-glucans[12–14]. In oat tissues[12] and in those of wheat and barley[13], there is a progressive reduction, with increasing plant maturity, in the values of the ratio of β-(1→3) to β-(1→4) linkages and in the d.p. for all of the glucan present in the total hemicelluloses. Kivilaan *et al.*[15] have shown that isolated cell-wall fractions from maize coleoptiles autolyse to give β-D-glucan fragments which contain both (1→3) and (1→4) linkages. This autolysis was ascribed to cell-wall bound enzyme systems.

EXPERIMENTAL

Paper chromatography was carried out on Schleicher and Schuell No. 2043b paper, and t.l.c. on Kieselgel G (Merck), using the following irrigants: (*1*) ethyl acetate–pyridine–water (8:2:1); (*2*) butan-1-ol–pyridine–benzene–water (5:3:1:3); (*3*) ethyl acetate–acetic acid–water (3:1:3); (*4*) butan-1-ol–ethanol–water–ammonia (4:1:5:trace); (*5*) butanone–water–ammonia (10:1:trace); (*6*) benzene–ethanol–acetic acid–water (200:47:1:15). Chromatographic detection reagents were alkaline silver nitrate, *p*-anisidine hydrochloride, or naphth-1-ol/conc. sulphuric acid. G.l.c. was carried out on a Perkin–Elmer F 30 chromatograph, using glass columns (2 m × 2 mm i.d.) containing (*A*) 10% *m*-bis(*m*-phenoxyphenoxy)benzene on AW DMCS Chromosorb W (100–120 mesh) and (*B*) 3% ECNSS-M on Gas Chrom Q (100–120 mesh). Electrophoretic examination of sugars was carried out in 0.1M borate buffer on glass paper at 15–20 volts/cm. Polysaccharides and oligosaccharides were hydrolysed in 0.5M sulphuric acid by heating for 1 h at 120° in an autoclave, and hydrolysates were neutralised with barium carbonate. Quantitative determinations of sugars in the hydrolysates were carried out by g.l.c. of their glycitol acetates[16] (column *B*).

Isolation of the hemicellulosic material. — Mature, maize stems were cut into small pieces and immediately immersed in boiling ethanol for 20 min. The air-dried plant material was ground, and a sample (50 g) was delignified by the method of Wise *et al.*[17]. The holocellulose (40 g) was successively treated with 5 and 24% aqueous potassium hydroxide (1 litre) for 1 day, and the total hemicellulose (14 g) was isolated.

Isolation of the glucan. — The total hemicellulose was separated into water-soluble and water-insoluble fractions. Ethanol was added to a 2% aqueous solution of the water-soluble material until there was a persistent turbidity. The solution was left for 2 h at 4°, and then the insoluble material was collected at the centrifuge. On acid hydrolysis, a sample of this material released glucose and xylose in the ratio ~1:1. The remainder of this material was dissolved in 10% aqueous sodium hydroxide (50 ml), and Fehling's solution was added until complexing was complete. The soluble and insoluble fractions were separated and treated with glacial acetic acid, and the solutions were exhaustively dialysed. The material which did not form an insoluble copper complex was predominantly a glucan. This material was suspended in water and washed at the centrifuge. The water-insoluble fraction (350 mg), on acid hydrolysis, released glucose and xylose in the ratio ~50:1 and is referred to as the glucan. The glucan had $[\alpha]_D^{23}$ −5.0° (*c* 1.2, M sodium hydroxide), gave no colour when treated with iodine–potassium iodide solution, and was not attacked by alpha- or beta-amylase. The i.r. spectrum (KBr disc) showed an absorption maximum at 895 cm^{-1}.

Periodate oxidation of the glucan. — A sample (15 mg) of the glucan was oxidised in the dark at 5° with 50mM sodium metaperiodate. The periodate consumed[18] after 25 days was 0.69 mole per glucose residue. The suspension was dialysed, and the oxopolysaccharide was treated with sodium borohydride (20 mg) for 2 days. After destruction of the excess of borohydride with acetic acid, the solution was redialysed.

The polyalcohol was hydrolysed and examined by paper chromatography (irrigants *1* and *2*); glucose, erythritol, and traces of glycerol were detected. A sample of the hydrolysate was reduced with sodium borohydride, and the borate was removed by codistillation with methanol after destruction of the excess of borohydride. G.l.c. examination of the material, after acetylation with sodium acetate and acetic anhydride, revealed components with retention times identical to the peracetates of glucitol, erythritol, and glycerol, in the molar proportions 1.0:2.0:trace.

Methylation of the glucan. — Attempted, direct methylation of the glucan by the method of Hakomori[8] was unsuccessful. A sample of the glucan (150 mg) was methylated three times by the method of Haworth[7] and once by the method of Hakomori[8]. The product was extracted with boiling light petroleum, and the insoluble material (150 mg), which dissolved in chloroform, showed no i.r. absorption at $\sim 3400\ cm^{-1}$. The d.p. of this material, determined by using a Knauer vapour-phase osmometer, was ~110.

A sample (~1 mg) was treated with 4% methanolic hydrogen chloride in a sealed tube for 16 h at 100°. The product was examined directly by g.l.c. (columns *A* and *B*). Peaks corresponding in retention times to the methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-glucose, and 2,3,6- and 2,4,6-tri-*O*-methyl-D-glucoses were detected, but resolution of the methyl glycosides of the tri-*O*-methylglucoses was not achieved. Traces of the methyl glycosides of 2,3-di-*O*-methyl-D-xylose, derived from the contaminating xylan, were also detected.

The remainder of the methylated glucan was hydrolysed by the formic acid–sulphuric acid method[9], and the hydrolysate was neutralised with barium carbonate, deionised with Dowex-50 (H^+) resin, and evaporated nearly to dryness at room temperature. A sample of the hydrolysate (~5 mg) in aqueous methanol was reduced with an excess of sodium borohydride. After 12 h, the remaining sodium borohydride was destroyed with acetic acid and the borate produced was removed by codistillation with successive volumes of methanol at room temperature. This material was acetylated with acetic anhydride (1 ml) and pyridine (1 ml) in a sealed tube for 30 min at 120°. The excess of acetic anhydride was hydrolysed by the addition of water, and the reaction mixture was extracted with chloroform. The products were examined by g.l.c. (column *B*), and the identities of the components were established by comparison with authentic compounds. The methylated glycitol peracetates of the following compounds were detected: 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose, and 2,4,6-tri-*O*-methyl-D-glucose.

The hydrolysate was also examined by paper chromatography (irrigants *4* and *5*) and by t.l.c. (irrigants *4* and *6*). In each case, it was possible to distinguish 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,6- and 2,4,6-tri-*O*-methyl-D-glucoses, but the resolution of the latter two components was difficult. The remainder of the hydrolysate was fractionated by preparative t.l.c. (irrigant *4*, 3 developments). There was insufficient tetramethylglucose for further characterisation.

The major component, 2,3,6-tri-*O*-methyl-D-glucose, was obtained as a syrup, $[\alpha]_D^{21}$ +72° (*c* 0.5, water), which crystallised from ether; m.p. 118–120°.

Neither the 2,4,6-tri-*O*-methyl-D-glucose, which was chromatographically homogeneous, nor its *N*-phenylglycosylamine derivative could be induced to crystallise.

Partial hydrolysis of the glucan. — A sample of the glucan (~100 mg) was heated with 25mM oxalic acid (20 ml) at 100° for 12 h. The neutralised (barium carbonate) hydrolysate was examined by paper chromatography (irrigants *1*, *2*, and *3*). The following sugars were identified by comparison with authentic compounds: glucose, cellobiose, cellotriose, cellotetraose, laminaribiose, and material with mobility similar to either 4-*O*-β-laminaribiosyl-D-glucose or 3-*O*-β-cellobiosyl-D-glucose in the ratio -:3:2:2:1:1 (determined by the method of Somogyi and Nelson[19]). The oligosaccharides were separated by paper chromatography (irrigant *2*) and further examined by electrophoresis[20]. In this way, it was possible to show that both 4-*O*-β-laminaribiosyl-D-glucose and 3-*O*-β-cellobiosyl-D-glucose were present. Each of the oligosaccharides released only glucose on acid hydrolysis, and treatment with the β-D-glucosidase from almond emulsin (Fluka) at pH 5.5 gave glucose and the appropriate lower oligosaccharides.

A sample of the glucan, at an intermediate stage of purification, was treated with the enzyme mixture[12] from *Cytophaga* at pH 5.5. A similar series of oligosaccharides was obtained, but electrophoresis showed the absence of 4-*O*-β-laminaribiosyl-D-glucose.

ACKNOWLEDGMENTS

Thanks are expressed to the Royal Society for the award of a European Fellowship to A. J. B., and to the Swiss National Research Foundation for financial support.

REFERENCES

1 C. G. Fraser and K. C. B. Wilkie, *Phytochem.*, 10 (1971) 199.
2 C. G. Fraser and K. C. B. Wilkie, *Phytochem.*, 10 (1971) 1539.
3 A. J. Buchala and K. C. B. Wilkie, *Naturwissenschaften*, 57 (1970) 496.
4 S. Wadi and P. M. Ray, *Plant Physiol.*, 38 (1963) XIII.
5 R. E. Gramera and R. L. Whistler, *Arch. Biochem. Biophys.*, 101 (1963) 75.
6 K. C. B. Wilkie and S. L. Woo, *Phytochemistry*, submitted.
7 W. N. Haworth, *J. Chem. Soc.*, 107 (1915) 8.
8 S. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205.
9 H. O. Bouveng, H. Kiessling, B. Lindberg, and J. E. McKay, *Acta Chem. Scand.*, 16 (1962) 615.
10 D. J. Manners and J. C. Patterson, *Biochem. J.*, 98 (1966) 19c.
11 G. O. Aspinall and C. T. Greenwood, *J. Inst. Brewing*, 58 (1962) 167.
12 A. J. Buchala and K. C. B. Wilkie, *Phytochem.*, 10 (1971) 2287.
13 A. J. Buchala and K. C. B. Wilkie, *Phytochemistry*, in press.
14 A. J. Buchala and H. Meier, *Planta*, submitted.
15 A. Kivilaan, R. S. Bandurski, and A. Schulze, *Plant Physiol.*, 48 (1971) 389.
16 A. J. Buchala, C. G. Fraser, and K. C. B. Wilkie, *Phytochem.*, 10 (1971) 1285.
17 L. E. Wise, M. Murphy, and A. A. D'Addieco, *Paper Trade J.*, 122 (1946) 35.
18 G. O. Aspinall and R. J. Ferrier, *Chem. Ind. (London)*, (1957) 1216.
19 M. Somogyi, *J. Biol. Chem.*, 195 (1952) 19.
20 I. A. Preece, N. K. Garg, and J. Hoggan, *J. Inst. Brewing*, 66 (1960) 331.