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A HEMICELLULOSIC β-GLUCAN FROM THE HYPOCOTYLS OF *PHASEOLUS AUREUS*

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Abstract—A glucan of $\overline{\text{DP}}_n$ *ca* 80 has been isolated from the hypocotyls of mung bean plants (*Phaseolus aureus*). Methylation analysis and periodate oxidation studies showed that the glucan has $(1 \rightarrow 3)$ and $(1 \rightarrow 4)$ linked D-glucopyranosyl residues in the molar ratio 1·0:1·7. Oligosaccharides containing both $\beta(1 \rightarrow 3)$ and $\beta(1 \rightarrow 4)$ linked residues were isolated from partial hydrolysates.

INTRODUCTION

HEMICELLULOSIC β-glucans have been recently isolated from oat leaf[1] and maize stem[2] and are known to be present in other monocotyledonous tissues.[3–5] Acidic β-glucans are also found in the compression wood of various Gymnospermae.[6] Using particulate enzyme preparations from the hypocotyls of *Phaseolus aureus* several groups of workers have synthesized β-glucans. It has been claimed that when GDP-glucose is used as precursor the product contains only $\beta(1 \rightarrow 4)$ linkages whereas $\beta(1 \rightarrow 3)$ linked glucans are produced when UDP-glucose is used as the precursor.[7] A product containing both $\beta(1 \rightarrow 4)$ and $\beta(1 \rightarrow 3)$ linkages has also been obtained with UDP-glucose.[8] The hemicelluloses from the hypocotyl of *P. aureus* have been examined in order to determine structural features of the naturally occurring polysaccharides which contain glucose residues.

RESULTS AND DISCUSSION

Previous studies showed that the young hypocotyls of *P. aureus* contain a high proportion of non-cellulosic glucan, other hemicelluloses, pectic substances and traces of starch.[9] The aqueous extract of cell wall preparations from the 2-day-old hypocotyls was found to contain a considerable quantity of glucan. This material was treated with α- and β-amylases and the water-soluble protein in the residue was removed by the Sevag method.[10] On acid hydrolysis the residual polysaccharide, which contained a negligible quantity of

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protein, gave glucose and traces of galactose. It gave no colouration with iodine and was not attacked by α- nor by β-amylase. The presence of β-glucosidic linkages was indicated by the low specific rotation ($-2°$) and by the presence of a peak at 890 cm^{-1} in the IR spectrum.

The glucan was methylated by the methods of Haworth[11] and Hakomori[12] to yield a product (*ca* 60%) which displayed no peak attributable to hydroxyl absorption in its IR spectrum. Hydrolysis of the methylated glucan by the $HCOOH/H_2SO_4$ method 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. GLC of the derived glycitol acetates and of the methyl glycosides showed that these sugars were present in the ratio of 1:28:52. The absence of any di-*O*-methyl derivatives shows that the glucan is unbranched and therefore the $\overline{DP}_n$ for the methylated glucan was *ca* 81.

The ratio of $(1 \rightarrow 3)$ to $(1 \rightarrow 4)$ linkages was also determined by periodate oxidation. On treatment with $NaIO_4$ the polysaccharide reduced 0·65 mol of the periodate per anhydro hexose residue. Hydrolysis of the reduced oxopolysaccharide gave glucose and erythritol in the molar ratio 1·0:1·6. The methylation analysis and the periodate oxidation values are in good agreement.

Partial acid hydrolysis of the glucan with 0·025 M oxalic acid or enzymic hydrolysis with an enzyme preparation from *Cytophaga*, containing $\beta(1 \rightarrow 3)$-glucanase activity, released oligosaccharides containing both $\beta(1 \rightarrow 3)$ or $\beta(1 \rightarrow 4)$ linkages and others with only $\beta(1 \rightarrow 3)$ or $\beta(1 \rightarrow 4)$ linkages. The oligosaccharides obtained by acid hydrolysis were isolated and further examined; the following were tentatively identified: cellobiose, cellotriose, cellotetraose, laminaribiose, 3-*O*-β-cellobiosylglucose and traces of 4-*O*-β-laminaribiosylglucose.

The β-glucan from *P. aureus* is structurally similar to the β-glucans found in the endospermic[13] and the non-endospermic tissues[1-4] of the Gramineae. Recently xyloglucans containing $\beta(1 \rightarrow 4)$ linked D-glucose residues have been shown to be present in primary cell walls[14,15] but the glucan obtained from the hypocotyl cell walls of *P. aureus* was free of xylose residues and in addition there was no evidence of a xyloglucan fraction. The differences may be due to the fact that the xyloglucans were obtained from the culture medium and the cell walls of suspension cultured cells where growth conditions are considerably different from those prevalent during natural growth. The *P. aureus* glucan which is present in considerable quantities only at the very early stages of growth[9] could either be degraded in the cell wall by the action of glucanases known to be present in cell walls[16,17] or it could be diluted by the formation of other hemicelluloses during plant maturity. Extraction of older cell walls from hypocotyl tissue however gave almost no hemicellulosic glucan in the water-soluble fraction. Thus the β-glucan containing both $(1 \rightarrow 3)$ and $(1 \rightarrow 4)$ linkages obtained after *in vitro* biosynthesis with UDP-glucose and particulate enzyme preparations at the same stages of development of this plant seems to be a naturally occurring polysaccharide.[8]

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EXPERIMENTAL

General methods. PC was on Schleicher and Schuell No. 2043b paper and TLC on Kieselgel G (Merck) using the following irrigants: *A*, EtOAc–pyridine–H_2O (8:2:1); *B*, EtOAc–pyridine–H_2O (2:1:2); *C*, *n*-BuOH–pyridine–C_6H_6–H_2O (5:3:1:3); *D*, EtOAc–HOAc–H_2O (3:1:3); *E*, *n*-BuOH–EtOH–H_2O–NH_3 (4:1:5:trace) and *F*, C_6H_6–EtOH–HOAc–H_2O (200:47:15:1). Chromatographic detection reagents were alkaline $AgNO_3$, *p*-anisidine HCl or naphth-1-ol/conc H_2SO_4. A Perkin–Elmer F-30 chromatograph was used for GLC with glass columns (2 m × 2 mm i.d.) containing *a*, 3% ECNSS-M on Gas Chrom Q (100–120 mesh) or *b*, 10% *m*-bis(*m*-phenoxyphenoxy)C_6H_6 on AW DMCS Chromosorb W (100–120 mesh). Polysaccharides and oligosaccharides were hydrolysed with 0·5 M H_2SO_4 in sealed tubes at 100° for 12–16 hr and the hydrolysates were neutralized with $BaCO_3$. The neutral sugars in hydrolysates were estimated by GLC of their glycitol acetates (column *a*).

Isolation of the cell wall material. Phaseolus aureus seeds were soaked overnight in H_2O and then allowed to germinate in the dark at *ca* 25° on moist sawdust. After 3 days the shoots were harvested, the hypocotyls were separated from the remainder of the seedlings and were washed with H_2O. They were then immersed in liquid N_2 and the frozen tissue was powdered in a precooled mortar. All subsequent isolation steps were carried out at 2–4°. The powdered material was suspended in 0·05 M phosphate buffer (2·5 vol.) at pH 6·8 and homogenized (Ultra-Turrax) for 5 min. The insoluble material was washed with the buffer (2 × 1 vol), H_2O (1 vol.) and resuspended in $CHCl_3$/MeOH (1:1, 2·5 vol.). The suspension was homogenized for 3 min and the insoluble material washed with $CHCl_3$/MeOH (1:1, 2 × 1 vol.) and with acetone (2 vol.). The cell wall material was dried over P_2O_5 in a desiccator. Microscopic examination of the material showed only traces of starch.

Isolation of the cell polysaccharides. Cell wall material (3·6 g) was heated with H_2O (400 ml) for 6 hr at 85° with constant stirring. The H_2O-soluble material gave a weak positive starch/iodine reaction and was treated with a mixture of α- and β-amylases for 8 hr at 37° in phosphate buffer (pH 5·8). After dialysis the non-diffusable material (230 mg) gave no colouration with I_2/KI soln. After removal of the H_2O-soluble protein by the method of Sevag[10] the material, on acid hydrolysis, gave glucose and traces of galactose and is referred to as the glucan. This polysaccharide had $[\alpha]_D^{24} - 2°$ (c. 0·9 in M NaOH) and it absorbed in the IR at 890 cm^{-1} (KBr disc). The hemicelluloses extracted from the residual plant material with 4 and 20% KOH released on acid hydrolysis, arabinose, galactose, glucose, xylose and acidic sugars. These alkali-soluble fractions were not studied in detail.

Periodate oxidation of the glucan. A sample (10 mg) of the glucan was oxidized in the dark at 5° with 0·05 M $NaIO_4$. The periodate consumed after 28 days was 0·65 mol per hexose residue. The solution was dialysed, and the oxopolysaccharide was treated with $NaBH_4$ (20 mg) for 2 days. After the destruction of the excess of borohydride with HOAc, the solution was redialysed. The polyalcohol was hydrolysed and examined by PC (irrigant *A*); glucose and erythritol were detected. A sample of the hydrolysate was reduced with $NaBH_4$, and the borate was removed by codistillation with MeOH after destruction of the excess of borohydride. GLC examination of the material, after acetylation with NaOAc/Ac_2O, revealed components with retention times identical to the peracetates of glucitol and erythritol, in the molar proportions 1·0:1·6.

Methylation of the glucan. A sample of the polysaccharide (50 mg) was methylated three times by the method of Haworth[11] and once by the method of Hakomori[12] to yield a product (35 mg) which showed no absorption attributable to hydroxyl in its IR spectrum. A sample was treated with 4% MeOH/HCl in a sealed tube (100°; 16 hr) and the product was examined directly by GLC (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. The remainder of the methylated glucan was hydrolysed and the neutralized ($BaCO_3$) and deionized (Dowex 50 H^+) hydrolysate was taken to dryness. A sample (5 mg) of the hydrolysate in aq. MeOH was reduced with $NaBH_4$ and acetylated with Ac_2O–pyridine (1:1). The products were examined by GLC (column *a*), 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*-methylglucose, 2,3,6-tri-*O*-methylglucose and 2,4,6-tri-*O*-methylglucose. The hydrolysate was also examined by PC (irrigants *E* and *F*) and by TLC (irrigants *E* and *F*) and the identities of the above sugars were further confirmed.

Partial hydrolysis of the glucan. A sample of the glucan (80 mg) was heated with 0·025 M oxalic acid (50 ml; 18 hr; 100°) and the cooled solution was neutralized ($BaCO_3$) and examined by PC (irrigants *B*, *C* and *D*). The following oligosaccharides were identified by comparison with authentic specimens and by comparison of their $R_{glucose}$ values;[1,18] cellobiose, cellotriose, cellotetraose, laminaribiose, 3-*O*-β-cellobiosylglucose and 4-*O*-β-laminaribiosylglucose. A similar series of oligosaccharides was obtained on treatment of the glucan with an enzyme preparation from *Cytophaga*. The oligosaccharides were isolated by preparative PC (irrigant *B*) and on acid hydrolysis released only glucose. Treatment of the oligosaccharides with almond emulsin (Fluka) gave glucose and the appropriate lower oligosaccharide.

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