Phytochemistry, 1972, Vol. 11, pp. 2559 to 2562. Pergamon Press. Printed in England.

SHORT COMMUNICATION

ARABINOGALACTAN FROM *PHASEOLUS ATROPURPUREUS* LEAVES

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(*Received* 3 *January* 1972, *in revised form* 10 *March* 1972)

Key Word Index—*Phaseolus atropurpureus*; Leguminosae; Siratro cultivar; arabinogalactan.

Abstract—Water-soluble polysaccharide material comprising D-galactose (53·0%), L-arabinose (33·2%) and D-glucuronic acid (13·8%) has been isolated from the leaves of *Phaseolus atropurpureus*. Acid hydrolysis, periodate oxidation and methylation have indicated a highly branched structure. The principal interglycosidic linkages have been tentatively identified as 1,3- and 1,6-linked D-galactopyranose and 1,3-linked L-arabinofuranose residues. In synthesising polysaccharide with these structural features, *P. atropurpureus* differs from other legumes such as soybean, lucerne and *Centrosema*.

INTRODUCTION

ARABINOGALACTANS occur widely as water-soluble polysaccharides in most coniferous woods.[1] In addition they have been isolated from such diverse sources as *Centrosema* seed,[2] soybean,[3] coffee,[4] lucerne,[5] saguaro cactus[6] and green seaweeds.[7] The polysaccharides are considered to have highly branched structures and to fall into two groups:[5] (i) those associated with pectin, having 1,4- linked D-galactopyranose residues; and (ii) the polymers from coniferous woods containing 1,3- and 1,6- linked D-galactopyranose residues. Attached to the galactose residues as end groups are either single L-arabinofuranose units or disaccharides of 3-*O*-*β*-L-arabinofuranose. The proportions of arabinose to galactose vary considerably, and 4-*O*-methyl-D-glucuronic acid has been reported from the arabinogalactan of the Bunya pine.[8] This paper describes structural investigations into the water-soluble polysaccharide from the leaf of Siratro, a cultivar of *Phaseolus atropurpureus*, which has been bred for use as a tropical pasture legume.[9]

RESULTS AND DISCUSSION

Hydrolysis and chromatographic analysis of the crude polysaccharide showed the presence of galactose and arabinose plus a small quantity of xylose. On DEAE-cellulose, the major carbohydrate component was eluted as one sharp peak, an indication of homogeneity. The isolated material had a carbohydrate content of 97%.[10] Hydrolysis and cochromatography with authentic sugars in solvents (A), (B), and on ionophoresis, established

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the identity of the sugars, and the ratio of arabinose to galactose as 1:1·6. No xylose was detected in this fraction. Ionophoresis in phosphate buffer (pH 7) indicated the presence of glucuronic acid in the hydrolysate.

The arabinogalactan was fractionated on a Sephadex G100 column with a void volume of 60 ml. Fractions containing carbohydrate were combined (eluted 70 and 150 ml, peak at 125 ml) excluding those which exhibited strong absorbance at 280 nm (peak at 225 ml). This material had a carbohydrate content of 98% and hydrolysis and chromatographic analysis indicated that it was identical to that isolated from DEAE-cellulose. Further confirmation of the identity of the sugars was obtained from GLC of the TMS derivatives. Good separations of all peaks were obtained under the conditions described. The proportions of arabinose to galactose, calculated from previous investigations into the peak areas produced from known mixtures, gave a ratio of 1:1·65, in good agreement with the paper chromatographic method. In addition GLC showed the presence of a small quantity (*ca.* 3%) of glucurone in the hydrolysate. Both fractionation procedures gave a yield of about 10% of the original crude polysaccharide. Subsequent purifications were performed on Sephadex. The purified arabinogalactan had $[\alpha]_D^{25} + 9°$ (*c*, 0·54 in water) and an equivalent of 1275. The latter figure corresponds to an anhydro-uronic acid content of 13·8%.

On treatment with periodate, the polysaccharide reduced 0·70 mol periodate per anhydrohexose unit after two days, with no further increase. This suggested that there was a considerable amount of uncleaved monosaccharide units left and this was confirmed by reduction of the oxopolysaccharide followed by hydrolysis. Chromatographic analysis of the hydrolysate indicated the presence of substantial quantities of arabinose, galactose and glycerol in addition to slower moving material.

TABLE 1. GLC ANALYSIS OF THE METHOLYSED, METHYLATED ARABINOGALACTAN

Identified component*	Relative retention times† Column (a)	Column (b)	Relative %
2,3,5-Tri-*O*-methyl-L-arabinose	0·54, 0·71	0·46, 0·62	2·1
2,3,4-Tri-*O*-methyl-L-arabinose	1·05	0·79	9·1
2,3,4,6-Tetra-*O*-methyl-D-galactose	Not resolved	1·50, 1·61	3·6
2,5-Di-*O*-methyl-L-arabinose	1·87, 3·53	0·67, 1·08	29·5
2,4,6-Tri-*O*-methyl-D-galactose	4·17, 4·79	2·14, 2·41	21·5
2,3,4-Tri-*O*-methyl-D-galactose	7·63	2·78, 2·93	12·6
2,4-Di-*O*-methyl-D-galactose	—	3·88, 4·47	11·4
2,3,4-Tri-*O*-methyl-D-glucuronic acid	2·50, 3·23	Not resolved	10·3

* As the methyl glycosides.
† β-Methyl-2,3,4,6-tetra-*O*-methyl glucoside = 1·00.

Methylation gave a final product having a methoxyl content of 36·8% which was not raised on further treatment. Methanolysis followed by GLC analysis gave peaks identified as listed in Table 1.

The tetramethylgalactose was masked by the dimethylarabinose on column (a), but identified on column (b). Allowance was made for the overlap, in calculation of the relative proportions. Equal detector responses were assumed for all the products. From Table 1 it can be calculated that the expected reduction of periodate, assuming all uronic acid is end group glucuronic acid, would be 0·73 mol periodate per anhydrohexose unit. This is close to the actual figure obtained. However, titration gave a higher uronic acid value than

that estimated by GLC giving a theoretical reduction of about 0·78 mol periodate. This figure would be depressed if some of the uronic acid existed as 4-*O*-methylglucuronic acid in the polymer. It is evident that the polymer has a highly branched structure with end group L-arabinofuranose, L-arabinopyranose and D-galactopyranose. The principal linkages are 1,3-linked D-galactopyranose units and 1,3-linked L-arabinofuranose units with an appreciable proportion of 1,6-linked D-galactopyranose. The presence of 2,4 di-*O*-methylgalactose indicates branching through the 6-position of D-galactose residues. Glycerol, detected in the hydrolysate of the reduced oxopolysaccharide, is consistent with methylation evidence, arising from end group L-arabinofuranose, D-galactopyranose and 1,6-linked D-galactopyranose. Likewise, the absence of threitol is consistent with the absence of 2,3,6-tri-*O*-methylgalactose.

The present results indicate that the type of polysaccharide occurring in Siratro, being devoid of 1,4-linked galactose residues, differs from soybean[3] which falls into category (i) (see Introduction). Siratro also differs from lucerne[5] and *Centrosema*[2] which contain all three intergalactose linkages, although the arabinogalactan from lucerne may comprise a mixture of polymers. It is of interest to note that subsequent oxalate extraction of the Siratro leaf used here yielded a substantial quantity of starch but no pectin.[11] This is in contrast to recently published work which quoted large amounts of pectin in Siratro leaf.[12]

EXPERIMENTAL

General experimental conditions. Paper chromatography was carried out on Whatman No. 1 and 3 MM paper in the following solvent systems: (A) EtOAc–pyridine–H_2O, 8:2:1, (B) EtOAc–HOAc–HCOOH–H_2O, 18:3:1:4. Zone ionophoresis was performed in 0·5 M sodium borate at 30 V/cm for 1·5 hr. Alkaline $AgNO_3$[13] and aniline oxalate were used in the detection of sugars on paper chromatograms. Methylation products were separated on the following GLC columns:[14] (a) Butane-1,4-diolsuccinate polyester (15%) on Chromosorb W (60–80 mesh) (152 by 0·2 cm i.d.) at 170°. (b) Polyphenyl ether (5 ring, 10%) on Chromosorb W (60–80 mesh) (152 by 0·2 cm i.d.) at 190°. Products from acid hydrolysis were separated as their trimethylsilylethers (TMS) on SE30 (3%) on Chromosorb W (80–100 mesh) (152 by 0·2 cm i.d.) at 140° programmed to rise at 2° per min using a flame ionisation detector. Solutions were concentrated under reduced pressure at 40° and quantitative determinations of carbohydrates were made with the phenol-sulphuric acid reagent.[10]

Isolation of the arabinogalactan. Siratro leaf (100 g dry wt), previously extracted with boiling 80% EtOH,[15] was further treated with H_2O (3 × 1·51) at room temp. for a total of 4 hr. Addition of EtOH (3 vol.) precipitated crude polysaccharide which on freeze-drying gave a pale brown solid, 3·0% by wt of original dry leaf (equivalent to 0·48 g pure polysaccharide).[10]

Purification. (a) *DEAE-cellulose.* Crude polysaccharide (1 g), dissolved in a minimum of H_2O was applied to a column of DEAE-cellulose (chloride form). A gradient elution of $H_2O \rightarrow$ 0·5 M KCl was set up, and fractions (5 ml) were collected from an effluent flow of 30 ml/hr. One sharp carbohydrate peak was eluted and isolated after dialysis and freeze-drying. (b) *Sephadex* G100. Crude polysaccharide (1 g), in H_2O, was applied to a column of Sephadex G100 previously swollen in H_2O. The column was irrigated with H_2O and effluent was monitored for carbohydrate and for absorption at 280 nm. The appropriate fractions were combined and freeze-dried.

Identification of hydrolysis products. Hydrolysis was carried out in N H_2SO_4 at 100° in a sealed tube. After neutralisation with $BaCO_3$ and removal of Ba^{2+} ions with cation exchange resin, the solution was analysed in two ways. (a) An aliquot corresponding to *ca.* 2 mg carbohydrate was dried *in vacuo* at 40° then over P_2O_5. The TMS derivatives were prepared by dissolving the syrup in TRI-SIL Z (Pierce Chem. Co.), and the mixture injected directly into the gas chromatograph. Standards prepared from known sugars were run concurrently. (b) The remaining hydrolysate sugars were applied to 3 MM paper, separated and analysed in the usual way.

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Determination of acidity. After cations were removed with Amberlite IR 120 (H) resin, the polysaccharide (45 mg) was dissolved in 0·01 N NaOH (10 ml) and heated in a flask closed with soda lime at 60° for 30 min. 10 ml 0·01 N NaOH was similarly treated in a parallel experiment. The solutions were then titrated with 0·01 N HCl.

Periodate oxidation. Purified polysaccharide (74·74 mg) was dissolved in sodium metaperiodate (25 ml, 0·015 M) and the reaction allowed to proceed in the dark at room temp. Reduction of periodate was measured from the absorption of the solution at 223 nm.[16]

Borohydride reduction. After excess periodate was destroyed with ethylene glycol, oxopolysaccharide from above was precipitated with EtOH. After one re-precipitation, the oxopolysaccharide was treated with excess sodium borohydride in H_2O for 24 hr. After destruction of remaining borohydride with HOAc, and removal of borate and Na^+ ions, the reduction product was hydrolysed and examined chromatographically.

Methylation. Polysaccharide (231 mg) was stirred overnight with sodium borohydride solution (20 ml, 1%), before being subjected to nine Haworth methylations followed by six Purdie methylations. The final product was metholysed with 3% methanolic HCl and analysed by GLC.

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