Note

Isolation and structural studies of a water-soluble galactan from potato (*Solanum tuberosum*) tubers*

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INTRODUCTION

The role of cell-wall and intercelluiar polysaccharides in the texture of the cooked potato has been the subject of much speculation[1–3]; but there is scant information on the structure of these components. Without this information, useful insight into features of organization within the plant, such as intercellular adhesion, is difficult. The presence in the potato tuber of large amounts of starch makes the extraction, purification, and identification of other polysaccharides a difficult task. Nevertheless, typical plant polysaccharides, such as the pectic group of substances, hemicelluloses, and cellulose, have been broadly identified[4].

β-(1→4)-Linked-D-galactopyranose residues are generally recognized as forming part of the pectic group of substances, but reports of β-(1→4)-linked D-galactans are relatively rare. Hirst *et al.*[5] described a linear β-(1→4)-linked-D-galactan from seeds of *Lupinus albus*, and similar polysaccharides, though possessing some branching, have since been isolated from *Strychnos nux-vomica* seeds[6] and from sugar-beet pectin[7]. We now report the isolation and structural studies of a water-soluble galactan present in tubers of the potato (*Solanum tuberosum*).

RESULTS AND DISCUSSION

The 80% ethanol-insoluble residues from four separate, tuber extractions, which showed variations in yield of only ±2% (total yield, 21% of peeled, diced tuber) were combined and extracted with cold water. Starch and some protein were removed from the water-soluble extract by treatment with the mixture of amylolytic and proteolytic enzymes, HT-1000, followed by filtration, dialysis, and precipitation with 80% ethanol. After removal of some insoluble, non-carbohydrate impurity, freeze-drying gave a crude polysaccharide product (0.06% of wet weight), showing sugar components characteristic of the pectic group of substances, namely galactose, arabinose,

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and uronic acid. Some glucose, presumed to derive from residual traces of starch or its degradation products, was also detected.

Fractionation of the crude polysaccharide on CM-cellulose produced fraction I (0.042% of wet weight) eluted by water, and fraction II (0.013%) eluted by acid salt. The latter fraction was largely protein, but also contained some polysaccharide which gave arabinose and lesser amounts of galactose and uronic acid on acid hydrolysis. Thus, elution from CM-cellulose, as well as allowing simple removal of protein, appeared to have fractionated small amounts of material rich in arabinan. The basis of such a fractionation is not clear, but suggests a close association between protein and carbohydrate.

The carbohydrate-enriched fraction I was further fractionated on DEAE-cellulose (borate form)[9] to give fraction III (0.015% of wet weight) eluted by water and which showed galactose–arabinose–glucose ratios of 15.2:1:1.3, and a number of fractions eluted by borate. The elution profile is shown in Fig. 1. In a survey[8] of the non-starch polysaccharides from tubers of the Netted Gem variety of potato grown in various locations in Canada, considerable variability was noted. Thus, elution profiles quite different to Fig. 1 were sometimes obtained, and some samples of tuber which contain very little of the title polysaccharide were found. These results will be reported elsewhere.

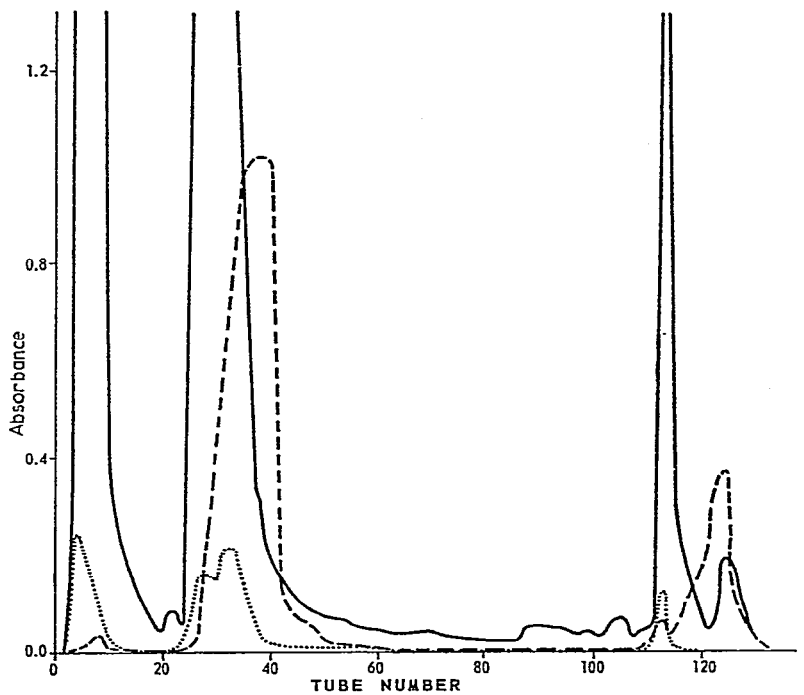

Fig. 1. Fractionation of fraction I on DEAE-cellulose (borate): ———, cysteine–sulphuric acid; – – – –, u.v. (280 nm); · · · · · ·, carbazole.

The results of fractional precipitation of fraction III with ethanol are shown in Table I. Essentially, the 43–62% ethanol fractions seemed to be the same, and were therefore combined. This material, the potato galactan, had $[\alpha]_D^{26}$ +87° (water), showed a single symmetrical peak on sedimentation analysis, and appeared to be homogeneous. Acid hydrolysis and g.l.c.[13] showed a galactose–arabinose ratio of 15.6:1, confirmed by the cysteine–sulphuric acid[10,14] colorimetric procedure (16:1). G.l.c.–m.s.[15] confirmed the sugars as galactose and arabinose.

TABLE I

FRACTIONAL PRECIPITATION BY ETHANOL OF FRACTION III

Ethanol concentration (%)	*Weight* (*mg*)	$[\alpha]_D^{25}$ (*water*) (*degrees*)	*Sugars present*
43	39.5	+78	Gal, Ara
50	11.3	+65	Gal, Ara
58	11.2	+76	Gal, Ara
62	5.3	+68	Gal, Ara
79	7.2		Gal, Ara, Glc
Supernatant (≥ 80)	53.4		Gal, Ara, Glc

The polysaccharide was difficult to methylate, a feature of β-(1→4)-linked D-galactans which has been reported previously. After repeated Haworth methylations, methylation by the method of Srivastava *et al.*[16] gave a pale-brown product which showed only a small i.r. hydroxyl absorption and had $[\alpha]_D^{27}$ −20.3° (chloroform).

The previously reported[6,7] difficulty in the methanolysis of methylated β-(1→4)-linked-D-galactans was also found for the potato galactan. Thus, 90% formic acid, followed by sulphuric acid, was required for hydrolysis. The identities of the methyl ethers were determined from their behaviour on paper chromatography and paper electrophoresis and from g.l.c. of their alditol acetates, which are tabulated in Table II. Where possible, conclusive identifications were made from the mass spectra of the methylated alditol acetates[15]. Positive, individual identifications of 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3-di-*O*-methyl-L-arabinose were difficult because of the overlapping of peaks. However, the mass spectra were consistent with a mixture of the alditol acetates of these two components. The presence of 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-galactitol, the major component, was confirmed by its mass spectrum. The tail from this component tended to "swamp" the peak of 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methyl-D-galactitol. Since the 2,6-di-*O*-methyl derivative gives no unique, additional fragments in the mass spectra, positive identification of this component is difficult, as reported previously by Aspinall and Cottrell[17]. However, the 2,6-di-*O*-methyl derivative gives a prominent fragment-ion of *m/e* 129, whereas a standard of the 2,3,6-tri-*O*-methyl derivative gave this ion as a minor fragment. Thus, despite the contamination of the 2,6-di-*O*-methyl derivative by the 2,3,6-tri-*O*-methyl derivative, the observed increase in relative intensity of the fragment ion of *m/e* 129 and the lack

of any fragment ions indicative of other di-*O*-methyl derivatives of galactose served to identify this component as the alditol acetate from 2,6-di-*O*-methyl-D-galactose.

TABLE II

G.L.C. OF METHYLATED ALDITOL ACETATES FROM METHYLATED POTATO GALACTAN

Methyl sugar	*Relative retention time*[a]	*Percentage molar composition*
2,3,4,6-Me_4-gal	1.20	1.2[b]
2,3,-Me_2-ara	1.30	2.6[b]
2,3,6-Me_3-gal	2.23	89
2,6-Me_2-gal	3.31	5.2
2,3-Me_2-gal	5.15	2.6

[a]Relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. [b]Overlapping peaks; molar percentages are approximate.

2,3-Di-*O*-methyl-D-galactose was present in small amount, and the derived alditol acetate emerged as a broad peak on g.l.c. When large amounts were injected, tailing from the 2,3,6-tri-*O*-methyl derivative prevented identification by mass spectrometry. Identification of 2,3-di-*O*-methyl-D-galactose therefore rests upon its M_G value and g.l.c. retention-time.

By analogy with other plant, pectic substances, the galactose was assumed to be the D-form and the arabinose the L-form. The optical rotations of the polysaccharide ($[\alpha]_D^{26}$ +87°) and methylated polysaccharide ($[\alpha]_D^{27}$ −20°) provide strong evidence for a large proportion of β-linked D-galactopyranose residues.

The loss of arabinose during methylation suggested inadvertent fractionation or degradation, a feature of arabinan-contaminated fractions reported previously[17]. Thus, it was likely that the 2,3-di-*O*-methyl-L-arabinose arose from contaminating arabinan and was not an integral part of the polysaccharide.

The galactan was not fully methylated, as shown by the persistence of a small i.r. hydroxyl absorption. By analogy with the report of Andrews *et al.*[6] on the methylated galactan from *Strychnos nux-vomica* seeds, all or most of the 2,6-di-*O*-methyl-D-galactose was attributed to undermethylation. The rather low percentage of 2,3,4,6-tetra-*O*-methyl-D-galactose may likewise be attributed to undermethylation, though some isolation losses are also possible.

Periodate-oxidation data showed a consumption of 1.08 moles of periodate and a production of 0.095 mole of formic acid per mole of polysaccharide; the arabinan contaminant must have accounted for the consumption of some of the periodate. Since the amount of polysaccharide was limited, only one assay was performed. These results suggested a galactan of about 60 β-(1→4)-linked D-galactopyranose residues containing 2 to 3 (1→4,6)-linkages. This required a molecular weight of just under 10,000 for the polysaccharide, which was consistent with the fact that a synthetic boundary cell was necessary for sedimentation analysis.

EXPERIMENTAL

Paper chromatography was performed by the descending method on Whatman No. 1 paper with (*A*) ethyl acetate–pyridine–water (8:2:1); (*B*) 1-butanol–ethanol–water (40:11:19). Paper electrophoresis was performed on Whatman No. 3MM paper with borate buffer[18] (pH 10) or 0.2M sodium acetate buffer (pH 5). Sugars were detected on paper chromatograms and electrophoretograms with aniline hydrogen phthalate. Unless otherwise stated, conditions for acid hydrolyses were M sulphuric acid at 100° for 3 h, followed by neutralization with barium carbonate. Rotations were measured on a Perkin–Elmer 141 polarimeter. Evaporations were carried out under diminished pressure at 35° on a rotary evaporator.

Extraction of polysaccharide. — Netted Gem tubers (*ca.* 7 kg) from New Brunswick were selected at random from sacks that had been stored for 6 months at 4°. The tubers were washed, peeled, and diced and, after mixing of the pieces, 4 batches of 250 g were extracted separately with 95% ethanol (1000 ml per batch) in a Waring Blendor at 17,000 r.p.m. for 5 min. The resultant slurries were filtered, washed twice with 80% ethanol (125 ml per batch), and further extracted with 80% ethanol (500 ml per batch) by stirring in a beaker at room temperature for 1 h. The slurries were filtered and air-dried for 48 h to give a total of 208 g of ethanol-insoluble residue (weight corrected for moisture).

The ethanol-insoluble material (203 g) was extracted for 24 h at 4° with water (2,500 ml) and centrifuged, the supernatant collected, and the residue washed with water (*ca.* 400 ml). The supernatant and washings were concentrated (to *ca.* 100 ml), centrifuged to remove a small amount of insoluble material, heated to *ca.* 85° for 1 h (solution becomes cloudy), and then cooled to 63°, and a filtered solution of Takamine HT-1000 (50 mg; Miles Chemical Company, Elkhart, Indiana, U. S. A.) was added. After 1 h, the solution was allowed to cool, left overnight under toluene vapour, and filtered. The filtrate was then dialysed against running tap-water in the presence of chloroform before precipitation of the crude polysaccharide material with 80% ethanol in the cold. The precipitate was collected by decantation and centrifugation, washed with 80% ethanol, dissolved in water (cloudy solution), concentrated, and freeze-dried. The freeze-dried material was re-dissolved in water (*ca.* 1% w/v) and insoluble material (showing only traces of sugars after acid hydrolysis) removed by centrifugation. The supernatant and washings were combined and freeze-dried to give 555 mg of crude polysaccharide extract. Acid hydrolysis and paper chromatography (solvent *A*) indicated the presence of galactose, glucose, uronic acid, and arabinose.

Fractionation of crude polysaccharide mixture. — The crude polysaccharide extract (500 mg) in water (*ca.* 5 ml) was eluted (1–2 ml/min) from a column (16 × 2.2 cm) of Whatman CM-32 cellulose (H^+ form) with water (*ca.* 200 ml) until the effluent gave a negative reaction in the anthrone[19] test for carbohydrate. Elution was continued with 4M sodium chloride (200 ml; adjusted to pH 1 with HCl). The aqueous fraction was concentrated and freeze-dried to give fraction I (357 mg). The salt fraction was dialysed, concentrated, and freeze-dried to give fraction II (112 mg).

Acid hydrolysis of fraction I and paper chromatography (solvent *A*) indicated galactose as the major sugar and small amounts of uronic acid, glucose, and arabinose. Similar treatment of fraction II revealed only traces of carbohydrate. A portion (50 mg) of fraction II was digested with Pronase B (2 mg; Calbiochemical Co. Ltd., California) in 0.1M acetate buffer (pH 7.5; 5 ml) containing Ca^{2+} (1mM) for 24 h under sterile conditions at 37°. The solution was then deproteinised three times with chloroform–pentyl alcohol (10:1) and the aqueous layer dialysed (24 h) against running tap-water in the presence of chloroform and then against two changes of distilled water. The solution was then concentrated and freeze-dried to give fraction II(*a*) (11.3 mg). Acid hydrolysis and paper chromatography (solvent *A*) showed arabinose and small amounts of galactose and uronic acid.

Fraction I (357 mg) in water (*ca.* 3 ml) was eluted (1.5 ml/min) from a column (24 × 2.3 cm) of Whatman DEAE-cellulose (borate form)[9], and 15-ml fractions were collected. Elution with water (20 fractions) was followed by a continuous gradient (0–0.5M) of sodium metaborate (90 fractions) and finally 4M sodium chloride adjusted to pH 1 with hydrochloric acid (20 fractions). The fractions were monitored by u.v. absorbance (280 nm), a cysteine–sulphuric acid reaction[10], and a modified carbazole reaction[11]. The elution profile is shown in Fig. 1.

Tubes 3–16 were combined, stirred with Rexyn 101 (H^+) resin, and concentrated. Methanol was evaporated five times from the residue which was then dissolved in water and freeze-dried to give white, fluffy material designated as fraction III (128 mg). Acid hydrolysis and paper chromatography (solvent *A*) showed the presence of galactose and small amounts of arabinose and glucose which were estimated by preparative, paper chromatography and colorimetry[12].

Fractions eluted by borate were acidified (pH 5) and dialysed. After five evaporations of methanol, the product was freeze-dried to give a number of fractions (total yield, 164 mg) which on acid hydrolysis and paper chromatography (solvent *A*) showed the presence of galactose, arabinose, and uronic acid. Similar material (4 mg) was recovered from the 4M sodium chloride (pH 1) fraction to give an overall recovery of 83%.

Fraction III (125 mg) was dissolved in water (19 ml) and centrifuged to remove traces of insoluble material. The supernatant and washings (2 × 0.5 ml) were combined and the polysaccharide was fractionally precipitated with ethanol. The results are summarised in Table I. The precipitates obtained at 43, 50, 58, and 62% ethanol were combined to give a white, fluffy material (58.1 mg), hereafter referred to as potato galactan. Acid hydrolysis and paper chromatography (solvent *A*) showed galactose and arabinose. No uronic acid was detected on paper electrophoresis in acetate buffer.

Analysis of potato galactan. — The potato galactan showed $[\alpha]_D^{26}$ +87° (*c* 0.1, water) and contained N, 0.86%. Sedimentation analysis of a 0.3% solution in 0.1M sodium tetraborate at 42,040 r.p.m. using a synthetic boundary cell, showed a single, symmetrical peak. The polysaccharide was hydrolysed and the galactose and arabinose content estimated by g.l.c. of the alditol acetates[13] and by a modification[14] of the cysteine–sulphuric acid reaction[10] (using readings at 420 and 380 nm and solving the

resultant simultaneous equations). A galactose–arabinose ratio of 15.6:1 (g.l.c.) and 16.0:1 (colorimetry) was found. G.l.c.–m.s.[15] of the alditol acetates confirmed the two sugars as an arabinose and a galactose. The i.r. spectrum (KBr disc) of the polysaccharide (1 mg/100 mg of KBr) showed absorption bands at 700, 755, 888, 945, 973, 1630, and 2900 cm^{-1}.

Methylation of the potato galactan .— The potato galactan (23 mg) was dissolved in freshly boiled and cooled, distilled water (1 ml), and methyl sulphate (1 ml) and 30% aqueous sodium hydroxide (2.8 ml) were added dropwise with cooling (ice–water) under a stream of nitrogen during 5 h. The reagents were added in equivalent amounts throughout. A further seven such additions were made at 24-h intervals at room temperature, and a little water was added from time to time to maintain the required dilution. At the end of eight days, the cloudy reaction mixture was cooled, neutralised with 20% sulphuric acid, and continuously extracted with chloroform for 4–5 h. The aqueous portion was dialysed for 4 days against running tap-water and for 2 h against distilled water, and then evaporated to dryness. The chloroform extract was concentrated, dried (Na_2SO_4), and concentrated under diminished pressure. The residue, dried *in vacuo* over phosphorus pentoxide–potassium hydroxide, was a pale-brown solid that was insoluble in methyl iodide, but soluble in chloroform (1 ml) and methyl iodide (8 ml). Silver oxide (80 mg) was added and the stirred mixture was boiled under reflux. A further two additions of silver oxide (80 mg) were made, and then the mixture was left overnight (20 h) and filtered, the silver oxide was washed with a little chloroform, and the filtrate and washings were combined and evaporated to dryness. The product was still insoluble in methyl iodide and was therefore again dissolved in chloroform–methyl iodide, but after a further methylation it remained insoluble in methyl iodide.

The material recovered from the aqueous and chloroform extracts from the first Haworth methylation was combined and subjected to a further seven additions of methyl sulphate and 30% sodium hydroxide. The reaction mixture was extracted as before to give a buff-coloured product (28.3 mg) which remained insoluble in methyl iodide.

The partially methylated polysaccharide (28.3 mg) was dissolved in dry methyl sulphoxide* (2 ml). Flakes of sodium hydroxide (300 mg) were added and the mixture stirred at room temperature under a flow of dry nitrogen. Methyl sulphate was added dropwise during 5 h and, after a further 22 h, the mixture was heated to 90° for 1 h to destroy excess of methyl sulphate. The mixture was cooled, water was added to dissolve the sodium hydroxide, and M sulphuric acid was added until the solution was neutral. The mixture was then extracted with chloroform and the extract was concentrated, washed with water, and dried (Na_2SO_4). The dried solution was then concentrated and the residue dried *in vacuo* to give a pale-brown product (32.4 mg), $[\alpha]_D^{27}$ −20.3° (*c* 1, chloroform), which showed only a small hydroxyl absorption in the infrared.

*Distilled under diminished pressure from calcium hydride and stored over molecular sieve type 4A (BDH, Poole, Dorset).

Hydrolysis of methylated galactan. — The methylated galactan (23.1 mg) was heated at 100° for 3 h in 90% formic acid (0.5 ml). Formic acid was then removed under diminished pressure, 0.25M sulphuric acid (1 ml) was added, and the sample was heated at 100° for 14 h. The hydrolysate was neutralised with barium carbonate, filtered, and concentrated to a syrup (25.2 mg).

Examination of methyl sugars. — Paper chromatography (solvent *B*) indicated a major component of 2,3,6-tri-*O*-methyl-D-galactose with small amounts of di-*O*-methyl-D-galactose. Paper electrophoresis in borate buffer revealed a component having the same mobility as 2,3-di-*O*-methyl-D-galactose, and additional material which did not complex with borate.

G.l.c. was carried out on a Pye 104 Gas Chromatograph using dual columns (5 ft × 0.25 in.) of Gas Chrom Q (100–120 mesh) coated with 3% (w/w) ECNSS-M (Applied Science Laboratories) at a nitrogen flow-rate of 45 ml/min at 180°. G.l.c.–m.s. was carried out with the same chromatographic system (but a single column) coupled to a Bell and Howell 21-490 Mass Spectrometer. The mass spectra were recorded at an inlet temperature of 200°, ionising potential of 70 eV, and a temperature of the ion source of 200°.

The methyl sugars (12.6 mg) were reduced and acetylated, essentially as described by Bjorndal *et al.*[20], but the duration of acetylation was extended to 1 h at 100° followed by storage overnight at room temperature. The acetylation mixture was diluted with water and evaporated under diminished pressure, and the residue was taken up in chloroform. Aliquots were injected into the gas chromatograph for g.l.c.–m.s.

Periodate oxidation. — Potato galactan (14.4 mg) was oxidised in 34mM sodium metaperiodate (10 ml) at room temperature in the dark. Aliquots were removed at 72 h and the consumption of periodate and production of formic acid determined[21]. Since the amount of polysaccharide was limited, only one assay was performed.

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