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CARBOHYDRATE COMPOSITION OF PARTICULATE PREPARATIONS FROM MUNG BEAN (*PHASEOLUS AUREUS*) SHOOTS*

R. W. BAILEY†, S. HAQ‡ and W. Z. HASSID

Department of Biochemistry, University of California, Berkeley, California

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Abstract—The carbohydrate compositions of mung bean (*Phaseolus aureus*) shoots and particulate preparations obtained from these plants, which synthesize polysaccharides *in vitro*, have been compared. Both types of tissue contained hot water soluble polysaccharides (starch, galactan and arabinan), pectin, hemicellulose and cellulose, but generally at much lower levels in the particulate preparations. Whole shoot hemicellulose contained D-galactose and D-glucose as the major monosaccharides, with a lesser amount of D-xylose and arabinose, and only traces of D-mannose. In contrast, the particulate hemicellulose contained D-glucose, D-galactose, arabinose and D-mannose in significant amounts, but only traces of D-xylose. The major lipid-bound sugar in the tissues was D-glucose, but lipid-bound D-galactose, L-rhamnose, and possibly arabinose and D-xylose were also present. A partial acid hydrolysis of the particulate preparation yielded the following oligosaccharides: cellobiose, 6-*O*-β-D-galactosyl-D-galactose, 3-*O*-β-L-arabinosyl-L-arabinose, di- and tri-D-galacturonic acids, D-galacturonosyl-L-rhamnose, D-glucuronosyl-D-galactose and D-glucuronosyl-D-galactosyl-D-mannose.

INTRODUCTION

MUNG bean (*Phaseolus aureus*) particulate preparations have been shown to synthesize, from nucleotide diphosphate sugars, polysaccharides which are apparently identical with several important plant cell-wall carbohydrates. Thus, D-glucose has been transferred from guanosine diphosphate D-glucose (GDP-D-glucose) to form cellulose,[1] D-mannose and D-glucose from guanosine diphosphate D-mannose (GDP-mannose) and GDP-glucose to form glucomannan,[1,2] and D-galacturonic acid from uridine diphosphate galacturonic acid (UDP-galacturonic) to synthesize the pectin chain.[3]

Little is known about the identity or composition of the particulate preparations involved in these syntheses, except that they are liberated by simple grinding of the shoots and, after their initial release, are sedimented below 30,000 *g*. They presumably contain polysaccharides possibly derived from cell walls which act as acceptors in the syntheses of the above-mentioned polysaccharides. In view of this latter possibility, a study has been made of the carbohydrate composition of active mung bean particulate preparations, which is the subject of the present paper. For comparative purposes the carbohydrate composition of whole mung bean shoots and of separate hypocotyls and roots has also been examined.

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† Permanent address: Plant Chemistry Division, D.S.I.R., Palmerston North, New Zealand.

‡ Deceased, June 18, 1965.

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RESULTS

Yields of Fractions from Mung Bean Shoots

The dry matter content of whole shoots and of separate hypocotyls and roots, respectively, and the yields of freeze-dried particulate fractions from the whole shoots are given in Table 1. Amounts of ethanol-insoluble and oxalate-insoluble residue obtained from each preparation are also listed. For the carbohydrate fractionation analysis, 1·2 g of freeze-dried 20,000 *g* particulate preparation was isolated from 1 kg of whole shoots. For the partial acid hydrolysis, 0·9 g of ethanol-extracted particulate preparation was obtained from approximately 1·5 kg of whole shoots.

Table 1. Dry matter content and yields of fractions from mung bean shoots

Fraction	(Yields in g dry wt. from 100 g wet wt. of plant tissue)		
	Total solid (freeze-dried)	Ethanol insoluble* residue	Oxalate extracted† residue
Whole shoots‡	6·35	2·0	1·0
Hypocotyls‡	5·6	2·5	1·56
Roots	7·6	2·4	1·6
1000 *g* fraction	0·55	0·48	0·32
20,000 *g* preparation	0·12	0·07	0·05
1000,000 *g* fraction	0·08	—	—

* Freeze-dried solid extracted successively with boiling 95% and 85% ethanol.

† Ethanol-insoluble residue extracted successively with boiling water and ammonium oxalate (0·5%) solution.

‡ Roots plus hypocotyls and hypocotyls from which the cotyledons were removed.

Carbohydrate Analysis of Mung Bean Preparations

Results obtained from the fractionation of the carbohydrates of the various mung bean preparations are listed in Table 2.

Table 2. Carbohydrate analyses of mung bean preparations

Fraction	Soluble sugars	Lipid-bound sugars	Water-soluble polysaccharide	Oxalate-soluble polysaccharide		Hemicellulose	Cellulose
				(*a*) Polyuronide	(*b*) Neutral polysaccharide		
	(Per cent of initial freeze-dried wt.)						
Whole shoots	23·0	0·60	1·2	3·22	1·80	3·5	4·13
Hypocotyls		0·41	1·46	3·02	1·90	5·9	3·42
Roots		0·31	0·65	2·07	0·71	1·52	3·30
1000 *g* fraction		0·06	3·20	0·60	4·15	1·76	1·08
20,000 *g* preparation	4·6	0·32	1·25	0·62	0·71	0·88	0·99

Monosaccharide Constituents of the Carbohydrate Fractions

Soluble sugars. Soluble sugars (mono-, di-, tri-saccharides, etc.) and glycolipids were extracted together in boiling ethanol (95%) and after removal of the ethanol were separated

by extracting the sugars with water. The lipid-bound sugars were then liberated from the insoluble residue by acid hydrolysis.[4] The soluble sugars were not examined in any detail.

Possible lipid-bound sugars. D-Galactose, D-glucose, L-rhamnose, D-xylose and arabinose were identified in the lipid hydrolysate. D-Glucose and D-galactose were always found to be the main sugars and present in approximately equal amounts in the hydrolysates of the root and 20,000 *g* lipids, but with much more D-glucose than D-galactose in those of the whole shoot, hypocotyl and 1000 *g* lipids. L-Rhamnose was present in hydrolysates of all of the lipids, arabinose in hydrolysates of the 1000 *g* and 20,000 *g* preparation lipids, and D-xylose was detected in hydrolysates of the root and hypocotyl lipids as a trace constituent only. The crude, dry lipids from whole shoots and from a 20,000 *g* preparation, representing 2 and 25 per cent, respectively, of the original dry weight, were extracted with boiling ether when 90–95 per cent was dissolved. Chromatograms of hydrolysates of the ether-soluble material showed the expected pattern of D-glucose, D-galactose and L-rhamnose but no pentoses. The total crude lipids gave no color with iodine showing the absence of starch contaminant.

The residues from the 95% ethanol extraction were extracted with boiling 80% ethanol. These extracts were also processed for soluble sugars and insoluble lipid-bound sugars. They were found to contain small amounts of soluble sugar, the values of which are included in Table 2. Only traces of sugar, which always included arabinose, were found in hydrolysates of the water-insoluble residues from these extracts.

Water-soluble polysaccharides. All of the hot-water extracts, except that from the 20,000 *g* preparation, gave a positive starch–iodine test, with the 1000 *g* extract giving the strongest color. After removal of this starch, the hydrolysates of these extracts from all of the preparations showed the same pattern of D-galactose and arabinose plus lesser amounts of D-glucose and traces of D-xylose. D-Ribose was also identified in the hydrolysates of the root and shoot extracts.

Oxalate extracts. Iodine tests indicated the presence of traces of starch in only the whole shoot and hypocotyl oxalate extracts, but all of the extracts were treated with salivary amylase before acid hydrolysis. All of the extracts gave hydrolysates with a similar pattern by paper chromatography of uronic acids, D-galactose and arabinose plus lesser amounts of D-glucose and D-ribose and traces of D-xylose. Free L-rhamnose was not detected in any of the hydrolysates. Control experiments with purified citrus pectin showed that under these hydrolysis conditions arabinose was not formed by the decarboxylation of liberated D-galacturonic acid.

Hemicellulose and cellulose hydrolysates. D-Galactose, D-glucose, arabinose, D-xylose and D-mannose, the latter usually present only in trace amounts, were identified in the 1 N acid hemicellulose hydrolysates. Uronic acid compounds, which were not identified, were also present but L-rhamnose was not detected. The cellulose hydrolysates contained the expected D-glucose together with small amounts of D-xylose and traces of D-mannose. The ratios of the neutral monosaccharides in the hemicellulose hydrolysates, corrected to include the D-xylose in the cellulose hydrolysates, are given in Table 3.

Hemicellulose A and B fractions were prepared from the oxalate extracted residue of hypocotyls, roots and 20,000 *g* preparation, respectively. D-Xylose and arabinose together with traces of D-galactose and uronic acids were identified in hydrolysates of the hemicellulose-A fractions. However, the 20,000 *g* hemicellulose-A fraction, which was very low in D-xylose, was heavily contaminated with dark red–brown alkali soluble material.

[4] R. W. BAILEY, *Anal. Biochem.* 3, 178 (1962).

D-Ga-lactose and D-glucose plus lesser amounts of arabinose, uronic acids and D-xylose were identified in the hydrolysates of the three hemicellulose-B fractions.

TABLE 3. MONOSACCHARIDE COMPOSITION OF HEMICELLULOSE OF MUNG BEAN PREPARATIONS

Fraction	Monosaccharides‡ D-Galactose	D-Glucose	D-Xylose*	D-Mannose†
Whole shoots	3·43	1·62	1·29	(trace)
Hypocotyls	3·50	4·38	0·92	(trace)
Roots	3·37	0·37	1·03	(trace)
1000 *g* fraction	0·90	3·01	0·1	—
20,000 *g* preparation	0·96	0·32	0·1	0·5

* Corrected to include D-xylose present in cellulose hydrolysate.
† Not measured quantitatively when a trace component.
‡ Ratios relative to arabinose (=1·0).

Sugars Isolated from a Stepwise Partial Acid Hydrolysate of Particulate Preparation

Neutral sugars. Eight monosaccharides were identified in the hydrolysate. The four main sugars were D-galactose, D-glucose, D-mannose, and arabinose, which were present in the ratio 5·3:4·6:3·0:5·4 (uncorrected for monosaccharide contained in the oligosaccharides). D-Ribose, D-xylose, L-rhamnose and L-fucose were present in trace amounts only.

Three disaccharides (*A*, *B* and *C*) were isolated in small amounts and the structures proposed for them are based on the results, summarized in Table 4, of: (1) acid hydrolysis, (2) reaction with spray reagents, (3) paper chromatographic comparison with authentic specimens in at least two solvents, and (4) paper electrophoresis of the borohydride-reduced compounds. Thus the movement on the chromatograms and electrophoretograms of the arabinobiose, Disaccharide *A*, was the same as that of authentic 3-O-β-L-arabinosyl-L-arabinose isolated from a partial hydrolysate of Tamarack arabino-galactan.[9] Electro-

TABLE 4. NEUTRAL DISACCHARIDES FROM A PARTIAL ACID HYDROLYSATE OF 20,000 *g* PARTICULATE PREPARATION

Disaccharide	$R_{arabinose}$ Solvent (*b*)	Solvent (*d*)	Color with *p*-anisidine phosphate	M_s* (electrophoresis)	Monosaccharides on acid hydrolysis	Tentative structure
A	0·33 (0·34)†	0·45 (0·44)	Pink	0	Arabinose	3-*O*-β-L-Arabinosyl-L-arabinose
B	0·16 (0·16)	0·23 (0·23)	Brown	0·43	Glucose	4-*O*-β-D-Glucosyl-D-glucose (cellobiose)
C	0·05 (0·05)	0·12 (0·11)	Brown	0·82	Galactose	6-*O*-β-D-Galactosyl-D-galactose

* $M_s = \frac{\text{Mobility of disaccharide alcohol}}{\text{Mobility of sorbitol}}$ in ammonium molybdate (0·008 M, pH 5·0).[7]
† $R_{arabinose}$ of authentic specimens in parenthesis.

[7] E. J. BOURNE, D. H. HUTSON and H. WEIGEL, *J. Chem. Soc.* 4252 (1960).

phoresis of the reduced glucobiose sugar (Disaccharide *B*, Table 4) indicated a 1→4 linkage, and in solvents (*b*) and (*d*) the sugar moved at the same rate as cellobiose (4-*O*-β-D-glucopyranosyl-D-glucose), but clearly different from maltose. Disaccharide *C* (Table 4) had the same chromatographic mobility as authentic 6-*O*-β-D-galactopyranosyl-D-galactose and, while electrophoresis of the sugar alcohol indicated a 1→2 or 1→6 linkage, a positive reaction with triphenyl tetrazolium chloride clearly eliminated the 1→2 linkage.[5,6]

Uronic acid compounds. The tentative identifications of the eight uronic acid compounds (I–VIII) isolated from the hydrolysate are listed in Table 5. Identification was based primarily on movement of the compounds or their hydrolysis products on chromatograms in solvents (*d*) and (*e*) and on electrophoretograms run in ammonium formate buffer. The hydrolysis products of the borohydride-reduced methyl ester methyl glycosides gave useful confirmation of proposed structures. Thus, mono-, di-, and tri-galacturonic acid yielded D-galactose; D-glucuronic acid yielded glucose, and glucuronosyl-galactose yielded D-glucose plus

TABLE 5. ACIDIC SUGARS FROM A PARTIAL ACID HYDROLYSATE OF 20,000 *g* PARTICULATE PREPARATION

Compound	$R_{arabinose}$ Solvent (*d*)	$R_{arabinose}$ Solvent (*e*)	M_p* (electrophoresis	Yield (μg/mg of particle)	Tentative structure
I	0·38	0·17	1·05	†	Trigalacturonic
II	0·38	0·17	0·40	66	D-Glucuronosyl-D-galactosyl-D-mannose
III	0·60	0·33	0·91	†	Digalacturonic acid
IV	0·60	0·39	0·54	46	D-Glucuronosyl-D-galactose
V	0·86	0·80	0·50	8	D-Galacturonosyl-L-rhamnose
VI	0·86	0·61	0·62	100	D-Galacturonic acid
VII	0·86	0·50	0·76	16	D-Glucuronic acid
VIII	1·04	0·91	0·53	†	4-*O*-Methyl-D-glucuronic acid

* $M_p = \frac{\text{Mobility of the compound}}{\text{Mobility of picric acid}}$ in ammonium formate (0·2 M, pH 3·6).

† Exact quantity not measured but present in significant amounts in hydrolysates.

D-galactose. Borohydride reduction and subsequent hydrolysis of the D-mannose containing aldotriouronic acid yielded D-glucuronic acid, D-galactose and D-mannitol, indicating that D-mannose was the reducing monosaccharide unit.

DISCUSSION

The 20,000 *g* particulate preparation from mung bean shoots contains all of the usual types of plant polysaccharides, although in some cases the levels are very low. The L-arabinose and D-galactose disaccharides obtained in the partial acid hydrolysis have been isolated previously from plant polysaccharides.[8,9,10] Their isolation here, together with the easily detectable amounts of L-arabinose and D-galactose found in hydrolysates of both the hot

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[8] J. K. N. JONES, *J. Chem. Soc.* 1672 (1953).
[9] S. HAQ and G. A. ADAMS, *Can. J. Chem.* **39**, 1563 (1961).
[10] G. O. ASPINALL, E. L. HIRST and E. RAMSTAD, *J. Chem. Soc.* 593 (1958).

water and oxalate extracts, suggests the presence of the arabinan-galactan complex isolated by Barrett and Northcote[11] from apple pectin. The di- and tri-galacturonic acids obtained indicate the presence of typical pectin polygalacturonic acid, while the isolation of a galacturonosyl-rhamnose aldobiouronic acid is in agreement with the suggested[11,12] presence of L-rhamnose in the pectin polygalacturonic acid chain. Presumably some of the D-galactose and arabinose found in the hydrolysate of the oxalate extract is also part of the pectin polymer.[11] Iodine tests showed that most of the starch present in the whole shoots is removed in the 1000 *g* fraction and very little, if any, is present in the 20,000 *g* preparation.

Compared with the intact shoots the levels of hemicellulose and cellulose in the particulate preparation are very low. D-Xylose is almost absent from hydrolysates of these fractions, although it is definitely present in the whole shoots and is the major monosaccharide of mature plant hemicellulose. The presence of D-glucose in the particulate hemicellulose and hemicellulose-B hydrolysates points to the presence of water-insoluble, alkali-soluble glucan which may contain $\beta 1 \rightarrow 4$ linkages as in lichenin.[13] This latter possibility is supported by the isolation of cellobiose from the partial acid hydrolysate of particulate preparation when the hydrolysis conditions would not normally have hydrolyzed cellulose itself. Hemicellulase fractions, which did not attack cellulose, have also been reported to liberate cellobiose from the glucan present in various leguminous hemicellulose fractions.[14] Glucuronosyl-galactose disaccharides have been prepared from many plant gums and several plant hemicelluloses,[15,16] but the D-glucuronosyl-D-galactosyl-D-mannose does not appear to have been isolated previously. The detection of this trisaccharide indicates the presence of D-mannose in polymers other than mannan or glucomannan. Oligosaccharides indicative of mannan or glucomannan were not found in the partial hydrolysate, although the relatively high levels of mannose in the hemicellulose hydrolysate and the fact that the particulate preparation can synthesize mannan and glucomannan[1,2] suggest the presence of these polysaccharides.

So far as the carbohydrate composition of the whole shoots, hypocotyls and roots is concerned, the results obtained are typical for leguminous plants. A possible exception is the proportion of D-xylose in the hemicellulose monosaccharides which, while much greater than that of the particulate preparation, is lower than values recorded for mature leguminous plants when the D-galactose:D-xylose:arabinose ratio is of the order of 0·5:6·0:1·0.[17,18]

The 20,000 *g* particulate preparation appears to be rich in lipids which contain D-glucose and L-rhamnose as well as D-galactose. D-Galactose is the major lipid-bound sugar present in green plant tissues,[19] but the crude lipid fraction in the whole mung bean shoots apparently contains much more D-glucose than D-galactose. L-Rhamnose does not appear to have been reported previously as a possible plant lipid-bound sugar.

From the point of view of polysaccharide synthesis all of the cell-wall polymers required as acceptors are present, although xylan and cellulose are present only at very low levels. Tests for cellulose synthesis depending on the liberation of cellobiose from synthesized polymer must allow for the possible presence of this repeating unit in glucan other than cellulose. Of most interest in connexion with cellulose synthesis is the presence of much

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possible glucolipid, as Colvin[20] suggested that such a compound played an essential role in cellulose synthesis in plants. Barber *et al.*[1] stated that various lipid fractions had no effect on the synthesis of cellulose from GDP-D-glucose by mung bean particulate preparation. This is not surprising as the present results suggest that the particles may be fully supplied with any lipid required in the synthesis.

EXPERIMENTAL

Mung Bean Preparations

Mung beans were germinated at room temperature and 100 per cent humidity for 4 days and the combined roots and hypocotyls, freed from cotyledons, were used to prepare the particulate fraction by the following procedure.[1] The shoots were ground at 0° in a mortar with sand and an equal weight of tris-HCl (0·1 M, pH 7·5) buffer, the macerate squeezed through cheese-cloth and decanted from any sand. After centrifuging at 1000 *g* for 20 min to remove coarse particles, the active particulate preparation was obtained by centrifuging the homogenate at 20,000 *g* for 30 min and dried either by dialyzing and freeze-drying or by washing successively with water, ethanol and ether. Portions of the material sedimenting at 1000 *g* and also at 100,000 *g*, after removal of the 20,000 *g* fraction, were also dialyzed and freeze-dried. Samples of the whole shoots (roots + hypocotyls) and of the separate hypocotyls and roots from the same batch of shoots were also freeze-dried and ground for analysis.

Carbohydrate Fractionation

Freeze-dried preparations (1–2 g) were first extracted with boiling ethanol (95% followed by 80%, 200 ml of each) and the filtrates processed to yield soluble sugar and lipid-bound sugar fractions.[4] The ethanol extracted residue was boiled with water (200 ml for 5 min) to dissolve water-soluble polysaccharides, then refluxed with ammonium oxalate (0·5%, 200 ml, 2 hr) to extract pectin. The residue was refluxed in 1 N H_2SO_4 (200 ml, 2 hr) to give a hemicellulose hydrolysate and cellulose in the acid-insoluble residue hydrolyzed by treatment first at room temperature for 6 hr with concentrated H_2SO_4 (72%, w/v, 5 ml) followed by dilution with water to 100 ml and refluxing for 4 hr.

Portions of oxalate-extracted residue were also extracted for 24 hr, under nitrogen, with aqueous KOH (10%, w/v). The alkaline filtrates were acidified with acetic acid (50%, v/v) to pH 4·7 to give hemicellulose-A precipitates, after which hemicellulose-B was precipitated from the acid supernatants with ethanol (2 v). The precipitates were dialyzed and freeze-dried.

Carbohydrate Analyses

The ethanol fractions were processed[4] to yield an aqueous-soluble sugar extract and an acid hydrolysate of the water-insoluble lipid fraction. Sugars in each fraction were determined with anthrone,[21,22] as were the neutral polysaccharide sugars in the hot water and oxalate extracts. Polyuronide in the oxalate extracts was estimated with carbazole[22,23] after de-esterification[24] and the results corrected for neutral hexose interference.[22] Sugars in the hemicellulose and cellulose hydrolysates were measured by the micro-cuprimetric method of

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Nelson.[25] D-Glucose standards were used in all analyses except those for polyuronide when D-galacturonic acid was used; all results were calculated as anhydro-sugar.

Individual monosaccharides in the hemicellulose and cellulose hydrolysates were measured by the quantitative paper chromatographic method of Wilson[26] using aniline hydrogen phosphate and solvents (*a*) or (*c*) below.

Paper Chromatography

Paper chromatograms were developed with one of the following solvents:[27] (*a*) ethyl acetate–pyridine–water (2:1:2), (*b*) ethyl acetate–pyridine–water (8:2:1), (*c*) ethyl acetate–acetic acid–formic acid–water (9:1·5:0·5:2), (*d*) ethyl acetate–acetic acid–water (9:2:2), and (*e*) the organic phase of a mixture of phenol (100 g), water (100 ml) and formic acid (88%, 1 ml). Spray reagents[27] were aniline hydrogen phthalate or phosphate, *p*-anisidine phosphate, triphenyltetrazolium chloride and alkaline acetone silver nitrate.

All monosaccharides in hydrolysates were identified by paper chromatography using comparisons with authentic specimens in at least two solvents.

Total Acid Hydrolyses of Carbohydrates

Hydrolysates in H_2SO_4 of the lipid, hemicellulose and cellulose fractions were prepared in the course of the carbohydrate fractionation. Water-soluble polysaccharide and oxalate extracts were first treated with human salivary amylase to solubilize the starch, dialyzed and freeze-dried. Portions (2·3 mg) of these preparations, of hemicellulose A and B and of the neutral disaccharides were hydrolyzed by heating at 100° for 1 or 2 hr in 1 N H_2SO_4 or 1 N or 2 N HCl (2 ml). Isolated uronic acid compounds and their borohydride reduced methyl ester methyl glycosides were hydrolyzed by heating them (20–40 mg) in 1 N H_2SO_4 (4 drops) at 100° in a sealed tube for 20 hr.

Sulphuric acid hydrolysates were desalted for paper chromatography by treatment successively with $BaCO_3$ and with mixed bed resin, except for the uronic acid hydrolysates which were separated into neutral and acidic sugars by fractionation on resin (see below). Hydrochloric acid hydrolysates were desalted by evaporation under vacuum over solid NaOH.

Partial Acid Hydrolysis of Particulate Fraction

The total 20,000 *g* particulate preparation was submitted to a stepwise, partial acid hydrolysis as follows. Ethanol–ether dried particulate material (0·9 g) was heated at 100° sequentially in 10 ml portions of H_2SO_4 as follows: 0·01 N (60 hr), 0·05 N (72 hr), 0·1 N (10 hr), 0·5 N (6 hr), and 1 N (4 hr). Following each hydrolysis the solution was poured into ethanol (4 v) and the precipitate separated by centrifugation for the next hydrolysis. The combined ethanol supernatants were evaporated under reduced pressure at 35° to about 50 ml, neutralized with $BaCO_3$, filtered and the filtrate and washings similarly evaporated to dryness to yield 0·289 g of solid; 0·175 g of acid insoluble solid was discarded.

The sugar mixture was first adsorbed on a resin column (Bio-Rad, Richmond, Calif., AG1-X8, 200–400 mesh, acetate form; 8 × 2 cm column) and the neutral sugars were eluted with water to separate them from the uronic acids which were then eluted with 6 N acetic acid. Neutral sugars were fractionated, on a charcoal–celite column, into monosaccharides

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[27] R. W. BAILEY and J. B. PRIDHAM, *Chromat. Rev.* **4**, 114 (1962).

which were eluted with water and disaccharides which were then eluted with aqueous ethanol. The three neutral disaccharides obtained in the latter fraction were further purified by preparative paper chromatography using solvent (*b*). The uronic acid mixture was first separated[16] into four zones by paper chromatography in solvent (*d*) and these zones were further fractionated and purified by paper chromatography in solvent (*e*) and paper electrophoresis in ammonium formate buffer (0·2 M, pH 3·6).

Examination of Uronic Acid Compounds

These were hydrolyzed and separated into neutral sugars and uronic acids which were identified by paper chromatography and paper electrophoresis as described above. Where the hydrolyzate was suspected to contain glucuronic acid, its neutral sugar fraction, in a drop of water, was made alkaline with NaOH (1 μl, 2%), kept at room temperature for 20–25 min and then submitted to paper electrophoresis. This was done to detect D-glucuronolactone which had passed through the resin column along with neutral sugars.

Uronic acids were methylated by refluxing in methanol–HCl (4·6% w/w) for 12 hr, then neutralized with Ag_2CO_3, centrifuged and evaporated to dryness under vacuum. The methylated sugar in water (0·5 ml) was reduced with sodium borohydride at room temperature for 18–20 hr, and after acidification with acetic acid (1 drop) Na^+ ions were removed with resin and borate by distillation with methanol.