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Note

An arabinogalactan from sugar cane

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Polysaccharides extracted into processing during the milling of sugar cane can seriously reduce the efficiency of both raw-sugar production and its refining. Some of these polysaccharides occur as components of sugar cane, whereas others are present as impurities of microbiological origin.

Our studies on polysaccharides from Australian sugar-cane varieties have shown an arabinogalactan to be a common constituent. American workers have reported a similar polysaccharide in freshly cut cane[1,2], and Hawes[3] isolated an arabinogalactan from the water-soluble polysaccharides from sugar cane. Methylation studies showed[3] it to be a structurally unusual arabinogalactan.

We now report the essential structural features of the arabinogalactan.

The crude polysaccharide was isolated from juice pressed from freshly cut cane of the variety Q80. Starch granules were removed by centrifugation of the juice, and addition of three volumes of ethanol led to the isolation of 1.3% of the dry matter of the cane. Only 36% of this was subsequently recovered as water-soluble material. The insoluble portion was largely denatured protein, ash, and adsorbed carbohydrate. The proportion of monosaccharides in the soluble material was determined by gas chromatography of the alditol acetate derivatives[4] and is shown in Table I.

The water-soluble material was crudely fractionated by addition of one and two volumes of ethanol, and relative neutral-monosaccharide compositions shown in Table I revealed that the 50–67.5% fraction was richest in arabinogalactan. Material not precipitated by two volumes of ethanol was significantly richer in arabinose; this suggests that the arabinose content can vary considerably and, as such, influences its water solubility.

The arabinogalactan was purified by a number of chromatographic procedures, which removed mainly protein as measured by u.v. absorption at 278 nm. Changes

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TABLE I

PROPORTIONS OF NEUTRAL MONOSACCHARIDES PRESENT IN THE VARIOUS FRACTIONS PRODUCED DURING THE ISOLATION OF AN ARABINOGALACTAN FROM SUGAR CANE

Sample origin	*Proportion of neutral monosaccharides (%)*[a]						
	Rha	Fuc	Ara	Xyl	Man	Gal	Glu
Crude plant extract (A)[b]	4	2	36	5	8	34	10
1 vol. ethanol ppt. of A	1	1	25	10	13	31	20
2 vol. ethanol ppt. of A	2		42	4	1	43	7
Fractions not pptd. by 2 vols.	1	1	38	6	18	24	13
G.p.c.[c] on Sepharose CL-6B	2		43	4		47	3
G.p.c. on Sephacryl S-300	2		43	4		51	

[a]Gas chromatography of the alditol acetate derivatives on 3% ECNSS-M at 185°. [b]Obtained by precipitation from juice by three volumes of ethanol. [c]Gel permeation chromatography.

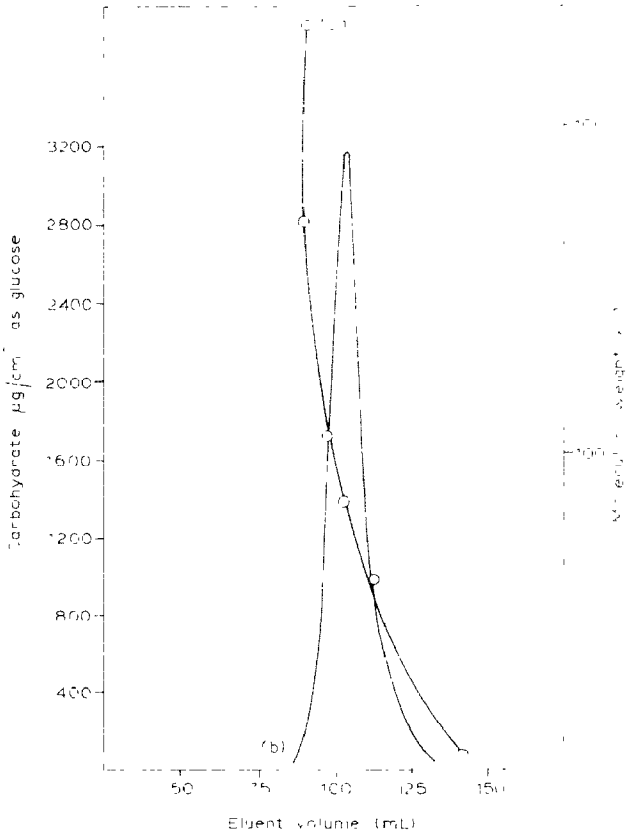

Fig. 1. (a) Molecular-weight calibration of a 95 × 1.5-cm column of Sephacryl S-300 using dextrans. (b) Elution profile of Q80 arabinogalactan on the same column

in the carbohydrate composition occurring throughout these steps are also recorded in Table I.

Initially, chromatography on Sepharose CL-6B in 7M urea removed the bulk of the protein, and complete removal was achieved by ion-exchange chromatography on macroporous resin. The polysaccharide was retained by the resin and eluted under the conditions of a linear gradient to 0.5M sodium chloride.

Subsequent chromatography on Sephacryl S-300 gave a Gaussian distribution of carbohydrate and indicated a molecular weight ~70.000, when referenced to standard dextrans chromatographed on the same column. This is shown in Fig. 1.

The homogeneity implied by chromatography on Sephacryl S-300 was not substantiated by chromatographic examination of the peak fractions on μBondagel E-Linear using high-pressure liquid chromatography (l.c.). Injections of 20 μL indicated two components of quite different molecular weight when referenced to

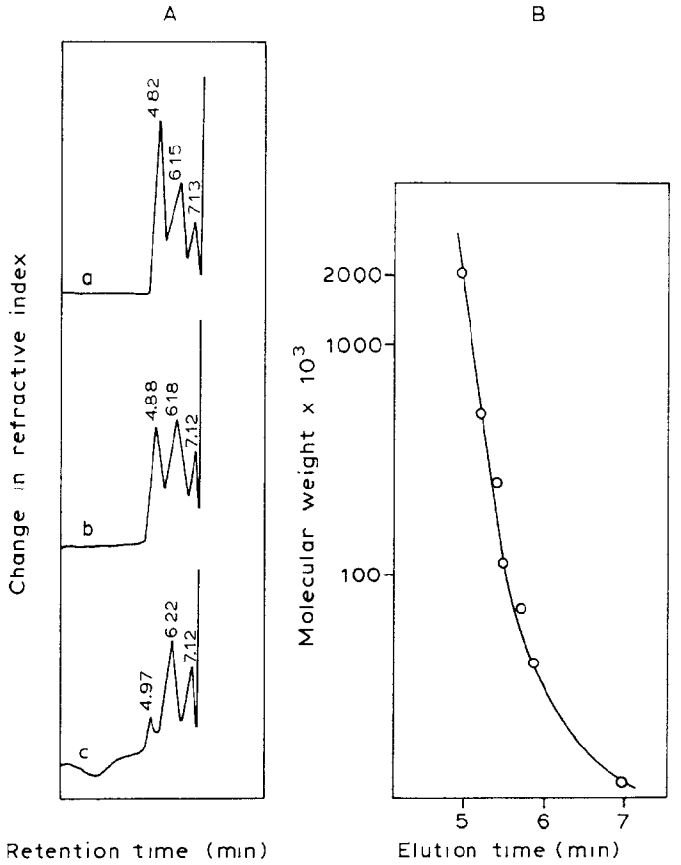

Fig. 2. A. L.c. on μBondagel E-Linear of peak fractions from Q80 arabinogalactan chromatographed on Sephacryl S-300 (Fig. 1). Curves a, b, and c correspond to fractions eluting at 97, 101, and 105 mL, respectively. B. The relationship of elution time as a function of molecular weight when dextrans are chromatographed on μBondagel E-Linear.

dextran. As indicated in Fig. 2, the molecular weight of the larger component was of the order of $1-2 \times 10^6$ whereas that of the smaller was $\sim 20-25 \times 10^3$. The increasing elution times recorded are consistent with the analysis of eluent taken from a gel-permeation separation (Sephacryl), as each sequential injection involves components of smaller molecular size. The third component (T_R = 7.12 min, Fig. 2) was present in all reference dextrans as well, and is not characteristic of the arabinogalactan.

Evidence for multicomponent arabinogalactans exists in the literature[5-7]. Arabinogalactans from larch have been demonstrated to contain two components that are readily separated by ultracentrifugation; the weight-average molecular weights were calculated at 100,000 and 16,000. Differences in arabinose/galactose ratios characterized these components, and viscosity studies have led to the suggestion of shape differences, one being essentially spherical and the other more nearly linear. Such shape differences, be they spherical, flexible coil, or rigid linear, would influence their molecular sieving properties, but this does not explain the chromatographic discrimination exhibited by cross-linked dextran and porous silica.

The monosaccharide proportions of the arabinogalactan recovered from the chromatography on Sephacryl S-300 are shown in Table I. This material was used in all structural studies, even though there are some doubts about its chemical homogeneity.

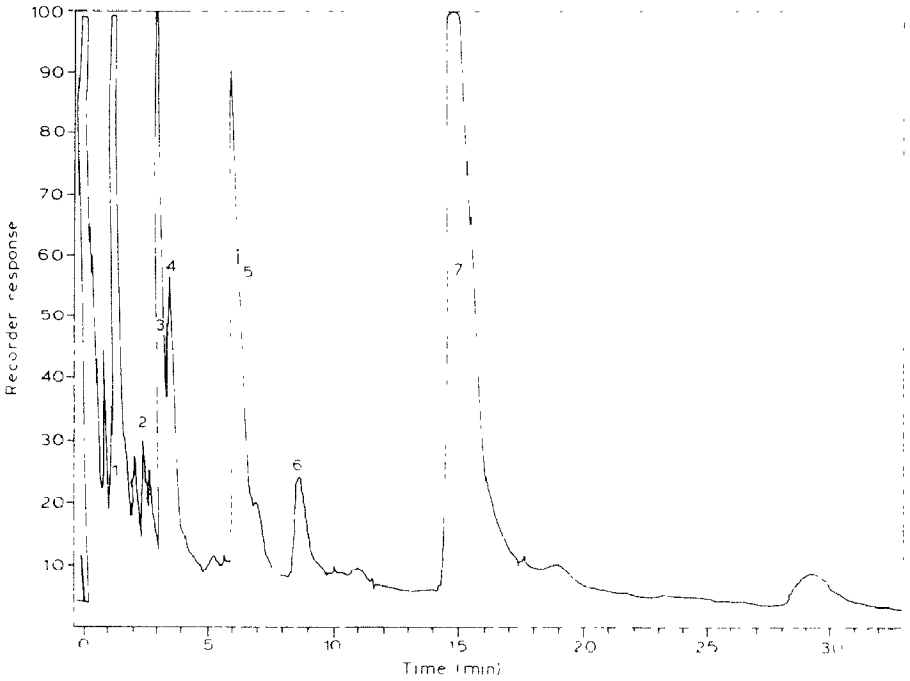

Fig. 3. Gas chromatogram of methylation products of the arabinogalactan from sugar cane on 3% OV-225 at 170°. Retention times of the numbered peaks are shown in Table II

TABLE II

METHYLATION ANALYSIS OF ARABINOGALACTAN FROM SUGAR CANE VARIETY Q80.

Peak No.[a]	*Methylated sugar*[b]	*T(OV-225)*[c]	*T(ECNSSM)*[c]	*Mole (%)*[d] *Q80*
1	2,3,5-Ara	0.50 (0.41)	0.52 (0.48)	35.6
2	3,5-Ara	0.81 (0.80)	0.93 (0.91)	1.5
3	2,3-Ara	1.06 (1.07)	1.25 (1.25)	8.8
4	2,3,4,6-Gal	1,19 (1.19)	1.25 (1.25)	6.0
5	2,4,6-Gal	2.00 (2.03)	2.25 (2.28)	13.1
6	2,3,4-Gal	2.74 (2.89)	3.32 (3.41)	4.5
7	2,4-Gal	5.00 (5.1)	6.4 (6.35)	30.5

[a]As labelled in Fig. 3. [b]2,3,5-Ara ≡ 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol etc. [c]Retention times of the corresponding alditol acetate relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on OV-225 at 170° and ECNSS-M at 150°. Times were actually calculated from the retention time of 2,3,4,6-Gal. Figures in parentheses are retention times from the literature[12]. [d]Calculated from integrated areas measured on OV-225.

Methylation data are summarized in Table II and are based on the separation shown in Fig. 3. The glycosidic linkages involved in the structure have been established, but their distribution and fine structure are not known. From the data, the arabinogalactan is envisaged as having a framework of (1→3)-linked β-D-galactose residues of which two out of every three carry a galactosyl or arabinosyl side-chain attached at O-6. L-Arabinofuranosyl residues as single units and small side-chains predominate at the branches. Galactosyl residues are attached in small chains linked β-(1→6).

The essential structural features of the arabinogalactan establishes it as a type

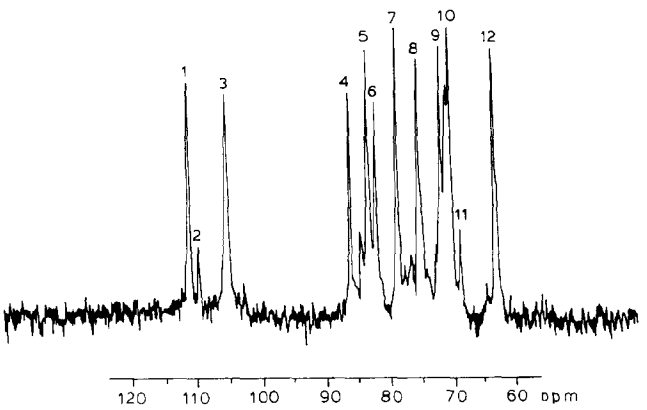

Fig. 4. ^{13}C-N.m.r. spectrum of Q80 arabinogalactan at 85°. Measured chemical shifts (p.p.m.) are shown for the numbered signals. (1) 110.9, (2) 109.6, (3) 105.1, (4) 86.0, (5) 83.2, (6) 82.0, (7) 78.7, (8) 75.4, (9) 71.8, (10) 70.4, (11) 69.0, and (12) 63.4.

widely distributed in the plant world[8]. The negative specific rotation ($[\alpha]_{5890}^{20}$ −56°) indicates the β configuration in D-galactose and α in L-arabinose.

The arabinose occurs only in the furanose form. This conclusion is supported by data from partial acid hydrolysis and ^{13}C-n.m.r. spectroscopy. In the latter spectrum (Fig. 4), signals at 110.9 and 109.6 p.p.m. arise from furanoside residues[9] whereas that at 105.1 p.p.m. would be from the anomeric carbon atom in β-(1→3)-linked galactose[10].

D-Glucuronic acid is present as a monosaccharide component in a concentration that enables retention on an ion-exchange resin but is insufficient to be detectable in the ^{13}C-n.m.r. spectrum. American workers[11] measured the concentration of this acid in their arabinogalactan studies but Hawes[3] could find no uronic acid in his work.

An aspect of the quantification of methylation data is the excess of terminal residues over branch points. This is unusual, for the greater volatility of the more methylated sugars frequently results in an apparent excess of branch-points in the final products. It is presumed that some area calculations in the gas-chromatographic evaluation are distorted by unresolved components.

In conclusion, the data suggest that the arabinogalactan from sugar cane may be classified as the 3,6-arabino disubstituted galactan type[8] found widely distributed in plant tissues. It is a little richer in arabinose than is normally found, and this sugar exists solely in the furanosidic form.

EXPERIMENTAL

Materials. — Cane stalks of variety Q80 were shredded and milled in a triple pass through a laboratory mill. Juice (18 L) was extracted from 28.6 kg of cane containing 72.4% of moisture. It was centrifuged at 2,000*g* for 15 min at 5° to remove starch. Three volumes of ethanol precipitated 45.7 g of material from the centrifuged juice (8 L) and 35.6% of this dissolved readily in water.

Purification. — The water-soluble portion obtained as just described was divided into 3 fractions by precipitation with ethanol; insolubles in 50% of aqueous ethanol, insolubles in the ethanol concentration range 50–67.5%, and those soluble in 67.5% ethanol. Of the water soluble fraction, 10.5% precipitated in ethanol in the concentration range 50–67.5%.

The compositions of the fractions obtained are shown in Table I. The major fraction, the 0–50% ethanol precipitate representing 67% of the starting material, was 64% protein (from the relationship elemental N × 6.25), and 2.2% carbohydrate (by phenol–sulphuric acid colorimetry against a reference of glucose).

Chromatography, twice, of the 50–67% ethanol fraction (0.44 g) on Sepharose CL-6B in 7M urea on a 90 × 2.5-cm column led to the recovery of 0.33 g of polysaccharide and a small proportion of material absorbing u.v. radiation at 278 nm.

Further purification was achieved by ion-exchange chromatography on Biorad macroporous resin AG MP-1 (PO_4). The sample was equilibrated by dialysis to

5mM phosphate, pH 7.5, for loading. After elution of unretained material from the column, the polysaccharide was released by a linear gradient to 0.5M sodium chloride prepared in phosphate buffer. Recovery was 0.19 g of polysaccharide, free from u.v.-absorbing material.

Chromatography on a column (95 × 1.5 cm) of Sephacryl S-300 in 0.02M phosphate pH 7.5, at a flow rate of 0.6 mL/min, gave a single peak of material as shown in Fig. 1. The column was calibrated by using dextrans of known molecular weight from Pharmacia Fine Chemicals (Uppsala, Sweden).

Fractions corresponding to elution volumes 97, 101, and 105 mL from Sephacryl S-300 were further examined by l.c. on a column (30 × 0.39 cm) of μBondagel E-Linear equilibrated to 6M urea. The liquid chromatograph was a Waters Associates unit incorporating a M6000A solvent-delivery system, a U6K sample injector, and an R401 differential refractive-index monitor. Injection size was 20 μL in eluent pumped at 0.4 mL/min. The column was calibrated from retention times of dextrans of known molecular weight. Results are shown in Fig. 2.

Structural analysis. — Methylation was effected by the Hakomori method and g.l.c.–m.s. analysis of the alditol acetates[12]. A chromatogram of the acetates on 3% OV-225 at 170° is shown in Fig. 3 and relevant data for identification and quantification are given in Table II.

Partial acid hydrolysis involved two procedures described by Adams[13,14]. In the first instance, the mild hydrolytic step released only arabinose, but significant quantities were also present in the total acid hydrolysate. In the second procedure, all arabinose was cleaved in the mild-acid stage, but some scission of galactose occurred as well. Oligosaccharides were also present when examined by paper chromatography, but failed to produce any pink colour characteristic of pentoses when sprayed with *p*-anisidine hydrochloride.

Glucuronic acid was isolated from the total acid hydrolysate by anion-exchange chromatography and was identified by chromatography on paper using 18:3:1:4 ethyl acetate–acetic acid–formic acid–water.

The ^{13}C-n.m.r. spectrum was obtained with the deuterated polysaccharide in deuterium oxide at a concentration of 50 mg/mL in a 10-mm probe and a JEOL FX-100 spectrometer at 85°. The collection was by overnight acquisition under deuterium lock and the spectrum is referenced to external tetramethylsilane. The spectrum is shown in Fig. 4, together with the measured chemical shifts.

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