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AN ACIDIC GALACTOARABINOXYLAN AND OTHER PURE HEMICELLULOSES IN OAT LEAF*

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Abstract—The total hemicellulose (with galactose:glucose:arabinose:xylose ratio 2·8:11·4:10:23·5) was extracted from the holocellulose of the leaves of young oat plants, *Avena sativa* (var. Blenda), by treatments with 5 and 24% KOH. The water-soluble part of the hemicellulosic material extracted by the 5% KOH was fractionated on a column of DEAE-cellulose irrigated by water and aqueous KOAc. The hemicellulosic material eluted by 0·5 M KOAc was subjected to further fractionation, to ultracentrifugal examination, to zone electrophoresis and to preliminary structural studies. A number of hydrolysis products from the methylated hemicellulosic material have been identified. The material is a pure acidic galactoarbinoxylan (galactose:arabinose:xylose, 2·8:10:18·5; uronic acids not less than 3–4 per cent). It is similar to the land-plant xylans but is unusual in having terminal and non-terminal residues of galactose.

INTRODUCTION

THIS paper is the second in a series dealing with the polysaccharides of the oat plant in relationship to plant growth. The terms pure and total hemicellulose and hemicellulosic material are defined and discussed earlier.[1] The composition of such preparations is there considered in terms of polydispersity, polymolecularity and polydiversity.

In preliminary work on oat leaf hemicellulosic material, fractions were obtained containing various proportions of galactose, glucose, arabinose and xylose residues.[1] All fractions appeared to be polydiverse. The variations in the proportions of glucose residue were consistent with the presence of a homoglucan and this has since been isolated. A pure acidic arabinoxylan isolated from oat straw[2] contained a lower proportion of arabinose to xylose residues than in the parent hemicellulosic material from the straw. The structural role of the galactose was unknown: a pure acidic galactoarabinoxylan has now been isolated.

RESULTS

The total hemicellulose isolated from the leaves of young oat plants contained galactose, glucose, arabinose and xylose residues (ratio 2·8:11·4:10:23·5),[3] small amounts of acidic sugars and a trace of rhamnose. A larger quantity of the same plant holocellulose was treated with 5 and 24% KOH to obtain the total hemicellulose. The material which was extracted by the 5% KOH and which was soluble in water (5S) was chromatographed on a column of DEAE-cellulose (acetate form). Four fractions were obtained on elution with water and with 0·1, 0·5 and 5 M KOAc, namely fractions *a*, *b*, *c* and *d* respectively. One of these, fraction *c*, contained no glucose residues; it accounted for 23 per cent of the material

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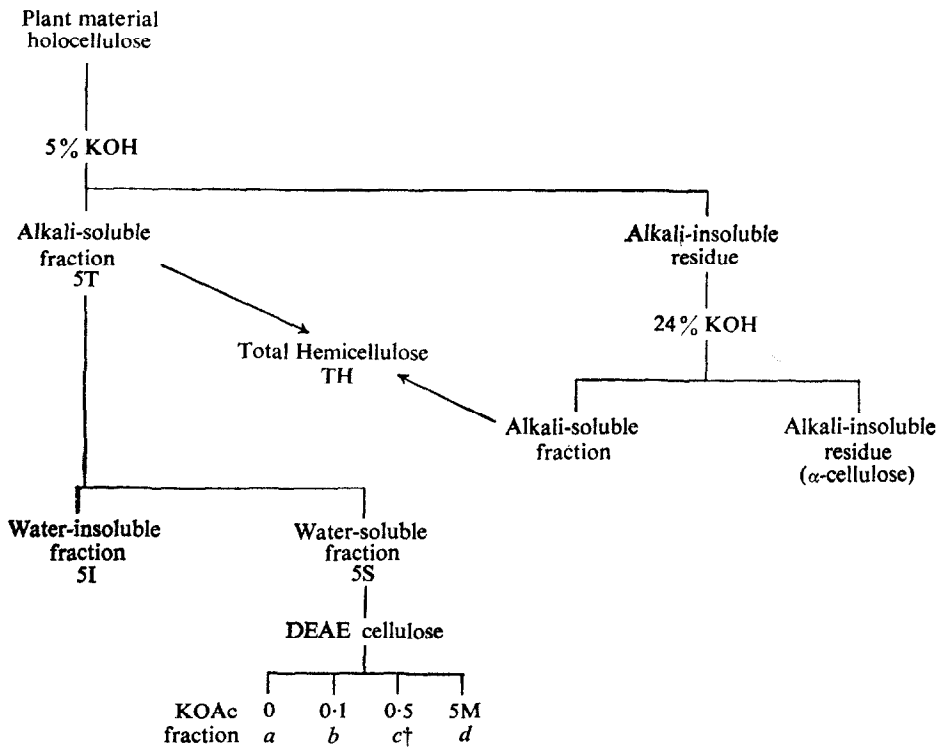
[1] J. S. G. REID and K. C. B. WILKIE, *Phytochem.* **8**, 2045 (1969).

[2] G. O. ASPINALL and K. C. B. WILKIE, *J. Chem. Soc.* 1072 (1956).

[3] F. G. FISCHER and H. DÖRFEL, *Z. Physiol. Chem.* **297**, 164 (1954).

applied to the column (15 per cent of the total hemicellulose). On hydrolysis it yielded galactose, arabinose and xylose (ratio 2·8:10:18·5), small amounts (*ca.* 3–4 per cent[3]) of glucuronic acid and of 4-*O*-methylglucuronic acid and a trace of rhamnose. The uronic acid content of the fraction *c* hemicellulosic material was 7·3 per cent[4] by decarboxylation.[5] The immediately relevant part of the fractionation of the hemicellulosic material from the plant holocellulose is outlined in Fig. 1.



	TH*	5T	5S	5I	*a*	*b*	*c**/†	*d*
% of TH composition:	100	—	64	13	3·1	3·5	15	3·8
Galactose	2·8	3	2	0	2	4	2·8	—
Arabinose	10	10	10	10	10	10	10	—
Xylose	23·5	20	15	55	26	14	18·5	—
Glucose	11·4	10	5	25	10	2	0	—

* Determinations by the method of Fischer and Dörfel[3] (other estimations by densitometry).
† The pure acidic galactoarabinoxylan subjected to structural analysis.

FIG. 1. ISOLATION AND FRACTIONATION OF OAT LEAF TOTAL HEMICELLULOSE.

There was no indication that fraction *c* was polydiverse. None of the fractionation methods listed in Table 1 caused any change in the ratio of the above three non-acidic sugar residues. It should be particularly noted that repetition of the DEAE-cellulose fractionation procedure resulted in the hemicellulosic material not being eluted until 0·5 M KOAc was again used; the material was then eluted unchanged. Zone electrophoresis on glass-fibre paper[6] or silica

[4] A. J. BUCHALA and K. C. B. WILKIE, to be published.
[5] D. M. W. ANDERSON, *Talanta* **12**, 73 (1959).
[6] D. R. BRIGGS, E. F. GARNER, R. MONTGOMERY and F. SMITH, *Nature* **178**, 154 (1956).

gel plates caused the hemicellulosic material to migrate as a single compact zone. On ultracentrifugal examination the material sedimented as a single symmetric peak. It was concluded that fraction *c* was a pure acidic galactoarabinoxylan and this was the material subsequently studied.

Proof that the galactose and xylose residues were present in the same molecule was provided by the isolation from an acid hydrolysate of an oligosaccharide containing both types of residue. The compound, almost certainly an aldotriouronic acid, contained residues of glucuronic acid, xylose and galactose (ratio of *ca.* 1:1:1). The galactose was at the reducing end of the molecule. The compound was probably identical to that isolated by Adams[7] from a hydrolysate of oat hull hemicellulosic material. Glucuronic acid, 4-*O*-methylglucuronic acid and a glucuronosylxylose were also found in a hydrolysate of fraction *c*.

A preliminary methylation analysis of the acidic galactoarabinoxylan has been carried out; further studies are in progress.[8] The fraction *c* material was unusually resistant to methylation but the final product had a methoxyl content probably higher than 33 per cent. Hydrolytic cleavage products of the methylated pure hemicellulose were separated by TLC and identified by GLC of their methyl glycosides or by other means. 2,3-Di-*O*-methylxylose, 2,3,5-tri-*O*-methylarabinose, 2,3,4,6-tetra-*O*-methylgalactose, 2-*O*-, and 3-*O*-methylxyloses and xylose accounted for *ca.* 85 per cent of the methylated sugars. The 2,3-di-*O*-methylxylose was present in high proportion but there was only a trace of xylose. There were also small or trace amounts of several unidentified methylated sugars which were probably derivatives of glucuronic acid, galactose and/or arabinose.

The ultracentrifugal sedimentation rate of the galactoarabinoxylan was compared with those of two dextran fractions of known molecular weight. It sedimented at approximately the same rate as a sample of "Dextran 10" (supplied by Pharmacia) which had M_n 6100 and M_w 9400.

The galactoarabinoxylan is similar to other land-plant xylans in having a backbone of 1→4 linked β-D-xylopyranose residues. Like most of the xylans of the Gramineae it possesses terminal non-reducing arabinofuranose residues but it is unusual in that it has galactose residues also. Other xylans having residues of galactose are those of maize hulls[9, 10] and of perennial rye grass.[11] These last have been shown to possess terminal non-reducing galactose residues. The acidic galactoarabinoxylan of oat leaf contains both terminal and non-terminal residues of galactose.

EXPERIMENTAL

General Methods

The chromatographic solvents were: A, ethyl acetate–pyridine–water (72:20:23); B, ethyl acetate–acetic acid–water (3:1:3); C, pyridine–ethyl acetate–acetic acid–water (5:5:1:3); D, ethyl acetate–acetic acid–formic acid–water (18:3:1:4); E, butan-2-one–water (10:1) to which a drop of 0·88 NH_4OH was added; and F, benzene–ethanol–water–acetic acid (200:47:15:1). The general detection reagents for chromatography were—triphenyltetrazolium chloride,[3] *p*-anisidine hydrochloride and $AgNO_3$–NaOH. For glass-fibre paper and silica-gel plates 1-naphthol/conc. H_2SO_4 was used as detection reagent. Densitometric determinations were carried out as described earlier.[1] Accurate quantitative determinations were by the method of Fischer and Dörfel.[3] A Pye Argon Chromatograph was used for GLC; methyl glycosides of methylated sugars were fractionated on a column (0·5 × 120 cm) of *m*-bis(*m*-phenoxyphenoxy)benzene on acid-washed

[7] E. L. FALCONER and G. A. ADAMS, *Can. J. Chem.* **34**, 338 (1956).
[8] C. G. FRASER and K. C. B. WILKIE, unpublished work.
[9] R. L. WHISTLER and W. M. CORBETT, *J. Am. Chem. Soc.* **77**, 6328 (1955).
[10] R. MONTGOMERY, F. SMITH and H. C. SRIVASTAVA, *J. Am. Chem. Soc.* **79**, 698 (1957).
[11] G. O. ASPINALL, I. M. CAIRNCROSS and K. M. ROSS, *J. Chem. Soc.* 1721 (1963).

celite (80–120 mesh) at 150–200° with argon flow rates of 30–60 ml/min.[12] Paper electrophoresis was on cellulose paper or on glass-fibre paper.[6] Thin-layer electrophoresis was on glass plates (5 × 20 cm) coated with silica gel (Kieselgel G—Merck). Strips of gold foil (5 × 20 cm) were cemented to the ends of the plates with araldite (CIBA). After coating the plates with silica-gel slurry they were air-dried and then wetted with buffer either by spraying or by allowing it to ascend. The polysaccharide solution was applied to the centre of the plates and electrodes attached to the gold strips. The plates were submerged in CCl_4 and 400 V applied for 6 hr. In an improved method ordinary silica gel plates were laid face down in contact with flat gold electrodes 5 cm wide and *ca.* 18 cm apart. Sedimentation analysis of polysaccharides in 1–3% aqueous solution were carried out in a Beckman Model E analytical ultracentrifuge. A titanium rotor was used with 12-mm sector-shaped cells at speeds of up to 48,000 rev/min. Hemicellulosic samples were hydrolysed in sealed tubes at 100° by heating with H_2SO_4 for 16–24 hr; neutralization was with $BaCO_3$ and, where appropriate, Amberlite IR 120 (H^+) was used prior to examination for acidic sugars. Solvents were removed by use of a rotary evaporator below 40°; water was removed on occasion by freeze-drying.

Plant Material

Oat plants, *Avena sativa* (var. Blenda), were harvested 76 days after planting when they were *ca.* 30–40 cm in height; the leaf tissue was immediately plunged in boiling ethanol and, after drying, was passed through a Casella Mill (No. 16 mesh).

The Total Hemicellulose

A sample (4·79 g) of the milled leaf was treated to isolate the total hemicellulose (0·92 g; 22 per cent of the leaf).[1]

Isolation of an Acidic Galactoarabinoxylan

After delignification the milled oat leaf (160 g) was treated with 5% and then with 24% KOH (3 l. of each) to extract the total hemicellulose. The hemicellulosic material extracted by 5% KOH was isolated without loss as described earlier[1] (Fraction 5T). A sample (10 g) of this material was macerated in water (400 ml) and the water-soluble fraction (5S; 8·3 g) and a water-insoluble fraction (5I; 1·7 g) were obtained after centrifugation and freeze-drying.

Fraction 5S was further fractionated on a column of DEAE-cellulose (Whatman DE 11; 375 g—acetate form) using water then 0·1, 0·5 and 5 M KOAc (3 l. of each) as eluants; hemicellulosic materials *a*, *b*, *c*, and *d* were isolated respectively.

Attempted Sub-Fractionation of Hemicellulosic Material c

TABLE 1. METHODS USED IN ATTEMPTED FRACTIONATION OF HEMICELLULOSIC MATERIAL *c*

Reagent	Method*	Result
DEAE-cellulose powder	C	No fractionation (see text)
Cetyltrimethylammonium bromide[13]	P	Precipitation but no fractionation
Cetylpyridinium chloride[13]	P	Precipitation but no fractionation
Cetyltrimethylammonium borate[13]	P	Precipitation but no fractionation
Bio-Rex 9 anion exchanger	C	No fractionation—most of hemicellulosic material lost
Methanol	E	No hemicellulose extracted
Ethanol	E	No hemicellulose extracted
Ethanol	FP	Precipitation but no fractionation
Ethanol in the presence of boric acid	FP	Precipitation but no fractionation
Water	E	No fractionation
Barium chloride[14]	P	No precipitation
Barium hydroxide[14]	P	No precipitation
Basic lead acetate	P	Precipitation but no fractionation
Fehling's solution[2]	P	No precipitation

* C—chromatography; P—precipitation; FP—fractional precipitation; E—extraction.

[12] G. O. ASPINALL, *J. Chem. Soc.* 1676 (1963).

[13] J. E. SCOTT, in *Methods in Carbohydrate Chemistry* (edited by R. L. WHISTLER), Vol. 5, p. 38, Academic Press, New York (1965).

[14] H. MEIER, in *Methods in Carbohydrate Chemistry* (edited by R. L. WHISTLER), Vol. 5, p. 45, Academic Press, New York (1965).

Acidic Compounds in a Hydrolysate of the Acidic Galactoarabinoxylan

A sample (100 mg) of the acidic galactoarabinoxylan was dissolved in N H_2SO_4 and heated at 100° for 16 hr. The neutralized ($BaCO_3$), deionized (Amberlite IR 120 H^+) hydrolysate was examined by paper chromatography (in solvent C). Components 1–5 were obtained and isolated by paper chromatography. They had $R_{galactose}$ in solvent C of 0·22, *ca.* 0·30, 0·38, 0·46 and 0·71; relative proportions (estimated visually with triphenyltetrazolium chloride) were 1 : trace : 0·2 : 1 : 1 respectively.

Component 1. This component was chromatographically pure (solvents B–D). A portion (*ca.* 0·2 mg) was hydrolysed. Chromatographic examination (solvent C) showed galactose, xylose and glucuronic acid (ratio *ca.* 1 : 1 : 1). A further sample (*ca.* 0·2 mg) was dissolved in water (2 ml) and reduced with $NaBH_4$ (5 mg). The borohydride was decomposed by treatment with Amberlite IR 120 (H^+) and the boric acid removed as the methyl borate. Chromatographic examination (solvent C) of its hydrolysate revealed glucuronic acid, xylose and galactitol; co-chromatographed galactose, galactitol, xylose and xylitol separated completely.

Component 2. As only a trace of this component was isolated, it was not investigable.

Component 3. The component was chromatographically homogeneous and on hydrolysis it yielded glucuronic acid and xylose (ratio *ca.* 1 : 1).

Component 4. The component was chromatographically homogeneous and indistinguishable from glucuronic acid.

Component 5. This was found on chromatographic examination to be a mixture of two components which were separated preparatively (solvent C). One was chromatographically homogeneous and was indistinguishable from 4-*O*-methylglucuronic acid; the other was also chromatographically homogeneous and on hydrolysis gave only xylose.

Methylation of the Acidic Galactoarabinoxylan

Unsuccessful attempts were made to methylate the pure hemicellulose using the methods of Srivastava[15] and of Anderson.[16] A sample (193 mg) of the acidic galactoarabinoxylan was methylated by one treatment with methyl sulphate (10 ml) and NaOH (20 ml; 42 per cent). Foaming led to a loss of about 10 per cent of the reaction mixture. The product (104 mg) was freed of salts by dialysis (dialysis of a sample of the non-methylated acidic galactoarabinoxylan under identical conditions led to the loss of about 30 per cent of the material). The dialysed and partially methylated hemicellulose was methylated by four successive treatments with sodium hydride, methyl iodide and dimethyl sulphoxide.[17] The product (70 mg) was a transparent film that was difficult to handle because of its lightness. It had a methoxyl content of over 33 per cent.

A portion (*ca.* 15 mg) was hydrolysed by the HCOOH–H_2SO_4 method.[18] The hydrolysate was examined by TLC on layers of microcrystalline cellulose (Avicel) and of silica gel (solvents E and F). On silica gel plates (solvent F) eight components were detected. A sample (5 mg) of the mixture was separated and removed from the plates in five fractions (A–E); one of these (D) was known to contain three components.

Fraction A was indistinguishable (TLC, solvent F) from 2,3,5-tri-*O*-methylarabinose. The material was treated with 3% methanolic HCl at 100° in a sealed tube to form the methyl glycosides. The neutralized (Ag_2CO_3) solution was taken to dryness and the product dissolved in a little $CHCl_3$. GLC examination showed two main peaks and a trace of a third. The two main peaks corresponded exactly in retention times and relative areas to those produced by the methyl glycosides of 2,3,5-tri-*O*-methylarabinose formed under identical conditions.

Fraction B, in very small amount, was indistinguishable (TLC, solvent F) from 2,3,4-tri-*O*-methylxylose.

Fraction C was indistinguishable (TLC, solvent F) from 2,3,4,6-tetra-*O*-methylgalactose. The methyl glycosides were formed as above and were examined by GLC. The two peaks produced corresponded in retention times and in relative areas to those given by the methyl glycosides of 2,3,4,6-tetra-*O*-methylgalactose formed under identical conditions.

Fraction D was known to contain three components. It was fractionated by TLC (solvent F; double irrigation) and yielded five components—four being present in only trace amount. The major component was isolated by preparative TLC and converted to its glycosides as earlier. GLC examination revealed two peaks corresponding in retention times and relative areas to the methyl glycosides of 2,3-di-*O*-methylxylose formed under identical conditions.

Fraction E was indistinguishable (TLC, solvent F) from 2-*O*-, and 3-*O*-methylxylose. It was examined by paper electrophoresis in borate buffer (200 V; 2–4 hr) with 2-*O*-, and 3-*O*-methylxyloses. Three components were detected—two being indistinguishable from the standards (the 2-*O*-methylxylose was the more abundant). The third component was not identified but migrated more rapidly than 3-*O*-methylxylose.

[15] H. C. Srivastava, S. N. Harshe and P. P. Singh, *Tetrahedron Letters* 1869 (1963).
[16] D. M. W. Anderson and G. M. Cree, *Carbohydrate Res.* **2**, 162 (1966).
[17] J. W. T. Craig, Ph.D. Thesis, University of Edinburgh, Edinburgh, Scotland (1966).
[18] H. O. Bouveng and B. Lindberg, in *Methods in Carbohydrate Chemistry* (edited by R. L. Whistler), Vol. 5, p. 296, Academic Press, New York (1965).

Fraction H was examined by paper chromatography (solvent A); only xylose was detected.

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