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A FUCOGALACTOXYLOGLUCAN FROM RAPESEED HULLS*

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ABSTRACT

A polysaccharide, isolated from rapeseed hulls by extraction with aqueous sodium hydroxide–sodium tetraborate, contained residues of L-arabinose, L-fucose, D-xylose, D-galactose, and D-glucose in the proportions of 2:8:25:13:52. Acetolysis furnished cellobiose, 6-*O*-α-D-xylopyranosyl-D-glucose, and 2-*O*-β-D-galactopyranosyl D-xylose. The cleavage products from the methylated polysaccharide were examined by g.l.c. of the methyl glycosides and g.l.c.–mass spectrometry of the partially methylated, alditol acetates. The results show that the polysaccharide is a member of the xyloglucan group in which additional fucose and galactose residues terminate some of the side-chains. For comparative purposes, aspects of the structures of xyloglucans from nasturtium seeds and suspension-cultured sycamore cells have been re-examined.

INTRODUCTION

In previous studies on the carbohydrate polymers of rapeseed hulls, a pectin was isolated and shown to be structurally similar to lemon-peel pectin[1]. In continuation of these investigations, we report an examination of a fucogalactoxyloglucan from the same source. Some features of the structurally related xyloglucans from nasturtium seeds[2], rapeseed meal[3], and from the extracellular polysaccharides elaborated by suspension-cultured sycamore cells[4] have been re-assessed.

RESULTS AND DISCUSSION

Rapeseed hull residues, after isolation of pectin[1], were delignified[5] and then extracted with disodium ethylenedinitrilotetraacetate at pH 4.5, giving small quantities of carbohydrate polymers that have not yet been examined in detail. Further extraction of the hulls with 10% aqueous sodium hydroxide containing 4% of boric acid furnished acid-soluble and acid-insoluble polysaccharide fractions. The major

*Dedicated to the memory of Professor J. K. N. Jones, F.R.S.
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component of the acid-soluble fraction was fractionated by chromatography on DEAE-Sephadex A-50 (formate form), followed by two precipitations via the insoluble copper complex, to give a polysaccharide whose sugar composition was unchanged on further fractionation. The procedures used ensured that the polysaccharide preparation was free from the most likely contaminants, namely, acidic polysaccharides including pectin and xylans of the type found in most plant cell-walls. The polysaccharide contained residues of arabinose (2%), fucose (8%), xylose (25%), galactose (13%), and glucose (52%).

The highly branched character of the rapeseed hull xyloglucan was shown, and the nature of the linkages was established, by qualitative identification of the constituents of the methylated polysaccharide by g.l.c. of their methyl glycosides (Table I). Quantitative estimation of the methylated sugars was carried out by g.l.c. of the derived, partially methylated alditol acetates (Table II), and the substitution patterns of the main components were confirmed by g.l.c.–m.s.[6] (see Table I). For comparison, the constituents of the methylated derivatives of xyloglucans from nasturtium seeds, rapeseed meal, and from the extracellular polysaccharides from suspension-cultured sycamore cells were examined by g.l.c., and the quantitative results, together with those reported by Bauer *et al.*[7], are summarized in Table II.

Partial acetolysis of rapeseed hull xyloglucan followed by *O*-deacetylation gave a mixture of sugars that was chromatographed to give the constituent monosaccharides, each of which was characterized by formation of crystalline derivatives, and three disaccharides. Cellobiose was characterized as the crystalline β-octaacetate. Assuming the enantiomeric configurations of the component sugars, optical rotations

TABLE I

G.L.C.–M.S. OF DERIVATIVES OF METHYLATED SUGARS FROM METHYLATED, RAPESEED HULL FUCOGALACTOXYLOGLUCAN[a]

Sugar	*Relative retention times of*				
	Methyl glycosides on column a		*Alditol acetates on columns* b	e	*Diagnostic fragment-ions*[b]
2,3,5-Me_3Ara	0.55	0.72	0.48	0.41	
2,3,4-Me_3Xyl	0.44	0.55	0.68 } [a]	0.54 } [a]	117(118) 161(162)
2,3,4-Me_3Fuc	0.72		0.68 ∫	0.58 ∫	117(118) 131 161(162) 175
2,3-Me_2Ara			1.32	1.07	
3,4-Me_2Xyl	1.10	1.23	1.54[a]	1.19	117 189(190)
2,3,4,6-Me_4Gal	1.72		1.25[a]	1.19	45 117(118) 161(162) 205
2,4,6-Me_3Gal			2.28	2.03	
3,4,6-Me_3Gal	3.20	4.82	2.50 } [a]	2.22 } [a]	45 161 189(190)
2,3,6-Me_3Glc	2.90	3.95	2.50 ∫	2.32 ∫	45 117(118) 233
2,3-Me_2Glc	7.72	10.0	5.39[a]	4.50[a]	117(118) 261

[a]Indicates g.l.c.-separated fractions whose mass spectra were recorded. [b]Figures in parentheses were obtained from alditols formed by reduction with $NaBD_4$.

TABLE II

COMPOSITIONS OF METHYLATED XYLOGLUCANS

Methyl ethers (%)[a]	*Sources of methylated xyloglucans*				
	Sycamore EP[b]	*Sycamore EP*[c]	*Rapeseed hulls*	*Rapeseed meal*	*Nasturtium seeds*
2,3,5-Me_3Ara	+	+	+	+	
2,3-Me_2Ara		+	+	+	
2,3,4-Me_3Fuc	5	5	6	8	
2,3,4-Me_3Xyl	28	16	17	21	14
3,4-Me_2Xyl	8	13	11	9	19
Total methyl ethers of xylose	36	29	28	30	33
2,3,4,6-Me_4Gal	2	6	6	3	19
2,4,6-Me_3Gal		+	+	+	
3,4,6-Me_3Gal	6	7	6	6	
Total methyl ethers of galactose	8	13	12	9	19
2,3,6-Me_3Glc	8	20	19	17	10
2,3-Me_2Glc	32	33	34	39	38
Total methyl ethers of glucose	40	53	53	56	48

[a]Minor components are not included in the calculations of the percentages of methylated sugars. [b]Sycamore extracellular polysaccharides described in ref. 7. [c]Sycamore extracellular polysaccharides in the present investigation and fractionated as described in ref. 4.

provided evidence for the glycosidic configurations of the other two disaccharides, 6-*O*-α-D-xylopyranosyl-D-glucose and 2-*O*-β-D-galactopyranosyl-D-xylose. The sequences of sugar residues and the sites of linkage in these two disaccharides were indicated by characteristic fragment-ions in the mass spectra of the derived, methylated disaccharide alditols. In the case of the latter disaccharide, alditol formation by using sodium borodeuteride effected labelling of the potentially symmetrical alditol residue.

In earlier studies on nasturtium seed xyloglucan, Hsu and Reeves[2] failed to isolate any galactose-containing disaccharides on partial fragmentation, and proposed that side-chains to the (1→4)-linked β-D-glucan core included single galactose residues and 2-*O*-α-D-xylopyranosyl-D-xylosyl groups. As this proposed arrangement of side-chains differs from those in other xyloglucans, we re-examined the disaccharides formed on acetolysis under the conditions used for the depolymerization of the rapeseed hull polysaccharide. Small-scale experiments showed that the same three disaccharides were formed. The available quantities of polysaccharide did not permit the preparative isolation of all the individual disaccharides, but small-scale experiments showed that mixtures of the same three disaccharides may be converted into the corresponding methylated disaccharide alditols, which may be partially separated by g.l.c. and analyzed by g.l.c.–m.s. (see Fig. 1). The disaccharide, 2-*O*-β-D-galactopyranosyl-D-xylose, whose derived methylated disaccharide alditol was not individual-

ly separated by g.l.c., was the only disaccharide liberated under milder conditions of acetolysis, and sufficient quantities of this disaccharide were isolated to complete its structural characterization.

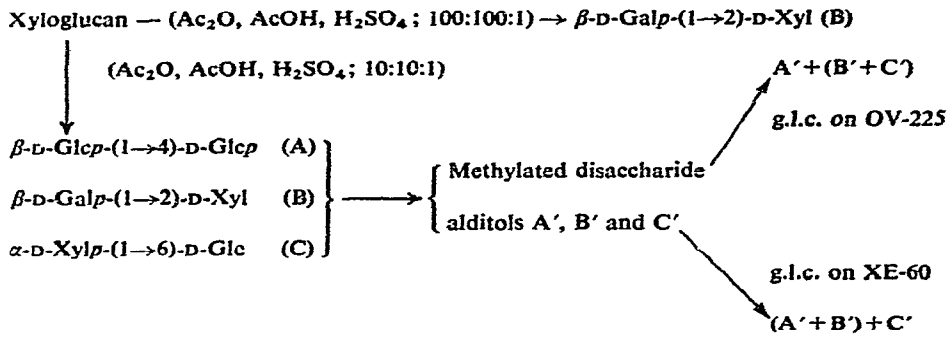

Fig. 1. Disaccharides from the partial acetolysis of nasturtium seed xyloglucan.

Figure 2 shows the main structural features of rapeseed hull fucogalactoxyloglucan in which residues in the cellulosic main chain carry side-chains of single α-D-xylopyranosyl groups, 2-*O*-β-D-galactopyranosyl-α-D-xylopyranosyl groups, and *O*-L-fucopyranosyl-(1→2)-*O*-β-D-galactopyranosyl-(1→2)-*O*-α-D-xylopyranosyl groups. Although there is no direct evidence for the site of attachment of terminal fucose groups, it is probable that they are placed as proposed for the structurally similar polysaccharide from sycamore cell-walls[7]. The results confirm the general similarity between the polysaccharides examined and other members of the xyloglucan family, such as the extensively studied polysaccharide from tamarind seeds[8,9]. The nasturtium seed and tamarind seed polysaccharides, like several other seed xyloglucans, do not contain L-fucose residues. When most of our experimental work had been completed, Courtois and Le Dizet[10] described similar studies on the xyloglucans from nasturtium seeds and other plant seeds. Their conclusions are completely in

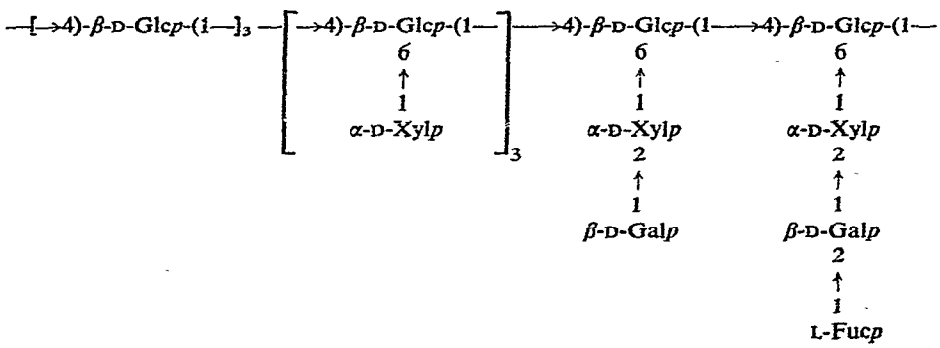

Fig. 2. The main structural features of rapeseed hull fucogalactoxyloglucan (relative proportions of residues are shown, but the sequence is purely arbitrary).

accord with our results in requiring a revision of the structure that was proposed by Hsu and Reeves[2] for the nasturtium xyloglucan.

The fucose-containing xyloglucans from rapeseed hulls, rapeseed meal, and sycamore cells form a distinct sub-group. The methylation results on the xyloglucan from the extracellular polysaccharides elaborated by suspension-cultured sycamore cells are in agreement with those of Albersheim and his collaborators[7] and require a revision of earlier results in that galactose was not recognized then as a constituent of the polysaccharide[4]. Whereas fucose was not reported as a constituent of the xyloglucan isolated from rapeseed meal by extraction with water[3], the polysaccharide, which was kindly provided by Dr. I. R. Siddiqui and was isolated at a later stage in the extraction sequence, is generally similar to the rapeseed hull polysaccharide.

The fucogalactoxyloglucans from rapeseed hulls, rapeseed meal, and from sycamore cells also contain small proportions of arabinose and some "additional" galactose residues. The structural significance of these sugar residues is uncertain, and further investigations will be necessary to determine whether these components arise from incomplete fractionation of the polysaccharide preparations or from residual, covalently attached stubs of another cell-wall component as suggested in the model for plant cell-wall structure proposed by Albersheim and his collaborators[11]*.

In the course of the present investigation, we observed that a commercial "hemicellulase" preparation hydrolyzed rapeseed hull xyloglucan with the liberation of the three disaccharides formed on acetolysis of the polysaccharide. The enzyme preparation as received contained lactose, and failure to remove this disaccharide could account for the reported isolation of lactose from the enzymic hydrolysis of tamarind seed xyloglucan by the same preparation[9].

EXPERIMENTAL

General methods. — Evaporations were carried out under diminished pressure at bath temperatures of 40° or less. Optical rotations were measured with a Perkin–Elmer model 141 polarimeter at 20 ±2°. Paper chromatography was performed on Whatman Nos. 1 and 3MM papers with the following solvent systems (v/v): (A) 6:4:3 1-butanol–pyridine–water; (B) 8:2:1 ethyl acetate–pyridine–water; and (C) 9:2:2 ethyl acetate–acetic acid–water.

G.l.c. was performed isothermally with Hewlett–Packard 5750 and Perkin–Elmer 990 chromatographs, using columns of Gas-Chrom Q coated with (*a*) 5% of neopentylglycol adipate (180°), (*b*) 3% of silicone–polyester copolymer ECNSS-M (180°), (*c*) 5% of silicone gum XE-60 (~200°), (*d*) 3% of silicone-polyester copolymer OV-225 (200–240°), and an OV-225 S.C.O.T. column (*e*). Retention times of methyl glycosides are given relative to that of methyl 2,3,4,6-tetra-*O*-methyl-β-D-gluco-pyranoside, and those of partially methylated alditol acetates relative to that of

*Since the completion of this investigation, Dr. Siddiqui has informed us that further fractionation of the rapeseed meal polysaccharide furnishes a xyloglucan from which the arabinose and "additional" galactose residues are absent.

1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. For g.l.c.–m.s., columns were attached to a Perkin–Elmer–Hitachi RMU-6 mass spectrometer, operated with an inlet temperature of 250°, an ionization potential of 70 eV, and an ion-source temperature of ~250°.

Sugar analyses of polysaccharide hydrolyzates were carried out by reduction with sodium borohydride, followed by acetylation and g.l.c. of the resulting alditol acetates on column *b*[12]. Unless otherwise stated, small-scale methylations of polysaccharides were performed as described by Lindberg *et al.*[13]. The sugar components of methylated polysaccharides were analyzed (a) as methyl glycosides formed on methanolysis by g.l.c. on column *a*, and (b) as partially methylated alditol acetates formed on hydrolysis, followed by reduction with sodium borohydride (or sodium borodeuteride) and acetylation[6], by g.l.c. alone or g.l.c.–m.s. on columns *b*, *d*, and *e*.

Nasturtium seed xyloglucan, isolated as described by Hsu and Reeves[2], had $[\alpha]_D$ +81° (*c* 1.0, M hydrochloric acid), and gave on hydrolysis xylose, galactose, and glucose in the molar proportions of 33:19:48. The xyloglucan from the extracellular polysaccharides from suspension-cultured sycamore cells was isolated as described previously[4], and gave on hydrolysis arabinose, fucose, xylose, galactose, and glucose in the molar proportions of 2:6:28:14:50. The sample of rapeseed meal xyloglucan was kindly provided by Dr. I. R. Siddiqui.

Isolation and fractionation of rapeseed hull xyloglucan. — Preliminary experiments indicated that polysaccharide fractions rich in xyloglucan were best isolated by extraction of the hulls with aqueous sodium hydroxide–sodium tetraborate after prior delignification and removal of pectic materials. Accordingly, rapeseed hulls (180 g), after previous extraction with ammonium oxalate[1], were delignified with acidified sodium chlorite solution[5], and the resulting holocellulose was extracted three times each with (a) aqueous 2% disodium ethylenedinitrilotetraacetate adjusted to pH 4.5 at 90° to remove residual pectic substances, and (b) aqueous 10% sodium hydroxide containing 4% of boric acid in an atmosphere of nitrogen. The alkaline extracts were acidified to pH 4.5 and the polysaccharide precipitate (1.8 g), which was washed, dispersed in water, and freeze-dried, contained some xyloglucan (small-scale methylation analysis) but was not examined in detail. The acidified supernatant solution was dialyzed to remove salts and concentrated, and polysaccharide (3.1 g) was precipitated by addition of ethanol, dispersed in water, and freeze-dried. The polysaccharide was chromatographed three times on columns of *O*-(2-diethylaminoethyl)-Sephadex A-50 (45 × 4 cm, formate form) to remove acidic polysaccharide contaminants, and the final aqueous eluate afforded crude xyloglucan (1.6 g), which was isolated after concentration, precipitation with ethanol, dispersal in water, and freeze-drying. Crude xyloglucan (1.6 g) was fractionated further by precipitation from aqueous solution (100 ml) by the addition of Fehling's solution (15 ml), dispersal of the resulting gelatinous precipitate in water (150 ml), and dropwise addition of 0.2M hydrochloric acid, and precipitation with ethanol. The fractionations were monitored by small-scale methylation studies in which methyl glycosides of the derived methylated sugars were examined by g.l.c.; the results

indicated clearly that any contaminating xylan had been removed, as derivatives of 2,3-di-*O*-methylxylose were absent. Repetition of this procedure gave no further change in sugar composition (directly or after methylation) and xyloglucan (0.8 g) was isolated by dispersal in water and freeze-drying. Xyloglucan had $[\alpha]_D$ +89° (*c* 0.68, water), and hydrolysis with 0.25M sulfuric acid for 16 h at 100° gave arabinose, fucose, xylose, galactose, and glucose in the molar proportions of 2:8:25:13:52.

Acetolysis of rapeseed hull fucogalactoxyloglucan. — The rapeseed polysaccharide (0.55 g) was converted into the acetylated derivative[14] (0.62 g). The polysaccharide acetate was added with stirring to a mixture of acetic anhydride (10 ml), acetic acid (10 ml), and concentrated sulfuric acid (1 ml) at 0°, and the mixture was shaken at room temperature for 8 h and then kept for a further 112 h. The resulting solution was poured into ice–water, the mixture was extracted with chloroform, and the chloroform extract was washed with aqueous sodium hydrogencarbonate, dried, and concentrated to a syrup. Methanolic 0.5M sodium methoxide was added to the syrupy acetates in methanol (10 ml) to maintain permanent alkalinity. The mixture was kept for 8 h and then poured into water with stirring. The solution was treated with Amberlite resin IR-120(H^+) to remove sodium ions, filtered, and concentrated to a syrup (350 mg). The mixture of sugars was absorbed on a column (3 × 18 cm) of 1:1 charcoal–Celite, and the column was eluted successively with water, and water containing 5 and 10% of ethanol.

The mixture of monosaccharides (220 mg), eluted with water, was separated by paper chromatography in solvents B and C to give: L-fucose, characterized as the *p*-tolylsulfonylhydrazone, m.p. and mixed m.p. 166–167°, $[\alpha]_D$ −12° (*c* 0.5, pyridine); L-arabinose, $[\alpha]_D$ +104° (equil., *c* 0.5, water), characterized as the *p*-tolylsulfonylhydrazone, m.p. and mixed m.p. 157°, $[\alpha]_D$ +5° (*c* 0.4, pyridine); D-xylose, $[\alpha]_D$ +18° (equil., *c* 0.5, water), characterized as the di-*O*-benzylidene dimethyl acetal, m.p. and mixed m.p. 212–214°, $[\alpha]_D$ −7° (*c* 1.0, chloroform); D-galactose, $[\alpha]_D$ +80° (equil., *c* 0.5, water), characterized as the β-D-pentaacetate, m.p. and mixed m.p. 141–142°, $[\alpha]_D$ +25° (*c* 0.8, chloroform); and D-glucose, $[\alpha]_D$ +51° (equil., *c* 1.0, water), characterized as the β-D-pentaacetate, m.p. and mixed m.p. 132°, $[\alpha]_D$ +4° (*c* 1.0, chloroform).

The syrup (35 mg) from elution of the column with water containing 5% of ethanol contained two disaccharides and traces of glucose and galactose; it was resolved by paper chromatography in solvent A. Disaccharide 1 (11 mg), R_{Xylose} 0.60 in solvent A, $[\alpha]_D$ +28° (*c* 0.4, water), gave galactose and xylose on hydrolysis. The disaccharide was reduced with sodium borodeuteride and the disaccharide alditol was methylated with methyl iodide and sodium hydride in *N*,*N*-dimethylformamide[15]. G.l.c. of the methylated disaccharide alditol on column *d* showed a major peak that was examined by g.l.c.–m.s. and gave characteristic fragment-ions at *m/e* 219 (8), 192 (55), 146 (32), 89 (33), 88 (100), and 45 (60). Methanolysis of the methylated disaccharide alditol gave compounds having the retention times of methyl glycosides of 2,3,4,6-tetra-*O*-methylgalactose (*T* 1.72 on column *a*) together with an additional substance (*T* 1.05, presumably 1,2,3,5-tetra-*O*-methylxylitol). Disaccharide 2 (9 mg),

R_{Xylose} 0.47 in solvent A, $[\alpha]_D$ +124° (*c* 0.25, water), gave xylose and glucose on hydrolysis. The disaccharide was reduced with sodium borohydride and then methylated[16]. G.l.c. of the methylated disaccharide alditol on column *d* showed a major peak, which was examined by g.l.c.–m.s. and gave characteristic fragment-ions at *m/e* 235 (45), 177 (22), 175 (13), 133 (40), 89 (21), 88 (100), and 45 (60). Methanolysis of the methylated disaccharide alditol gave compounds having the retention times of methyl glycosides of 2,3,4-tri-*O*-methylxylose (*T* 0.44 and 0.55 on column *a*), together with an additional substance (*T* 3.49, presumably 1,2,3,4,5-penta-*O*-methylglucitol).

The syrup (40 mg) from elution of the column with water containing 10% of ethanol contained a major component that was chromatographically indistinguishable from cellobiose. The disaccharide, after purification by paper chromatography in solvent A, gave glucose only on hydrolysis, and was characterized by conversion into β-cellobiose octaacetate, m.p. and mixed m.p. 199°, $[\alpha]_D$ −14° (*c* 0.5, chloroform).

Acetolysis of nasturtium seed galactoxyloglucan. — *A.* Acetylated xyloglucan (100 mg) was treated with 10:10:1 acetic anhydride–acetic acid–sulfuric acid as described for the rapeseed polysaccharide, the products were *O*-deacetylated, and the resulting sugars were partially fractionated on charcoal–Celite. The fraction eluted with water containing 10% of ethanol contained three disaccharides, R_{Xylose} 0.60, 0.47, and 0.38 in solvent A. The mixture of disaccharides was reduced with sodium borohydride, and the disaccharide alditols were methylated with methyl iodide and sodium hydride in methyl sulfoxide. The permethylated disaccharide alditols were analyzed by g.l.c.–m.s. on columns *c* and *e*.

B. Similar treatment of acetylated xyloglucan (500 mg) with 100:100:1 acetic anhydride–acetic acid–sulfuric acid furnished a disaccharide fraction containing a single major component, R_{Xylose} 0.61. Paper chromatography afforded homogeneous disaccharide (15 mg), $[\alpha]_D$ +15° (*c* 0.6, water), which gave galactose and xylose on hydrolysis. Reduction of the disaccharide with sodium borodeuteride followed by methylation furnished the methylated disaccharide alditol, n.m.r. (chloroform-*d*): δ 4.45 (1-proton doublet, $J_{1,2}$ 6 Hz, H-1′), which gave a single peak on g.l.c. on column *c* and whose mass spectrum was identical to that of the derivative from the rapeseed hull polysaccharide.

Methylation of xyloglucan. — Rapeseed hull xyloglucan (100 mg) was methylated with methyl iodide and sodium hydride in methyl sulfoxide[16] to give methylated xyloglucan (95 mg), $[\alpha]_D$ +52° (*c* 1.0, chloroform) (Found: OMe, 43.5%).

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