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STRUCTURAL INVESTIGATION OF SODIUM HYDROXIDE-SOLUBLE RAPESEED (*Brassica campestris*) POLYSACCHARIDES

PART V: FUCOAMYLOID*†

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ABSTRACT

Sedimentation analysis indicated that a fucoamyloid isolated from sodium hydroxide-soluble rapeseed polysaccharides was homogeneous. The fucoamyloid contained D-galactose, D-glucose, D-xylose, and L-fucose residues in molar proportions of 3:14:10:2. Methylation studies showed that the polysaccharide had a highly branched structure. Hydrolysis of the methylated fucoamyloid yielded 2,3,4-tri-*O*-methyl-D-xylose (8 mol.), 2,3,4-tri-*O*-methyl-L-fucose (2 mol.), 2,3,4,6-tetra-*O*-methyl-D-glucose (0.2 mol.), 2,3,4,6-tetra-*O*-methyl-D-galactose (1 mol.), 3,4-di-*O*-methyl-D-xylose (2 mol.), 2,3,6-tri-*O*-methyl-D-glucose (4 mol.), 3,4,6-tri-*O*-methyl-D-galactose (2 mol.), and 2,3-di-*O*-methyl-D-glucose (12 mol.). The general structural features of the fucoamyloid are discussed and compared with those of other fucoamyloids.

INTRODUCTION

In previous papers[1–4], the isolation and characterization of an amyloid, an acidic arabinogalactan, an arabinan, and pectic polysaccharides from rapeseed cotyledon meal were described. A sodium hydroxide extraction of the meal has yielded an acidic xylan and a polysaccharide designated as fucoamyloid, and we now report on the main structural features of the latter.

RESULTS AND DISCUSSION

The residue from the oxalate extraction[3] of rapeseed meal was extracted under nitrogen with 10% sodium hydroxide, yielding a major polysaccharide fraction (hemicellulose B) which was almost free from protein; acid hydrolysis gave galactose, glucose, fucose, xylose, uronic acid, and small proportions of mannose and arabinose. The hemicellulose A and C fractions, also isolated, were composed largely of proteins and showed only minute amounts of the same sugars as the hemicellulose B fraction.

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The fact that only a minute amount of hemicellulose A was present was consistent with the view that seeds of the dicotyledon variety contain either very little or no hemicellulose A.

Fractionation of the hemicellulose B fraction on DEAE-cellulose[5] (CO_3^{2-}) yielded a major, neutral fraction (45%, eluted with water) and a series of acidic fractions comprising 12, 6, 10, 9, and 2% of the sodium hydroxide-soluble hemicellulose B fraction. Acid hydrolysis of the water-eluted fraction gave mainly galactose, glucose, and xylose, together with small proportions of mannose, fucose, and uronic acid, and traces of rhamnose. On the basis of hydrolysis results, these fractions appeared to be similar to the pectin fraction reported earlier[4]. The acidic fractions 4 and 5, following hydrolysis, showed major proportions of galactose, glucose, and xylose, together with minor proportions of mannose, arabinose, fucose, and uronic acid; rhamnose was not detected.

The water-eluted fraction was further purified by fractionation on DEAE-cellulose (borate form)[6], yielding a water-eluted fraction (18%) and a series of borate-eluted fractions, B_1–B_4 (14, 50, 2, and 2%). The water-eluted fraction and fraction B_1 were essentially identical on the basis of their specific optical rotations and their behaviour on hydrolysis; each gave mainly glucose and xylose, together with comparatively smaller amounts of galactose and fucose. These fractions were combined to yield the fucoamyloid Hydrolysis of fractions B_2–B_4 gave, in addition to the above sugars, increasing (traces–minor–major) amounts of mannose. These fractions, therefore, contained the fucoamyloid admixed with small proportions of a mannose-containing polysaccharide. However, attempts to isolate such a polymer from fraction B_4 were not successful.

The fucoamyloid had $[\alpha]_D^{26}$ +54° and showed a single, symmetrical peak on sedimentation analysis[7]. Analysis revealed the constituent sugars to be galactose, glucose, xylose, and fucose in the molar ratios 1.6:5.2:5.1:1.0

Hydrolysis of the fucoamyloid, and fractionation of the resulting sugars on paper chromatograms, gave crystalline D-galactose and D-xylose. D-Glucose was identified as the crystalline *N*-*p*-nitrophenyl-β-D-glucopyranosylamine dihydrate, and L-fucose as the methylphenylhydrazone. The fucoamyloid was methylated[8], and then methanolysed and hydrolysed, and the identities and molar ratios of the products were determined by g.l.c. There was poor correspondence between the proportions of end-groups and branch-points, and also between the ratios of the sugars determined before and after methylation.

Consequently, a sample of the fucoamyloid was further purified by high-speed centrifugation, deionization, precipitation with methanol, and dialysis. The product thus recovered had $[\alpha]_D^{24}$ +56° and showed a single, symmetrical peak on sedimentation analysis. However, a small shoulder appeared at ~32,000 r.p.m., but sedimented before the emergence of the major peak. Graded hydrolysis (3–12 h) of the purified material showed that the hydrolysis of fucose, galactose, and xylose residues was essentially complete at 6–9 h, but that the amount of glucose continued to increase and was still not constant after 12 h.

A second methylation of the fucoamyloid, using a clear, centrifuged solution in methyl sulphoxide, yielded a fully methylated product which was subjected to methanolysis and hydrolysis. Paper chromatography of the hydrolysis products gave 5 fractions, and the identities of the sugars in each fraction were determined by g.l.c.–m.s.[9,10] of the derived alditol acetates. The molar proportions of the sugars (determined by g.l.c.) were as follows: 2,3,4-tri-*O*-methyl-D-xylose, 8; 2,3,4-tri-*O*-methyl-L-fucose, 2; 2,3,4,6-tetra-*O*-methyl-D-glucose, 0.2; 2,3,4,6-tetra-*O*-methyl-D-galactose, 1; 3,4-di-*O*-methyl-D-xylose, 2; 2,3,6-tri-*O*-methyl-D-glucose, 4, 3,4,6-tri-*O*-methyl-D-galactose, 2; and 2,3-di-*O*-methyl-D-glucose, 12. The molar ratios 3:16:10:2 for galactose, glucose, xylose, and fucose, calculated from the proportions of methylated sugars, were in good agreement with those (3:14:10:2) found for the original polysaccharide after hydrolysis for 12 h. The deficiency of two mol. of glucose in the unmethylated polysaccharide is ascribed to the slow hydrolysis of the residual cellulose backbone, since hydrolysis with 72% sulphuric acid of the material that remained after hydrolysis with M sulphuric acid showed only glucose.

The methylation analysis data show that the fucoamyloid has a highly branched structure. From the above molar proportions, it is seen that, on average, for every 31 sugar residues, there are 11 terminal, non-reducing end-groups (8 D-xylose, 2 L-fucose, and 1 D-galactose.) There are 11–12 residues of D-glucose involved in branching, through positions 4 and 6. The remaining 8 non-terminal residues consist of two (1→2)-linked D-xylose units, four (1→4)-linked D-glucose units, and two (1→2)-linked D-galactose units.

Amyloids of seed origin which have been studied in detail fall into two classes. The first class includes those that contain D-glucose, D-galactose, and D-xylose residues, and have been adequately discussed in a previous communication[1]. The second group, termed fucoamyloids, contain L-fucose residues in addition to the

TABLE I

SUGARS (MOL.) OBTAINED ON HYDROLYSIS OF THE METHYLATED FUCOAMYLOIDS

Sugar	*Mustard Ref. 11*	*Sycamore*[a] *Refs. 12 and 13*	*Rapeseed Present study*
2,3,4,6-Tetra-*O*-methyl-D-glucose	–	—	0.2
2,3,4,6-Tetra-*O*-methyl-D-galactose	+	—	1
2,3,4-Tri-*O*-methyl-D-xylose	+	5	8
2,3,4-Tri-*O*-methyl-L-fucose	+	2	2
2,3,6-Tri-*O*-methyl-D-glucose	+	3	4
3,4,6-Tri-*O*-methyl-D-galactose	–	2	2
3,4-Di-*O*-methyl-D-xylose	–	2	2
2,3-Di-*O*-methyl-D-xylose	+	—	—
2,4-Di-*O*-methyl-D-xylose	+	—	—
2-*O*-Methyl-D-xylose	+	—	—
2,3-Di-*O*-methyl-D-glucose	+	6	12
3-*O*-Methyl-D-glucose	–	1	—
2,3,4-Tri-*O*-methyl-L-arabinose	–	1	—

[a]Calculated on the basis of structure proposed.

above sugars. Fucoamyloids are found in mustard seed[11] and in suspension-cultured sycamore cells[12,13]. Rapeseed apparently contains both types, the former being present in small amounts only[1]. A detailed comparison of rape- and mustard-seed fucoamyloids is not possible in view of the lack of quantitative data for the latter. However, the presence of xylose residues linked through positions 3 and 4, rather than position 2, warrants a closer examination of the methylated products from the soluble- and insoluble-fucoamyloids of mustard. It is possible that these fragments originate either from the insoluble-fucoamyloids or are derived from a contaminating xylan. The absence of both (1→2)-linked xylose[11] and galactose units is difficult to understand. The distinction between the fucoamyloids (Table I) of sycamore[12,13] and rape is based on differences in the ratio of the sugar moieties. Another significant variation noted in the present study is the presence of 1 mol. of D-galactose as a non-reducing end-group. Finally, since our preparation of fucoamyloid, following rigorous purification, showed no arabinose, the presence of a non-reducing arabinose end-group and a triply-linked-glucose unit is definitely ruled out[12,13].

EXPERIMENTAL

The general experimental methods have been reported previously[1].

Extraction with sodium hydroxide. — The oxalate-insoluble residue[3] (500 g) was extracted with 10% sodium hydroxide (10 l) in an atmosphere of nitrogen for 24 h, yielding an insoluble residue (95.2 g, 19%; N, 4.55; ash, 5.88; moisture, 6.45%), and a soluble extract which was adjusted to pH 4.5 with 50% acetic acid. The precipitated material (63.5 g, 13%; N, 13.29; ash, 3.08; moisture 5.18%) was recovered by centrifugation and the supernatant solution was mixed with ethanol (2 vol.), yielding insoluble material (hemicellulose B, 44.9 g, 9%; N, 1.35; ash, 13.11; moisture, 5.52%). The alcohol supernatant was freeze-dried, following removal of ethanol and dialysis, to yield a fourth fraction (74.4 g, 15%; N, 13.35; ash, 5.69; moisture 7.3%). Acid hydrolysates (M sulphuric acid, 100°, 3 h) prepared from the four fractions showed major amounts of sugars consisting mainly of galactose, glucose, xylose, fucose, and uronic acids, with small amounts of mannose and traces of arabinose in the case of hemicellulose B fraction. The hydrolysates from the earlier two fractions showed only traces of these sugars. Acid hydrolysis (70% H_2SO_4) of the residue showed mainly glucose.

Fractionation of polysaccharide. — The hemicellulose B fraction (15 g) was fractionated on a column (4 × 16 in.) of DEAE-cellulose[5] (CO_3^{2-}). Elution with water (7 l, fraction 1), followed by stepwise elution with 0.5M ammonium carbonate (6 l, fraction 2), and 0.5M sodium hydroxide (3 l, 2 l, 1 l, 1 l, fractions 3–6), yielded a total of six fractions which were recovered by freeze-drying, following neutralization, dialysis, and concentration. The hydrolysis and analytical data are shown in Table II.

Fractionation of the neutral polysaccharide. — Fraction 1 (2 g) was further fractionated on a column (2 × 16 in.) of DEAE-cellulose (borate form). Elution with water (3 l) removed a fraction (0.36 g, 18%), and gradient elution with sodium meta-

TABLE II

FRACTIONATION OF SODIUM HYDROXIDE-SOLUBLE RAPESEED-MEAL POLYSACCHARIDES ON DEAE-CELLULOSE (CO_3^{2-})

Fraction	*Yield* (g)	$[\alpha]_D$ (*water*) (*degrees*)	$[\alpha]_D$ (*0.5M NaOH*)	*Hydrolysis results*								*Analytical data* (%)			
				Gal	*Glc*	*Man*	*Ara*	*Xyl*	*Fuc*	*Rha*	*Uronic acid*	*N*	*Ash*	*Moisture*	*Uronic acid*
1	6.7	+40.4	—	major	major	minor	trace	major	minor	−ve	−ve	0.23	3.52	5.57	nil
2	1.8	+10.8	+2.7	major	major	minor	major	major	trace	minor	+ve	0.73	11.13	5.79	14.07
3	0.94	—	+0.83	major	major	minor	major	major	trace	minor	+ve	4.2	12.98	4.14	11.91
4	1.5	—	+9.2	major	major	minor	minor	major	minor	trace	trace	1.12	3.52	3.82	2.28
5	1.4	—	+14.3	major	major	major	minor	major	minor	−ve	+ve	1.55	8.52	5.44	6.8
6	0.33	—	—	trace	major	major	trace	major	−ve	−ve	−ve	0.39	30.88	3.14	nil

borate (0→0.5M, 4 l) yielded 4 fractions which were dialyzed for 48 h against running tap-water and for 4 h against two changes of distilled water, and freeze-dried to yield fractions B_1 (0.27 g, 13.6%), B_2 (0.99 g, 49.7%), B_3 (0.037 g, 1.6%), and B_4 (0.04 g, 1.7%).

Acid hydrolysis (M sulphuric acid, 100°, 3 h) of the water-eluted fraction gave mainly glucose and xylose, with smaller amounts of galactose and fucose. Fraction B, following hydrolysis, showed the same results. On hydrolysis, fractions B_2, B_3, and B_4 gave, in addition to the above sugars, increasing amounts (trace to minor to major) of mannose. The water-eluted fraction, $[\alpha]_D^{23}$ +55° (*c* 1.1, water), and the borate-eluted fraction B_1, $[\alpha]_D^{23}$ +49° (*c* 7, water), were combined to yield the pure fucoamyloid (0.63 g, 31.5%).

Analysis of the fucoamyloid. — The fucoamyloid had $[\alpha]_D^{26}$ +54° (*c* 0.37, water) (Found: N, 0.38; ash, 1.93; moisture, 5.16%). Sedimentation analysis[7] of a 0.5% solution in 0.1M sodium tetraborate at 44,000 r.p.m. showed a single, symmetrical peak. The fucoamyloid (5 mg) was hydrolysed with M sulphuric acid for 3 h at 100°. The hydrolysate was neutralized ($BaCO_3$), filtered, and evaporated to dryness. The residue was dissolved in M ammonium hydroxide, and sodium borohydride (20 mg) was added[14]. After 20 h at room temperature, excess borohydride was decomposed with glacial acetic acid, the solution was evaporated to dryness (codistillation with methanol–benzene once, followed by methanol four times), and the residue was dried *in vacuo* over KOH overnight. The resulting mixture of alditols was acetylated with acetic anhydride at 100° for 1.5 h. Following removal of acetic anhydride by codistillation with water *in vacuo* and drying overnight over KOH, the mixture of alditol acetates was taken up in dichloromethane for analysis by g.l.c. G.l.c. was carried out on a Pye 104 Gas chromatograph, using dual columns (5 ft) of 3% of OV-225 on Chromosorb W-HP (80–100 mesh), with temperature programming at 2°/min from 180–250°, and a nitrogen flow-rate of 45 ml/min. Peak areas, evaluated by a CSI digital integrator (model 208), gave molar ratios of glucose, galactose, xylose, and fucose of 5.2:1.6:5.1:1.0.

Acid hydrolysis of the fucoamyloid. — The fucoamyloid (200 mg) was hydrolysed with M sulphuric acid (10 ml) for 3 h at 100°. Neutralization of the hydrolysate ($BaCO_3$), filtration, and evaporation yielded a mixture of sugars which was separated on six sheets (18 × 22 in.) of Whatman No. 1 paper, using solvent *A*, to give fractions 1, 2, and 3, corresponding to galactose, glucose, and a mixture of xylose and fucose. Fraction 3 was further fractionated in 1-butanol–acetic acid–water (4:1:5) to give fractions 3a (xylose) and 3b (fucose). Fractions 1 and 3a, on crystallization and recrystallization from 90–95% ethanol in the cold, gave D-galactose, m.p. and mixture m.p. 164–166°, $[\alpha]_D^{26}$ +101° (12 min)→+75° (equil.) (*c* 10, water), and D-xylose, m.p. and mixture m.p. 145–146°, $[\alpha]_D^{25}$ +67° (3 min)→18° (equil.) (*c* 0.49, water). Fraction 2 (D-glucose) was identified as *N*-*p*-nitrophenyl-β-D-glucopyranosylamine dihydrate, m.p. and mixture m.p. 183–184°, $[\alpha]_D^{27}$ −204° (2 min)→206° (equil.) (*c* 0.29, pyridine). Fraction 3b (L-fucose) was characterized as the methylphenylhydrazone, m.p. and mixture m.p. 172–173°, $[\alpha]_D^{28}$ +5° (*c* 0.04, pyridine).

Methylation analysis of the fucoamyloid. — The fucoamyloid (100 mg) was dissolved in dry methyl sulphoxide[8] (15 ml) by stirring for 12 h at room temperature. The solution was flushed with nitrogen, and a 2M solution (1 ml) of methylsulphinyl carbanion in methyl sulphoxide was added dropwise. The resulting, viscous solution was stirred for 10 h at room temperature. Methyl iodide (1 ml) was then added dropwise, with external cooling, and the mixture was stirred at room temperature for 4 h, then poured into water (50 ml), dialysed for 20 h against running tap-water, concentrated, and extracted continuously with chloroform. The extract was dried (Na_2SO_4) and concentrated. The solid residue (67 mg) showed a small but definite i.r. absorption for hydroxyl. The residue from the chloroform extraction was dialysed against running tap-water for 4 days and freeze-dried to yield material (45 mg) which was further methylated in methyl sulphoxide (5 ml) using 2M methylsulphinyl carbanion (1 ml) and methyl iodide (0.1 ml). Following the addition of methyl iodide, a second portion (1 ml) of methylsulphinyl carbanion was added, and the methylation process repeated except that excess of methyl iodide (1 ml) was added. The solution was poured into water; recovery of the product, as before, yielded a material (41 mg) which also showed a small i.r. absorption for hydroxyl.

The combined, methylated product (105 mg) was dissolved in methyl iodide (20 ml), silver oxide (1 g) was added, and the mixture was boiled under reflux for 24 h. After four more methylations by this procedure, a product (94 mg) was recovered which still showed a small i.r. absorption for hydroxyl. Consequently, the methylated product (90 mg) was dissolved in methyl sulphoxide (5 ml) to which crushed pellets of sodium hydroxide (1 g) were added, followed by methyl sulphate (0.5 ml) during 5 h. The suspension was stirred for 24 h before excess of methyl sulphate was decomposed by heating for 1 h at 90°. Water was added to dissolve the sodium hydroxide, the pH was adjusted to 7, and the solution was extracted 4 times with equal volumes of chloroform. The extract (75 ml) was dried (Na_2SO_4), filtered, and evaporated to a solid (90 mg) which showed only a weak i.r. absorption for hydroxyl and had $[\alpha]_D^{26}$ + 15° (*c* 0.78, chloroform).

The solution of methylated polysaccharide (90 mg) in 2% methanolic hydrogen chloride (50 ml) was boiled for 24 h, then neutralized (Ag_2CO_3), and concentrated. The syrupy product was hydrolysed with 0.5M sulphuric acid (3 ml) for 40 h at 100°. The hydrolysate was neutralised ($BaCO_3$), filtered, and evaporated to a syrup (67 mg).

A portion (28 mg) of the methylated sugars was fractionated on four sheets of Whatman No. 1 paper (7 × 22 in.) with solvent B, giving 5 fractions. Portions (1–2 mg) of each fraction were demethylated with boron trichloride and the products were examined by p.c. Portions (1–4 mg) of each fraction were reduced with sodium borohydride[14], and the products were acetylated and examined by g.l.c., as described above (temperature programming from 100 → 250° at 2°/min). G.l.c.–m.s. was carried out on a combined Finnigan 3100 D GC/MS, equipped with a U-shaped column (5 ft × 0.25 in.) of 3% of OV-225 on Chromosorb W-HP (80–100 mesh), with temperature programming from 100 → 200° at 2°/min. The separator temperature was 250°, analyser temperature 90°, and ionizing electron energy 70 eV. The spectra

were recorded as bar graphs by means of the Finnigan 6000 MS Data System. The results are summarized in Table III.

TABLE III

PAPER-CHROMATOGRAPHIC, ELECTROPHORETIC, DEMETHYLATION, AND G.L.C.–M.S. DATA FOR SUGARS FROM THE METHYLATED FUCOAMYLOID

Fraction	R_F (*Solvent* B)	M_G (*Borate buffer, pH 10*)	*Parent sugar*[a]	*Reduced product*[b]
1	0.80	0.00	Xylose, glucose (trace)	2,3,4-Tri-*O*-methylxylitol 2,3,4,6-Tetra-*O*-methylglucitol
2	0.65	0.00	Galactose and fucose	2,3,4,6-Tetra-*O*-methylgalactitol 2,3,4-Tri-*O*-methylfucitol
3	0.48	0.00 0.32	Glucose and xylose	2,3,6-Tri-*O*-methylglucitol 3,4-Di-*O*-methylxylitol
4	0.30	0.24	Galactose, glucose (trace)	3,4,6-Tri-*O*-methylgalactitol 2,3-Di-*O*-methylglucitol
5	0.26	0.17	Glucose	2,3-Di-*O*-methylglucitol

[a]Identified by demethylation. [b]Identified by g.l.c.–m.s. of the acetate

A second portion (5 mg) was similarly reduced, acetylated, and examined by g.l.c. The molar proportions (to the nearest whole number) of the methylated alditol acetates with respect to 2,3,4,6-tetra-*O*-methylgalactitol diacetate were: 2,3,4-tri-*O*-methylxylitol, 6; 2,3,4-tri-*O*-methylfucitol, 2; 2,3,4,6-tetra-*O*-methylgalactitol, 1; 3,4-di-*O*-methylxylitol, 1; 2,3,6-tri-*O*-methylglucitol, 5; 3,4,6-tri-*O*-methylgalactitol, 2; and 2,3-di-*O*-methylglucitol, 14.

Purification and re-examination of the fucoamyloid. — The fucoamyloid (316 mg) was dissolved in water (25 ml), and the solution was centrifuged at 10,000 r.p.m. for 0.5 h. A minute amount of precipitate was discarded, and the clear, supernatant solution was mixed with Rexyn 101(H^+) resin, filtered, and then mixed with methanol (3 vol.). The precipitate was recovered by centrifugation, dissolved in water, dialysed for 24 h against running tap-water and for 4 h against distilled water, concentrated, and freeze-dried to yield a product (260 mg) which showed $[\alpha]_D^{24}$ +56° (*c* 0.48, water).

(*a*) *Sedimentation analysis.* The fucoamyloid, as a 1% solution in 0.1M sodium tetraborate, at 44,000 r.p.m. again showed a single, symmetrical peak. However, at ~32,000 r.p.m., a tiny shoulder appeared, but it sedimented ahead of the main peak.

(*b*) *Sugar ratios.* The fucoamyloid (10 mg) was hydrolysed in M sulphuric acid (1 ml) at 100°. Aliquot portions (0.2 ml) were withdrawn at intervals of 3, 6, 9, and 12 h, neutralized ($BaCO_3$), filtered, evaporated to dryness, reduced with borohydride, acetylated, and examined by g.l.c. as before. The molar ratios for the alditol acetates of fucose–galactose–xylose–glucose were: 3 h, 1:1.8:7.7:7.6; 6 h, 1:1.4:5.0:5.9; 9 h, 1:1.6:5.2:6.6; and 12 h, 1:1.6:5.2:6.8.

A second portion (15 mg) was hydrolysed in M sulphuric acid (1.5 ml) and the hydrolysis was followed polarimetrically. The $[\alpha]_D^{25}$ values at 2, 3, 4, 5, 6, 7, and 8 h

were +31.1°, +32.0°, +34.4°, +36.7°, +37.8°, +38.5°, and +38.9°. After 8 h, the product was isolated, and hydrolysed with cold 72% sulphuric acid (0.5 ml) at room temperature for 1.5 h. The reaction mixture was then diluted (4 ml), hydrolysed for 3 h at 100°, neutralized ($BaCO_3$), filtered, and evaporated to dryness. Paper chromatography showed only glucose.

(*c*) *Methylation analysis.* The fucoamyloid (54 mg) was dissolved in dry methyl sulphoxide (5 ml), and the solution was centrifuged at 10,000 r.p.m. for 0.5 h. A small amount of precipitate (~5 mg) was discarded, the solution flushed with nitrogen, and a 2M solution (1 ml) of methylsulphinyl carbanion in methyl sulphoxide added dropwise. The resulting solution was stirred for 8 h at room temperature. Methyl iodide (0.1 ml) was added dropwise with external cooling (ice–water), and the mixture was stirred overnight. Following a second methylation, using methylsulphinyl carbanion solution (0.5 ml) and methyl iodide (1 ml), the reaction mixture was poured into water (50 ml), dialysed for 24 h against tap water, concentrated, and extracted continuously with chloroform. The solid residue (55 mg) from the chloroform extract showed a small i.r. absorption for hydroxyl. The methylated product (55 mg) was dissolved in methyl iodide (10 ml), silver oxide (500 mg) was added, the mixture was refluxed overnight and filtered, and the product recovered. After five further methylations, a product (49 mg) was recovered which still showed a small i.r. absorption for hydroxyl. After a final methylation by the Hakomori[3] procedure as above, the product (48 mg) had $[\alpha]_D^{25}$ +14° (*c* 0.22, chloroform) and showed only a weak absorption band for hydroxyl. Methanolysis, hydrolysis, reduction, acetylation, and g.l.c. gave the results summarized in Table IV.

TABLE IV

ANALYTICAL DATA FOR THE METHYLATED SUGARS (2nd METHYLATION)

Acetates of	*Retention time (min)*	*Molar ratio*
2,3,4-Tri-*O*-methylxylitol	0.66	8
2,3,4-Tri-*O*-methylfucitol	0.70	2
2,3,4,6-Tetra-*O*-methylglucitol	1.00	0.2
2,3,4,6-Tetra-*O*-methylgalactitol	1.06	1
3,4-Di-*O*-methylxylitol	1.10	2
2,3,6-Tri-*O*-methylglucitol	1.43	4
3,4,6-Tri-*O*-methylgalactitol[a]	1.43	2
2,3-Di-*O*-methylglucitol	1.75	12

[a]Calculated from the ratio of 3,4,6-tri-*O*-methylgalactitol and 2,3-di-*O*-methylglucitol following reduction, acetylation, and g.l.c. of the combined fractions 4 and 5 obtained by p.c.

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REFERENCES

1 I. R. SIDDIQUI AND P. J. WOOD, *Carbohydr. Res.*, 17 (1971) 97–108.
2 I. R. SIDDIQUI AND P. J. WOOD, *Carbohydr. Res.*, 24 (1972) 1–9.
3 I. R SIDDIQUI AND P. J. WOOD, *Carbohydr. Res.*, 36 (1976) 35–44.
4 Part IV. I. R. SIDDIQUI AND P. J. WOOD, *Carbohydr. Res.*, 50 (1976) 97–107
5 I R SIDDIQUI AND P. J WOOD, *Carbohydr. Res.*, 16 (1971) 452–454.
6 H. NEUKOM, H. DEUEL, W. J. HERI, AND W. KUNDIG, *Helv Chim Acta*, 43 (1960) 64–71.
7 T. SVEDBERG AND K. O. PEDERSEN, *The Ultracentrifuge*, Clarendon Press, Oxford, 1940
8 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–207.
9 H. BJÖRNDAL, B. LINDBERG, AND S. SVENSSON, *Carbohydr. Res.*, 5 (1967) 433–440.
10 H. BJORNDAL, B LINDBERG, AND S. SVENSSON, *Acta Chem. Scand*, 21 (1967) 1801–1804.
11 E. SHEILA, B GOULD, D. A. REES, AND N. J. W. WRIGHT, *Biochem. J.*, 124 (1971) 47–53.
12 W. D. BAUER, K. W. TALMADGE, K. KEEGSTRA, AND P. ALBERSHEIM, *Plant Physiol.*, 51 (1973) 174–187.
13 P. ALBERSHEIM, *Scientific American*, 232 (1975) 80–95.
14 L. J. GRIGGS, A. POST, E. R. WHITE, J. A. FINKELSTEIN, W. E. MOECKEL, K. G. HOLDEN, J. E. ZAREMBO, AND J. A. WEISBACK, *Anal. Biochem.*, 43 (1971) 369–381.
15 S. ALLEN, T. G. BONNER, E. J. BOURNE, AND N. M. SAVILLE, *Chem. Ind. (London)*, (1958) 630.