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POLYSACCHARIDES OF THE RED ALGA *Chaetangium erinaceum*

PART I. ISOLATION AND CHARACTERISATION OF THE WATER-SOLUBLE XYLAN

J. R. NUNN, H. PAROLIS*, AND I. RUSSELL
Chemistry Department, Rhodes University, Grahamstown (South Africa)
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ABSTRACT

Hot-water extraction of the red alga *Chaetangium erinaceum* yielded a mixture of polysaccharides from which was isolated a xylan. Methylation and partial hydrolysis analysis of the xylan showed it to be a linear molecule with no evidence of branching, and composed exclusively of β-(1→3)- and β-(1→4)-linked D-xylopyranose residues in the ratio of 2:9. Evidence was obtained for regions of adjacent (1→4)-linkages interdispersed with (1→3) linkages, but none for contiguous (1→3)-linkages in the molecule. The following oligosaccharides were isolated from the partial, acid hydrolysate and identified: xylobiose, rhodymenabiose, xylotriose, *O*-β-D-xylopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose, *O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→3)-D-xylose, xylotetraose, and xylopentaose.

INTRODUCTION

The major water-soluble polysaccharide elaborated by most red algae appears to be a galactan sulfate. In contrast, the major water-soluble polysaccharides elaborated by the red seaweeds *Rhodymenia palmata*[1,2] and *Chaetangium fastigiatum*[3] are xylans, containing β-(1→4)- and β-(1→3)-linked D-xylopyranose residues. In continuing our work on red-seaweed polysaccharides, we now report on the isolation and characterisation of the water-soluble xylan elaborated by the red alga *Chaetangium erinaceum*.

RESULTS AND DISCUSSION

Chaetangium erinaceum, a red alga belonging to the *Chaetangiaceae* (order *Nemalionales*), was collected at Port Alfred, South Africa, in October, 1966. It is a striking seaweed, with thin, flat fronds bearing many, branching outgrowths and is often found with *Gelidium pristoides* on exposed surfaces of rocks and wave-cut platforms in the mid-tidal range. Hot-water extraction of the fresh weed, after careful separation from contaminating *Gelidium pristoides*, afforded a mucilage which was precipitated with ethanol. The crude product (7.3% on a wet-weight basis) was shown,

*To whom correspondence should be addressed.

by paper chromatography, to contain mainly xylose with small proportions of galactose and mannose. A portion of the crude product was "purified" by ethanol precipitation. Quantitative determination of the constituent units in the "purified product" revealed the molar ratios of xylose–galactose–mannose to be 56.4:3:4, and a sulphate content of 2.85%.

Treatment of the hot-water extract with Fehling's solution afforded a crude xylan (~50%) and a sulphated polysaccharide fraction which gave galactose, mannose, and xylose on acid hydrolysis. The xylan was purified by further treatment with Fehling's solution and finally by dissolution in water, centrifugation, and precipitation with ethanol. The xylan had a specific rotation of $-112°$, indicating a predominance of β-D linkages. Oxidation with periodate in unbuffered solution at room temperature ceased after 96 h (Table I), when 0.812 mole of periodate had been consumed per "anhydro-pentose" unit, showing that ~80% of the xylose residues possess α-glycol groups which are linked through C-1 and C-2 or C-4, if the the presence of furanose residues is discounted. The resistance to attack of the remaining 20% can be explained by the presence of (1→3) linkages.

TABLE I

PERIODATE OXIDATION OF THE XYLAN

Time (h)	*5*	*12*	*24*	*48*	*72*	*96*	*120*
Oxidant consumption[a]	0.594	0.664	0.712	0.760	0.800	0.812	0.813

[a]Mole/"anhydro-pentose" unit.

Methylation of the polysaccharide, by the method of Srivastava *et al.*[4], afforded a partially methylated product which was fractionated on silica gel with chloroform–methanol. Four fractions were obtained, all of which gave very similar patterns of methylated sugars on hydrolysis; the fractions with lower methoxyl values gave larger quantities of mono-*O*-methylxyloses and unmethylated xylose. The largest fraction (71% of the total, recovered polymer) had a methoxyl content of 34.9%, which was increased to 38.3% on treatment with Purdie's reagents[5]. The infrared spectrum of this material had no hydroxyl peak. The methylated polysaccharide was hydrolysed by using the sulphuric acid method of Garegg and Lindberg[6]. The procedure was modified, in that the acid hydrolysate was extracted with dichloromethane before neutralisation of the aqueous phase with barium carbonate because of the volatility of tri-*O*-methylxylose[7]. The products of hydrolysis were separated by paper chromatography to give 2,3,4-tri-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose, 2,4-di-*O*-methyl-D-xylose, and a mono-*O*-methyl fraction. The 2,3-di-*O*-methyl- and 2,4-di-*O*-methyl-D-xyloses were identified as crystalline derivatives, whereas the 2,3,4-tri-*O*-methyl-D-xylose was identified on the combined evidence of paper and gas–liquid chromatography. The low specific rotation found for the 2,3,4-tri-*O*-methyl-D-xylose is considered to be due to contamination with degradation products arising

during hydrolysis of the methylated polymer. These are extracted with dichloromethane and have a high mobility on paper (as does 2,3,4-tri-*O*-methyl-D-xylose).

Hydrolysis of the methylated polysaccharide, followed by conversion of the derived monomers into their acetylated nitriles[8] and examination by g.l.c., gave molar ratios of 2,3,4-tri-, 2,4-di-, 2,3-di-, and mono-*O*-methylxyloses of 0.08:1:4.56:0.27. The mono-*O*-methylxyloses probably have no structural significance and are believed to be the result of undermethylation and also demethylation during hydrolysis. The methylation analysis, therefore, indicates that the xylan is essentially linear. The ratio between β-(1→4) and β-(1→3) linkages in the polysaccharide, as determined by methylation analysis, is in good agreement with the values obtained by periodate oxidation. The average chain-length of the xylan, estimated from the relative, molar proportion of 2,3,4-tri-*O*-methyl-D-xylose, is ~74 xylose units. However, this estimate may not be an accurate reflection of the length of the xylan chain because of the volatility of the 2,3,4-tri-*O*-methyl-D-xylose, although all possible precautions to counteract this were taken during the analytical determination. The relative, molar ratios of the *O*-methylxyloses, isolated by paper chromatography of the hydrolysed, methylated polymer, agree fairly well with the ratios obtained by g.l.c. analysis (see Table II).

TABLE II

O-METHYLXYLOSES (MOLAR %) FROM METHYLATED XYLAN

	2,3,4-Tri-	*2,4-Di-*	*2,3-Di-*	*Mono-*
G.l.c. analysis	1.35	16.9	77.9	4.5
From paper separation	3.1	16.3	74.6	6.0

Partial, acid hydrolysis of the polysaccharide, followed by separation of the products on a charcoal–Celite column, yielded D-xylose and several oligosaccharides. The D-xylose was obtained in crystalline form and was further characterised by conversion into the di-*O*-benzylidene dimethyl acetal derivative. The structures of seven of the oligosaccharides were elucidated, these being xylobiose (**1**), rhodymenabiose (**2**), xylotriose (**3**), *O*-β-D-xylopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose (**4**), *O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→3)-D-xylose (**5**), xylotetraose (**6**), and xylopentaose (**8**). Oligosaccharides **1**, **3**, **6**, and **8** were obtained crystalline, and the structures of **1**–**3**, **6**, and **8** were elucidated by partial, acid hydrolysis to the component sugars, and by methylation studies. A portion of each methylated oligosaccharide was methanolysed and the product mixture examined by g.l.c. The remainder was hydrolysed and the derived, methylated sugars were converted into their acetylated nitriles and analysed by g.l.c. The structures of oligosaccharides **4** and **5** were elucidated in this way, but, in addition, they were reduced with sodium borohydride, and the partially hydrolysed products were examined by paper chromatography. This procedure established the nature of the reducing end-group. The acetylated nitriles from each methylated oligosaccharide, with the exception of

oligosaccharide **8**, were quantitatively determined. The results of these analyses (Table III) are consistent with the structures of the oligosaccharides as formulated in the first column. The type of glycosidic linkage assigned to the oligosaccharides was based on optical rotation or comparison with the optical rotation of authentic compounds.

TABLE III

STRUCTURAL ANALYSIS OF OLIGOSACCHARIDES

Oligosaccharide	*Partial hydrolysis products*	*Partial hydrolysis products of reduced oligosaccharide*	*Acetylated nitriles of hydrolysed, methylated oligosaccharide*[a]
β-D-Xyl-(1→4)-D-Xyl (**1**)	—		*A*:*B* = 1:0.9
β-D-Xyl-(1→4)-β-D-Xyl-(1→4)-D-Xyl (**3**)	**1**		*A*:*B* = 0.9:2.2
β-D-Xyl-(1→4)-β-D-Xyl-(1→4)-β-D-Xyl-(1→4)-D-Xyl (**6**)	**1, 3**		*A*:*B* = 0.9:3.1
β-D-Xyl-(1→4)-β-D-Xyl-(1→4)-β-D-Xyl-(1→4)-β-D-Xyl-(1→4)-D-Xyl (**8**)	**1, 3, 6**		*A*, *B*
β-D-Xyl-(1→3)-D-Xyl (**2**)	—		*A*:*C* = 1:1.1
β-D-Xyl-(1→4)-β-D-Xyl-(1→3)-D-Xyl (**5**)	**1, 2**	**1**	*A*:*B*:*C* = 0.9:1.0:1.1
β-D-Xyl-(1→3)-β-D-Xyl-(1→4)-D-Xyl (**4**)	**1, 2**	**2**	*A*:*B*:*C* = 1.1:0.9:1.0

[a] *A* = 2,3,4-Tri-*O*-methylxylose; *B* = 2,3-di-*O*-methylxylose; *C* = 2,4-di-*O*-methylxylose.

"Oligosaccharide" **7**, although chromatographically homogeneous, was shown by electrophoresis to be a mixture of at least two components. Partial, acid hydrolysis, before and after treatment with sodium borohydride, indicated that **7** was probably a mixture of *O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→3)-D-xylose, *O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose, *O*-β-D-xylopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose, and/or *O*-β-D-xylopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→3)-D-xylose.

When log $(1/R_F - 1)$ of the oligosaccharides present in the partial hydrolysis (calculated from a chromatogram in solvent *A*) is plotted against d.p., the points fall on two nearly parallel, straight lines (Fig. 1). The R_F values were calculated from the R_{XYL} values of the oligosaccharides multiplied by the R_F of xylose (0.21). The upper line represents the oligosaccharides containing only (1→4) linkages, and the lower line those containing both (1→4) and (1→3) linkages. It is of interest to note that the major component of Fraction 9 [which was not structurally elucidated and had R_{XYL} 0.03 (solvent *A*)] falls on the lower line if it is assumed to be a mixture of pentasaccharides containing both (1→4) and (1→3) linkages.

The isolation, in substantial yield, of two pure trisaccharides containing both a (1→4) and a (1→3) linkage affords conclusive proof that the polysaccharide molecule contains both types of linkage.

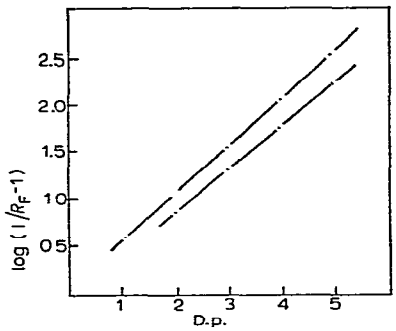

Fig. 1. Relationship between chromatographic mobility and d.p. of xylose oligosaccharides.

The above results suggest that the xylan is essentially linear and is composed of β-(1→3)- and β-(1→4)-D-xylopyranose residues in the ratio 2:9. There are regions of adjacent (1→4)-linkages interspersed with (1→3) linkages. No evidence was obtained for the presence of contiguous (1→3)-linkages in the macromolecule.

The calculated specific rotation[3] of the xylan (−107.8°) is in good agreement with the measured value (−112°). This suggests that all residues have the β-D configuration, and that the polysaccharide has a random-coil structure. This implies that the (1→3) linkages are interspersed throughout the structure, rather than grouped contiguously.

The xylan from *Chaetangium erinaceum* resembles markedly the xylans isolated from the red algae *Rhodymenia palmata*[1,2] and *Chaetangium fastigiatum*[3], and is another example of a "mixed linkage" homoxylan-type polymer[9].

EXPERIMENTAL

Paper chromatography was carried out on Whatman No. 1 paper with the following solvent systems: (*A*) ethyl acetate–acetic acid–formic acid–water (18:3:1:4), and (*B*) ethyl acetate–pyridine–water (8:2:1). Spray reagents used were (*1*) *p*-anisidine hydrochloride[10] and (*2*) 20% sulphuric acid in ethanol. R_{XYL} values refer to rates of movement relative to that of D-xylose. Electrophoresis was carried out on Whatman No. 1 paper, using 0.4M sodium tetraborate buffer (pH 10). M_{XYL} values refer to rates of movement relative to that of D-xylose. The degree of polymerization (d.p.) of oligosaccharides was determined by the phenol–sulphuric acid method[11]. Infrared spectra were recorded on a Beckman IR-8 spectrophotometer. Concentration of solutions was carried out at 40°/20 torr, and specific rotations were measured in water, unless otherwise stated. Thin-layer chromatography (t.l.c.) was carried out with silica gel G (containing calcium sulphate as binder) and butanone–water (85:7). R_{TMG} values of methylated sugars refer to rates of travel relative to that of 2,3,4,6-tetra-*O*-methyl-D-galactose. Gas–liquid chromatography (g.l.c.) was carried out on a Beckman GC-4 chromatograph equipped with dual flame-ionisation detectors and

nitrogen as carrier gas, using the following columns: (*a*) 15% w/w poly(butane-1,4-diol succinate) on acid-washed Celite (80–100 mesh) at 175° for methyl glycosides, and (*b*) 3% w/w ECNSS-M on Chromosorb W (100–120 mesh) at 180° for acetylated nitriles. Retention times T and T_X are relative to those of methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside and 3,5-di-*O*-acetyl-2,4-di-*O*-methyl-D-xylononitrile, respectively.

Extraction of Chaetangium erinaceum. — The wet seaweed (6 kg) was exhaustively extracted with hot water which was maintained at pH 6 by the addition of acetic acid. Centrifugation of the combined, aqueous extracts, followed by precipitation of the mucilage in ethanol (5 vol.), afforded, after filtration and drying, an off-white product (440 g) (Found: N, 1.8%). Paper-chromatographic examination of an acid hydrolysate revealed (solvents *A* and *B*) the presence of xylose (major), mannose, and galactose. A portion of the polysaccharide mixture was purified by dialysis of its aqueous solution, followed by precipitation by pouring into ethanol [Found (on material dried at 60°/0.5 torr): OMe, 0.0; N, 0.0; SO_4^{2-}, 2.85%].

Separation and purification of a xylan from the polysaccharide mixture. — The polysaccharide mixture (400 g) was dissolved in hot water, and the centrifuged solution was treated with Fehling's solution. The copper complex was collected by decantation, washed with water, and decomposed by maceration in a blender for 1 min at 0° with ethanol which contained 5% (v/v) of hydrochloric acid. The residue was washed with ethanol and finally acetone, and dried *in vacuo* (yield, 192 g). Paper-chromatographic examination of an acid hydrolysate revealed the presence of xylose and a small proportion each of galactose and mannose. The xylan was further purified *via* its copper complex, and finally by dissolution in water, centrifugation, and precipitation by pouring into ethanol. The recovered polysaccharide was washed with ether, and dried *in vacuo* at 45° (yield, 130 g); $[\alpha]_D^{20}$ −112° (*c* 0.91). Paper-chromatographic examination (solvents *A* and *B*) of a hydrolysate revealed the presence of xylose only.

The solution remaining after removal of the xylan was neutralised with acetic acid and dialysed against running tap-water (5 days). The residue was concentrated and passed through Amberlite IR-120(H^+) resin. The eluate was neutralised with sodium hydroxide, concentrated, and poured into ethanol (5 vol.). The resulting polysaccharide mixture was collected by centrifugation, washed with ethanol, and dried *in vacuo*, affording an off-white, fibrous product (70 g). Chromatographic examination (solvents *A* and *B*) of a hydrolysate of the product revealed the presence of galactose, mannose, and xylose as the major constituents.

Periodate oxidation of the polysaccharide. — Polysaccharide (478.6 mg) dried at 70° (0.1 torr for 24 h) was dissolved in 0.1M sodium metaperiodate (100 ml) and set aside at room temperature in the dark. Aliquots (5 ml) were withdrawn at regular intervals, and the reduction of periodate was determined titrimetrically[12] (Table I).

Methylation of the polysaccharide. — To polysaccharide (4.8 g) dissolved in methyl sulphoxide (500 ml) was added powdered sodium hydroxide (200 g) and methyl sulphate (175 ml), with stirring over a period of 8 h under nitrogen[4]. During the first 2 h, the reaction was carried out at 20°. After stirring for another 16 h, the

mixture was heated on a boiling-water bath for 1.5 h to decompose the methyl sulphate. Water (200 ml) was added to dissolve the sodium hydroxide, and the mixture was cooled to 5° and neutralised with 5M sulphuric acid. The sodium sulphate which precipitated was filtered off and washed with chloroform. The aqueous filtrate was extracted with chloroform (2 l; in portions), and the combined chloroform extracts were dried (Na_2SO_4) and evaporated to a brown syrup *in vacuo*. The partially methylated xylan was fractionated by elution from a column (48 × 3 cm) of silica gel (60–120 mesh, 150 g) with a chloroform–methanol gradient.

To the main fraction (3.62 g; OMe, 34.9%) dissolved in methyl iodide (85 ml) and *N*,*N*-dimethylformamide (3 ml), was added dry silver oxide (30 g, in portions). The mixture was gently refluxed for 8 h with stirring. After filtration, the silver salts were extracted with boiling chloroform. Concentration of the combined filtrate and extracts yielded the methylated polysaccharide (3.35 g) (Found: OMe, 38.3. Calc.: OMe, 38.75%). The i.r. spectrum of the product (in dry $CHCl_3$) showed no hydroxyl peak. Further treatment with Purdie's reagents[5] failed to increase the methoxyl content.

Hydrolysis of the methylated polysaccharide. — The methylated polysaccharide {0.5 g; $[\alpha]_D^{20}$ −73° (*c* 0.82, chloroform)} was dissolved with cooling in 72% sulphuric acid[6] (5 ml), and the solution was kept at room temperature for 1 h. Water (40 ml) was then added, and the solution was kept at 100° for 4 h. The cooled hydrolysate was extracted with freshly distilled dichloromethane[7] (4 × 50 ml portions) to give fraction (*i*), and a further 4 × 50 ml portions to give fraction (*ii*). The aqueous phase was neutralised with barium carbonate. The solids were separated by centrifugation and carefully washed with water. The centrifugate and washings were concentrated to a syrup under reduced pressure at a bath temperature of 35° to give fraction (*iii*). Fractions (*i*) and (*ii*) were shaken with solid sodium hydrogen carbonate, dried (Na_2SO_4), and allowed to evaporate to dryness at room temperature in a dust-free atmosphere. Fractions (*i*) (58 mg), (*ii*) (25 mg), and (*iii*) (411 mg) were applied to Whatman No. 1 paper and separated (solvent *B*) into the following components.

2,3,4-Tri-*O*-methyl-D-xylose. The syrup (12 mg), R_{TMG} 1.11 (solvent *B*), had $[\alpha]_D^{20}$ +9.6° (*c* 1.04); lit.[13] $[\alpha]_D^{15}$ +20.3° (*c* 1.1). A portion (0.5 mg) was refluxed with 3% methanolic hydrogen chloride for 6 h, and the derived methyl glycosides were examined by g.l.c. Peaks corresponding to 2,3,4-tri-*O*-methylxylose (*T* 0.49, 0.58) were observed.

2,3-Di-*O*-methyl-D-xylose. The syrup (269 mg), R_{TMG} 0.93 (solvent *B*), $[\alpha]_D^{20}$ +22° (*c* 0.50), was not revealed on spraying with triphenyltetrazolium hydroxide. Chanda *et al.*[14] reported $[\alpha]_D^{15}$ +23° (*c* 1.0) for 2,3-di-*O*-methyl-D-xylose. The aniline derivative had m.p. and mixed m.p. 147–148° with authentic 2,3-di-*O*-methyl-*N*-phenyl-D-xylosylamine, $[\alpha]_D^{20}$ +180° (*c* 0.56, ethyl acetate); lit.[15] m.p. 146°, $[\alpha]_D^{19}$ +185° (*c* 0.76, ethyl acetate).

2,4-Di-*O*-methyl-D-xylose. The syrup (59 mg), R_{TMG} 0.86 (solvent *B*), had $[\alpha]_D^{20}$ +22° (*c* 0.60); lit.[16] $[\alpha]_D^{17}$ +21.5° (*c* 0.5). The sugar was not revealed on spraying with triphenyltetrazolium hydroxide and the aniline derivative, after several recrystal-

lisations from ethyl acetate–pentane, had m.p. 163–164°, $[\alpha]_D^{20}$ −85° (*c* 0.47, 1,4-dioxane). Barker *et al.*[17] reported m.p. 170°, $[\alpha]_D^{20}$ −82° (1,4-dioxane) for 2,4-di-*O*-methyl-*N*-phenyl-D-xylosylamine.

Mono-*O*-methylxyloses. The syrup (20 mg) contained (paper chromatography, solvent *B*) two components, R_{XYL} 2.32 and 2.57. The mono-*O*-methylxyloses were not further investigated.

The hydrolysate of the methylated polysaccharide was devoid of unmethylated xylose.

Quantitative determination of the relative molar percentages of O-*methylxyloses from the methylated polysaccharide.* — The methylated polysaccharide (10 mg) was hydrolysed in a sealed tube by the sulphuric acid method of Garegg and Lindberg[6]. The hydrolysate was diluted with water and shaken with Amberlite IRA-400 (acetate) resin. The more-volatile components were separated by extraction with dichloromethane. The combined extracts were shaken with solid sodium hydrogen carbonate, dried ($MgSO_4$), filtered, and allowed to evaporate to dryness at room temperature in a dust-free atmosphere to give fraction (*a*). The aqueous phase was concentrated to a syrup under reduced pressure at a bath temperature of 30° to give fraction (*b*). Fractions (*a*) and (*b*) were combined (7.5 mg), dissolved in pyridine (12 drops), and treated[8] with hydroxylamine hydrochloride (7.5 mg) at 90° for 1 h. Acetic anhydride (40 drops) was then added and heating continued for another hour. The cooled solution was subjected to g.l.c. analysis on column (*b*) at 180° and a nitrogen flow-rate of 50 ml/min. The molar percentages (estimated conventionally from peak areas for the acetylated nitriles) for 2,3,4-tri-, 2,4-di-, 2,3-di-, and mono-*O*-methylxyloses were 1.74:1.0:1.07:0.44.

Partial hydrolysis of the polysaccharide. — In order to determine the optimal conditions for the production of oligosaccharides of low molecular weight, the polysaccharide (0.5 g) was heated on a boiling-water bath with 50mM sulphuric acid (20 ml). Aliquots (1 ml) were withdrawn at regular intervals, neutralised with barium carbonate, and analysed by paper chromatography (solvents *A* and *B*; spray 1). Hydrolysis for 2.75 h gave the maximal concentration of oligosaccharides. Thus, the polysaccharide (10 g) in 50mM sulphuric acid (400 ml) was heated on a boiling-water bath for 2.75 h. The hydrolysate was neutralised (barium carbonate), centrifuged, and evaporated. The syrupy residue was dissolved in the minimal quantity of water and applied to a charcoal–Celite column (5.4 × 60 cm). The xylose was eluted with water, and the oligosaccharides with an aqueous ethanol gradient. Fractions (~30 ml) were analysed by paper chromatography and combined into nine major fractions.

Fraction 1. The syrup (1.84 g), eluted with water (2.7 l), crystallised from ethanol and, after recrystallisation first from methanol and then ethanol, had $[\alpha]_D^{19}$ +88 (3 min)→ +19° (*c* 0.51), m.p. and mixed m.p. 144–145° with authentic D-xylose. The derived di-*O*-benzylidene dimethyl acetal[18] had m.p. and mixed m.p. 210–211°, $[\alpha]_D^{15}$ −9° (*c* 1.04, chloroform).

Fraction 2. The syrup (523 mg), eluted with 0–5% aqueous ethanol (4.7 l), was shown (paper chromatography) to be a mixture of xylose (major) and two oligo-

saccharides, R_{XYL} 0.38 and 0.68 (trace) (solvent *A*). Separation of this mixture on Whatman No. 1 paper (solvent *A*; 19 h) yielded a chromatographically pure syrup (112 mg), R_{XYL} 0.38 (solvent *A*), 0.33 (solvent *B*), M_{XYL} 0.26. The syrup failed to crystallise until seeded with authentic xylobiose. Recrystallisation from aqueous ethanol–ethyl acetate gave xylobiose (**1**) as needles, m.p. and mixed m.p. 190–191°, $[\alpha]_D^{20}$ −27° (*c* 0.52); lit.[19] m.p. 190° $[\alpha]_D$ −25.8°. Complete, acid hydrolysis (0.5M sulphuric acid, 100°, 2 h) yielded only xylose, and chromatograms of partial, acid hydrolysates (50mM sulphuric acid, 100°, 15 min) showed no sugars other than the starting material and xylose. A solution of the saccharide (6 mg) in *N*,*N*-dimethylformamide (0.6 ml) was cooled to 0°, and methyl iodide (1.0 ml) and dry silver oxide (0.6 g) were added[20]. The mixture was stirred in the dark for 3 h at 0° and then for 21 h at room temperature to give a partially methylated product which, after one treatment with Purdie's reagents[5], was found to be completely methylated (t.l.c., spray *2*). A portion of the methylated product was refluxed with 3% methanolic hydrogen chloride for 6 h, and the derived methyl glycosides were examined by g.l.c. Peaks corresponding to 2,3,4-tri-*O*-methylxylose (*T* 0.49, 0.62) and 2,3-di-*O*-methylxylose (*T* 1.54, 1.78) were observed. The remainder of the methylated product was hydrolysed with 50mM sulphuric acid in a sealed tube for 5 h at 100°. The solution was diluted, shaken with Amberlite IRA-400 (acetate) resin, and extracted with dichloromethane (3 × 8 ml). The extract was neutralised with solid sodium hydrogen carbonate, dried ($MgSO_4$), filtered, and evaporated to dryness at room temperature in a dust-free atmosphere. The aqueous phase was concentrated to a syrup under reduced pressure at 30° (bath). The dichloromethane extract was redissolved in a small quantity of dichloromethane and added to the syrup from the aqueous phase, and the whole was allowed to evaporate to dryness at room temperature in a dust-free atmosphere. The acetylated nitrile derivatives were prepared[8], and examined by g.l.c. Peaks corresponding to 2,3,4-tri-*O*-methylxylose (T_X 0.63) and 2,3-di-*O*-methylxylose (T_X 1.43), in the molar ratio 1.0:0.9, were observed.

Fraction 3. The syrup (13.5 mg), eluted with 5–7.5% aqueous ethanol (2.2 l), was a mixture of xylose (trace) and two oligosaccharides, R_{XYL} 0.68 and 0.38 (trace) (solvent *A*). The main component (10 mg), after separation of the mixture on Whatman No. 1 paper (solvent *A*; 16 h), had R_{XYL} 0.68 (solvent *A*), 0.78 (solvent *B*), M_{XYL} 0.47, $[\alpha]_D^{20}$ −17° (*c* 0.75). Complete, acid hydrolysis yielded only xylose; partial hydrolysis gave only xylose and the starting material. The sugar (4 mg) was methylated and methanolysed as described above, and the derived methyl glycosides were examined by g.l.c. Peaks corresponding to 2,3,4-tri-*O*-methylxylose (*T* 0.48, 0.61) and 2,4-di-*O*-methylxylose (*T* 1.53, 1.97) were observed. G.l.c. examination of the acetylated nitrile derivatives, prepared[8] from a hydrolysed sample of the methylated oligosaccharide, yielded peaks corresponding to 2,3,4-tri-*O*-methylxylose (T_X 0.64) and 2,4-di-*O*-methylxylose (T_X 1.00), in the molar ratio 1.0:1.1. Fraction 3 is thus 3-*O*-β-D-xylopyranosyl-D-xylose (**2**, rhodymenabiose); lit.[19] $[\alpha]_D^{22}$ −18.4° ±0.6° (*c* 3.25).

Fraction 4. The syrup (450 mg), eluted with 7.5–10% aqueous ethanol (15 l),

was shown by paper chromatography (solvents *A* and *B*) to contain xylose (trace) and two oligosaccharides, R_{XYL} 0.13 and 0.38 (trace) (solvent *A*). The major component, R_{XYL} 0.13 (solvent *A*), 0.09 (solvent *B*), M_{XYL} 0.22, crystallised readily from aqueous ethanol–ethyl acetate and, after recrystallisation from 85% aqueous ethanol, had m.p. 216–217° alone and on admixture with authentic xylotriose, $[\alpha]_D^{20}$ −51° (*c* 0.45); lit.[21] $[\alpha]_D^{25}$ −47°, m.p. 205–206°. Paper chromatography of a partial, acid hydrolysate revealed the presence of xylose, xylobiose, and the original material, and complete hydrolysis gave xylose. G.l.c. examination of the methanolysed, methylated oligosaccharide showed peaks corresponding to 2,3,4-tri- (*T* 0.48, 0.60) and 2,3-di-*O*-methylxylose (*T* 1.50, 1.76). A portion of the methylated oligosaccharide was hydrolysed, and the derived monosaccharides were converted into their acetylated nitriles[8]. G.l.c. analysis of the acetylated nitriles showed peaks corresponding to 2,3,4-tri- (T_X 0.63) and 2,3-di-*O*-methylxylose (T_X 1.43) in the molar ratio 0.9:2.2.

Fraction 5. The syrup (37 mg), eluted with 10–15% aqueous ethanol (4.5 l), was shown by chromatography in solvent *A* to contain a sugar having R_{XYL} 0.23, and traces of three other sugars, R_{XYL} 0.38, 0.13, and 0.05. Separation on Whatman No. 1 paper (solvent *A*, 65 h) afforded a syrup (30 mg), which was shown to be a mixture by electrophoresis and by chromatography in solvent *B*: M_{XYL} 0.17 and 0.35, R_{XYL} 0.20 and 0.23. The syrup was thus further fractionated by paper chromatography (solvent *B*, 5 days) into fraction 5a, a syrup (8 mg), $[\alpha]_D^{20}$ −38° (*c* 0.42), R_{XYL} 0.20 (solvent *B*, pink, spray *1*), M_{XYL} 0.17; and 5b, a syrup (11 mg), $[\alpha]_D^{20}$ −37° (*c* 0.50), R_{XYL} 0.23 (solvent *B*; orange–pink, spray *1*), M_{XYL} 0.35. Paper chromatography of a partial, acid hydrolysate of fraction 5a revealed the presence of xylose, rhodymenabiose, xylobiose, and the original material. The sugar (1 mg) was dissolved in water (1 ml), sodium borohydride (2 mg) was added, and the mixture was allowed to stand for 16 h. The solution was then treated with Amberlite IR-120(H^+) resin and evaporated, and methanol was distilled from the residue to remove borate. Hydrolysis of the non-reducing syrup, followed by paper chromatography (solvent *A*), revealed the presence of xylose and rhodymenabiose. A portion, after methylation and methanolysis, gave g.l.c. peaks with the same retention times as those of methyl 2,3,4-tri-*O*-methylxylosides (*T* 0.48, 0.58), methyl 2,4-di-*O*-methylxylosides (*T* 1.55, 2.03), and methyl 2,3-di-*O*-methylxylosides (*T* 1.55, 1.74). A further portion, after methylation and hydrolysis, was converted into the acetylated nitriles[8] which showed g.l.c. peaks corresponding to 2,3,4-tri-*O*-methylxylose (T_X 0.64), 2,4-di-*O*-methylxylose (T_X 1.00), and 2,3-di-*O*-methylxylose (T_X 1.41), in the molar ratios 1.1:1.0:0.9. The above evidence suggests that fraction 5a is *O*-β-D-xylopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose (**4**).

Partial hydrolysis of a portion of fraction 5b gave xylose, rhodymenabiose, xylobiose, and the original material (paper chromatography). Reduction, followed by hydrolysis and paper chromatography, showed xylose and xylobiose as the only reducing sugars. Methylation, followed by methanolysis and g.l.c. of the methyl glycosides, showed the presence of components with the same retention times as methyl 2,3,4-tri-*O*-methyl- (*T* 0.48, 0.60), 2,4-di-*O*-methyl- (*T* 1.53, 1.99), and 2,3-di-

O-methylxylosides (*T* 1.53, 1.77). G.l.c. examination of the acetylated nitrile derivatives[8], prepared from a hydrolysed sample of the methylated oligosaccharide, yielded peaks corresponding to 2,3,4-tri-*O*-methylxylose (T_X 0.62), 2,4-di-*O*-methylxylose (T_X 1.00), and 2,3-di-*O*-methylxylose (T_X 1.41), in the molar ratios 0.9:1.1:1.0. These results indicate that this oligosaccharide is *O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→3)-D-xylose (**5**).

Fraction 6. The syrup (514 mg), eluted with 15% aqueous ethanol (12 l), consisted mainly of two sugars, R_{XYL} 0.05 and 0.23, together with traces of three other sugars, R_{XYL} 0.38, 0.13, and 0.08 (solvent *A*). Separation of a portion of this fraction (193 mg), on Whatman No. 1 paper in solvent *A* for 70 h, yielded a chromatographically homogeneous syrup (105 mg), R_{XYL} 0.05 (solvent *A*), 0.02 (solvent *B*), M_{XYL} 0.19. The syrup crystallised from aqueous methanol–ethyl acetate and, on recrystallisation from the same solvent, had m.p. 214–215°, $[\alpha]_D^{20}$ −60° (*c* 0.55); lit.[21] m.p. 219–220°, $[\alpha]_D^{25}$ −60°, for xylotetraose. Partial hydrolysis of a portion (50mM sulphuric acid, 100°, 20 min) gave xylose, xylobiose, xylotriose, and the original material (paper chromatography, solvent *A*). Methylation, followed by methanolysis and g.l.c. examination of the methyl glycosides, gave peaks with the same retention times as those of methyl 2,3,4-tri-*O*-methyl- (*T* 0.48, 0.61) and 2,3-di-*O*-methyl-xylosides (*T* 1.51, 1.76). Analysis of the acetylated nitriles[8], prepared after methylation and hydrolysis of the saccharide, showed peaks corresponding to 2,3,4-tri-*O*-methylxylose (T_X 0.63) and 2,3-di-*O*-methylxylose (T_X 1.43), in the molar ratio 0.9:3.15.

Fraction 7. The syrup (335 mg), eluted with 15–16% aqueous ethanol (25.1 l), contained four oligosaccharides, R_{XYL} 0.08 (major), 0.05, 0.13, and 0.23 (trace) (paper chromatography, solvent *A*). Fractionation on Whatman No. 1 paper (solvent *A*, 90 h) gave a syrup (64 mg), d.p. 3.7, R_{XYL} 0.08 (solvent *A*) and 0.06 (solvent *B*), which was a mixture by electrophoresis, M_{XYL} 0.33 and 0.18. Partial hydrolysis of the syrup, followed by paper chromatography (solvents *A* and *B*), revealed the presence of xylose, rhodymenabiose, xylobiose, *O*-β-D-xylopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose, *O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→3)-D-xylose, xylotriose, and the original material. Reduction, followed by hydrolysis and paper chromatography (spray *1*), revealed all the above sugars with the exception of the starting material. This suggests that the syrup is a mixture of *O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→3)-D-xylose, *O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose, *O*-β-D-xylopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose, and/or *O*-β-D-xylopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→3)-D-xylose (**7**).

Fraction 8. The syrup (323 mg), eluted with 16–20% aqueous ethanol (17.4 l), was shown (paper chromatography, solvent *A*) to contain four oligosaccharides, R_{XYL} 0.015 (major), 0.08, 0.05 (trace), and 0.13 (trace). The syrup, dissolved in 85% aqueous methanol, crystallised on the addition of a few drops of ethyl acetate. The crystals (64 mg), after recrystallisation from 85% aqueous methanol–ethyl acetate, had R_{XYL} 0.015 (solvent *A*), M_{XYL} 0.16, m.p. 221–222°, $[\alpha]_D^{20}$ −66° (*c* 0.48). Partial hydrolysis

of this sugar, followed by paper chromatography, revealed the presence of xylose, xylobiose, xylotriose, xylotetraose, and the original material. Methylation, followed by methanolysis and g.l.c. examination of the methyl glycosides, showed the presence of components with the same retention times as methyl 2,3,4-tri-*O*-methyl- (T 0.48, 0.58) and 2,3-di-*O*-methylxylosides (T 1.52, 1.76). G.l.c. investigation of the acetylated nitriles[8], prepared from a hydrolysed sample of the methylated oligosaccharide, showed peaks corresponding to 2,3,4-tri-*O*-methylxylose (T_X 0.63) and 2,3-di-*O*-methylxylose (T_X 1.43). These results indicate that this saccharide is xylopentaose (8). Whistler and Tu[21] reported m.p. 231–232°, $[\alpha]_D^{25}$ −66°, for xylopentaose hemihydrate.

Fraction 9. The syrup (190 mg), eluted with 20–30% aqueous ethanol (21.6 l), consisted mainly of a saccharide having R_{XYL} 0.03, together with traces of three other saccharides, R_{XYL} 0.13, 0.08, and 0.015 (solvent *A*). This fraction was not further investigated.

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