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SULPHATED POLYSACCHARIDES OF THE *Solieriaceae* FAMILY

PART II*. THE ACIDIC COMPONENTS OF THE POLYSACCHARIDE FROM THE RED ALGA *Anatheca dentata*

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ABSTRACT

Fractionation of a partial, acid hydrolysate of the polysaccharide from the red alga *Anatheca dentata* yielded 4-*O*-(α-D-glucopyranosyluronic acid)-L-galactose, 4,6-*O*-(1-carboxyethylidene)-D-galactose, and 4-*O*-[4,6-*O*-(1-carboxyethylidene)-β-D-galactopyranosyl]-L-galactose. A third disaccharide, probably an *O*-[4,6-*O*-(1-carboxyethylidene)galactopyranosyl]-galactose, was isolated in too small quantity for full identification. Free pyruvic acid was identified in a total, acid hydrolysate. Treatment with alkali revealed that only a small part of the sulphate is suitably placed for formation of 3,6-anhydro rings. Periodate oxidation showed the xylose residues to be either (1→4)-linked, (1→2)-linked, and/or present as non-reducing end-groups, and that the xylose residues are free of sulphate. *Anatheca dentata* polysaccharide is compared with the agars in the light of a recent analysis of the latter[2], and shown to be surprisingly similar in many respects.

INTRODUCTION

The water-soluble, highly sulphated polysaccharide from *Anatheca dentata*[1], shown to have an alternating sequence of α-L-(1→3)- and β-D-(1→4)-galactosidic links as its main structural feature, was also shown to have xylose and small proportions of 3-*O*-methylgalactose and uronic acid as integral parts of the macromolecule, thus belying the apparent simplicity of its structure. We now describe results of the analysis of the acidic fragments isolated from total and partial acid hydrolysates of the polysaccharide, as well as the results of periodate oxidation and alkali treatment of the polysaccharide.

RESULTS AND DISCUSSION

Ether extraction of an acid hydrolysate of the polysaccharide yielded a residue which on treatment with 2,4-dinitrophenylhydrazine gave the 2,4-dinitrophenylhydrazone of pyruvic acid. The pyruvic acid content of the polysaccharide has so far not

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been estimated. The presence of pyruvic acid in polysaccharides from red seaweeds was first demonstrated by Hirase[3,4] in 1957 for commercial agar. Subsequently, the isolation[5] and characterization[4,6] of 3,6-anhydro-4-0-[4,6-*O*-(1-carboxyethylidene)-β-D-galactopyranosyl]-L-galactose dimethyl acetal from a methanolysate of commercial agar demonstrated its mode of attachment in the macromolecule. More recently, Yaphe and co-workers[7] have demonstrated that pyruvate is a common component of agars from different agarophytes. Pyruvic acid has also been isolated from the sulphated polysaccharide of *Phyllymenia cornea* (*Grateloupiaceae*)[8]. There is also evidence that it is present in the sulphated polysaccharides of other members of the *Grateloupiaceae, viz. Aeodes ulvoidea* and *Aeodes orbitosa*[8]. The presence of pyruvate in these highly sulphated polymers suggests that it may well be of more widespread occurrence in red-algal polymers than was previously thought.

The acidic components of a partial, acid hydrolyate of the polysaccharide were fractionated on a charcoal–Celite column to remove inorganic salts and materials of high molecular weight. Successive separations on paper of the acidic fragments of low molecular weight yielded nine chromatographically homogeneous compounds, as their ammonium salts. The only sulphated sugars isolated were L-galactose 3-sulphate (**1**) and L-galactose 6-sulphate (**2**). Methylation and methanolysis of **1** and **2** gave (g.l.c.) products corresponding to methyl 2,4,6-tri-*O*-methyl- and 2,3,4-tri-*O*-methylgalactosides, respectively. The sulphated sugars both had a negative optical rotation, indicating that the galactose was present as the L-isomer. It is apparent from previous work[1] that the L-galactose residues present in the polymer are linked through position 4. Sulphate on positions 3 or 6 of such units would be alkali-labile. It follows that, since the sulphate units in the polymer are all alkali-stable, those L-galactose residues carrying sulphate must either be the site of branch points, or be present as trisulphated residues. The latter units would not be expected to be alkali-labile since base hydrolysis (so far unrecorded in carbohydrate sulphates) of one of the sulphate groups would be necessary to produce the oxide ion required for the formation of 3,6- or 2,3-anhydro derivatives. The molar ratio of L-galactose to sulphate in the polymer is 1:3.

In addition to the above compounds, an aldobiouronic acid, identified as 4-*O*-(α-D-glucopyranosyluronic acid)-L-galactose (**3**) was isolated from the partial, acid hydrolysate. Hydrolysis of **3** gave galactose and glucuronic acid. Reduction of the carboxyl group of the uronic acid moeity and subsequent hydrolysis yielded L-galactose and D-glucose in the ratio 1.0:1.08. Methylation and methanolysis of the reduced disaccharide gave (g.l.c.) products corresponding to methyl 2,3,4,6-tetra-*O*-methylglucosides and methyl 2,3,6-tri-*O*-methylgalactosides. When the aldobiouronic acid was methylated, followed by reduction of the carboxyl group and methanolysis, products corresponding (g.l.c.) to methyl 2,3,4-tri-*O*-methylglucosides and methyl 2,3,6-tri-*O*-methylgalactosides were obtained. The α-D-configuration of the glycosidic linkage of **3** was assumed from the specific rotation; the β-D-linked aldobiouronic acid would be expected to have a negative specific rotation. A small amount of free glucuronic acid was also obtained from the partial hydrolysate.

Three pyruvate-containing saccharides were also isolated, two of which were identified as 4,6-*O*-(1-carboxyethylidene)-D-galactose (**4**) and 4-*O*-[4,6-*O*-(1-carboxyethylidene)-β-D-galactopyranosyl]-L-galactose (**5**). The component sugars of **4** and **5** were determined by total and partial hydrolysis with acid, and the presence of pyruvic acid was confirmed by g.l.c. of methanolysates. The positions of the glycosidic linkage and of attachment of the pyruvic acid were determined by methylation of each sugar followed by g.l.c. examination of the derived methyl glycosides and/or glycitol acetates. The third pyruvate-containing sugar (**6**) is thought to be an *O*-[4,6-*O*-(1-carboxyethylidene)galactopyranosyl]-galactose, but insufficient material was available for complete structural elucidation. Two further acidic sugars (**8** and **9**) were isolated in very small amounts from among the fragments of partial, acid hydrolysis. Both sugars were devoid of sulphate and were unchanged by acid hydrolysis.

Treatment of the sulphated polysaccharides from *Anatheca dentata* with alkali, in the presence of sodium borohydride[9] to prevent end-group degradation, led to the elimination of only 0.85% of the sulphate, with negligible increase in the 3,6-anhydride content of the polysaccharide. When the polymer was re-treated with alkali, the sulphate content decreased by a further 1.1% and the 3,6-anhydride content increased from 0.70% to 1.3%. Thus, only a small amount of the sulphate in the polymer is suitably situated for 3,6-anhydride formation. In addition, no monomethyl-pentose or -hexose was formed when the polysaccharide was treated with hot sodium methoxide.

Oxidation of the polysaccharide with periodate was followed titrimetrically[10] and ceased after 96 h at room temperature when 0.277 mole of periodate had been consumed per C-6 "anhydro unit". The resulting oxopolysaccharide was reduced with borohydride. Complete, acid hydrolysis of the polyalcohol gave (paper chromatography) galactose only. G.l.c. of the acetylated glycitols prepared from the hydrolysate confirmed the absence of xylose. The xylose residues in the polysaccharide must, therefore, be either (1→4)-linked, (1→2)-linked, and/or present as a non-reducing end-group. Furthermore, these results indicate that the xylose residues in the polymer are not sulphated since they are cleaved by periodate, and no monomethylpentoses are formed when the polymer is treated with sodium methoxide.

When the known, structural features of *Anatheca dentata* polysaccharides are considered in the light of recent results and conclusions[2] on the agars, some interesting comparisons are evident. Duckworth and Yaphe[2] questioned the concept of agar's being composed of a mixture of two distinct polysaccharides, *viz.*, agarose and agaropectin. They believed this to be an over-simplification of the situation and, on the basis of careful fractionation, postulated three extreme agar structures; the ratio of each extreme structure is different for agars from different species. One extreme type is a non-gelling, sulphated galactose in which the 3,6-anhydro-L-galactose residues have largely been replaced by L-galactose sulphate residues and which contains little or no 4,6-*O*-(1-carboxyethylidene)-D-galactose residues.

Anatheca dentata polysaccharide has the "back-bone" structure of a "Yaphe type-three"agar. However, the high sulphate content (~30%) and the presence of xylose appear to place it beyond the region of the true agars. The content of 3-*O*-methylgalactose and D-glucuronic acid might still be reconciled with an agar-type structure, because mono-*O*-methylgalactoses[11] and D-glucuronic acid[12] have been reported to be present in some agars.

EXPERIMENTAL

The analytical methods have been previously described[1]. In addition, the chromatographic solvent *D*, ethyl acetate–pyridine–water (10:4:3), was used. The degree of polymerisation (d.p.) was determined by the phenol–sulphuric acid method[13]. G.l.c. packings described previously for methyl glycosides and alditol acetates[1] are columns (*a*) and (*b*), respectively; in addition, column (*c*) 3% w/w ECNSS-M on Chromosorb W (100–120 mesh) operated at 180°, was used. Retention times T_E are relative to that of erythritol tetra-acetate.

Isolation of pyruvic acid from the polysaccharide. — Polysaccharide (5.0 g) was heated on a boiling-water bath with M sulphuric acid (20 ml) for 9 h. After neutralisation (barium carbonate) and centrifugation, the solution was shaken with Amberlite IR-120(H$^+$) resin and filtered, and the acidic solution was extracted with ether (400 ml; in portions). The ether was removed by distillation and a portion of the residue was treated with boiling 3% methanolic hydrogen chloride for 3 h. The solution was neutralised (lead carbonate) and filtered. G.l.c. showed a peak having a retention time identical with that of methanolysed pyruvic acid [column (*c*) at 100°, with a nitrogen flow-rate of 25 ml/min]. This product had the same retention time as that of methanolysed 2-furaldehyde on columns (*a*) and (*b*) but was separated from it on column (*c*) under the above conditions [retention times on column (*c*): methanolysed pyruvic acid, 2.1 min; methanolysed 2-furaldehyde, 1.9 min].

The remainder of the ether extract was treated with a methanolic solution of 2,4-dinitrophenylhydrazine and the product was fractionated by preparative t.l.c. (ethyl acetate). The appropriate zones were scraped off and extracted with methanol, and the extract was treated with Amberlite IR-120(H$^+$) resin and evaporated to dryness. The residue (12 mg) crystallised from ether and had m.p. 218–219° alone and in admixture with pyruvic acid 2,4-dinitrophenylhydrazone. The infrared spectrum (KBr disc) was identical with that of the authentic derivative.

Action of alkali on the polysaccharide. — A solution of polysaccharide (1 g) in water (150 ml) containing sodium borohydride[9] (0.2 g) was set aside for 48 h at room temperature. Sodium hydroxide (7 g) and sodium borohydride (1 g) were then added, and the mixture was maintained at $82° \pm 2°$. After 4.5 h, a further amount of sodium borohydride (1 g) was added, and, after 10.5 h, the solution was cooled and made slightly acid with hydrochloric acid. The mixture was dialysed against frequently changed distilled water, concentrated, and freeze-dried, yielding a white foam (0.76 g), $[\alpha]_D^{20}$ $-36.4°$ (*c* 0.66) (Found: SO_4^{2-}, 35.3; 3,6-anhydrogalactose[14], 0.70%). A portion of the product (0.40 g) was subjected to a second treatment with alkali as above, giving a white solid (0.38 g), $[\alpha]_D^{20}$ $-33°$ (*c* 0.61) (Found: SO_4^{2-}, 34.8; 3,6-anhydrogalactose, 1.31%). Chromatography of a neutralised, acid hydrolysate (solvents *A* and *B*) revealed the presence of galactose and xylose.

Treatment of the polysaccharide with sodium methoxide. — To a solution of polysaccharide (0.5 g) in water (25 ml) was added sodium borohydride (0.1 g). The mixture was allowed to stand for 24 h with occasional shaking, after which time further sodium borohydride (0.1 g) was added and the solution was left for another 24 h. The solution of polyalcohol was then dialysed against frequently changed distilled water, concentrated, and freeze-dried. The polyalcohol (dried at 60° over P_2O_5 *in vacuo* for 24 h) was added to a solution of sodium methoxide prepared from sodium (1.5 g) and absolute methanol (70 ml), and the mixture was boiled for 24 h in an atmosphere of nitrogen. The insoluble material was collected by centrifugation, washed with methanol, and dissolved in water (50 ml), and the solution was dialysed against frequently changed distilled water for 3 days. Concentration of the solution, followed by freeze-drying, yielded a white foam (0.43 g). Chromatography of a neutralised, acid hydrolysate (solvents *A* and *B*) showed the presence of galactose and xylose. No artefacts (*e.g.* methyl ethers) could be detected.

Separation and characterization of the acidic components of the partial hydrolysate. — An aqueous solution of the acidic sugars[1] (10 g) as the ammonium salts was applied to a charcoal–Celite column (1:1; 27×4 cm) and eluted with water and then aqueous ethanol to give three main fractions. The first fraction (6.82 g), eluted with water (600 ml), consisted of inorganic material. The third fraction (1.65 g), eluted with aqueous ethanol (0–20%; 9 l), was shown by paper chromatography (solvents *A* and *D*) to contain acidic fragments of high molecular weight. These fractions were not further investigated. The second fraction (0.58 g), eluted with water (2.1 l), was separated on Whatman No. 1 paper for 16 h, using solvent *A*. The appropriate portions of the papers were extracted with 50% aqueous methanol. The fractions, after the addition of ammonia to pH 8, were concentrated to dryness and re-chromatographed on Whatman No. 1 paper (solvent *D*; 16 h). The following chromatographically homogeneous compounds (solvents *A* and *D*) were obtained as their ammonium salts.

Acidic sugar **1**. The syrup (50 mg) had R_{GAL} 0.75 (solvent *D*; yellow, spray 1), $[\alpha]_D^{21}$ $-32°$ (*c* 0.56) calculated as the ammonium salt of a hexose monosulphate from the sugar concentration found by the method of Dubois *et al.*[15]. A neutralised, acid hydrolysate (0.5M sulphuric acid; 100°; 12 h) gave a single spot with the mobility of galactose (paper chromatography; solvents *A* and *B*). The sugar had a d.p. of 1.0 and a molar ratio of galactose[15] to sulphate of 1.0:1.13. A portion of the sugar (8.5 mg) in redistilled *N*,*N*-dimethylformamide (1.0 ml) was cooled to 0°, and redistilled methyl iodide (1.0 ml) and dry silver oxide (1.0 g) were added[16]. The mixture was stirred in the dark for 3 h at 0° and then for 21 h at room temperature. The mixture was filtered, and the silver salts were thoroughly washed with chloroform. The filtrate and washings were concentrated to dryness, and traces of *N*,*N*-dimethylformamide were removed under vacuum (0.1 torr; 40°; 5 min). The dry residue was then given one treatment with Purdie's reagents[17]. T.l.c. (spray 4) of the product indicated that methylation was complete. The methylated product was refluxed with 3% methanolic hydrogen chloride for 6 h and g.l.c. examination of the derived methyl glycosides revealed peaks characteristic of methyl 2,4,6-tri-*O*-methylgalactosides (*T* 4.05, 4.50). Hydrolysis (0.5M sulphuric acid) of a portion of the methylated sugar and examination of the product by paper chromatography (solvent *A* and *B*) revealed a single spot having the mobility of 2,4,6-tri-*O*-methylgalactose. The above evidence indicates that this sulphated monosaccharide is L-galactose 3-sulphate. Peat *et al.*[18] reported $[\alpha]_D^{18}$ +45° for authentic D-galactose 3-sulphate (barium salt).

Acidic sugar 2. The syrup (25 mg) had R_{GAL} 0.56 (solvent *D*; orange-brown, spray 1), $[\alpha]_D^{20}$ −43° (*c* 0.56) calculated as the ammonium salt of a hexose monosulphate. Hydrolysis (0.5M sulphuric acid; 100°; 12 h), followed by neutralisation (barium carbonate) and paper chromatography (solvent *A* and *B*), revealed a single spot with the mobility of galactose. The sugar had a d.p. of 1.0 and a molar ratio of galactose[15] to sulphate of 1.0:1.18. A sample (6 mg) was methylated as above and the product methanolysed for 6 h. T.l.c. of the methyl glycosides (spray 4) revealed two spots with mobilities identical with those of authentic methyl 2,3,4-tri-*O*-methylgalactosides, and g.l.c. revealed a single peak (*T* 7.38) corresponding to methyl 2,3,4-tri-*O*-methylgalactosides. These results indicate that this sulphated monosaccharide is L-galactose 6-sulphate. Turvey and Williams[19] reported $[\alpha]_D$+47° for authentic D-galactose 6-sulphate (sodium salt).

Acidic sugar 3. The syrup (69 mg) had R_{GAL} 0.31 (solvent *A*; orange-brown, spray 1), $[\alpha]_D^{20}$ +24° (*c* 0.50; free-acid form). The sugar gave a positive reaction with triphenyltetrazolium hydroxide, and on complete hydrolysis with acid yielded galactose and glucuronic acid (paper chromatography; solvents *A* and *B*). The saccharide (36 mg) was shaken with Amberlite IR-120(H^+) resin and then boiled with 2% methanolic hydrogen chloride (10 ml) for 6 h. The solution was neutralised with silver carbonate, filtered, and concentrated to dryness, yielding the methyl ester methyl glycoside (*i*) (30 mg). Part of (*i*) (27 mg) was treated with sodium borohydride (60 mg) in water (5 ml) for 16 h at room temperature. The solution was shaken with Amberlite IR-120(H^+) resin, and boric acid was removed by repeated co-distillation with methanol. A portion (19 mg) of the product (*ii*) was hydrolysed (0.5M sulphuric acid; 100°; 4 h) to yield galactose and glucose in the ratio 1.0:1.08 (estimated by g.l.c. of the derived glycitol acetates[20]). The galactose/glucose mixture was separated on Whatman No. 1 paper (solvent *B*; 40 h), giving D-glucose (4 mg), $[\alpha]_D^{21}$ +40° (*c* 0.40), and L-galactose (4 mg), $[\alpha]_D^{21}$ −65° (*c* 0.40). The remainder of (*ii*) (2.5 mg) was methylated, and the product was methanolysed for 6 h. G.l.c. of the derived methyl glycosides showed peaks corresponding to methyl 2,3,4,6-tetra-*O*-methylglucosides (*T* 0.98, 1.45) and methyl 2,3,6-tri-*O*-methylgalactosides (*T* 3.30, 4.29, 4.68).

The remaining methyl ester methyl glycoside (*i*) (2 mg) was methylated and subjected to borohydride reduction. The product was methanolysed and on g.l.c. gave peaks corresponding to methyl 2,3,4-tri-*O*-methylglucosides (*T* 2.58, 3.69) and methyl 2,3,6-tri-*O*-methylgalactosides (*T* 3.17, 4.29, 4.60). The above evidence suggests that this aldobiouronic acid is 4-*O*-(α-D-glucopyranosyluronic acid)-L-galactose. The anomeric configuration follows from the specific rotation.

Acidic sugar 4. The syrup (30 mg), R_{GAL} 2.18 (solvent *A*), 0.20 (solvent *D*:

orange, spray 1), was devoid of sulphate, had $[\alpha]_D^{20}+51°$ (*c* 0.43) (ammonium salt), and on total hydrolysis with acid yielded galactose as the only reducing sugar. The sugar (0.6 mg) was refluxed with 3% methanolic hydrogen chloride for 3 h. After neutralisation with lead carbonate, the product was examined by g.l.c. on column (*c*) and a peak corresponding to that of methyl pyruvate dimethyl acetal was observed. A portion (5 mg) of the sugar was methylated and hydrolysed, and paper-chromatographic examination of the product (solvents *A* and *B*) revealed the presence of 2,3-di-*O*-methylgalactose. The hydrolysate of the methylated sugar was reduced (sodium borohydride) and acetylated[20], and g.l.c. then showed a peak with retention time identical with that of the glycitol acetate prepared from authentic 2,3-di-*O*-methylgalactose (T_E 4.42, column (*c*)]. These results indicate that this sugar is 4,6-*O*-(1-carboxyethylidene)-D-galactose.

Acidic sugar **5**. The syrup (43 mg) was devoid of sulphate and had R_{GAL} 0.67 (solvent *A*; yellow, spray 1), $[\alpha]_D^{20}-37.4°$ (*c* 0.60) (ammonium salt). Total hydrolysis (0.5M sulphuric acid; 100°; 16 h), followed by paper chromatography (solvents *A* and *B*), revealed galactose as the only reducing sugar. Partial hydrolysis (0.5M sulphuric acid; 100°; 10 min), followed by paper chromatography, revealed the presence of galactose, acidic sugar **4**, 4-*O*-β-D-galactopyranosyl-L-galactose, and the original sugar. A portion (0.9 mg) of the sugar was methanolysed for 3 h, and g.l.c. [column (*c*)] of the neutralised product showed a peak corresponding to that of methyl pyruvate dimethyl acetal. The sugar was methylated; a portion of the product was methanolysed, and the remainder was hydrolysed and converted into the alditol acetates[20]. G.l.c. showed peaks corresponding to methyl 2,3,6-tri-*O*-methylgalactosides [*T* 3.23, 4.02, 4.28, 4.70; column (*a*)] and 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methylgalactitol [T_E 4.42; column (*c*)]. The presence of these sugars was confirmed by paper chromatography (solvents *A* and *B*) of an acid hydrolysate of a sample of the fully methylated oligosaccharide. These results indicate that this sugar is 4-*O*-[4,6-*O*-(1-carboxyethylidene)-β-D-galactopyranosyl]-L-galactose.

Acidic sugar **6**. The syrup (15 mg), $[\alpha]_D^{20}+4°$ (*c* 0.50) (ammonium salt), was chromatographically different from **5** having R_{GAL} 1.15 (solvent *A*), 0.12 (solvent *D*; yellow, spray 1). The sugar was devoid of sulphate, and total hydrolysis gave galactose as the only reducing sugar. Partial, acid hydrolysis, followed by paper chromatography (solvents *A* and *B*), revealed the presence of galactose, acidic sugar **4**, an unidentified sugar having R_{GAL} 0.33 (solvent *A*), and the original material. Methanolysis of a portion of the saccharide, followed by g.l.c. examination [column (*c*)] of the products showed a peak corresponding to that of methyl pyruvate dimethyl acetal. The sugar was methylated and hydrolysed, and paper-chromatographic examination (solvents *A* and *B*) of the hydrolysate revealed spots with the mobilities of 2,3-di-*O*-methyl- and 2,3,6-tri-*O*-methylgalactose. Insufficient material remained for further investigations.

Acidic sugar **7**. The syrup (3.3 mg) was chromatographically identical with glucuronic acid, R_{GAL} 0.92 (solvent *A*), 0.09 (solvent *D*; yellow, then red, spray 1). An optically clear solution of the sugar could not be obtained. The sugar was shaken with

Amberlite IR-120(H^+) resin and methanolysed and the derived methyl ester methyl glycoside was treated with sodium borohydride. A portion of the product was hydrolysed (0.5M sulphuric acid; 100°; 4 h) and paper chromatography revealed glucose (solvents *A* and *B*). The remaining product was methylated, and peaks corresponding to 2,3,4,6-tetra-*O*-methylglucose (*T* 1.02, 1.46) were observed on g.l.c.

Two further acidic sugars were obtained: **8** (2 mg), R_{GAL} 1.80 (solvent *A*), 0.30 (solvent *D*; yellow, spray 1); and **9** (3 mg), R_{GAL} 2.20 (solvent *A*), 0.32 (solvent *D*; yellow, spray 1). These sugars were devoid of sulphate and were unchanged by total, acid hydrolysis (0.5M sulphuric acid; 100°; 16 h).

Periodate oxidation of polysaccharides. — To a solution of polysaccharide (368.9 mg; dried at 60° over P_2O_5 *in vacuo* for 48 h) in water (50 ml) was added 60mM sodium metaperiodate (50 ml), and the mixture was allowed to stand in the dark at room temperature. Aliquots (5 ml) were withdrawn at regular intervals, and the reduction of periodate, measured by the arsenite method[10], was as follows:

Time (h)	4	12	24	48	72	96	120	144
Periodate reduced (mmoles/C-6 "anhydro unit")	144	212	223	248	263	276	277	277

After 144 h, the remaining solution was treated with an excess of ethylene glycol. Sodium borohydride (0.4 g) was then added and the mixture was allowed to stand overnight. The polysaccharide alcohol (194 mg) was isolated by freeze-drying after dialysis. Paper chromatography of a neutralised, acid hydrolysate of the polysaccharide alcohol revealed galactose only (solvents *A* and *B*; spray 1). A portion of the polysaccharide alcohol (20 mg) was hydrolysed, reduced (sodium borohydride), acetylated[20], and examined by g.l.c. No peak corresponding to xylitol acetate was detected.

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REFERENCES

1 J. R. Nunn, H. Parolis, and I. Russell, *Carbohyd. Res.*, 20 (1971) 205.
2 M. Duckworth and W. Yaphe, *Carbohyd. Res.*, 16 (1971) 189.
3 S. Hirase, *Bull. Chem. Soc. Japan*, 30 (1957) 68.
4 S. Hirase, *Mem. Fac. Ind. Arts Kyoto Tech. Univ. Sci. Technol.*, (1957) 17.
5 S. Hirase, *Bull. Chem. Soc. Japan*, 30 (1957) 70.
6 S. Hirase, *Bull. Chem. Soc. Japan*, 30 (1957) 75.
7 K. Young, M. Duckworth, and W. Yaphe, *Carbohyd. Res.*, 16 (1971) 446.
8 I. Russell, Ph. D. thesis (Rhodes University) 1971.
9 D. A. Rees, *J. Chem. Soc.*, (1961) 5168.
10 P. Fleury and J. Lange, *J. Pharm. Chim.*, 17 (1933) 107.

11 C. Araki, *J. Chem. Soc. Japan*, 59 (1937) 1214.

12 C. Araki, K. Arai, and S. Hirase, *Bull. Chem. Soc. Japan*, 40 (1967) 959; S. Hirase and C. Araki, *ibid.*, 34 (1961) 1048.

13 S. Peat, W. J. Whelan, and J. G. Roberts, *J. Chem. Soc.*, (1956) 2258; T. E. Timell, *Sv. Papperstidn.*, 63 (1960) 668.

14 W. Yaphe, *Anal. Chem.*, 32 (1960) 1327.

15 M. Dubois, K. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350.

16 Q. N. Haq and E. Percival, in H. Barnes (Ed.), *Some Contemporary Studies in Marine Science*, Allen and Unwin, London, 1966, p. 355.

17 T. Purdie and J. C. Irvine, *J. Chem. Soc.*, (1903) 1021.

18 S. Peat, D. M. Bowker, and J. R. Turvey, *Carbohyd. Res.*, 7 (1968) 225.

19 J. R. Turvey and T. P. Williams, *J. Chem. Soc.*, 83 (1962) 2119.

20 D. M. Bowker and J. R. Turvey, *J. Chem. Soc., C*, (1968) 983.