ISOLATION OF THREE OLIGOSACCHARIDES FROM THE MUCILAGE FROM THE BARK OF *Ulmus fulva* (SLIPPERY-ELM MUCILAGE). SYNTHESIS OF *O*-(3-*O*-METHYL-β-D-GALACTOPYRANOSYL)-(1→4)-L-RHAMNOSE

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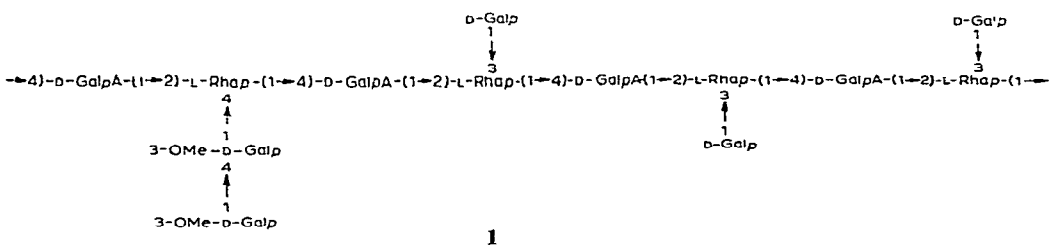
(Received March 15th, 1971)

ABSTRACT

Borohydride reduction of the periodate-oxidized polysaccharide obtained from slippery-elm mucilage, affords, on partial hydrolysis with hot acid, three oligosaccharides: *O*-(3-*O*-methyl-β-D-galactopyranosyl)-(1→4)-*O*-(3-*O*-methyl-β-D-galactopyranosyl)-(1→4)-L-rhamnose (**2**), *O*-(3-*O*-methyl-β-D-galactopyranosyl)-(1→4)-L-rhamnose (**3**), and *O*-(3-*O*-methyl-β-D-galactopyranosyl)-(1→4)-3-*O*-methyl-D-galactose (**4**). The structure assigned to **3** was corroborated by a synthesis of the disaccharide.

INTRODUCTION

On the basis of results described in a previous publication[1], a possible structural fragment (**1**) has been proposed for a water-soluble polysaccharide obtained from slippery-elm mucilage. The results of methylation analysis on the methylated poly-



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saccharide, in conjunction with the isolation of an oligosaccharide containing 3-*O*-methyl-D-galactose and L-rhamnose, from the partial, acid hydrolyzate of the polyalcohol afforded on borohydride reduction of the periodate-oxidized polysaccharide, indicated that chains of 3-*O*-methyl-D-galactosyl residues are attached to O-4 of some of the L-rhamnose residues. The oligosaccharide was originally formulated as the tetrasaccharide *O*-(3-*O*-methyl-D-galactopyranosyl)-(1→4)-*O*-(3-*O*-

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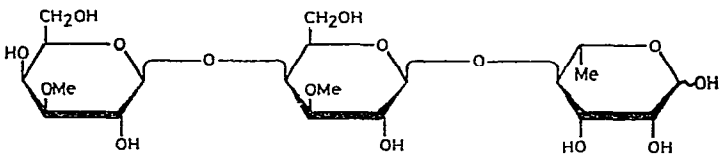
methyl-D-galactopyranosyl)-(1→4)-*O*-(3-*O*-methyl-D-galactopyranosyl)-(1→4)-L-rhamnose. The results of subsequent work have, however, suggested that the oligosaccharide is in fact the trisaccharide *O*-(3-*O*-methyl-β-D-galactopyranosyl)-(1→4)-*O*-(3-*O*-methyl-β-D-galactopyranosyl)-(1→4)-L-rhamnose (**2**). The evidence for this structural assignment is now presented. In addition, the isolation of the two corresponding disaccharides, namely, *O*-(3-*O*-methyl-β-D-galactopyranosyl)-(1→4)-L-rhamnose (**3**) and *O*-(3-*O*-methyl-β-D-galactopyranosyl)-(1→4)-3-*O*-methyl-D-galactose (**4**), is reported; the former assignment has been corroborated by a synthesis of disaccharide **3**.



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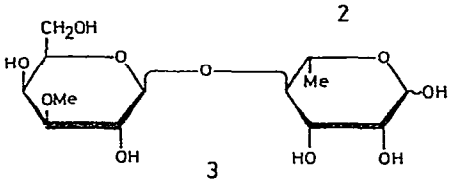

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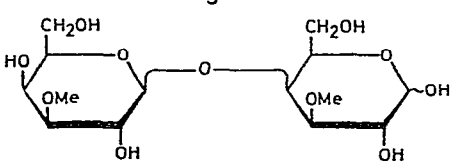

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RESULTS AND DISCUSSION

A polysaccharide product was extracted from elm bark as described previously[1], and separated into three fractions. The major fraction (see Experimental section), obtained by precipitation with hexadecyltrimethylammonium hydroxide, migrated as a single peak on free-boundary electrophoresis, and was used for the structural investigations. From the partial, acid hydrolyzate of the polyalcohol afforded on borohydride reduction of the periodate-oxidized polysaccharide[1], three oligosaccharides have now been isolated, to which structures **2**, **3**, and **4** have been assigned by n.m.r. spectroscopic and chemical methods.

The n.m.r. spectra of **2**, **3**, and **4** in deuterium oxide are shown in Figs. 1, 2, and 3, respectively. The doublet at τ 8.58 in the spectra of **2** and **3** is assigned to the methyl group of the L-rhamnose residue in each of these oligosaccharides. In each of the spectra of **2** and **4**, there are two methoxyl signals, at τ 6.38 and 6.30, from the 3-*O*-methylgalactose residues; only one methoxyl signal is observed at τ 6.39 in the spectrum of **3**. The spectra of the three oligosaccharides were compared with those of L-rhamnose and 3-*O*-methyl-D-galactose. The signals at τ 6.05 and 5.56 in the

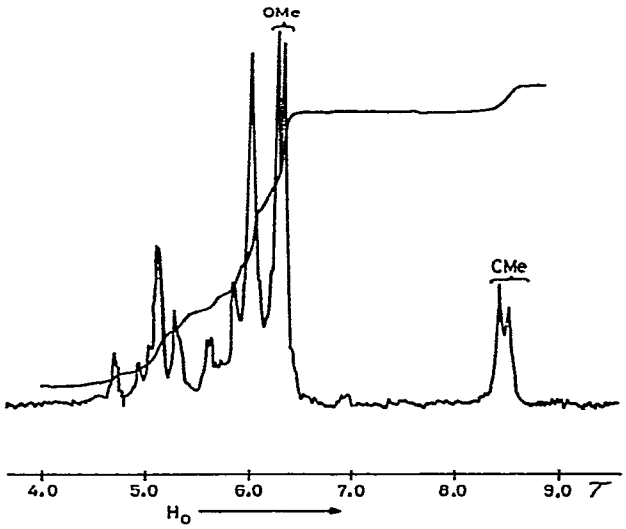

Fig. 1. The 60-MHz n.m.r. spectrum of *O*-(3-*O*-methyl-β-D-galactopyranosyl)-(1→4)-*O*-(3-*O*-methyl-β-D-galactopyranosyl)-(1→4)-L-rhamnose (**2**) in deuterium oxide.

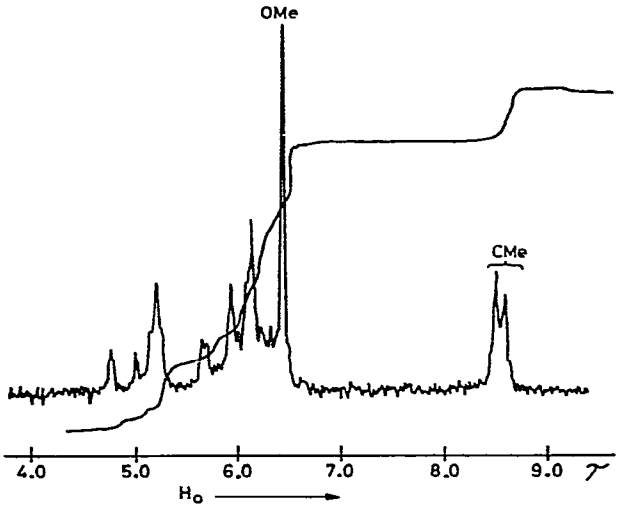

Fig. 2. The 60-MHz n.m.r. spectrum of *O*-(3-*O*-methyl-β-D-galactopyranosyl)-(1→4)-L-rhamnose (**3**) in deuterium oxide.

spectra of the three oligosaccharides were characteristic of 3-*O*-methyl-D-galactose, whereas that at τ 5.93 in the spectra of **2** and **3** was characteristic of L-rhamnose. Although the preceding, limited spectral interpretations were consistent with the structural formulation assigned the three oligosaccharides, an unequivocal assignment of the number of monosaccharide residues in each of them could not be made by integration of the spectra, partly because of the appearance of an HOD signal at τ 5.2.

Evidence for the formulation of **2** and **3** as a trisaccharide and a disaccharide, respectively, came from the experimentally determined molecular proportions of

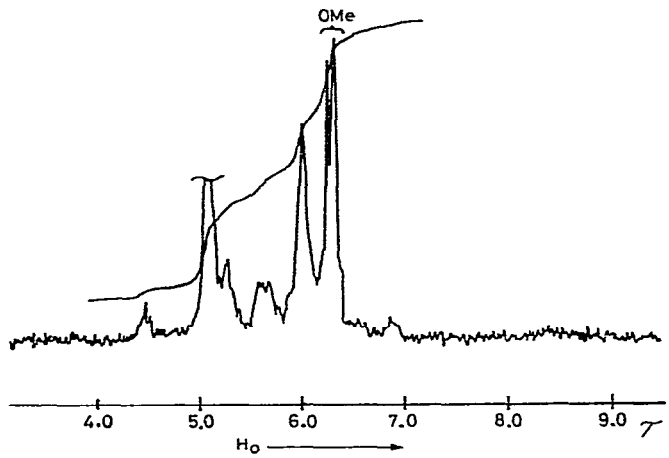

Fig. 3. The 60-MHz n.m.r. spectrum of *O*-(3-*O*-methyl-β-D-galactopyranosyl)-(1→4)-3-*O*-methyl-D-galactose (**4**) in deuterium oxide.

3-*O*-methyl-D-galactose to L-rhamnose; the ratios were 2.34:1 for **2**, and 1.32:1 for **3**. Reduction of **2** and **3** with sodium borohydride, followed by acid-catalyzed hydrolysis of the resultant products, gave rhamnitol and 3-*O*-methyl-D-galactose from each; the reducing end-groups in **2** and **3** are, therefore, rhamnose residues. Methylation analysis showed that the *O*-methyl derivative of **2** contained approximately equimolar proportions of 2,3,4,6-tetra- and 2,3,6-tri-*O*-methyl-D-galactose and 2,3-di-*O*-methyl-L-rhamnose, whereas the *O*-methyl derivative of **3** contained approximately equimolar proportions of 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3-di-*O*-methyl-L-rhamnose. Both **2** and **3** were found to be homogeneous on paper chromatograms in a number of solvent systems. The low, positive values for the specific rotations (+12° for **2**, and +7° for **3**) suggested that the configuration of the glycosidic linkages is β-D. Assuming that all of the residues are in the pyranoid form, the experimental evidence is consistent with the formulation of **2** as *O*-(3-*O*-methyl-β-D-galactopyranosyl)-(1→4)-*O*-(3-*O*-methyl-β-D-galactopyranosyl)-(1→4)-L-rhamnose and of **3** as *O*-(3-*O*-methyl-β-D-galactopyranosyl)-(1→4)-L-rhamnose.

On borohydride reduction of oligosaccharide **4**, followed by acid-catalyzed hydrolysis of the resultant product, approximately equimolar amounts of 3-*O*-methyl-D-galactitol and 3-*O*-methyl-D-galactose were formed; methylation analysis showed that the *O*-methyl derivative of **4** contained approximately equimolar proportions of 2,3,4,6-tetra- and 2,3,6-tri-*O*-methyl-D-galactose. The value of the specific rotation (+60°) suggested that the configuration of the glycosidic linkage in **4** is also β-D. Again, assuming that the residues are in the pyranoid form, the experimental evidence is consistent with the formulation of **4** as *O*-(3-*O*-methyl-β-D-galactopyranosyl)-(1→4)-3-*O*-methyl-D-galactose.

In the present work, the formulation of **3** as *O*-(3-*O*-methyl-β-D-galactopyranosyl)-(1→4)-L-rhamnose was corroborated by comparison of the naturally derived product with a sample obtained by a structurally definitive synthesis. 3-*O*-

Methyl-D-galactose was prepared by the method of Ball and Jones[2], namely, by methylation of 4,6-*O*-ethylidene-1,2-*O*-isopropylidene-D-galactose[2,3], followed by removal of the acetal groups by acid-catalyzed hydrolysis*. The 3-*O*-methyl-D-galactose was treated with acetic anhydride–pyridine, and the syrupy peracetylated product** was converted into 2,4,6-tri-*O*-acetyl-3-*O*-methyl-α-D-galactopyranosyl chloride by treatment with zinc chloride–thionyl chloride[8] in benzene. The high, positive value (+154°) for the specific rotation, and the magnitude of the splitting (2.0 Hz) of the H-1 signal in the n.m.r. spectrum, suggested that the anomeric configuration of the glycosyl chloride was α-D. Treatment of 2,4,6-tri-*O*-acetyl-3-*O*-methyl-α-D-galactopyranosyl chloride with benzyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside[9] in the presence of mercuric cyanide in nitromethane afforded, after chromatographic fractionation of the product and appropriate removal of the protecting groups (see Experimental section), *O*-(3-*O*-methyl β-D-galactopyranosyl)-(1→4)-L-rhamnose. The n.m.r. spectrum of **3** (see Fig. 2) was indistinguishable from that of the synthetic disaccharide, and their optical rotations were in agreement.

The work described in the present paper supports our earlier[1] conclusion that the polysaccharide from slippery-elm mucilage contains chains of 3-*O*-methyl-D-galactosyl residues attached to O-4 of certain L-rhamnose residues, as shown in structural fragment **1**.

EXPERIMENTAL

General methods. — Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer Model 141 polarimeter at 23 ±1°. N.m.r. spectra were recorded at 60 MHz in deuterium oxide with sodium 4,4-dimethyl-4-silapentane-1-sulfonate (τ 10.00) as the external standard, or in chloroform-*d* with tetramethylsilane (τ 10.00) as the internal standard. Paper chromatography was conducted on Whatman Nos. 1 and 3MM papers, with the following solvent systems (v/v): (*a*) 18:3:1:4 ethyl acetate–acetic acid–formic acid–water; (*b*) 3:1:1 butyl alcohol–ethanol–water; (*c*) butyl alcohol–pyridine–water–benzene (5:3:3:1, upper layer); (*d*) 200:17:1 butanone–water–ammonia (*d* 0.88); (*e*) butyl alcohol–ethanol–water (4:1:5, upper layer); (*f*) butyl alcohol–acetic acid–water (4:1:5, upper layer); and (*g*) 10:4:3 ethyl acetate–pyridine–water. Values of R_{Gal} and R_{Rha} refer to distances moved, relative to those of galactose and rhamnose, respectively; R_G values of *O*-methyl sugars refer to distances moved relative to 2,3,4,6-tetra-*O*-methyl-D-glucose. Thin-layer chromatography (t.l.c.) was

*Several syntheses of 3-*O*-methyl-D-galactose have been reported[4]. In addition to its occurrence in slippery-elm mucilage and sassafras polysaccharide[5], the sugar has recently been identified[6] in the whole-cell hydrolyzates of certain filamentous bacteria known as actinomycetes; obtained from the last source, it has been called madurose[7].

**A low yield of a crystalline compound could be isolated from the syrup; it was assigned the structure of 1,2,4,6-tetra-*O*-acetyl-3-*O*-methyl-β-D-galactopyranose. The β-D configuration was indicated by the magnitude of the specific rotation (+43°) and of the coupling of H-1 and H-2 ($J_{1,2}$ 8.5 Hz) in the n.m.r. spectrum.

performed with Silica Gel G as the adsorbent, and 1:1 (v/v) acetone–toluene as the developing solvent. For detection, the developed plates were air-dried, sprayed with 5% ethanolic sulfuric acid, and heated at about 150°. Gas–liquid partition chromatography (g.l.c.) (F and M chromatograph, type 402) of methyl glycosides of *O*-methyl sugars was performed at 175° with helium flow-rates of ~100 ml. min^{-1} on columns (110 × 0.4 cm) of (*i*) 15% (wt./wt.) 1,4-butanediol succinate polyester on acid-washed Chromosorb W (60–80 mesh), and (*ii*) 15% (wt./wt.) ethylene glycol adipate polyester on acid-washed Chromosorb W (60–80 mesh). Retention times (*T*) are given relative to that of methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside as the standard. Molar ratios of neutral sugars were determined by conversion[10] into their acetylated alditols, which were examined by g.l.c. on column (*i*) at 260°. Peak areas were measured by triangulation, and a standard curve was obtained for known molar ratios of 3-*O*-methylgalactose to rhamnose after conversion into their acetylated alditols. Unless otherwise stated, oligosaccharides were methylated successively with methyl iodide and silver oxide in *N*,*N*-dimethylformamide, and with methyl iodide and silver oxide. Methanolyses were performed with 4% methanolic hydrogen chloride for 6 h under reflux. Authentic samples of 2,3,4-tri-, 2,3- and 3,4-di-, and 3- and 4-*O*-methyl-L-rhamnose, and 2,3,4,6-tetra- and 2,3,6- and 2,4,6-tri-*O*-methyl-D-galactose were available, for purposes of comparison in g.l.c., after conversion into their methyl glycosides.

Isolation of polysaccharide from elm bark. — A polysaccharide product was extracted from chips of elm bark as described previously[1]; $[\alpha]_D$ +60° (*c* 1.5, water); (Found: N, 0.12; galacturonic acid "anhydride", 36%; molar ratios of rhamnose: galactose: 3-*O*-methylgalactose, 1.00:2.70:2.08). The product gave a blue color with iodine, and paper-chromatographic examination [solvents (*a*) and (*e*)] of the sugars liberated on acid-catalyzed hydrolysis of a small sample indicated the presence of traces of glucose and arabinose. The remainder of the product in water (1200 ml) was passed through Rexyn-101 (H^+) ion-exchange resin, and the effluent was concentrated to a volume of 1.75 liters (pH 2.9). A 5% solution of hexadecyltrimethylammonium hydroxide [prepared by passing a solution of hexadecyltrimethylammonium bromide through a column of Duolite A-4 (OH^-) ion-exchange resin] was added dropwise, with stirring, until precipitation of the complex was complete (300 ml). The precipitate was immediately collected by centrifugation, and washed twice with water. A polysaccharide fraction (90% of protein-free polysaccharide product) was obtained as described[1] for the isolation of the first polysaccharide product from its Cetavlon complex. This major fraction (galacturonic acid "anhydride", 34%) did not give a blue color with iodine, and paper-chromatographic examination of the sugars liberated on acid-catalyzed hydrolysis (0.75M sulfuric acid for 8 h) of a small sample (~8 mg) did not show the presence of any glucose. After dialysis of a 1% solution against 50 mM sodium tetraborate for 48 h, free-boundary electrophoresis in a Tiselius apparatus (Perkin–Elmer Model 38-A) at 150 V and 20 mA indicated the presence of one component. This polysaccharide fraction was used for the structural investigations.

Isolation of oligosaccharides. — A polyalcohol was obtained by reduction of the

periodate-oxidized polysaccharide with sodium borohydride as described previously[1], $[\alpha]_D$ −28° (*c* 1.7, water); (Found: galacturonic acid "anhydride", 7.7%; rhamnose: 3-*O*-methylgalactose, 1.8:1). The polyalcohol (2.5 g) was hydrolyzed with 0.25M sulfuric acid (100 ml) for 1 h on a steam-bath. The solution was cooled, and made neutral with barium carbonate, and the suspension was filtered; the filtrate was treated with Rexyn-101 (H^+) ion-exchange resin, and then evaporated to a syrup (1.44 g). An aqueous solution (50 ml) of the syrup was passed through a column of Duolite A-4 (OH^-) ion-exchange resin, to remove the acidic components. The eluate was evaporated to a syrup (895 mg) which, by paper-chromatographic examination in solvent *c*, showed the presence of three major components, R_{Gal} 1.05, 1.27, and 1.63, and R_{Rha} 0.55, 0.67, and 0.86, in addition to 3-*O*-methylgalactose and rhamnose, and a trace of galactose. The three major components were isolated by chromatography on Whatman No. 3MM paper, as described for each of them, and were identified as the oligosaccharides **4**, **2**, and **3**, respectively.

O-(*3*-O-*Methyl*-β-D-*galactopyranosyl*)-(*1*→*4*)-L-*rhamnose* (**3**). — Disaccharide **3** was purified by separation on Whatman No. 3MM paper in solvents *b* and *c* successively; yield 11.0 mg, $[\alpha]_D$ +6.7° (*c* 0.9, water). This material was homogeneous on paper chromatograms in two solvents (R_{Rha} 0.86 in solvent *c*, and 0.63 in solvent *b*; R_{Gal} 1.63 and 1.50 in these solvents, respectively). The n.m.r. spectrum is shown in Fig. 2. On hydrolysis with 0.5M sulfuric acid for 7 h at 100°, the oligosaccharide yielded 3-*O*-methylgalactose and rhamnose in the molar proportions of 1.32:1. Reduction of the oligosaccharide with sodium borohydride, followed by acid-catalyzed hydrolysis, acetylation, and g.l.c. of the derived acetates, showed the presence of rhamnitol and 3-*O*-methylgalactose in the approximate ratio of 1:1. A portion (~4 mg) of the oligosaccharide was methylated; g.l.c. of the methanolysis products revealed peaks having retention times corresponding to those of the methyl glycosides of 2,3-di-*O*-methyl-L-rhamnose [(*i*) *T* 1.42; (*ii*) 1.38] and 2,3,4,6-tetra-*O*-methyl-D-galactose [(*i*) *T* 1.68; (*ii*) 1.66]. Hydrolysis of the methyl glycosides, followed by paper chromatography in solvent *d*, confirmed the presence of 2,3-di-*O*-methyl-L-rhamnose (R_G 0.70) and 2,3,4,6-tetra-*O*-methyl-D-galactose (R_G 0.85).

O-(*3*-O-*Methyl*-β-D-*galactopyranosyl*)-(*1*→*4*)-O-(*3*-O-*methyl*-β-D-*galactopyranosyl*)-(*1*→*4*)-L-*rhamnose* (**2**). — Trisaccharide **2** was purified by separation on Whatman No. 3MM paper in solvents *b*, and *a*, successively; yield 10 mg, $[\alpha]_D$ +13° (*c* 1.0, water). This material was homogeneous on paper chromatograms in four solvents (R_{Rha} 0.27 in solvent *b*, 0.59 in solvent *c*, 0.25 in solvent *e*, and 0.86 in solvent *g*). The n.m.r. spectrum is shown in Fig. 1. On hydrolysis with 0.5M sulfuric acid for 7 h at 100°, the oligosaccharide yielded 3-*O*-methylgalactose and rhamnose in the molar proportions of 2.34:1. Borohydride reduction of the oligosaccharide, followed by acid hydrolysis, acetylation, and g.l.c. of the derived acetates, showed the presence of rhamnitol and 3-*O*-methylgalactose. A portion (6 mg) of the oligosaccharide was methylated; g.l.c. of the methanolysis products revealed peaks having retention times corresponding to those of the methyl glycosides of 2,3-di-*O*-methyl-L-rhamnose [(*i*) *T* 1.45; (*ii*) 1.43], and 2,3,4,6-tetra- [(*i*) *T* 1.73; (*ii*) 1.71] and

2,3,6-tri-*O*-methyl-D-galactose [(*i*) *T* 3.00, 4.75; (*ii*) 2.76, 4.05]. Hydrolysis of the methyl glycosides, followed by paper chromatography in solvent *d*, confirmed the presence of 2,3,4,6-tetra- (R_G 0.84) and 2,3,6-tri-*O*-methyl-D-galactose (R_G 0.58), and 2,3-di-*O*-methyl-L-rhamnose (R_G 0.68).

O-(*3*-O-*Methyl-β*-D-*galactopyranosyl*)-(*1→4*)-*3*-O-*methyl*-D-*galactose* (**4**). — Disaccharide **4** was purified by separation on Whatman No. 3MM paper in solvents *b* *c*, and *a* successively; yield 10 mg, $[\alpha]_D$ +60° (*c* 0.7, water). This material was homogeneous on paper chromatograms in three solvents (R_{Rha} 0.57 in solvent *a*, and 0.55 in solvent *c*; R_{Gal} 1.27 in solvent *a*, 0.50 in solvent *b*, and 1.05 in solvent *c*). The n.m.r. spectrum is shown in Fig. 3. After hydrolysis of the oligosaccharide with 0.5M sulfuric acid for 7 h at 100°, only the presence of 3-*O*-methylgalactose was detected by paper chromatography in solvents *c* and *e*. Reduction of the oligosaccharide with borohydride, followed by acid hydrolysis, acetylation, and g.l.c. of the derived acetates, showed the presence of 3-*O*-methylgalactitol and 3-*O*-methylgalactose in the approximate ratio of 1:1. A portion (~3.5 mg) of the oligosaccharide was methylated; g.l.c.* of the methanolysis products revealed peaks having retention times corresponding to those of the methyl glycosides of 2,3,4,6-tetra- [(*i*) *T* 1.73; (*ii*) 1.75] and 2,3,6-tri-*O*-methyl-D-galactose [(*i*) *T* 3.05, 4.75; (*ii*) 2.76, 4.00]. Hydrolysis of the methyl glycosides, followed by paper chromatography in solvent *d*, confirmed the presence of 2,3,4,6-tetra- (R_G 0.84) and 2,3,6-tri-*O*-methyl-D-galactose (R_G 0.58).

3-O-*Methyl*-D-*galactose*. — This compound was prepared by the method of Ball and Jones[2]: D-galactose was converted into 4,6-*O*-ethylidene-D-galactose, which was condensed with acetone in the presence of zinc chloride to give 4,6-*O*-ethylidene-1,2-*O*-isopropylidene-D-galactose[3]; treatment with methyl iodide–silver oxide for 24 h at reflux temperature, followed by acid-catalyzed hydrolysis of the methylated product, gave 3-*O*-methyl-D-galactose, which migrated as a single component with an authentic sample[2] on paper chromatograms in solvents *a*, *b*, and *c*.

Acetylation of 3-O-*methyl*-D-*galactose*. — 3-*O*-Methyl-D-galactose (1.52 g) was treated with 1:1 (v/v) acetic anhydride–pyridine (20 ml) for 12 h, the solution was evaporated to a syrup, and traces of the reagents were removed by repeated addition and evaporation of dry toluene. Crystalline product was obtained from a solution of a portion (500 mg) of the syrup in chloroform–petroleum ether (b.p. 30–60°), yield 80 mg, m.p. 123–124°, $[\alpha]_D$ +43° (*c* 1.8, chloroform); n.m.r. data (chloroform-*d*): τ 4.38 (1-proton doublet, $J_{1,2}$ 8.5 Hz, H-1), 6.60 (3-proton singlet, OMe), 7.85, 7.90, and 7.95 (12 protons, 4 OAc).

Anal. Calc. for $C_{15}H_{22}O_{10}$: C, 49.8; H, 6.1. Found: C, 49.8; H, 6.0.

2,4,6-Tri-O-*acetyl-3*-O-*methyl-α*-D-*galactopyranosyl chloride*. — To a solution of 1.0 g of syrupy tetra-*O*-acetyl-3-*O*-methyl-D-galactose in dry benzene (30 ml) was

*G.l.c. of the methanolysis products derived from the oligosaccharides **2**, **3**, and **4** showed, for each, several peaks in addition to those mentioned for the methyl glycosides of the methylated sugars. By a blank experiment, all of these peaks were shown to be due to the methylation and methanolysis reagents.

added zinc chloride (2.5 g), and then thionyl chloride (2.2 g), dropwise, with stirring, the reactants being protected from atmospheric moisture by means of a calcium chloride drying-tube. The progress of the reaction was monitored by t.l.c. When the reaction was complete, the mixture was filtered through silica gel (15 g); the filtrate was evaporated to dryness, and the residue was coevaporated three times with benzene to afford the glycosyl chloride as a syrup; this was kept for 6 h at ~25°/0.03 torr to remove the last traces of solvent; yield 0.76 mg (76%), $[\alpha]_D$ +154° (*c* 1.1, chloroform); n.m.r. data (chloroform-*d*): τ 3.70 (1-proton doublet, $J_{1,2}$ 2.0 Hz, H-1), 6.58 (3-proton singlet, OMe), 7.80–8.00 (9 protons, 3 AcO).

Benzyl 2,3-O-isopropylidene-α-L-rhamnopyranoside. — This compound was prepared from L-rhamnose monohydrate (19 g) essentially as described by Brimacombe and Tucker[9]; yield 9.5 g. The product was purified by distillation at 145–155°/0.05 torr; the distillate crystallized on standing, m.p. 72–73°, $[\alpha]_D$ −55° (*c* 1.2, chloroform); lit.[9] m.p. 73–75°, $[\alpha]_D$ −55° (*c* 1, chloroform).

Synthesis of O-(3-O-methyl-β-D-galactopyranosyl)-(1→4)-L-rhamnose. — A mixture of 2,4,6-tri-*O*-acetyl-3-*O*-methyl-α-D-galactopyranosyl chloride (550 mg), benzyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (450 mg), and mercuric cyanide (500 mg) in nitromethane was stirred for 10 days at room temperature. Chloroform (50 ml) was added, the mixture was filtered through Celite, the filtrate was evaporated to a syrup (980 mg), and the product was treated with sodium methoxide solution to remove the acetyl groups. The progress of the de-*O*-acetylation was monitored by t.l.c.; a new major component having R_F 0.33 appeared. On completion of the reaction, the solution was de-ionized by successively passing it through a column of Rexyn-101 (H^+) and a column of Duolite A-4 (OH^-) ion-exchange resin. The eluate and washings were evaporated to a syrup which, on fractionation on silica gel, with 1:1 acetone–toluene as the eluant, gave the component having R_F 0.33 as a syrup, yield 26 mg, $[\alpha]_D$ −35° (*c* 1.0, chloroform); n.m.r. data (chloroform-*d*): τ 2.60 (5-proton singlet, aromatic H), 6.39 (3-proton singlet, OMe), 8.35–8.60 (9 protons, CMe_2 and C-5 Me). The isopropylidene group was removed by treatment of the syrup with 1:1 (v/v) acetic acid–water at 80°, and the solution was evaporated to a syrup. The benzyl group was removed by hydrogenolysis in ethanol in the presence of palladium on carbon. The catalyst was removed by filtration, and the filtrate was evaporated to a syrup (10 mg). Paper-chromatographic examination (solvent *c*) showed the presence of a major component having R_{Gal} 1.64 and a minor component having R_{Gal} 1.20. The major component was purified by separation on Whatman No. 3MM paper in solvent *c*, yield 7 mg, $[\alpha]_D$ +8° (*c* 0.7, water). The n.m.r. spectrum was indistinguishable from that of disaccharide **3** (see Fig. 2). On hydrolysis with 0.5M sulfuric acid for 7 h at 100°, the synthetic product yielded 3-*O*-methyl-D-galactose and L-rhamnose. A portion (4 mg) of the product was methylated; g.l.c. of the methanolysis products revealed peaks having retention times corresponding to those of the methyl glycosides of 2,3-di-*O*-methyl-L-rhamnose [(*i*) *T* 1.41] and 2,3,4,6-tetra-*O*-methyl-D-galactose [(*i*) *T* 1.65].

ACKNOWLEDGMENTS

The authors thank the National Research Council of Canada for the award of a scholarship (to R. J. B.) and for a grant which made this work possible.

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