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STRUCTURAL STUDIES OF THE CAPSULAR POLYSACCHARIDE OF *Klebsiella* TYPE 28

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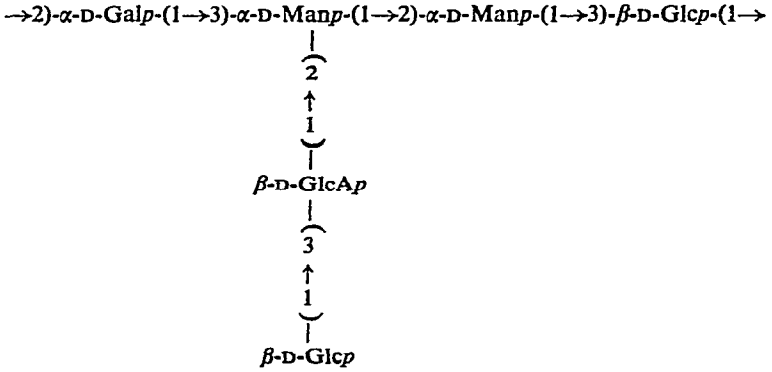
ABSTRACT

The capsular polysaccharide from *Klebsiella* type 28 has been studied by methylation analysis, a modified Smith-degradation procedure, and uronic acid degradation with subsequent oxidation and elimination of the substituents of the oxidized residue. The polysaccharide contained the hexasaccharide repeating-unit shown below. The terminal D-glucopyranose residue was hydrolysed by emulsin, indicating a β linkage. The anomeric natures of other glycosidic linkages were determined by characterization of fragments obtained during the degradative studies. The D-galactopyranose residue was not present in any fragment, but is assumed to be α-linked from optical-rotation considerations.



INTRODUCTION

The capsular polysaccharide (K28) from *Klebsiella* type 28 was investigated by Nimmich[1], who found that it contained mannose, galactose, glucose, and glucuronic acid. Several of the *Klebsiella* capsular-polysaccharides contain these sugars[1,2], and

structural investigations of two of these, type 7[3] and type 59[4], have been communicated. We now report structural studies of K28.

RESULTS AND DISCUSSION

The polysaccharide, isolated as previously described[1], had $[\alpha]_{589}$ +47°, and contained traces of nitrogen but no phosphorus. From the i.r. and n.m.r. spectra, it was concluded that no *O*-acetyl or other *O*-acyl groups or acetal-linked pyruvic acid were present. Acid hydrolysates of the original and carboxyl-reduced[5] K28 contained mannose, galactose, and glucose in the proportions 28:29:43 and 34:17:50, respectively. These figures indicated that K28 contained mannose, galactose, glucose, and glucuronic acid residues in the proportions 2:1:2:1. The sugars were isolated from a hydrolysate of the carboxyl-reduced polysaccharide and, from their optical rotations, all belonged to the D-series.

Methylation analyses[6,7] of the original and carboxyl-reduced K28 (Table I, columns *A* and *B*) indicated that K28 is composed of hexasaccharide repeating-units with two D-glucose residues (one terminal and the other linked to O-3), two D-mannose residues (one linked to O-2 and the other to O-2 and O-3), one D-galactose residue (linked to O-2), and one D-glucuronic acid residue (liked to O-3). The methylated sugars were analysed by g.l.c.–m.s. of their alditol acetates[7,8]. The methylation patterns are evident from the mass spectra, which also demonstrated that all the sugars were pyranosidic. Since all D-glucose, D-galactose, and D-mannose di- and tri-methyl ether derivatives with the observed methylation patterns are separated on the SP-1000 capillary column used, the identifications of these components were unambiguous. However, the alditol acetates from the 2,3,4,6-tetra-*O*-methyl derivatives of D-glucose and D-mannose are not separated. As all the D-mannose residues in K28 have been

TABLE I

METHYLATION ANALYSES OF ORIGINAL, CARBOXYL-REDUCED, AND ENZYMICALLY DEGRADED *Klebsiella* TYPE 28 CAPSULAR POLYSACCHARIDES

Methylated sugar[a]	T[b]	T[c]	*Mole %*[d]			
			A	*B*	*C*	*D*
2,3,4,6-Glc	1.00	1.00	26	19	—	18[e]
3,4,6-Man	1.95	1.67	24	17	25	18
2,4,6-Glc	1.95	1.74	18	34[e]	27	27[f]
3,4,6-Gal	2.50	1.98	22	15	25	19
4,6-Man	3.29	2.70	11	15	23	19

[a]2,3,4,6-Glc = 2,3,4,6-tetra-*O*-methyl-D-glucose, etc. [b]Retention time of the corresponding alditol acetate relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on an ECNSS-M column at 170°. [c]As in *b*, but on an SP-1000 column at 220°. [d]Polysaccharide: *A*, original; *B*, carboxyl-reduced (using deuterated reagent) before methylation; *C*, enzymically degraded, original polysaccharide; *D*, enzymically degraded, carboxyl-reduced polysaccharide. [e]~50% dideuterated at C-6. [f]~30% dideuterated at C-6.

accounted for as other methyl ethers, the tetra-*O*-methyl derivative must have been a D-glucose residue. Moreover, treatment of original K28 or carboxyl-reduced K28 with emulsin released D-glucose, demonstrating that the tetramethyl ether came from this sugar. As the enzyme preparation had no α-D-glucosidase activity, the result further demonstrated that the terminal D-glucose residue is β-linked. Methylation analyses of enzyme-treated original and carboxyl-reduced (using deuterated reagent) K28 demonstrated that the terminal D-glucose residue is linked to D-glucuronic acid (Table I, columns *C* and *D*). Enzymic hydrolysis of carboxyl-reduced K28 removed only ~50% of the terminal D-glucose residues, possibly because of the low solubility of the polysaccharide. The penultimate unit in carboxyl-reduced K28 is also a β-D-glucopyranose residue (see below) and, as it derives from the D-glucuronic acid, dideuterated at C-6. It was, however, not hydrolysed by the enzyme. This resistance to enzymic hydrolysis is not uncommon for terminal units linked directly to the chain. The sequence **1** in the polysaccharide is established by these experiments.

β-D-Glc*p*-(1→3)-D-GlcA*p*-(1→

1

Fully methylated K28 was subjected to a uronic acid degradation[9], that is, sequential treatment with base and acid using mild conditions. The polymeric product was isolated by gel-permeation chromatography, and remethylated using trideuteriomethyl iodide. A hydrolysate of this material contained the sugars listed in Table II, column *A*. These results demonstrated that the side chains removed during the

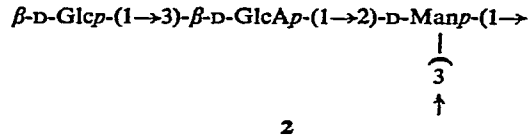
TABLE II

METHYLATION ANALYSES OF CHEMICALLY DEGRADED *Klebsiella* TYPE 28 CAPSULAR POLYSACCHARIDES

Methylated sugar[a]	T[b]	T[c]	*Mole %*[d]		
			A	*B*	*C*
1,3,4,5,6-Gal[e]	0.35	0.50	—	—	24[g]
2,3,4,6-Man	1.00	1.00	—	—	33[f]
2,3,4,6-Glc	1.00	1.00	6	3	2
3,4,6-Man	1.95	1.67	23	33	3
2,4,6-Glc	1.95	1.74	25	37	34
2,4,6-Man	2.09	1.86	22[f]	—	—
3,4,6-Gal	2.50	1.98	23	27	3
4,6-Man	3.29	2.70	1	—	—

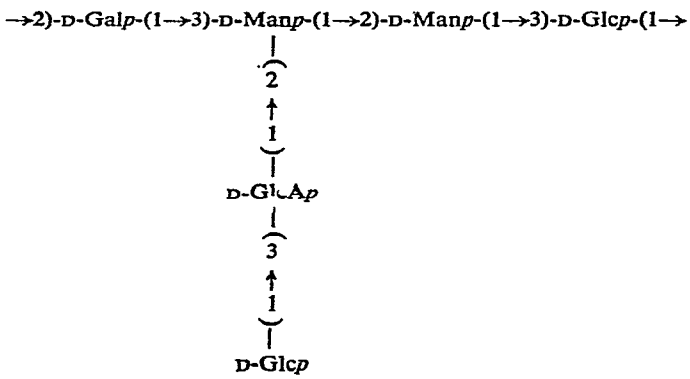
[a–c]See Table I, footnotes *a–c*. [d]Polysaccharide: *A*, degraded, trideuteriomethylated polysaccharide (uronic acid degradation, see Text); *B*, degraded and oxidized polysaccharide (see Text); *C*, polysaccharide *B*, treated with base and acid, reduced with sodium borodeuteride, and trideuteriomethylated (see Text). [e]Part of this volatile ether and its acetate was probably lost during concentrations. [f]Trideuteriomethylated at O-2. [g]Monodeuterated at C-1, and trideuteriomethylated at O-1 and O-5.

degradation consist of D-glucopyranosyl-D-glucuronic acid residues. These were linked to O-2 of the branching D-mannose residue, as 4,6-di-*O*-methyl-D-mannose in the analysis of the original K28 was replaced by 2,4,6-tri-*O*-methyl-D-mannose having a trideuteriomethyl group at O-2. From the difference in optical rotations of methylated K28, $[\alpha]_{589}$ +53°, and of the methylated, degraded product, $[\alpha]_{589}$ +101°, it is concluded that both sugar residues in the side chain are *β*-linked, as shown in partial structure **2**.



2

The polymeric product formed by uronic acid degradation contained free hydroxyl groups at C-2 of the originally branched D-mannose residues. This product was further degraded by oxidation of the alcohol groups, and then sequential treatment with base and acid using mild conditions. This method has been tested[10] on methylated polymers having free hydroxyl groups at C-3. Model experiments with alkylated glycosides having a carbonyl function at C-2 have demonstrated that the substituent at C-4 is eliminated on treatment with base[11], and those at C-1 and C-3 on subsequent treatment with acid using mild conditions[12]. Analysis of a hydrolysate of the oxidized product prior to base treatment verified that the oxidation was complete, as no 4,6-di-*O*-methyl-D-mannose was detected (Table II, column *B*). The degraded material was reduced with sodium borodeuteride, remethylated using trideuteriomethyl iodide, hydrolysed, and analysed (Table II, column *C*). The mixture contained three main components: unchanged 2,4,6-tri-*O*-methyl-D-glucose, 2,3,4,6-tetra-*O*-methyl-D-mannose trideuteriomethylated at O-2, and 1,3,4,5,6-penta-*O*-methyl-D-galactitol monodeuterated at C-1 and trideuteriomethylated at O-1 and O-5. These results, in conjunction with those reported above, demonstrated the sequence of the six sugar residues in the repeating unit **3**.



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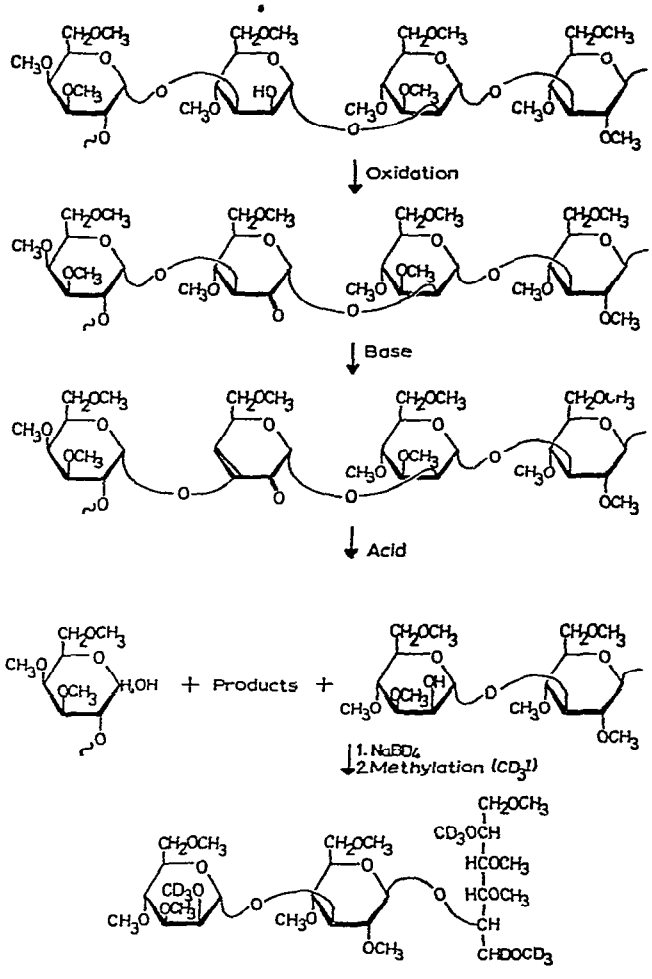
The degradation sequence is summarized in Scheme 1, where the correct anomeric configurations, determined as described below, are also given.



Scheme 1. Degradation of the product obtained on uronic acid degradation of methylated K28 followed by reduction and remethylation.

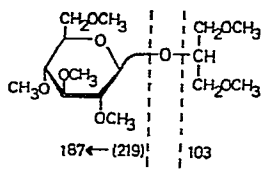
The degradation was repeated on a larger scale, using non-deuterated reagents, and the fully methylated trisaccharide alditol **4** was isolated by high-speed liquid chromatography. Its mass spectrum[8] and the products of acid hydrolysis agreed with the postulated structure. The optical rotation, $[\alpha]_{589}$ +42°, suggested that one sugar residue was α-linked and the other β-linked. In agreement with this, the n.m.r. spectrum showed two signals in the region for anomeric protons, one at δ 5.32, $J_{1,2}$ 1.5 Hz, assigned to an α-D-mannopyranose residue, and another at δ 4.34, $J_{1,2}$ 7.2 Hz, assigned to a β-D-glucopyranose residue.

α-D-Man*p*-(1→3)-β-D-Glc*p*-(1→2)-D-Galactitol

4

Carboxyl-reduced K28 (reduced with deuterated reagent) was subjected to a modified Smith-degradation, that is, periodate oxidation, borohydride reduction, methylation, hydrolysis under mild conditions, borohydride reduction, and re-methylation[4,13–15]. Hydrolysis of the product obtained after the first borohydride reduction yielded D-glucose and D-mannose in the ratio 2:1. Hydrolysis of the product after the first methylation yielded 2,4,6-tri-*O*-methyl-D-glucose (~50% dideuterated at C-6) and 4,6-di-*O*-methyl-D-mannose in the same ratio. These analyses indicated that the periodate oxidation had gone to completion. After the final step, *i.e.*, methylation using trideuteriomethyl iodide, hydrolysis of the product yielded 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4,6-tri-*O*-methyl-D-mannose, again in the same proportion. Both sugars were trideuteriomethylated at O-3, and ~50% of the former was dideuterated at C-6, demonstrating that one-half of the amount found came from the uronic acid residue and one-half from the chain D-glucose residue. Furthermore, the results indicated that the hydrolysis of non-cyclic acetals was complete and no intact sugar residue had been hydrolysed.

The modified Smith-degradation was repeated on a larger scale, using non-labelled chemicals, and the degraded material was separated by high-speed liquid chromatography, yielding two methylated glycosides, **5** and **6**, the structures of which were evident from analyses of hydrolysates and from their mass spectra[8]. The origins of some pertinent fragments are depicted in the formulae. The glycerol moieties of these glycosides obviously derived from the D-galactose residue and the D-mannose chain-residue, respectively. The former (**5**), $[\alpha]_{589}$ −8°, showed an n.m.r. signal for the anomeric proton at δ 4.45, $J_{1,2}$ 7.0 Hz, and was consequently a β-D-glucopyranoside. The second (**6**), $[\alpha]_{589}$ +6°, was not chromatographically pure, but contained a minor, non-carbohydrate contaminant. It showed n.m.r. signals for two anomeric protons, one at δ 5.07, $J_{1,2}$ 1.6 Hz, assigned to an α-D-mannopyranose residue, and the other at δ 4.25, $J_{1,2}$ 7.0 Hz, assigned to a β-D-glucopyranose residue. The D-glucose residue in this glycoside derived from the uronic acid residue.



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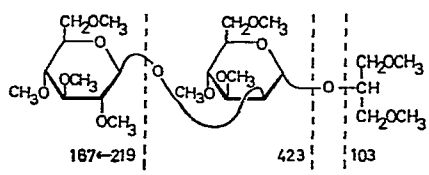

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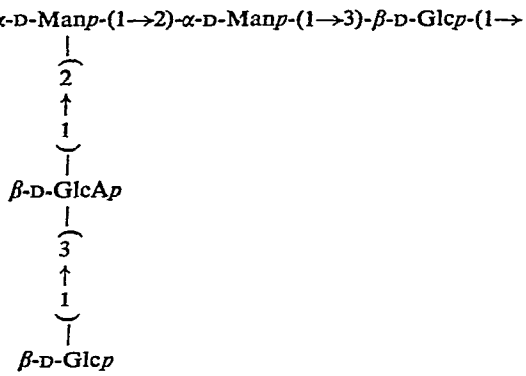
The results from the Smith degradation gave independent support to the sequence and anomeric natures proposed from the two-step degradative procedure described above, and further demonstrated that the branching D-mannose residue was α-linked.

From the studies reported above, the anomeric natures of all the sugar residues, except the D-galactose residue, have been determined. The product obtained after methylation and uronic acid degradation was linear and composed of tetrasaccharide repeating-units (7), methylated in all positions except O-2 of the 3-linked D-mannose residue. From the high optical rotation of the product, $[\alpha]_{589}$ +101°, the D-galactose residue was most probably α-linked. The 100-MHz Fourier-transform n.m.r. spectrum of the original K28 supported this assumption. In the region for anomeric protons, there were two overlapping signals at δ 5.42 (2 H) and one signal at δ 5.16, $J_{1,2}$ ~2 Hz (1 H), indicative of 3 α-linked residues, and a multiplet (from overlapping doublets) at δ 4.48–4.84 (3 H), indicative of β-linkages in sugars giving high values (~7 Hz) for $J_{1,2}$.

→2)-D-Gal*p*-(1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→3)-β-D-Glc*p*-(1→

7

From the combined evidence presented above, it is proposed that K28 is composed of hexasaccharide repeating-units with the structure **8**.



8

EXPERIMENTAL

General methods. — Equipment and columns for g.l.c., g.l.c.–m.s., and high-speed liquid chromatography were the same as in the investigations of the *Klebsiella* type 59[4] and type 81[15] capsular polysaccharides.

Isolation of the polysaccharide from Klebsiella *K-type 28 (strain 5758).* — This was performed as described[1] earlier. The polysaccharide had $[\alpha]_{589}^{22}$ +47° (*c* 0.2, water). In the i.r. (KBr), no absorption was observed around 1735 cm^{-1} (*O*-acyl region). The 100-MHz n.m.r. spectrum of K28 was measured on a solution containing 10 mg/ml of D_2O at 85°. An FID acquisition time of 1.5 s was used, and the Fourier transform was computed from 2000 transients. No absorptions from *O*-acetyl or pyruvic acid residues were observed. K28 contained nitrogen (0.92%) but no phosphorus.

Carboxyl-reduction of the native polysaccharide. — This was performed by the procedure of Taylor and Conrad[5], and one treatment resulted in complete reduction. Sodium borodeuteride was used as the reductive agent in some experiments.

Sugar and methylation analyses. — These were performed essentially as described before[6–9,16]. The partially methylated and trideuteriomethylated alditol acetates gave mass spectra in which fragments containing a trideuteriomethoxyl group were recognized by the shift of three mass-units. Mannose, $[\alpha]_{578}$ +19°; galactose, $[\alpha]^{22}_{578}$ +82°; and glucose, $[\alpha]^{22}_{578}$ +56° (all rotations in water, *c* 0.2) were isolated from a hydrolysate of carboxyl-reduced polysaccharide by paper chromatography.

Enzymic degradation of original and carboxyl-reduced polysaccharide. — In a pilot experiment, it was demonstrated by g.l.c. that methyl α-D-glucopyranoside (10 mg) in 0.1M sodium acetate buffer of pH 4.8 (5 ml) was unaffected by the emulsin preparation (20 mg, Sigma Chemical Company) used. Original K28 (15 mg) was dissolved in 0.1M sodium acetate buffer of pH 4.8 (10 ml). Emulsin (40 mg) was added and the mixture was incubated at 37°. Samples were withdrawn, reduced, acetylated, and examined by g.l.c. When the release of D-glucose had ceased, the mixture was heated for 10 min at 100° and then centrifuged, and the supernatant solution was dialysed. The non-dialysable material was recovered by freeze-drying and subjected to methylation analysis (Table I, column *C*). Carboxyl-reduced polysaccharide (14 mg, reduced with sodium borodeuteride) was treated likewise with emulsin (40 mg) for 48 h at 37° and, after purification, subjected to methylation analysis (Table I, column *D*).

Uronic acid degradation of methylated polysaccharide. — In a typical experiment, a solution of carefully dried, methylated K28 (70 mg) in a mixture (12 ml) of methyl sulphoxide and 2,2-dimethoxypropane (19:1) was prepared in a serum vial sealed with a rubber cap. The vial was flushed with nitrogen and kept in an ultrasonic bath for 30 min. Methylsulphinyl anion in methyl sulphoxide (2M, 6 ml) was added and the solution sonicated for another 30 min. The vial was kept at room temperature overnight, an excess of 50% aqueous acetic acid added, and the product dialysed. The non-dialysable material was treated with 75% aqueous acetic acid (50 ml) for 2 h at 100°. The reaction mixture was concentrated to dryness, and the residue was suspended in water and freeze-dried. The recovered material was fractionated on a Sephadex LH-20 column (30 × 3 cm) eluted with chloroform–acetone (3:1). The eluate was monitored polarimetrically, and the material eluted with the void volume was collected. The yield of degraded polysaccharide, $[\alpha]^{22}_{589}$ +101° (*c* 0.3, chloroform), was 40 mg. Methylated, original K28 had $[\alpha]^{22}_{589}$ +53° (*c* 0.4, chloroform). Part (6 mg) of the degraded material was trideuteriomethylated, dialysed, and hydrolysed, and the resulting sugars were analysed as their alditol acetates by g.l.c.–m.s[7,8] (Table II, column *A*).

Oxidation and degradation of degraded polysaccharide. — The oxidation agent[17] was prepared, under anhydrous conditions, at −45° by dropwise addition of methyl sulphoxide (1.8 ml) to a stirred solution of chlorine in dichloromethane (M, 5 ml)[17]. Degraded polysaccharide (15 mg of the material described above) in methyl

sulphoxide (2 ml) was added dropwise with the aid of a syringe to the stirred, cooled oxidation mixture. The reaction mixture was kept at −45°, with stirring, for 5 h and then triethylamine (10 ml) was added dropwise. The reaction mixture was warmed to room temperature and dialysed, and the product was recovered by freeze-drying. Part (1/3) of the recovered material was hydrolysed, and the resulting sugars were analysed as their alditol acetates (Table II, column *B*). Another part (2/3) was dissolved in dichloromethane (4 ml), and sodium ethoxide (M, 4 ml) was added. The reaction mixture was kept at room temperature for 30 min, neutralised with 90% aqueous acetic acid, and evaporated to dryness. The product was treated with 50% aqueous acetic acid (10 ml) at 100° overnight, evaporated to dryness, and partitioned between chloroform and water. The resulting material was reduced with sodium borodeuteride (50 mg) in *p*-dioxane–ethanol (8:3, 5 ml) overnight. The reaction mixture was treated with Dowex-50 (H^+, prewashed with *p*-dioxane–ethanol), and boric acid was removed by repeated distillations with methanol. The product was trideuteriomethylated, and then partitioned between chloroform and water. The chloroform-soluble product was hydrolysed, and the resulting sugars were analysed as their alditol acetates (Table II, column *C*).

In order to isolate an oligomeric product, a larger amount of degraded K28 (120 mg) was oxidized as described above, using appropriate amounts of reagents. The product was treated with sodium ethoxide (0.5M in ethanol–dichloromethane, 40 ml), neutralised, treated with 50% aqueous acetic acid (50 ml), and reduced with sodium borohydride (500 mg) in *p*-dioxane–ethanol (30 ml). The resulting material was methylated, and recovered by partition between chloroform and water. The material from the chloroform phase was fractionated by liquid chromatography on two Merck silica gel 60 (size A) columns connected in series and eluted with hexane–acetone (2:3), yielding permethylated α-D-Man*p*-(1→3)-β-D-Glc*p*-(1→2)-D-galactitol (**4**, 15.3 mg). On t.l.c. (silica gel; hexane–acetone, 2:3), the compound showed R_F 0.80; and on g.l.c. (OV-1 column at 250°), T_{MEL} 7.1 (retention time relative to permethylated melibiitol). The mass spectrum[8] showed, *inter alia*, the following fragments (relative intensities in brackets): *m/e* 88 (64), 101 (100), 133 (7), 155 (24), 187 (71), 219 (28), 235 (21), 423 (0.5). In the n.m.r. spectrum ($CDCl_3$), the signals from the anomeric protons appeared at δ 5.32 ($J_{1,2}$ 1.5 Hz) and 4.34 ($J_{1,2}$ 7.2 Hz). The compound had $[\alpha]_{589}^{22}$ +42° (*c* 0.2, chloroform), and hydrolysis of part of the material yielded 1,3,4,5,6-penta-*O*-methyl-D-galactitol, 2,3,4,6-tetra-*O*-methyl-D-mannose, and 2,4,6-tri-*O*-methyl-D-glucose in comparable amounts.

Smith degradation of the polysaccharide. — Carboxyl-reduced K28 (20 mg, reduced with sodium borodeuteride) was dissolved in 0.1M sodium acetate buffer of pH 3.9 (13 ml), and 0.2M sodium metaperiodate solution (3.3 ml) was added. The reaction mixture was kept in the dark at 5° for 120 h. Excess of periodate was reduced by adding ethylene glycol (1 ml), and the mixture was dialysed. The solution was concentrated to 40 ml and sodium borohydride (200 mg) was added. After stirring for 9 h at room temperature, the excess of borohydride was destroyed by adding 50% acetic acid. The solution was dialysed, and part (1/4) of the recovered material was

hydrolysed and converted into alditol acetates. Analysis by g.l.c. yielded D-mannose and D-glucose in the ratio 1:2. The remainder of the recovered material (3/4) was methylated, and recovered by dialysis. Part (1/3) of this product was hydrolysed, and the sugars were analysed as alditol acetates. 2,4,6-Tri-*O*-methyl-D-glucose (~50% dideuterated at C-6) and 4,6-di-*O*-methyl-D-mannose were obtained in the ratio 2:1. The remainder of the methylated material (2/3) was treated with 90% formic acid (3 ml) for 1 h at 40°. The solution was evaporated to dryness, and the residue was suspended in water and freeze-dried. The recovered product was dissolved in a mixture (5 ml) of *p*-dioxane–ethanol (8:3). Sodium borohydride (50 mg) was added and the reaction mixture was stirred overnight. After conventional work-up, the material was trideuteriomethylated, partitioned between chloroform and water, and hydrolysed, and the sugars were analysed as their alditol acetates. 2,3,4,6-Tetra-*O*-methyl-D-glucose (trideuteriomethylated at O-3 and ~50% dideuterated at C-6) and 3,4,6-tri-*O*-methyl-D-mannose (trideuteriomethylated at O-3) were obtained in the ratio 2:1.

In order to isolate oligomeric products, a larger amount of carboxyl-reduced K28 (160 mg, reduced with sodium borohydride) was oxidized, reduced, methylated, hydrolysed, reduced, and remethylated as described above, using appropriate amounts of non-deuterated reagents. The product after the final methylation was recovered by partition between chloroform and water, and the products were fractionated by liquid chromatography on two Waters Microporasil columns connected in series and eluted with ethyl acetate. Permethylated β D-Glc*p*-(1→2)-glycerol (**5**, 3.8 mg) and permethylated β-D-Glc*p*-(1→2)-α-D-Man*p*-(1→2)-glycerol (**6**, 11.0 mg) were obtained. Compound **5** had R_F 0.47 on t.l.c. (silica gel, ethyl acetate), and t'_{GG} 0.94 (retention time relative to permethylated α-D-Gal*p*-(1→2)-glycerol) on g.l.c. (OV-1 column at 180°). The mass spectrum[8] showed, *inter alia*, the following fragments: 88 (100), 101 (34), 103 (25), 163 (32), 187 (2). In the n.m.r. spectrum ($CDCl_3$), the signal from the anomeric proton occurred at δ 4.45 ($J_{1,2}$ 7.0 Hz). The compound had $[\alpha]_{589}^{22}$ −8° (*c* 0.2, chloroform). Part of the material was hydrolysed, and the sugars were analysed. Only 2,3,4,6-tetra-*O*-methyl-D-glucose was obtained. Compound **6** had R_F 0.21 on t.l.c. (silica gel, ethyl acetate), and T_{MEL} 2.0 on g.l.c. (OV-1 column at 225°). The mass spectrum[8] showed, *inter alia*, the following fragments: 88 (100), 101 (64), 103 (26), 155 (20), 163 (20), 187 (53), 219 (5), 275 (31), 391 (1). In the n.m.r. spectrum ($CDCl_3$), the signals from the anomeric protons occurred at δ 5.07 ($J_{1,2}$ 1.6 Hz) and 4.25 ($J_{1,2}$ 7.0 Hz). The compound had $[\alpha]_{589}^{22}$ +6° (*c* 0.3, chloroform). Part of the material was hydrolysed, and the analysis indicated 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4,6-tri-*O*-methyl-D-mannose in equimolecular proportions.

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