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THE STRUCTURE OF THE EXTRACELLULAR POLYSACCHARIDE FROM *Mycobacterium lacticolum* STRAIN 121[1]

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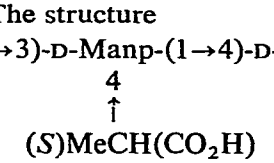
ABSTRACT

The structure



has been assigned to the extracellular polysaccharide from *M lacticolum* strain 121, and is based on methylation analysis, alkaline degradation, oxidation of the reduced polysaccharide acetate by chromium trioxide–acetic anhydride, and ^{13}C-n m r spectroscopy. The structure of the new acidic monosaccharide 4-*O*-[(*S*)-1-carboxyethyl]-D-mannose, elucidated by chemical transformations and spectroscopic data, was confirmed unequivocally by synthesis

INTRODUCTION

Extracellular polysaccharides are produced by various micro-organisms[2–4] The data available indicate a great variety of monosaccharide constituents and types of linkage, extracellular polysaccharides of many micro-organisms are structurally unique and often contain rare-sugar constituents We now report on the structure of the extracellular polysaccharide from the culture medium of *Mycobacterium lacticolum* strain 121

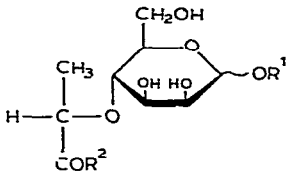
RESULTS AND DISCUSSION

The extracellular polysaccharide was isolated from the culture medium by precipitation with cetylpyridinium bromide Its homogeneity was demonstrated by high-pressure liquid chromatography and the molecular weight thereby estimated to be >150,000

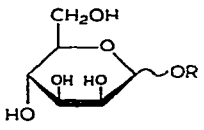
D-Glucose and D-glucuronic acid were identified in an acid hydrolysate by p c , together with an unknown acidic monosaccharide (**1**) which was shown to belong to a new class of acidic monosaccharide[5–8] The D configurations of glucose and

glucuronic acid were determined by treatment of an acid hydrolysate of the reduced[9] polysaccharide with D-glucose oxidase

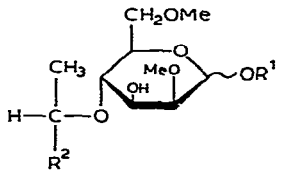
The monosaccharide **1**, isolated by adsorption onto Dowex-1 X8 (AcO^-) resin, had $[\alpha]_D^{20}$ +15° (*c* 2, water) Treatment of **1** with 1% methanolic hydrogen chloride gave the methyl ester methyl glycoside, from which the amide **2** was obtained by reaction with dry ammonia in methanol Cleavage of **2** with sodium hypochlorite[10] and hydrolysis of the resulting methyl glycoside **3** afforded D-mannose (**4**), which was identified by ion-exchange chromatography and p c , and as the hexitol hexa-acetate by g l c



1 R^1 = H, R^2 = OH
2 R^1 = Me, R^2 = NH_2



3 R = Me
4 R = H

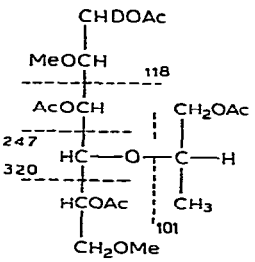


5 R^1 = Me, R^2 = CO_2Me
6 R^1 = H, R^2 = CH_2OH

The glycoside **3** had $[\alpha]_D^{20}$ +67°. Since the $[\alpha]_D$ values[11,12] of methyl α- and β-D-mannopyranoside are +79° and −49°, respectively, and since the α anomer is preferentially formed during the methanolysis of D-mannose[13], the D configuration can be assigned to the mannose residue in **1**

Methanolysis of the methylated polysaccharide, followed by chromatography of the products on silica gel, gave the methyl glycoside **5**, the p m r spectrum of which contained a doublet at δ 1.47 (3 H, *J* 7 Hz) for the methyl group of the lactic acid residue, and singlets at δ 3 36, 3 43, 3.51, and 3 80, corresponding to MeO-1,2,6 and the methoxycarbonyl group

Reduction of **5** with lithium aluminium hydride, followed by hydrolysis and reduction of the resulting sugar **6** with sodium borodeuteride and then acetylation, gave the mannitol derivative **7**, the structure of which was confirmed by its mass spectrum[14] Hence, the lactic acid residue is located at position 4 in the monosaccharide **1**, which, in turn, is linked to the polysaccharide chain through position 3



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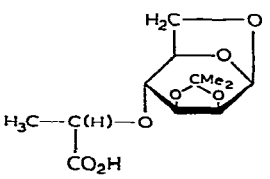
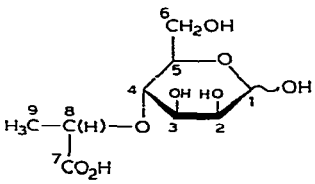


8a (*S*)
8b (*R*)



9a (*S*), +14° (water)
9b (*R*), +57° (water)

TABLE I

^{13}C-N M R DATA[a] FOR 4-*O*-(1-CARBOXYETHYL)-D-MANNOSE [9(*R*) AND 9(*S*)]

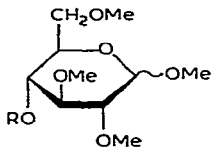
	Compound	
	1, 9(S)	9(R)
C-1α,β	94 5	94 5
C-4α,β, C-5β, C-8α,β	76 35, 76 0	75 3, 75 1, 74 6
C-3β	73 8	73 8
C-2α,β, C-3α, C-5α	72 2, 70 8, 70 2	72 3, 71 8, 71 7, 71 5
C-6α,β	60 8	61 7
C-9α,β	19 5	19 5
C-7α,β	181 6	181 7

[a]At pH 7

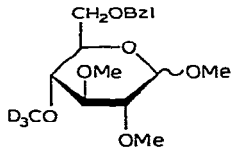
The diastereomeric acids **9** were synthesised by condensation of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose[15,16] and (*R*)- and (*S*)-α-chloropropionic acids[17], followed by acid hydrolysis of the products (**8**) Comparison of the $[\alpha]_D$ value for **1** (isolated from the polysaccharide, +15°) and those for **9**(*S*) and **9**(*R*) indicates unequivocally the (*S*)-configuration for the lactic acid residue in **1** The ^{13}C-n m r spectrum of **1** at pH 7 was identical to that of **9**(*S*), but different from that of **9**(*R*) (see Table I)

The acetates of glucitol and the alditol derived from **1** were present in the ratio 2 1 (determined by g l c –m s) in a hydrolysate of the reduced[9] polysaccharide Hence the polysaccharide contains D-glucose, D-glucuronic acid, and 4-*O*-[(*S*)-1-carboxyethyl]-D-mannose in the ratios 1 1 1

The ratios of monosaccharides in the polysaccharide were also determined on a methylated sample (Hakomori methylation[18]) which was reduced with lithium aluminium hydride and then benzylated (for identification of the D-glucopyranose residue by g l c –m s) Methanolysis of the product, followed by trideuteriomethylation and analysis by g l c –m s , allowed **10–12** to be identified in the ratios 0 85 1 1 The low value for **10** may be explained by losses due to its relatively high volatility



10 R = CD_3
13 R = H



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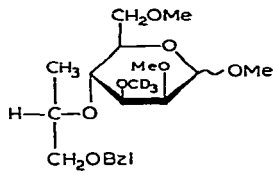

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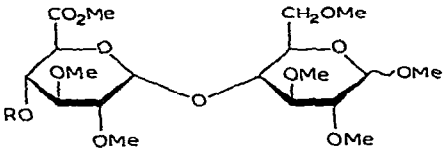
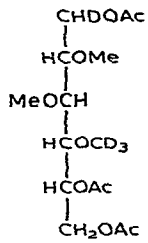
Methanolysis of the permethylated polysaccharide, followed by chromatography of the products on silica gel, gave methyl 2,3,6-tri-*O*-methyl-D-glucopyranoside (**13**, identified by g l c –m s as its 4-*O*-trideuteriomethyl derivative[19]), methyl 4-*O*-



14 R = H
15 R = CD_3



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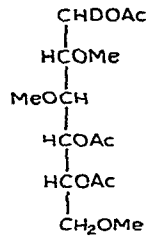

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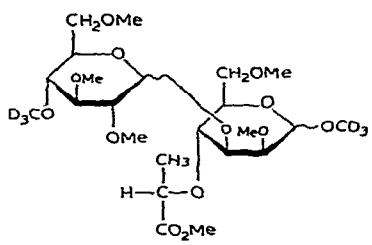
[(*S*)-1-(methoxycarbonyl)ethyl]-2,6-di-*O*-methyl-D-mannopyranoside (**5**), and the aldobiouronic ester **14** The structure of **14** was elucidated by conversion of its trideuteriomethyl derivative **15** into the alditols **16** and **17** The structures of **15–17** were confirmed by mass spectrometry[14 20] Hence, D-glucose, D-glucuronic acid, and **1** are (1→4)-, (1→4)-, and (1→3)-linked, respectively, in the polysaccharide.

The methylated polysaccharide was subjected to alkaline degradation[21] The disaccharide **18** was obtained by treatment of the methylated polysaccharide with sodium methylsulphinylmethanide[22] followed by mild hydrolysis with acid and trideuteriomethylation (with low yield owing to further degradation of **18** in the alkaline conditions)



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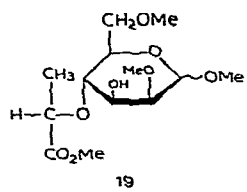

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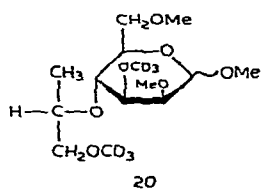

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Methanolysis of **18** afforded **10** and **19** in the ratio 1 : 1, which were identified by g l c –m s Reduction of **18** with lithium aluminium hydride, followed by methanolysis and trideuteriomethylation of the resulting mixture, gave **10** and **20** in the ratio 1 . 1, also identified by g l.c –m s Isolation of the disaccharide **18** and the aldobiouronic ester **14** demonstrates unequivocally the following sequence of monosaccharide units in the polysaccharide

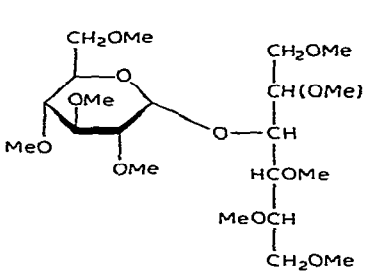
→3)-D-Man*p*-(1→4)-D-GlcA*p*-(1→4)-D-Glc*p*-(1→
4
↑
(*S*)MeCH(CO_2H)

Information on the configurations of the glycosidic bonds in the polysaccharide was obtained from ^{13}C-n m r data There are two peaks in the spectrum at 102 7 and 99 9 p.p m , corresponding to anomeric carbon atoms with relative intensities 1 2 Taking into account that C-1 of the mannosyl residue (either α or β) should

resonate[23] at ~100 p p m , the peak at 102 7 p p m is assigned to C-1 of either β-D-glucosyl or β-D-glucosyluronic acid residues Hence, the latter two residues must have different configurations at their anomeric centres Since there are no peaks near 72 p p m in the spectrum of the polysaccharide, corresponding to the C-5 resonance in a 4-*O*-substituted α-D-mannose[24], this unit must have the β-D configuration

For the elucidation of the configurations at C-1 in the D-glucosyl and D-glucosyluronic acid residues, the acetate of the polysaccharide was reduced with diborane[9], and then oxidised[25] with CrO_3–Ac_2O under conditions where only β-linked pyranose residues are oxidised to 5-hexulosonate residues[21] The product was reduced with lithium aluminium hydride and methylated to give the D-glucopyranosyl-hexitol derivative **21** The mass spectrum of **21** was identical with that reported earlier[26]. Methanolysis of **21** gave **22** and **23** in the ratio 1 1 which were identified by g l c –m s The isolation of the disaccharide **21** supported the sequence of monosaccharide units proposed above and indicated the α-D configuration of glucuronic acid and the β-D configurations of glucose and **1** in the polysaccharide

On the basis of the foregoing data, the repeating-unit **24** can be assigned to the polysaccharide



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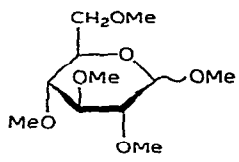

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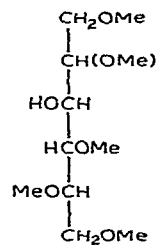

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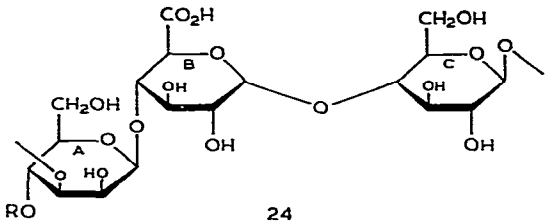
TABLE II

^{13}C-N M R DATA FOR THE EXTRACELLULAR POLYSACCHARIDE FROM *M lacticolum* STRAIN 121

Chemical shift (p p m)	*Integrated intensity*[a]	*Assignment*[b]	*Chemical shift (p p m)*	*Integrated intensity*[a]	*Assignment*[b]
18 1	1	1R	77 9	1	4A
60 9	2	6A, 6C	79 4	2	3A, 4C
68 5	1	2A	80 5	1	4B
70 6	1	5B	99 9	2	1A, 1B
73 4	2	2B, 3B	102 7	1	1C
74 6, 75 0, 75 8, 76 6	5	2C, 3C, 5C, 5A, 2R	176 2	1*	6B
			182 0	1*	3R

[a]The integrated values refer to comparison of carbon atoms linked to H atom(s), except for the entries marked with an asterisk (*) which refer to comparison of carbon atoms linked to carbonyl carbon atoms [b]The assignments refer to formula **24**



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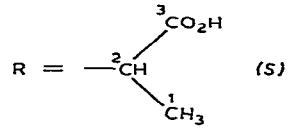

Determination of the structure of the polysaccharide allowed the ^{13}C-n m r. data to be interpreted fully (see Table II) These data may be useful in studies of similar polysaccharides produced by related strains of micro-organisms Comparison with the spectra of model compounds, 2-methoxypropionic acid, methyl (methyl 4-*O*-methyl-α-D-glucopyranosid)uronate[27], methyl β-maltoside[28], and the polysaccharide containing (1→3)-linked β-D-mannosyl residues[29], was used for this purpose Corrections reflecting the effect of the substitution at position 4 by the lactic acid residue were made in the last compound

EXPERIMENTAL

Mass spectra were measured with a Varian MAT CH-6 instrument at 70 eV A Varian MAT CH-111 Gnom instrument was used for g l c–m s G l c was performed on a Varian 1700 chromatograph fitted with columns (1–2 m) of 5% of NPGS, 5% of SE, or 3% of ECNSS-3M on Chromosorb W The temperature was programmed from 80–100° up to the highest possible value for the liquid phase used Optical rotations were measured with a Perkin–Elmer 141 instrument at 589 nm

^{13}C-N m r spectra were recorded for solutions in D_2O (internal Me_2SO) at 35–70° with a Bruker WP-60 instrument at 15 08 MHz The value 39 445 p p m (relative to internal Me_4Si) was established for the chemical shift of Me_2SO Pulse angles of 90° were used for polymers, and 30° for compounds of low molecular weight The acquisition time was 1 1 sec

Cells were grown as described previously[30]

Isolation of the polysaccharide — The polysaccharide was precipitated by using 5 65% aqueous cetylpyridinium bromide (0 5 L per L of culture medium) at 40° The precipitate was collected by centrifugation at 7000 r p m and washed with 0 1% aqueous cetylpyridinium bromide and water, and a solution in 4M NaCl was washed with chloroform (3 × 200 ml), and then dialysed for 3 days against tap water Acetone (4 vol) was added to the concentrated dialysate The precipitate was collected, dissolved in water, reprecipitated with acetone, and dissolved in water, and the solution was dialysed against distilled water for 3 days and then freeze-dried The yield was 4 9 g/L of culture medium, and the product had $[\alpha]_D^{20}$ −16° (*c* 0 75, water)

High-pressure liquid chromatography (Chromatronix Spectra-Physics Model 3500 instrument) on porous glass CPG-10 (Electro-Nucleonics) (3-m column, 0 4 ml of water/min, u v detection at 254 nm) at 20° showed the polysaccharide to be homogeneous, with molecular weight 150,000 (relative to that of dextran)

Hydrolysis of the polysaccharide (M H_2SO_4, 100°, 8 h) afforded glucose and glucuronic acid (identified by p c) together with the acid **1** (R_{Glc} 0 70) The polysaccharide, when reduced with diborane[9] and then hydrolysed, gave glucose and carboxyl-reduced **1** (detected by p c) The hydrolysis products were reduced with $NaBH_4$, acetylated with Ac_2O–pyridine, and identified by g l c–m s as glucitol acetate and the alditol acetate **24** formed in the ratio 2 1

The absolute configurations of glucose and glucuronic acid were determined by using D-glucose oxidase[31] and the hydrolysate of the carboxyl-reduced polysaccharide No glucose was detected in the oxidation products by p c or g l c of the alditol acetates

Identification of the monosaccharide units and determination of the absolute configuration of the acid **1** — (*a*) *Isolation of* **1** The polysaccharide (0 6 g) was hydrolysed with M H_2SO_4 (100°, 8 h) The hydrolysate was neutralised with Amberlite CG-400 (HCO_3^-) resin, which was washed with water until there was a negative reaction for sugars in the washings (phenol–H_2SO_4) and then with 20% acetic acid Preparative p c of the acidic monosaccharides on FN-11 paper, with 1-butanol–pyridine–water (6 4 3) or pyridine–ethyl acetate–acetic acid–water (5 5 1 3), yielded **1** (0 052 g), $[\alpha]_D^{20}$ +15° (*c* 2, water)

(*b*) The acid **1** (0 04 g) was heated with 2% methanolic HCl in a sealed tube (4 h, 100°) The solution was neutralised with NH_3 and then concentrated to dryness The residue was dissolved in methanol, saturated with NH_3, and left for 24 h at 0° The solution was concentrated, 10% aqueous NaOCl (5 ml) was added to the residue, and the mixture was kept for 4 h at 20° (see Ref 10) Then $NaBH_4$ (0 1 g) was added, and the solution was neutralised with AcOH and concentrated to dryness The residue was acetylated with Ac_2O–pyridine The acetylated products, extracted with $CHCl_3$, had $[\alpha]_D^{20}$ +34° (*c* 2 3, chloroform) Zemplén deacetylation then yielded methyl αβ-D-mannopyranoside (**3**, 0 022 g), $[\alpha]_D^{20}$ +67° (*c* 2, water) Hydrolysis of **3** (M H_2SO_4, 100°, 5 h) afforded D-mannose which was identified by p c and ion-exchange chromatography (Technicon SC-2), and by g l c of its alditol acetate

(*c*) *4-O-(1-Carboxyethyl)-D-mannose* (**9**) — To 1,6-anhydro-2 3-*O*-isopropylidene-β-D-mannopyranose[15 16] (1 g) in 1,4-dioxane (70 ml), NaH (0 58 g, 50% suspension in paraffin oil) was added with vigorous stirring Stirring was continued at 95° for 1 h, the mixture was then cooled to 65°, and (*S*)- or (*R*)-chloropropionic acid[17] (1 4 g) was added After stirring for 1 h, NaH (2 3 g) and 1,4-dioxane (30 ml) were added, and stirring was continued for 14 h at 65° The mixture was then cooled and water (100 ml) was added carefully to decompose excess of NaH 1,4-Dioxane was evaporated *in vacuo*, and the aqueous solution was extracted with $CHCl_3$ to remove mineral oil and unreacted anhydride, acidified with 2 5M HCl to pH 3, and

extracted with $CHCl_3$ (3 × 100 ml) The organic layer was washed with water, dried (Na_2SO_4), and concentrated The residue was eluted from a column of silica gel to yield **8a** (0 11 g, 11%), $[\alpha]_D^{20}$ −11 5° (*c* 2, chloroform), and **8b** (0 15 g, 15%), $[\alpha]_D^{20}$ +11° (*c* 1)

Compound **8a** (0 2 g) was treated with boiling 3% HCl (10 ml) for 4 h. The cooled mixture was neutralised with Amberlite CG-400 (HCO_3^-) resin, which was washed with water and then with 20% AcOH The acidic eluate was concentrated to dryness, and a solution of the residue in water was treated with KU-2 (H^+) resin, filtered, and concentrated to give **9a** (0 16 g, 82%), $[\alpha]_D^{20}$ +14° (*c* 5 9, water) Similar hydrolysis of **8b** gave **9b**, $[\alpha]_D^{20}$ +57° (*c* 3 9, water)

(*d*) 2-Methoxypropionic acid was prepared by heating chloropropionic acid with methanolic MeONa (100°, 5 h) ^{13}C-N m r data (D_2O) pH 7 C-1, 179 7, C-2, 77 94, C-3, 18 44, and OMe, 57 3, pH 1 C-1, 177 3, C-2, 76 54, C-3, 18 13, and OMe, 57 7 p p m

Methylation analysis — The polysaccharide (1 g) was methylated by the Hakomori method[18] The reaction mixture was dialysed against tap water for 3 days and then concentrated *in vacuo* The product (1 22 g) had $[\alpha]_D^{20}$ −18 5° (*c* 2 43, chloroform)

The methylated polysaccharide (0 5 g) was treated with 1% methanolic HCl (100°, 4 h) in a sealed tube The solution was neutralised with aqueous ammonia and concentrated *in vacuo*, and the residue was extracted with $CHCl_3$

The products were fractionated by elution from a column of silica gel with a chloroform–acetone gradient Methyl 2,3,6-tri-*O*-methyl-α,β-D-glucopyranoside (**13**, 0.073 g), the aldobiouronic ester **14** (0 047 g), and the derivative **5** (0 067 g) of **1** were obtained Compound **13** was identified by g l c–m s[19] after conversion into the 4-*O*-CD_3 derivative

After deuteriomethylation[32], the aldobiouronic ester **14**[20] gave the following mass spectrum *m/e* 279 (6%), 236 (4), 219 (9), 204 (20), 201 (4), 187 (8), 172 (3 5), 169 (4), 155 (10), 149 (14), 145 (11), 131 (6), 127 (11), 115 (9), 111 (10), 104 (61), 101 (27), 88 (100), 75 (68), 73 (14), 71 (28), and 45 (35)

The 4-*O*-CD_3 derivative (**15**) of **14** and compound **5** were both reduced with $LiAlH_4$ in boiling ether (8 h), following by formolysis, hydrolysis, and reduction with $NaBD_4$ The resulting alditols were acetylated, and analysed by g l c–m s 1,5,6-Tri-*O*-acetyl-2,3-di-*O*-methyl-4-*O*-trideuteriomethyl and 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl derivatives of D-glucitol-*l*-*d* were identified as degradation products of **14**

4-*O*-(1-Acetoxy-2-propyl)-1,3,5-tri-*O*-acetyl-2,6-di-*O*-methyl-D-mannitol-*l*-*d* formed from **5** was characterised by its mass spectrum *m/e* 320 (65%), 247 (57), 218 (7), 187 (30), 160 (20), 158 (18), 154 (16), 147 (22), 129 (60), 126 (42), 118 (90), 115 (43), 101 (100), 91 (30), 87 (50), 59 (50), 45 (54), and 43 (95)

The methylated polysaccharide (0 1 g) was dried over P_2O_5 (10 h, 70°), and a solution in CH_2Cl_2 (5 ml) and ether (60 ml) was boiled under reflux with $LiAlH_4$ (0 5 g) for 8 h The reduced polysaccharide, isolated in the usual way, was benzylated with benzyl chloride according to the Hakomori procedure[18], neutralised with acetic

acid, and dialysed against tap water for 3 days The precipitate was collected, and reprecipitated several times with light petroleum from solution in chloroform The benzyl ether (30 mg) was treated with 2 5% methanolic HCl (4 h, 100°, sealed tube) The solution was neutralised with aqueous ammonia and concentrated to dryness After trideuteriomethylation of the residue, methyl 2,3,6-tri-*O*-methyl-4-*O*-trideuteriomethyl-D-glucopyranoside[19] (**10**), methyl 6-*O*-benzyl-2,3-di-*O*-methyl-4-*O*-trideuteriomethyl-D-glucopyranoside (**11**), and methyl 4-*O*-(1-benzyloxy-2-propyl)-2,6-di-*O*-methyl-3-*O*-trideuteriomethyl-D-mannopyranoside (**12**) were identified in the ratios 0 85 1 1 by g l c –m s Mass spectra **11**, *m/e* 298 (10%), 266 (2), 265 (2), 225 (25), 134 (10), 104 (77), 101 (52), 91 (95), 88 (100), 75 (90), 45 (30), and 44 (40), **12** *m/e* 356 (4%), 266 (14), 235 (46), 222 (13), 208 (11), 190 (21), 189 (8), 187 (7), 178 (7), 177 (5), 176 (6), 158 (18), 155 (13), 148 (30), 144 (45), 134 (27), 130 (23), 127 (14), 111 (18), 104 (46), 91 (100), 88 (64), 78 (67), 75 (60), 45 (62), and 44 (60)

Alkaline degradation of the methylated polysaccharide[21,22] — To a solution of the methylated polysaccharide (0 36 g, dried at 50° over P_2O_5 *in vacuo* for 6 h) in methyl sulphoxide (20 ml) were added 2,2-dimethoxypropane (1 ml) and toluene-*p*-sulphonic acid (5 mg) The mixture was stirred under nitrogen for 30 min, a solution of sodium methylsulphinylmethanide (from 0 5 g of NaH and 30 ml of Me_2SO) was added, and the mixture was left overnight at ambient temperature After neutralisation with AcOH, the solution was concentrated *in vacuo* at 50–55° and the residue was treated with boiling 10% AcOH for 1 h Solvents were evaporated, and the remaining solid was dissolved in *N*,*N*-dimethylformamide and treated[32] with CD_3I and Ag_2O After the usual work-up, the products were separated by chromatography on silica gel to give the disaccharide **18** (0 067 g)

A solution of **18** (0 01 g) in 3% methanolic HCl (5 ml) was heated in a sealed tube at 100° for 4 h, neutralised with aqueous ammonia, and concentrated A solution of the residue in chloroform was filtered and concentrated to give a mixture of methyl 2,3,6-tri-*O*-methyl-4-*O*-trideuteriomethyl-α,β-D-glucopyranoside (**10**) and methyl 4-*O*-[(*S*)-1-(methoxycarbonyl)ethyl]-2,6-di-*O*-methyl-α,β-D-mannopyranoside (**19**), which were identified by g l c –m s[19] in the ratio 1 1 The mass spectrum of **19** was as follows *m/e* 277 (1 5%), 245 (4), 244 (4), 217 (4), 216 (3 5), 203 (6), 200 (3), 199 (2 6), 187 (4), 185 (8), 159 (7), 158 (5), 157 (5), 156 (10), 155 (14), 143 (100), 128 (54), 127 (28), 115 (96), 99 (54), 97 (80), 88 (40), 87 (76), 85 (52), 83 (43), 75 (94), 74 (44), 73 (70), 72 (30), 71 (100), 59 (>100), and 45 (60)

Conventional reduction of **18** with $LiAlH_4$ in ether (8 h, boiling under reflux) and treatment of the products with methanolic HCl, as described above, was followed by Kuhn methylation[32] with CD_3I The product mixture contained, *inter alia*, methyl 2,3,6-tri-*O*-methyl-4-*O*-trideuteriomethyl-α,β-D-glucopyranoside (**10**) and methyl 2,6-di-*O*-methyl-4-*O*-[(*S*)-1-trideuteriomethoxy-2-propyl]-3-*O*-trideuteriomethyl-α,β-D-mannopyranoside (**20**), which were identified by g l c –m s in the ratio 1 1 The mass spectrum of **20** was as follows *m/e* 266 (73%), 248 (5), 240 (6), 234 (5), 222 (41), 208 (32), 201 (8), 190 (55), 162 (100), 158 (34), 155 (31), 148 (64),

145 (24), 134 (56), 130 (39), 127 (30), 111 (38), 104 (60), 102 (25), 101 (27), 91 (72), 88 (59), 87 (53), 78 (50), 76 (73), 71 (37), 48 (29), and 45 (25)

Oxidation[25] of the polysaccharide acetate — The acidic polysaccharide was reduced with diborane[9] and then treated with Ac_2O in pyridine–formamide To a solution of the product (0 2 g) in Ac_2O (3 ml) was added a solution of CrO_3 (0 6 g) in Ac_2O. The mixture was stirred at 50° for 2 h and then cooled, and water (20 ml) was added The mixture was neutralised with $CaCO_3$ and extracted with $CHCl_3$ (5 × 50 ml) The combined extracts were concentrated *in vacuo*, and the residue was dissolved in 50% aqueous methanol (20 ml) and reduced with $NaBH_4$ (0 2 g) for 10 h at room temperature The mixture was treated with KU-2 (H^+) resin and then concentrated, and the residual syrup was methylated (Hakomori[18]) The products were subjected to chromatography on silica gel The mass spectrum of the resulting 4-*O*-D-glucosylhexitol **21** was identical to that reported earlier[26] Methyl 2,3,4,6-tetra-*O*-methyl-α,β-D-glucopyranoside (**22**) and 1,2,3,5,6-penta-*O*-methylhexitol (**23**) were identified amongst the methanolysis products of **21** by g l c –m s in the ratio 1 1

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