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SOMATIC ANTIGENS OF SHIGELLA: THE STRUCTURE OF THE POLYSACCHARIDE CHAIN OF *Shigella boydii* TYPE 2 LIPOPOLYSACCHARIDE

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ABSTRACT

The structure of the polysaccharide chain of *Shigella boydii* type 2 lipopolysaccharide was established using mainly ^{13}C-n.m.r. spectroscopy, partial hydrolysis, Smith degradation, and methylation analysis. The repeating unit of the polysaccharide was concluded to be a branched hexasaccharide, as follows.

→2)-β-D-Rib*f*-(1→4)-α-D-Gal*p*A-(1→3)-α-D-Glc*p*NAc-(1→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→
3
↑
1
β-D-Gal*f*

Acetaldehyde was detected in the hydrolysate of the lipopolysaccharide, but no evidence was obtained to indicate that acetaldehyde is located in the polysaccharide moiety.

INTRODUCTION

Systematic studies of the specific polysaccharide chains of the antigenic lipopolysaccharides (LPS) from *Shigella* have established the structures for the majority of the serotypes in three serological groups, namely, *Sh. dysenteriae*[1], *Sh. flexneri*[2], and *Sh. sonnei*[3]. The structural data on the specific polysaccharides of the fourth group, *Sh. boydii,* which consists of fifteen non-cross-reacting serotypes, are incomplete. In continuing our chemical and immunochemical studies[4,5] of *Shigella,* aimed at the structural analysis of the *Sh. boydii* and pathogenic *E. coli* species, we now report on the polysaccharide chain of the *Sh. boydii* type 2 specific LPS.

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RESULTS AND DISCUSSION

Characterisation of the polysaccharide. — LPS was isolated from dried bacterial cells by the standard extraction with hot, aqueous phenol followed by removal of nucleic acids by precipitation with Cetavlon[6]. The preparation exhibited the properties of the type-2 specific somatic antigen and was highly active in serological tests (passive haemagglutination and its inhibition) with homologous antiserum. Mild, acid degradation (aqueous 1% acetic acid, 100°, 1.5 h) of the LPS followed by fractionation of the products on Sephadex G-50 gave polysaccharide and oligosaccharide fractions. The latter were not investigated further.

The polysaccharide had $[\alpha]_D$ +30.5° (water), M_{GalA} 0.44 in paper electrophoresis (pH 8.0), and i.r. absorptions at 1560 and 1650 (Amide I and II) and 1728 cm^{-1} (carbonyl). The ^{1}H-n.m.r. spectrum contained, *inter alia,* signals for six anomeric protons (overlapping resonances, 4.48–5.15 p.p.m.), two *C*-methyl groups of 6-deoxyhexoses (superposed doublets at 1.1 p.p.m., *J* 6.5 Hz), and one acetamido group (1.85 p.p.m.). The ^{13}C-n.m.r. spectrum of the polysaccharide (see Table I) contained 37 signals, including six for anomeric carbons (96–110 p.p.m.), three for hydroxymethyl groups (61.5, 63.7, and 64.1 p.p.m.), two for an acetamido group (23.1 and 174 p.p.m.), one for a carbon carrying the acetamido group (53.1 p.p.m.), two for methyl groups of, presumably, 6-deoxyhexoses (17.9 and 18.0 p.p.m.), and one for a carbonyl group (176 p.p.m.), which were readily assigned.

The data presented indicated that the repeating unit of the polysaccharide is a hexasaccharide containing two 6-deoxy sugars, two aldoses, one 2-acetamido-2-deoxyhexose, and one uronic acid.

Monosaccharide composition. — Analysis of the polysaccharide hydrolysate (2M HCl, 100°, 3 h) by p.c., ion-exchange chromatography (including amino acid analysis), and paper electrophoresis revealed rhamnose, ribose, and galactose in the ratios 2:1:1, together with smaller amounts of 2-amino-2-deoxyglucose and galacturonic acid. Analysis of the hydrolysate by the deamination–g.l.c. procedure[7] gave ratios of 2:1:1:0.7 for rhamnitol, ribitol, galactitol, and 2,5-anhydromannitol. Similar analysis of the carboxyl-reduced polysaccharide revealed the same alditols, but in the ratios 2:1:2:0.9. The five sugar constituents were identified and quantified on one chromatogram after methanolysis (M HCl, 100°, 24 h) of the polysaccharide followed by *N,O*-acetylation and g.l.c. The D configuration of the 2-amino-2-deoxyglucose, galactose, and ribose and the L configuration of the rhamnose were assigned from optical rotation data for the monosaccharides isolated from the hydrolysate by p.c. The D configuration of the galacturonic acid followed from the susceptibility of all of the galactose in the hydrolysate of the carboxyl-reduced polysaccharide to D-galactose oxidase.

Methylation analysis. — The polysaccharide was methylated by successive application of the Hakomori and Purdie procedures. A second methylation by the Hakomori method caused extensive degradation with loss of the methylated de-

TABLE I

^{13}C-N.M.R DATA FOR *Shigella boydii* TYPE 2 POLYSACCHARIDE, GLYCOSIDE **2**, AND MODEL SACCHARIDES

Compound	*Constituent*	*Chemical shifts*[a]							
		C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C=O*[b]	*Ref.*
Native polysaccharide	β-D-Gal*f*-(1→	109.8 (175.5)	82.6	77.6	83.45	72.05	64.1		
	→2)-β-D-Rib*f*-(1→	107.75 (181.3)	81.3	71.3	83.7	63.7			
	→4)-α-D-Gal*p*A-(1→	100.5 (174.7)	71.7	71.3	78.6	71.6	175.05		
	→3)-α-D-Glc*p*NAc-(1→	96.6 (171.1)	53.1	79.1	70.7	72.9	61.5	174	
	→2)-α-L-Rha*p*-(1→	99.75 (172.9)	76.5	76.9	72.75		18.0		
	3								
	→2)-α-L-Rha*p*-(1→	101.7 (175.7)	81.85	70.7	73.5	70.4	17.9		
Disaccharide **2**	-D-Glc*p*NAc-(1→	96.8 (171.0)	54.8	71.6	71.1	73.2	61.7	175.4	13
	→2)-α-L-Rha*p*-(1→	97.9 (172.2)	77.2	70.7	73.2	70.3	17.8		
	→2)-Gro-al[e]	90.35 (165.0)[d]	81.35	60.9					
Methyl β-D-galactofuranoside		109.2	81.9	77.8	84.0	72.0	63.9		12
Methyl 2-*O*-methyl-β-D-ribofuranoside		106.4	84.5	71.1	84.5	63.6			12
Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside		99.3	54.85	72.4	71.3	72.9	61.9	175.6	19[c]
Methyl 2-acetamido-2-deoxy-3-*O*-methyl-α-D-glucopyranoside		99.4	53.2	81.6	70.5	72.9	61.7	175.6	19[c]
2-*O*-β-D-Glucopyranosyl-α-L-rhamnopyranose	(Rha)[f]	94.0	82.1	70.9	73.5	69.3	17.9		
3-*O*-β-D-Glucopyranosyl-α-L-rhamnopyranose	(Rha)[f]	95.0	71.8	81.0	72.5	69.5	18.0		

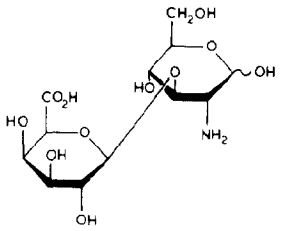
[a] $J_{C\text{-}1,H\text{-}1}$ values (Hz) in brackets. [b] Chemical shifts for the CH_3 carbon of the group of amino sugars were within the range 2.0–2.15 p.p.m. [c] Data corrected for the solvent and temperature of our measurement. [d] Signal of C-1 of glyceraldehyde residue; not given by **2a**. The spectrum of **2a** contained signals for $3CH_2OH$ groups from GlcN and glycerol in the range 61.7–62.6 p.p.m. [e] Gro-al = glyceraldehyde residue. [f] Chemical shifts for the rhamnose residue are given.

rivatives of galacturonic acid and ribose. Hydrolysis of the methylated polysaccharide followed by conventional conversion of the products into alditol acetates yielded 1,2,4-tri-*O*-acetyl-3,5-di-*O*-methylribitol, 1,4-di-*O*-acetyl-2,3,5,6-tetra-*O*-methylgalactitol, 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methylrhamnitol, and 1,2,3,5-tetra-*O*-acetyl-4-*O*-methylrhamnitol, which were identified[8] by g.l.c.–m.s. Methanolysis of the methylated polysaccharide and *N,O*-acetylation of the products followed by g.l.c.–m.s. gave, *inter alia*, methyl 3-*O*-acetyl-2-deoxy-4,6-di-*O*-methyl-2-(*N*-methylacetamido)-α(β)-glucopyranoside, the mass spectrum of which was identical to that published[9]. Reduction of the products in the foregoing methanolysate with lithium aluminium hydride followed by hydrolysis and conversion of the methylated aldoses into their alditol acetates gave 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methylgalactitol (from galacturonic acid) in addition to the alditols mentioned above.

These results suggest that the polysaccharide has a branched structure with the side chain terminated by a galactofuranosyl group, and with branch points at one of two rhamnosyl residues. Further, ribofuranose is substituted at position 2, 2-amino-2-deoxyglucose at position 3, one rhamnose at position 2, galacturonic acid at position 4, and the second rhamnose at positions 2 and 3.

Analysis of the degraded, methylated polysaccharide, formed after two successive Hakomori methylations, revealed the methylated sugars noted above, except that 2,3-di-*O*-methylgalacturonic acid and 3,5-di-*O*-methylribose were absent. This result points to the sequence →2)-Rib*f*-(1→4)-GalA-(1→ in the polysaccharide chain, and that this sequence is destroyed by a β-elimination mechanism[10].

Analysis of the above polysaccharide hydrolysate with an amino acid analyser revealed a ninhydrin-positive material that was eluted faster than 2-amino-2-deoxyglucose and could be separated from the latter by p.p.c. This product was an amphoteric disaccharide (**1**), which was immobile in paper electrophoresis at pH 4.5 (pyridine acetate buffer) and migrated towards the anode at pH 8 (triethylammonium hydrogencarbonate buffer). Quantification of the galacturonic acid by the carbazole method[11] and of 2-amino-2-deoxyglucose, using the amino acid analyser after hydrolysis (4M HCl, 100°, 16 h), revealed an equimolar ratio of the constituents. Oxidation of **1** with bromine followed by hydrolysis showed that the amino sugar had reacted and hence must have been the reducing moiety.



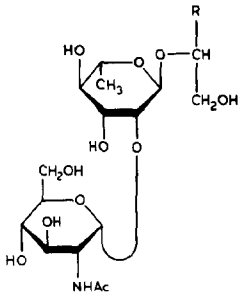
1

Partial hydrolysis. — The polysaccharide was hydrolysed with 25mM oxalic acid (100°, 3 h) to cleave the terminal galactofuranosyl groups. Gel filtration of the hydrolysate on Sephadex G-50 gave polymeric material and galactose.

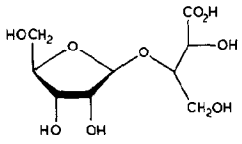
This modification of the polysaccharide caused a marked decrease in the intensities of the resonances at 109.80, 83.45, 82.60, 77.60, 72.05, and 64.10 p.p.m. in the ^{13}C-n.m.r. spectrum. These resonances corresponded to those for methyl β-D-galactofuranoside[12]. Methylation analysis of the modified polysaccharide revealed the absence of 2,3,5,6-tetra-*O*-methylgalactose and 4-*O*-methylrhamnose, whereas the content of 3,4-di-*O*-methylrhamnose was doubled and that of the other methylated sugars remained unchanged. These data establish that the branches in the polysaccharide are galactofuranosyl groups attached to O-3 of rhamnosyl residues in the main chain and that the modified polysaccharide contains the following sequence.

→2)-D-Rib*f*-(1→4)-D-Gal*p*A-(1→3)-D-Glc*p*NAc-(1→2)-L-Rha*p*-(1→2)-L-Rha*p*-(1→

Smith degradation. — The polysaccharide was subjected, in sequence, to periodate oxidation, borohydride reduction, and mild acid hydrolysis. Fractionation of the products on Sephadex G-15 gave the glycosides **2** and **3**.



2 R = CHO
2a R = CHDOH



3

Reduction of **2** with sodium borodeuteride followed by acid hydrolysis and deamination gave rhamnose and 2,5-anhydromannose in the ratio 1:1. Hydrolysis of reduced **2** with methanolic hydrogen chloride and acetylation of the products gave (g.l.c.–m.s.) [1-^{2}H]glycerol triacetate, indicating that **2** contained a 2-substituted glyceraldehyde residue. On the other hand, comparison of the ^{13}C-n.m.r. spectra of the polysaccharide and **2** revealed a downfield shift (~1.7 p.p.m.) of the C-2 resonance in the 2-acetamido-2-deoxyglucose residue, indicating that the latter was unsubstituted in **2** and, hence, was the non-reducing terminus. These data proved that the rhamnosyl residue in **2** was substituted by a galactofuranosyl group in the polysaccharide.

Glycoside **3** was purified by p.p.c.; it then had R_{Rib} 0.34, and migrated towards the anode in paper electrophoresis at pH 8 (M_{GalA} 1.45). Hydrolysis of **3** gave ribose, and methanolysis followed by acetylation gave methyl 2,3,4-triacetoxybutyrate and methyl 2,3,4-tri-*O*-acetyl-$\alpha(\beta)$-ribopyranoside in the ratio 1:1, providing additional proof that, in the polysaccharide, ribose was attached to galacturonic acid.

Linkage configurations. — In the ^{13}C-n.m.r. spectrum of the original polysaccharide, the first low-field signal at 109.80 p.p.m. was assigned to C-1 of a β-D-galactofuranosyl group, because this signal is absent from the spectrum of the modified, galactose-free polysaccharide. The second low-field signal at 107.75 p.p.m. was assigned to C-1 of a β-D-ribofuranosyl residue, because C-1 atoms of β-D-ribofuranosides resonate in the region 106.5–109.5 p.p.m., whereas those in α-D-ribofuranosides resonate[13] in the range 103–104 p.p.m.

The remaining four residues are α-pyranosides since the $J_{C\text{-}1,H\text{-}1}$ values were 171–175 Hz; values not exceeding 160 Hz would be expected for β-pyranosides[14].

The furanoid form for galacturonic acid is not excluded by the methylation analysis data, but the pyranoid form is indicated by the ^{13}C-n.m.r. data and the resistance of **1** to acid hydrolysis. The ^{13}C-n.m.r. data for the polysaccharide and related substances are given in Table I. The assignments are unequivocal only for those atoms the resonances of which were proved either by the chemical transformations performed during the structural analysis or by comparison with literature

data for model glycosides and disaccharides. Only for a few signals in a narrow range of chemical shifts are the assignments uncertain.

Detection of acetaldehyde. — The polysaccharide was inactive in the serological tests, whereas both the native lipopolysaccharides and the alkali-treated (0.5M NaOH, 100°, 30 min) lipopolysaccharide exhibited high activity. When the lipopolysaccharide was hydrolysed with acid and the products were treated with 2,4-dinitrophenylhydrazine, the hydrazone of acetaldehyde was formed, and identified by g.l.c.–m.s. The content of acetaldehyde in the lipopolysaccharide was comparable to that of each sugar in the repeating unit of the polysaccharide. However, the ^{13}C-n.m.r. data indicated that the acetaldehyde is not attached to the polysaccharide moiety of the lipopolysaccharide molecule, and its location remains obscure as does the reason for the loss of serological activity by the polysaccharide.

EXPERIMENTAL

General methods. — P.c. was performed on Whatman No. 1 paper with *A*, 1-butanol–pyridine–water (6:4:3); and *B*, ethyl acetate–acetic acid–formic acid–water (18:3:1:4). Electrophoresis was performed on the same paper with *A*, 25mM pyridine–acetic acid buffer (pH 4.5); and *B*, 0.05M triethylammonium hydrogencarbonate buffer (pH 8) at 27 V/cm. Reducing sugars were detected with alkaline silver nitrate, and non-reducing sugars after pretreatment with KIO_4; amino sugars were detected with ninhydrin. Ion-exchange chromatography was effected with a Technicon SC-2 analyser, and sodium borate buffers *C*, 0.3M (pH 7.1); and *D*, 0.5M (pH 8.9). Amino sugars were analysed on an amino acid analyser BC-200 (BIO-CAL), using a column (27 × 0.9) of Chromex UA-8 resin, and elution with 0.35M sodium citrate buffer (pH 5.28) at 65°. G.l.c. was performed with a Pye Unicam 104 gas chromatograph, and glass columns containing *A*, ECNSS-M on Gas Chrom Q (100–200 mesh); and *B*, OV-17; and *C*, a steel column containing 3% of SE-30 on diatomite. Gel chromatography was performed on Sephadex G-50 and G-15, using a pyridine–acetate buffer (pH 4.5). I.r. spectra were recorded for KBr pellets or for solutions in $CHCl_3$, using a UR-20 spectrometer. Optical rotations were determined with a Perkin–Elmer Model 141 polarimeter. N.m.r. spectra (^{13}C,^{1}H) were recorded with a Bruker-Physik WP 250 spectrometer. G.l.c.–m.s. was performed on a Varian-MAT GNOM 111 instrument, using the above liquid phases. Solvents were evaporated under diminished pressure at below 40°. Serological tests were accomplished by a published method[15].

Lipopolysaccharide and polysaccharide preparations. — Dry cells of *Shigella boydii* type 2 were extracted with aqueous 45% phenol at 70°, nucleic acids were precipitated with Cetavlon, and the LPS was precipitated by ultracentrifugation (4 h, 105,000*g*); yield, 6.2%. A suspension of LPS (2 g) in aqueous 1% acetic acid (200 mL) was heated for 1.5 h at 100°, the precipitate of lipid was separated by ultracentrifugation (0.5 h, 105,000*g*), the supernatant was freeze-dried, and the residue was eluted from a column (67 × 4 cm) of Sephadex G-50, to give polysac-

charide (420 mg) and oligosaccharides (120 mg). The polysaccharide migrated as a single zone towards the anode in electrophoresis (buffer *A*). LPS and alkali-treated LPS (0.5M NaOH, 100°, 0.5 h) inhibited passive haemagglutination in the range 6.75–7.80 μg with antiserum to a live culture.

Composition of polysaccharide. — The polysaccharide (5 mg) was hydrolysed (100°, 3 h) with 2M HCl (1 mL). P.c. of the hydrolysate revealed rhamnose, ribose, galactose, and 2-amino-2-deoxyglucose (solvent *A*), and galacturonic acid (solvent *B*). Use of the sugar analyser showed the ratio of rhamnose to ribose to be 2:1 (buffer *C*), and that of rhamnose + ribose to galactose to be 3:1 (buffer *D*). G.l.c. after deamination revealed the ratio of 2-amino-2-deoxyglucose to galactose to be 0.7:1. A solution of the polysaccharide (50 mg) in dimethyl sulphoxide (1 mL) was treated with ethereal diazomethane, to give a methyl ester that was further reduced with $NaBH_4$ in borate buffer[16]. A hydrolysate of the reduced polysaccharide contained the above monosaccharides, but the ratio of galactose to rhamnose was 1:1. Galactose isolated from the hydrolysate by p.p.c., reacted completely when incubated with D-galactose oxidase for 2 h. The polysaccharide (25 mg) was hydrolysed (100°, 3 h) with 2M HCl (10 mL). The hydrolysate was concentrated *in vacuo* over NaOH, and the residue was subjected to paper electrophoresis (buffer *B*). The zone with M_{GalA} 0.8 was excised and eluted with water to give disaccharide **1**, R_{Glc} 0.17 (p.c., solvent *A*); the retention time T_{GlcN} on the amino acid analyser was 0.3. To a solution of **1** in water (10 mL) were added $BaCO_3$ (30 mg) and bromine (0.1 mL). The mixture was kept for 2 days in the dark with occasional shaking. Bromine was removed by using a stream of nitrogen, and the solution was freeze-dried. The residue was hydrolysed (6M HCl, 100°, 6 h). The hydrolysate did not contain 2-amino-2-deoxyglucose, whereas **1** contained 2-amino-2-deoxyglucose and uronic acid in equimolar amounts. The uronic acid was determined by the carbazole reaction[11].

Modified polysaccharide. — A solution of the polysaccharide (150 mg) in 25mM oxalic acid (100 mL) was heated for 3 h at 100°, cooled, and freeze-dried. The residue was eluted from a column (90 × 3 cm) of Sephadex G-50 with an aqueous solution of pyridine (4 mL/L) and acetic acid (10 mL/L), to give modified polysaccharide (100 mg) and monosaccharide that consisted of galactose together with small proportions of rhamnose and ribose. Separate solutions of the native and modified polysaccharides (5 mg) in 0.2M KIO_4 (5 mL) were kept in the dark for 2 days, and then $NaBH_4$ (50 mg) was added to each solution. After 3 h, each mixture was neutralised with acetic acid and freeze-dried. Each residue was desalted by passing through a column (30 × 1.7 cm) of Sephadex G-50 and then hydrolysed (2M HCl, 100°, 2 h), and the products were analysed by ion-exchange chromatography. The ratio of rhamnose to ribose was 1:1 for the native polysaccharide, whereas the modified polysaccharide contained ribose and only traces of rhamnose.

Methylation analysis. — The polysaccharide (50 mg) was dried over P_2O_5 (50°, 3 h), and methylated by the standard Hakomori[17] method. After removal of

dimethyl sulphoxide *in vacuo* at 40–50°, the methylated polysaccharide was eluted from a column (30 × 1.7 cm) of Sephadex G-50 with an aqueous solution of pyridine (4 mL/L) and acetic acid (10 mL/L), to give a syrup (45 mg) that exhibited i.r. absorption for hydroxyl at 3200–3600 cm^{-1}. After an additional Purdie methylation[18], there was no such i.r. absorption. Alditol acetates were prepared conventionally from the methylated polysaccharide, and analysed by g.l.c. (column *A*, 150°) and g.l.c.–m.s. (relative retention-time in brackets). The following fully acetylated derivatives were identified: 3,5-di-*O*-methylribitol (1.00), 3,4-di-*O*-methylrhamnitol (1.24), 2,3,5,6-tetra-*O*-methylgalactitol (1.59), and 4-*O*-methylrhamnitol (2.17).

The methylated polysaccharide was treated in a sealed ampoule with methanolic M hydrogen chloride (100°, 20 h). The mixture was concentrated *in vacuo* at ~20°. The residue was acetylated, and the products were analysed by g.l.c.–m.s. Among other glycosides, only one derivative of an amino sugar was identified, namely, methyl 3-*O*-acetyl-2-deoxy-4,6-di-*O*-methyl-2-(*N*-methylacetamido)-D-glucopyranoside: *m/z* 43, 45, 71, 73, 86, 98, 101, 115, 126, 142, 157, 169, 186, 187, 214, 228, and 246 (*cf.* ref. 9).

Smith degradation. — A solution of the polysaccharide (500 mg) in 0.2M KIO_4 (66 mL) was kept in the dark for 2 days, $NaBH_4$ (3.5 g) was added, and, after 3 h, the mixture was neutralised with acetic acid and freeze-dried. The residue was desalted by using a column (62 × 4 cm) of Sephadex G-50, and the product (450 mg) was hydrolysed with 0.5M HCl (150 mL) at room temperature for 20 h. The hydrolysate was freeze-dried, and the residue was eluted from a column (90 × 1.7 cm) of Sephadex G-15 with aqueous pyridine–acetic acid (see above), to give glycoside **2** (80 mg), $[\alpha]_D$ +61° (*c* 1, water), and glycoside **3** (40 mg), which was slightly contaminated with ribose.

Glycoside **2** (80 mg in 2 mL of water) was reduced with $NaBD_4$ (~20°, 1 day) to give, after neutralisation with KU-2(H^+) resin followed by gel chromatography on Sephadex G-15, reduced glycoside **2a** (72 mg), $[\alpha]_D$ +84° (*c* 1, water), R_F 0.27 (solvent *A*). After hydrolysis and deamination, both **2** and **2a** gave rhamnose and 2,5-anhydromannose in the ratio 1:1.

Reduced glycoside **2a** (2 mg) was heated (100°, 3 h) with methanolic M hydrogen chloride (1 mL). The methanolysate was concentrated *in vacuo*, the residue was acetylated, and the product analysed by g.l.c.–m.s. The mixture contained [1-^{2}H]glycerol triacetate (columns *A* and *B*, 120°); *m/z*, *inter alia*, 103, 104, 145, and 146.

Glycoside **3** was freed from ribose by p.p.c. (solvent *A*, R_{Rib} 0.34), and migrated towards the anode in paper electrophoresis as a single zone (buffer *B*, detection with alkaline $AgNO_3$ by heating the paper over boiling water). A hydrolysate (M HCl, 100°, 2 h) of **3** contained (p.c., ion-exchange chromatography) ribose as the only sugar.

Methanolysis (100°, 3 h) of **3** (methanolic M hydrogen chloride) followed by acetylation gave (g.l.c., column *B*, 140°) a mixture of methyl 2,3,4-triacetoxybuty-

rate and methyl 2,3,4-tri-*O*-acetyl-$\alpha(\beta)$-riboside. The latter derivative was indistinguishable from an authentic sample. Mass spectrum of the former, *m/z*: 217 (C-2,3,4), 203 (C-1,2,3), 175 (217 − CH_2=C=O), 174 (M − Ac_2O), 161 (203 − CH_2=C=O), 145 (C-3,4), 143 (203 − AcOH), 131 (C-1,2), 115 (175 − AcOH), and 43.

Detection of acetaldehyde. — A solution of LPS (50 mg) in 0.5M HCl (2 mL) was kept in a sealed tube for 15 min at 100°, cooled, and treated with saturated 2,4-dinitrophenylhydrazine in 2M HCl (10 mL) for 1 h. The mixture was extracted with chloroform, the extract was concentrated, and the residue was analysed by g.l.c. (column *C*, 196°). Acetaldehyde dinitrophenylhydrazone was detected, and identified by g.l.c.–m.s.: *m/z* 51, 63, 64, 77, 79, 91, 104, 105, 122, 152, 180, 207, and 224). An analogous result was obtained with the alkali-treated LPS. No acetaldehyde was found in the polysaccharide.

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