Carbohydrate Research, 114 (1983) 267–276
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

STRUCTURAL INVESTIGATIONS ON THE CORE OLIGOSACCHARIDE OF *Aeromonas hydrophila* (CHEMOTYPE II) LIPOPOLYSACCHARIDE

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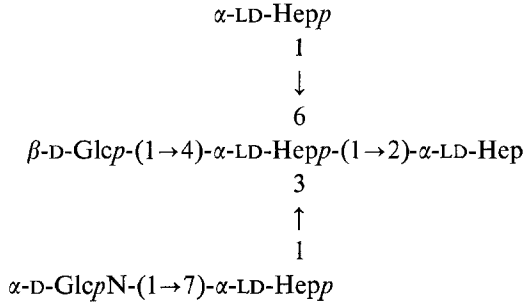
(Received October 14th, 1982; accepted for publication, November 4th, 1982)

ABSTRACT

The structure of the core oligosaccharide of *Aeromonas hydrophila* (Chemotype II) lipopolysaccharide has been investigated. The studies involved the use of nuclear magnetic resonance spectroscopy, methylation analysis, partial hydrolysis with acid, periodate oxidation, Smith degradation, nitrous acid deamination, and oxidation with chromium trioxide. As a result of these studies the following structure is proposed (abbreviation: α-LD-Hep = L-*glycero*-α-D-*manno*-heptose).



INTRODUCTION

Aeromonas hydrophila is a pathogenic bacterium normally associated with outbreaks of disease in fresh-water fish, particularly salmonid species, where the disease is usually described as motile Aeromonas septicemia[1]. Recently, however, strains of this bacterium have been isolated as primary pathogens of serious consequence in humans; on occasion, death has resulted from infection therewith[2]. Studies conducted on the serological identity of members of the *Aeromonas hydrophila* group indicated an extraordinary amount of heterogeneity[3]. Recent investigations by traditional biochemical reactions[4], and by analysis of the composition of the core

oligosaccharide of the cell-surface lipopolysaccharide[5], have indicated three major sub-groups.

Interest in the structure and immunological properties of the cell-surface polysaccharide of the different chemotypes of this Gram-negative bacterium has increased, as little is known of the biochemical basis of pathogenicity of these species. The detailed core-structure of *Aeromonas hydrophila* Chemotype III has recently been reported[6]. In the present investigation, we report the structure of the core oligosaccharide of Chemotype II.

RESULTS AND DISCUSSION

The lipopolysaccharide (LPS) was isolated by the aqueous phenol method of Westphal and Jann[7], and purified by electrodialysis[8]. The core oligosaccharide was obtained by mild hydrolysis of the lipopolysaccharide with acetic acid, and purified by chromatography on columns of Sephadex G-50 and Sephadex G-15. As with the lipopolysaccharide[6] of *Aeromonas hydrophila* Chemotype III, the core oligosaccharide of this strain of Chemotype II is the main constituent of the polysaccharide portion of the LPS.

The purified core-oligosaccharide was a white, water-soluble powder that had $[\alpha]_D^{23}$ +85° (*c* 0.5, water) and was essentially free from nucleic acid. Analysis of the core oligosaccharide indicated that it was composed of residues of D-glucose, L-*glycero*-D-*manno*-heptose, and 2-amino-2-deoxy-D-glucose in the molar ratios of 1:4:0.8. The optical rotations of the sugars isolated from the hydrolyzate established that the glucose and the 2-amino-2-deoxyglucose had the D configuration. The heptose was identified as L-*glycero*-D-*manno*-heptose by the retention time of its alditol acetate in g.l.c. Analytical studies on the core oligosaccharide indicated that it did not contain phosphorus[9], fatty acid[10], 2-aminoethanol[11], protein[12], or 3-deoxy-D-*manno*-2-octulosonic acid[13] (KDO).

The ^{1}H-n.m.r. spectrum of the core oligosaccharide[14] showed, *inter alia*, a signal at 7.26 p.p.m. assigned to the proton of the NH of 2-amino-2-deoxy-D-glucose, five anomeric signals indicating an α configuration ($J_{1,2} \sim 2$ Hz), at 5.68 (1 H), 5.53 (1 H), 5.43 (1 H), and 5.34 (2 H) p.p.m., and one anomeric signal at 4.61 p.p.m. ($J_{1,2}$ 8 Hz) indicating a β configuration. There were no signals for pyruvate, acetate, or the methyl protons of o-deoxy sugars.

The ^{13}C-n.m.r. spectrum showed, *inter alia*, six anomeric carbon atoms resonating at 103.10, 102.50, 100.47, 100.20 (double signal), and 96.5 p.p.m. The chemical shift of the anomeric carbon atom at 103.10 p.p.m. was attributed to C-1 of the D-glucosyl unit, and the $^1J_{CH}$ value was 160.2 Hz, suggesting that it had the β configuration[15]. Due to the lack of reported chemical-shifts for L-*glycero*-D-*manno*-heptose, no attempt has been made to assign the anomeric signals between 102.50 and 96.5 p.p.m., but their $^1J_{CH}$ values lay between 170.0 and 170.4 Hz, suggesting the α configuration[15]. The chemical shifts at 61.60 and 61.95 p.p.m. correspond to the non-linked C-6

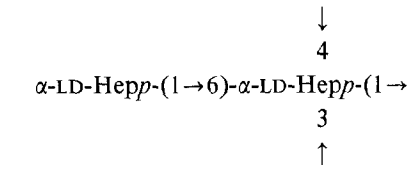
atom of the primary hydroxymethyl group of hexoses. The chemical shift at 54.95 p.p.m. corresponds to the C-2 deoxy function of 2-amino-2-deoxy-D-glucose.

Methylation of the core oligosaccharide by the Hakomori method[16], followed by hydrolysis with 2M trifluoroacetic acid, reduction, and derivatization as the alditol acetates, afforded 2,3,4,6-tetra-*O*-methylglucose, 2,3,4,6,7-penta-*O*-methylheptose, 3,4,6,7-tetra-*O*-methylheptose, and 2,7-di-*O*-methylheptose, in the molar ratios of ~1 : 1 : 1 : 1, identified by g.l.c.–m.s.[17–19] (see Table II).

The presence of a 2,7-di-*O*-methylheptose is attributable to a double branch-point, and that of 2,3,4,6-tetra-*O*-methylglucose and 2,3,4,6,7-penta-*O*-methylheptose, to two nonreducing, terminal groups. The presence of 3,4,6,7-tetra-*O*-methylheptose is assigned to a residue linked through O-2. It is clear from the stoichiometric composition of the hydrolysis products of the methylated core-oligosaccharide that alditol acetates derived from 2-amino-2-deoxy-D-glucose and 1 mol of L-*glycero*-D-*manno*-heptose are missing. This result suggested that these two residues were glycosidically linked by a linkage resistant to the hydrolysis conditions used.

When gas–liquid chromatography of the partially methylated alditol acetates was conducted under programmed temperature conditions [190° (32 min), 16° per min to 270° (32 min)], a slow-moving component was observed whose identity was established by mass spectrometry as 1,5-di-*O*-acetyl-7-*O*-[2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-D-glucopyranosyl]-2,3,4,6-tetra-*O*-methyl-L-*glycero*-D-*manno*-heptitol. This structure was confirmed by the fragmentation pattern, which gave primary ions at *m/z* 260 (for the nonreducing 2-amino-2-deoxyglucosyl group) and *m/z* 335 (for the heptitol residue), together with secondary ions, *inter alia*, *m/z* 228 (260 — 32), 196 (228 — 32), 161, 154 (196 — 32), 142, 129, 117, 101, 88, 87, 45, and 43. This result indicated that this disaccharide-alditol consists of a terminal, non-reducing 2-amino-2-deoxy-D-glucosyl group linked to O-7 of L-*glycero*-D-*manno*-heptitol.

Partial hydrolysis of the core oligosaccharide with 0.5M sulfuric acid for 1 h at 100°, followed by purification on Sephadex G-15, yielded a degraded oligosaccharide. Methylation of the degraded oligosaccharide, followed by hydrolysis, reduction, and g.l.c.–m.s. of the alditol acetates, yielded 2,3,4,6-tetra-*O*-methylglucose, 3,4,6,7-tetra-*O*-methylheptose, and 2,6,7-tri-*O*-methylheptose in the molar ratios of ~1 : 1 : 1. This indicated that the 2,7-di-*O*-methylheptose found on methylation and hydrolysis of the original material had been replaced by 2,6,7-tri-*O*-methylheptose, and that the terminal LD-heptosyl unit is linked to the branched L-*glycero*-D-*mannose*-heptose residue through O-6, as in the partial structure **1**.



1

TABLE I

SUGAR ANALYSIS OF THE CORE OLIGOSACCHARIDE AND DERIVED PRODUCTS[a]

Oligosaccharide	D-*Glc*	D-*GlcN*[b]	LD-*Hep*	2,5-*Anhydro*-D-*Man*	*Glycerol*	*Threitol*
Original core	16.5	14.2	69.3			-
Degraded oligosaccharide	21.2	15.0	63.8	-		
Periodate-oxidized core	-		36.5		49.5	16.0
Smith-degraded core (**2**)			71.5		28.5	-
Deaminated core (**3**)	18.4	-	69.9	11.7		-
Disaccharide **4** isolated from 2M HCl hydrolysis	-	41.0	59.0			
Deaminated disaccharide **4**, reduced, acetylated	---	--	61.0	39.0		--
Chromium trioxide-oxidized core (**5**)		16.0	84.0			-

[a]In mole percent, as alditol acetate derivative. [b]Determined by analytical assays

The core oligosaccharide was oxidized with periodate, and the product was reduced, and the product purified by chromatography on Sephadex G-15. Sugar analysis of the purified polyol indicated the presence of L-*glycero*-D-*manno*-heptose, glycerol, and threitol in the molar ratios of ~1:5:1. It is evident, that the 7-*O*-substituted L-*glycero*-D-*manno*-heptose unit is the one responsible for the formation of the threitol residue. This result showed that 1 mol of D-glucose, 1 mol of 2-amino-2-deoxy-D-glucose, and 3 mol of L-*glycero*-D-*manno*-heptose had been oxidized, thus confirming the results of the methylation analysis.

The core oligosaccharide was subjected to Smith degradation, that is, periodate oxidation, borohydride reduction, and mild hydrolysis with acid. The Smith-degraded product was purified by chromatography on Sephadex G-15. Sugar analysis of the resulting product indicated the presence of L-*glycero*-D-*manno*-heptose and glycerol (see Table I).

Methylation analysis of the Smith-degraded product yielded 2,3,4,6,7-penta-*O*-methylheptose and 1,3-di-*O*-methylglycerol in the molar ratio of ~1:1. This result indicated that the 2,7-di-*O*-methylheptose found in the analysis of the original material had been replaced by 2,3,4,6,7-penta-*O*-methylheptose, and that the original, branched heptosyl residue was linked through O-2 of the L-*glycero*-D-*manno*-heptose unit that yielded the 1,3-di-*O*-methylglycerol. Hence, the structure of the Smith-degraded product (**2**) is as shown.

α-LD-Hep*p*-(1→2)-glycerol

2

In order to establish the sequence and anomeric configuration of the glycosidic linkages of the sugar units of the core oligosaccharide, and to corroborate the

TABLE II

METHYLATION ANALYSIS OF THE CORE OLIGOSACCHARIDE AND DERIVED PRODUCTS

Methylated sugar[a]	*Core (original)*	*Degraded oligo-saccharide*	*Deaminated core* (3)	*CrO_3-oxidized core* (5)	*Linkages*
2,3,4,6-Me_4-D-Glc	26.73	36.1	21.2	3.0	D-Glc(1→
2,3,4,6,7-Me_5-LD-Hep	28.0	—	33.5	27.5	LD-Hep(1→
3,4,6,7-Me_4-LD-Hep	25.7	34.2	23.1	31.5	→2)-LD-Hep-(1→
2,3,4,6-Me_4-LD-Hep	—	—	7.0	13.0	→7)-LD-Hep-(1→
					↓ 4
2,6,7-Me_3-LD-Hep	—	29.7	—	—	→3)-LD-Hep-(1→
2,4,7-Me_3-LD-Hep	—	—	—	23.0	→3)-LD-Hep-(1→
					4 ↑
					↓ 6
2,7-Me_2-LD-Hep	19.8	—	15.2	2.0	→4)-LD-Hep-(1→
					3 ↑

[a]In mole percent, as alditol acetate derivative. 2,3,4,6-Me_4-D-Glc = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, *etc.*

existence of the 2-amino-2-deoxy-D-glucosyl group, subsequent work was directed towards characterization of the oligosaccharide obtained by nitrous acid deamination[20], hydrolysis with hydrochloric acid, and oxidation with chromium trioxide[21].

The core oligosaccharide was treated with nitrous acid, followed by reduction of the product with borohydride. The 2,5-anhydromannose released was acetylated, and the ester identified by g.l.c.–m.s. Sugar analysis gave D-glucose and L-*glycero*-D-*manno*-heptose (1 : 4). The deaminated core-oligosaccharide (**3**) was purified by chromatography on Sephadex G-15, and the resulting product was methylated; subsequent hydrolysis, reduction, and g.l.c.–m.s. of the alditol acetates yielded 2,3,4,6-tetra-*O*-methylglucose, 2,3,4,6,7-penta-*O*-methylheptose, 3,4,6,7-tetra-*O*-methylheptose, 2,3,4,6-tetra-*O*-methylheptose, and 2,7-di-*O*-methylheptose in the molar ratios of ~1 : 1.7 : 1 : 0.3 : 1 (see Table II). The limited proportion (0.3) of 2,3,4,6-tetra-*O*-methylheptose arising from the 7-*O*-substituted LD-heptosyl unit, considered together with the increase in the molar proportion (1.7) of 2,3,4,6,7-penta-*O*-methylheptose, reconfirmed that the 2-amino-2-deoxy-D-glucosyl group is linked to O-7 of this L-*glycero*-D-*manno*-heptose unit, which is, in turn, linked to the branched L-*glycero*-D-*manno*-heptose unit, either through O-4 or O-3. Hence, the structure of the deaminated core-oligosaccharide (**3**) is as shown.

α-LD-Hep*p*
|
↓
6
α-LD-Hep*p*-(1→4/3)-α-LD-Hep*p*-(1→2)-α-LD-Hep*p*
3/4
↑
|
β-D-Glc*p*
3

Attempts were made to isolate an oligosaccharide containing the 2-amino-2-deoxy-D-glucose unit. Thus, partial hydrolysis of the core oligosaccharide with 2M hydrochloric acid for 1 h at 100°, followed by purification by high-voltage electrophoresis, yielded a disaccharide (**4**) that had M_{GlcN} 0.6. When disaccharide **4** was subjected to nitrous acid deamination and the products reduced and acetylated, it afforded 1,3,4,6-tetra-*O*-acetyl-2,5-anhydro-D-mannose and the alditol acetate of LD-heptose in stoichiometric proportions. Acetylation of disaccharide **4**, followed by methylation, hydrolysis, reduction, acetylation, and g.l.c.–m.s. of the alditol acetates gave 2,3,4,6-tetra-*O*-methylheptose, reconfirming the results of the nitrous acid deamination. Hence, the structure of disaccharide **4** is as shown.

α-D-Glc*p*N-(1→7)-α-LD-Hep*p*
4

The anomeric configurations of the various glycosyl groups were also ascertained. The core oligosaccharide was acetylated, and the product subjected to oxidation with chromium trioxide[21]. During the oxidation, only the D-glucose unit was oxidized, indicating that it was β-linked. The oxidized core-oligosaccharide was purified by chromatography on Sephadex LH-20. Methylation analysis of the purified, oxidized core-oligosaccharide gave 2,3,4,6,7-penta-*O*-methylheptose, 3,4,6,7-tetra-*O*-methylheptose, 2,3,4,6-tetra-*O*-methylheptose, and 2,4,7-tri-*O*-methylheptose in the molar ratios of ~1:1:0.5:0.8 (see Table II), together with 1,5-di-*O*-acetyl-7-*O*-[2-deoxy-3,4,6-tri-*O*-methyl-2-(methylacetamido)-D-glucopyranosyl]-2,3,4,6-tetra-*O*-methyl-L-*glycero*-D-*manno*-heptitol (0.5).

This result indicated that the 2,7-di-*O*-methylheptose found in the methylated, original material had been replaced by 2,4,7-tri-*O*-methylheptose, and that the β-D-glucosyl terminal unit was linked through O-4 of the branched L-*glycero*-D-*manno*-heptose residue. It also indicated that disaccharide **4** is linked through O-3 of the branched L-*glycero*-D-*manno*-heptose residue. Hence, the partial structure of the chromium trioxide-oxidized, core oligosaccharide (**5**) is as shown

α-LD-Hep*p*
1
↓
6
α-D-Glc*p*N-(1→7)-α-LD-Hep*p*-(1→3)-α-LD-Hep*p*-(1→2)-α-LD-Hep*p*
5

The oligosaccharide degraded with 0.5M sulfuric acid was also acetylated, and the product submitted to oxidation with chromium trioxide. During the oxidation, only the D-glucose was oxidized, reconfirming the fact that it was β-linked.

In the methylation analysis of the oxidized, degraded oligosaccharide, the disappearance of 2,6,7-tri-*O*-methylheptose, and the appearance of 2,4,6,7-tetra-*O*-methylheptose, indicated that the β-D-glucosyl group was linked to the branched LD-heptose residue through O-4.

In most of the lipopolysaccharide structures studied so far, three molecules of KDO (3-deoxy-D-*manno*-2-octulosonic acid) have been found to join the core oligosaccharide to the lipid A. As previously reported[6], in the structure of Chemotype III, KDO also appears to be absent in the core oligosaccharide of *Aeromonas hydrophila* Chemotype II. From the combined evidence, the core oligosaccharide from *Aeromonas hydrophila* Chemotype II has the following structure.

α-LD-Hep*p*
1
↓
6
β-D-Glc*p*-(1→4)-α-LD-Hep*p*-(1→2)-α-LD-Hep*p*
3
↑
1
α-D-Glc*p*N-(1→7)-α-LD-Hep*p*

EXPERIMENTAL

General methods. — Analytical paper-chromatography was performed on Whatman No. 1 paper, and Whatman No. 3 MM paper was used for preparative purposes; in both cases, 8:2:1 ethyl acetate–pyridine–water was the developing solvent. Chromatograms were developed with alkaline silver nitrate. High-voltage paper electrophoresis was conducted for 90 min in a Shandon flat-bed, high-voltage, electrophoresis apparatus, using a buffer of 5:2:43 pyridine–acetic acid–water at pH 5.4 (2 kV, 170 mA). The amino sugar was detected by spraying with ninhydrin, or by using the alkaline silver nitrate reagent. Thin-layer chromatography was conducted on plates precoated with cellulose (100 μm; E. Merck, Darmstadt)

with 5:5:1:3 ethyl acetate–pyridine–acetic acid–water in an atmosphere saturated with 40:11:6 ethyl acetate–pyridine–water.

Gas–liquid chromatography of the acetylated alditols and partially methylated alditol acetates was performed on packed columns (1.83 m × 2 mm i.d.) of 1.5% of Silar 7CP on Gas Chrom Q (100–120 mesh) in a Perkin–Elmer Model 3920 gas chromatograph operated isothermally at various temperatures ranging from 190 to 220° with a helium flow of 40 mL/min. G.l.c. was also performed in a 25-m, W.C.O.T. CP-Sil 5 (0.25-μm) column (Chrompack, the Netherlands) at 180°. Gas–liquid chromatography–mass spectrometry was performed in a Hewlett–Packard Model 5981A GC/MS instrument controlled by a 5934A data system, with a membrane separator, a source temperature of 200°, and an ionizing voltage of 70 eV. ^{13}C- and ^{1}H-N.m.r. spectra were recorded at 62.86 and 250 MHz, respectively, in the pulsed, Fourier-transform mode with a CAMECA 250-MHz spectrometer. The ^{1}H-n.m.r. spectra were recorded for solutions in D_2O, and the chemical shifts are expressed relative to sodium 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentanoate. Carbon-13 chemical-shifts are given in p.p.m., and are expressed relative to internal acetone (δ 31.07), with complete proton-decoupling. Coupling constants ($^{1}J_{^{13}C,^{1}H}$), expressed in Hz, were determined by the gated-decoupling technique[15].

Bacterial culture. – The culture was from a strain of *A. hydrophila* originally isolated from canned milk, and was a gift from the National Collection of Marine Bacteria, Aberdeen, Scotland (strain NCMB86). The cells used to isolate the lipopolysaccharide were grown to the stationary phase in Trypticase Soy Broth, without added dextrose (Baltimore Biological Laboratories).

Isolation, and purification, of the core oligosaccharide. – The lipopolysaccharide was isolated by the aqueous phenol method of Westphal and Jann[7], and the core oligosaccharide was isolated as previously described[5,6].

Hydrolysis of the core oligosaccharide, and sugar analysis. – For the neutral glycose analysis, the core oligosaccharide (1 mg) was hydrolyzed with either 2M trifluoroacetic acid or 0.5M sulfuric acid for 8 h at 100°, followed by neutralization and concentration. The monosaccharides liberated were converted into alditol acetates by reduction and acetylation, and these were identified by g.l.c.–m.s. analysis using *myo*-inositol as the internal standard. For the analysis of 2-amino-2-deoxy-D-glucose, the sample (1 mg) was hydrolyzed for 8 h at 100° with 4M hydrochloric acid. The 2-amino-2-deoxy-D-glucose was also determined with an amino acid analyzer[22], and assayed according to the method of Strominger *et al.*[23]. D-Glucose was isolated by paper chromatography and thin-layer chromatography, and had $[\alpha]_D^{23}$ +49° (*c* 0.01, water). The identity of L-*glycero*-D-*manno*-heptose was established by experimental comparison of its g.l.c. retention-time and its mass spectrum with those of an authentic sample.

Methylation analysis. – All compounds were methylated by the Hakomori method[16]. The methylated oligosaccharides were hydrolyzed with 2M trifluoroacetic acid for 12 h at 100°. The resulting, partially methylated sugars were reduced, the

alditols acetylated, and the alditol acetate derivatives analyzed by g.l.c.–m.s. (see Table II).

Partial hydrolysis of the core oligosaccharide with acid. — The core oligosaccharide (10 mg) was partially hydrolyzed with 0.5M sulfuric acid for 1 h at 100°, and the solution made neutral with $BaCO_3$, and evaporated. The product, purified by chromatography on Sephadex G-15 using 47mM pyridinium acetate buffer, pH 4.26, afforded a major fraction. The degraded oligosaccharide was subjected to sugar analysis, and to methylation. The permethylated product was hydrolyzed with 2M trifluoroacetic acid for 12 h at 100°, and the sugars were reduced with borohydride, the alditols acetylated, and the acetates analyzed by g.l.c.–m.s. (see Table II).

Oxidation of the core oligosaccharide with periodate. — The core oligosaccharide (50 mg) was dissolved in distilled water (10 mL), 0.1M sodium metaperiodate (10 mL) was added, and the solution was kept in the dark at 5°. After 72 h, $BaCO_3$ (25 mg) was added, and the suspension was agitated overnight. After filtration of the insoluble salts, the product was reduced with sodium borohydride, and the base neutralized. The polyol was purified by chromatography on a column of Sephadex G-15 eluted with pyridinium acetate buffer, pH 4.26, and subjected to sugar analysis (see Table I).

Smith degradation of the core oligosaccharide. — The polyol (20 mg) obtained from the periodate oxidation of the core oligosaccharide was hydrolyzed with 0.5M trifluoroacetic acid for 3 h at 100°. After evaporation of the acid, the residue was purified by column chromatography on Sephadex G-15 eluted with pyridinium acetate buffer, pH 4.26. The pure, Smith-degraded product (**2**) was methylated, followed by hydrolysis, reduction, acetylation, and g.l.c.–m.s. analysis.

Deamination of the core oligosaccharide with nitrous acid. — The core oligosaccharide (10 mg) in water (1 mL) was treated with 30% acetic acid (1 mL) and fresh 5% sodium nitrite solution (1.8 mL)[20], and the solution was kept for 60 min at room temperature, and then passed through a column of Rexyn 101 (H^+) resin. The deaminated oligosaccharide was reduced with sodium borodeuteride, and the base neutralized with acetic acid. The product was purified by chromatography on Sephadex G-15 with pyridinium acetate buffer, pH 4.26. The purified, deaminated oligosaccharide (**3**) was methylated, the product hydrolyzed with 2M trifluoroacetic acid for 10 h at 100°, the sugars reduced, the alditols acetylated, and the acetates analyzed by g.l.c.–m.s. (see Table II).

Hydrolysis of the core oligosaccharide with 2M hydrochloric acid. — The core oligosaccharide (30 mg) was hydrolyzed with 2M hydrochloric acid for 1 h at 100°, followed by evaporation. The residue was purified by high-voltage electrophoresis using a pyridinium acetate buffer. It afforded a fraction (disaccharide **4**) that had M_{GlcN} 0.6; this was isolated from the electrophoretogram by elution with 0.05% HCl solution. Disaccharide **4** was acetylated, followed by methylation, hydrolysis, reduction, acetylation, and g.l.c.–m.s. analysis. Disaccharide **4** was subjected to nitrous acid deamination, followed by borohydride reduction, acetylation, and g.l.c.–m.s. (see Table I).

Oxidation with chromium trioxide. — The core oligosaccharide (20 mg) was

dissolved in *N*,*N*-dimethylformamide (1.5 mL), and acetic anhydride (1.5 mL) and pyridine (1.5 mL) were added. The solution was stirred for 16 h at room temperature, and evaporated to dryness[21]. The residue was dissolved in chloroform, *myo*-inositol hexaacetate (2 mg) was added as the internal standard, and the solution was divided into two parts, and these were evaporated. One part was dissolved in glacial acetic acid (2 mL), and powdered chromium trioxide (40 mg) was added; the mixture was then agitated ultrasonically for 3 h at 50°, poured into water, extracted with chloroform (10 mL), and the extract washed twice with water, and evaporated to dryness. The oxidized core and the remaining, original unoxidized material were subjected to sugar analysis (see Table I). The oxidized core oligosaccharide (**5**) was purified by chromatography on Sephadex LH-20. The resulting product was methylated, hydrolyzed with 2M trifluoroacetic acid for 12 h at 100°, the sugars reduced, the alditols acetylated, and the acetates analyzed by g.l.c.–m.s. (see Table II).

ACKNOWLEDGMENTS

The authors thank Dr. Michel R. Vignon (C.N.R.S.-CERMAV, Grenoble, France) for recording the n.m.r. spectra, Dr. David R. Bundle (NRC, Ottawa) for helpful discussions, and Mr. Howard J. Hodder for technical assistance.

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