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Structure of the L5 Lipopolysaccharide Core Oligosaccharides of *Neisseria meningitidis**

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Three different oligosaccharides were isolated by mild acid hydrolysis of the lipopolysaccharides, obtained from *Neisseria meningitidis* serotype 5, and their structures were elucidated by combined chemical and physical techniques. The use of 500-MHz ^{1}H NMR in both one-dimensional and two-dimensional modes as well as nuclear Overhauser effect experiments were employed. To assist in the structural assignments the purified oligosaccharides were also degraded by chemical and enzymatic procedures to smaller fragments. The largest of the three original oligosaccharides is a triantennary partially *O*-acetylated decasaccharide in which the largest antenna terminates in a lacto-*N*-neotetraose unit. The smaller oligosaccharides (heptasaccharide and octasaccharide) except for terminal glycose deletions from the longest antenna are structural replicas of the larger.

The meningococcal LPS[1] has been implicated in the immune response to natural infection (1), and at least 11 serotypes (L1-L11) have been identified (2, 3). There is no apparent correlation between meningococcal serogroup, designated by meningococci having a common capsular polysaccharide, and LPS serotype, except that the L10 and L11 serotypes are exclusively associated with serogroup A organisms (4). The LPS serotype epitopes are located in the glycose moieties of the LPS (5), the latter having been identified as low molecular weight oligosaccharides of the R-type (6, 7). By injecting rabbits with protein conjugates of the above oligosaccharides it has also been demonstrated (5) that they contain bactericidal epitopes. Structural studies (7, 8) on the largest of the oligosaccharides obtained from some individual meningococcal serotypes, including the one obtained from the L5 serotype (8), have identified regions of structural similarity and structural difference in them which are probably responsible for both the serotype specificity and cross-reactivity exhibited by meningococci (3, 5).

However, the above structural studies did not address the phenomenon of heterogeneity among the LPS oligosaccharides associated with individual meningococcal serotypes (9, 10), which is probably the basis of even further immunologic diversity. This heterogeneity is generated either by structurally similar oligosaccharides having phosphoethanolamine groups in differing locations (8) or by glycose deletions from the oligosaccharides. This latter phenomenon was hypothesized to explain the molecular size heterogeneity exhibited by the LPS of individual meningococcal serotypes when run in sodium dodecyl sulfate gels (11, 12), and this hypothesis has since been confirmed by chromatographic procedures on the isolated oligosaccharides (8, 13). The isolation and structural determination of three different sized but structurally related oligosaccharides from the meningococcal L5 serotype confirms the above hypothesis.

EXPERIMENTAL PROCEDURES[2]

RESULTS

Isolation of Core Oligosaccharides—The heterogeneous nature of the core oligosaccharides of the L5 determinant was confirmed when 1% acetic acid hydrolysis of the LPS and gel filtration chromatography of the hydrolysate on Bio-Gel P-4 yielded three distinct products with K_{av} 0.44, 0.59, and 0.65, designated oligosaccharides **1**, **2**, and **3** in order of decreasing size (Fig. 1).

*Structure of Oligosaccharides **1**, **2**, and **3***—The structures of oligosaccharides **1**, **2**, and **3** are shown in Fig. 2. Sugar analysis of **1** indicated that it was composed of D-galactose, D-glucose, 2-acetamido-2-deoxy-D-glucose, L-*glycero*-D-*manno*-heptose, and 3-deoxy-D-*manno*-octulosonic acid (KDO) in the molar ratio of 2:3:2:2:1. In addition the ^{1}H NMR spectrum of **1** indicated that it also contained *O*-acetyl groups (δ = 2.19 ppm) in a molar ratio of ~0.4. Following removal of these groups with sodium hydroxide, the ^{1}H NMR of de-*O*-acetylated **1** (Table I) was in agreement with the sugar analysis, *i.e.* two signals at δ 2.041 and δ 2.119 ppm were indicative of *N*-acetyl groups assigned to the two D-glucosamine residues **b** and **i**, respectively. Also, nine signals in the proton anomeric region indicated that **1** contained at least 9 sugar residues with 5 of them, at δ 4.485, 4.756, 4.456, 4.541, and 4.576, having large $^3J_{1,2}$ vicinal coupling constants (~7-8 Hz) indicating that they were in the β-anomeric configuration. The remaining 4 residues at δ 5.075, 5.424, 5.328, and 5.187 ppm (Table I) had small (~1-3 Hz) $^3J_{1,2}$ coupling constants, and except for those having the *manno*-configuration, could

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[1] The abbreviations used are: LPS, lipopolysaccharide; COSY, correlated spectroscopy; KDO, 3-deoxy-D-*manno*-octulosonic acid; NOE, nuclear Overhauser effect; FAB-MS, fast atom bombardment-mass spectroscopy.

[2] Portions of this paper (including "Experimental Procedures," Fig. 1, and Tables I-IV) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.

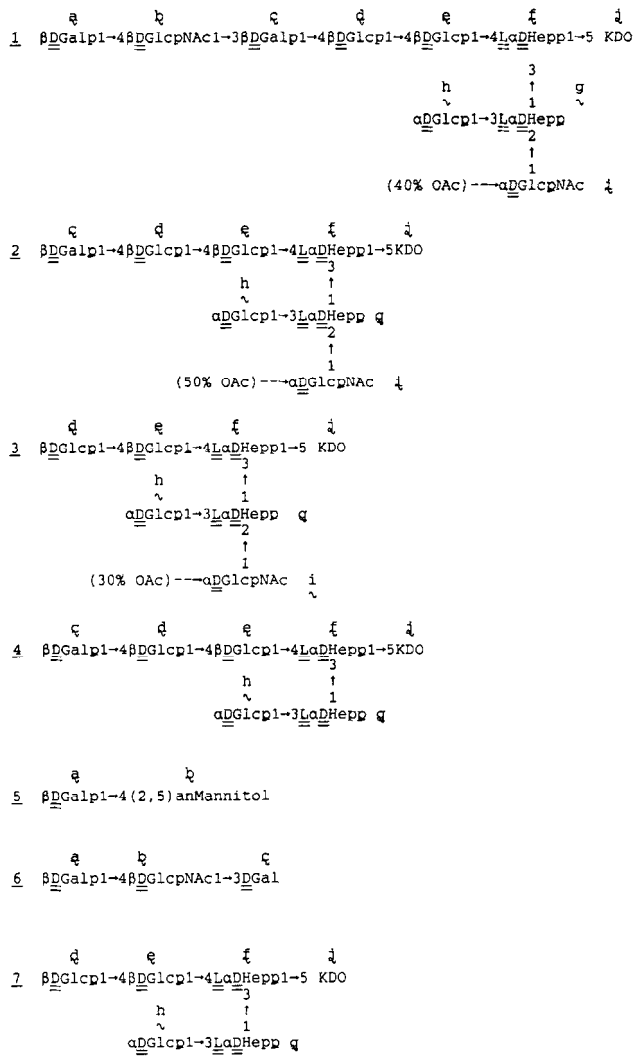

FIG. 2. **Structures of the L5 serotype oligosaccharides (*1–3*) and degraded L5 serotype oligosaccharides (*4–7*).**

be assigned the α-anomeric configuration.

Methylation analysis of **1** (Table II) indicated that it contained terminal nonreducing D-galactose, D-glucose, and 2-acetamido-2-deoxy-D-glucose residues, D-galactose linked at *O*-3, two D-glucose residues, and one 2-acetamido-2-deoxy-D-glucose residue linked at *O*-4. Both heptoses were branch points, one of them substituted at *O*-3 and *O*-4, the other one at *O*-2 and *O*-3. KDO was linked at *O*-5 as it is in the L3 determinant (7). In order to obtain information on the sequence, a number of chemical and enzymatic degradations were performed on **1**. First, it was anticipated from the structural information obtained for the L3 core determinant and from the results of the methylation analysis of **1** that **1** would also contain the same lacto-*N*-neotetraose unit from which β-D-Gal*p*-1→4-β-D-Glc*p*-NAc-1→3-D-Gal*p* would be hydrolyzed by *endo*-β-galactosidase of *Escherichia freundii* (23).

Indeed when **1** was treated with the enzyme, two products of hydrolysis could be isolated by gel filtration on Bio-Gel P4 and identified following methylation and ^{1}H NMR analyses. The fastest moving (larger) component had K_{av} = 0.65 identical to that of **3**, and ^{1}H NMR analysis of this compound indicated that indeed it did have a structure identical to that of **3** (Fig. 2). The slowest moving (smaller) component (**6**) was shown to be a reducing trisaccharide having the structure β-D-Gal*p*-1→4-β-D-Glc*p*NAc-1→3-D-Gal*p* by methylation (Table II) and ^{1}H NMR analysis (Table I).

As shown above, **3** is a product resulting from both the partial hydrolysis (1% acetic acid) of the native L5 LPS and the *endo*-β-galactosidase treatment of oligosaccharide **1**. Methylation analysis of **3** indicated that it contained all methylated sugars present in **1** (Table II) except for those corresponding to unit **6** and for the appearance of one terminal glucose unit and disappearance of the *O*-4-linked glucose residue present in **1**. These results were in agreement with the known specificity of *endo*-β-galactosidase from *E. freundii* which cleaves the β-D-Gal*p*-1→4-β-D-Glc*p* linkage present in various glycosphingolipids (23).

The sequence and anomeric configurations of the individual residues of **3** (its *O*-deacetylated and $NaBH_4$ reduced form) were ascertained from ^{1}H NMR (NOE) data. The chemical shifts of some of the protons associated with the individual residues of modified **3** were assigned by two-dimensional (H,H) COSY using both one-step (14) and two-step relayed coherent transfer (15) and are listed in Table III. Following assignments, the individual anomeric signals of each of the residues (**g, h, i, f, e, d**) in **3** (Fig. 2) were selectively irradiated, and the observed NOE values are listed in Table IV. Irradiation of H-1 (**d**) gave enhancements on its own H-3 and H-5 protons as well as on H-4 (**e**) indicating that the terminal glucopyranosyl residue (**d**) is in the β-D-configuration and is linked to *O*-4 of the adjacent D-glucopyranosyl residue (**e**). When H-1 (**e**) was irradiated it gave enhancements on H-3 (**e**) and H-5 (**e**) consistent with **e** being in the β-D-configuration and on H-4 (**f**) indicating that **e** was linked to *O*-4 of the next L-*glycero*-D-*manno*-heptopyranosyl residue (**f**). Irradiation of H-1 (**f**) gave enhancements on H-2 (**f**) indicating that **f** is in the α-D-*manno* configuration; other enhancements were observed that could belong to the borohydride-reduced (open chain) form of the KDO residue (**j**), but we could not be certain of the assignments of these signals. When H-1 (**h**) was irradiated, it gave enhancements on H-2 (**h**) consistent with **h** being in the α-D-configuration and on H-3 (**g**) indicating that the terminal α-D-glucopyranosyl residue **h** was linked to *O*-3 of its adjacent L-*glycero*-α-D-*manno*-heptopyranosyl residue (**g**). Now when H-1 (**g**) was irradiated enhancements on both H-2 (**g**) and H-3 (**f**) were observed, indicating that **g** is in the α-D-*manno* configuration and that it is linked to *O*-3 of the L-*glycero*-D-*manno*-heptopyranosyl branch point residue (**f**). Finally when H-1 (**i**) was irradiated, enhancements on both H-2 (**i**) and H-2 (**g**) were observed consistent with the terminal 2-acetamido-2-deoxy-D-glucopyranosyl residue **i** being in the α-D-configuration and linked through *O*-2 of its adjacent L-*glycero*-D-*manno*-heptopyranosyl residue (**g**). The NOE data support the sequence of glycoses of **3** shown in Fig. 2. It is interesting to note that the structure of **3** is part of that of the L3 core determinant (7) except for an additional α-D-glucopyranosyl residue linked at *O*-3 of the heptopyranosyl side chain residue (**g**) and also for an additional β-D-glucopyranosyl residue linking the lacto-*N*-neotetraose unit to the inner core of **1**. The "native" oligosaccharide **3** as well as the resulting digestion product of **1** by *endo*-β-galactosidase are both *O*-acetylated (~30%) on an as yet undetermined position on the terminal 2-acetamido-2-deoxy-α-D-glucopyranosyl residue (**i**); this chemical evidence was originally obtained for **1**, **2**, and **3** by FAB-MS upon analysis of their (positive mode) mass spectra (24). The final structure of **1**, shown in Fig. 2, is of course the result of linking the reducing trisaccharide **6** to heptasaccharide **3**. To confirm this we performed deamination studies on *N*-deacetylated **1**. Following treatment with sodium nitrite in acetic acid, the products of deamination of *N*-deacetylated **1** were purified on Bio-Gel

P4, and two major products were obtained. The largest fragment identified as **4** (Fig. 2) was analyzed by methylation (Table II) and ^{1}H NMR analyses (Table I). By comparison with the methylation analysis of **3**, that of **4** contained one more additional terminal galactose residue, and in addition one of the original 2,3,4,6-tetramethylglucose residues found originally in **3** was transformed into a 2,3,6-trimethylglucose indicating that the terminal galactopyranosyl residue (**c**) is linked to *O*-4 of the glucopyranosyl residue (**d**). Also, the 4,6,7-trimethylheptopyranosyl residue present in the methylation analysis of **3** was now replaced by a 2,4,6,7-tetramethylheptopyranosyl residue indicating that the terminal 2-acetamido-2-deoxy-glucopyranosyl residue (**i**) was indeed linked to *O*-2 of residue **g** as previously indicated by ^{1}H NMR (NOE) data on **3**. The smallest deamination product identified as **5** was shown to have the structure depicted in Fig. 2 following methylation and ^{1}H NMR analyses.

Additional confirmatory evidence that terminal galactose (**c**) of **4** (Fig. 2) was linked to *O*-4 of the glucopyranosyl residue (**d**) was obtained when **4** was treated with a β-D-galactosidase. Indeed after treatment with the enzyme, **4** has its terminal β-D-galactose residue (**c**) removed, and a new product identified as **7** was characterized following methylation and ^{1}H NMR analyses. It had the structure depicted in Fig. 2, where the *O*-4-linked glucose residue (**d**) in **4** had now become a terminal nonreducing end.

Based on the structural results obtained from both treatment of **1** with *endo*-β-galactosidase and deamination studies, a single structure for **1** as depicted in Fig. 2 was proposed. The entire structure differs from that of the L3 core (7) by having an additional *O*-4-linked β-D-glucopyranosyl internal residue (**e**) and an additional terminal α-D-glucopyranosyl residue (**h**) linked to *O*-2 of the heptopyranosyl side chain residue (**g**). The oligosaccharide is partially *O*-acetylated on residue (**i**) and does not contain phosphoryl substituents as in the case of the L3 core determinant (7). Finally the third product with K_{av} 0.59 identified as **2** obtained from the acetic acid hydrolysis of the L5 LPS is an oligosaccharide whose structure depicted in Fig. 2 is an intermediate between that of **1** and that of **3**. It differs from that of **1** by lacking the terminal β-D-Gal1→4β-D-GlcpNAc disaccharide unit. The compositional analysis as well as the sequence of residues in oligosaccharides **1**, **2**, and **3** was totally (for the composition) and in part (for the sequence) confirmed by FAB-MS studies (24).

DISCUSSION

The structures of the three oligosaccharides isolated from the L5 serotype LPS are shown in Fig. 2. The structure of the largest oligosaccharide has been previously reported (8) and has terminal lacto-*N*-neotetraose on its longest antenna. This structural feature has also been identified on the oligosaccharides obtained from the LPS of the L2 (8) and L3 (7) serotypes and the fact that the LPS from which they were obtained exhibit predominant serotype specificity (5) confirms that lacto-*N*-neotetraose, despite its large size, is not immunodominant. Thus the serotype epitopes of the meningococcal LPS reside in the heptose-containing inner core of its oligosaccharide moieties (8). The nonimmunogenicity of the lacto-*N*-neotetraose unit is probably due to immune tolerance because it is a known human and animal tissue antigen (25, 26). The largest of the L5 oligosaccharides differs in structure from that of L3 (7) by having two additional D-glucopyranosyl residues linked to its inner core. A terminal α-D-glucopyranosyl residue is linked to *O*-3 of one L-α-D-heptopyranosyl residue, and an interchain β-D-glucopyranosyl residue is linked to *O*-4 of the other L-α-D-heptopyranosyl residue. The lacto-*N*-neotetraose unit is linked to *O*-4 at this latter β-D-glucopyranosyl residue. These structural features play a dominant role in both the serotype specificity of the L5 LPS and also account for cross-reactions with both the L2 and L10 serotype LPS (5).

The isolation of three different oligosaccharides from the LPS of the L5 serotype confirms the heterogeneity of these structures on LPS obtained from meningococci of the same serotype. This phenomenon had been hypothesized to explain the heterogeneity exhibited by the individual serotype LPS of both meningococci (9, 10) and gonococci (12) when subjected to sodium dodecyl sulfate gel chromatography (9, 12), and confirmation of this hypothesis was later obtained by analytical and oligosaccharide chromatographic procedures (8, 13). However, the structural basis of this heterogeneity was not previously established. The three oligosaccharides from the LPS of the L5 serotype are structurally identical except for glycose deletions. Interestingly these deletions occur exclusively from the longest antenna of the L5 oligosaccharide whereas glycose deletions from the inner heptose core would have produced oligosaccharides associated with LPS of different serotypes (8). Also, it is interesting to note that glycose deletions from the long antenna of the L5 oligosaccharide create new and probably more immunogenic epitopes due to the destruction of the lacto-*N*-tetraose unit. This is consistent with the fact that of all the meningococcal serotype LPS, only in the case of L5 serotype is the smallest component on SDS gels the major antigenic component (27).

All the three oligosaccharides obtained from the L5 serotype LPS are partially *O*-acetylated on their terminal 2-acetamido-2-deoxy-α-D-glucopyranosyl residues. This is the first report of the presence of *O*-acetyl substituents in the neisserial LPS, the identification of which suggests a further mechanism by which meningococci modulate their surface glycose structures. The identification of *O*-acetyl substituents also raises the question as to whether in the L5 LPS the oligosaccharides are completely *O*-acetylated. Certainly it is reasonable to assume that *O*-acetyl groups could be partially removed under the mild hydrolytic conditions used to obtain the oligosaccharides from the LPS.

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Supplemental material to:

Structure of the L5 lipopolysaccharide core oligosaccharides of Neisseria meningitidis.

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EXPERIMENTAL PROCEDURES

Materials

Group B Neisseria meningitidis strain 981 (serotype L5), was grown in Neisseria chemically defined medium (General Biochemicals Inc., Chagrin Falls, Ohio), and the LPS isolated previously described (7).

Analytical methods

Solutions were evaporated under diminished pressure below 40° in a rotatory evaporator. Gel filtration was done on columns (1.6 x 90 cm) of Bio-Gel P4 (Bio-Rad Laboratories, minus 400 mesh) and Sephadex G-15 (2.0 x 80 cm) (Pharmacia) at 20°C using pyridinium acetate buffer (0.02 M, pH 5.4) and water as the respective eluents at a flow rate of ~ 12 mL/h. Individual fractions were monitored using a Waters R403 differential refractometer.

Instrumental methods

Gas-liquid chromatography (g.l.c.) was performed on a Hewlett-Packard model 5830A instrument equipped with a flame ionization detector and a model 3380A electronic integrator. The following conditions were used with capillary column (0.32 mm x 25 m) of OV-17 (Quadrex Corp.). Program A for alditol acetates: from 180°C to 240°C at 4°C/min and program B for methylated alditol acetates: from 200°C to 240°C at 1°C/min. Combined gas-liquid chromatography - mass spectrometry (g.l.c.-m.s.) was carried out on a Hewlett-Packard 5985B instrument using the above capillary column and employing programs A and B as described above and an ionization potential of 70 eV. ^{1}H-n.m.r. spectra were recorded at 500 MHz and at 343 K with a Bruker AM500 spectrometer, in the pulsed Fourier transform mode. The chemical shifts (δ) are quoted relative to internal acetone (0.1%, v/v); δ = 2.225 p.p.m. downfield from sodium 4,4-dimethyl-4-silapentane-1-sulfonate (D.S.S.). Proton homonuclear shift correlated 2D-n.m.r. experiments, COSY (14) and relayed COSY (15), were performed by using the standard pulse sequences provided by Bruker (DISB87). Quadrature detection in both dimensions was employed. The initial (t_1,t_2) matrices of 256 x 1024 data points were zero filled to 512 x 2048 points, providing 1.6 Hz/point digital resolution in the second domain. Resolution enhancement in both dimensions was done by the unshifted sine bell window function prior to Fourier transformation. Magnitude spectra symmetrized about the diagonal were used to represent data. The nuclear Overhauser enhancement (n.O.e.) experiments were performed in the difference mode with sequential irradiation of each line in a multiplet (16,17). Total irradiation time per multiplet was 1 sec.

Isolation of oligosaccharides

The core oligosaccharides were obtained by heating the LPS (10 mg/mL) in 1% acetic acid for 2 h at 100°. The insoluble lipid A was eliminated from the hydrolysis mixture by centrifugation at 10.000 r.p.m. The cores (yield ~ 50%) were then purified by gel filtration on Bio-Gel P4 using pyridinium acetate buffer as eluent. Three main oligosaccharide fractions identified as products 1 - 7 were obtained which had K_{av} of 0.44 for the largest (1) and K_{av} of 0.59 and 0.65 for the two other slower fractions 2 and 3 respectively. Each fraction was individually re-chromatographed using the same conditions in order to afford a product free of neighbouring fractions. Each K_{av} value was calculated after a sample (~ 1 mg) of each purified oligosaccharide had been chromatographed together with dextran (2 mg; mw 17,900; Sigma Laboratories) and glucose (2 mg), as standards for V_0 and V_T determination respectively. A portion of each fraction was de-O-acetylated in 0.1N NH_4OH at r.t. for 16 hrs. De-O-acetylated fractions 1 and 3 had K_{av} of 0.44 and 0.67 respectively.

Glycose analysis

Sugar and methylation analysis were conducted essentially as previously described (6). Following their deamination, and conversion into alditol acetates, the sugars were identified, and quantified, by g.l.c. Methylations were performed by the method of Hakomori (18), the products were purified with C18 Sep-Pak cartridges (Waters Associates) (19). Methylated products were hydrolysed with 0.5 N H_2SO_4 in 95% acetic acid for 18 h at 80°C and following a two-fold dilution with water, samples were hydrolysed for an additional 5 h at 80°C (20). The individual methylated components were identified, and quantified by g.l.c.-m.s. after conversion into alditol acetates. The absolute configurations of the sugars obtained by hydrolysis of oligosaccharide (1) were determined by the method of Leontein et al. (21) by g.l.c. analysis of their silylated (-) 2 - butyl- glycosides. Authentic L-glycero-D-manno-heptose was obtained by hydrolysis of the L3 N. meningitidis core oligosaccharide (7).

N-Deacetylation and deamination of oligosaccharide 1

Oligosaccharide 1 (15 mg) was dissolved in hydrazine (5 ml) containing hydrazine sulfate (50 mg). The solution was heated at 105°C for 10 h. Hydrazine was removed by coevaporation with toluene. The residue was dissolved in water (2 ml) and desalted on a Sephadex G-15 (Pharmacia) column using water as eluent. Void volume fractions were pooled and freeze-dried. The N-deacetylated material (5.5 mg) was then deaminated essentially by the method of Dmitriev et al. (22) with 2% sodium nitrite in 11% acetic acid at r.t. for 1 hr. The products of the deamination were fractionated on a Bio-Gel P4 column using conditions described above. A major fraction (0.8 mg) was identified as oligosaccharide (4) by methylation and ^{1}H-n.m.r. analysis. Another component (small fragment) was identified as disaccharide (5) after $NaBH_4$ reduction and methylation analysis.

Treatment of oligosaccharide (1) with endo-β-galactosidase.

Oligosaccharide 1 (5 mg) was dissolved in 0.1 M sodium acetate buffer pH 6.0 (300 µl), and to this solution 20 mU of endo-β-galactosidase (Miles Laboratories) from E. freundii (23) were added. A few drops of toluene were added to the solution and incubation was carried out at 37°C for 18 h. Digestion products were purified by gel filtration on Bio-Gel P4 using conditions described above. Two major fractions besides undegraded 1 were identified as oligosaccharides 3 and 6 by methylation and ^{1}H-n.m.r. analysis.

Treatment of deaminated (4) with β-galactosidase

Oligosaccharide (4) (0.5 mg) was dissolved in 1 ml of water to which 0.1 ml of 0.01 M tris buffer pH 7.6 containing 0.5 mg of β-galactosidase from E. coli (Grade VI, Sigma Laboratories) was added. PH of the solution was adjusted to 7.0 with 1 M NaOH and a few drops of toluene were added. Incubation was carried out at 37° for 1d, and digestion products were purified by gel filtration on Bio-Gel P4. Oligosaccharide (7) was identified as the main degradation product by methylation and ^{1}H-n.m.r. analysis.

Table I. ^{1}H-n.m.r. data[a] for de O-acetylated L5 core oligosaccharides and derived products.

Proton[b]	1[d]	2[d]	3[d]	4	6	7
1 - α	4.485	-	-	-	4.487	-
1 - β	4.756	-	-	-	4.770	-
1 - ζ	4.456	4.461	-	4.462	5.243	-
					4.575	
1 - α	4.541	4.527	4.537	4.527	-	4.498
1 - β	4.576	4.603	4.574	4.605	-	4.605
1 - ζ	5.075	5.065	5.077	5.066	-	5.065
1 - α	5.424	5.416	5.416	5.323	-	5.321
1 - β	5.328	5.325	5.330	5.305	-	5.304
1 - ε	5.187	5.187	5.187	-	-	-
NAc-β	2.041	-	-	-	2.043 (0.7)	-
					2.054 (0.3)	
NAc-ε	2.119	2.120	2.120	-	-	-

[a] Measured in p.p.m. at 343 K in D_2O with acetone (0.1% v/v) as internal chemical shift reference: δ=2.225 p.p.m. downfield from D.S.S. [b] α - ε letters refer to residues α - ε of compounds 1 - 7 reported in Figures 1 and 3. [c] Twin signals (integrales in parenthesis) due to the anomeric equilibrium of the reducing galactose residue (ζ). [d] Native oligosaccharides 1, 2 and 3 had O-acetyl signal at 2.19 p.p.m. in their spectra.

Table II. Methylation analysis of oligosaccharides derived from the L5 lipopolysaccharide cores.[a]

	Molar ratios						
	1	2	3	4	5	6	7
Methylated Sugar[b,c]							
2,3,4,6 - Me$_4$ Glc	1.0	1.0	2.0	1.0	-	-	2.0
2,3,4,6 - Me$_4$ Gal	0.9	1.0	-	0.9	1.0	1.0	-
2,3,6 - Me$_3$ Glc	1.8	1.8	0.9	1.8	-	-	0.8
2,4,6 - Me$_3$ Gal	1.0	-	-	-	-	-	-
2,4,6,7 - Me$_4$ Hep	-	-	-	0.9	-	-	0.9
2,6,7 - Me$_3$ Hep	1.1	1.1	1.0	1.1	-	-	0.9
4,6,7 - Me$_3$ Hep	1.0	1.0	1.1	-	-	-	-
3,4,6 - Me$_3$ Glc NMeAc	+	+	+	-	-	-	-
3,6 - Me$_2$ Glc NMeAc	+		-	-	-	+	-
1,3,6 - Me$_3$-2,5-anMannitol	-	-	-	-	0.8	-	-
1,2,4,5,6-Me$_5$ Gal	-	-	-	-	-	0.7	-

[a] (+) slight non quantitative response (-) not detected. [b] As the methylated alditol acetates. [c] KDO was detected in 1 as its 1,2,4,6,7,8, hexamethyl-3-deoxy-5-OAc derivatives as described in Jennings et al. (6).

Table III. Proton chemical shifts[a] of some of the signals for borohydride reduced 3.

Residue	Proton H-1	H-2	H-3	H-4	H-5	NAc
g	5.48	4.18	4.14	-	-	
h	5.36	3.59	3.70	3.48	3.70	
i	5.18	3.94	3.79	3.56	3.64	2.12
e	5.05	4.04[b]	4.08[b]	4.28	4.12	
		4.16	4.20			
f	4.54	3.44	3.63	3.52	3.56	
d	4.50	3.32	3.51	3.42	3.51	

[a] Measured at 300K and 500 MHz, assignments made from data obtained by homonuclear 2D shift correlated (H,H) COSY and two-step relayed cosy.
[b] Separate signals due to two isomers of KDO-ol.

Table IV. Nuclear Overhauser enhancements for borohydride reduced 3.

Saturated signal[a]	Negative n.O.e. observed Signal	% n.O.e.
1-g	2-g	11
	2-h,3-h	11
1-h	2-h	12
	3-g	10
1-i	2-i	11
	2-g	10
1-e	2-e	4
	3-e	11
	5-e	19
	4-f	14
	5-f	13
1-d	2-d	2
	3-d,5-d,4-e	23

[a] Letters g - d refer to residues of 3 as depicted in Fig. 2.

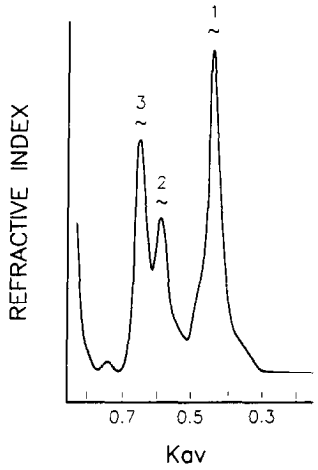

Fig. 1. Elution profile (Bio-Gel P-4) of the water soluble hydrolyzate of the meningococcal serotype L5 LPS.