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Carbohydrate Research 298 (1997) 219–227

CARBOHYDRATE RESEARCH

Structural studies of the O-specific chain of *Hafnia alvei* strain PCM 1190 lipopolysaccharide

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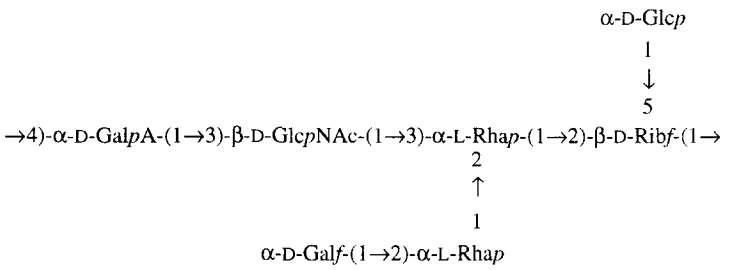
Received 13 September 1996; accepted 13 November 1996

Abstract

The structure of the O-specific side-chain of the lipopolysaccharide of *Hafnia alvei* strain PCM 1190 has been investigated. Methylation analysis, partial acid hydrolysis, Smith degradation, NMR spectroscopy, MALDI-TOF, and FAB mass spectrometry in combination with collision-induced-decomposition MS/MS were the principal methods used. It was concluded that the polysaccharide is composed of heptasaccharide repeating units having the following structure:



Only 80% of the repeating units are complete, whereas 20% of them are lacking the α-D-glucopyranosyl group.

Keywords: *Hafnia alvei*; Lipopolysaccharide; O-Antigen; MS/MS

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PII S0008-6215(96)00311-4

1. Introduction

Hafnia alvei, a member of the *Enterobacteriaceae*, is an opportunistic pathogen found in some incidences of nosocomial infections and cases of septicemia caused by these bacteria have also been reported. The serotyping scheme of *H. alvei* includes 39 O-serotypes [1] and a preliminary chemical characterization of lipopolysaccharides isolated from 33 strains of this genus has been published [2]. Recently, the structures of the O-specific polysaccharides from a number of serologically different strains of *H. alvei* have been reported [3,4].

We now report on structural studies of the O-specific polysaccharide of the *H. alvei* strain PCM 1190 lipopolysaccharide.

2. Results and discussion

The lipopolysaccharide (LPS) was extracted from bacterial cells by the hot phenol–water method [5], purified by ultracentrifugation and freeze-dried. In the SDS-PAGE analysis [6] the pattern indicated different lengths of the O-polysaccharide chains. Delipidation of the LPS by treatment with mild acid yielded a polysaccharide (PS), which was isolated by gel filtration on Bio-Gel P-10. Sugar analysis of the PS gave L-rhamnose, D-glucose, D-galactose, D-ribose, and D-glucosamine in the relative proportions 1.9:0.8:1.0:1.0:0.9, determined as their alditol acetates on GLC–MS. The absolute configurations of the sugars were determined as devised by Gerwig et al. [7,8]. The ^{1}H, ^{13}C, and $^{1}H,^{1}H$-COSY NMR spectra indicated, however, a repeating unit of seven sugars, and in addition to the previously identified six sugars, the presence of a uronic acid was evident from a carbonyl signal and from a spin-system involving one anomeric proton that only consisted of five protons. Thus the PS was carboxyl-reduced [9] and in the sugar analysis of this material the relative proportion of D-galactose increased from 1.0 to 1.9, indicating the presence of a D-galacturonic acid residue in the PS repeating unit. The methylation analysis of the PS (Table 1) showed the presence of 2-substituted rhamnose, 2,3-disubstituted rhamnose, 3-substituted *N*-acetylglucosamine (*N*-acetyl from NMR), and terminal glucose (78%) as pyranosyl residues. In addition, terminal galactose, 2-substituted ribose (25%), and 2,5-disubstituted ribose (76%) were found in their furanose forms (see below). When the PS was methylated and then reduced with 'Superdeuteride' ($LiB(C_2H_5)_3D$) [10] the analysis also gave 2,3-di-*O*-methylgalactose-*6*-d_2 (Table 1).

Table 1
Methylation analysis of O-specific polysaccharide from *Hafnia alvei* PCM 1190 before and after chemical modifications

Methylated sugars (as alditol acetates)	t_R [a]	Detector response (%)		
		A [b]	B	C
3,5-Me_2Rib [c]	0.76	0.25	0.26	1.00
3,4-Me_2Rha	0.89	1.10	1.12	–
2,4-Me_2Rha	0.93	– [d]	–	1.30
2,3,4,6-Me_4Glc	1.00	0.78	0.80	–
2,3,5,6-Me_4Gal	1.02	1.00	1.00	–
3-MeRib	1.07	0.75	0.74	–
4-MeRha	1.09	1.15	1.10	–
2,3-Me_2Gal-6-d_2 [e]	1.50	–	1.18	–
3,4,6-Me_3GlcNAc	1.68	–	–	1.30
4,6-Me_2GlcNAc	1.90	0.68	1.14	–

[a] t_R = retention time for the corresponding alditol acetate relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol (t_R = 1.00) on an HP-1 glass capillary column at 150–270 °C, 8 °C/min.
[b] A, original; B, methylated and ester reduced; C, Smith-degraded polysaccharide.
[c] 3,5-Me_2Rib = 1,2,4-tri-*O*-acetyl-3,5-di-*O*-methyl-D-ribitol, etc.
[d] A dash (–) indicates that the component is not present.
[e] 1,4,6-tri-*O*-acetyl-6,6-dideutero-2,3-di-*O*-methyl-D-galactitol.

The 1D ^{1}H (Fig. 1), 1D ^{13}C, and 2D ^{1}H–^{13}C HMQC NMR spectra of the PS contained main signals for the anomeric atoms of seven sugar residues at δ_H/δ_C 5.48/107.0, $J_{H\text{-}1,H\text{-}2} < 2$ Hz (residue **I**, 80% intensity); δ_H/δ_C 5.34/100.9, $J_{H\text{-}1,H\text{-}2} < 2$ Hz (**II**); δ_H/δ_C 5.25/100.4, $J_{H\text{-}1,H\text{-}2} < 2$ Hz (**III**); δ_H/δ_C 5.15/102.5, $J_{H\text{-}1,H\text{-}2}$ 3 Hz (**IV**); δ_H/δ_C 5.04/100.1, $J_{H\text{-}1,H\text{-}2} < 2$ Hz (**V**); δ_H/δ_C 5.00/99.1, $J_{H\text{-}1,H\text{-}2}$ 3 Hz (**VI**, 80% intensity); and δ_H/δ_C 4.73/103.6, $J_{H\text{-}1,H\text{-}2}$ 8.2 Hz (**VII**), supporting a heptasaccharide repeating unit. In addition, a signal at δ_H/δ_C 5.40/107.3, $J_{H\text{-}1,H\text{-}2} < 2$ Hz (**I'**), having 20% of the intensity of the other signals, was also observed. The $^{1}J_{C,H}$-values obtained from HMQC experiments showed that four of the pyranosidic sugars had the α-configuration ($^{1}J_{C,H}$ 173, 175, 175, and 174 Hz for residues **II**, **III**, **V**, and **VI**, respectively) and one the β-configuration ($^{1}J_{C,H}$ 160 Hz for residue **VII**). The two furanosidic residues, **I** and **IV**, had $^{1}J_{C,H}$-values of 181 and 180 Hz, respectively. As the ^{1}H NMR spectrum was complex the assignments of the major signals and spin-systems were made by different 2D COSY experiments. By these techniques eight spin-systems,

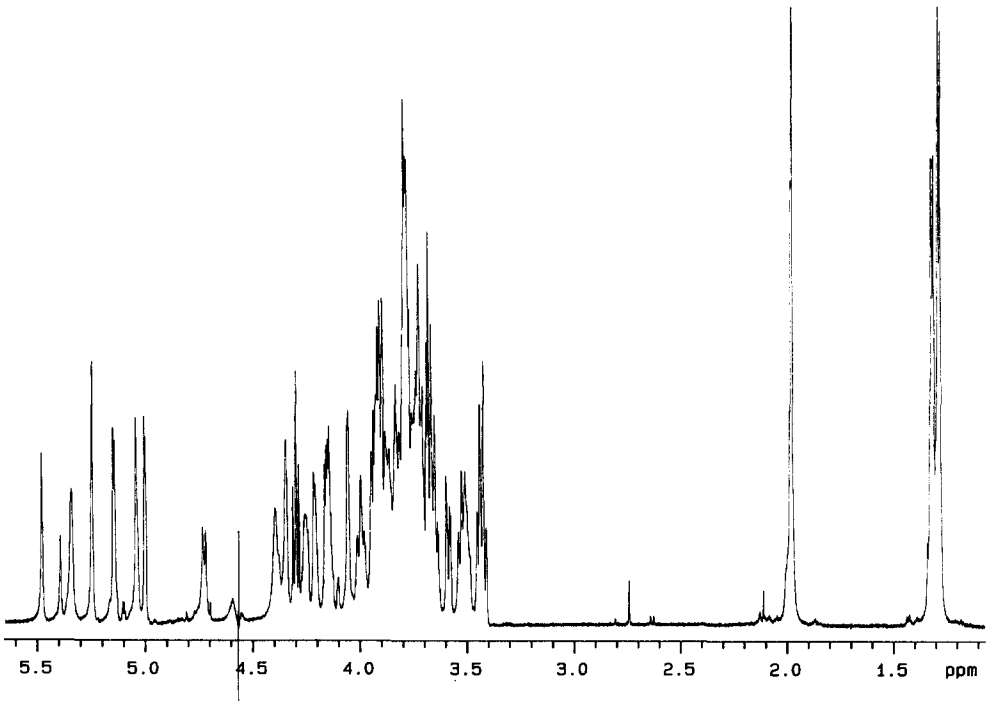


Fig. 1. The 600-MHz ^{1}H NMR spectrum of *H. alvei* strain PCM 1190 polysaccharide.

starting with the signals for the anomeric protons, could be determined. From the assigned proton signals and using the one-bond C–H-connectivities observed in the HMQC spectrum, the carbon signals were assigned. By comparison of the ^{1}H and ^{13}C chemical shifts (Table 2) with earlier published NMR data for the respective monosaccharides [11–13], and taking the $^{3}J_{H,H}$-values for the coupling between the ring protons into consideration, the type of sugar, anomeric configuration, and linkage position were determined. Residue **I** was recognized as → 2,5)-β-D-Rib*f*-(1 → due to the high chemical shifts of the

Table 2
^{1}H and ^{13}C NMR chemical shifts of the *H. alvei* strain PCM 1190 O-specific polysaccharide

Residue		H-1/C-1	H-2/C-2	H-3/C-3	H-4/C-4	H-5/C-5	H-6a/C-6	H-6b
I	→ 2,5)-β-D-Rib*f*-(1 →	5.48	4.22	4.26	4.14	3.72; 3.86		
		107.0	81.1	71.6	80.9	69.8		
I′	→ 2)-β-D-Rib*f*-(1 →	5.40	4.20	4.27	3.99	3.80		
		107.3	81.4	70.8	80.9	63.2		
II	→ 4)-α-D-Gal*p*A-(1 →	5.34	3.87	4.00	4.40	4.46 [a]		
		100.9	69.0	70.1	77.2	71.4 [a]	173.4 [a]	
III	→ 2)-α-L-Rha*p*-(1 →	5.25	4.06	3.83	3.43	3.73	1.28	
		100.4	79.5	70.0	72.9	69.8	17.0	
IV	α-D-Gal*f*-(1 →	5.15	4.16	4.30	3.89	3.80	3.69	3.66
		102.5	76.6	73.7	81.4	70.6	63.4	
V	→ 2,3)-α-L-Rha*p*-(1 →	5.04	4.35	3.91	3.53	3.77	1.31	
		100.1	76.9	80.9	71.6	70.0	17.0	
VI	α-D-Glc*p*-(1 →	5.00	3.59	3.79	3.44	3.81	3.79	3.94 [b]
		99.1	72.2	73.7	71.3	74.4	61.9	
VII	→ 3)-β-D-Glc*p*NAc-(1 →	4.73	3.84	3.73	3.66	3.51	3.75	3.93 [b]
		103.6	55.0	82.4	71.4	75.8	62.2	

[a] The chemical shifts of these signals varied to some extent depending on the pD of the solution.
[b] Assignment could be reversed.

C-1 (δ 107.0), C-2 (δ 81.1), C-4 (δ 80.9), and C-5 (δ 69.8) signals, the similarities of the chemical shifts of the proton signals to those of β-D-Rib*f* [13], and the weak couplings between all ring protons. The minor residue **I'** was recognized as → 2)-β-D-Rib*f*-(1 → as the chemical shift and coupling pattern for all signals were similar to those of **I** but for the C-5 signal, which occurred at δ 63.2. Residue **II** was assigned as → 4)-α-D-Gal*p*A-(1 → as this spin-system consisted of only five protons with a high chemical shift of the H-5 signal (δ 4.46), weak couplings between H-3, H-4, and H-5 and a high chemical shift of the C-4 signal (δ 77.2). Residue **III** was recognized as → 2)-α-L-Rha*p*-(1 → due to signals for an exocyclic-CH_3 group, the weak couplings between H-1, H-2, and H-3 and the high chemical shift of the C-2 signal (δ 79.5). Residue **IV** was recognized as α-D-Gal*f*-(1 → as the chemical shifts of the carbon signals were similar to those of methyl α-D-galactofuranoside [11]. Residue **V** was determined as → 2,3)-α-L-Rha*p*-(1 → due to weak couplings between H-1, H-2, and H-3, signals for an exocyclic $-CH_3$ group, and the high chemical shifts of the C-2 (δ 76.9) and C-3 (δ 80.9) signals. Residue **VI** was recognized as α-D-Glc*p*-(1 → on basis of the strong couplings between H-2, H-3, H-4, and H-5, and the similar chemical shifts to those of α-D-Glc*p* [12]. Residue **VII** was assigned as → 3)-β-D-Glc*p*NAc-(1 → from a low chemical shift of the C-2 signal (δ 55.0) and signals for an *N*-acetyl group, the large $J_{H\text{-}1,\,H\text{-}2}$-value (8.2 Hz), strong couplings between all the ring protons, and a high chemical shift of the C-3 signal (δ 82.4). These data agree with the substitutions and the relative amounts found for the sugar residues in the methylation analyses.

In order to obtain information on the sequence of the sugars in the repeating unit the PS was subjected to a Smith degradation [14], and the products were separated on Bio-Gel P-2. The main product was eluted in the trisaccharide region and a sugar analysis of this material showed that it consisted of glucosamine, rhamnose, ribose, and threonic acid. The latter was identified in the sugar analysis as threitol-1,1-d_2 tetraacetate which was obtained by reduction with $NaBD_4$ of the threonic acid lactone formed during the acid hydrolysis. Methylation analysis of

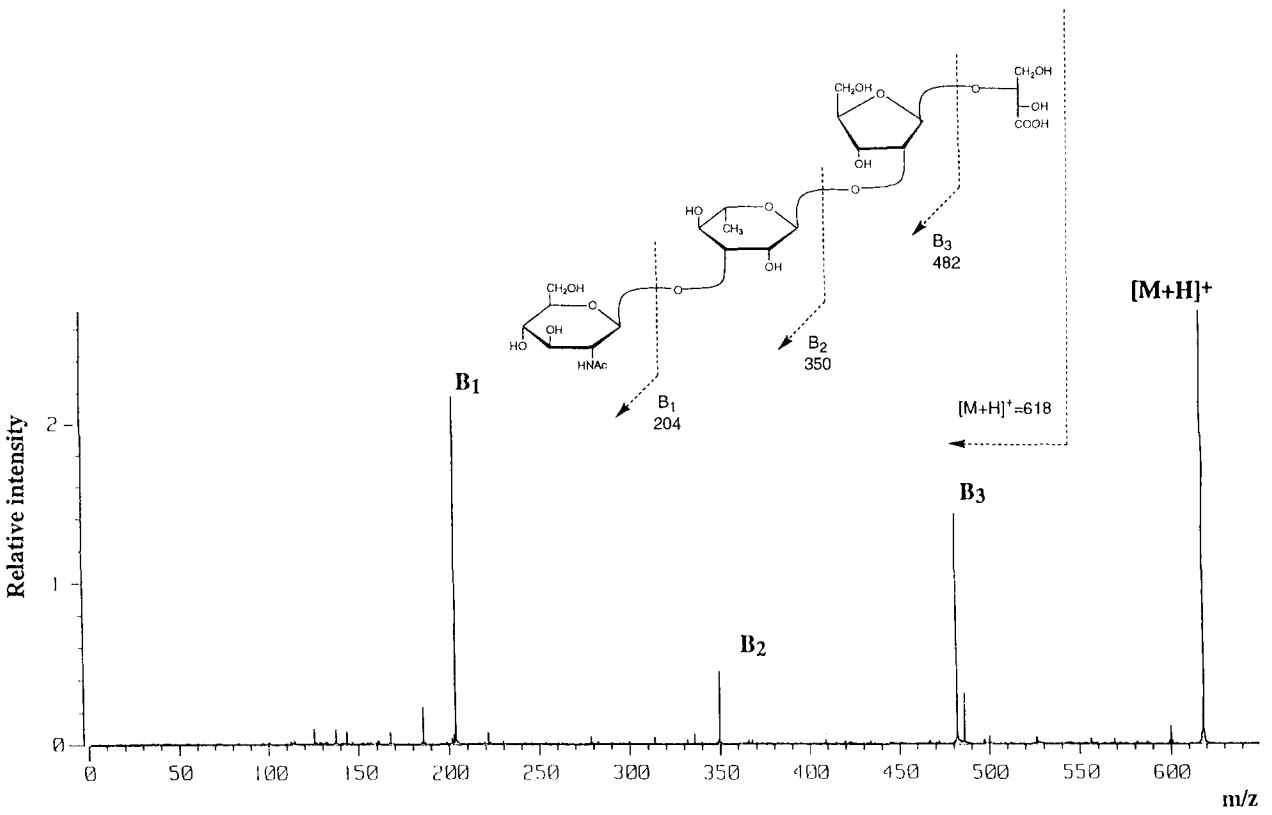


Fig. 2. Collision-induced decomposition fragment ions observed in an MS/MS experiment using the $[M + H]^+$ ion at *m/z* 618, obtained in positive FABMS. The trisaccharide was obtained by Smith degradation of the polysaccharide.

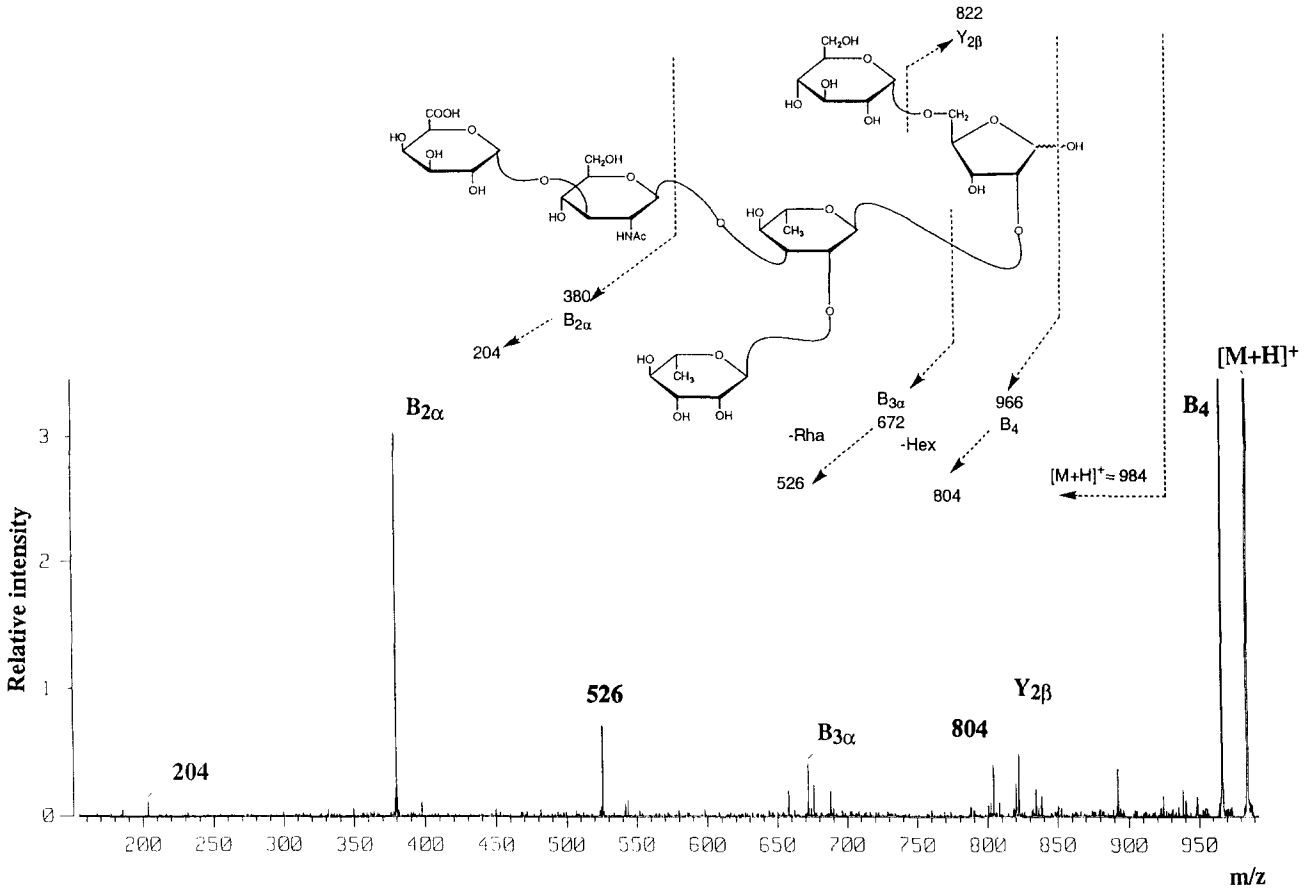
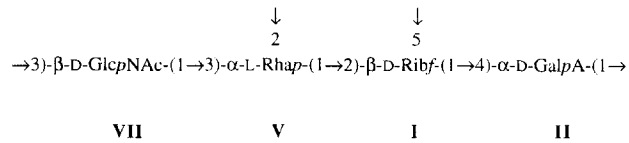


Fig. 3. Collision-induced decomposition fragment ions observed in an MS/MS experiment using the $[M + H]^+$ ion at m/z 984, obtained in positive FABMS. The hexasaccharide was obtained by partial acid hydrolysis of the polysaccharide.

the oligosaccharide gave terminal *N*-acetyl-glucosamine, 3-substituted rhamnose, and 2-substituted ribose in the molar ratios 1.3:1.3:1.0 (Table 1). FABMS of the oligosaccharide produced an $[M + H]^+$ ion at m/z 618 and the high-energy collision-induced-decomposition mass spectrum of this ion showed the characteristic B-type fragments (Domon and Costello nomenclature [15]) with ions at m/z 204 (B_1), m/z 350 (B_2), and m/z 482 (B_3) (Fig. 2). This indicates a sequence of HexNAc-deoxyHex-Pent-Tetronic acid for the oligosaccharide obtained by Smith degradation of the PS. These results combined with results from methylation analysis and NMR spectroscopy of the PS suggest the following structure as a part of the repeating unit:

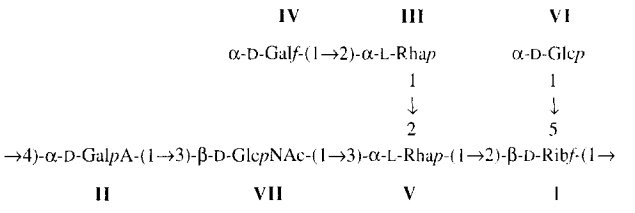


As the PS contains two acid-sensitive furanosidic linkages [16], it was subjected to partial acid hydrolysis to obtain oligosaccharides which could give additional information on the sequence. The PS was treated with 48% HF at room temperature for 24 h and the hydrolysis products were analyzed by MALDI-TOF mass spectrometry. The main $[M + Na]^+$ ion present was m/z 1006, which corresponds to one repeating unit minus one hexose residue. No ions corresponding to one complete repeating unit or larger oligosaccharides were found, indicating that all furanosidic linkages were cleaved and consequently the oligosaccharide obtained lacks the α-furanosidic galactose. The hexasaccharide was analyzed by FABMS and the $[M + H]^+$ ion at m/z 984 was analyzed by high-energy collision-induced decomposition (Fig. 3). The partial structure (shown above), obtained from the Smith degradation, allowed the assignment of most of the fragments observed in the spectrum. The masses of the $B_{2\alpha}$ and the $B_{3\alpha}$ fragments show that the first consists of the GalA and

GlcNAc residues and the second fragment of additional two Rha residues indicating that the branched Rha residue in the main chain is substituted with another Rha. The difference in mass between the B_4 and the $B_{3\alpha}$ fragments corresponds to one pentose and one hexose residue, and thus the α-D-Glcp-(1 → group is substituted to the branched Rib residue in the PS. The combined data also suggest that the main chain consists of only the four sugars shown above, and that the other terminal group, α-D-Gal*f*-(1 → , is linked to → 2)-α-L-Rha*p*-(1 → in the PS.

The established structures of the oligosaccharides were confirmed and the sequence of the total repeating unit was determined by the inter-residual connectivities observed by NOESY and HMBC experiments, on the PS. Inter-residue NOEs (Table 3), observed as cross-peaks in the NOESY spectrum, were found between H-1 of **I** and H-4 of **II** (δ 5.48/4.40), H-1 of **II** and H-3 of **VII** (δ 5.34/3.73), H-1 of **VII** and H-3 of **V** (δ 4.73/3.91), H-1 of **V** and H-2 of **I** (δ 5.04/4.22), H-1 of **IV** and H-2 of **III** (δ 5.15/4.06), H-1 of **III** and H-2 of **V** (δ 5.25/4.35), H-1 of **VI** and H-5 of **I** (δ 5.00/3.72). In addition to these, inter-residual connectivities were observed between the anomeric protons of residues **IV** and **III**, **III** and **V**, and **V** and **I**, respectively, and between H-5 of the two rhamnosides, **III** and **V**, and H-1 of the next sugar residue, **V** and **I**, respectively. These observations are typical for these linkage types [17].

The inter-residue $^3J_{H,C}$ connectivities between the anomeric protons and the linkage carbons and between the anomeric carbons and the protons on the linkage carbons were observed by HMBC experiments, and the results (Table 4) supported the NOE data. The NOE and HMBC results are in agreement with data from methylation analyses and with the structures of the oligosaccharides obtained by partial acid hydrolysis and Smith degradation. The combined results thus suggest the following structure for the repeating unit of the *H. alvei* strain PCM 1190 O-specific polysaccharide:



Oligosaccharides containing the α-D-Gal*f*-(1 → group were also obtained by treatment of the PS at some milder conditions, 48% HF for 24 h at 4 °C. The MALDI-TOF mass spectrum of the hydrolysis products showed ions corresponding to oligosaccharides consisting of up to three repeating units. The $[M + Na]^+$ ion at *m/z* 1168, corresponding to one complete repeating unit, was the most abundant ion in the spectrum.

Only 80% of the repeating units are complete, whereas 20% of them are lacking the α-D-glucopyranosyl group. The structure has several similarities with that of *H. alvei* strain 1222 [18]. All sugars but D-glucose are components in both and the sugars have the same ring forms. Only the anomeric configurations of D-GlcNAc and terminal D-Gal*f* are different. The oligosaccharide of the main chain, → 2,3)-α-L-Rha*p*-(1 → 2)-β-D-Rib*f*-(1 → 4)-α-D-Gal*p*A-(1 → 3)-β-D-Glc*p*NAc-(1 → , is the same. However, the additional α-L-Rha residue in the side chain of strain

Table 3
The significant NOEs observed for the anomeric protons of the O-specific polysaccharide of *Hafnia alvei* strain PCM 1190 lipopolysaccharide

Anomeric proton			NOE to proton		
Residue		δ_H	δ_H	Residue	Atom
I	→ 2,5)-β-D-Rib*f*-(1 →	5.48	4.22	**I**	H-2
			3.77	**V**	H-5
			4.40	**II**	H-4
			4.46	**II**	H-5
			5.04	**V**	H-1
II	→ 4)-α-D-Gal*p*A-(1 →	5.34	3.87	**II**	H-2
			3.73	**VII**	H-3
III	→ 2)-α-L-Rha*p*-(1 →	5.25	4.06	**III**	H-2
			4.35	**V**	H-2
			5.04	**V**	H-1
			5.15	**IV**	H-1
IV	α-D-Gal*f*-(1 →	5.15	4.16	**IV**	H-2
			4.06	**III**	H-2
			5.25	**III**	H-1
V	→ 2,3)-α-L-Rha*p*-(1 →	5.04	4.35	**V**	H-2
			3.73	**III**	H-5
			4.22	**I**	H-2
			5.25	**III**	H-1
VI	α-D-Glc*p*-(1 →	5.00	3.59	**VI**	H-2
			3.72	**I**	H-5
VII	→ 3)-β-D-Glc*p*NAc-(1 →	4.73	3.51	**VII**	H-5
			3.73	**VII**	H-3
			3.91	**V**	H-3
			4.35	**V**	H-2

Table 4
The significant $^3J_{H,C}$-connectivities, observed in an HMBC spectrum, for the anomeric protons/carbons of the sugar residues of the O-specific polysaccharide of *Hafnia alvei* strain PCM 1190 lipopolysaccharide

Residue		H-1/C-1	Observed connectivities		
		δ_H/δ_C	δ_C/δ_H	Residue	Atom
I	→ 2,5)-β-D-Rib*f*-(1 →	5.48	80.9	**I**	C-4
			71.6	**I**	C-3
			77.2	**II**	C-4
		107.0	4.40	**II**	H-4
II	→ 4)-α-D-Gal*p*A-(1 →	5.34	71.4	**II**	C-5
			70.1	**II**	C-3
			82.4	**VII**	C-3
		100.9	3.73	**VII**	H-3
III	→ 2)-α-L-Rha*p*-(1 →	5.25	79.5	**III**	C-2
			≈ 70	**III**	C-3/5
			76.9	**V**	C-2
IV	α-D-Gal*f*-(1 →	5.15	81.4	**IV**	C-4
			73.7	**IV**	C-3
			79.6	**III**	C-2
		102.5	3.89	**IV**	H-4
			4.06	**III**	H-2
V	→ 2,3)-α-L-Rha*p*-(1 →	5.04	80.9	**V**	C-3
			76.9	**V**	C-2
			70.0	**V**	C-5
			81.1	**I**	C-2
		100.1	4.22	**I**	H-2
VI	α-D-Glc*p*-(1 →	5.00	73.7	**VI**	C-3
			72.2	**VI**	C-2
			69.8	**I**	C-5
		99.1	3.79	**VI**	H-3
			3.72	**I**	H-5
			3.86	**I**	H-5′
VII	→ 3)-β-D-Glc*p*NAc-(1 →	4.73	80.9	**V**	C-3

1190 is in the main chain of strain 1222, and only the D-Gal*f* residue constitutes the side chain of this strain.

3. Experimental

General methods.—GLC–MS was carried out with a Hewlett–Packard 5971A system using an HP-1 glass capillary column (0.2 mm × 12 m) and a temperature program of 150 → 270 °C at 8 °C/min. Gel-permeation chromatography was performed on columns (100 × 1.6 cm) of Bio-Gel P-2 or P-10, equilibrated with a 0.05 M pyridine–HOAc buffer (pH 5.6). Eluates were monitored with a Knauer differential refractometer and all fractions checked by ^{1}H NMR spectroscopy.

Mass spectrometry.—MALDI-TOF mass spectrometry was performed in the positive-ion mode on an LDI-1700XS time-of-flight instrument using 2,5-dihydroxybenzoic acid as a matrix. FABMS spectra were recorded on a JEOL JMS-SX/SX-102A four sectors tandem mass spectrometer by bombardment of samples, dissolved in a glycerol matrix, with Xe atoms of the average translational energy of 6 keV. The mass spectrometer was operated at an accelerating voltage of 10 kV. Tandem mass spectrometry was conducted using the first two sectors (B_1E_1) to select the precursor ions and the second mass spectrometer (B_2E_2) to analyze the product ions. A resolution of 1000 was used to separate the ^{12}C peak of the $[M + H]^+$ precursor ion. Helium was used as the collision gas at a pressure sufficient to attenuate the precursor ion by approximately 50%. All samples were treated with Dowex-50 (H^+) ion-exchange resin to remove Na^+ ions prior to analysis.

NMR spectroscopy.—NMR spectra were obtained for D_2O solutions at 50 °C on Varian Unity 600 and VXR-400 spectrometers using sodium 3-trimethylsilylpropionate-d_4 (δ_H 0.00) and acetone (δ_C 31.00) as internal references. The signals were assigned by one- and two-dimensional experiments (dq-COSY, relayed COSY, double relayed COSY, NOESY,

HMQC, and HMBC). In relayed COSY experiments τ was 50 and 100 ms. The delay time in the HMBC and the mixing time in the NOESY experiments were 80 and 200 ms, respectively. The HMQC experiments were performed both with and without carbon decoupling.

Preparation of LPS and PS.—*H. alvei* strain PCM 1190 bacteria were obtained from the collection of the Institute of Immunology and Experimental Therapy, Wrocław, Poland. LPS was prepared by phenol–water extraction [5] of bacterial cells and purified by repeated ultracentrifugation (100,000 × *g*, 3 × 6 h). LPS was analyzed by SDS-PAGE [6] and the LPS bands were detected by silver staining [19]. LPS was degraded by mild acid hydrolysis using 1% HOAc at 100 °C for 30–45 min, the solution cooled and centrifuged. The PS was separated from core oligosaccharides by chromatography on Bio-Gel P-10.

Sugar and methylation analyses.—A solution of the sample (~ 0.5 mg) in 2 M aq CF_3CO_2H (1.0 mL) was kept in a closed vial at 120 °C for 2 h. The sugars in the hydrolysate were then converted into alditol acetates by conventional methods. The absolute configurations of the sugars were determined as described by Gerwig et al. [7,8] using (+)-2-butanol for glycosylation.

Methylations were performed according to the method of Hakomori [20]. Products were recovered using Sep-Pak C_{18} cartridges [21]. The permethylated material was hydrolysed with 2 M aq CF_3CO_2H at 120 °C for 2 h. The partially methylated sugars in the hydrolysate were then converted into alditol acetates by conventional methods. Reduction of ester groups with 'Superdeuteride' ($LiB(C_2H_5)_3D$) after methylation of the polysaccharide was carried out according to Bhat et al. [10].

Carboxyl reduction of PS.—PS (10 mg) was dissolved in water (1.5 mL) and treated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (50 mg) at pH 4.75 and then with $NaBH_4$ (60 mg) at 37 °C for 16 h according to the method of Taylor et al. [9]. The excess of $NaBH_4$ was destroyed by adding aq 50% HOAc and the sample was dialyzed against distilled water and freeze-dried (8 mg).

Smith degradation of PS.—PS (10 mg) was treated with 0.05 M $NaIO_4$ in 0.1 M NaOAc buffer (pH 3.9) at 4 °C for 48 h. Excess of ethyleneglycol was added, the oxidized product purified on a column of Bio-Gel P-2, freeze-dried, and then reduced with $NaBH_4$. The reaction mixture was acidified (pH 6.0) by adding aq 50% HOAc and the product was isolated by gel-permeation chromatography on Bio-Gel P-2. Reduced material was degraded by treatment with 2% HOAc at 100 °C for 1.5 h, and the products were separated on a column of Bio-Gel P-2 yielding an oligosaccharide product (2 mg) after freeze-drying.

Partial acid hydrolysis.—*Procedure 1.* The PS (1 mg) was treated with aq 48% HF (0.5 mL) for 24 h at room temperature. The HF was removed under a stream of N_2. The product was analyzed by MALDI-TOF mass spectrometry and FAB in combination with high energy collision-induced-decomposition mass spectrometry. *Procedure 2.* The same conditions were used as in procedure 1 but for a temperature of 4 °C.

Acknowledgements

This work was supported by grants from the Swedish Natural Science Research Council and the Swedish Council of Forestry and Agricultural Research. A scholarship from the University supporting the collaboration between the Swedish and Polish groups and the Swedish NMR Center for the use of the 600 MHz instrument are acknowledged.

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