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Structural studies of the exocellular polysaccharide from *Sphingomonas paucimobilis* strain I-886

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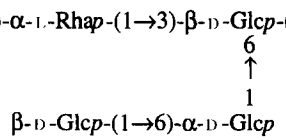
Abstract

The exocellular polysaccharide from *Sphingomonas paucimobilis* strain I-886 has been studied using methylation analysis, Smith degradation, partial acid hydrolysis, NMR spectroscopy, and mass spectrometry as the principal methods. It is concluded that the repeating unit has the following structure:



The absolute configuration of the uronic acid was deduced from ^{1}H NMR chemical shifts and is most likely D. Some preparations of the polysaccharide also contain phosphate and *O*-acyl groups which have not been identified or localised. © 1996 Elsevier Science Ltd.

Keywords: *Sphingomonas paucimobilis*; Capsular polysaccharide structure; Deoxyglucuronic acid

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1. Introduction

Polymers with viscous properties are of great importance in many places in nature. For instance, in mammals, hyaluronic acid is present in the vitreous body of the eye and together with collagen is the supportive agent to the retina; it has also been found to work as a lubricating agent in joints. In bacteria, viscous capsular polysaccharides surround the cell and prevent attack and dehydration.

Several polysaccharides of bacterial origin are used in different formulations because of their favourable rheological properties. Xanthan gum has been on the market for a long time and is now produced in multi-ton quantities. Gellan gum and welan gum are others that have useful properties and compete with xanthan gum. The exocellular polysaccharide from *Sphingomonas paucimobilis* strain I-886 has, in many respects, properties that could make it a good competitor to xanthan gum. Thus, it is easily prepared and also retains its properties at high ionic concentration and high temperature. It should therefore be a candidate for numerous applications in agriculture, pharmacy, and other fields. Preliminary studies have shown that the polysaccharide contains glucose, rhamnose, and *O*-acetyl groups, and a structure has been proposed [1].

2. Results and discussion

Sphingomonas paucimobilis strain I-886 was grown and harvested as previously described [2]. Subsequent standard workup yielded the exocellular polysaccharide hereafter referred to as I-886. A hydrolysate obtained using trifluoroacetic acid contained glucose and rhamnose in the ratio 4.9:1.0, as the only detectable sugars. GLC analysis of the acetylated 2-butyl glycosides [3,4] demonstrated that the sugars had the D and the L configuration, respectively. Methylation analysis revealed that one residue each of terminal, 4-substituted, 6-substituted, and 3,6-substituted glucose were present in addition to one residue of 4-substituted rhamnose (Table 1). This is in agreement with previous data. For some batches it could be shown that a significant amount of

Table 1
Methylation analysis data from *Sphingomonas paucimobilis* I-886 [a]

Sugar residue		Detector response			
	T [b]	A	B	C	D
2,3-Rha [c]	0.89	25	21		
2,3,4,6-Glc	1.00	20	35		25
2,3,6-Glc	1.32	24		44	
2,3,4-Glc	1.39	16	32		75
2,4-Glc	1.75	14	13	56	

[a] Key: A, Native I-886; B, partially hydrolysed I-886; C, deacylated–periodate-oxidised–borohydride-reduced I-886; D, OS2 obtained on partial acid hydrolysis.
[b] Retention times relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol (1.00) on a DB-5 column.
[c] 2,3-Rha = 2,3-di-*O*-methyl-L-rhamnose, etc.

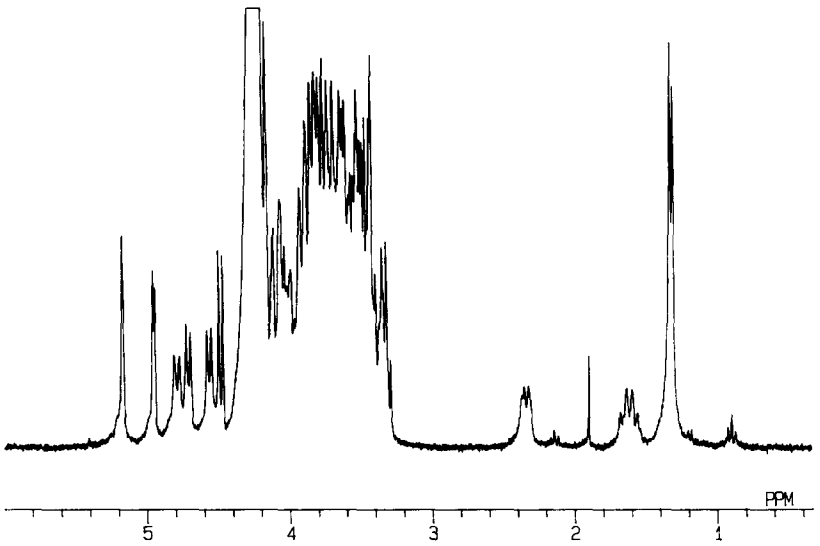


Fig. 1. ^{1}H NMR spectrum of *O*-deacylated I-886.

phosphorus was also present (~ 1.6%). As it turned out the phosphate was very labile and was removed in all chemical modifications made to the polysaccharide, and its location was not determined. The content of *O*-acyl groups was also variable and found to be in one case acetyl groups and in another acyl groups giving resonances at δ 2.4–2.5 in the NMR spectrum. Because of the wide distribution their location was not determined.

Native I-886 gave viscous solutions, but heating overnight at 100 °C at approximately neutral pH removed the *O*-acyl groups and made the sample more suitable for NMR. The ^{1}H NMR spectrum of the resulting material (Fig. 1) showed that at least one further residue was present in the repeating unit, in addition to those five indicated in the methylation analysis. Thus, in the anomeric region signals for six protons could be observed. Three of these, at δ 4.50, 4.58, and 4.73, had a $J_{\text{H-1,H-2}}$ value of 7.8 Hz each and were attributed to β-D-glucopyranose residues; one signal, at δ 4.96, had a coupling of 3.5 Hz and was attributed to an α-D-glucopyranose residue. The signal at δ 5.17 had a $J_{\text{H-1,H-2}}$ value of 1.7 Hz and was therefore assigned to the L-rhamnopyranose residue. The sixth signal at δ 4.81 was an apparent doublet with a $J_{\text{H-1,H-2}}$ value of 8.9 Hz. In the high-field region the signal for the CH_3 group in L-rhamnose appeared at δ 1.33 and the signals for a methylene group at δ 1.62 and 2.35. In agreement with a repeating unit of six sugars the ^{13}C NMR spectrum had signals for anomeric carbons at δ 99.0, 100.7, 101.4, 103.5 (2C), and 103.9. Signals at δ 17.6, 38.0, and 175.7 indicated the presence of a Rha methyl, a methylene, and a carboxyl group. The conclusion from these data was that an acidic deoxy sugar was present. By subsequent NMR spectroscopy, it could be assigned to a 2-deoxy-*arabino*-Hex*p*A residue (2-deoxyglucuronic acid). Attempts to hydrolyse I-886 in order to obtain oligosaccharides showed that the additional sugar was acid-labile and that the deoxy function therefore probably was in the 2-position.

Table 2
^{1}H NMR data for partially hydrolysed *Sphingomonas paucimobilis* I-886

Sugar residue	Chemical shifts [a] (δ)						
	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
β-D-Glc *p*-(1 →	4.50 [7.8]	3.33	3.52	~ 3.45	~ 3.45	3.74	3.92
A	(−0.14)	(0.08)	(0.02)	(0.03)	(−0.01)	(0.02)	(0.02)
→ 3,6)-β-D-Glc *p*-(1 →	4.58 [7.8]	3.45	3.65	3.5—3.6		~ 3.8	~ 3.9
B	(−0.06)	(0.20)	(0.15)				
→ 4)-β-D-Glc *p*-(1 →	4.73 [7.8]	3.35	3.63	3.54	3.45	3.76	3.93
C	(0.09)	(0.10)	(0.13)	(0.12)	(−0.01)	(0.04)	(0.03)
→ 6)-α-D-Glc *p*-(1 →	4.96 [3.5]	3.56	3.78	3.50	3.84	3.92	4.16
D	(−0.27)	(0.02)	(0.06)	(0.08)	(0)	(0.16)	(0.32)
→ 4)-α-L-Rha *p*-(1 →	5.17 [~ 2]	4.07	3.99	3.71	4.06	1.33	
E	(0.05)	(0.15)	(0.18)	(0.26)	(0.20)	(0.05)	
	H-1	H-2ax	H-2eq	H-3	H-4	H-5	
→ 4)-2-Deoxy-β-D-*arabino*-Hex *p*A-(1 →	4.81 [8.9]	1.62	2.35	3.80	3.64	3.37	
F	(−0.11) [b]	(0.11)	(0.09)	(0.09)	(0.36)		
2-Deoxy-α-D-*arabino*-Hex *p*	5.37	1.71	2.13	3.93	3.36	3.81	
2-Deoxy-β-D-*arabino*-Hex *p*	4.92	1.51	2.26	3.71	3.28	3.36	

[a] Chemical shift differences compared to monomers are given in parentheses and $^3J_{H,H}$ values [Hz] are given in square brackets.
[b] Chemical shift differences compared to 2-deoxy-β-D-*arabino*-Hex *p* are given in parentheses.

Unfortunately, the yield of oligosaccharides was low and furthermore the hydrolysate appeared to be heterogeneous, as indicated by ^{1}H NMR spectroscopy. Also, a significant part of the material precipitated and could not be redissolved.

Analysis of the *O*-deacylated I-886 with one-dimensional (1D) HOHAHA and various 2D NMR experiments yielded most of the ^{1}H NMR chemical shifts (Table 2). Residues **A**, **B**, **C**, and **D** were assigned to the D-glucose residues, **E** to the L-rhamnose residue, and **F** to the deoxyglucuronic acid. Because of the low spectral quality no reliable sequence data were obtained. However, from the data it is clear that one of the β-D-Glc residues is terminal as no significant chemical shift displacements were observed for any proton signal. It was also clear from the chemical shift of the anomeric proton signal (δ 4.96) that the α-D-glucose residue was linked to the 6-position of another glucose residue [5]. This is of importance in the determination of the structure of the main chain (see below).

Upon mild acid hydrolysis the signal at δ 4.81 diminished and a new signal at δ 5.40 appeared. This was apparently a signal from a reducing deoxyglucuronic acid which also caused splitting of the signal at δ 4.73 to give a pair of doublets interpreted as due to a β-D-glucose residue next to the acid. Methylation analysis of the resulting material (Table 1, column B) showed an increase in the content of 2,3,4,6-Glc and disappearance of 2,3,6-Glc. The deoxyglucuronic acid is thus linked to the 4-position of a D-glucose

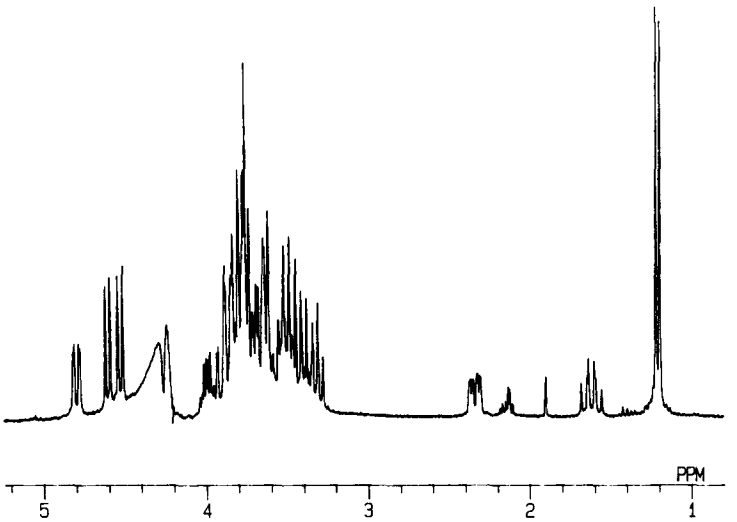


Fig. 2. ^{1}H NMR spectrum of the Smith degradation product of I-886.

residue. An increase in the amount of 2,3,4-Glc also indicates that some rhamnosyl linkages are cleaved.

Since 2-deoxy sugars are prone to degradation, a carboxyl reduction [6,7] followed by hydrolysis with trifluoroacetic acid in the presence of 4-methylmorpholinoborane as a reducing agent [8] was performed. Upon borohydride reduction and acetylation a significant amount of a 2-deoxyhexitol acetate that co-chromatographed on two different GLC columns with the alditol acetate from authentic 2-deoxy-D-*arabino*-hexose (2-deoxyglucose) was formed. The determination of the absolute configuration of the sugar is discussed below.

To determine the sequence of the sugars and to verify the identity and linkage position of the acid a Smith degradation [9], that is, periodate oxidation, borohydride reduction, and mild acid hydrolysis, was carried out. A methylation analysis of the material that had been oxidised and reduced showed that the branch-point residue and, unexpectedly, the 4-substituted D-glucose residue had survived. Following the mild acid hydrolysis the material was purified by gel filtration. The major part of the material was eluted as a symmetrical peak at approximately 1.6 void volumes. The ^{1}H NMR spectrum (Fig. 2) clearly showed that the deoxy sugar was also intact. The spectrum showed inter alia three signals in the anomeric region, at δ 4.53 ($J_{\text{H-1,H-2}}$ 7.9 Hz), 4.62 ($J_{\text{H-1,H-2}}$ 7.9 Hz), and 4.81 ($J_{\text{H-1,H-2}}$ 9.6 Hz), and signals for deoxy protons at δ 1.21, 1.62, and 2.35. Thus, in addition to the deoxy sugar two β-D-glucose residues were present, and the modified L-rhamnose residue, now being a 3-substituted 1-deoxyerythritol. The ^{1}H/^{13}C HMQC spectrum contained signals in the anomeric region at δ 100.6 ($J_{\text{C-1,H-1}}$ 163 Hz), 102.9 ($J_{\text{C-1,H-1}}$ 162 Hz), and 103.4 ($J_{\text{C-1,H-1}}$ 162 Hz) in agreement with the ^{1}H NMR data and with three β anomers. The ^{13}C NMR spectrum contained 22 major signals in accord with an oligosaccharide-alditol containing two hexoses, one deoxyhexuronic acid, and a 1-deoxyerythritol. By the use of various 2D experiments the ^{1}H and ^{13}C

Table 3
^{1}H NMR data for the product from the Smith degradation of *Sphingomonas paucimobilis* I-886

Sugar residue	Chemical shifts [a] (δ)						
	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
β-D-Glc *p*-(1 →	4.53 [7.9]	3.31	3.51	3.43	3.54	3.73	3.92
B′	(−0.11)	(0.06)	(0.01)	(0.01)	(0.08)	(0.01)	(0.02)
→ 4)-β-D-Glc *p*-(1 →	4.62 [7.9]	3.38	3.65	3.64	3.53	3.73	3.88
C	(−0.02)	(0.13)	(0.15)	(0.22)	(0.07)	(0.01)	(−0.02)
→ 3)-1-Deoxyerythritol	1.21	4.02	3.78	n.d.			
E′							
	H-1	H-2ax	H-2eq	H-3	H-4		
→ 4)-2-Deoxy-β-D-*arabino*-Hex *p*A-(1 →	4.81 [9.6]	1.62	2.35	3.82	3.64 [b]		
F	(−0.11) [b]	(0.11)	(0.09)	(0.11)	(0.36)		

[a] Chemical shift differences compared to monomers are given in parentheses and $^3J_{H,H}$ values [Hz] are given in square brackets.
[b] Chemical shift differences compared to the monomers are given in parentheses for **F** as compared to 2-deoxy-β-D-*arabino*-Hex *p*.

NMR signals were assigned (Tables 3 and 4). The modified residues **B** and **E** are now called residues **B′** and **E′**. All the coupling constants of residue **F** are large, in accord with an *arabino* configuration of the deoxyhexuronic acid. A ^{1}H-detected HMBC experiment was used in order to obtain sequence information (Table 5). A correlation

Table 4
^{13}C NMR data for the product from the Smith degradation of *Sphingomonas paucimobilis* I-886

Sugar residue	Chemical shifts [a] (δ)					
	C-1	C-2	C-3	C-4	C-5	C-6
β-D-Glc *p*-(1 →	103.4 [163]	74.1	76.4	70.4	76.8	61.3 [b]
B′	(6.6)	(−1.1)	(−0.4)	(−0.3)	(0.0)	(−0.5)
→ 4)-β-D-Glc *p*-(1 →	102.9 [162]	74.1	75.0	79.6	75.4	61.4 [b]
C	(6.1)	(−1.1)	(−1.8)	(8.9)	(−1.4)	(−0.4)
→ 3)-1-Deoxyerythritol	18.2	68.2	84.9	61.1 [b]		
E′						
→ 4)-2-Deoxy-β-D-*arabino*-Hex *p*A-(1 →	100.6 [163]	37.9	69.9	82.8	76.5	175.6
F	(6.3) [c]	(−2.5)	(−1.5)	(10.5)	(−0.3)	
2-Deoxy-α-D-*arabino*-Hex *p*	92.2	38.1	68.8	71.9	72.9	61.8 [b]
2-Deoxy-β-D-*arabino*-Hex *p*	94.3	40.4	71.4	72.3	76.8	62.0 [b]

[a] Chemical shift differences compared to monomers are given in parentheses and $^1J_{C,H}$ values [Hz] are given in square brackets.
[b] Tentative assignments.
[c] Chemical shift differences compared to the monomers are given in parentheses for **F** as compared to 2-deoxy-β-D-*arabino*-Hex *p*.

Table 5
Observed $^{2}J_{H,C}$ and $^{3}J_{H,C}$ connectivities in an HMBC experiment for the anomeric atoms of the product from the Smith degradation of *Sphingomonas paucimobilis* I-886

Residue	Anomeric atom		$J_{H,C}$ connectivities to		
	$\delta(^{1}H)$	$\delta(^{13}C)$	$\delta(^{13}C)$	$\delta(^{1}H)$	Residue, atom
β-D-Glc *p*-(1 →	4.53		82.8		**F**, C-4
B′		103.4		3.64	**F**, H-4/**C**, H-4
				3.31	**B′**, H-2
→ 4)-2-Deoxy-β-D-*arabino*-Hexp*A*-(1 →	4.81		79.6		**C**, C-4
F					
→ 4)-β-D-Glc *p*-(1 →	4.62		84.9		**E′**, C-3
C					

from δ 4.53 (H-1 in residue **B′**) to a resonance at δ 82.8 (C-4 in residue **F**) established the element **B′–F**. Another between δ 103.4 (C-1 in residue **B′**) and δ 3.64 (H-4 in residue **F**) corroborated the element. The anomeric protons of residues **F** (δ 4.81) and **C** (δ 4.62) showed connectivities to C-4 (δ 79.6) of residue **C** and to C-3 (δ 84.9) of residue **E′**, establishing the elements **F–C** and **C–E′**. In addition to the correlations given, a number of intra-residual correlations were obtained.

The sequence of sugars was further confirmed with a NOESY experiment (Table 6). Thus, NOE between H-1 in residue **B′** (δ 4.53) and H-4 in residue **F** (δ 3.64) again gave the **B′–F** element. The anomeric proton in residue **F** (δ 4.81) had NOE to H-4 in residue **C** (δ 3.64) and that of residue **C** (δ 4.62) to H-3 in residue **E′** (δ 3.78). This corroborates the elements **F–C** and **C–E′**.

For the determination of the absolute configuration of the deoxyhexuronic acid, the glycosylation shift of its anomeric proton signal, in a comparison with 2-deoxy-β-D-*arabino*-hexose, was used. In two model disaccharides, β-D-Glc *p*-(1 → 4)-β-D-Glc *p*-OMe [10] and β-L-Fuc *p*-(1 → 4)-α-D-Glc *p*-OMe [10], each glycosidic linkage is similar

Table 6
NOE data for the product from the Smith degradation of *Sphingomonas paucimobilis* I-886

Residue	Anomeric proton	NOE to proton		
	δ	δ	Intensity [a]	Residue, atom
β-D-Glc *p*-(1 →	4.53	3.51	m	**B′**, H-3
B′		3.64	s	**F**, H-4/**C**, H-4
→ 4)-2-Deoxy-β-D-*arabino*-Hex *p*A-(1 →	4.81	3.64	m	**C**, H-4
F		3.82	s	**F**, H-3
→ 4)-β-D-Glc *p*-(1 →	4.62	3.53	m	**C**, H-5
C		3.65	m	**C**, H-3
		3.78	s	**E′**, H-3

[a] s = strong, m = medium.

to that in either of the alternatives in I-886; that is, the acid is D or L. The first disaccharide has, for the H-1 signal of the glycosyl group, a negative glycosylation shift of −0.11 ppm (shifts upfield) as compared to the second which is positive, 0.06 ppm (downfield). The glycosylation shift in the Smith product of I-886 is −0.11 ppm and it is therefore concluded that it is 2-deoxy-D-*arabino*-hexuronic acid. Attempts to determine the configuration of 2-deoxyglucose from reduced I-886, by analysis of the 2-octyl glycosides, were unsuccessful as it could be shown that the conditions needed for complete hydrolysis or solvolysis of the polysaccharide destroyed the 2-deoxyglucose. From the combined data it is evident that the oligosaccharide has structure **1**:

B' **F** **C** **E'**

β-D-Glc*p*-(1→4)-2-deoxy-β-D-*arabino*-Hex*p*A-(1→4)-β-D-Glc*p*-(1→3)-1-deoxy-D-erythritol

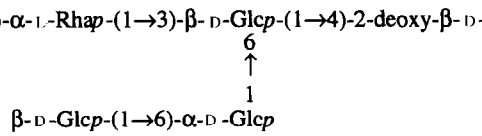
1

Two sugars have not yet been accounted for, the terminal group and the 6-substituted α-D-glucose residue. From the linkage pattern it is evident that residue **B'** is the former 3,6-substituted branch-point residue. The terminal glucosyl group will constitute the end of the side chain but the 6-substituted α-D-glucose residue could be either in the side chain or in the main chain. To resolve this problem a partial acid hydrolysis with 48% hydrofluoric acid was attempted as trifluoroacetic acid did not give conclusive results (see above). After removal of HF, and chromatography on Bio-Gel P-4 and Superdex 30, two compounds were obtained. The smaller of these gave glucose and rhamnose on hydrolysis, showed a signal with a large coupling in the ^{1}H NMR spectrum (δ 4.71, ~ 8 Hz) and two smaller signals for reducing rhamnose in the NMR spectrum, and was therefore assigned to the **C**–**E** disaccharide, β-D-Glc*p*-(1 → 4)-L-Rha. The larger of the two on methylation analysis showed the presence of terminal and 6-substituted glucose in the proportions 1:3 (Table 1). The ^{1}H NMR spectrum showed two signals with a large coupling (~ 8 Hz) (i.e., of *β-gluco* type) at δ 4.49 and 4.54, one signal with an intermediate size coupling (for an α anomer) at δ 4.95, and a signal at δ 5.40 attributed to the reducing deoxyglucuronic acid. A negative FAB mass spectrum of the material that had been analysed by NMR spectroscopy and clearly had the methylene protons still in the deuterium form [11] showed a pseudomolecular ion at *m/z* 666 corresponding to a tetrasaccharide with the formula Hex$_3$deoxyHexA-d_2. The oligosaccharide thus contains a terminal and two 6-substituted Glc residues in addition to the deoxyglucuronic acid. From data on the native I-886 it was evident that the terminal sugar is β-linked, and from hydrolysis and Smith degradation data that a β-D-Glc residue was located next to the acid. Thus the oligosaccharide has structure **2**:

β-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→4)-2-deoxy-D-*arabino*-Hex*p*A

2

The side chain must therefore be comprised of two sugar residues and these are linked to the branch-point residue in the 6-position. The repeating unit of I-886 therefore has structure **3**:



3

The deoxy sugar in this polysaccharide, 2-deoxy-D-*arabino*-hexuronic acid, is the first 2-deoxy sugar to be found in a bacterial polysaccharide. Several 6-deoxy sugars occur and six sugars that have a ring carbon that is devoid of oxygen have been found, namely the 3,6-dideoxyhexoses having D-*ribo* (paratose), D-*arabino* (tyvelose), L-*arabino* (ascarylose), D-*xylo* (abequose), and L-*xylo* (colitose) configurations, and 4-deoxy-D-*arabino*-hexose, which has been found only in *Citrobacter* [12].

3. Experimental

General methods.—Evaporations were performed by flushing with air at 50 °C. For GLC, a Hewlett–Packard 5890 instrument fitted with a flame-ionisation detector was used. Separations of alditol acetates were performed on a DB-5 capillary column (15 m × 0.25 mm) or on a DB-225 capillary column (15 m × 0.25 mm), using a temperature programme from 160 °C (1 min) → 250 °C at 3°/min. GLC–MS (EI) was performed on a Hewlett–Packard 5970 MSD instrument or on a Delsi Di200 GC-Nermag R10-10H quadrupole MS. FAB mass spectra obtained in the negative mode were recorded on a Nermag R10-10L instrument, using Xe atoms (6 keV) and a matrix of glycerol. Partially methylated alditol acetates were separated on a DB-5 capillary column or on a DB-225 capillary column, using a temperature programme from 130 °C (1 min) → 250 °C at 3 °C/min. Absolute configurations of the sugars were determined using the 2-butyl glycosides according to the procedures of Leontein et al. [3], and of Gerwig et al. [4], using acetates instead of Me_3Si ethers. Gel permeation chromatography was performed on Bio-Gel P-4 or P-2 (Bio-Rad, USA) and Superdex 30 columns (Pharmacia, Sweden) using water as eluent. Fractions were monitored by a differential refractometer.

Extraction and purification of I-886.—The strain I-886 was isolated at the Centre de Pedologie Biologique (Vandoeuvre les Nancy, France). It is available from the CNCM collection (Paris, France) under the identity I-886. The polysaccharide is obtained after centrifugation of the broth and precipitation from the supernatant solution with EtOH. The purification was performed according to the usual method [2].

NMR spectroscopy.—NMR spectra of solutions in deuterium oxide were recorded at 70 (^{13}C) and 60 or 85 °C (^{1}H) with a JEOL EX270 instrument. Chemical shifts are reported in ppm, using acetone (δ_C 31.00) and sodium 3-trimethylsilylpropanoate-d_4

(δ_H 0.00) as internal references. ^{1}H,^{1}H-COSY and NOESY experiments were performed using JEOL standard pulse-sequences. ^{1}H,^{1}H-HOHAHA experiments were performed in the phase-sensitive mode. The mixing time in the NOESY experiment was 500 ms and ^{1}H,^{1}H-HOHAHA experiments were obtained using mixing times of 40, 80, 120, and 180 ms. Proton–carbon-correlated spectra (HMQC) were obtained with or without decoupling and the long-range proton–carbon-correlated spectra (HMBC) were performed using a delay time of 62.5 ms.

Sugar and methylation analysis.—Hydrolysis of native and methylated I-886 was performed by treatment with 2 M CF_3CO_2H at 120 °C for 2 h or at 80 °C for 16 h. The sugars in the hydrolysates were converted into alditol acetates and partially methylated alditol acetates. The methylated products were purified by absorbing them on Sep-Pak C_{18}-cartridges and subsequently eluting them with acetonitrile.

Carboxyl reduction.—The native polysaccharide (2 mg) was dissolved in 0.2 M 2-(4-morpholino)ethanesulfonic acid [6,7], the pH was adjusted to 4.75, and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (150 mg) was added. The solution was stirred at room temperature for 90 min whereupon $NaBH_4$ (80 mg) was added and the solution was kept at room temperature for 60 min. The pH was adjusted to 7 and the solution was dialysed overnight.

Sugar analysis with morpholinoborane.—Carboxyl-reduced I-886 was hydrolysed with 0.5 M CF_3CO_2H at 100 °C for 2 h. During the hydrolysis 10 equivalents of borane–4-methylmorpholine complex were added for instant reduction [8]. The sugars in the hydrolysate were then converted into alditol acetates.

Preparation of O-deacylated I-886.—The polysaccharide was dissolved in 0.1 M NaOH and kept at room temperature for 16 h. After neutralisation the *O*-deacylated polysaccharide was dialysed and freeze-dried.

Partial degradation.—Native polysaccharide was dissolved in distilled water and the pH was adjusted to neutral. The solution was heated to 100 °C for 18 h. After dialysis with running tap water the partially degraded polysaccharide was recovered by gel filtration on a Bio-Gel P-2 column.

Smith degradation.—*O*-Deacylated I-886 was treated with 0.03 M sodium periodate in the dark at 4 °C for 72 h. The resulting product was reduced with sodium borohydride (3 mg per mg polysaccharide), followed by hydrolysis with 0.5 M CF_3CO_2H at room temperature for 24 h [9]. The Smith product was recovered by gel filtration on Bio-Gel P-4 and Superdex 30 columns.

Partial acid hydrolysis of I-886.—Native I-886 (10 mg) in aq 48% HF (1 mL) was kept for 48 h at 4 °C. The acid was removed by flushing with air. Undissolved material was removed by centrifugation. The resulting mixture of oligosaccharides was then purified by gel filtration on a Bio-Gel P-4 column.

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References

[1] J. Balandreau, B. Gueniot, and K.P. Hebbar, French Pat. 8809999 (1988).
[2] K.P. Hebbar, B. Gueniot, A. Heyraud, P. Colin-Morel, T. Heulin, J. Balandreau, and M. Rinaudo, *Appl. Microbiol. Biotechnol.*, 38 (1992) 248–253.
[3] K. Leontein, B. Lindberg, and J. Lönngren, *Carbohydr. Res.*, 62 (1978) 359–362.
[4] G.J. Gerwig, J.P. Kamerling, and J.F.G. Vliegenthart, *Carbohydr. Res.*, 62 (1978) 349–357.
[5] M. Forsgren, P.-E. Jansson, and L. Kenne., *J. Chem. Soc., Perkin Trans. 1*, (1985) 2383–2388.
[6] R.L. Taylor, J.E. Shively, and H.E. Conrad, *Methods Carbohydr. Chem.*, 7 (1976) 149–151.
[7] M. McNeil, W. Szalecki, and P. Albersheim, *Carbohydr. Res.*, 131 (1984) 139–148.
[8] P.J. Garegg, P. Konradsson, I. Kvarnström, and B. Lindberg, *Carbohydr. Res.*, 176 (1988) 145–148.
[9] I.J. Goldstein, G.W. Hay, B.A. Lewis, and F. Smith, *Methods Carbohydr. Chem.*, 5 (1965) 361–370.
[10] I. Backman, B. Erbing, P.-E. Jansson, and L. Kenne, *J. Chem. Soc., Perkin Trans. 1*, (1988) 889–898.
[11] P. Edebrink, P.-E. Jansson, M.M. Rahman, G. Widmalm, T. Holme, and M. Rahman, *Carbohydr. Res.*, 266 (1995) 237–261.
[12] B. Lindberg, *Adv. Carbohydr. Chem. Biochem.*, 48 (1990) 279–318.