Carbohydrate Research, 215 (1991) 303–314
Elsevier Science Publishers B.V., Amsterdam

Structural studies of the *Vibrio cholerae* O:3 O-antigen polysaccharide

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(Received October 11th, 1990; accepted for publication, November 26th, 1990)

ABSTRACT

The structure of the *Vibrio cholerae* O:3 O-antigen polysaccharide has been investigated, mainly by n.m.r. spectroscopy, mass spectrometry, sugar and methylation analysis, and specific degradations, and is proposed to involve the following tetrasaccharide repeating-unit.

→2)-D-α-D-Hep*p*-(1→4)-α-L-Fuc*p*NAc-(1→3)-β-Sug*p*-(1→
3
↑
1
α-Asc*p*

In this structure, D-D-Hep is D-*glycero*-D-*manno*-heptose, Asc is 3,6-dideoxy-L-*arabino*-hexose (ascarylose), and Sug is 2,4-diamino-2,4,6-trideoxy-D-glucose (bacillosamine) in which N-2 is acetylated and N-4 is acylated with a 3,5-dihydroxyhexanoic acid. That the 2,4-diamino-2,4,6-trideoxy-D-glucose residue is linked through O-3 and not through one of the hydroxyl groups in the 3,5-dihydroxyhexanoyl group is indicated but not definitely proved. The configuration of the latter group has not been determined. The f.a.b.-mass spectrum of the methylated O-antigen indicates that the structure given above also represents the biological repeating-unit.

INTRODUCTION

There are some 80 serogroups of *Vibrio cholerae*[1]. *V. cholerae* O:1 is the cause of Asian cholera, and the other serogroups, commonly referred to as non-O:1 or non-agglutinating (NAG), cause similar but less severe diseases. The three O-antigens that have been investigated in more detail all contain unusual components. The O:1 O-antigen is a homopolysaccharide of 4-amino-4,6-dideoxy-D-mannose, *N*-acylated with (*S*)-2,4-dihydroxybutanoic acid[2]. The O:2 O-antigen contains a 5-acetamidino-7-acetamido-3,5,7,9-tetradeoxy-L-*glycero*-L-*manno*-nonulosonic acid[3] and the O:21 O-antigen a D-*glycero*-D-*manno*-heptose[4] residue in their oligosaccharide repeating-units. We now report structural studies of the O:3 O-antigen polysaccharide.

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0008-6215/91/$ 03.50

RESULTS AND DISCUSSION

Treatment of the lipopolysaccharide from *V. cholerae* O:3 with aqueous 1% acetic acid at 100° for 2 h, centrifugation, and chromatography of the material in the supernatant solution on a column of Bio-Gel P-10 yielded the O-antigen polysaccharide (PS), which was eluted with 1.1–1.6 void volumes in a yield of 6%. The void fraction contained incompletely delipidated material together with ~1% of another polysaccharide. The pellet obtained on delipidation and centrifugation was further hydrolysed with 0.1M trifluoroacetic acid at 100° for 2 h and yielded a modified polysaccharide, formed by loss of the most acid-labile sugar component (ascarylose, see below).

An improved yield of PS (16%) was obtained when the LPS was *O*-deacylated with aqueous sodium hydroxide in the presence of sodium dodecyl sulfate (SDS), followed by hydrolysis at pH 3.6 for 2 h. The detergent permits delipidation under milder conditions[5]. Furthermore, the initial treatment with sodium hydroxide increased the solubility of the LPS and depolymerised the contaminating RNA. The insoluble lipid part did not contain any polysaccharide material after this treatment.

Two major sugar components, namely, 2-amino-2,6-dideoxy-L-galactose (fucosamine) and D-*glycero*-D-*manno*-heptose, were found in the sugar analysis. Smaller amounts of 3,6-dideoxy-L-*arabino*-hexose (ascarylose), fructose, glucose, and a heptose, tentatively identified as L-*glycero*-D-*manno*-heptose, were also found. Ascarylose is a component of the repeating unit of the PS (see below), but was partially released on delipidation and was also degraded during the hydrolysis under acidic conditions. The other sugars, present in small proportions, were also found in the core of the O:1 antigen[6]. The absolute configurations of these sugars were determined as devised by Gerwig *et al.*[7].

The anomeric regions in the ^{1}H-n.m.r. (Fig. 1) and the ^{13}C-n.m.r. (Fig. 2) spectra of the PS each show four major signals, indicating a tetrasaccharide repeating-unit. The chemical shifts of the signals from the anomeric carbons (Table I) show that each component is pyranosidic. The $^1J_{C,H}$ and $^3J_{H,H}$ values (Table I) show that three sugar residues have an axial O-1 and one has an equatorial O-1. Most of the signals from each of the four sugar residues could be assigned from the COSY spectrum. The sets of signals obtained were assigned to the sugar residues according to chemical shifts, coupling constants estimated from the COSY spectrum, and the presence of amino groups as judged from a H,C-COSY spectrum. Thus, three sugar residues with H-1 signals at δ 4.86, 5.07, and 5.25 could be assigned to the 3,6-dideoxy-α-L-*arabino*-hexopyranosyl, 2-amino-2-deoxy-α-L-fucopyranosyl, and D-*glycero*-α-D-*manno*-D-heptopyranosyl residues, respectively. The fourth H-1 signal, at δ 4.37 ($J_{1,2}$ 8.3 Hz), could be assigned to a 2,4-diamino-2,4,6-trideoxy-D-glucopyranosyl residue (see below) that is β-linked.

The ^{13}C-n.m.r. spectrum contained, *inter alia*, signals for carbonyl and hydroxymethyl groups, *N*-linked and methylene carbons, and *N*-acetyl and CH*Me* groups in the proportions 3:1:3:3:2:4. All of these signals could not be accounted for by the three sugars ascarylose, L-fucosamine, and D-*glycero*-D-*manno*-heptose. An additional spin

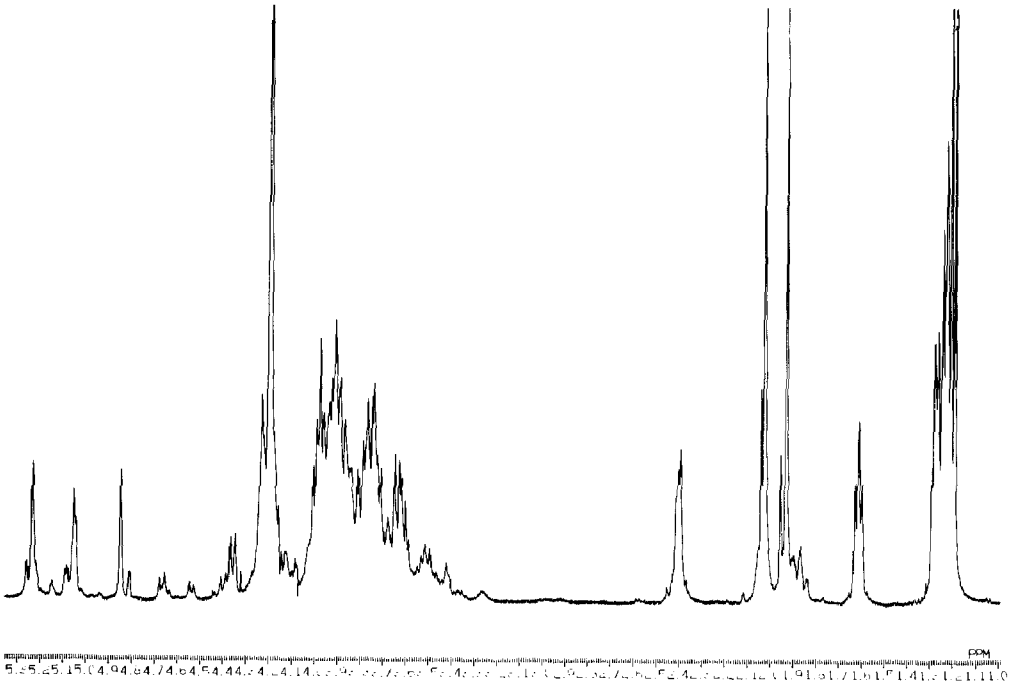


Fig. 1. ^{1}H-N.m.r. spectrum of the *V. cholerae* O:3 O-antigen.

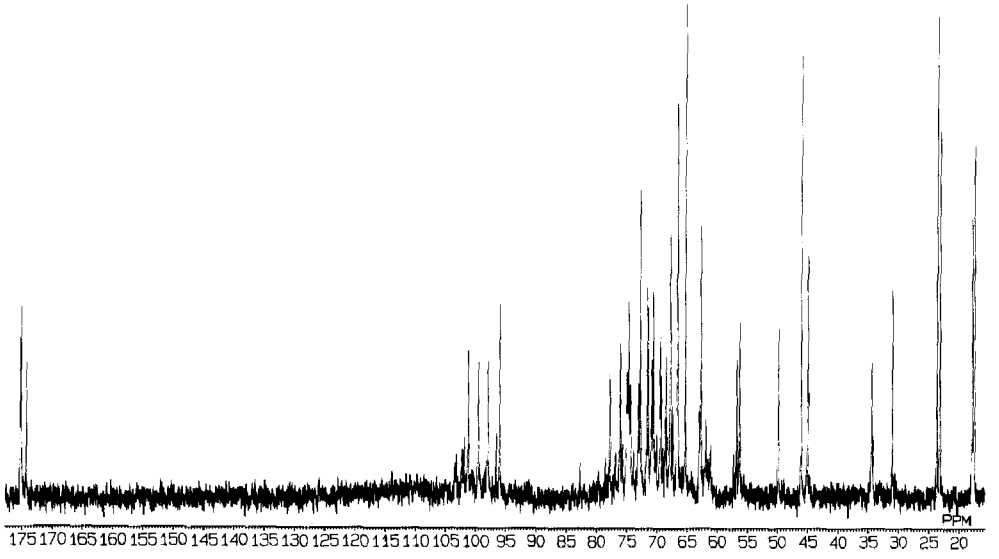


Fig. 2. ^{13}C-N.m.r. spectrum of the *V. cholerae* O:3 O-antigen.

TABLE I

^{1}H- and ^{13}C-n.m.r. chemical shift data[a] for the *Vibrio cholerae* O:3 O-antigen and derivatives

Material	*→3)-α-Hepp-(1→*[b]								*→4)-α-L-FucpNAc-(1→*[c]						
	H/C								*H/C*						
	1	*2*	*3*	*4*	*5*	*6*	*7*	*7'*	*1*	*2*	*NH*	*3*	*4*	*5*	*6*
Native VCO3[d]	5.25(n.r.)	4.25	3.97	3.79	3.84	4.01	3.64	3.78	5.07(n.r.)	4.23		3.90	3.73	3.53	1.25
	99.6[174]	74.8				72.8[e]	62.7		98.1[171]	49.9			76.9	71.5	17.6
Acid-treated VCO3	5.25	4.14	3.87			3.97	3.64	3.77	5.07	4.21		3.89	3.76	3.57	1.24
	99.3						62.9		98.0	49.6					
Alditol C	4.95	4.07	3.80			3.98	3.67	3.78				4.02	3.54	4.16	1.31
Peracetylated C	4.82	4.24	5.01	5.32	4.06	5.11	4.30	4.19	4.09, 3.80	5.52	5.73	5.20	3.67	5.04	1.28
		(3.4)	(10.3)	(10.3)	(< 1)		(12.0, 7.9)	(12.0, 4.3)	(6.2, 7.6)	(< 1)	(10.1)	(9.3)	(2.3)		(6.6)

Material	*3,5-Dihydroxyhexanamido group*							*→3)-β-Sugp-(1→*							
	H/C							*H/C*							
	2	*2'*	*3*	*4*	*4'*	*5*	*6*	*1*	*2*	*NH*	*3*	*4*	*NH*	*5*	*6*
Native VCO3[d]	~2.41,d		4.20	~1.64,dd		4.00	1.23	4.37 (8.3)	3.89		3.76	3.74		3.94	1.31
	45.0		66.7	46.1		65.3[e]	23.7	101.3 [166]	56.8			56.4			17.9
Acid-treated VCO3	~2.41,d		4.19	~1.63,dd		4.00	1.23	4.42	3.90		3.77			3.96	1.28
	45.0			46.1					56.9			56.3			
Alditol C	2.48		4.22	1.65		4.02	1.22	4.82	3.71			4.06		3.66[f]	1.26
Peracetylated C	2.44	2.33	5.12	1.89	1.79	4.91	1.22	4.72	3.96	6.94	4.99	3.91	5.61	3.64	1.22
								(8.5)		(9.3)			(9.4)		(6.3)
Methyl α-Sug								4.64	3.98		3.8–3.5		3.8–3.5	3.8–3.5	1.17
Methyl α-SugAc								4.66	4.34	5.58	4.99	3.97	5.24		1.24
								(3.7)	(10.1)	(9.52)	(10.3)	(9.9)	(9.5)		(6.6)

TABLE I (continued)

Material	*αAsc-(1→*						
	H/C						
	1	*2*	*3*	*3'*	*4*	*5*	*6*
Native VCO3	4.86(n.r) 96.0[170]	3.99 68.5[e]	~1.86 34.5		3.64 67.8	3.91	1.27 17.6

[a] $^{3}J_{H,H}$ values in parentheses and $^{1}J_{C,H}$ values in brackets. [b] Hep = D-*glycero*-D-*manno*-heptose. [c] →4)-FucNAc-ol-*1-d* for alditol *C*. [d] NAc*Me* δ 23.2, NAcC=O δ 175.3, 175.2, 174.4. [e] Tentative assignments. [f] Assigned by analogy with peracetylated *C*.

system, CH_3–CH–CH_2–CH–CH_2–C, was detected in the COSY spectrum and was proved (see below) to be derived from a 3,5-dihydroxyhexanoyl group. The unassigned signals thus indicated that the fourth sugar residue is a 6-deoxyhexose with two amino groups. Of the three amino groups (one in L-fucosamine), two are acetylated and the third is acylated with 3,5-dihydroxyhexanoic acid.

Methanolysis of the PS, *N*-acetylation, and chromatography of the product on columns of Bio-Gel P-2 and Bondapak C18 yielded, *inter alia*, two glycosides containing *N*-acetyl and CH*Me* groups, as judged from their ^{1}H-n.m.r. spectra. A compound from the gel filtration, eluted in the disaccharide region, was identified as methyl 2-acetamido-2-deoxy-α-L-fucopyranoside. The ^{1}H-n.m.r. spectrum of a compound eluted in the trisaccharide region contained signals for equatorial H-1, NAc, and CH*Me* in the proportions 1:6:3, indicating that it was derived from a 6-deoxyhexose that contained two amino groups. The signals for H-3–H-5 gave a higher order spectrum, and therefore the compound was acetylated. Assignment of its ^{1}H-n.m.r. signals by a COSY experiment demonstrated that it was the acetate of methyl 2,4-diamino-2,4,6-trideoxy-α-D-glucopyranoside. Because of the small amount of material available, an accurate value for the optical rotation of the *N*-acetylated derivative could not be determined, but it was definitely positive. Benzyl 2,4-diacetamido-2,4,6-trideoxy-α-D-glucopyranoside[9] has $[\alpha]_D$ +163°, and it is concluded that the parent sugar is 2,4-diamino-2,4,6-trideoxy-D-glucose.

Methylation analysis of the PS showed terminal ascarylose, fucosamine linked through O-4, and heptose linked through O-2 and O-3 in the proportions 1:1.2:1. No derivative of 2,4-diamino-2,4,6-trideoxy-D-glucose was detected in this analysis.

The integrals of the anomeric signals assigned to the ascarylose and 2,4-diamino-2,4,6-trideoxy-β-D-glucose residues (Fig. 3) were smaller than those of the other two sugars. Some ascarylose was also released on delipidation of the LPS. In the ^{1}H-n.m.r. spectrum of the material from which all of the ascarylose had been cleaved by hydrolysis, the signal for the anomeric proton of the 2,4-diamino-2,4,6-trideoxy-β-D-glucose residue had shifted from δ 4.37 to 4.41. A signal at δ 4.41, corresponding to the deficit in ascarylose, was also given by the native PS, indicating equimolar amounts of 2,4-diamino-2,4,6-trideoxy-D-glucose, fucosamine, and heptose. Other weak signals (Fig. 3) most probably are derived from the core sugars. For each of the strong anomeric signals, however, a neighbouring weak signal was detected. It was evident, from the COSY spectrum, that the H-2 signals corresponding to the strong and weak anomeric signals were also close and that the $J_{1,2}$ values for each pair of signals were of the same magnitude. The ascarylose-free PS showed the same low-intensity signals. The integration of the signals from the *N*-acyl groups showed that these were present in equimolar amounts. The most reasonable explanation of these weak signals is that a small portion of the glycosidic linkages in the chain of the PS was hydrolysed during its preparation.

When the PS was hydrolysed with acid under mild conditions, in order to remove the 3,6-dideoxyhexosyl groups, some depolymerisation also occurred and three products, as their alditol-*1d* derivatives *A*–*C*, were isolated from the hydrolysate.

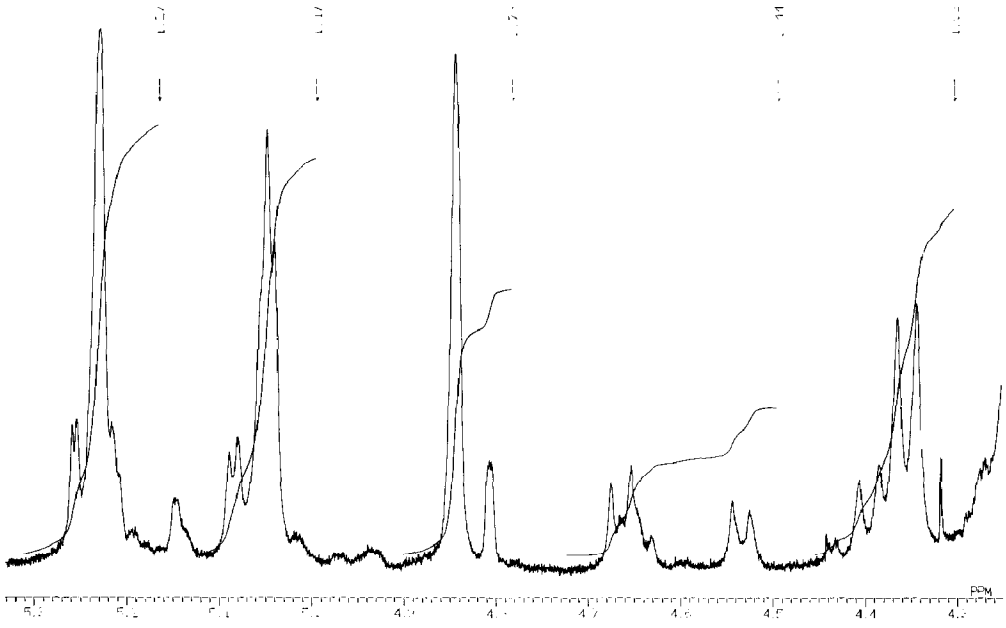
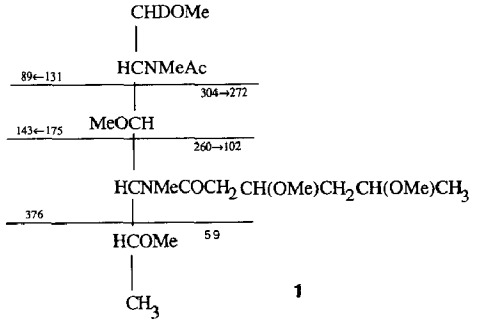


Fig. 1. ^{1}H-N.m.r. spectrum of the *V. cholerae* O:3 O-antigen.

The ^{1}H-n.m.r.spectrum of *A* contained signals from two methylene, one *N*-acetyl, and two CH*Me* groups, but no signals from anomeric protons. In a COSY-spectrum, two spin systems could be observed. One of these could be attributed to protons from a carbon chain with two methylene groups and a methyl group, as was also observed for the PS. The $(M-H)^-$ ion (*m/z* 336) in the negative-mode f.a.b.-mass spectrum, in conjunction with evidence discussed above, indicated that it was a 2,4-diamino-2,4,6-trideoxy-D-glucitol-*1-d*, *N*-acylated with acetic acid and a 3,5-dihydroxyhexanoic acid. The e.i. mass spectrum of permethylated *A* (**1**) confirmed this conclusion and further determined the positions of the acyl groups.



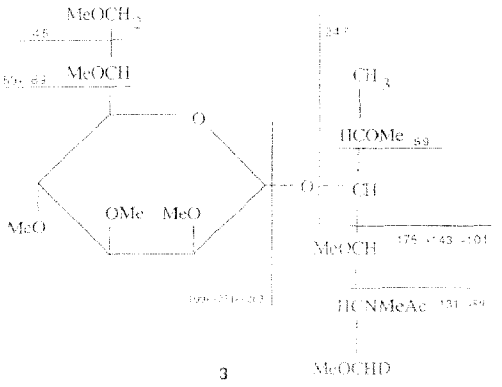
1

Alditol *B* gave pseudomolecular ions at *m/z* 399 for $(M-H)^-$, 401 for $(M+H)^+$ and 423 for $(M+Na)^+$ on f.a.b.-m.s. in the negative and positive mode, respectively, indicating that it was derived from a disaccharide composed of 2-acetamido-2-deoxy-fucose and heptose. Hydrolysis yielded D-*glycero*-D-*manno*-heptose and 2-amino-2-

deoxy-L-fucitol-*1-d*. This was confirmed by the ^{1}H-n.m.r. spectrum, which, *inter alia*, showed a signal for one anomeric proton at δ 4.92 ($J_{1,2}$ 1.8 Hz). These results, in conjunction with evidence discussed above, show that *B* has structure **2**. This was further confirmed by the e.i.-mass spectrum of permethylated *B* (**3**).

D-α-D-Hep*p*-(1→4)-L-FucNAc-ol-*1-d*

2



3

Hydrolysis of *C* yielded 2-amino-2-deoxy-L-fucitol-*1-d* and D-*glycero*-D-*manno*-heptose, but the ^{1}H-n.m.r. spectrum demonstrated that it also contained the *N*-acylated 2,4-diamino-2,4,6-trideoxy-β-D-glucopyranosyl residue (Sug) and, consequently, has structure **4** or a similar structure in which the heptose is linked through O-3.

β-Sug*p*-(1→2)-D-α-D-Hep*p*-(1→4)-L-FucNAc-ol-*1-d*.

4

The ^{1}H-n.m.r. spectrum of *C* was assigned, and further confirmed the configuration of the heptose and the diamino sugar residues. By comparison of ^{1}H-n.m.r. chemical shifts of the native and the peracetylated *C* (Table I), the linkage positions could also be determined. Thus, signals for H-2 in the heptosyl residue and H-4 in the 2-acetamido-2-deoxyfucosyl residue were not significantly shifted downfield upon acetylation, indicating that the residues are linked through the 2- and 4-positions, respectively. Therefore, the ascarylose group must be linked to O-3 in the heptose residue. This conclusion was confirmed by methylation analysis of the ascarylose-free PS, which showed heptose linked through O-2 and fucosamine linked through O-4 in the proportions 1:1.5.

The combined evidence discussed above indicated that the PS is composed of tetrasaccharide repeating-units with the structure **5**.

→2)-D-α-D-Hep*p*-(1→4)-α-L-Fuc*p*NAc-(1→3)-β-Sug*p*-(1→

3

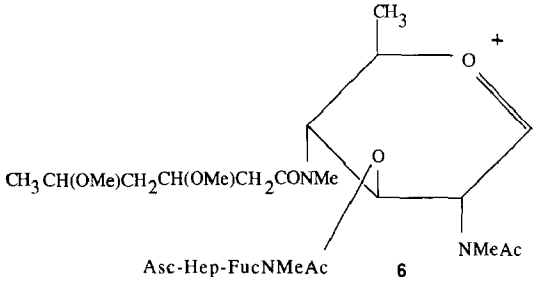
↑

α-Asc*p*

5

If the L-FucNAc is linked to the 2,4-diamino-2,4,6-trideoxy-β-D-glucose residue, as in **5**, it has to be linked to O-3. Another possibility, that it is linked to one of the oxygens of the 3,5-dihydroxyhexanoyl moiety, is not excluded, and examples of this type of linkage are known[9,10]. The complete structure of the 3,5-dihydroxyhexanoic acid was not determined, as it decomposed on attempted isolation by treatment of PS with acid or base.

On f.a.b.-m.s., in the positive mode, of permethylated high molecular weight glycoconjugates that contain 2-acetamido-2-deoxyhexosyl residues, A_1-type ions are obtained by fragmentation at these residues[11]. Similarily, f.a.b.-m.s. of the fully methylated PS yielded, *inter alia*, fragment ions with *m/z* 994 and 622. These are the values expected for the ions **6** and **7**, respectively, which are A_1 ions formed from the non-reducing end of the PS by fission at either of the amino sugar residues. These results support the sequence of the sugars in the tetrasaccharide repeating-unit **5** given above, and indicate that **5** is actually the biological repeating-unit.



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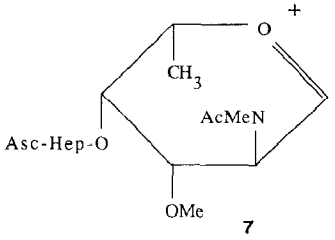
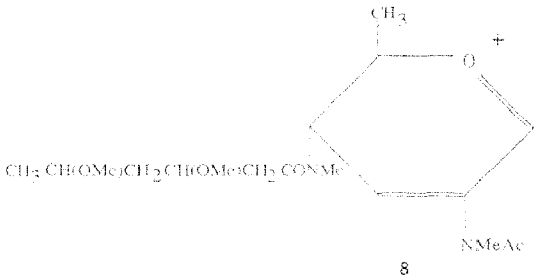


7

As discussed above, some glycosidic linkages were most probably cleaved during delipidation. That the non-reducing end giving rise to the ions **6** and **7** should be formed exclusively by such cleavage is, however, not supported by the results of the partial hydrolysis of the PS.

Secondary fragments are not common in f.a.b.-m.s. of carbohydrates. One exception, however, involves the A_1-type fragments discussed above, which eliminate the substituent in the 3-position, either methanol or a methylated mono- or oligosaccharide[11]. A strong fragment with *m/z* 355 probably had structure **8** and was derived from **6** and larger ions, formed by fission at a 2-acetamido-4-(3,5-dihydroxyhexanamido)-2,4,6-trideoxy-D-glucosyl residue. This result therefore implies that this residue is actually linked through the 3-position, as indicated in **5**.



8

2,4-Diamino-2,4,6-trideoxy-D-glucose (bacillosamine) is a component of cell walls of *Bacillus licheniformis*[12]. It is also a component of the O-antigen from *Pseudomonas aeruginosa* O:3, in which it is acylated by acetic acid at N-2 and by (*S*)-3-hydroxybutyric acid at N-4 (ref. 13). 3(*S*),5(*S*)-Dihydroxyhexanoic acid has been found in berries of *Sorbus aucuparia* as the β-D-glucopyranoside of its δ-lactone[14].

EXPERIMENTAL

General methods. — Concentrations were performed under diminished pressure at < 40° or by flushing with air at room temperature. For g.l.c., a Hewlett–Packard 5830 instrument fitted with a flame-ionisation detector was used. G.l.c.–m.s. (e.i.) was performed on a Hewlett–Packard 5970 MSD. A JEOL SX102 instrument was used for the f.a.b.-m.s. Ions were produced by a beam of xenon atoms (4–6 keV), using a matrix consisting of glycerol for oligosaccharides, glycerol–thioglycerol (1:1) for peracetylated oligosaccharides, and thioglycerol for the permethylated PS.

Fractionation of alditol acetates was performed on an HP-5 capillary column (25 m × 0.20 mm × 0.33 μm), using a temperature programme 200° (3 min) →250° at 2°/min, and of permethylated oligosaccharide-alditols on an HP-1 column (12 m × 0.20 mm × 0.33 μm), using a temperature programme 235° (2 min) →350° at 5°/min.

Gel filtration was performed using water containing 1% of 1-butanol as irrigant and was monitored with a differential refractometer. H.p.l.c. was performed on a Shimadzu LC6A system including a u.v. detector (195 nm). All separations were isocratic, using a reversed phase C18 (μBondapak) column eluted with water containing up to 10% of acetonitrile.

Preparation of LPS and PS. — The LPS was prepared as previously described[1]. The LPS (800 mg) was suspended in aqueous 0.1M sodium hydroxide containing 2% of SDS, sonicated for 5 min, and kept at room temperature for 15 h. Glacial acetic acid was added to pH 3.6 and the solution was kept at 100° for 2 h. After lyophilisation, the material was washed three times with ethanol, suspended in water, and centrifuged. The supernatant solution was then applied to a column (95 × 2.5 cm) of Bio-Gel P-10. The PS (125 mg) was eluted at 1.1–1.6 void volumes.

Sugar and methylation analysis. — A solution of the native or permethylated PS in anhydrous hydrogen fluoride was kept for 3 h at room temperature. The hydrogen fluoride was evaporated and the residue treated with 2M trifluoroacetic acid at 120° for 3

h. The sugars in the hydrolysates were then converted into the alditol acetates. The absolute configurations of the sugars were determined as described by Gerwig *et al.*[7].

N.m.r. spectroscopy. — N.m.r. spectra of solutions in deuterium oxide were recorded at 70° (^{13}C) and 85° (^{1}H), using a JEOL GX-400 or GSX-270 instrument. Chemical shifts are reported in p.p.m., using sodium 3-trimethylsilylpropanoate-d_4 (δ 0.00) for ^{1}H and acetone (δ 31.07) for ^{13}C as internal references. Phase-sensitive double-quantum filter H,H-COSY, relayed H,H-COSY, and H,C-COSY experiments were performed using JEOL standard pulse sequences. The double-relayed H,H-COSY experiment was performed according to Bax and Drobny[15]. Relayed H,H-COSY spectra were obtained using a 30- or 60-ms delay time. For the double-relayed H,H-COSY experiments, values of 30 ms for both delay times or 30 ms and 60 ms for delay times one and two, respectively, were used. The $^{1}J_{C,H}$ values for the anomeric carbon signals were determined by an INEPT experiment using a 1.8-ms delay, and the multiplicities for ^{13}C resonances were established via a ^{1}H decoupled DEPT experiment, using P_0 135° and a delay time of 3.58 ms.

Partial hydrolysis. — The PS (120 mg) was treated with 0.1 M trifluoroacetic acid at 100° for 1 h. Chromatography on a column (95 × 2.5 cm) of Bio-Gel P-10 gave a polymeric, an intermediate, and a low molecular weight fraction. The last fraction, eluted at 2.4–3.9 void volumes, was applied to a column (90 × 2.5 cm) of Bio-Gel P-2. Compounds eluted in the range 1.3–2.1 void volumes were reduced with sodium borodeuteride, rechromatographed on Bio-Gel P-2, and further purified by h.p.l.c., using a reversed phase C18 column.

Methanolysis. — The fraction of intermediate molecular weight (see above), eluted at 1.6–2.4 void volumes, was dissolved in dry methanolic M hydrogen chloride (20 mL). The solution was kept at 85° for 15 h and then neutralised with silver carbonate. Acetic anhydride (1 mL) was added, the solution was kept at room temperature for 4 h and then centrifuged, and the supernatant solution was concentrated. The residue was fractionated on a column (90 × 2.5 cm) of Bio-Gel P-2. Fractions were investigated by ^{1}H-n.m.r. spectroscopy and those containing acetamido sugars further purified by h.p.l.c.

ACKNOWLEDGMENTS

This investigation was supported by grants from the Swedish Medical Research Council (03X-02522) and the Swedish National Board for Technical Development. A maintenance grant from the International Program in the Chemical Sciences, Uppsala University (to T.A.C.) is also gratefully acknowledged.

REFERENCES

1 R. Sakazaki and J. Donovan, *Methods Microbiol.*, 16 (1984) 271–289.
2 L. Kenne, B. Lindberg, P. Unger, B. Gustafsson, and T. Holme, *Carbohydr. Res.*, 100 (1982) 341–349.
3 L. Kenne, B. Lindberg, E. Schweda, B. Gustafsson, and T. Holme, *Carbohydr. Res.*, 180 (1988) 285-294.
4 A. A Ansari, L. Kenne, B. Lindberg, B. Gustafsson, and T. Holme, *Carbohydr. Res.*, 150 (1986) 213–219.
5 M. Caroff, A. Tacken, and L. Szabo, *Carbohydr. Res.*, 175 (1988) 273–282.

6 B. Jann, K. Jann, and G. O. Beyaert, *Eur. J. Biochem.*, 37 (1973) 531–534.
7 G. J. Gerwig, J. P. Kamerling, and J. F. G. Vliegenthart, *Carbohydr. Res.*, 62 (1978) 349–357.
8 A. Liav, I. Jacobsson, M. Sheinblatt, and N. Sharon, *Carbohydr. Res.*, 66 (1978) 95–101.
9 P.-E. Jansson, B. Lindberg, M. Spellman, T. Hofstad, and N. Skaug, *Carbohydr. Res.*, 137 (1985) 197–203.
10 Yu. A. Knirel, E. V. Vinogradov, A. S. Shashkov, B. A. Dmitriev, N. K. Kochetkov, E. S. Stanislavsky, and G. M. Mashilova, *Eur. J. Biochem.*, 157 (1986) 129–138.
11 A. Dell, *Adv. Carbohydr. Chem. Biochem.*, 45 (1987) 19–72.
12 U. Zehavi and N. Sharon, *J. Biol. Chem.*, 248 (1973) 433–438.
13 Yu. A. Knirel, E. V. Vinogradov, A. S. Shashkov, S. G. Wilkinson, Y. Tahara, B. A. Dmitriev, N. K. Kochetkov, E. S. Stanislavsky, and G. M. Mashilova, *Eur. J. Biochem.*, 155 (1986) 659–669.
14 R. Tschesche, H.-J. Hoppe, G. Snatzke, G. Wulff, and H.-W. Fehlhaber, *Chem. Ber.*, 104 (1971) 1420–1428.
15 A. Bax and G. Drobny, *J. Magn. Reson.*, 61 (1985) 306–320.