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Structure of the O-polysaccharide chain of the lipopolysaccharide of *Vibrio anguillarum* V-123 *

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ABSTRACT

The O-polysaccharide chain (PS-1), released by mild acidic treatment of the LPS of *V. anguillarum* V-123 (serogroup JO-2), a pathogenic bacterium of marine and estuarine fish, consists of 2-amino-2-deoxy-D-galacturonic acid, 2-amino-2,6-dideoxy-D-glucose (D-quinovosamine), and 4-amino-4,6-dideoxy-D-glucose (D-viosamine) *N*-acylated with 2,4-dihydroxy-3,3,4-trimethylpyroglutamic acid. Strong-acid hydrolysis of PS-1 afforded α-GalNA-(1 → 4)-α-GalNA-(1 → 3)-QuiN (A1) and α-GalNA-(1 → 3)-QuiN (A2), and hydrolysis with hydrogen fluoride gave *N*-acetylated A1 and 4-amino-4,6-dideoxy-D-glucose *N*-acylated by 2,4-dihydroxy-3,3,4-trimethylpyroglutamic acid. Mild treatment of PS-1 with alkali removed the *N*-formyl substituents and Smith degradation of the product gave α-QuiNAc-(1 → 3)-β-VioNAcyl-(1 → 3)-α-GalNAcA-(1 → 3)-2,3,4-trihydroxybutanoic acid (S1) and S2 in which the carboxyl group of the GalNAcA residue was amidated. Thus, the repeating unit of the O-polysaccharide is → 3)-α-GalNAcA(amido)-(1 → 4)-α-GalNFoA-(1 → 3)-α-QuiNAc-(1 → 3)-β-VioNAcyl-(1 → in which the *N*-Acyl group is 2,4-dihydroxy-3,3,4-trimethylpyroglutamic acid and Fo is formyl.

INTRODUCTION

Information on the structure of lipopolysaccharides (LPSs) of *V. anguillarum* is limited. There have been reports[1–6] on the structure of the LPS of *V. anguillarum* strain ST-40, which contains 3-acetamido-3,6-dideoxy-L-glucose as an immunodeterminant group at the non-reducing terminal position of the O-polysaccharide chain. In the preceding paper[7], the O-polysaccharide preparation from the LPS of *V. anguillarum* V-123 was shown to consist of 2-amino-2-deoxy-D-galacturonic acid

* Studies of the Structure of the Lipopolysaccharide of *Vibrio anguillarum* V-123, Part II. For Part I, see ref. 7.

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(GalNA), D-quinovosamine (QuiN), and 4-amino-4,6-dideoxy-D-glucose (D-viosamine, VioN) with 2,4-dihydroxy-3,3,4-trimethylpyroglutamic acid, formic acid, and acetic acid as the *N*-acyl substituents.

We now report on the structure of the O-polysaccharide.

EXPERIMENTAL

Preparation of the O-polysaccharide.—The cultivation of *V. anguillarum* V-123 (serogroup JO-2), isolation of the LPS, and preparation of the O-polysaccharide were as described[7]. From a 135-L culture, 377 mg of O-polysaccharide (PS-1) and 435 mg of capsular polysaccharide (PS-2) were obtained.

Isolation of oligosaccharide fragments.—PS-1 (11 mg) was hydrolysed in 10 M HCl at 80° for 0.5 h (→ the trisaccharide A1) or in 4 M HCl at 100° for 1 h (→ the disaccharide A2). The products of hydrolysis were fractionated into hexosamine-containing components (1–4) by gel chromatography on a column (1 × 145 cm) of Cellulofine GCL-25-m in 50 mM pyridine–acetic acid–water (pH 5.0). The respective fractions I and II (see Fig. 3 in the preceding paper[7]) were further purified to give A1 (6.9 mg) and A2 (2.5 mg) from I, and A1 (1.0 mg) and A2 (5.8 mg) from II.

N-*Deformylation and isolation of Smith-degradation products.*—PS-1 (150 mg) was treated with 0.1 M NaOH (20 mL) at 30° for 18 h. The solution was neutralised with 2 M HCl, dialysed, and lyophilised to give the *N*-deformylated product (128 mg; no ^{1}H signal at 8.16 ppm for NCHO groups). This product (100 mg) was oxidised with 0.1 M $NaIO_4$ in 0.1 M NaOAc buffer (10 mL, pH 5.0) at 4° for 3 days in the dark. The excess of $NaIO_4$ was reduced with 1,2-ethanediol, the product was reduced with $NaBH_4$, and the solution was neutralised with acetic acid and deionised by passing through a colum of Dowex 50W (H^+) resin. The product was treated with 0.5 M HCl at 20° for 3 days, and the products were fractionated into hexosamine-containing components by gel chromatography on Cellulofine GCL-25-m, namely, S1 (36.2 mg), S2 (36.4 mg), and a large product that reflected incomplete hydrolysis of the acetal bonds.

Analytical methods.—Unless otherwise indicated, the methods and materials used were those in the previous paper[7]. Total hexosamine was assayed by the method of Tsuji et al.[8] with 2-amino-2-deoxy-D-glucose as the standard after acid hydrolysis (2 M HCl, 100°, 2 h). Total hexose was determined by the phenol–H_2SO_4 method[9] with D-glucose as the standard. For determination of amino sugars and their alditols, GLC was performed with a Shimadzu Gas-Chromatograph GC-7AG, using glass columns (4 mm × 100 cm) of 5% SE-52 on Chromosorb WAW and 1% OV-17 on Gas-Chrom Q[10,11]. Amide nitrogen was determined by assay of the ammonia released by acid hydrolysis (2 M HCl, 100°, 2 h) of deionised samples[12]. Amino sugars and 2,4-dihydroxy-3,3,4-trimethylpyroglutamic acid were determined with an amino acid analyser[7]. For *N*-acetylation, samples were treated twice with 2% acetic anhydride in aq 8% triethylamine at 25° for 90 min. Carboxyl groups were reduced by the method of Taylor and Conrad[13]. Methylation was carried out

by the method of Ciucanu and Kerek[14]. The ^{1}H- and ^{13}C-NMR spectroscopy and GLC–MS were carried out as described in the previous paper[7]. 2D ^{1}H–^{1}H correlation NMR spectroscopy (COSY) was carried out for the precise assignment of complicated signals.

RESULTS

Constituents of PS-1.—In the previous paper[7], the occurrence of 2,4-dihydroxy-3,3,4-trimethylpyroglutamic acid as the *N*-acyl substituent of 4-amino-4,6-dideoxy-D-glucose (VioN) residues of the O-polysaccharide (PS-1) released from the LPS of *V. anguillarum* V-123 by mild treatment with acid was reported. In the chemical analysis of PS-1, only a qualitative result was obtained, owing to the presence of uronosidic linkages that were resistant to acid hydrolysis. Repeated carboxyl-reduction effected incomplete conversion of GalNA into GalN, probably owing to the presence of ~25% of the amide form of GalNA. As shown in Fig. 1, the ^{1}H- and ^{13}C-NMR spectra of PS-1 were complex, probably due to the presence of both acid and amide forms of GalNA, 6-deoxyHexN residues (QuiN and VioN), and various *N*-acyl groups (acetyl, formyl, and 2,4-dihydroxy-3,3,4-trimethylpyroglutamyl). However, several signals could be assigned: ^{1}H, NCHO (8.14 ppm), H-1 (4.65, 4.81, 5.08, and 5.36 ppm), NAc (2.00 and 2.05 ppm), CMe of 2,4-dihydroxy-3,3,4-trimethylpyroglutamic acid residues (1.25 ppm), and CMe of 6-deoxyHexN (1.19 and 1.24 ppm); ^{13}C, 5 or 6 C=O (174–179 ppm), NCHO (165.9 ppm), C-1 (97.0, 99.0, 99.5, and 104.2 ppm), 16–18 C–O (61.7–81.9 ppm), 4–5 C–N (48.7–60.0 ppm), 2 $NCOCH_3$ (22.85 ppm), 2 CMe of 6-deoxyHexN (16.73 and 17.32 ppm), and 3 CMe of 2,4-dihydroxy-3,3,4-trimethylpyroglutamic acid residues (16.15, 16.50, and 19.23 ppm). These assignments were confirmed by the following findings.

The above NMR data, together with the results of qualitative analysis and the characterisation of amino sugar components[7], suggest that PS-1 has a repeating tetrasaccharide unit which consists of GalNA, QuiN, and VioN in the molar ratios 2:1:1. In addition, PS-1 contained *N*-formyl, *N*-acetyl, and *N*-2,4-dihydroxy-3,3,4-trimethylpyroglutamyl groups, in the molar ratios 1:2:1.

Characterisation of oligosaccharide fragments obtained after acid hydrolysis of PS-1.—As described in the previous paper[7], acid hydrolysis (4 M HCl, 100°, 1 h) of PS-1 gave a disaccharide (A2) as the major fragment. However, hydrolysis in 10 M HCl at 80° for 0.5 h gave a trisaccharide (A1). When each fragment was *N*-acetylated, reduced with carbodi-imide/$NaBH_4$, and hydrolysed with acid, A1 gave GalN and QuiN-ol in the molar ratio 2:1, whereas A2 gave the same components in the ratio 1:1. Thus, QuiN was the reducing terminus in each fragment.

Methylation analysis (GLC–MS) of carboxyl-reduced A1 gave equimolar proportions of GalN-ol, 4-substituted GalN-ol, and 3-substituted QuiN-ol, and of carboxyl-reduced A2 gave equimolar proportions of GalN-ol and 3-substituted QuiN-ol. The ^{1}H- and ^{13}C-NMR spectra of *N*-acetylated A1 and A2 indicated the

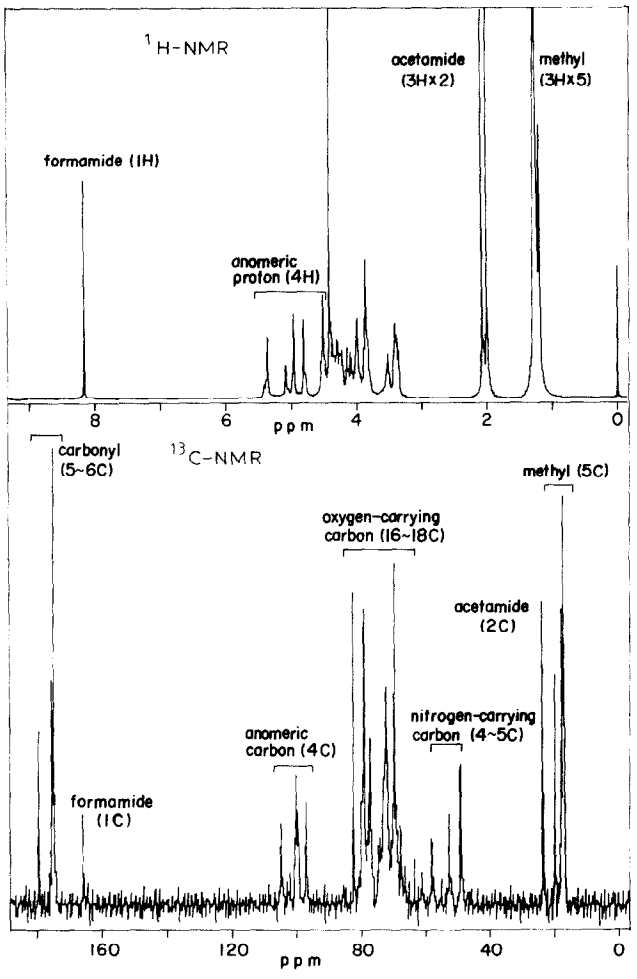


Fig. 1. ^{1}H- and ^{13}C-NMR spectra of PS-1.

presence of two and one α-GalNA residues, respectively. Furthermore, the data for *N*-acetylated A1 accorded with those of the authentic trisaccharide, α-GalNAcA-(1 → 4)-α-GalNAcA-(1 → 3)-QuiNAc, isolated[10] from the acid hydrolysate of the O-polysaccharide of *Pseudomonas aeruginosa* IID 1008 LPS. Therefore, the most likely structure of A2 is α-GalNA-(1 → 3)-QuiN.

Characterisation of the oligosaccharide fragment obtained by hydrolysis of PS-1 with hydrogen fluoride.—As described in the previous paper[7], gel chromatography on Cellulofine GCL-25-m of the HF hydrolysate of PS-1 gave 4-amino-4,6-dideoxy-D-glucose *N*-acylated with 2,4-dihydroxy-3,3,4-tri-methylpyroglutamic acid, together with a major oligosaccharide (H2). In the ^{1}H-NMR spectra, the signals for H-1 and the other ring protons of H2 were almost in agreement with those from *N*-acetylated A1, but signals for the *N*-acyl group differed by the presence of a

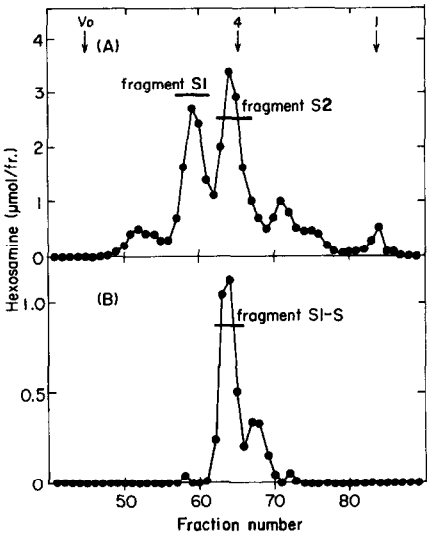


Fig. 2. Fractionation of the Smith-degradation products of *N*-deformylated PS-1. (A) *N*-Deformylated PS-1 (100 mg) was subjected to Smith degradation (see Experimental) and the products were chromatographed on a column (1×145 cm) of Cellulofine GCL-25-m in 50 mM $(NH_4)_2CO_3$. Fractions (1 mL) were assayed for total hexosamine. Fractions indicated by bars were combined to give S1 and S2. (B) S1 was subjected to Smith degradation and the product was chromatographed as in (A) to give S1-S.

signal for NCHO (8.16 ppm, 0.5 H) for H2. The smaller integrated value of this signal reflected partial removal of the *N*-formyl group during the treatment with HF. Thus, H2 is a trisaccharide with the same sugar sequence as in A1, but the *N*-formyl group seemed to be present on either amino sugar residue.

Characterisation of Smith-degradation products.—Mild treatment of PS-1 with alkali (0.1 M NaOH, 30°, 18 h) caused *N*-deformylation (loss of the signal at 8.16 ppm) without any cleavage of the polysaccharide chain. *N*-Deformylated PS-1 was oxidised with $NaIO_4$, whereas PS-1 was unaffected. Smith degradation of *N*-deformylated PS-1 followed by gel chromatography on Cellulofine GCL-25-m (Fig. 2A) gave two major fragments (S1 and S2) and one minor fragment which reflected incomplete hydrolysis of acetal linkages. Acid hydrolysis of S1 and S2 each gave GalNA, QuiN, VioN, 2,4-dihydroxy-3,3,4-trimethylpyroglutamic acid, acetic acid, and 2,3,4-trihydroxybutanoic acid in the molar ratios 1:1:1:1:2:1. The butanoic acid derivative seems to arise from the *N*-deformylated GalNA by oxidative cleavage of the C-2–C-3 bond. Thus, one of the two GalNA residues in the repeating unit of PS-1 was *N*-formylated. In addition, analysis of amide nitrogen revealed that S2 contained one amide nitrogen per QuiN, whereas S1 did not contain detectable amide nitrogen. Therefore, both carboxyl groups in S1 were present in the free form, whereas, in S2, the carboxyl group of either GalNA or 2,3,4-trihydroxybutanoic acid was amidated.

A second Smith-degradation of S1 followed by gel chromatography on Cellulofine GCL-25-m gave a fragment (S1-S) smaller than S1 (Fig. 2B), and chemical

analysis of S1-S revealed that only the QuiN had been oxidised by $NaIO_4$. Thus, the QuiN was the non-reducing terminus. In addition, the 2,3,4-trihydroxybutanoic acid residue was unoxidised, indicating the GalNA to be 4-substituted. Thus, the *N*-deformylated GalNA (yielding 2,3,4-trihydroxybutanoic acid upon the first Smith-degradation) was 4-substituted.

On the basis of the structures of A1, S1, and H1, the partial structure of the repeating unit of PS-1 is inferred to be → 3)-QuiNAc-VioNAcyl-α-GalNAcA-(1 → 4)-α-GalNFoA-(1 →, in which *N*-Acyl is the 2,4-dihydroxy-3,3,4-trimethylpyroglutamyl group[7].

COSY spectroscopy.—The COSY spectrum of S1 (Fig. 3) revealed the presence of three H-1 signals. Taking account of the NMR data for A1 and S1-S (see below), all proton signals for GalNA, QuiN, 2,3,4-trihydroxybutanoic acid, and the *N*-acyl moiety were assigned from the ^1H–^1H correlation. Table I summarises the assignments for S1 and S2. The chemical shifts and coupling constants are

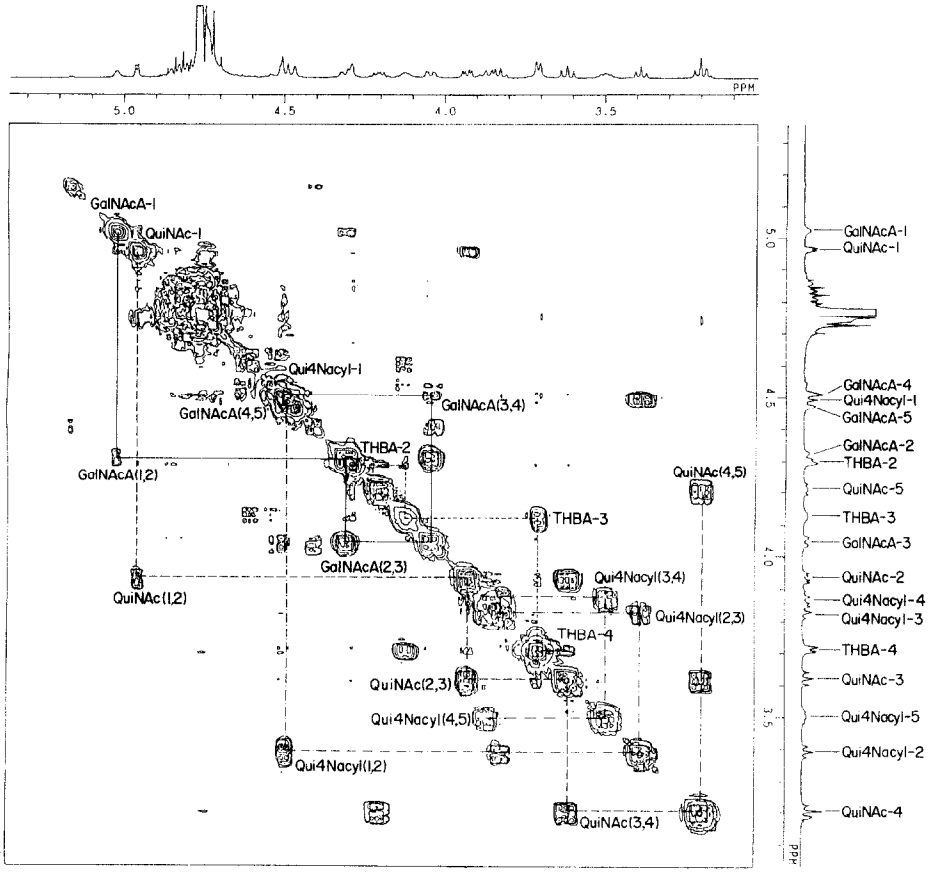


Fig. 3. The COSY spectrum of S1. THBA indicates the 2,3,4-trihydroxybutanoic acid residue.

TABLE I

^{1}H-NMR data [a] (δ in ppm, J in Hz) for S1 and S2

Residue	Chemical shift (J, multiplicity)	
	S1	S2
α-QuiNAc-(1 →		
H-1	4.959 ($J_{1,2}$ 3.90, d)	4.975 ($J_{1,2}$ 3.91, d)
H-2	3.931 ($J_{2,3}$ 10.37, dd)	3.947 ($J_{2,3}$ 9.28, dd)
H-3	3.617 ($J_{3,4}$ 9.77, t)	3.631 ($J_{3,4}$ 9.28, t)
H-4	3.201 ($J_{4,5}$ 9.77, t)	3.218 ($J_{4,5}$ 9.76, t)
H-5	4.206 ($J_{5,6}$ 6.35, m)	4.210 ($J_{5,6}$ 5.86, m)
H-6	1.243 ($J_{5,6}$ 6.10, d)	1.243 ($J_{5,6}$ 6.40, d)
→ 3)-β-VioNAcyl-(1 →		
H-1	4.498 ($J_{1,2}$ 8.30, d)	4.512 ($J_{1,2}$ 8.30, d)
H-2	3.388 ($J_{2,3}$ 8.30, t)	3.403 ($J_{2,3}$ 7.91, t)
H-3	3.835 ($J_{3,4}$ 8.78, dd)	3.848 ($J_{3,4}$ 8.79, dd)
H-4	3.863 ($J_{4,5}$ 9.28, dd)	3.879 ($J_{4,5}$ 9.77, dd)
H-5	3.499 ($J_{5,6}$ 5.87, d)	3.513 ($J_{5,6}$ 5.86, d)
H-6	1.195 ($J_{5,6}$ 6.10, d)	1.207 ($J_{5,6}$ 5.86, d)
→ 3)-α-GalNAcA-(1 →		
H-1	5.021 ($J_{1,2}$ 3.66, d)	5.078 ($J_{1,2}$ 3.41, d)
H-2	4.310 ($J_{2,3}$ 11.30, dd)	4.353 ($J_{2,3}$ 11.23, dd)
H-3	4.042 ($J_{3,4}$ 2.45, dd)	4.070 ($J_{3,4}$ 2.45, dd)
H-4	4.506 (s)	4.520 (s)
H-5	4.468 (s)	4.610 (s)
2,3,4-Trihydroxybutanoic acid		
H-2	4.289 (s)	4.215 (s)
H-3	4.123 ($J_{3,4}$ 6.35, dd)	4.150 ($J_{3,4}$ 6.35, dd)
H-4	3.707 ($J_{3,4}$ 6.35, d)	3.730 ($J_{3,4}$ 6.35, d)

[a] Other signals: S1, 2.050 ppm (s, 6 H, 2 NAc); S2, 1.297 and 1.464 ppm (s, 6 and 3 H, 3 CMe).

consistent with α-GalNA, α-QuiN, and β-VioN. Since the GalNA in A1 is α, the *N*-deformylated GalNA in the O-polysaccharide chain was also inferred to be α. The difference in H-5 signals for the GalNA residues in S1 and S2 (Table I) reflected the presence of an amide group on the GalNA residue in S2. A similar change in the chemical shift by amidation has been reported[10].

^{13}C-NMR spectroscopy.—The ^{13}C-NMR spectra of S1 and S2 were complex because of the overlapping signals. However, S1-S (second Smith-degradation product) had a much simpler spectrum owing to the removal of the non-reducing QuiN residue. Taking account of the ^{13}C-NMR data for H1, all of the ^{13}C resonances from S1-S were assigned tentatively as shown in Table II. The chemical shifts (97.54 and 102.87 ppm) of the C-1 resonances supported the above inference that the GalNA and VioN are α and β, respectively. Comparison of the chemical shift data[10] for GalNAcA with those for the corresponding residue in S1-S showed a large downfield shift of the C-3 signal (66.8–69.6 to 78.75 ppm), indicating 3-substitution. Furthermore, a large downfield shift was observed for the C-3 signal of 2,3,4-trihydroxybutanoic acid, consistent with the result of the second Smith-de-

TABLE II

^{13}C-NMR data [a] for products of the first (S1 and S2) and second (S1-S) Smith-degradation of *N*-deformylated PS-1

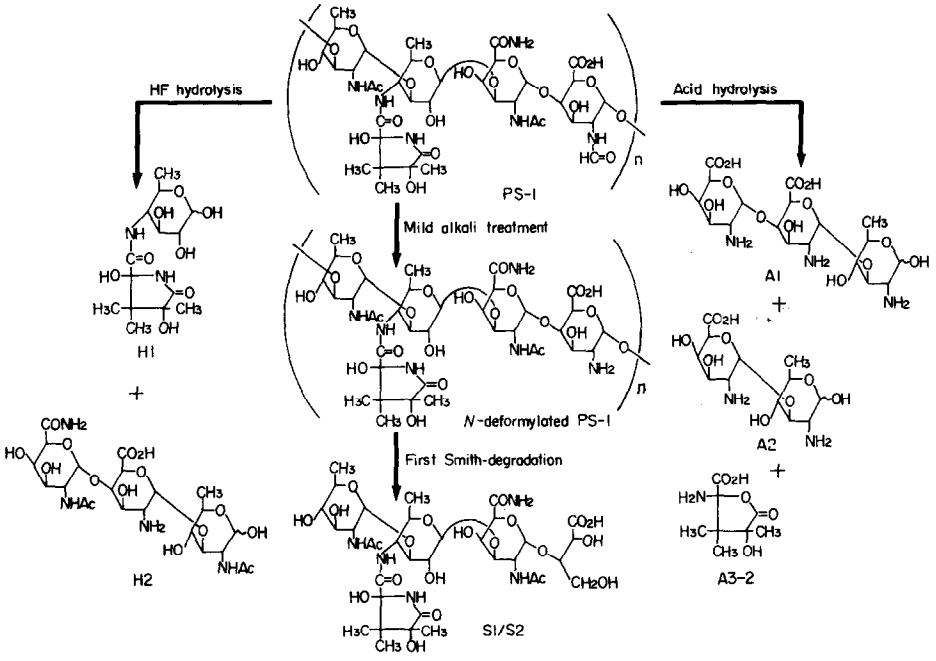
Residue	Chemical shifts (ppm)					
	C-1	C-2	C-3	C-4	C-5	C-6
S1						
α-QuiNAc-(1 →	97.03	53.80	75.62	77.15	68.51	16.73
→ 3)-β-VioNAcyl-(1 →	104.17	72.69	*78.05* [b]	57.60	71.96	17.32
→ 3)-α-GalNAcA-(1 →	99.55	48.65	*78.51*	69.12	71.46	carboxyl
→ 3)-2,3,4-Trihydroxy-butanoic acid	carboxyl	70.32	*81.89*	61.52		
S2						
α-QuiNAc-(1 →	97.04	53.87	75.72	77.42	68.58	17.00
→ 3)-β-VioNAcyl-(1 →	104.24	72.77	*77.74*	57.66	72.03	17.42
→ 3)-α-GalNAcA(amido)-(1 →	99.03	48.60	*78.56*	69.27	71.51	carboxyl
→ 3)-2,3,4-Trihydroxy-butanoic acid	carboxyl	69.50	*81.92*	61.91		
S1-S						
β-VioNAcyl-(1 →	102.87	73.43	74.04	57.40	72.11	17.32
→ 3)-α-GalNAcA-(1 →	97.54	48.71	*78.75*	69.04	71.29	carboxyl
→ 3)-2,3,4-Trihydroxy-butanoic acid	carboxyl	70.41	*81.63*	61.52		

[a] Other signals: S1, 16.15, 16.50, and 19.28 (3 CMe), 22.85 (2 NCO*C*H$_3$), 174.72, 175.07, 177.76, and 179.12 ppm (4 C=O); S2, 16.25 (1 C), 16.66 (1 C), 19.35 (1 C), 22.95 (2 C), 174.12 (1 C), 174.71 (1 C), 175.12 (1 C), 177.38 (1 C), and 179.21 ppm (1 C), assignable as for S1; S1-S, 16.8–19.8 (3 C), 22.28 (1 C), and 175–179 ppm (4 C). [b] Italicised values indicate downfield shifts due to *O*-glycosylation.

gradation. Taking account of the above assignments for S1-S, all of the ^{13}C resonances for S1 and S2 were assigned firmly as shown in Table II. These downfield shifts may be explained by the α-effects of glycosylation, indicating that the VioN in each Smith-degradation product is 3-substituted. On the other hand, the signals ascribable to the VioN in S1-S were in accordance with those arising from H1, indicating its non-reducing terminal position. Thus, the VioN in S1 and S2 is 3-substituted by QuiN residues, which were oxidised during the second Smith-degradation.

As shown in Table II, all of the assigned signals for the respective sugar residues, their *N*-acyl moieties, and the terminal 2,3,4-trihydroxybutanoic acid residues in S1 and S1-S were in accord, except for the C-1 signals of GalNA, which reflects the presence of an amide group in S2 and is consistent with similar observations on amidated polysaccharides[15,16].

From the above assignments together with the plausible structure α-GalNA-(1 → 4)-α-GalNA-(1 → 3)-QuiN of A1, it is concluded that the possible structures of S1 and S1-S are α-QuiNAc-(1 → 3)-β-VioNAcyl-(1 → 3)-α-GalNAcA-(1 → 3)-2,3,4-trihydroxybutanoic acid and β-VioNAcyl-(1 → 3)-α-GalNAcA-(1 → 3)-2,3,4-trihydroxybutanoic acid, in which *N*-Acyl is 2,4-dihydroxy-3,3,4-trimethylpyro-



Scheme 1. Scheme for fragmentation of PS-1 by acid hydrolysis, HF hydrolysis, and Smith degradation.

glutamyl. S1 and S2 have the same structure, except that the GalNAcA residue in the latter is amidated. Taking account of the formation of 2,3,4-trihydroxybutanoic acid from *N*-deformylated GalNA during the first Smith-degradation, the most likely structure of the repeating unit of the O-polysaccharide chain is →3)-α-QuiNAc-(1 → 3)-β-VioNAcyl-(1 → 3)-α-GalNAcA-(1 → 4)-α-GalNFoA-(1 → . From the proportions of S1 and S2 found in the first Smith-degradation, ~ 50% of the 3-substituted GalNAcA residues in the O-polysaccharide chain are amidated.

DISCUSSION

The results of analysis of PS-1 and its various fragments obtained by strong-acid hydrolysis, hydrolysis with HF, and Smith degradation led to the most likely structure for the repeating unit of the O-polysaccharide chain of *V. anguillarum* V-123 lipopolysaccharide shown in Scheme 1. The repeating QuiN-VioN-GalNA-GalNA was deduced primarily from the structure of A1 and the Smith-degradation products (S1 and S2) of *N*-deformylated PS-1. The formation of 2,3,4-trihydroxybutanoic acid on Smith degradation of *N*-deformylated PS-1 occurs through the oxidative cleavage on the C-2–C-3 bond of the *N*-deformylated GalNA residue; therefore, position 3 of the butanoic acid moiety corresponds to position 4 of the GalNA residue. The isolation of α-GalNA-(1 → 4)-α-GalNA-(1 → 3)-QuiN (A1) from the acid hydrolysate of PS-1 and α-GalNAcA-(1 → 4)-α-GalNFoA-

(1 → 3)-QuiNAc (H2) from the HF hydrolysate of PS-1 strongly supports the above deduction. The downfield shifts of several ^{13}C signals in the NMR spectra of PS-1 and the various fragments (Table II) occur by the α-effects of *O*-glycosylation, indicating that the GalNAcA, QuiNAc, VioNAcyl, and GalNFoA residues are 3-, 3-, 3-, and 4-substituted, respectively.

The locations of the *N*-formyl, *N*-acyl, and two *N*-acetyl groups on the amino sugar residues were supported by the isolation of H1 and S1 (or S2). After *N*-deformylation by mild treatment with alkali, PS-1 was changed to a periodate-sensitive form, and one of two GalNA residues was changed to 2,3,4-trihydroxybutanoic acid upon Smith degradation, indicating *N*-formylation. As reported in the preceding paper[7], H1 is a 4-amino-4,6-dideoxyglucose (VioN) *N*-acylated with 2,4-dihydroxy-3,3,4-trimethylpyroglutamic acid. Therefore, two *N*-acetyl groups are located on the other amino sugars, GalNA and QuiN. The occurrence of *N*-formyl groups is rare in Nature, although 2-deoxy-2-formamido-D-galacturonic acid[10,17,18] and 4,6-dideoxy-4-formamido-L-mannose have been found in the O-polysaccharides of *P. aeruginosa* strains and *Yersinia enterocolitica* 09[19], respectively.

Mild treatment of PS-1 with alkali did not lead to any cleavage of the polysaccharide chains through β-elimination, supporting the location of amide groups on 3-substituted GalNA but not on the 4-substituted GalNA. In the ^1H-NMR spectra of S1 and S2 (Table I), the chemical shifts of the H-5 resonances of the GalNAcA residues were different, whereas the signals ascribable to the 2,3,4-trihydroxybutanoic acid residues were similar. The downfield shift of the H-5 resonance in S2 may be explained by the amidation of this sugar residue. A similar change in the resonance of H-5 has been reported in several polysaccharides containing uronosyl residues substituted by amide and amino acids[12,15,16]. Hydrolysis of PS-1 with 10 M HCl at 80° for 0.5 h or with 4 M HCl at 100° for 1 h yielded α-GalNA-(1 → 4)-α-GalNA-(1 → 3)-QuiN (A1) or α-GalNA-(1 → 3)-QuiN (A2), respectively, as the major product. The formation of A1 and A2 may reflect the different sensitivities of uronosidic bonds towards acid in amidated and 3-substituted GalNAcA residues and the unamidated and 4-substituted GalNAcA residues.

The repeating units of the O-polysaccharides of *V. anguillarum* V-123 and *P. aeruginosa* IID 1008 are similar in part, namely, α-GalNAcA(amido)-(1 → 4)-α-GalNFoA-(1 → 3)-α-QuiNAc-(1 → . Moreover, similar trisaccharide units have been reported in several other *P. aeruginosa* serotypes[20], *Shigella dysenteriae* type 7[21], and *Francisella tularensis* strain 15[22]. Comparison of the repeating units of the O-polysaccharide chains of the LPSs of *V. anguillarum* V-123 and *P. aeruginosa* IID 1008 shows that the fourth sugar residue in the former is β-VioN and α-Rha in the latter. In a preliminary experiment, the competitive ELISA assay using a commercial antiserum specific to group G antigen of *P. aeruginosa* indicated that the O-polysaccharide of *V. anguillarum* and its fragments cross-reacted with the antiserum specific to group G antigen. The details will be reported elsewhere.

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