Carbohydrate Research, 208 (1990) 293–300
Elsevier Science Publishers B.V., Amsterdam

Note

Structural studies of the O-specific polysaccharide from *Salmonella kentucky* strain 98/39 (O:8, H:*i*, Z_6)

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(Received December 6th, 1989; accepted for publication, April 3rd, 1990)

O-Specific polysaccharides of *Salmonella* are among the most extensively investigated bacterial polysaccharides[1]. More than 15 years ago, the structure **1** was reported[2,3] for the polymer from *S. kentucky* strain I.S.98 (serogroup C_3). At that time, the conclusion that the L-rhamnose residue was β was based on the results of CrO_3-oxidation which may be misleading. In connection with our studies of the synthesis of oligosaccharide fragments and precursors in the biosynthesis of this polysaccharide[4–6], the structure has been investigated. The results now presented are consistent with the structure **2** for the polymer, which differs from **1** by the absence of OAc groups and by the location of the D-Glc branch at HO-2 (instead of HO-4) of the D-galactose residue.

$$\rightarrow 3)\text{-}\beta\text{-D-Gal-}(1\rightarrow 4)\text{-}\beta\text{-L-Rha-}(1\rightarrow 2)\text{-}\alpha\text{-D-Man-}(1\rightarrow 2)\text{-}\alpha\text{-D-Man-}(1\rightarrow$$
$$\qquad\qquad\qquad\quad |\qquad\qquad\quad |$$
$$\qquad\qquad\qquad R^1\qquad\qquad R^2$$

1 R^1 = 2-O-Ac-α-D-Glc-(1→4), R^2 = α-D-Abe-(1→3)-
2 R^1 = (75%) α-D-Glc-(1→2), R^2 = α-D-Abe-(1→3)-

The microorganism used in our investigation was *S. kentucky* strain 98/39 (O:8, H:*i*, Z_6), obtained from the collection of A. A. Tarasevich (All-Union Institute of Control Cultures). The lipopolysaccharide isolated from the dry bacterial cells by the Westphal procedure[7] was hydrolysed with aqueous 1% AcOH (1 h, 100°) followed by gel filtration on Sephadex G-50 to give the O-specific polysaccharide (PS-I). The monosaccharide composition of PS-I (Rha:Man:Gal:Glc:Abe 1:2:1:1:0.8) accorded with previous data[2,3]. Methylation analysis[8] of PS-I (Table I) revealed terminal Abe and Glc residues, 4-substituted and 3,4-disubstituted Rha, 2-substituted Man, and 3-substituted and 2,3-disubstituted Gal. The absence of 3,4-disubstituted Gal is noteworthy. The ^{13}C-n.m.r. data (Table II) showed the irregularity of PS-I and the absence of OAc groups.

Treatment of PS-I with aqueous 1% AcOH (8 h, 100°) selectively cleaved the

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0008-6215/90/$ 03.50

TABLE I

Methylation analysis of PS-I and PS-II

Component	*Molar ratio*[a]	
	PS-I	*PS-II*
2,4-Me_2-Abe-ol[b]	+	
2,3-Me_2-Rha-ol	0.68	1.07
2,3,4,6-Me_4-Glc-ol	1.0	0.93
2-Me-Rha-ol	0.93	
3,4,6-Me_3-Man-ol	3.2	2.13
2,4,6-Me_3-Gal-ol	0.73 }	= 1
4,6-Me_2-Gal-ol	1.3 }	

[a] Calculated as in ref. 16. [b] 2,4-Me_2-Abe-ol = 2,4-di-*O*-methylabequitol *etc.*

TABLE II

^{13}C-n.m.r. assignments for PS-I and PS-II

PS-I	*PS-II*	*Residue*	*Carbon atom*	*Presence in polysaccharide*	
				Glc	*Abe*
105.07	105.01	D-Gal	C-1	–	–
104.76	104.70	D-Gal	C-1	+	–
104.10		D-Gal	C-1	+	+
102.76	102.66	D-Man II	C-1	±	±
		L-Rha	C-1	±	–
102.37		L-Rha	C-1	±	+
99.19		D-Abe, D-Glc	C-1?	+	+
98.17	98.12	D-Glc	C-1	+	–
97.98		D-Glc, D-Abe	C-1?	+	+
96.63	96.56	D-Man I	C-1	–	+
95.69	95.69	D-Man I	C-1	+	±
94.32		D-Abe	C-1?	–	+
82.60	82.50	L-Rha	C-4	–	–
81.16		L-Rha	C-4	–	+
80.30–79.5 multiplet	80.29	D-Man I and II	C-2	±	±
	80.16	L-Rha	C-4	+	–
	79.91	L-Rha	C-3 (?)	±	+
	79.65				
78.67	78.42	D-Gal	C-3	–	±
77.04		L-Rha	C-3 or C-4	+	±
				±	+
76.46					
76.28	76.28	D-Gal	C-5	±	±
75.96	75.90	D-Gal	C-3	+	±
75.62					
74.71	74.64	D-Man I and II	C-5	±	±
74.32	74.51	L-Rha, D-Glc	C-3	±	–
	74.15				

TABLE II (continued)

PS-I	*PS-II*	*Residue*	*Carbon atom*	*Presence in polysaccharide*	
				Glc	*Abe*
73.41	73.51	D-Glc	C-5	+	±
73.12	73.11	D-Glc	C-2	+	±
72.88	72.83	D-Gal	C-2	+	±
		L-Rha	C-5	±	+
72.25	72.16	D-Man I and II	C-3	±	±
71.55	71.49	L-Rha	C-2 and C-5	±	–
70.97		D-Gal	C-2	–	±
		D-Glc	C-4	+	±
69.51		D-Abe	C-4	±	+
69.41					
68.86	68.82	D-Man II and I	C-4	±	±
68.53	68.46				
68.24		D-Abe	C-5	±	+
68.11					
67.24		L-Rha	C-2	±	+
66.27	66.18	D-Gal	C-4	–	±
65.62		D-Gal	C-4	+	+
65.48	65.44	D-Gal	C-4	+	–
64.55		D-Abe	C-2	±	+
64.36					
62.45	62.40	D-Gal	C-6	±	±
62.22	62.31	D-Man I	C-6	±	±
62.12	62.07	D-Man II	C-6	±	±
34.56		D-Abe	C-3	±	+
19.00	18.61	L-Rha	C-6	±	±
18.65	18.35				
17.15		D-Abe	C-6	±	+
16.49					

abequosyl residues, and the resulting polymer (PS-II) was isolated by gel chromatography on Fractogel TSK-40(S). Comparison of the methylation analysis data for PS-I and PS-II (Table I) clearly showed that the terminal abequose residue was 3-linked to rhamnose.

Hydrolysis of PS-I with 0.05M trifluoroacetic acid (4 h, 100°), followed by gel filtration on Fractogel TSK-40(S), gave several oligosaccharides. Four main products (OS-I/IV, Fig. 1) were isolated by borate ion-exchange chromatography[9]. The structures of OS-I–IV were established by monosaccharide analysis and ^{13}C-n.m.r. spectroscopy of the oligosaccharides and their alditol derivatives. The microcomputer program "ANMROL"[10] and glycosylation effects[11] were used to interpret the n.m.r. data.

The spectra of OS-III, OS-II, and OS-IV were consistent only with the structures α-D-Man-(1→2)-α-D-Man-(1→3)-D-Gal (**3**, OS-III), α-D-Man-(1→2)-α-D-Man-(1→3)-β-D-Gal-(1→4)-L-Rha (**4**, OS-II), and α-D-Man-(1→2)-α-D-Man-(1→3)-[α-D-Glc-(1→2)]-β-D-Gal-(1→4)-L-Rha (**5**, OS-IV) (see Table III). The synthetic compounds

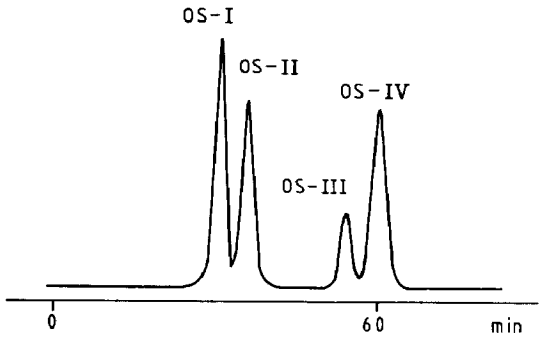


Fig. 1. Ion-exchange chromatography of the partial hydrolysate of PS-I on a column (250 × 4 mm) of Durrum DAx4 resin; elution at 18 mL.h^{-1} with 0.5M borate buffer (pH 8.5) and 70°.

TABLE III

^{13}C-n.m.r. data for oligosaccharides

α-D-*Man-(1→2)*-α-D-*Man-(1→3)*-α,β-D-*Gal* (**3**, *OS-III*)
II I

		C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
α-D-Man	II	103.49	71.20	71.49	68.20	74.46	62.40
α-D-Man	I	95.88 95.60	80.29	71.12	68.07	73.88	62.08
D-Gal	α	93.40	67.90	74.46	66.38	71.49	62.40
	β	97.52	71.49	77.78	65.80	76.06	62.15

α-D-*Man-(1→2)*-α-D-*Man-(1→3)*-β-D-*Gal-(1→4)*-α,β-L-*Rha* (**4**, *OS-II*)
II I

α-D-Man	II	103.24	70.87[a]	71.02[a]	67.94	74.21	61.81[b]
α-D-Man	I	95.65	80.02	70.87	67.82	73.67	61.81[b]
β-D-Gal		104.44	71.24[a]	77.52	65.38	75.85	62.15[b]
L-Rha	α	94.61	71.70	70.81	82.21	67.82	17.89
	β	94.28	72.18	73.67	81.74	71.49	17.89

α-D-*Man-(1→2)*-α-D-*Man-(1→3)*-[α-D-*Glc-(1→2)*]-β-D-*Gal-(1→4)*-α,β-L-*Rha* (**5**, *OS-IV*)
II I

α-D-Man	II	103.12	70.60[a]	71.20	67.75	74.06	61.63[d]
α-D-Man	I	96.00	79.49	71.00[a]	67.75	73.72	61.63
β-D-Gal		104.20	72.41[b]	75.13[c]	64.50	75.29[c]	61.95[d]
α-D-Glc		97.46	72.00[b]	73.72	70.60	72.20[b]	61.26[d]
L-Rha	α	94.45	72.00	70.60	79.85	68.10	17.91
	β	94.10	72.20	73.72	79.15	71.40	17.91

TABLE III (continued)

α-D-*Man-(1→2)*-α-D-*Man-(1→3)*-[α-D-*Glc-(1→2)*]-*Gal-ol*
II *I*

α-D-Man	II	103.10	71.19	71.45	68.05	74.73	62.29
α-D-Man	I	100.88	79.78	71.19	68.05	74.34	62.13
α-D-Glc		101.67	72.79	74.17	70.87	73.54	61.70
Gal-ol		62.64	80.47	79.63	70.43	71.10	64.15

α-D-*Man-(1→2)*-α-D-*Man-(1→3)-Gal-ol*
II *I*

α-D-Man	II	102.79	71.10	71.20	67.62	74.42	61.91
α-D-Man	I	100.22	79.54	70.73	67.62	73.95	61.69
Gal-ol		63.83	70.73	78.89	70.30	71.95	63.82

α-D-*Man-(1→2)*-α-D-*Man-(1→3)*-[α-D-*Glc-(1→2)*]-β-D-*Gal-(1→4)*-L-*Rha-ol*
II *I*

α-D-Man	II	103.13	70.91	71.20	68.26	64.18	61.85
α-D-Man	I	94.91	79.44	70.77	67.90	73.91	61.79
β-D-Gal		103.83	75.72	72.72	64.90	75.61	62.11
α-D-Glc		97.82	71.80	73.91	70.77	72.20	61.49
L-Rha-ol		63.61	72.57	71.36	80.34	69.15	18.61

α-D-*Abe-(1→3)*-[β-D-*Gal-(1→4)*]-β-L-*Rha-(1→2)-X* (7, *S-OS-I*)

β-D-Gal	103.90	72.60	74.19	70.05	76.50	62.32
α-D-Abe	93.60	64.35	34.30	69.63	67.84	16.50
β-L-Rha	100.40	67.10	76.22	79.74	72.47	18.50
X	104.10	77.94	60.81	67.63	76.30	63.00

[a-d] Assignments may be interchanged.

α-D-Man-(1→3)-D-Gal[12] and α-D-Man-(1→3)-[α-D-Glc-(1→2)]-β-D-Gal-OMe[13] were used as standards. The signal at 64.5 p.p.m. in the spectra of OS-IV and the latter standard reflected the presence of a 2,3-disubstituted β-Gal residue because, in the spectrum of synthetic α-D-Man-(1→3)-[α-D-Glc-(1→4)]-β-D-Gal-OMe[14], signals in the range 62–67.5 p.p.m. were absent.

The structure α-D-Man-(1→2)-α-D-Man-(1→3)-[α-D-Glc-(1→2)]-D-Gal (**6**) was suggested for OS-I on the basis of the ^{13}C-n.m.r. spectrum of the corresponding oligosaccharide-alditol, since the spectrum of the parent oligosaccharide could not be interpreted because of the presence of significant proportions of furanose and pyranose forms.

In order to elucidate the configuration of the rhamnosyl–mannose linkage, PS-I was subjected to Smith degradation. After mild hydrolysis followed by gel filtration, an oligosaccharide fraction (S-OS) that contained a mixture of tri-, tetra- and pentasaccharides was obtained. Borate ion-exchange chromatography of S-OS (Fig. 2), as for OS-I–IV, revealed a main product (S-OS-I), sugar analysis of which indicated a trisaccharide (Abe:Rha:Gal 1:1:1). However, borohydride reduction of S-OS-I did not change the monosaccharide composition or chromatographic mobility.

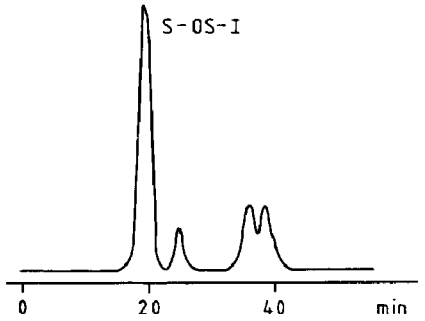
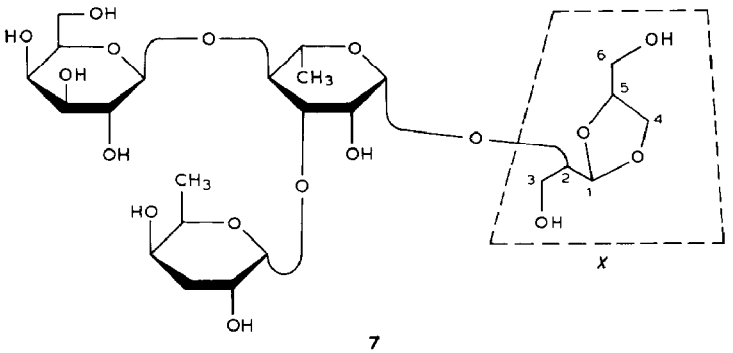


Fig. 2. Ion-exchange chromatography of the Smith-degradation products of PS-I; conditions as in Fig. 1.



7

TABLE IV

^1H- and ^{13}C-n.m.r. assignments for **7** (S-OS-I)

Unit	*C-1*	*H-1*	$J_{1,2}$ *(Hz)*	$J_{C\text{-}1,H\text{-}1}$ *(Hz)*
X	104.1	5.13	3.75	172
β-D-Gal	103.9	4.70	8.0	162.8
β-L-Rha	100.4	4.87	1.0	160.9
α-D-Abe	93.6	5.13	3.75	168.3

In the ^{13}C-n.m.r. spectrum of S-OS-I (Table III), 18 signals corresponding to three monosaccharide units (Abe, Rha, and Gal) were identified in addition to the signals of a C_6 fragment with two CH_2OH groups. The ^1H- and ^{13}C-n.m.r. spectra of the product (Table IV) suggested the structure **7**, which was confirmed by n.O.e. experiments that involved the irradiation of H-1 of the β-galactose and α-abequose residues.

Thus, the main chain of PS-I is composed of the tetrasaccharide repeating-unit **4** with a β-rhamnosyl-(1→2)-mannose linkage, and α-D-Abe and α-D-Glc branches linked to the main chain in non-stoichiometric ratio (see structure **2**). The non-equimolar amount of abequose per repeating unit is probably due to partial hydrolysis of the acid-labile glycoside bond of the 3,6-dideoxy sugar during the mild acid hydrolysis

involved in the preparation of PS-I. The ^{13}C-n.m.r. data of PS-I and PS-II (see Table II) also accord with the structure **2** for PS-I, and PS-II seems to consist of repeating units **4** and **5** in the ratio 1:3.

The differences between structures **1** and **2** may be due to the different strains of *S. kentucky* used.

EXPERIMENTAL

N.m.r. spectra were recorded with a Bruker AM-300 spectrometer for solutions in D_2O (internal MeOH) at 30° for oligosaccharides and 60° for polysaccharides. Monosaccharide analysis[9], ion-exchange chromatography of oligosaccharides[9], cultivation of bacteria[15], and methylation analysis[8] were performed as reported. Solutions were concentrated *in vacuo* at 40°.

Isolation of PS-I. — Ground cells (30 g) of *S. kentucky* (O:8, H:*i*, Z_6, GISK 98/39 strain) were stirred for 20 min in a mixture of water (500 mL) and aqueous 90% phenol (500 mL) at 60–75°. The solution was then chilled and centrifuged at 2000*g*, the aqueous layer was dialysed against tap water, nucleic acids were precipitated with Cetavlon, the resulting suspension was centrifuged, and the supernatant solution was dialysed against distilled water, then freeze-dried to give LPS (2 g, 6.6%).

A solution of LPS (1 g) in aqueous 1% AcOH (100 mL) was heated for 1 h at 100°, and the suspension was chilled and centrifuged. The supernatant solution was concentrated to 15 mL, and subjected to gel filtration on Sephadex G-50 (column 30 × 600 mm, elution with pyridine–acetate buffer) to give PS-I (500 mg).

Isolation of PS-II. — A solution of PS-I (200 mg) in aqueous 1% AcOH (20 mL) was heated for 8 h at 100°, then chilled, neutralised with Et_3N, concentrated to 1 mL, and subjected to gel filtration on a column (16 × 900 mm) of TSK-40S by elution with water to give PS-II (100 mg).

Partial acid hydrolysis of PS-I. — A solution of PS-I (200 mg) in 0.05M CF_3COOH (20 mL) was heated for 4 h at 100°, then chilled, neutralised, concentrated, and subjected to gel chromatography on TSK-40(S) (16 × 900 mm, elution with water). The resulting oligosaccharide fraction was eluted from a column of Durrum DAx4 resin in borate buffer[9] (see Fig. 1) followed by desalting to give fractions OS-I (*T* 30 min, 4 mg), OS-II (*T* 35 min, 6 mg), OS-III (*T* 50 min, 5 mg), and OS-IV (*T* 60 min, 9 mg).

Reduction of the oligosaccharides. — A solution of each oligosaccharide (1–5 mg) in water (1 mL) was treated with $NaBH_4$ (2 × 5 mg) for 16 h at 20°, then neutralised with AcOH, and the product was isolated by gel chromatography on TSK-40(S) (16 × 900 mm, elution with water).

Smith degradation. — A solution of PS-I (100 mg) in 0.1M $NaIO_4$ (5 mL) was kept for 48 h at 20° in the dark, then ethylene glycol (0.1 mL) was added, and, after 20 min, an excess of $NaBH_4$ was added. The mixture was kept for 2 h at 20°, then neutralised with AcOH, and the polymeric material was isolated by gel chromatography on TSK-40(S) and hydrolysed with 0.5M HCl for 48 h at 20°. Further neutralisation with Et_3N, concentration, and gel chromatography on TSK-40(S) (16 × 900 mm, elution with

water) gave the combined oligosaccharide fraction. Borate ion-exchange chromatography[9] of the oligosaccharide fraction (see Fig. 2) followed by gel chromatography on TSK-40(S) (16 × 900 mm, elution with water) gave S-OS-I (T 20 min, 3 mg).

ACKNOWLEDGMENTS

We thank Dr. Yu A. Knirel and Dr. G. M. Lipkind for helpful discussion, and Professor N. K. Kochetkov for his interest and encouragement.

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