Structure of the O-specific polysaccharide of *Salmonella arizonae* O45
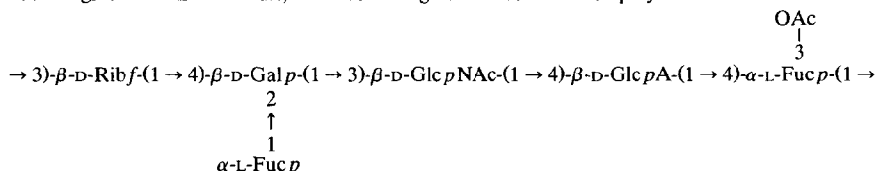
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ABSTRACT

The O-specific polysaccharide of *Salmonella arizonae* O45 (*Arizona* 11) is acidic and has a branched hexasaccharide repeating unit containing two residues of L-fucose, one residue each of D-galactose, D-ribose, D-glucuronic acid, and 2-acetamido-2-deoxy-D-glucose, and an *O*-acetyl group. It was studied with the help of ^{1}H and ^{13}C NMR spectroscopy, including 1D selective spin-decoupling and homonuclear Hartmann–Hahn spectroscopy, 2D homonuclear and [13]C–[1]H heteronuclear shift-correlated (COSY) and NOE (ROESY) spectroscopy, as well as by methylation analysis, and selective cleavages with anhydrous HF (or dilute HCl) and lithium in ethylenediamine to yield two different tetrasaccharide fragments. As a result, the following structure of the polysaccharide was established:

$$
\begin{array}{c}
\text{OAc} \\
| \\
3
\end{array}
$$

→ 3)-β-D-Rib$f$-(1 → 4)-β-D-Gal$p$-(1 → 3)-β-D-Glc$p$NAc-(1 → 4)-β-D-Glc$p$A-(1 → 4)-α-L-Fuc$p$-(1 →
                    2
                    ↑
                    1
                α-L-Fuc$p$

Anomalous ^{13}C chemical shifts were observed in the spectrum of the trisaccharide fragment α-L-Fuc$p$-(1 → 2)-β-D-Gal$p$-(1 → 3)-β-D-Glc$p$NAc, structurally related to the Le[d] blood-group determinant, and rationalised by inter-residue proton–proton interactions.

INTRODUCTION

Recently[1], we determined the structure of the O-specific polysaccharide of *S. arizonae* O61, which was the first O-antigen among *Salmonella* spp. containing an

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acidic sugar (a di-*N*-acyl derivative of 5,7-diamino-3,5,7,9-tetradeoxy-D-*glycero*-L-*galacto*-nonulosonic acid). We report now the structure of another acidic O-specific polysaccharide from *S. arizonae* O45 (*Arizona* 11), which was established by ^{1}H and [13] NMR spectroscopy and confirmed by chemical methods.

RESULTS AND DISCUSSION

O-Specific polysaccharide (PS-I) was obtained by mild acid degradation of *S. arizonae* O45 lipopolysaccharide isolated from dried cells by phenol–water extraction[2].

The ^{1}H NMR spectrum of PS-I displayed signals for two Me–C groups of 6-deoxy sugars at 1.223 and 1.312 ppm (both d, $J_{5,6} \sim 6.5$ Hz), and two Ac groups at 2.040 and 2.102 ppm (both s). In the downfield region 4.35–5.30 ppm, there were seven signals, six of which were typical of anomeric protons (a broadened singlet at 5.295 ppm and doublets with $J_{1,2} \sim 4$ or ~ 8 Hz). The seventh signal (dd at 5.002 ppm), with the coupling constants 3 and 11 Hz, probably belonged to a proton attached to a carbon carrying the OAc group with a resonance shifted downfield due to strong deshielding.

The ^{13}C NMR spectrum of PS-I contained signals for six anomeric carbons at 100.3–109.9 ppm, one carbon bearing nitrogen at 55.8 ppm, two Me–C groups (C-6 of 6-deoxy sugars) at 16.0 and 16.2 ppm, three $HOCH_2$–C groups (C-5 and C-6 of pentoses and hexoses, respectively) at 61.8–63.8 ppm, one carboxyl group (C-6 of a hexuronic acid) at 170.5 ppm, one OAc and one NAc group (Me at 21.4 and 23.5 ppm, respectively, and CO at 174.5 and 174.6 ppm). A number of minor signals were observed in the spectrum, which may be due to incomplete O-acetylation.

These data and the total number of sugar carbon signals (35) in the ^{13}C NMR spectrum showed that PS-I has a hexasaccharide repeating unit which included one residue each of a hexose, a pentose, a hexuronic acid, and an *N*-acetylated hexosamine, two residues of a 6-deoxy hexose, and an O-acetyl group.

Treatment of PS-I with M NaOH at room temperature resulted in an O-deacetylated polysaccharide (PS-II), which was strictly regular according to the ^{1}H and ^{13}C NMR spectral data. The spectra lacked the signals for the OAc group and underwent several other changes; in particular, the signal at 5.002 ppm (dd) disappeared from the ^{1}H NMR spectrum.

Hydrolysis of PS-II with 2 M CF₃COOH revealed the presence of fucose, ribose, and galactose, identified by GLC as alditol acetates, and 2-amino-2-deoxyglucose and glucuronic acid, identified with the use of an amino acid analyzer and a sugar analyzer, respectively. Neutral sugars were isolated from the hydrolysate by anion-exchange chromatography in a borate buffer; ribose and galactose were shown to be D and fucose L by GLC of the corresponding O-acetylated (*S*)-2-octyl glycosides[3]. The D configuration of 2-amino-2-deoxyglucose and of glucuronic acid was established by detailed analysis of the effects of glycosylation in the ^{13}C NMR spectrum of PS-II (see below).

TABLE I

Data of the ^{1}H NMR spectrum of O-deacetylated polysaccharide (δ in ppm, J in Hz)

	H-1	H-2	H-3	H-4	H-5	H-6
$\rightarrow 3)$-β-D-Rib$f$-$(1 \rightarrow$ (unit A)						
δ	5.301	4.282	4.123	4.170	3.846 ^{a}	
					3.700 ^{b}	
J	$J_{1,2} < 2$	$J_{2,3}$ 4.7	$J_{3,4}$ 7.4	$J_{4,5a}$ 3.6	$J_{4,5b}$ 7.0	
$\rightarrow 4)$-β-D-Gal$p$-$(1 \rightarrow$ (unit B)						
$\overset{\uparrow}{2}$						
δ	4.629	3.592	3.939	4.020	3.73	3.7–3.8
J	$J_{1,2}$ 7.6	$J_{2,3}$ 10.0	$J_{3,4}$ 3.8	$J_{4,5} < 2$		
$\rightarrow 3)$-β-D-Glc$p$NAc-$(1 \rightarrow$ (unit C)						
δ	4.530	3.775	3.97	3.51	3.51	3.92
J	$J_{1,2}$ 8.0	$J_{2,3}$ 9.0	$J_{3,4} > 9$			3.96
$\rightarrow 4)$-β-D-Glc$p$A-$(1 \rightarrow$ (unit D)						
δ	4.488	3.476	3.612	3.790	3.755	
J	$J_{1,2}$ 7.6	$J_{2,3}$ 9.0	$J_{3,4}$ 9.0	$J_{4,5}$ 9.0		
$\rightarrow 4)$-α-L-Fuc$p$-$(1 \rightarrow$ (unit E)						
δ	5.020	3.791	3.878	4.005	4.292	1.302
J	$J_{1,2}$ 3.8	$J_{2,3}$ 10.1	$J_{3,4}$ 3.0	$J_{4,5} < 2$	$J_{5,6}$ 6.5	
α-L-Fuc$p$-$(1 \rightarrow$ (unit F)						
δ	5.180	3.770	3.708	3.770	4.271	1.219
J	$J_{1,2}$ 3.7	$J_{2,3}$ 10.0	$J_{3,4}$ 3.8	$J_{4,5} < 2$	$J_{5,6}$ 6.5	

^{a} H-5a, $J_{5a,5b}$ 9.0 Hz. ^{b} H-5b.

The ^{1}H NMR spectrum of PS-II was well-resolved at 70°C and was completely assigned with the use of a modified[4], sequential, selective spin-decoupling procedure, 1D homonuclear Hartmann–Hahn spectroscopy (TOCSY-HOHAHA)[5], and 2D homonuclear shift-correlated (COSY), and relayed coherence transfer (COSYRCT) spectroscopy (Table I). As judged by the coupling constants[6,7], determined from the spectrum, the Rib residue was furanoid and β-linked, whereas the other sugar residues were pyranoid. Both Fuc residues were α-linked, and the residues of Gal, GlcA, and GlcNAc were β-linked. The presence of the β-Rib$f$ residue was confirmed by the low-field position (110 ppm) of one of the anomeric carbon signals in the ^{13}C NMR spectrum of PS-II characteristic[8] of furanosides with *trans*-orientation of substituents at C-1 and C-2.

Information on the linkage positions and sequence of the sugar residues was obtained from the rotating-frame 2D NOE (ROESY) spectrum of PS-II (Fig. 1). H-1 of both of the α-pyranosides gave an intense cross-peak with H-2 of the same sugar residue and a smaller one with H-3. For the β-pyranosides, there were observed intense cross-peaks of H-1 with H-3 and H-5 and a smaller one with H-2; β-Rib$f$ H-1 gave a cross-peak with H-2 only.
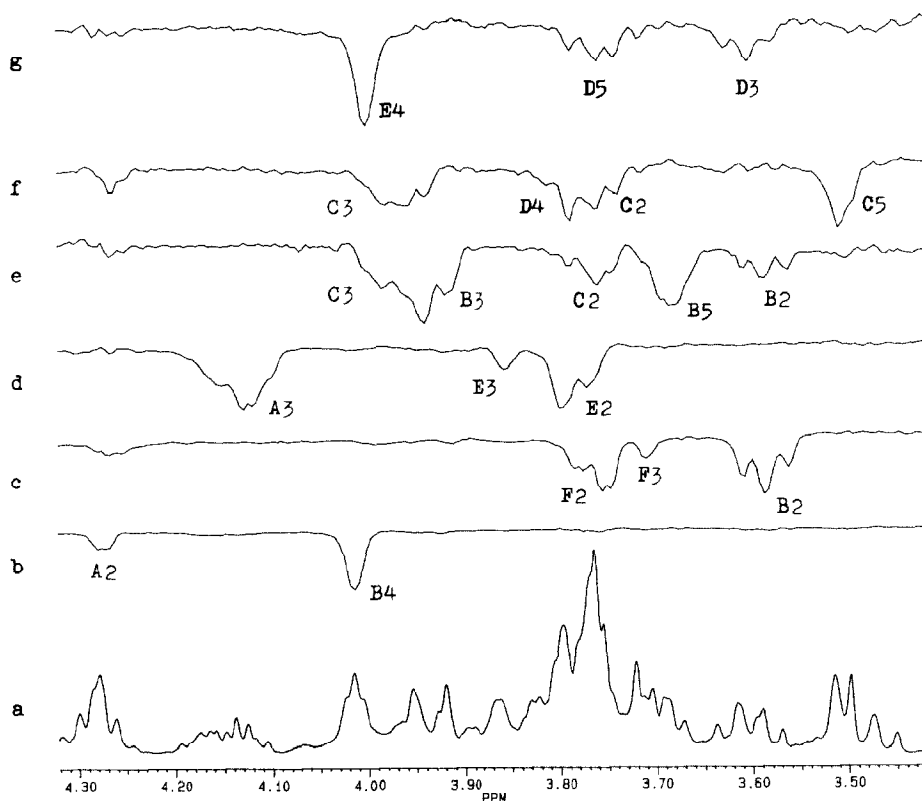
Fig. 1. Part of the 500-MHz ^{1}H NMR spectra of the O-deacetylated polysaccharide (PS-II). (a) One-dimensional ^{1}H NMR spectrum. (b–g) NOE subspectra, as obtained from cross-sections through H-1 resonances of (b) Rib **A**, (c) Fuc **F**, (d) Fuc **E**, (e) Gal **B**, (f) GlcNAc **C**, and (g) GlcA **D** in the 2D ROESY spectrum. Arabic numbers refer to the protons in the sugar residues denoted by letters.

In addition, inter-residue connections were observed for H-1 of each of the sugar residues. They were between Rib (unit **A**) H-1 and Gal (unit **B**) H-4, Gal H-1 and GlcNAc (unit **C**) H-3 (major) and H-2 (minor), GlcNAc H-1 and GlcA (unit **D**) H-4; GlcA H-1 and Fuc (unit **E**) H-4 and H-6 (the cross-peak H-1 GlcA/H-6 Fuc **E** is not shown in Fig. 1); Fuc **E** H-1 and Rib H-3 (major) and H-2 (minor); and Fuc (unit **F**) H-1 and Gal H-2. The appearance of the minor inter-residue as well as intra-residue cross-peaks is caused by spin diffusion, which is characteristic of NOE in large molecules. Therefore, the residues of Rib and GlcNAc were 3-substituted, GlcA and one of the Fuc residues 4-substituted, the Gal residue 2,4-disubstituted, and the second Fuc residue unsubstituted.

Complete assignment of the ^{13}C NMR spectrum of PS-II (Fig. 2, Table II), performed by using 2D heteronuclear ^{13}C–^{1}H shift-correlated spectroscopy, proved that the major NOEs just discussed were those determining the linkage positions. Thus, the relatively low-field positions of the signals for Rib C-3, Gal C-2 and C-4, GlcNAc C-3, GlcA C-4 and Fuc **E** H-4, as compared with those in the correspond-
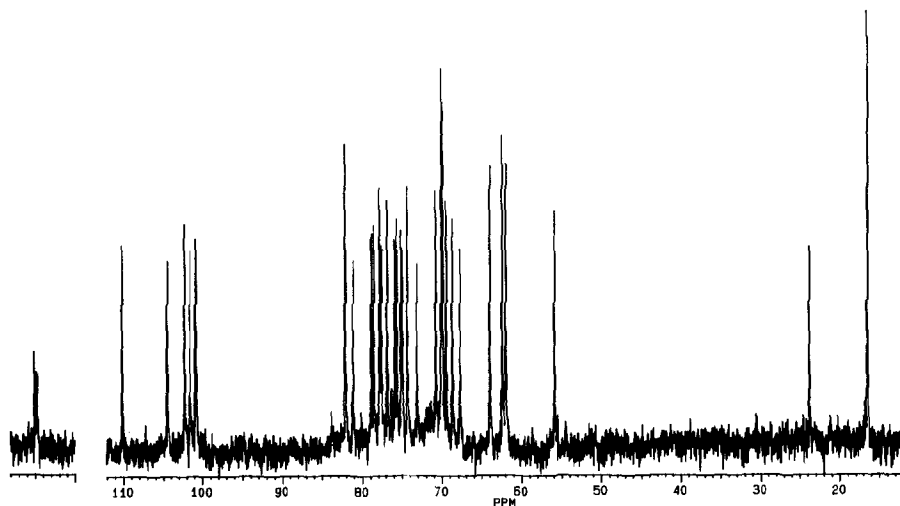
Fig. 2. 90-MHz ^{13}C NMR spectrum of the O-deacetylated polysaccharide (PS-II).
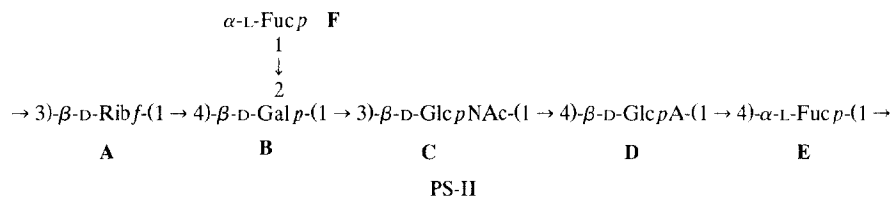
ing unsubstituted monosaccharides[8], confirmed the substitution pattern deduced from the NOE data, in particular, the lateral position of Fuc F and the position of the Gal residue at the branching point.

Analysis of changes of ^{13}C chemical shifts caused by glycosylation[9] and inter-residue NOEs[10] in PS-II confirmed the absolute configurations of galactose, ribose, and fucose, and revealed the D configuration of two other constituent monosaccharides.

Thus, a positive (+1 ppm) β-effect of glycosylation on Gal$p$ C-3, caused by its 2-substitution by the α-Fuc$p$ F, is characteristic of different absolute configurations of the constituent monosaccharides[9], consistent with D-galactose and L-fucose. A negative and relatively high β-effect of glycosylation of -1.4 ppm on Glc$p$NAc C-4 proved that it had the same (D) configuration as the glycosylating β-Gal$p$ residue. In the same way, a β-effect of -2 ppm on Glc$p$A C-3 in the disaccharide fragment β-D-Glc$p$NAc-(1 → 4)-Glc$p$A indicated that the glycosylated monosaccharide, like the glycosylating one, had the D configuration.
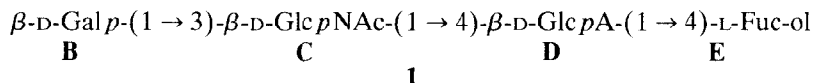
The NOEs from H-1 of GlcA to both H-4 and H-6 of Fuc$p$ E indicated[10] the different absolute configurations of these monosaccharides and, hence, the L configuration of the second Fuc residue. On the contrary, the absence of a NOE Rib$f$ H-1/Gal$p$ H-6,6' was evidence of the same absolute configuration of these two sugars (both D). It is noteworthy that no conclusion about the absolute configuration of Fuc E and Rib could be made by consideration of the glycosylation effects on ^{13}C chemical shifts, since these effects are not sensitive to the relative absolute configuration of the constituents in disaccharides with a 4-substituted Gal (or Fuc) residue[9].

On the basis of the data obtained, it was concluded that PS-II had the following structure:
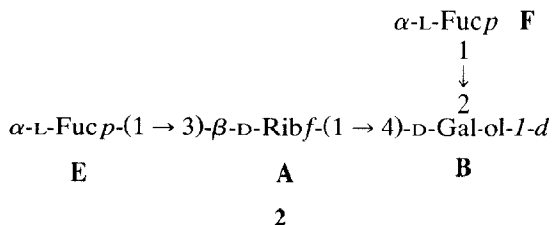
$$\alpha\text{-L-Fuc}p \quad \mathbf{F}$$
$$1$$
$$\downarrow$$
$$2$$
$$\rightarrow 3)\text{-}\beta\text{-D-Rib}f\text{-}(1 \rightarrow 4)\text{-}\beta\text{-D-Gal}p\text{-}(1 \rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{NAc-}(1 \rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{A-}(1 \rightarrow 4)\text{-}\alpha\text{-L-Fuc}p\text{-}(1 \rightarrow$$

$$\mathbf{A} \qquad\qquad \mathbf{B} \qquad\qquad\quad \mathbf{C} \qquad\qquad\qquad \mathbf{D} \qquad\qquad\qquad \mathbf{E}$$

$$\text{PS-II}$$

This structure was confirmed by methylation analysis which led to 2,5-di-O-methylribose, 2,3,4-tri-O-methylfucose, 2,3-di-O-methylfucose, 3,6-di-O-methyl-galactose, and 2-deoxy-4,6-di-O-methyl-2-(N-methylamino) glucose identified by GLC–MS of the alditol acetates. Carboxyl-reduction of the methylated PS-II with LiBH$_4$ resulted in identification of an additional partially methylated sugar, 2,3-di-O-methylglucose, derived from the 4-substituted GlcA residue.

In order to confirm the sequence of the monosaccharide residues, PS-II was solvolysed with anhydrous HF at −30°C and the resulting oligosaccharide was purified by gel-permeation chromatography on TSK HW 40, and reduced with NaBH$_4$. Sugar analysis, methylation analysis, and a computer-assisted 1D ^{13}C NMR study[11] (Table II) showed that this product represented a linear tetrasaccha-ride-alditol with the fucitol residue (Fuc-ol) as aglycon, and has structure 1. Its formation was a result of selective cleavage of glycosidic linkages of the residues of Rib and Fuc, which are most labile[12] towards anhydrous HF. The same oligosac-charide was isolated from PS-II by partial hydrolysis with 0.1 M HCl followed by borohydride reduction.

$$\beta\text{-D-Gal}p\text{-}(1 \rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{NAc-}(1 \rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{A-}(1 \rightarrow 4)\text{-L-Fuc-ol}$$
$$\quad \mathbf{B} \qquad\qquad\qquad \mathbf{C} \qquad\qquad\qquad\qquad \mathbf{D} \qquad\qquad \mathbf{E}$$
$$\mathbf{1}$$

Another oligosaccharide was prepared by successive alkaline degradation of PS-II with lithium in ehtylenediamine and reduction with NaBD$_4$. It was analysed as for oligosaccharide 1 and found to have the structure 2 (for the ^{13}C NMR data, see Table II). The formation of this branched tetrasaccharide may be accounted for by β-elimination from the GlcA residue, to release a reducing 3-substituted GlcNAc residue which underwent an alkaline degradation.

$$\alpha\text{-L-Fuc}p \quad \mathbf{F}$$
$$1$$
$$\downarrow$$
$$2$$
$$\alpha\text{-L-Fuc}p\text{-}(1 \rightarrow 3)\text{-}\beta\text{-D-Rib}f\text{-}(1 \rightarrow 4)\text{-D-Gal-ol-}1\text{-}d$$

$$\quad \mathbf{E} \qquad\qquad\qquad \mathbf{A} \qquad\qquad\quad \mathbf{B}$$

$$\mathbf{2}$$

The structures of both oligosaccharide fragments obtained were in accord with the proposed structure of PS-II.

TABLE II

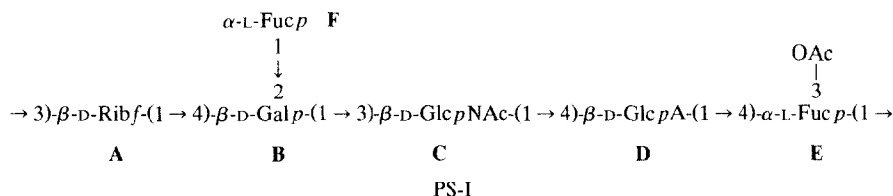Chemical shifts in the ^{13}C NMR spectra (δ in ppm) [a]

Compound	C-1	C-2	C-3	C-4	C-5	C-6
Unit **A** (*β-ribofuranose*)						
PS-I	109.9	75.4	78.6	81.9	63.8	
PS-II	110.0	75.0	78.4	82.0	63.8	
2	109.4	75.7	77.8	81.9	64.0	
Unit **B** (*β-galactopyranose or galactitol*)						
PS-I	101.3	77.7	74.9	77.4	75.8	62.3
PS-II	101.4	77.7	75.0	77.5	75.8	62.3
1	104.4	72.0	74.3	69.9	76.4	62.1
	(104.4)	(71.7)	(74.1)	(70.0)	(76.3)	(62.2)
2	[b]	79.4	71.3	80.2	70.5	62.8
3	101.5	78.0	74.9	70.5	76.5	62.4
4	104.1	79.6	74.7	70.1	76.2	62.3
5	104.7	72.1	73.9	69.9	76.6	62.2
Unit **C** (*2-acetamido-2-deoxy-β-glucopyranose*)						
PS-I	102.7	55.7	78.8	69.7	76.6	61.8
PS-II	102.1	55.8	78.8	69.8	76.7	61.8
1	101.8	55.6	83.8	69.8	76.7	62.0
	(101.9)	(55.8)	(83.8)	(69.8)	(77.0)	(62.1)
3	103.9	56.0	79.0	70.1	76.9	62.2
5	102.9	55.7	84.1	70.2	76.8	62.2
Unit **D** (*β-glucopyranuronic acid*)						
PS-I	104.9	74.0	74.7	81.4	75.4	170.5
PS-II	104.3	74.2	74.8	81.0	76.2	[c]
1	104.6	73.8	75.1	81.1	75.9	[d]
		(74.1)	(75.1)	(81.3)	(76.0)	
Unit **E** (*α-fucopyranose or fucitol*)						
PS-I	100.4	66.7	72.9	78.6	68.0	16.0
PS-II	100.7	69.9	69.9	82.0	68.6	16.3
1	64.2	71.0	71.0	82.3	67.8	19.7
2	100.5	79.5	70.5	72.8	68.3	16.3
Unit **F** (*α-fucopyranose*)						
PS-I	100.7	69.3	70.7	72.9	67.6	16.2
PS-II	100.7	69.4	70.7	73.0	67.0	16.3
2	101.4	69.1	70.5	72.8	68.0	16.4
3	100.8	69.5	70.1	73.1	67.8	16.4
4	101.4	69.9	71.1	73.3	68.1	16.4

[a] Additional signals: NAc at 23.0–23.6 (Me) and 174.5–175.9 (CO) ppm; OAc at 21.4 (Me) and 174.5 or 174.6 (CO) ppm; OMe at 58.3–58.4 ppm. The values calculated for 1 by the published method[11] are given in parentheses. [b] The signal was absent from the spectrum due to attachment of deuterium to C-1. [c] 174.7 or 175.0 ppm. [d] Not found.

Interpretation of the ^{1}H NMR spectrum of PS-I, performed as described above for PS-II, allowed assignment of the low-field signal at 5.002 ppm as belonging to H-3 of the 4-substituted Fuc **E**, which resonated at 3.878 ppm in the spectrum of the *O*-deacetylated PS-II. The displacements of the signals for C-2,3,4 of Fuc **E**

from 69.9, 69.9, and 82.0 ppm, respectively, in the ^{13}C NMR spectrum of PS-II to 66.7, 72.9, and 78.6 ppm in the spectrum of PS-I (Table II) corresponded to the α- and β-effects of O-acetylation[13] and confirmed the location of the OAc group at position 3 of the 4-substituted Fuc residue. The presence of minor signals in the spectrum of PS-I, but not PS-II, indicated that O-acetylation was not stoichiometric, the degree of O-acetylation being estimated as $\geqslant 80\%$.

Therefore, the O-specific polysaccharide of *S. arizonae* O45 has the following structure:

α-L-Fuc$p$ **F**

1

↓

2   OAc

|

3

→ 3)-β-D-Rib$f$-(1 → 4)-β-D-Gal$p$-(1 → 3)-β-D-Glc$p$NAc-(1 → 4)-β-D-Glc$p$A-(1 → 4)-α-L-Fuc$p$-(1 →

      A   **B**   **C**   **D**   **E**

PS-I

Apparently, *S. arizonae* O45 is the first representative of *Salmonella* reported which produces an O-antigen containing a hexuronic acid. An interesting feature of this O-antigen is the presence of the trisaccharide fragment α-L-Fuc$p$-(1 → 2)-β-D-Gal$p$-(1 → 3)-β-D-Glc$p$NAc with the structure of the Le^d blood-group determinant. Remarkably, for both of the polymers PS-I and PS-II, the ^{13}C resonances of C-1 of the Gal residue and C-3 of the GlcNAc residue were shifted by −3.2 and −5.1 ppm, respectively, as compared with the same resonances obtained for the oligosaccharide **1**, where the β-D-Gal$p$-(1 → 3)-β-D-Glc$p$NAc disaccharide unit was terminal (Table II).

There have been many NMR and conformational studies of the Le^d and related oligosaccharides[14–20], and analogous anomalies in ^{13}C chemical shifts have been observed[14]. Marked deviations in ^{13}C chemical shifts have also been observed[21,22] for several other trisaccharides which included a (1 → 2) linkage. In branched trisaccharides, such anomalies were accounted for by changes of the valence angle τ of the glycosidic linkage[14] or the torsion angles ϕ and ψ in the preferred or "mean statistical" conformer (or conformers)[23]. We reinvestigated the Le^d oligosaccharide with the help of NOE spectroscopy in order to attempt the correlation of ^{13}C chemical shift changes with possible conformational changes.

The Le^d trisaccharide glycoside **3** and the glycosides of the corresponding disaccharides **4** and **5** were synthesised[24,25], and the anomaly in question was observed again for the disaccharide fragment β-D-Gal$p$-(1 → 3)-β-D-Glc$p$NAc in **3** as compared with **5** (Table II).

α-L-Fuc$p$-(1 → 2)-β-D-Gal$p$-(1 → 3)-β-D-Glc$p$NAc-(1-OMe

3

α-L-Fuc$p$-(1 → 2)-β-D-Gal$p$-(1-OMe
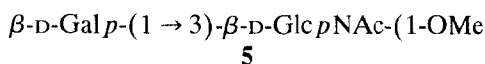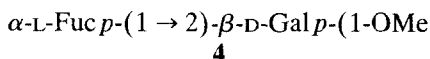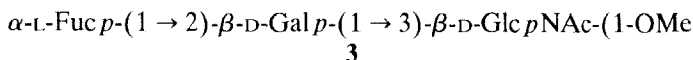
4

β-D-Gal$p$-(1 → 3)-β-D-Glc$p$NAc-(1-OMe

5

TABLE III

NOE data (in % of the intensity of the signal for the pre-irradiated proton)

Compound	Pre-irradiated unit	proton	NOEs observed on Fuc			Gal				GlcNAc	
			H-2+H-3	H-4	H-5	H-2	H-3		H-5	H-3	H-4
3	Fuc	H-1	14.5			14.6					
				(1:1)							
	Gal	H-1					6.0		7.8	8.1	
							(1:1.3:1.35)				
	Fuc	H-6		1.8	3.0						1.1
				(1.6:2.7:1)							
4	Fuc	H-1	15.2			15.3					
				(1:1)							
5	Gal	H-1					5.3		7.1	14.2	
							(1:1.3:2.7)				

Since the chemical shifts for the transglycosidic carbons were shown in our previous work[9] to depend on the distance between the attached protons, the relevant distances were estimated by comparing the appropriate NOEs. The NOE observed for H-2 of the Gal residue upon pre-irradiation of H-1 of the Fuc residue was practically identical in the case of 3 and 4 (Table III). In contrast, the relative NOE on H-3 of the GlcNAc residue obtained by pre-irradiation of H-1 of the Gal residue in 3 was half that in 5. These results were in accord with published data[18,19] and proved that the conformation around the α-L-Fuc$p$-$(1 \rightarrow 2)$-β-D-Gal$p$ glycosidic linkage was similar in 4 and 3, whereas the conformation of the β-D-Gal$p$-$(1 \rightarrow 3)$-β-D-Glc$p$NAc fragment was different in 5 and 3, the distance between H-1 of the Gal residue and H-3 of the GlcNAc residue being larger in 3 than in 5. These data demonstrated directly the influence of the interaction between protons at 1,3-carbons on the ^{13}C chemical shifts for disaccharides, suggested by us earlier[9,26].

It should be added that the observation of an NOE on H-4 of the GlcNAc residue, as a result of pre-irradiation of H-6 of the Fuc residue, was in agreement with the changes of the torsion angles ϕ and ψ in 3 inferred from the Gal H-1/GlcNAc H-3 NOE and ^{13}C chemical shifts just discussed.

EXPERIMENTAL

General methods.—Optical rotations were measured with a Jasco DIP 360 polarimeter at 30°C in water. Solutions were freeze-dried or evaporated in vacuo at $\leqslant$ 40°C.

Gel-permeation chromatography was done on a column (70 × 3 cm) of Sephadex G-50 in pyridine–acetic acid buffer (pH 5.5) or a column (80 × 1.6 cm) of TSK HW 40 (S) in water, and monitored with a Knauer differential refractometer. Anion-ex-

change chromatography was performed on a column (20 × 0.6 cm) of Durrum DAx4 resin in 0.5 M sodium borate buffer (pH 9) at 65°C and the elution was monitored by using a Technicon Autoanalyzer II. GLC was carried out with a Hewlett–Packard 5890 instrument equipped with a glass capillary column (25 m × 0.2 mm) coated with Ultra 1 stationary phase.

Strain No 40014 of *S. arizonae* O45 : H $_{Z_{29}}$ (*Arizona* 11 : 16, 17, 18 : -) was kindly provided by B. Lanyi and M.K. Thage (National Institute of Hygiene, Budapest) from the Hungarian National Collection of Medical Bacteria. The growth of bacteria[27] and the isolation of lipopolysaccharide[2] and O-specific polysaccharide[28] were performed as described.

NMR spectroscopy.—^{13}C NMR spectra of oligosaccharides were recorded with a Bruker AM-300 instrument for solutions in D_2O at 30°C. ^{13}C NMR spectra of polysaccharides were recorded with a Bruker AM-360 instrument for solutions in D_2O at 70°C. ^{1}H NMR spectra were recorded with a Bruker AM-500 instrument for solutions in D_2O at 70°C. Acetone was used as an internal standard (δ_H 2.225 ppm, δ_C 31.45 ppm).

The 1D TOCSY-HOHAHA experiments were performed as described[29], with variable mixing time during accumulation; the DANTE pulse sequence[30] was used for selective excitation. Bruker standard software was applied for 2D COSY spectra; the time-domain data matrix containing 2K × 512 points were multiplied before the magnitude-mode Fourier transformation by the sine-bell function and zero-filled in t_1. The 2D ROESY spectrum was recorded in the phase-sensitive mode by using the method of time-porportional phase incrementation[31] and the pulse sequence proposed by Rance[32]; the time-domain data matrix (2K × 256 points) was zero-filled, multiplied by a squared cosine-bell function, and Fourier-transformed.

Degradative methods.—The O-specific polysaccharide (PS-I) was *O*-deacetylated with M NaOH at room temperature overnight, and the *O*-deacetylated polysaccharide (PS-II) was isolated by gel chromatography on Sephadex G-50.

For sugar analysis, PS-II was hydrolysed with 2 M CF_3COOH for 4 h at 100°C, and the hydrolysate was concentrated and used in anion-exchange chromatography on Durrum DAx4. The monosaccharides obtained were deionised by treatment with KU-2 cation-exchange resin (H^+ form) and, after removal of boric acid by evaporation with MeOH, converted into (*S*)-2-octyl glycoside acetates as described[3].

Methylation analysis was performed according to the Hakomori procedure[33] and the products were recovered by using a Sep-Pak cartridge[34]. Carboxyl-reduction of the methylated PS-II was performed as described[35].

Solvolysis of PS-II with anhyd HF was carried out for 30 min at −30°C, the reaction mixture was treated as described[35], and the oligosaccharide product was isolated by gel-permeation chromatography on a column of TSK HW 40 and reduced conventionally with $NaBH_4$ to yield oligosaccharide **1** desalted on the same column.

Partial acid hydrolysis of PS-II was performed with 0.1 M HCl for 1 h at 100°C, and oligosaccharide 1 was isolated in the same manner as in HF solvolysis.

Alkaline degradation of PS-II was done with lithium in anhyd ethylenediamine for 1 h (uncontrolled temperature) as described[36]. The product was isolated, reduced with $NaBD_4$, and desalted as described above to give oligosaccharide 2.

REFERENCES

1 E.V. Vinogradov, A.S. Shashkov, Y.A. Knirel, N.K. Kochetkov, J. Dabrowksi, H. Grosskurth, E.S. Stanislavsky, and E.V. Kholodkova, *Carbohydr. Res.*, 231 (1992) 1–11.
2 O. Westphal and K. Jann, *Methods Carbohydr. Chem.*, 5 (1965) 83–89.
3 K. Leontein, B. Lindberg, and J. Lönngrenn, *Carbohydr. Res.*, 62 (1978) 359–362.
4 N.A. Kocharova, Y.A. Knirel, A.S. Shashkov, N.K. Kochetkov, and G.B. Pier, *J. Biol. Chem.*, 263 (1988) 11291–11295.
5 A. Bax and D.G. Davis, *J. Magn. Reson.*, 65 (1985) 355–360.
6 S.J. Angyal, *Carbohydr. Res.*, 77 (1979) 37–50.
7 C. Altona and C.A.G. Haasnoot, *Org. Magn. Reson.*, 13 (1980) 417–429.
8 K. Bock and C. Pedersen, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 27–65.
9 A.S. Shashkov, G.M. Lipkind, Y.A. Knirel, and N.K. Kochetkov, *Magn. Reson. Chem.*, 26 (1988) 735–747.
10 G.M. Lipkind, A.S. Shashkov, S.S. Mamyan, and N.K. Kochetkov, *Carbohydr. Res.*, 181 (1989) 1–12.
11 G.M. Lipkind, A.S. Shashkov, Y.A. Knirel, E.V. Vinogradov, and N.K. Kochetkov, *Carbohydr. Res.*, 175 (1989) 59–75.
12 Y.A. Knirel, E.V. Vinogradov, and A.J. Mort, *Adv. Carbohydr. Chem. Biochem.*, 47 (1989) 167–202.
13 P.-E. Jansson, L. Kenne, and E. Schweda, *J. Chem. Soc., Perkin Trans. 1*, (1987) 377–383.
14 R.U. Lemieux, K. Bock, L.T.J. Delbaere, S. Koto, and V.S. Rao, *Can. J. Chem.*, 58 (1980) 631–653.
15 M. Biswas and V.S. Rao, *Biopolymers*, 19 (1980) 1555–1556.
16 J. Dabrowski, P. Hanfland, H. Egge, and U. Dabrowski, *Arch. Biochem. Biophys.*, 210 (1981) 405–411.
17 R.U. Lemieux and K. Bock, *Arch. Biochem. Biophys.*, 221 (1983) 125–134.
18 B.N.N. Rao, V.K. Dua, And C. Allen, *Biopolymers*, 24 (1985) 2207–2229.
19 Z.-Y. Yan, B.N.N. Rao, and C.A. Bush, *J. Am. Chem. Soc.*, 109 (1987) 7663–7669.
20 J. Breg, D. Romijn, J.F.G. Vliegenthart, G. Strecker, and J. Montreuil, *Carbohydr. Res.*, 183 (1988) 19–34.
21 P.-E. Jansson, L. Kenne, and G. Widmalm, *Carbohydr. Res.*, 188 (1989) 169–191.
22 Z. Sidorczyk, E.V. Vinogradov, A.S. Shashkov, K. Zych, Y.A. Knirel, and N.K. Kochetkov, *Eur. J. Biochem.*, in preparation.
23 N.K. Kochetkov, G.M. Lipkind, A.S. Shashkov, and N.E. Nifant'ev, *Carbohydr. Res.*, 221 (1991) 145–168.
24 N.E. Nifant'ev, A.S. Shashkov, and N.K. Kochetkov, *Carbohydr. Res.*, 226 (1992) 331–336.
25 N.E. Nifant'ev, V.Y. Amochaeva, A.S. Shashkov, and N.K. Kochetkov, *Carbohydr. Res.*, in preparation.
26 N.K. Kochetkov, O.S. Chizhov, and N.K. Kochetkov, *Carbohydr. Res.*, 133 (1984) 173–185.
27 E.S. Stanislawsky, L.S. Edvabnaya, O.A. Bandman, V.F. Boolk, M.I. Zhvanetskaya, and A.K. Vargina, *Vaccine*, 7 (1987) 562–566.
28 Y.A. Knirel, E.V. Vinogradov, A.S. Shashkov, B.A. Dmitriev, N.K. Kochetkov, E.S. Stanislawsky, and G.M. Mashilova, *Eur. J. Biochem.*, 163 (1987) 627–637.

29 S. Subramanian and A. Bax, *J. Magn. Reson.*, 71 (1987) 325–330.
30 G.A. Morris and R. Freeman, *J. Magn. Reson.*, 29 (1978) 433–462.
31 D. Morrion and K. Wuthrich, *Biochem. Biophys. Res. Commun.*, 113 (1983) 967–970.
32 M.R. Rance, *J. Magn. Reson.*, 64 (1985) 533–535.
33 S. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
34 T.J. Waeghe, A.G. Darvill, M. McNeil, and P. Albersheim, *Carbohydr. Res.*, 123 (1983) 281–304.
35 E.V. Vinogradov, Z. Sidorczyk, A. Swierzko, A. Rozalski, E.D. Daeva, A.S. Shashkov, Y.A. Knirel, and N.K. Kochetkov, *Eur. J. Biochem.*, 197 (1991) 93–103.
36 J.R. Thomas, A.G. Darvill, and P. Albersheim, *Carbohydr. Res.*, 185 (1989) 279–305.