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Carbohydrate Research 278 (1995) 155–165

CARBOHYDRATE
RESEARCH

NMR investigation of the 6-deoxy-L-talose-containing O45, O45-related (O45rel), and O66 polysaccharides of *Escherichia coli*

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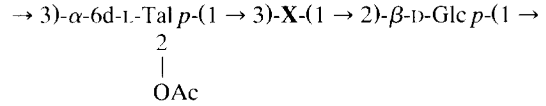
Received 27 February 1995; accepted 15 June 1995

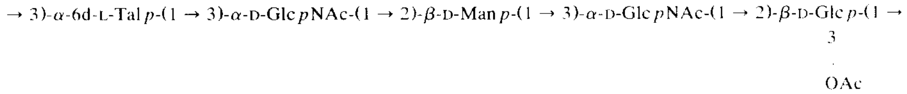
Abstract

The structures of the 6-deoxytalose-containing O-specific polysaccharides from the O45 antigen, an O45-related antigen (O45rel), and the O66 antigen (lipopolysaccharides, LPSs) of *Escherichia coli* were elucidated by chemical characterization and by one- and two-dimensional ^{1}H and ^{13}C NMR spectroscopy. The O45 and O45-related polysaccharides have the following general structure:



$$\rightarrow 3)\text{-}\alpha\text{-6d-L-}\underset{\underset{\text{OAc}}{|}}{\overset{}{\text{Tal}}}{}^{2}p\text{-}(1 \rightarrow 3)\text{-}\mathbf{X}\text{-}(1 \rightarrow 2)\text{-}\beta\text{-D-Glc}p\text{-}(1 \rightarrow$$

For the O45 antigen, **X** is α-D-Fuc*p*NAc and for the O45-related antigen, **X** is β-D-Glc*p*NAc. The structure of the O66 polysaccharide is



$$\rightarrow 3)\text{-}\alpha\text{-6d-L-Tal}p\text{-}(1 \rightarrow 3)\text{-}\alpha\text{-D-Glc}p\text{NAc-}(1 \rightarrow 2)\text{-}\beta\text{-D-Man}p\text{-}(1 \rightarrow 3)\text{-}\alpha\text{-D-Glc}p\text{NAc-}(1 \rightarrow 2)\text{-}\beta\text{-D-}\underset{\underset{\text{OAc}}{|}}{\text{Glc}}{}^{3}p\text{-}(1 \rightarrow$$

Keywords: Escherichia coli O45 and O66 antigens; Polysaccharide structures; 6-Deoxy-L-talose; NMR analysis

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SSDI 0008-6215(95)00243-X

1. Introduction

The O antigens of *Escherichia coli* are lipopolysaccharides (LPSs) which consist of a lipid moiety (lipid A), an oligosaccharide region (core), and a polysaccharide moiety. The latter expresses the serological O-specificity of the bacteria and is termed the O-specific polysaccharide [1,2]. Over 150 distinct *E. coli* O groups are known today which are defined by the epitope structure of the respective O-specific polysaccharide moiety of their LPS. It was found that some O groups can be further divided into subgroups [3–5]. We have recently elucidated the structures of three O1 antigens, two O4 antigens, and four O18 antigens [6–10]. While analysing a number of *E. coli* strains of clinical origin, we found that the LPSs from *E. coli* strains 3332, 4610, and P1a, which were reactive in anti-O45 antisera, exhibited different patterns in SDS-PAGE. Chemical analyses had shown that all three polysaccharides contained 6-deoxytalose, a characteristic constituent of the O45 and O66 antigens [11]. Serological studies revealed that *E. coli* 4610 belonged to serogroup O45, whereas strain 3332 was related to, but not identical with, O45 (termed O45rel). *E. coli* P1a was the O66 test strain, cross-reacting with *E. coli* O45 [2]. We have now elucidated the structures of these polysaccharides. The results are presented in this communication.

2. Results and discussion

Isolation and characterization of the O-specific polysaccharides from E. coli *strains 4610, 3332, and P1a.*—The LPSs were obtained by extraction of the bacteria with aq 45% phenol and subsequent ultracentrifugation of the materials from the aqueous phases [12].

The polysaccharides, as obtained from the sedimented LPSs by mild acid hydrolysis, were purified by gel permeation chromatography on Sephadex G-50. They were eluted with water immediately after the void volume (K_D 0.9–0.95). The chromatographed materials did not contain undegraded LPS, as shown by ultracentrifugation (no pellets obtained) and by the absence of fatty acids.

The sugar composition of the polysaccharides was determined by GLC. The O45 polysaccharide contained glucose (Glc), *N*-acetylfucosamine (FucNAc), and 6-deoxytalose (6dTal) in equimolar ratios; the O45rel polysaccharide also contained Glc and 6dTal, and had *N*-acetylglucosamine (GlcNAc) instead of FucNAc as a constituent. The O66 polysaccharide contained Glc, GlcNAc, Man, and 6dTal in the approximate molar ratios of 1:2:1:1. Glc, present in all polysaccharides, was also determined with glucose oxidase. This characterized it as the D enantiomer, information helpful in the NMR analysis of the absolute configurations of the sugar constituents in the polysaccharides. All three polysaccharides contained one acetyl group per repeating unit. These results are corroborated by the NMR analysis (see below). Periodate oxidation of the O45 and O45rel polysaccharides, before and after *O*-deacetylation, destroyed only the glucose residue and left the others intact. Periodate oxidation of the O66 polysaccharide destroyed the mannose residue and, after *O*-deacetylation, glucose in addition.

NMR analysis of the O45 polysaccharide.—The ^{13}C NMR spectrum showed signals

Table 1
^{1}H NMR and ^{13}C NMR data for the O45 polysaccharide before (PS) and after *O*-deacetylation (PS_{da})

Residue	Proton	δ		$J_{H,H}$	Hz	Carbon	δ			
		PS	PS_{da}				PS	AE [a]	PS_{da}	GE [b]
→ 3)-α-6d-L-Tal*p*-(1 →	H-1	4.97	5.02	$J_{1,2}$	1.6	C-1	101.2	−2.8	104.0	+8.6
(A)	H-2	5.01	3.92	$J_{2,3}$	3.5	C-2	71.2	+0.3	70.9	−0.3
	H-3	4.13	3.98	$J_{3,4}$	3.5	C-3	77.8	−0.9	78.7	
	H-4	3.67	3.68	$J_{4,5}$	~1	C-4	72.6		71.7	−1.4
	H-5	4.17	4.15	$J_{5,6}$	6.5	C-5	68.7		69.1	
	H-6	1.27	1.27			C-6	16.5		16.5	
→ 3)-α-D-Fuc*p*NAc-(1 →	H-1	5.32	5.41	$J_{1,2}$	3.8	C-1	97.7		97.7	
(B)	H-2	4.36	4.35	$J_{2,3}$	10.8	C-2	49.3		49.4	−1.3
	H-3	3.93	3.97	$J_{3,4}$	3.2	C-3	75.5		76.5	+7.8
	H-4	3.83	3.87	$J_{4,5}$	~1	C-4	70.2		72.6	+0.2
	H-5	4.41	4.43	$J_{5,6}$	6.5	C-5	68.1		68.2	
	H-6	1.22	1.24			C-6	16.5		16.7	
→ 2)-β-D-Glc*p*-(1 →	H-1	4.75	4.77	$J_{1,2}$	7.6	C-1	104.2		103.9	
(C)	H-2	3.45	3.51	$J_{2,3}$	9	C-2	79.0		78.0	
	H-3	3.59	3.62	$J_{3,4}$	9	C-3	75.9		76.0	−1.0
	H-4	3.50	3.51	$J_{4,5}$	9	C-4	70.6		70.8	
	H-5	3.43	3.46	$J_{5,6a}$	2.4	C-5	76.9		77.0	
	H-6a	3.80	3.90	$J_{6a,6b}$	12	C-6	62.0		62.0	
	H-6b	3.76	3.78	$J_{5,6b}$	5					

[a] Acetylation effect.

[b] Glycosylation effect. Other signals are: CH_3–CO–O at δ 21.8 and 175.4; CH_3–CO–NH at δ 23.7 and 175.1; CH_3–CO–NH at δ 23.6 and 174.8.

at δ 21.8 and 175.4, due to *O*-acetyl groups. After *O*-deacetylation, the ^{13}C NMR spectrum exhibited three signals in the region of anomeric carbon atoms (δ 97.7, 103.9, and 104.0), as well as signals for a nitrogen-bearing carbon atom (δ 49.3), an *N*-acetyl group (δ 23.6 and 175.4), and the methyl group of a 6-deoxyhexose (δ 16.5) (Table 1). These data indicated a trisaccharide repeating unit.

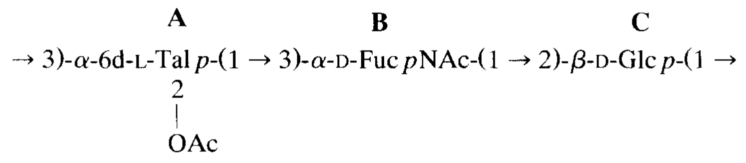
The ^{1}H spectrum of the polysaccharide contained one signal for a β-anomeric proton in the *gluco/galacto* configuration (δ 4.75; $J_{1,2}$ 7.6), one signal for an α-anomeric proton in the *gluco/galacto* configuration (δ 5.41; $J_{1,2}$ 3.8), and one signal characteristic of a proton in the *manno* configuration (δ 4.97; $J_{1,2} < 2$). Further signals indicative of the methyl groups from an acetamido substituent, from an *O*-acetyl substituent (which was absent after alkali treatment of the polysaccharide), and from a 6-deoxy sugar were present. The signals of the ^{1}H spectra from the original and the *O*-deacetylated polysaccharide (Table 1) were assigned using 2D COSY one- and two-step H-relayed coherence transfer (COSYRCT) 2D spectra [13,14] and 1D homonuclear double resonance in the difference mode [15]. An analysis of the coupling constants [16,17] and chemical shifts indicated the presence of β-Glc, α-FucNAc, and 6dTal. The anomeric configuration of α-6dTal became evident from the results of a gated decoupling experiment ($J_{C,H}$ 173 Hz). Gated decoupling also corroborated the α configuration of FucNAc ($J_{C,H}$ 173 Hz) and the β configuration of Glc ($J_{C,H}$ 163 Hz). For the assignments of the signals of the ^{13}C NMR spectra, 2D heteronuclear ^{13}C/^{1}H NMR spectra were used.

The sequence and types of substitution were determined in a series of 1D NOE experiments with preirradiation of the anomeric protons (Table 2). After irradiation of H-1 of α-6dTal*p* (residue **A**) an NOE response was observed for H-2 of the same residue and for H-3 of α-D-Fuc*p*NAc (residue **B**), indicative of an α-**A**-(1 → 3)-**B** linkage. Preirradiation of the anomeric proton of residue **B** also resulted in a response of H-2 of the same residue and of H-2 of β-D-Glc*p* (residue **C**), Taken together with the periodate sensitivity of the glucose residue, this indicated an α-**B**-(1 → 2)-**C** linkage. Preirradiation of the anomeric proton of residue **C** resulted in a response of H-3 of

Table 2
NOE data for the *O*-deacetylated O45 polysaccharide

NOE observed on residue	Proton	Preirradiated proton			
		A,H-1	**B**,H-1	**C**,H-1	**B**(CH_3COND)
→ 3)-α-6d-L-Tal*p*-(1 →	H-1				+
(**A**)	H-2	+			+
	H-3			+	+
	H-5				+
→ 3)-α-D-Fuc*p*NAc-(1 →	H-1				+
(**B**)	H-2		+		+
	H-3	+			+
→ 2)-β-D-Glc*p*-(1 →	H-1				+
(**C**)	H-2		+	+	+
	H-3			+	
	H-5			+	

residue **A**. Together with the glycosylation effect, this indicated a β-**C**-(1 → 3)-**A** linkage. The glycosylation effects [18–20] (Table 1) were used to determine the absolute configurations of all sugar residues. The large negative glycosylation effect on C-4 and the very small effect on C-2 of the 3-linked α-6dTal*p* (residue **A**) is characteristic of an absolute configuration of this glycosylated sugar which is opposite to that of a β-D-pyranose (β-D-Glc*p*, residue **C**) glycosylating it at position 3. This showed that residue **A** was α-6d-L-Tal*p*. Similarly, the large positive glycosylation effect on C-1 of α-6d-L-Tal*p* and on C-3 of α-Fuc*p*NAc together with the small effect on C-4 of Fuc*p*NAc are typical for opposite absolute configuations of these sugars, Thus α-Fuc*p*NAc had the D configuration. This was borne out further by the NOE response pattern of residue **A** after irradiation of the methyl protons of the acetamido group of residue **B** (Table 2). The chemical shifts of the carbon signals before and after *O*-deacetylation of the polysaccharide and the acetylation effects given in Table 3 showed that α-6d-L-Tal*p* was *O*-acetylated at C-2 and that the O45 polysaccharide of *E. coli* 4610 can be formulated as **1**.



$$\overset{\mathbf{A}}{\rightarrow 3)\text{-}\alpha\text{-6d-L-Tal}p\text{-}(1} \rightarrow \overset{\mathbf{B}}{3)\text{-}\alpha\text{-D-Fuc}p\text{NAc-}(1} \rightarrow \overset{\mathbf{C}}{2)\text{-}\beta\text{-D-Glc}p\text{-}(1} \rightarrow$$
$$\underset{\substack{|\\ \text{OAc}}}{2}$$

1

NMR analysis of the O45rel polysaccharide.—The ^{13}C NMR spectrum contained signals at δ 21.7 and 174.3 due to *O*-acetyl groups. After *O*-deacetylation, the ^{13}C NMR spectrum exhibited three signals in the region of anomeric carbon atoms (δ 101.5, 102.2, and 103.1), as well as signals for a nitrogen-bearing carbon atom (δ 56.9), an *N*-acetyl group (δ 23.7 and 175.8), and a 6-deoxyhexose (δ 16.4). These data indicated a trisaccharide repeating unit.

The ^{1}H NMR spectrum of the *O*-deacetylated polysaccharide contained two signals for β-anomeric protons in the *gluco / galacto* configuration (δ 4.67 and 4.92, both with $J_{1,2}$ 7.5 Hz) and one signal characteristic of an anomeric proton in the *manno* configuration. The spectrum also contained signals for protons of methyl groups from acetamido and 6-deoxy sugars.

For the interpretation of the NMR spectra of the polysaccharide the same methods were used as described for the O45 polysaccharide. The signal assignments of the original and the *O*-deacetylated polysaccharide (Table 3) and the results of the NOE spectra after irradiation of the anomeric protons (Table 4) showed that residue **A** was → 3)-α-6d-L-Tal*p*, residue **B** was → 3)-α-D-Glc*p*NAc, and residue **C** was → 2)-β-D-Glc*p*. The absolute configurations (relative to D-Glc*p*) were derived from the glycosylation effects (Table 3).

From the results obtained, the O45rel polysaccharide from *E. coli* 3332 can be formulated as **2**.

Table 3
^{1}H NMR and ^{13}C NMR data for the O45rel polysaccharide before (PS) and after *O*-deacetylation (PS$_{da}$)

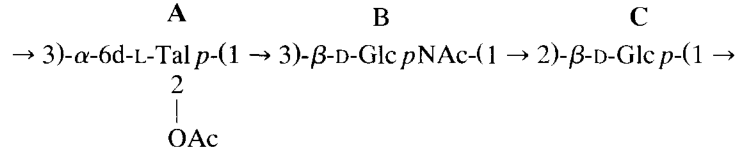
Residue	Proton	δ		$J_{H,H}$	Hz	Carbon	δ			
		PS	PS$_{da}$				PS	AE [a]	PS$_{da}$	GE [b]
→ 3)-*α*-6d-L-Tal*p*-(1 → **(A)**	H-1	4.95	4.98	$J_{1,2}$	< 2	C-1	100.3	−2.8	103.1	
	H-2	5.02	3.85	$J_{2,3}$	3.5	C-2	71.2	−0.1	71.3	−0.4
	H-3	4.15	3.99	$J_{3,4}$	3.5	C-3	75.3	−0.4	75.7	
	H-4	3.95	3.96	$J_{4,5}$	< 2	C-4	69.9		70.9	−2.1
	H-5	4.35	4.29	$J_{5,6}$	6	C-5	68.1		68.4	
	H-6	1.26	1.25			C-6	16.4		16.7	
→ 3)-*β*-D-Glc*p*NAc-(1 → **(B)**	H-1	4.92	4.92	$J_{1,2}$	7.5	C-1	101.6		102.2	
	H-2	3.82	3.82	$J_{2,3}$	9	C-2	56.9		57.1	
	H-3	3.65	3.67	$J_{3,4}$	9	C-3	83.0		82.8	
	H-4	3.49	3.47	$J_{4,5}$	9	C-4	69.9		69.8	−1.4
	H-5	3.47	3.47	$J_{5,6a}$	1.5	C-5	77.4		77.2	
	H-6a	3.84	3.90	$J_{6a,6b}$	12	C-6	62.3		62.2	
	H-6b	3.74	3.74	$J_{5,6b}$	4.5					
→ 2)-*β*-D-Glc*p*-(1 → **(C)**	H-1	4.65	4.67	$J_{1,2}$	7.5	C-1	102.5		101.5	
	H-2	3.56	3.57	$J_{2,3}$	–	C-2	81.0		81.6	
	H-3	3.56	3.57	$J_{3,4}$	–	C-3	77.3		77.2	+0.2
	H-4	3.45	3.43	$J_{4,5}$	9	C-4	70.5		71.0	
	H-5	3.42	3.43	$J_{5,6a}$	2	C-5	76.8		76.9	
	H-6a	3.93	3.90	$J_{6a,6b}$	12	C-6	61.7		61.9	
	H-6b	3.75	3.76	$J_{5,6b}$	5					

[a] Acetylation effect.

[b] Glycosylation effect. Other signals are: CH_3–CO–O at δ 21.7 and 174.3; CH_3–CO–NH at δ 23.7 and 175.8.

Table 4
NOE data for the *O*-deacetylated O45rel polysaccharide

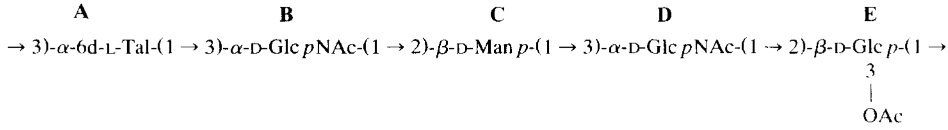
NOE observed on residue	Proton	Preirradiated proton		
		A,H-1	**B**,H-1	**C**,H-1
→ 3)-α-6d-L-Tal*p*-(1 →	H-2	+		
(A)	H-3			+
→ 3)-β-D-Glc*p*NAc-(1 →	H-2		+	
(B)	H-3	+	+	
	H-5		+	
→ 2)-β-D-Glc*p*-(1 →	H-2,3		+	+
(C)	H-5			+



$$\rightarrow \underset{\substack{2\\|\\\text{OAc}}}{\overset{\textbf{A}}{3)\text{-}\alpha\text{-6d-L-Tal}p}}\text{-}(1 \rightarrow \overset{\textbf{B}}{3)\text{-}\beta\text{-D-Glc}p\text{NAc}}\text{-}(1 \rightarrow \overset{\textbf{C}}{2)\text{-}\beta\text{-D-Glc}p}\text{-}(1 \rightarrow$$

2

NMR analysis of the O66 polysaccharide.—The assignments of the ^{1}H and ^{13}C NMR spectra for the polysaccharide and its *O*-deacetylated form are shown in Table 5. The interpretation of the NMR data was the same as described for the O45 polysaccharide. The relatively large negative glycosylation effects on C-4 of the 3-substituted α-Glc*p*NAc (residues **B** and **D**) and that on C-3 of the 2-substituted β-Glc*p* (residue **E**) indicated the D configuration for residues **B**, **C**, **D**, and **E**. As shown above, the large negative glycosylation effect at C-4 and the very small effect on C-2 of the 3-linked α-6d-Tal*p* showed that this residue (residue **A**) had the L configuration. The linkage sequence in the polysaccharide was determined in a series of NOE spectra after irradiation of the anomeric protons (Table 6). After irradiation of H-1 of residue α-6d-L-Tal*p* (residue **A**) a response of H-3 of α-Glc*p*NAc (residue **B**) indicated an **A**-(1 → 3)-**B** linkage. After irradiation of H-1 of residue **B** a response of H-2 of β-D-Man*p* (residue **C**) was observed which indicated a **B**-(1 → 2)-**C** linkage. Preirradiation of the complex signal given by the anomeric protons of **C** and of β-Glc*p* (residue **E**) resulted in responses of H-3/H-4 of residue **A** and H-1 and H-3 of α-D-Glc*p*NAc (residue **D**). When the low-field component of this signal, due to H-1 of residue **E**, was irradiated separately, the response was on H-3/H-4 of residue **A**. Finally, preirradiation of H-1 of residue **D** resulted in a response of H-1 and H-2 of residue **E**. This indicated the sequence **A** → **B** → **C** → **D** → **E** and corroborated the types of linkages described above, thus the O66 polysaccharide from *E. coli* P1a has structure **3**.



$$\rightarrow \overset{\textbf{A}}{3)\text{-}\alpha\text{-6d-L-Tal}}\text{-}(1 \rightarrow \overset{\textbf{B}}{3)\text{-}\alpha\text{-D-Glc}p\text{NAc}}\text{-}(1 \rightarrow \overset{\textbf{C}}{2)\text{-}\beta\text{-D-Man}p}\text{-}(1 \rightarrow \overset{\textbf{D}}{3)\text{-}\alpha\text{-D-Glc}p\text{NAc}}\text{-}(1 \rightarrow \underset{\substack{3\\|\\\text{OAc}}}{\overset{\textbf{E}}{2)\text{-}\beta\text{-D-Glc}p}}\text{-}(1 \rightarrow$$

3

Table 5
^{1}H NMR and ^{13}C NMR data for the O66 polysaccharide before (PS) and after *O*-deacetylation (PS_{da})

Residue	Proton	δ PS_{da}	$J_{H,H}$	Hz	Carbon	δ			
						PS	AE [a]	PS_{da}	GE [b]
→ 3)-*α*-6d-L-Tal*p*-(1 → (A)	H-1	5.02	$J_{1,2}$	< 2	C-1	102.8		102.8	
	H-2	3.89	$J_{2,3}$	3.5	C-2	70.2		70.9	+0.2
	H-3	3.87	$J_{3,4}$	3.5	C-3	76.8		76.5	
	H-4	3.64	$J_{4,5}$	< 2	C-4	72.0		71.7	−1.3
	H-5	4.24	$J_{5,6}$	6.5	C-5	69.3		68.9	
	H-6	1.21			C-6	16.3		16.7	
→ 3)-*α*-D-Glc*p*NAc-(1 → (B)	H-1	5.11	$J_{1,2}$	3.5	C-1	99.6		99.6	
	H-2	3.98	$J_{2,3}$	11	C-2	55.1		55.0	
	H-3	3.88	$J_{3,4}$	9	C-3	81.3		81.0	
	H-4	3.56	$J_{4,5}$	9	C-4	69.3		69.3	−2.1
	H-5	4.02			C-5	73.2		73.1	
	H-6a,6b	~ 3.82			C-6	61.6		61.7	
→ 2)-*β*-D-Man*p*-(1 → (C)	H-1	4.73	$J_{1,2}$	< 2	C-1	101.4		101.4	
	H-2	3.92	$J_{2,3}$	3.5	C-2	78.3		78.1	
	H-3	3.76	$J_{3,4}$	9.5	C-3	74.9		74.9	
	H-4	3.71	$J_{4,5}$	9.5	C-4	68.1		68.2	
	H-5	3.40	$J_{5,6a}$	2.5	C-5	78.1		78.1	
	H-6a	3.94	$J_{6a,6b}$	11	C-6	62.0		62.0	
	H-6b	3.80	$J_{5,6b}$	2.5					

→ 3)-α-D-Glc*p*NAc-(1 →	H-1	5.43	$J_{1,2}$	3.5	C-1	97.2		97.2	
(D)	H-2	4.06	$J_{2,3}$	10.5	C-2	53.9		55.0	
	H-3	3.95	$J_{3,4}$	9	C-3	81.3		81.4	
	H-4	3.56	$J_{4,5}$	9	C-4	69.3		69.5	−1.9
	H-5	4.10			C-5	73.1		73.1	
	H-6a,6b	~ 3.80			C-6	61.6		61.7	
→ 2)-β-D-Glc*p*-(1 →	H-1	4.74	$J_{1,2}$	7.5	C-1	104.8	+0.9	103.9	
(E)	H-2	3.52	$J_{2,3}$	9	C-2	75.7	−1.8	77.5	
	H-3	3.59	$J_{3,4}$	9	C-3	77.4	+1.6	75.8	−1.1
	H-4	3.47	$J_{4,5}$	9	C-4	67.0	−3.5	70.5	
	H-5	3.43	$J_{5,6a}$	6	C-5	76.8		76.9	
	H-6a	3.77	$J_{6a,6b}$	12	C-6	61.6		61.5	
	H-6b	3.72	$J_{5,6b}$	3.5					

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[a] Acetylation effect.

[b] Glycosylation effect. Other signals are: CH_3–CO–NH at δ 23.3 (23.4) and 174.7 (175.2); CH_3–CO–NH at δ 23.3 (23.4) and 174.4 (175.1); CH_3–CO–O at δ 21.45 and 173.9.

Table 6
NOE data for the *O*-deacetylated O66 polysaccharide

NOE observed on residue	Proton	Preirradiated proton				
		A,H-1	**B**,H-1	**C**,H-1 + **E**,H-1	**E**,H-1 [a]	**D**,H-1
→ 3)-α-6d-L-Tal*p*-(1 →	H-2	+				
(A)	H-3			+	+	
	H-4			+	+	
→ 3)-α-D-Glc*p*NAc-(1 →	H-2		+			
(B)	H-3	+				
→ 2)-β-D-Man*p*-(1 →	H-2		+	+		
(C)	H-3			+		
	H-5			+		
→ 3)-α-D-Glc*p*NAc-(1 →	H-1			+	+	
(D)	H-2					+
	H-3			+		
→ 2)-β-D-Glc*p*-(1 →	H-1					+
(E)	H-2			+		+
	H-3			+	+	
	H-4,5			+	+	

[a] Pre-irradiation of the low-field component only.

The common partial structure of polysaccharides **1–3** is the disaccharide unit → 2)-β-D-Glc*p*-(1 → 3)-α-6d-L-Tal*p*-(1 → 3)-. This substructure is likely to be a common epitope of the three polysaccharides, resulting in their cross-reactivity. Preliminary serological studies (Flemming Scheutz, Statens Seruminstitut, Copenhagen, personal communication) indicate that the O45rel antigen has to be classified as a new *E. coli* O antigen. This would be in keeping with the results of our structural analysis.

3. Experimental

Bacteria and cultivation.—*E. coli* strains 4610 (O45:K1:H10) and 3332 (O45rel:K:H7) were from the collection of the Robert Koch Institut Wernigerode, and strain P1a (O66:K$^-$:H25) is the *E. coli* O66 test strain from the Statens Seruminstitut, Copenhagen. The bacteria were grown to the stationary phase (5–6 h) in 14-L batch cultures at 37°C in a medium containing, per L, tryptone (7.5 g), yeast extract (10 g), D-glucose (10 g), NaCl (3 g), $Na_2HPO_4 \cdot 12\ H_2O$ (8 g), $MgSO_4 \cdot 7\ H_2O$ (0.2 g), and poly(ethylene glycol) (0.3 g). D-Glucose and $MgSO_4$ were sterilized separately. At the end of the cultivation, the bacteria were killed with phenol (1% final concentration) and harvested by centrifugation.

Isolation of the LPSs and preparation of the polysaccharides.—The LPSs were isolated from the bacteria with aq 45% phenol at 65°C (10 min) and the material obtained from the aqueous phase was purified by repeated ultracentrifugation as described [12]. The polysaccharides were obtained from the LPSs by hydrolysis in aq 1% AcOH (100°C, 90 min) and purified by chromatography on Sephadex G-50.

Periodate oxidation and *O*-deacetylation have been described previously [10,21].

Analytical procedures.—Glucose and mannose were determined as their alditol acetates by GLC on ECNSS-M at 170°C; glucosamine and fucosamine were determined [22] as alditol acetates by GLC on PolyA103 at 220°C. The absolute configuration of glucose was determined with the D-Glc-specific glucose oxidase.

NMR spectroscopy.—^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker WM-300 spectrometer at 90°C in D_2O, using acetone (δ_H 2.225; δ_C 31.45) as the internal standard. Homonuclear 2D COSY spectra, H-relayed H,H-COSY spectra (one and two step), and heteronuclear ^{13}C/^{1}H-COSY spectra were obtained by using standard Bruker software for ASPEC 2000 (COSYHG, COSYRCT, COSYRCT2, and XHCORD, respectively). NOE experiments were performed in the truncated driven (TOE) mode [23] with the Bruker NOEMULT program. The relaxation delay was 1 s, the irradiation time of every component of multiplets was 0.1 s, and the total preirradiation time of whole multiplets was 1.0–1.2 s.

References

[1] K. Jann and B. Jann, in E.T. Rietschel (Ed.), *Handbook of Endotoxins*, Vol. 1, *Chemistry of Endotoxin*, Elsevier, Amsterdam, 1984, pp 138–186.

[2] I. Ørskov, F. Ørskov, B. Jann, and K. Jann, *Bacteriol. Rev.*, 41 (1977) 667–710.

[3] M. Achtman, A. Mercer, B. Kusecek, A. Pohl, M. Heuzenroeder, W. Aaronson, A. Sutton, and M.R.P. Silver, *Infect. Immun.*, 39 (1983) 315–335.

[4] B. Kusecek, H. Wloch, A. Mercer, V. Väisanen, G. Pluschke, T. Korhonen, and M. Achtman, *Infect. Immun.*, 43 (1984) 368–379.

[5] A. Moll, B. Kusecek, G. Pluschke, G. Morelli, M. Kamke, B. Jann, K. Jann, and M. Achtman, *Infect. Immun.*, 53 (1986) 257–263.

[6] B. Jann, A.S. Shashkov, D.S. Gupta, S.M. Panasenko, and K. Jann, *Carbohydr. Polym.*, 18 (1992) 51–57.

[7] D.S. Gupta, A.S. Shashkov, B. Jann, and K. Jann, *J. Bacteriol.*, 174 (1992) 7963–7970.

[8] B. Jann, A.S. Shashkov, H. Kochanowski, and K. Jann, *Carbohydr. Res.*, 248 (1993) 241–250.

[9] B. Jann, A.S. Shashkov, D.S. Gupta, and K. Jann, *Eur. J. Biochem.*, 210 (1992) 241–248.

[10] D.S. Gupta, B. Jann, and K. Jann, *Infect. Immun.*, 45 (1984) 203–209.

[11] F. Ørskov, I. Ørskov, B. Jann, K. Jann, E. Müller-Seitz, and O. Westphal, *Acta Pathol. Microbiol. Scand.*, 71 (1967) 339–358.

[12] O. Westphal and K. Jann, *Methods Carbohydr. Chem.*, 5 (1965) 83–91.

[13] J. Jeener, B.H. Meier, P. Bachmann, and E.E. Ernst, *J. Chem. Phys.*, 71 (1979) 4546–4553.

[14] J. Dabrowski, *Methods Stereochem. Anal.*, 9 (1987) 349–386.

[15] N.A. Kocharowa, Y.A. Knirel, A.S. Shashkov, N.K. Kochetkov, and G.B. Pier, *J. Biol. Chem.*, 263 (1988) 11291–11295.

[16] C. Altona and C.A.G. Haasnoot, *Org. Magn. Reson.*, 13 (1980) 417–429.

[17] Y.A. Knirel, N.A. Paramonov, A.S. Shashkov, N.K. Kochetkov, R.G. Yarrulin, S.M. Farber, and V.I. Efremenko, *Carbohydr. Res.*, 233 (1992) 185–193.

[18] A.S. Shashkov, G.M. Lipkind, Y.A. Knirel, and N.K. Kochetkov, *Magn. Reson. Chem.*, 26 (1988) 735–747.

[19] G.M. Lipkind, A.S. Shashkov, Y.A. Knirel, E.V. Vinogradov, and N.K. Kochetkov, *Carbohydr. Res.*, 175 (1988) 59–75.

[20] G.M. Lipkind, A.S. Shashkov, S.S. Mamyan, and N.K. Kochetkov, *Carbohydr. Res.*, 181 (1988) 1–12.

[21] M. Bartelt, A.S. Shashkov, H. Kochanowski, B. Jann, and K. Jann, *Carbohydr. Res.*, 254 (1994) 203–212.

[22] W. Niedermayer and M. Thomana, *Anal. Biochem.*, 57 (1974) 363–368.

[23] G. Wagner and K. Wüthrich, *J. Magn. Reson.*, 33 (1979) 675–680.