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STRUCTURE OF THE 3-DEOXY-D-*manno*-OCTULOSONIC ACID-CONTAINING POLYSACCHARIDE (K6 ANTIGEN) FROM *Escherichia coli* LP 1092*†

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ABSTRACT

The acidic polysaccharide (K6) antigen from *Escherichia coli* LP 1092 contains D-ribose and 3-deoxy-D-*manno*-octulosonic acid in the molar ratio of 2 : 1, respectively. Spectroscopic data (^{13}C- and ^{1}H-n.m.r.), methylation analyses, and periodate oxidation indicate that the polysaccharide is composed of the foregoing components essentially in the following trisaccharide sequence:

→2)-β-D-Rib*f*-(1→2)-β-D-Rib*f*-(1→7)-α-D-KDO-(2→

The polysaccharide also contains *O*-acetyl substituents (~0.2–0.3 mol per KDO residue).

INTRODUCTION

The occurrence of 3-deoxy-D-*manno*-octulosonic acid (KDO) in bacterial polysaccharides other than lipopolysaccharides was almost simultaneously reported in the *E. coli* LP 1092 (K6) antigen[1] and the *N. meningitidis* group 29e polysaccharide antigen[2]. The KDO residue of this latter antigen was later demonstrated[3] to be in the β-D configuration. Further identification of KDO in polysaccharides of bacterial origin has also been more recently reported[4] in the *E. coli* K12 and K13 antigens. We have previously demonstrated[3,5,6] the potential of ^{13}C- and ^{1}H-n.m.r. techniques in the elucidation of the structures of polysaccharides containing such complex saccharides as KDO, and this paper reports extension of these techniques to the elucidation of the primary structure of the K6 antigen, in which KDO probably occurs in the α-D configuration. In preliminary communications, Unger and coworkers[7,8] have postulated a Rib*f*-(1→2)-β-Rib*f*-(1→7)-KDO trisaccharide as the repeating unit of the K6 antigen, with which we concur. However while we have

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proposed an essentially linear structure for the K6 antigen, they have postulated[7] it to be highly branched. It is possible that this structural diversity could be attributed to strain difference or phenotypic variation in the organism*.

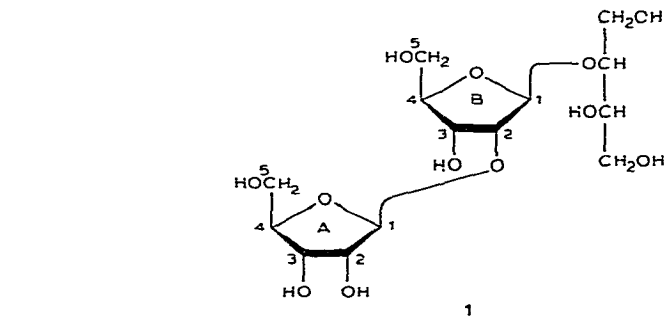
RESULTS AND DISCUSSION

Component sugars of the K6 polysaccharide antigen. — The K6 polysaccharide was shown to contain ribose and KDO in the molar ratio of 1.9:1, as previously reported by Messmer and Unger[7]. The individual components were further characterized by hydrolysis (0.5M hydrochloric acid for 3 h at 80°) of the polysaccharide and separation of the neutral and acidic hydrolysis products on a Rexyn 201 (carbonate form) ion-exchange column as previously described[3] for the meningococcal group 29e polysaccharide. The neutral component was shown to be ribose by g.l.c.–m.s. after conversion[9] into its ribitol acetate derivative, and was assigned the D configuration by g.l.c. of its (+)-2-octyl glycoside as described by Leontein *et al.*[10]. The acid component was eluted from the column with 0.5M ammonium hydrogencarbonate, and the inorganic salt was removed by repeated lyophilizations from water. The residue was recrystallized from ethanol–water to yield crystals of authentic ammonium 3-deoxy-D-*manno*-octulosonate, m.p. 120–122°, undepressed on admixture with an authentic sample[3].

Oligosaccharide **1**. — Smith degradation[11] of the *O*-deacetylated K6 antigen yielded **1**, which on hydrolysis (0.5M hydrochloric acid for 3 h at 80°) was shown to contain ribose and erythritol in the molar ratio of 2:1 by g.l.c.–m.s. of their alditol acetates[9]. Methylation of **1** with subsequent hydrolysis of the permethylated product yielded three methylated components identified by g.l.c.–m.s. of their alditol acetate derivatives as 2,3,5-tri-*O*-methylribitol, 3,5-di-*O*-methylribitol, and 1,3,4-tri-*O*-methylerythritol. The fact that the erythritol residues originated from a 7-linked KDO moiety in the native K6 antigen was ascertained when the only major product of the mild acid hydrolysis (2% acetic acid for 3 h at 100°) of the K6 antigen was a trisaccharide containing ribose and KDO in the molar ratio of 2:1. The trisaccharide was reduced with sodium borohydride and 1-ethyl-3-(dimethylaminopropyl)carbodiimide hydrochloride as previously described[24] prior to being methylated. The methylated trisaccharide shown in Fig. 1 was subjected to g.l.c.–m.s., and the fragmentation pattern observed was consistent only with the original trisaccharide's having a Rib*f*-Rib*f*-KDO sequence, the KDO moiety being linked at O-7. The isolation of this trisaccharide has been previously reported[7] by Messner and Unger, and its methylation analysis yielded the same methylated ribitol acetate derivatives as **1**.

Assignment of the linkage configurations of the ribofuranosyl residues was

*This was ascertained by the detection of significant chemical-shift differences in the ^{13}C-n.m.r. spectra of both antigens. This comparison was made possible by Dr. F. Unger's kindly making available to us the ^{13}C-n.m.r. spectrum of his K6 antigen.



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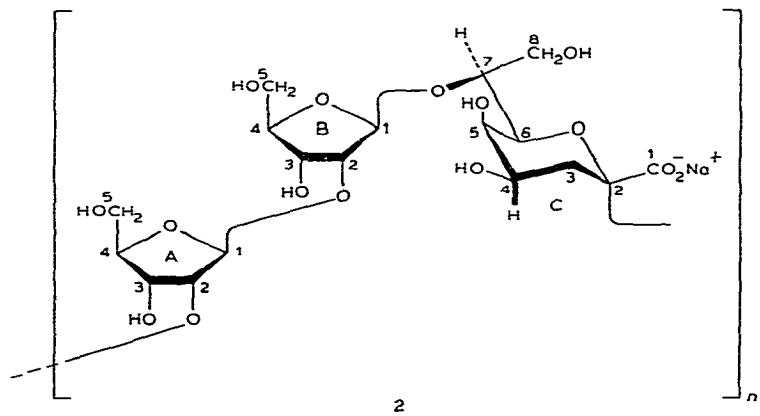

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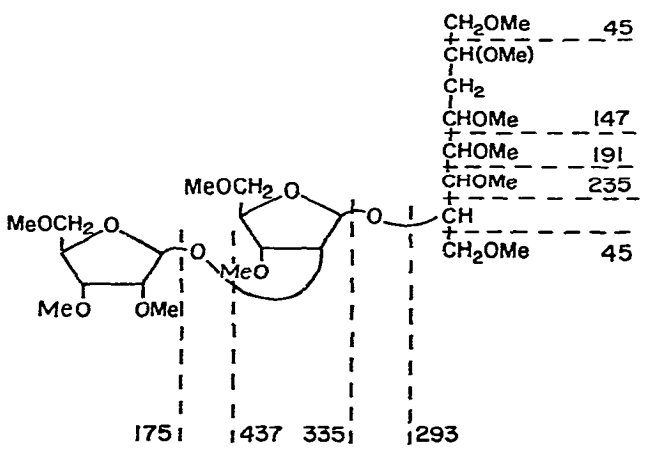

Fig. 1. Reduced and methylated oligosaccharide obtained from the K6 antigen by mild acid hydrolysis, with some primary mass fragments.

performed by ^{13}C-n.m.r. spectroscopy. The ^{13}C-n.m.r. spectrum of **1** is shown in Fig. 2 and some of the assigned chemical shifts are also listed in Table I. These assignments are based on previous assignments made on the model compounds methyl α- and β-D-ribofuranosides[12], and the spectrum is only consistent with both ribofuranosyl residues being in the β-D configuration. The low-field signal at 109.0 p.p.m. was assigned to the anomeric carbon atom of the nonreducing end-group β-D-ribofuranosyl group (1A) and the signal at 106.1 p.p.m. was assigned to the anomeric carbon atom of the internal β-D-ribofuranosyl residue (1B). The substantially upfield position of this latter signal in comparison with that of 1A is consistent with the 2-linkage (2B) being responsible for a characteristic, upfield displacement[5,6] (2–3 p.p.m.) on the vicinal anomeric carbon (1B). Substantiating evidence for the β-D configuration of both ribofuranosyl residues may be obtained from the signals at 84.1 and 83.8 p.p.m. On the basis[12] of model studies, these signals may be assigned to 4A and 4B, respectively. The 4-carbon is the lowest-field signal of the ribofuranosyl ring in either configuration, and, because of the sensitivity of this carbon atom to changes in anomeric configuration, the 4-carbon of the α-D anomer being the most deshielded, the assignment of the α-D configuration to either of the ribofuranosyl residues would have required the presence of a signal at ~85.5 p.p.m. in the ^{13}C-n.m.r. spectrum of **1**. Confirmation of the foregoing carbon assignments was obtained by the periodate oxidation and reduction ($NaBH_4$) of **1**. In this oxidation, the ring of the end-

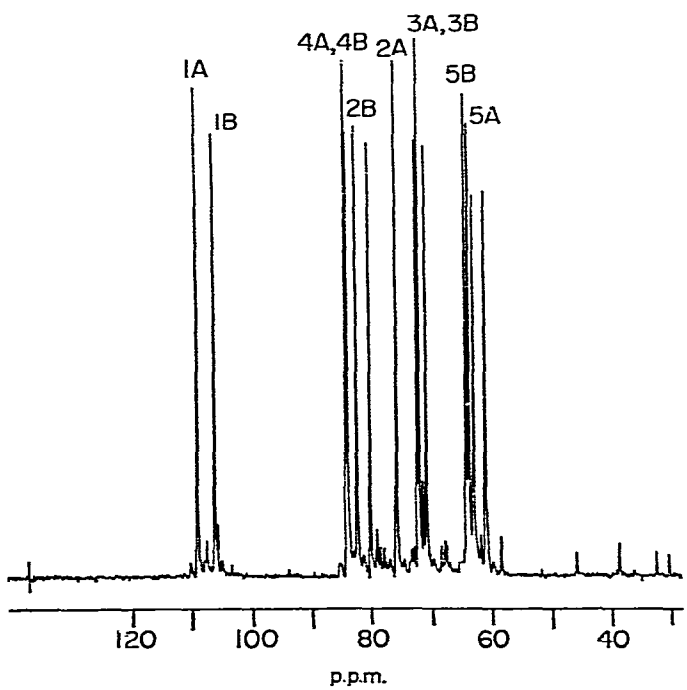

Fig. 2. Fourier-transformed ^{13}C-n.m.r. spectrum (20 MHz) of **1** taken with an acquisition time of 0.5 s and spectral width of 5 kHz. The number of free-induction decays was 104,000.

TABLE I

SOME CHEMICAL SHIFTS OF THE NATIVE AND MODIFIED *E. coli* K6 ANTIGEN AND DERIVED OLIGOSACCHARIDES

Compound	^{13}C-*chemical shift (p.p.m.) of*										
	1C	*1A*	*1B*	*2C*	*4A*	*4B*	*2A*	*2B*	*7C*	*6C*	*3C*
Oligosaccharide **1**		109.0	106.1		84.1[a]	83.8[a]	75.6	82.3[a]			
Periodate-oxidized and reduced **1**		104.4	106.6		NA[b]	83.8	NA[b]	NA[b]			
Native K6 antigen	176.1[c]	107.9[d]	104.8[e]	100.9	85.0[a]	84.0[a]	77.1[a]	83.1[a]	75.6[a]	71.6[a]	35.5
Periodate-oxidized and reduced K6 antigen	175.3	108.0	106.2	105.5	85.2[a]	84.0[a]	79.7[a]	82.6[a]	75.8[a]	NA[b]	38.3
KDO moiety of *N. meningitidis* 29e polysaccharide[f]	174.5[g]			103.9					78.9	73.3	36.1

[a]Tentative assignments. [b]NA Not assigned. [c]$^3J_{C\text{-}1,H\text{-}3a} \sim 9$ Hz (coupling measured at half-height of signal; width of signal 6 Hz in decoupled spectrum). [d]$^2J_{C\text{-}1,H\text{-}1} = 177$ Hz. [e]$^2J_{C\text{-}1,H\text{-}1} = 179$ Hz. [f]Values taken from ref. 3. [g]$^3J_{C\text{-}1,H\text{-}3a} \sim 12$ Hz (coupling measured at half-height of signal; width of signal 6 Hz in decoupled spectrum).

group ribofuranosyl group (A) is cleaved, causing a major chemical shift in the 1A and 4A signals, whereas the 1B and 4B signals remained virtually unaltered (Table I). At the same time, the erythritol residue of **1** is converted into a glycerol residue. Unfortunately, the anomeric configuration of the internal ribofuranosyl residue (B) could not be unambiguously obtained by the hydrolysis of the foregoing, intermediate Smith-degradation[11] product because, even under the mildest conditions of hydrolysis, both the 2-substituent and the glycerol aglycon were simultaneously removed from the intermediate. This result is in contrast to the relative acid stability of the β-D-glycosidic bonds of the ribofuranosyl residues of the intermediate Smith-degradation product of the K6 antigen. On the basis of model studies[12] and making an allowance for the linkage-effect[5,6], the signals at 82.3 and 75.6 p.p.m. were assigned to 2B and 2A, respectively. The foregoing evidence is in complete accord with the structure of the trisaccharide repeating-unit proposed by Messner and Unger[7] for the K6 antigen, but the fact that we were able to isolate **1** from the Smith degradation[11] of the K6 antigen plus additional evidence (see later) means that we differ markedly with their proposal[7] as to how this repeating unit is incorporated in the K6 antigen.

Structure of the K6 polysaccharide antigen. — Methylation of the K6 antigen, followed by hydrolysis, and conversion of the products into their alditol acetates yielded only one major, g.l.c.-detectable methylated component (3,5-di-*O*-methyl-D-ribitol), which was identified by g.l.c.–m.s. Because of the importance of this determination to the postulation of the final structure of the K6 antigen, the homogeneity of the peak given by this derivative in the gas chromatograph was checked by multiple-m.s. scanning. No 2,5-di-*O*-methyl-D-ribitol derivative could be detected. This result suggests that the K6 antigen has essentially a linear structure [→2-β-Rib*f*-(1→2)-β-Rib*f*-(1→7)-KDO] and not the branched structure as proposed by Messner and Unger[7]. This proposal was also confirmed by ^{13}C-n.m.r. spectroscopy on the native and periodate-oxidized, native K6 antigen.

^{13}C-N.m.r. spectroscopy. — The ^{13}C-n.m.r. spectrum of the native K6 polysaccharide is shown in Fig. 3 and is consistent with the K6 antigen having a structure based essentially on repeating unit **2**. The signal of low intensity at 21.6 p.p.m. is indicative[3,5,6] of the presence of an additional small quantity of *O*-acetyl substituents (~0.2–0.3 mol of *O*-acetyl per KDO residue) in the native K6 antigen, the exact location of which we were unable to determine from the ^{13}C-n.m.r. spectrum of the *O*-deacetylated K6 antigen. The assignment of other resonances important to the structural elucidation of the K6 antigen are also listed in Table I, and some of these signals have been previously assigned[8]. The three sharp, anomeric signals, compatible with repeating unit **2**, at 107.9, 104.8, and 100.9 p.p.m., were assigned to residues A, B, and C in **2**, respectively. Periodate oxidation of the *O*-deacetylated K6 antigen with subsequent reduction of the oxidized product yielded a polymeric material which gave an eighteen-signal ^{13}C-n.m.r. spectrum corresponding to the eighteen original carbon atoms of **2**. The spectrum exhibited three anomeric carbon atoms at 108.0, 106.2, and 105.5 p.p.m. (Table I) and also five hydroxymethyl signals at 63.8, 62.9, 62.2, 61.6, and 58.4 p.p.m.; there were two additional hydroxymethyl signals to those

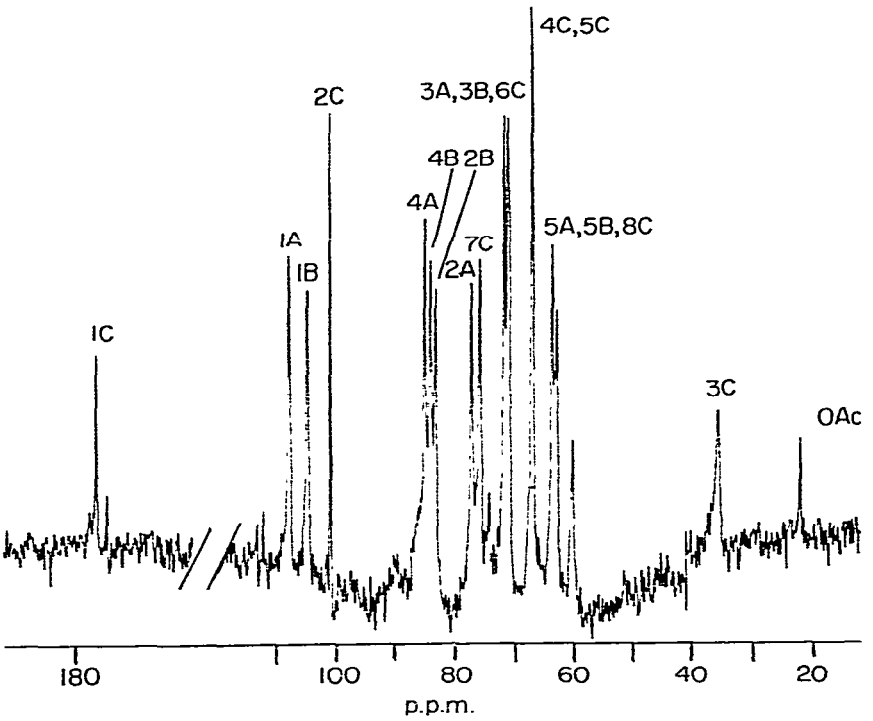

Fig. 3. Fourier-transformed, ^{13}C-n.m.r. spectrum (20 MHz) of the native K6 antigen taken with an acquisition time of 0.5 s and spectral width 5 kHz. The number of free-induction decays was 114,000.

exhibited in the spectrum of the native K6 antigen, at 63.7, 63.3, and 62.9 p.p.m. These two additional signals result from the oxidation of only one pair of vicinal hydroxyl groups in the original K6 antigen, which is consistent with the eventual formation of **1** as the final Smith-degradation[11] product. This evidence is entirely in accord with the K6 antigen having structure **2**, which is different from the structure proposed by Messner and Unger[7].

Compared with **1**, the anomeric-signal residue (A) of the K6 antigen is predictably[5,6] displaced upfield by 1.1 p.p.m. because of the effect of the 2-linkage. Interestingly, when compared to the anomeric carbon signals of the model methyl α- and β-D-ribofuranosides[12] (109.0 and 104.2 p.p.m.), 1B has a chemical shift more compatible with residue B having the α-D configuration. However we must adhere to our original assignment of the β-D configuration to this same residue (B) in **1** and thus attribute this unusual shielding of the 1B signal in the K6 antigen to an aglycon effect. This effect is probably associated with the ring structure of the KDO residue, because when the native K6 antigen is subjected to Smith degradation, the ring structure of this residue (C) is broken and 1B of the intermediate, reduced product has a chemical shift (106.2 p.p.m.) almost identical to that of 1B in **1** (106.1 p.p.m.). Another anomalous chemical shift is also found in the signal of 2A of the K6 antigen, which again could be due to the same type of shielding. A substantial downfield

displacement in this signal as compared with 2A of **1** when 2A becomes a linkage point in the K6 antigen would normally be anticipated[5,6]. Based on model studies[12], the equivalent 2B signal undergoes a downfield displacement of +7.8 p.p.m. which, if representative of a 2-linkage, should result in the appearance of an additional downfield signal (2A) at approximately 83.4 p.p.m. in the ^{13}C-n.m.r. spectrum of the K6 antigen. However the lowest-field signal other than those previously assigned to 4A, 4B, and 2B of **2** is at 77.1 p.p.m., which represents a downfield displacement of only +1.5 p.p.m. in 2A when compared with 2A of **1**. On the basis of this result, the possibility of a 3-linkage to residue *A* of the K6 antigen might be considered, but the methylation analysis and other ^{13}C-n.m.r. evidence (see later) are consistent only with a 2-linkage. These results emphasize the care that must be exercised in making assignments based solely on the chemical shifts of individual carbon signals.

The remaining feature of the K6 antigen to be assigned is the anomeric configuration of the KDO residue (C). Unger *et al.*[8], have remained noncommital on this assignment, but our cumulative evidence would suggest that this residue has the α-D configuration. Although on the basis of previous anomeric carbon assignments[12] for the methyl α-D-(101.7 p.p.m.)* and methyl β-D-(102.4 p.p.m.) glycopyranosides of KDO, it was not possible to make an assignment, it could be accomplished by using the characteristic chemical shift of the 1C signal of **2** at 176.1 p.p.m. Previous studies[3] have indicated that this chemical shift is characteristic of KDO in the α-D configuration (with an equatorially oriented carboxylate group) (**2**). This general rule was first developed for the sterically related, sialic acid residues[13] and was used to assign[3] the β-D configuration to the KDO residues of the meningococcal group 29e polysaccharide. The validity of the assignment of the α-D configuration to the KDO residues of the K6 antigen by this rule is also supported by other n.m.r. evidence.

The $^{3}J_{C-1,H-3a}$ coupling constant of the KDO residues of the K6 antigen is much smaller than that of the KDO residues of the 29e polysaccharide. Although these coupling constants could not be measured directly from the proton-coupled ^{13}C-n.m.r. spectra of the two polysaccharides, because of signal broadening, this difference could be deduced from the increased line-width (measured at the half-height) of the 1C signal of the 29e polysaccharide (12 Hz) over that of the equivalent signal of the K6 antigen (9 Hz); the line width of the 1C signals of both polysaccharides in their proton-decoupled spectra being identical ($\Delta\nu$ = 6 Hz). This difference (3 Hz) is attributable to the fact that the KDO residues of the 29e polysaccharide are in the β-D configuration[3] and therefore 1C is *trans*-coupled to H-3a (J_t), whereas the KDO residues of the K6 antigen are in the opposite configuration (α-D) and therefore 1C is *gauche*-coupled to H-3a (J_g) in this case. Using values for J_g and J_t (1 and 4 Hz, respectively) obtained from the proton-coupled ^{13}C-n.m.r. spectra of the respective methyl α- and β-D-glycosides of KDO[15], and assuming the α-D configuration of the KDO residues in the K6 antigen, the calculated line-widths for 1C in the proton-coupled spectra of the polysaccharides are 11 Hz ($\Delta\nu + J_t + J_g$) for the 29e poly-

*Corrected from the previous assignment in ref. 3.

TABLE II

CHEMICAL SHIFTS OF THE H-3e AND H-3a PROTONS OF THE KDO MOIETIES OF THE *E. coli* K6 ANTIGEN AND *N. meningitidis* 29e ANTIGEN

KDO moiety of	*Chemical shift (p.p.m.)*	
	H-3e	*H-3a*
Native K6 antigen	2.23	1.84
Native 29e antigen[a]	2.44	1.83
Methyl α-D-KDO[a]	2.13	1.79
Methyl β-D-KDO[a]	2.40	1.75

[a]Prepared as described in ref. 3.

saccharide and 8 Hz ($\Delta\nu + 2J_g$) for the K6 antigen. These values compare favorably with the observed values in the proton-coupled, ^{13}C-n.m.r. spectrum of the 29e and K6 polysaccharides (12 and 9 Hz, respectively).

It has been demonstrated by ^{1}H-n.m.r. spectroscopy that the chemical shift of the equatorial, geminal proton (H-3e) signals of sialic acid[16] and KDO[17] are sensitive to change in anomeric configuration. The chemical shifts of the H-3e and H-3a signals associated with the KDO residues of the K6 antigen and meningococcal group 29e polysaccharide are listed in Table II, and the difference between the H-3e signals (0.21 p.p.m.) is of sufficient magnitude, as demonstrated by the differences in the chemical shifts of the equivalent H-3e signals of the methyl α-D and β-D anomers of KDO (0.27 p.p.m.), to indicate that they exist in different anomeric configurations. The actual values of these chemical shifts are also consistent with the assignment of the α-D configuration to the KDO residues of the K6 antigen and the β-D configuration to those of the 29e polysaccharide, based on their close similarity with the equivalent chemical shifts exhibited in the ^{1}H-n.m.r. spectra of the respective methyl α-D- and β-D-glycosides of KDO (Table II).

Whereas the assignment of the α-D configuration to the KDO residues of the K6 antigen is not yet definitive, the foregoing cumulative evidence is highly supportive of this assignment. This assignment means that KDO probably occurs in bacterial polysaccharides in both anomeric configurations, whereas the sterically related sialic acid has been found only to occur in one (α-D anomer).

CONCLUSION

We propose that the structure of the *Escherichia coli* LP 1092 (K6) antigen is an essentially linear repeating-unit of →2)-β-D-Rib*f*-(1→2)-β-D-Rib*f*-(1→7)-α-D-KDO-(2→, with approximately one *O*-acetyl substituent per five repeating units. A different, branched structure has also been proposed[7] for this antigen, which could be attributed to strain difference or of phenotypic variation in the organism.

EXPERIMENTAL

Growth of the organism. — The *Escherichia coli* LP 1092 (K6) organisms were kindly supplied by Dr. P. W. Taylor of the School of Medicine, the University of Leeds, England, and were grown in a fully synthetic mineral salt–citric acid–D-glucose (0.5% wt/vol) medium[18] adjusted to pH 7.5 prior to sterilization. A 150-L fermenter containing 130 L of this medium was inoculated with 10 L of an 18-h culture of *E. coli* LP 1092 grown in the same medium. During growth at 37°, agitation and aeration were maintained at 175 r.p.m. and 130 $L.min^{-1}$, respectively. The culture pH was kept constant at pH 7.5 throughout growth by the automatic addition of 5M potassium hydroxide or 6M sulfuric acid. The cells were harvested by centrifugation near the end of the exponential-growth phase (7–8 h).

Isolation of the K6 polysaccharide. — The polysaccharide was isolated by a modified phenol-extraction procedure[19] in which the organisms were disrupted with glass beads in an Eppenbach colloid mill for 20 min at 4° prior to phenol extraction. The pooled, aqueous layers from the phenol extraction were dialyzed against water and lyophilized. The crude polysaccharide was then suspended at 40 mg/mL in 10mM sodium phosphate buffer (pH 7.0) containing 1mM magnesium chloride and treated with RNase and DNase at a final concentration of 50 μg/mL for 1 h at 37°. Following nuclease digestion, the suspension was dialyzed against distilled water and the dialyzate was ultracentrifuged at 105,000*g* for 18 h to sediment the lipopolysaccharide. The polysaccharide was then precipitated from the supernatant liquid by the addition of Cetavlon (to a final concentration of 0.1%) as described by Gotschlich *et al.*[20], and the supernatant liquid was decanted from the sticky, Cetavlon–polysaccharide complex. This complex was dissolved in 0.9M calcium chloride and the crude polysaccharide precipitated with ethanol. The precipitate was obtained by low-speed centrifugation and was redissolved in water and lyophilized. The polysaccharide had u.v. absorption at 260 nm and was purified by ion-exchange chromatography.

The native K6 polysaccharide was applied to a column (15 × 2 cm) of DEAE-Sephadex A-25 pre-equilibrated with 0.1M Tris buffer (pH 8.5), and the column was washed with the same buffer. The acidic polysaccharide was eluted in a linear gradient of 0–0.6M sodium chloride in the foregoing buffer, and the eluate was monitored by using the phenol–sulfuric acid method. The K6 polysaccharide was eluted as a single component at a gradient strength of 0.3M sodium chloride, and was recovered by dialysis and lyophilization. Although passage of the K6 polysaccharide through the DEAE-Sephadex column considerably decreased its u.v. absorption at 264 nm, the absorption could not be completely eradicated, even after repeated passage through the same column. The native K6 antigen was *O*-deacetylated as previously described[3].

Analytical methods. — Solutions were concentrated under diminished pressure <40°. Quantification of KDO was performed by the method of Weisbach and Hurwitz[21] and of ribose by the phenol–sulfuric acid method[22].

G.l.c. was performed with a Hewlett–Packard 5830A instrument equipped with

a flame-ionization detector and a model 18850A electronic integrator. The glass columns (180 × 0.15 cm) used contained the following liquid phases on Gas-Chrom Q: (*i*) 3% ECNSS-M at 190° (alditol acetates) and 170° (partially methylated alditol acetates, and (*ii*) 3% OV-1 at 230° (methylated oligosaccharides). Glass-capillary chromatography was performed by using SP-1000 W.C.O.T. columns. Combined g.l.c.–m.s. was effected with a Finnegan 3100D instrument with columns (*i*) and (*ii*) and an ionization potential of 70 eV.

A Bausch and Lomb (Spectronic 200 u.v.) scanning spectrophotometer was used for u.v. measurements made on the K6 antigen (concentration 100 μg/mL).

^{13}C-N.m.r. spectra were recorded (in 10-mm tubes) at 37° with a Varian CFT 20 spectrometer operating at 20 MHz in the pulsed, Fourier-transform mode and with complete proton decoupling. Chemical shifts are reported in p.p.m. downfield from internal tetramethylsilane, and the ^{2}H resonance of deuterium oxide was used as a field–frequency lock-signal. The polysaccharide and oligosaccharides were examined as deuterium oxide solutions at concentrations of 25–80 mg/mL. Proton-coupled spectra were obtained with a Bruker WH400 instrument (Regional n.m.r. Facility, University of Montreal, Montreal, Quebec). The gated spectrum was obtained with a delay of 5 s between measurements.

^{1}H-N.m.r. spectra were recorded with Varian CFT 20 and Bruker WH 400 spectrometers operated at 80 and 400 MHz, respectively, at 85°, both in the pulsed Fourier-transform mode, the former with a 30° pulse-angle and an acquisition time of 1.5 s, and the latter with a spectral width of 2 kHz and a 90° pulse angle with a 3-2 pulse delay. These parameters ensured that saturation of the resonances was avoided. Samples were lyophilized (twice) from 99.7% deuterium oxide and examined in the same solvent, and the chemical shifts are reported in p.p.m. downfield from internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate.

Methylation analysis. — The native K6 polysaccharide and related oligosaccharides were methylated according to the Hakomori procedure[23]. The methylated oligosaccharides were analyzed directly by g.l.c.–m.s. with column (*ii*) and also, as for the methylated polysaccharide were successively hydrolyzed, reduced ($NaBH_4$), and acetylated as previously described[24]. The partially methylated alditol acetates were then analyzed[25] by g.l.c.–m.s. with column (*i*).

Periodate oxidation of the K6 antigen. — The *O*-deacetylated native K6 polysaccharide (1 g) was dissolved in 0.1M sodium metaperiodate (200 mL) and the solution was kept in the dark for 16 h. The excess of periodate was decomposed by the addition of ethylene glycol, and the solution was dialyzed against water for 24 h. The solution was then concentrated to 25 mL before the addition of sodium borohydride (0.5 g). After 4 h, the solution was made acidic (pH 4.5) with acetic acid, dialyzed, and lyophilized to yield an intermediate, polymeric product of the Smith degradation[11] (0.8 g). The polymeric material (0.5 g) was then hydrolyzed with 2% acetic acid for 3 h at 100° and the solution was lyophilized. The residue was redissolved in water (1 mL) and fractionated on a column (80 × 4 cm) of Bio-gel P2, which was eluted with water and monitored by using a Waters model R403 differential

refractometer. The elution profile indicated the formation of one major product, which was recovered by lyophilization and shown to be **1** (0.4 g).

An attempt was made to conduct a further Smith degradation[11] of **1** (0.2 g), and the mixture was applied directly to a column of Sephadex G-25 eluted with water. The partially resolved, higher molecular-weight fraction was then further fractionated by passage through a column of Bio-gel P2. The polyaldehyde intermediate was reduced with sodium borohydride, made neutral with Dowex 50 (H^+) ion-exchange resin at 4°, and filtered. The solution was lyophilized, methanol was distilled six times from the residue, and the product was refractionated on the column of Bio-gel P2 to yield the polyalcohol intermediate (80 mg). Hydrolysis of this intermediate to form the ribofuranosyl-glycerol Smith-degration[11] product proved to be impossible. All hydrolytic conditions that removed the glyceraldehyde residue from O-2 of the ribofuranosyl residue also simultaneously cleaved the glycerol aglycon.

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