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INVESTIGATION OF THE STRUCTURE OF THE CAPSULAR POLYSACCHARIDE OF *Escherichia coli* K55 USING *Klebsiella* BACTERIOPHAGE ϕ5*

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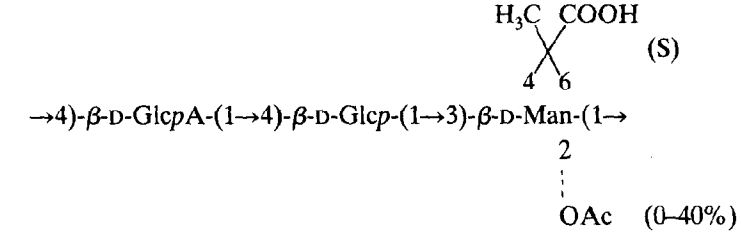
ABSTRACT

The structure of the capsular polysaccharide from *Escherichia coli* O9:K55 (N 24c) has been studied, using methylation analysis, degradation by bacteriophage, and n.m.r. spectroscopy. Depolymerisation of the K55 polysaccharide, using the lyase enzyme borne by *Klebsiella* ϕ5, yielded a tri- and a hexa-saccharide, analysis of which indicated the following repeating unit.



This structure differs from that for the repeating unit of the capsular polysaccharide of *Klebsiella* K5 only in the position of acetylation (position 2 of the glucose residue).

INTRODUCTION

Degradation by bacteriophages[1] is now an established technique in the elucidation of the structure of the capsular polysaccharides of enterobacteria. The technique has been applied[2–7] to the K-antigens of *E. coli*, using bacteriophage enzymes with endoglycanase activity. Bacteriophage enzymes with lyase activity have been reported in studies of *Klebsiella* capsular polysaccharides[8–10], but similar enzyme activity has not been reported for the *E. coli* series. We now report the

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elucidation of the structure of the repeating unit of the capsular polysaccharide of *E. coli* K55 by degradation with the lyase[8] borne by *Klebsiella* bacteriophage $\phi 5$.

RESULTS AND DISCUSSION

Composition of the K55 polysaccharide. — The acidic polysaccharide was isolated by precipitation with cetyltrimethylammonium bromide (CTAB) from five batches of *E. coli* K55 bacteria grown on Mueller–Hinton agar. The isolates formed viscous aqueous solutions and were polydisperse in gel-permeation chromatography (g.p.c.) with M_r 3.7×10^4–1.4×10^7. Treatment of the isolate with Amberlite IR-120 (H^+) resin did not alter the molecular mass range significantly, as occurs with the polysaccharides of *E. coli* K37[11] and *Klebsiella* K39[12]. Sugar analysis of the isolates from each batch (Table I), after carboxyl reduction, showed that the amount of Gal present decreased as the time of incubation for growth was decreased, although the ratio of Man–Glc remained reasonably constant. These results suggested that Gal was not part of the primary structure of the K55 capsular polysaccharide and was part of a separate galactan. These assumptions were borne out by the results of the study of the degradation effected by the bacteriophage reported below. The production of a Gal-rich polysaccharide has been reported[12] in the study of the capsular polysaccharide of *Klebsiella* K39.

The isolates from batches 3–5 were designated as the K55 polysaccharide. Sugar analyses of the K55 polysaccharide (Table II, columns I and II), with and without prior carboxyl reduction, indicated the presence of equimolar amounts of Man, Glc, and GlcA. The sugar residues were shown to be D by g.l.c. of the acetylated (−)-2-octyl glycosides prepared from an acid hydrolysate of the polysaccharide, with and without prior carboxyl reduction. The ^{1}H-n.m.r. spectrum of K55 polysaccharide from batch 3 contained H-1 signals for β linkages at δ 4.55 ($J_{1,2}$ 7.0 Hz), 4.67 ($J_{1,2}$ 8.0 Hz), and 4.72 (unresolved) (Table III), and a signal for the methyl group of a pyruvate acetal (δ 1.47). The ^{13}C-n.m.r. data accorded with the ^{1}H-n.m.r. data. The K55 polysaccharide from batch 5 was shown by ^{1}H-n.m.r. spectroscopy to be ~40% *O*-acetylated (signal at δ 2.18, 1.2 H) and gave a complex

TABLE I

SUGAR ANALYSES OF *E. coli* K55 POLYSACCHARIDE BATCHES 1–5

Sugar (as acetylated aldononitrile)	*Analysis (%)*[a,b]				
	I (84)[c]	*II (48)*	*III (36)*	*IV (36)*	*V (36)*
Man	29	31	32	32	32
Glc	38	52	64	55	57
Gal	33	16	3	2	0

[a]Determined on a capillary DB-225 column at 225°. [b]I–V, carboxyl-reduced polysaccharides from batches 1–5, respectively. [c]Figures in parentheses represent the incubation time (h) for each batch.

spectrum which was assigned on the basis of the resonances of the oligosaccharides (see below).

Methylation analysis. — Data for the methylation analyses of K55 polysaccharide, given in Table IV (columns I and II), show the presence of 4-linked Glc, trisubstituted Man, and, after carboxyl reduction, 4-linked GlcA. Methylation analysis of a depyruvylated sample of the K55 polysaccharide gave 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylmannitol, indicating that the pyruvate group was attached to positions 4 and 6 of Man (column III). The above results, in conjunction with the n.m.r. data, suggested the K55 polysaccharide to be linear and composed of pyruvylated trisaccharide repeating-units containing various amounts of *O*-acetyl groups. It was assumed, from the low molar yield of the 2,3,6-tri-*O*-methylglucitol derivative in the methylation analysis of K55 polysaccharide (column I), that GlcA was linked to Glc. This assumption suggested that the structure of the K55 polysaccharide was similar to that of the capsular polysaccharide[14] of *Klebsiella* K5.

Degradation by bacteriophage. — Depolymerisation of the capsular polysaccharide of *Klebsiella* K5 by *Klebsiella* bacteriophage $\phi 5$ established[8] that the bacteriophage carried a lyase enzyme. Preliminary experiments indicated that $\phi 5$ was capable of lysing *E. coli* K55 bacteria. Therefore, the isolate from batch 5 was degraded on a preparative scale (495 mg) with $\phi 5$ (titre, 1.5×10^{13} plaque forming units) and yielded two oligosaccharides, P1 (104.8 mg) and P2 (119.5 mg). G.p.c. indicated P1 to be a trisaccharide and P2 to be a hexasaccharide.

Sugar analysis of P1, P1-alditol, and carboxyl-reduced P1 (Table II, columns III–V) showed that P1 contained Glc and reducing Man, but no GlcA. A positive thiobarbituric acid test for P1 and P2 indicated that the 4-linked β-D-GlcA in the polysaccharide (negative thiobarbituric acid test) had been converted into a 4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid terminal group in P1 and P2 by elimination of the Man residue. Analysis of P1-alditol ($NaBD_4$ reduced; Table IV, column V), after Hakomori methylation[16,17], revealed 4-linked Glc and a derivative (3,4,6-tri-*O*-acetyl-1,3,5-tri-*O*-methylmannitol) arising from a 3-linked 4,6-pyruvylated Man. Incomplete reduction of P1 resulted in the formation of a small amount

TABLE II

SUGAR ANALYSES OF *E. coli* K55 POLYSACCHARIDE AND DERIVED PRODUCTS

Sugar (as peracetylated aldononitrile)	*Molar ratio*[a,b]						
	I[c]	*II*[c]	*III*	*IV*	*V*	*VI*	*VII*
Man	1.00	1.00	1.00		1.00	1.00	1.00
Glc	0.49	1.78	1.00	1.00	1.06	0.81	2.93
Man-ol[d]				0.92			1.35

[a]Determined on a capillary DB-225 column at 225°. [b]I, K55 polysaccharide; II, carboxyl-reduced K55 polysaccharide; III, P1; IV, P1-alditol; V, carboxyl-reduced P1; VI, P2; and VII, carboxyl-reduced P2-alditol. [c]Batch 5 used. [d]Mannitol hexa-acetate.

TABLE III

N.M.R. DATA (500 MHz) FOR *E. coli* K55 POLYSACCHARIDE AND DERIVED PRODUCTS

^{1}H-N.m.r. data				*^{13}C-N.m.r. data*	
δ[a] (p.p.m.)	*$J_{1,2}$ (Hz)*	*Integral (no. of H)*	*Assignment[c]*	*δ[d] (p.p.m.)*	*Assignments[c]*
K55 polysaccharide, batch 3[f]					
4)-β-GlcA-(1→4)-β-Glc-(1→3)-4,6-pyr-β-Man-(1→					
4.72	n.o.[b]	1.0	3-β-Man	176.11	C-3 of pyruvate
4.67	8.0	1.0	4-β-Glc	175.62	C-6 of 4-β-GlcA
4.55	7.0	1.0	4-β-GlcA	103.12	4-β-GlcA
1.47		3.0	Me of pyruvate	102.74	4-β-Glc
				100.94	C-2 of pyruvate
				99.99	3-β-Man
				60.94	C-6 of 4-β-Glc
				25.48	C-1 of pyruvate
K55 polysaccharide, batch 5					
4)-β-GlcA-(1→4)-β-Glc-(1→3)-2-OAc-4,6-pyr-β-Man-(1→					
5.60	n.o.	0.4	H-2 of 3-β-Man(2OAc)		
4.88	n.o.	0.4	3-β-Man(2OAc)		
4.72	n.o.	0.6	3-β-Man		
4.66	7.0	0.6	4-β-Glc-(1→3)-β-Man		
4.59	n.o.	0.4	4-β-Glc-(1→3)-β-Man(2OAc)		
4.55	6.0	0.6	4-β-GlcA-→3)-β-Man		
4.51	n.o.	0.4	4-β-GlcA-→3)-β-Man(2OAc)		
2.18		1.2	Me of acetate		
1.48		3.0	Me of pyruvate		
α-Hex-4-enA-(1→4)-β-Glc-(1→3)-2-OAc(70%)-4,6-pyr-Man (P1)					
6.21	3.5	1.0	H-4 of Hex-4-enA	174.02	CO of acetate
5.64	3.2	0.3	H-2 of 3-β-Man(2OAc)	173.63	C-3 of pyruvate
5.36		0.4	3-α-Man(2OAc)	173.59	Man α,β
5.23			α-Hex-4-enA	165.92	C-6 of Hex-4-enA
to		1.6	H-2 of 3-α-Man(2OAc)	141.19	C-5 of Hex-4-enA
5.22			3-α-Man	112.92	C-4 of Hex-4-enA

5.11	n.o.	0.3	3-β-Man(2OAc)	101.29	α-Hex-4-enA
4.96	n.o.	0.1	3-β-Man	100.91	C-2 of pyruvate
4.65	8.0	0.1	4-β-Glc-(1→3)-β-Man	100.11	4-β-Glc-(1→3)-α,β-Man
4.64	8.0	0.2	4-β-Glc-(1→3)-α-Man	99.87	
4.57	8.0	0.4	4-β-Glc-(1→3)-α-Man(2OAc)	99.49	4-β-Glc-(1→3)-α,β-Man(2OAc)
4.56	8.0	0.3	4-β-Glc-(1→3)-β-Man(2OAc)	99.00	
4.47	3.3 9.9	0.4	H-3 of 3-α-Man(2OAc)	95.60	3-α-Man
4.38	3.3 9.9	0.3	H-3 of 3-β-Man(2OAc)	95.05	3-β-Man
4.25		1.0	H-3 of Hex-4-enA	93.95	3-α-Man(2OAc)
4.24		0.1	H-2 of 3-β-Man	93.37	3-β-Man(2OAc)
4.21		0.2	H-2 of 3-α-Man	60.84	C-6 of 4-β-Glc-Man(2OAc)
3.75		1.0	H-2 of Hex-4-enA	60.79	C-6 of 4-β-Glc
2.18		0.8	Me of acetate	25.35	Me of pyruvate
2.15		1.3	3-α,β-Man	21.10	Me of acetate
1.59		1.4	Me of pyruvate		
1.58		1.6	3-α,β-Man		
α-Hex-4-enA-(1→4)-β-Glc-(1→3)-4,6-pyr-Man (O-deacetylated P1)					
6.2	3.9	1.0	H-4 of Hex-4-enA	173.86	C-3 of pyruvate
5.23	5.2	1.0	α-Hex-4-enA	173.79	Man α,β
5.22	n.o.	0.7	3-α-Man	166.06	C-6 of Hex-4-enA
4.96	n.o.	0.3	3-β-Man	141.31	C-5 of Hex-4-enA
				112.79	C-4 of Hex-4-enA
4.65	8.0	0.3	4-β-Glc-(1→3)-β-Man	101.29	α-Hex-4-enA
4.64	8.0	0.7	4-β-Glc-(1→3)-α-Man	101.08	C-2 of pyruvate
4.25		1.0	H-3 of Hex-4-enA	100.14	4-β-Glc-(1→3)-α,β-Man
1.57		2.1	Me of pyruvate	99.89	
1.54		0.9	3-α,β-Man	95.60	3-α-Man
				95.05	3-β-Man
				60.87	C-6 of 4-β-Glc
				25.36	Me of pyruvate

[a]Measured with reference to internal acetone, δ 2.23. [b]Not observed. [c]4-β-Glc refers to H-1 of a 4-linked β-glucopyranosyl residue. [d]As in *a* with acetone 31.07 p.p.m. [e]As in *c*, but for anomeric ^{13}C nuclei. [f]For origin of compounds, see text.

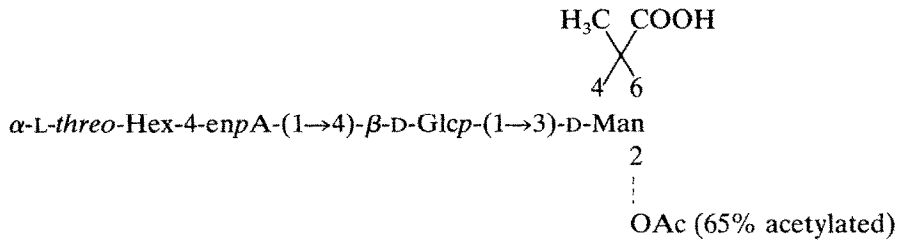
TABLE IV

METHYLATION ANALYSIS OF *E. coli* K55 POLYSACCHARIDE AND DERIVED PRODUCTS

Methylated sugar[a] *(as alditol acetate)*	T_R[b]	*Molar ratio*[c,d]					
		I	*II*	*III*	*IV*	*V*	*VI*
1,2,5-Man	1.45					0.67	0.73
2,4,6-Man	1.57			0.65			0.12
2,3,6-Glc	1.82	1.00	1.00	1.00	1.00	1.00	1.00
2,3-Glc	3.08		1.03				0.90
2-Man	3.81	2.65	1.27	0.30	0.51	0.16	0.71
Man	4.35				0.88		

[a]1,2,5-Man = 3,4,6-tri-*O*-acetyl-1,2,5-tri-*O*-methylmannitol, *etc.*; all substitution patterns were confirmed by g.l.c.–m.s. [b]Retention time relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol. [c]Determined on a capillary DB-225 column at 205°. [d]I, K55 polysaccharide; II, carboxyl-reduced K55 polysaccharide; III, depyruvylated K55 polysaccharide; IV, P1; V, P1-alditol; and VI, carboxyl-reduced P2-alditol.

of the 2-*O*-methylmannitol derivative. Methylation of P1 by the Prehm method[18] and subsequent analysis (Table IV, column IV) located the acetyl group at position 2 of 65% of the Man (from the identification of mannitol hexa-acetate). The n.m.r. data for P1 accorded with the above results (see below). Therefore, the following structure for P1 can be proposed.



N.m.r. data. — The 1D ^1H- and ^{13}C-n.m.r. spectra and a 2D ^1H–^{13}C shift-correlated (Hetcorr) spectrum for P1-alditol (acid form) gave the data in Table V. The ^1H resonances at δ 5.17, 3.87, 4.25, and 6.13 could be assigned readily to H-1/4 of the hex-4-enuronic acid residue (sugar C). These resonances correlated with signals at 101.35, 70.65, 66.82, and 112.25 p.p.m., respectively, in the Hetcorr spectrum. The signals for C-5 (141.92 p.p.m.) and C-6 (166.59 p.p.m.) were assigned from the 1D ^{13}C-n.m.r. spectrum. The ^1H and ^{13}C assignments and $^3J_{H,H}$ constants were in good agreement with those reported[19] for the α-L-*threo*-hex-4-enopyranosyluronic acid residue produced by the action of lyase on the *Klebsiella* K14 polysaccharide. The ^{13}C signals for the 4-linked Glc (Table V, sugar B) were assigned after consideration of published data[20]. Comparison of these assignments with those published for methyl β-D-glucopyranoside[21] shows a downfield shift (α-

effect) for the C-4 resonance (70.6 →79.72 p.p.m.) and upfield shifts (β-effects) for the signals of C-3 (76.8 →74.79 p.p.m.) and C-5 (76.8 →75.31 p.p.m.). The assignment of the ^{1}H resonances for sugar B were obtained from the Hetcorr spectrum. Thus, the six remaining ^{13}C signals arise from the mannitol residue (Table V, sugar A) which is 3-linked and carries the 4,6-acetal group. The resonances for C-1 (62.81 p.p.m.) and C-6 (67.39 p.p.m.) were assigned readily, since each correlated with two ^{1}H peaks in the Hetcorr spectrum. The signal to lower field was assigned to C-6 because of its linkage to pyruvate. The ^{13}C signal at 71.26 p.p.m. was assigned to

TABLE V

N.M.R. DATA (500 MHz) FOR P1-ALDITOL

δ (^{1}H)[a] *(p.p.m.)*	J *(Hz)*	*δ (^{13}C)*[b] *(p.p.m.)*	*Assignments*
P1-alditol[c]			
α-L-threo-*Hex-4-en*p*A-(1→4)-β-*D-*Glc*p-*(1→3)-4,6-pyr-*D-*mannitol*			
(C)	(B)	(A)	
Sugar A			
3.77	12.0	62.81	H/C 1′
3.94	2.7	62.81	H/C 1
4.01		71.26	H/C 2
4.06	1.6	76.80	H/C 3
3.76	8.3	77.34	H/C 4
4.08	2.0	60.77	H/C 5
3.52	12.5	67.39	H/C 6′
4.07		67.39	H/C 6
Sugar B			
4.52	8.0	103.89	H/C 1
3.44	9.2	73.87	H/C 2
3.65	9.0	74.79	H/C 3
3.70	9.1	79.72	H/C 4
3.61	2.7	75.31	H/C 5
3.76	12.2	61.46	H/C 6′
3.97		61.46	H/C 6
Sugar C			
5.17	5.3	101.35	H/C 1
3.87	4.4	70.65	H/C 2
4.25	3.8	66.82	H/C 3
6.13		112.25	H/C 4
		141.92	C-5
		166.59	C-6
Pyruvate acetal			
		175.51	C-3
		101.00	C-2
1.51		25.12	H/C Me

[a]Chemical shift relative to that of internal acetone, δ 2.23. [b]Chemical shift relative to that of internal acetone, 31.07 p.p.m. [c]For origin of compound, see text.

C-2 of the mannitol residue (72.2 p.p.m. for mannitol)[21] and appears upfield due to glycosylation at C-3 (β-effect). Of the three unassigned ^{13}C resonances, the two resonating downfield (76.80 and 77.34 p.p.m.) from those for mannitol[21] must be due to the carbons involved in glycosidic (C-3) and pyruvate (C-4) linkages. These resonances correlated with ^{1}H signals at δ 4.06 and 3.76, respectively. The ^{1}H resonance at δ 3.76 was assigned to H-4 on the basis of the $J_{4,5}$ value (8.3 Hz); H-4,5 are *trans*-diaxial and should have a coupling of 8–10 Hz. The resonances at δ 4.06 and 76.80 p.p.m. may be assigned to H-3 and C-3 of sugar A, respectively. The small value (1.6 Hz) of $J_{3,4}$ is consistent with H-3,4 being *gauche*. The remaining signals at δ 4.08 and 60.77 p.p.m. may be assigned to H-5 and C-5, respectively, of sugar A. The signal at 60.77 p.p.m. is to much higher field than that of C-5 of mannitol (72.2 p.p.m.)[21]. This is due, at least partially, to the dual β-effects exerted by the 4,6-acetal.

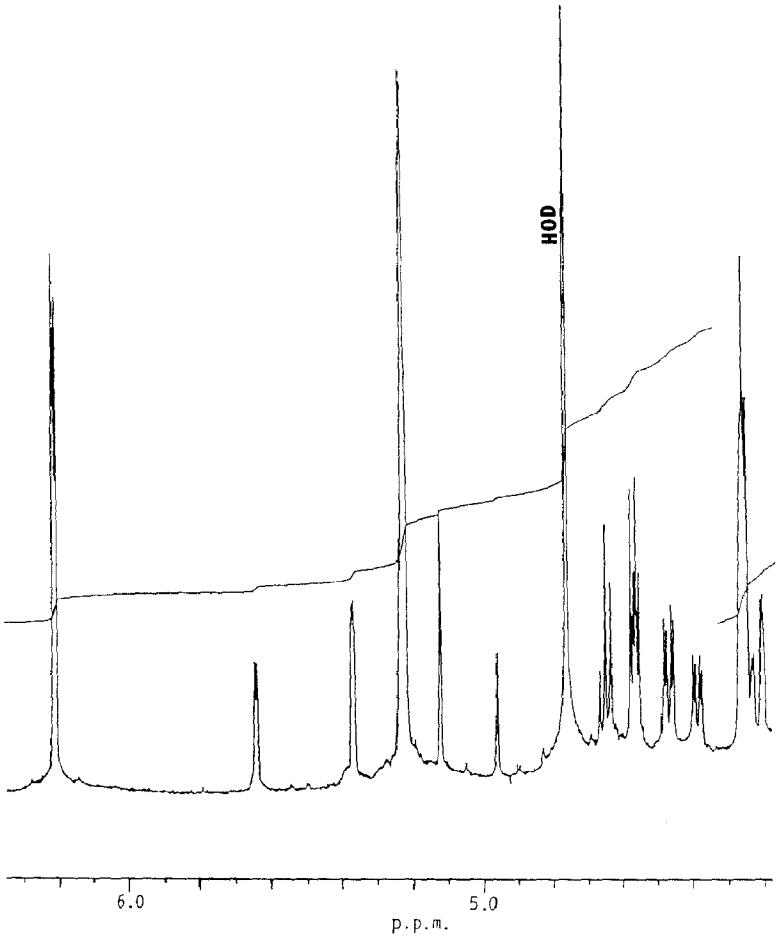


Fig. 1. ^{1}H-N.m.r. spectrum (region for anomeric protons) of P1.

The ^1H and ^{13}C signals for the pyruvate acetal (Table V) were assigned from the 1D spectra and establish[22,23] the *S* configuration for this group.

The n.m.r. data for P1 and *O*-deacetylated P1 are presented in Table III. The assignments of the ^1H and ^{13}C signals for *O*-deacetylated P1 follow from the assignments for P1-alditol. Due to the mutarotation of the reducing Man, the ^1H signal for the 4,6-pyruvate acetal and the signals for H-1 and C-1 of β-Glc are twinned. The ^1H- and ^{13}C-n.m.r. spectra of P1 are complex because ~70% of the molecules are *O*-acetylated. Fig. 1 shows the region δ 4.2–6.3 of the ^1H-n.m.r. spectrum of P1. The ^1H signals for the α and β anomers of the non-acetylated molecules in P1 were assigned readily by comparison with the assignments for *O*-deacetylated P1. Thus, the remaining signals are due to the α and β anomers of the *O*-acetylated molecules in P1. Protons attached to carbon atoms carrying *O*-acetyl groups are deshielded by 1.1–1.5 p.p.m. and often resonate in the region for anomeric protons[24]. Protons on adjacent carbon atoms are also deshielded, but to a much lesser extent. Thus, the signals for H-1 of the α- and β-Man in the acetylated molecules occur at δ 5.36 (0.4 H, +0.14 p.p.m.) and 5.11 (0.3 H, +0.15 p.p.m.), respectively. The corresponding signals for H-3 of the *O*-acetylated Man occur at δ 4.47 (α anomer, 0.4 H) and 4.38 (β anomer, 0.3 H). These signals occur appreciably downfield from their usual positions (δ 3.83 for α and δ 3.64 for β; values corrected for acetone, δ 2.23)[25] because H-3 of Man is also deshielded by the glycosidic linkage at position 3 and the pyruvate linkage at position 4. The signals for H-2 of the *O*-acetylated Man occur at δ 5.64 (0.3 H, β anomer, +1.40 p.p.m.) and 5.22–5.23 (α anomer, +1.01–1.02 p.p.m.). The latter resonance is at the same position as those of H-1 of the hex-4-enuronic acid and the α-Man in non-acetylated P1. Finally, the signals at δ 4.57 and 4.56 may be assigned to the H-1 of β-Glc in the α and β anomers of the *O*-acetylated trisaccharide. The ^{13}C signals for the acetylated trisaccharide in P1 were assigned readily after the signals for the non-acetylated compound were identified by reference to the data for *O*-deacetylated P1.

The sugar (Table II, columns VI and VII) and methylation (Table IV, column VI) analyses for P2 were consistent with that of a hexasaccharide corresponding to the double repeating-unit of K55 polysaccharide, but with a 4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid rather than a glucuronic acid non-reducing end-group.

The above results permit the structure shown in the Abstract to be written for the repeating unit of the capsular polysaccharide of *E. coli* K55. This structure is identical to that reported[14] for the repeating unit of the capsular polysaccharide of *Klebsiella* K5, with the exception that the latter has the acetyl group at position 2 of the glucose residue.

EXPERIMENTAL

General methods. — Analytical g.l.c. was performed using a Hewlett–Packard 5890A gas chromatograph, fitted with flame-ionisation detectors, and a 3392A recording integrator. A J & W Scientific fused-silica DB-225 bonded-phase

capillary column (30 m × 0.25 mm) having a film thickness of 0.25 μm was used, with helium as the carrier gas, and operated at 205°, 225°, or 230°. Retention times of partially methylated alditol acetates relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol were compared with those reported[26]. G.l.c.–m.s. was conducted on a Hewlett–Packard 5988A mass spectrometer with an ionisation energy of 70 eV and an ion-source temperature of 200°. N.m.r. spectra were recorded with a Bruker WM-500 Ft spectrometer at 30° or at 95°. Samples were deuterium-exchanged by freeze-drying solutions in D_2O, and acetone was used as the internal standard (δ 2.23 for ^1H and 31.07 p.p.m. for ^{13}C) measured against external sodium 4,4-dimethyl-4-silapentane-1-sulfonate. G.p.c. of K55 polysaccharides was performed using a column (1.6 × 65 cm) of dextran-calibrated Sephacryl S-500 with 0.1M pyridinium acetate as eluent. G.p.c. of the bacteriophage digest was performed on columns (1.6 × 40 cm and 2.6 × 70 cm) of Bio-Gel P-4, using the same eluent. All fractions were analysed by the phenol–sulfuric acid method[27], using a Beckman model DB spectrophotometer (490 nm).

Samples were hydrolysed using 2M trifluoroacetic acid at 100°. Acid hydrolysates were co-concentrated with water, and solutions were concentrated under reduced pressure at ≤40° (bath). Acetylated aldononitrile derivatives were prepared by the method of McGinnis[28]. Alditol acetates were prepared by the reduction of aqueous solutions of hydrolysates with sodium borohydride or sodium borodeuteride and acetylation of the products with 1:1 acetic anhydride–pyridine for 1 h at 100°. Carboxyl reduction was achieved by treating the sample with dry methanolic 3% hydrogen chloride under reflux for 16–20 h, the mixture was then neutralized ($PbCO_3$), and the products were reduced with sodium borohydride in anhydrous methanol. Unless otherwise stated, methylation of samples was carried out by the Hakomori method as modified by Sandford and Conrad[16] using potassium dimsyl[17]. The procedure proposed by Hascall *et al.*[15] for the thiobarbituric acid test was followed, using 1-mg samples of P1, P2, and the K55 polysaccharide, and the resulting solutions were analysed by spectrophotometry.

Preparation and properties of K55 polysaccharide. — An authentic culture of *E. coli* O9:K55 (N 24c) was obtained from Dr. I. Ørskov (Copenhagen). Five batches of the bacteria were grown[29] on Mueller–Hinton agar and incubated for the periods specified in Table I. The acidic polysaccharides were isolated by precipitation with CTAB to give yields of 513, 674, 461, 496, and 980 mg for batches 1–5, respectively. Solutions of samples of K55 polysaccharide from batches 3 (17.2 mg) and 5 (19.2 mg) in water (2 mL) were ultrasonicated for 0.5 h in order to reduce the viscosity prior to n.m.r. spectroscopy (Table III).

Methylation analysis. — K55 polysaccharide (33.8 mg, acid form) was methylated once by the Hakomori method[16,17] and then by the Kuhn method[30]. The product (14.0 mg) was methanolysed (19 h). One portion of the methanolysate was hydrolysed (8 h) and the products were derivatised (alditol acetates); the second portion was carboxyl-reduced ($NaBH_4$) prior to hydrolysis (8 h) and derivatisation of the products (alditol acetates). A solution of the K55 polysaccharide (6.1 mg,

acid form) in water (5 mL) was heated for 40 min at 100°, then filtered, dialysed against running tap water (3500 mol. wt. cut-off, 1 day), and freeze-dried to give depyruvylated K55 polysaccharide (4.0 mg). Methylation of this product followed by hydrolysis (16 h) and derivatisation of the products (alditol acetates) gave the results in Table IV, column III.

Degradation by the bacteriophage. — A sample of *Klebsiella* bacteriophage $\phi 5$ was obtained from Professor S. Stirm (Giessen) and propagated[1] on *E. coli* K55 in nutrient broth to give a purified bacteriophage solution (1.5×10^{13} PFU, 230 mL) to which was added K55 polysaccharide (batch 5, 495 mg). The degradation was allowed to proceed for 5 days at 30° in the presence of chloroform, and the products, P1 (104.8 mg) and P2 (119.5 mg), were isolated by g.p.c. as described above. P1 (5.5 mg) was reduced with sodium borodeuteride, and a sample of the product (P1-alditol, 1.0 mg) was analysed by the Morrison method[31] (Table VI, column IV).

Determination of absolute configuration. — The K55 polysaccharide (5.2 mg) was hydrolysed (18 h) and the products were converted into the acetylated (−)-2-octyl glycosides[13]. A second sample (6.4 mg) was methanolysed and carboxyl-reduced, and the products were derivatised similarly. These derivatives were analysed by g.l.c. at 230°.

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