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STRUCTURE OF THE CAPSULAR POLYSACCHARIDE OF *Escherichia coli* O9:K32(A):H19

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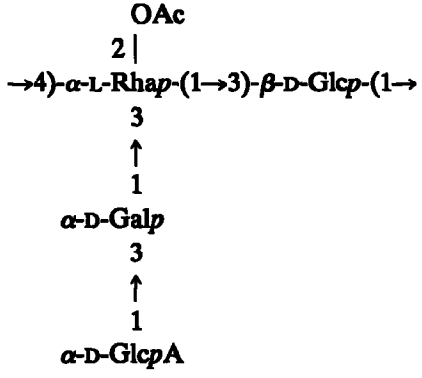
ABSTRACT

The capsular polysaccharide of the bacterium *Escherichia coli* O9:K32(A):H19 was analyzed using chemical methods (hydrolysis, sequential Smith degradation, methylation analysis) together with ^{1}H- and ^{13}C-n.m.r. spectroscopy. ^{13}C-N.m.r. spectroscopy and chemical analyses indicated that the K32 polysaccharide is composed of equimolar proportions of glucose, galactose, rhamnose, and glucuronic acid, and carries *O*-acetyl groups. ^{1}H-N.m.r. analysis of native K32 polysaccharide revealed five resonances in the anomeric region (δ 5.52, 5.16, 5.12, 5.02, and 4.73) and the presence of an acetyl group (δ 2.18). *O*-Deacetylation of the polysaccharide resulted in the loss of the resonance at δ 2.18 and one of the resonances (δ 5.52) in the anomeric region. The "extra" anomeric resonance in the ^{1}H-n.m.r. spectrum of the native K32 polymer was assigned to H-2 of rhamnose, which experiences a large downfield shift when the 2-position is *O*-acetylated. This was confirmed by a 2D-COSY n.m.r. experiment and studies of model compounds. The K32 capsular polysaccharide is of the "2 + 2" type, comprised of the following repeating unit:



This structure is identical to that of *Klebsiella* K55 capsular polysaccharide.

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INTRODUCTION

Escherichia coli, a member of the *Enterobacteriaceae*, is of medical interest due to its involvement in physiopathological processes[1]. The microorganism has been classified into many serological types on the basis of the nature of the surface antigens. Approximately 100 "K" (capsular), 164 "O" (somatic), and 56 "H" (flagellar) antigens are currently recognized[2]. Most of the *E. coli* K antigens are acidic capsular polysaccharides, and some are under systematic investigation in our laboratory. This paper presents the structure of the K antigen of *E. coli* K32.

RESULTS AND DISCUSSION

Sugar analysis. — The acidic capsular polysaccharide produced by *E. coli* K32 grown on solid Mueller–Hinton agar was shown to be homogeneous by gel-permeation chromatography, with $M_r = 9 \times 10^6$. Analysis of the polysaccharide by total acid hydrolysis revealed the presence of three neutral sugars (glucose, galactose, and rhamnose). Methanolysis and uronic-ester reduction prior to hydrolysis increased the level of glucose found, indicating that glucuronic acid was also present (Table I). Sugar analysis of the carboxyl-reduced polysaccharide confirmed this, and thus the sugars in the K32 polysaccharide were determined to be glucose, galactose, rhamnose, and glucuronic acid in the ratio 1:1:1:1.

An investigation into the effect of hydrolysis time on the ratio of sugars in the hydrolysate showed that rhamnose is released rapidly from the polymer and is subject to degradation. Deoxy sugars are known to be less stable in acid conditions than other hexoses[3]. The gradual appearance of galactose in the hydrolysate is evidence for this residue being adjacent to the glucuronic acid, as the glycosidic link of uronic acids is stabilised by the presence of the carboxyl group[4].

Methylation analysis. — The formation of 2,4,6-tri-*O*-methylglucose, 2,4,6-

TABLE I

SUGAR ANALYSIS OF K32 POLYSACCHARIDE AND DERIVATIVES

Sugar	*Molar proportions[a] after hydrolysis times shown (h)*							
	I[b]				*II*	*III*	*IV*	*V*
	3[c]	*12*	*16*	*24*	*18*	*16*	*16*	*16*
Glucose	0.40	0.45	0.37	0.49	0.46	0.45	0.34	0.43
Galactose	0.10	0.17	0.35	0.37	0.30	0.25	0.36	0.16
Rhamnose	0.50	0.37	0.29	0.13	0.24	0.30	0.29	0.41

[a]Determined using program 1. [b]I, K32 polysaccharide; II, K32 polysaccharide, methanolyzed and reduced prior to hydrolysis; III, carboxyl-reduced K32 polysaccharide; IV, SM1 polysaccharide; V, SM2 polysaccharide. [c]Time of hydrolysis.

TABLE II

METHYLATION ANALYSIS OF K32 POLYSACCHARIDE AND DERIVATIVES

Methylated sugars[a] *(as alditol acetates)*	*Molar proportions*[b]				
	I[c]	*II*	*III*	*IV*	*V*
2,4,6-Glc	0.44	0.25	0.19	0.29	0.48
2,3,4,6-Glc	—	0.31	0.27	0.04	—
2,4,6-Gal	0.09	0.18	0.24	—	—
2,3,4,6-Gal	—	—	—	0.22	0.14
2-Rha	0.46	0.26	0.30	0.45	0.20
2,3-Rha	—	—	—	—	0.18

[a]2,4,6-Glc = 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylglucitol, *etc.* [b]Determined using program 2; values corrected using the effective carbon response factors given by Sweet *et al.*[22]. [c]I, K32 polysaccharide; II, K32 polysaccharide, uronic ester reduced after methylation, then remethylated; III, carboxyl-reduced K32 polysaccharide; IV, SM1 polysaccharide; V, SM2 polysaccharide.

tri-*O*-methylgalactose, and 2-*O*-methylrhamnose in the methylation analysis of K32 polysaccharide indicates that the polymer contains glucose and galactose linked at O-3 and rhamnose linked at O-3 and O-4 (Table II, column I). Thus, rhamnose forms a branch point in the polysaccharide. The low level of galactose detected again indicates that it is probably attached to the uronic acid. Uronic-ester reduction followed by remethylation increased the levels of 2,4,6-tri-*O*-methylgalactose and also resulted in the formation of 2,3,4,6-tetra-*O*-methylglucose (Table II, column II). Similar results were seen when the carboxyl-reduced polysaccharide was subjected to methylation analysis. From this it can be deduced that the glucuronic acid is the terminal residue in the side chain of the K32 polysaccharide.

Sequential Smith degradation of K32 polysaccharide. — Smith degradation of the carboxyl-reduced K32 polysaccharide gave a polymer (**SM1**) that contained glucose, galactose, and rhamnose in the ratio 1:1:1 (Table I). The fact that these residues survived the Smith degradation confirms that each is linked through O-3 in the native polymer. Methylation analysis of **SM1** again indicated that rhamnose forms a branch point (as 2-*O*-methylrhamnose was observed) and furthermore, the detection of 2,3,4,6-tri-*O*-methylgalactose suggested that the galactose is also present in the side chain and in **SM1** exists as a terminal residue. Thus in native K32 polysaccharide the side chain must consist of a terminal glucuronic acid attached through galactose to the main chain of rhamnose and glucose. This was confirmed when **SM1** was subjected to a second Smith degradation, which removed the terminal galactose to leave the intact rhamnose–glucose main chain (**SM2**). During methylation analysis of this latter polymer, a large proportion of 2,3-di-*O*-methylrhamnose was detected, indicating that the side chain is linked to O-3 of rhamnose. Hydrolysis and sugar analysis of **SM2** showed that the second Smith degradation was not complete (Table I, column V).

TABLE III

^{13}C-N.M.R. DATA FOR K32 POLYSACCHARIDE AND DERIVATIVES

Polysaccharide	*Chemical shift[a] in p.p.m. ($J_{C,H}$ in Hz)*	*Assignment[b]*
Native K32 polysaccharide	176.20	C=O of acetate
	174.00	C=O of GlcA
	103.12	-3Glcβ-
	101.43[c]	-3,4Rhaα-
	99.62	-3,4Rha(2-OAc)α-
	95.72	GlcAα-
	93.43	-3Galα-
	85.91	unassigned
	21.12	-CH_3 of acetate
	17.91	-CH_3 of Rha
K32 polysaccharide, *O*-deacetylated	103.00 (163)	-3Glcβ-
	101.75 (171)	-3,4Rhaα-
	96.65 (171)	GlcAα-
	94.80 (173)	-3Galα-
	18.00	-CH_3 of Rha
SM1[d]	103.14	-3Glcβ-
	101.61	-3,4Rhaα-
	94.20	Galα-
	17.61	-CH_3 of Rha
SM2	104.07	-3Glcβ- (3Rhaα-)[e]
	103.01[c]	-3Glcβ- (3,4Rhaα-)
	101.62	-3,4Rhaα-; -4Rhaα-
	94.28[c]	Galα-
	17.61	-CH_3 of Rha

[a]Measured at 75 MHz. [b]The numerical prefix indicates the position in which the sugar is substituted; the α or β indicates the configuration of the glycosidic bond. Thus, -3Glcβ- refers to the anomeric carbon of a 3-linked glucosyl residue in the β-anomeric configuration. The absence of a numerical prefix indicates a terminal (non-reducing) group. [c]Minor peak. [d]For the source of **SM1** and **SM2** see text. [e]Parentheses indicate identity of neighboring residue at reducing end.

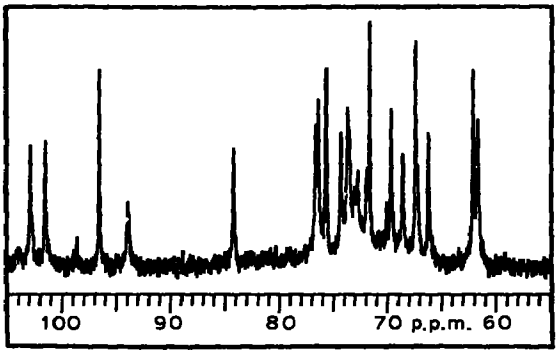

Fig. 1. Broadband decoupled, 75 MHz ^{13}C-n.m.r. spectrum of *O*-deacetylated K32 polysaccharide.

TABLE IV

^{1}H-N.M.R. DATA FOR K32 POLYSACCHARIDE AND DERIVATIVES

Polysaccharide	δ[a]	$J_{1,2}$ (Hz)	*Integral (no. of H)*	*Assignment*[b]	*Polysaccharide*	δ[a]	$J_{1,2}$ (Hz)	*Integral (no. of H)*	*Assignment*[b]
Native K32	5.52		1	-3,4Rha(2-OAc)α- (H-2)	K32 polysaccharide,	5.26	3–4	1	-3Galα-
polysaccharide	5.16		1	-3,4Rha(2-OAc)α-	*O*-deacetylated	5.20		1	-3,4Rhaα-
	5.12		1	-3Glcα-		5.11	3–4	1	GlcAα-
	5.02		1	GlcAα-		4.72	8–10	1	-3Glcβ-
	4.73		1	-3Glcβ-		1.34	8–10	3	-CH_3 of Rha
	2.18		3	-CH_3 of acetate					
	1.35	8–10	3	-CH_3 of Rha	K32 polysaccharide,	5.26		1	-3Galα-
					carboxyl-reduced	5.20		1	-3,4Rhaα-
K32	5.52		1	-3,4Rha(2-OAc)α- (H-2)		5.08		1	Glcα-
polysaccharide,	5.16		1	-3,4Rha(2-OAc)α-		4.74	8–10	1	-3Glcβ-
autohydrolyzed	5.12[c]	3.5	1	-3Glcα-		1.34	8–10	3	-CH_3 of Rha
	5.06[c]	3.2	1	GlcAα-					
	4.70[c]	7.8	1	-3Glcβ-	SM1[d]	5.25		1	Galα-
	4.44	10	1	Ring proton		5.21		1	-3,4Rhaα-
	2.19		3	-CH_3 of acetate		5.16		tr[e]	Unassigned
	1.34	8–10	3	-CH_3 of Rha		4.70	8–10	1	-3Glcβ-
						1.34	8–10	3	-CH_3 of Rha
					SM2[d]	5.25		0.28	Galα-
						5.20		0.28	-3,4Rhaα-
						5.15		0.72	-4Rhaα-
						4.72	8–10	1	-3Glcβ-
						1.34	8–10	3	-CH_3 of Rha

[a]Determined at 400 MHz, except as otherwise noted. [b]Refers to the anomeric proton, unless indicated otherwise. Substitution and configuration of residues is indicated as described in footnote to Table III. [c]Determined at 500 MHz. [d]For the source of SM1 and SM2 see text. [e]Trace, minor peak.

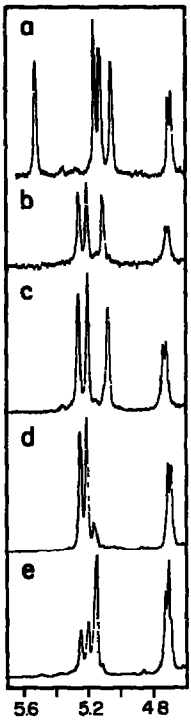

Fig. 2. ^{1}H-N.m.r. spectra (anomeric region) at 400 MHz of: (*a*) autohydrolyzed K32 polysaccharide; (*b*) *O*-deacetylated K32 polysaccharide; (*c*) carboxyl-reduced K32 polysaccharide; (*d*) **SM1** polysaccharide; (*e*) **SM2** polysaccharide.

N.m.r.-spectroscopic analysis. — ^{13}C-N.m.r. analysis of the native K32 polysaccharide showed the presence of four major resonances in the anomeric region (90–105 p.p.m.; Table III, Fig. 1). Measurement of the $J_{C,H}$ coupling of each resonance indicated that there are three α- and one β-glycosidic linkages[5]. In addition, resonances at 17.91, 21.12, 174.00, and 176.20 p.p.m. in the ^{13}C-n.m.r. spectrum suggested the presence of a 6-deoxyhexose, an acetate group, and a uronic acid residue. The ^{1}H-n.m.r. spectrum of the *O*-deacetylated K32 polysaccharide (Table IV, Fig. 2b) has four resonances in the anomeric region. Three resonances are downfield of δ 5.00 and one upfield, indicating again that the polysaccharide has three α- and one β-glycosidic linkages. The resonance at δ 1.34 is characteristic of the methyl protons of a 6-deoxyhexose. Thus, both the ^{1}H- and ^{13}C-n.m.r. data support the results of the chemical analyses of the K32 polysaccharide and show that the polymer is comprised of a four-sugar repeating unit.

N.m.r. examination of the polymeric derivatives of the K32 polysaccharide (**SM1** and **SM2**) and comparison of their spectra with those of the *O*-deacetylated K32 polysaccharide enabled us to make complete assignments of the anomeric resonances in both the ^{1}H- and the ^{13}C-n.m.r. spectra. The resonance at δ 5.11 in the ^{1}H-n.m.r. spectrum of *O*-deacetylated K32 polysaccharide is absent from the

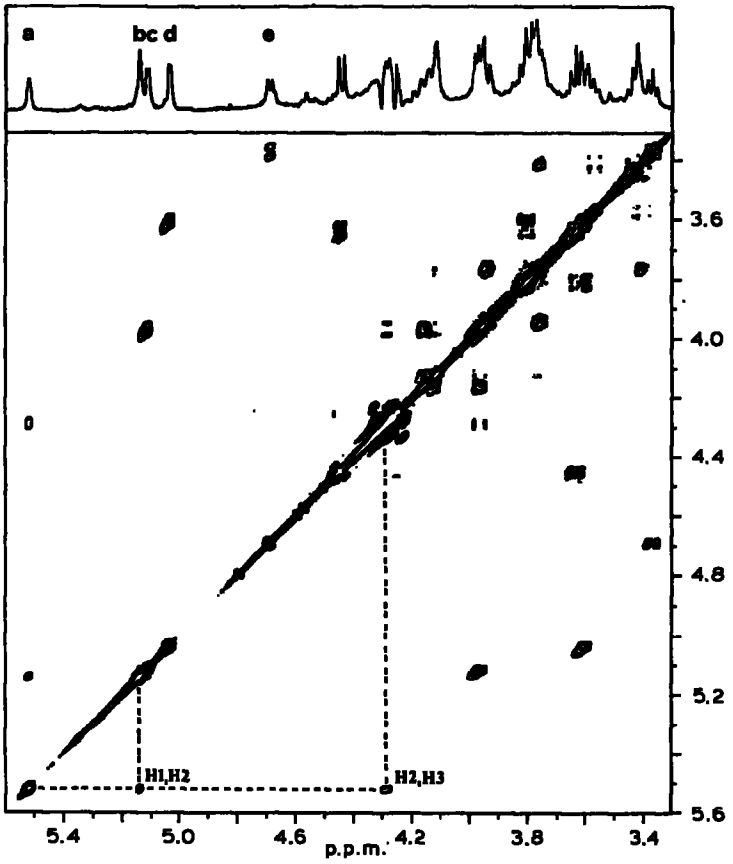

Fig. 3. 2D ^{1}H-n.m.r. (COSY) spectrum at 500 MHz of autohydrolyzed K32 polysaccharide. Labeled resonances are: (*a*) α-Rha, H-2; (*b*) α-Rha, H-1; (*c*) α-Gal, H-1; (*d*) α-GlcA, H-1; (*e*) β-Glc, H-1. Connectivities between α-Rha protons are indicated (——).

spectrum of **SM1** and was therefore attributed to D-glucuronic acid. In the ^{1}H-n.m.r. spectrum of the **SM2** sample, which contained some 16% of **SM1**, resonances at δ 5.26 and δ 5.20 are greatly reduced and, on the basis of their coupling constants ($J_{1,2}$ 3 Hz and ~0 Hz), were assigned to side-chain galactose and substituted rhamnose, respectively, in the residual **SM1**. The new signal at δ 5.15 ($J_{1,2}$ ~0 Hz), associated with **SM2**, was assigned to the rhamnose which is no longer substituted at O-3 by galactose. The remaining resonance at δ 4.72 could then be confidently assigned to D-glucose in β-glycosidic linkage.

Comparison of the ^{13}C-spectra of **SM1** and **SM2** with that of *O*-deacetylated K32 revealed that the sequential Smith degradations resulted in the loss of one resonance (δ 96.62) and a great reduction in intensity of another (δ 94.80). These resonances were therefore assigned to D-glucuronic acid and D-galactose, respectively. In the ^{13}C-spectrum of *O*-deacetylated K32 polysaccharide only the resonance at 103.00 p.p.m. had a $J_{C,H}$ value close to 160 Hz, which is indicative of a β-

anomeric configuration. Thus, this resonance was assigned to the D-glucose residue. The remaining resonance at 101.75 p.p.m. can therefore be attributed to L-rhamnose, and this completes the assignments of the anomeric resonances in the ^{13}C-n.m.r. spectra of *O*-deacetylated K32 polysaccharide and its polymeric derivatives. In the ^{13}C-spectrum of the native polysaccharide a resonance at δ 99.62 is observed in addition to a weak resonance at δ 101.43. The upfield shift of the anomeric resonance indicates that C-1 of the rhamnose is β to the position of the *O*-acetyl substitution[6]. Thus, the *O*-acetyl resides at position 2 of the rhamnose.

To confirm the position of the *O*-acetyl group, a 2D-COSY ^1H-n.m.r. experiment[7] was carried out on the native K32 polysaccharide. In the 2D-COSY spectrum (Fig. 3) connectivities between resonances at δ 5.14 and δ 5.52 and between those at δ 5.52 and δ ~4.28 (under HOD) can be seen. In keeping with previous observations of large downfield shifts of ring protons at positions of *O*-acetylation in polysaccharides[8,9], the resonance at δ 5.52 could be attributed to H-2 of rhamnose, and indeed this low field resonance is not present in the spectrum of the *O*-deacetylated K32 polysaccharide. To confirm that the H-2 of rhamnose is shifted downfield on acetylation at O-2, methyl 3,4-di-*O*-methyl-α-L-rhamnoside and methyl 2-*O*-acetyl-3,4-di-*O*-methyl-α-L-rhamnoside were synthesized and analysed by ^1H-n.m.r. *O*-Acetylation at position 2 resulted in the H-2 resonance being shifted downfield from δ 4.01 to δ 5.25, which agrees closely with the data reported by Toman *et al.*[10] from a similar study. The fact that H-2 of acetylated rhamnose is shifted downfield to a greater degree in the K32 polysaccharide than in the model compounds suggests an additional deshielding effect of the *O*-substitution of rhamnose by galactose at position 3.

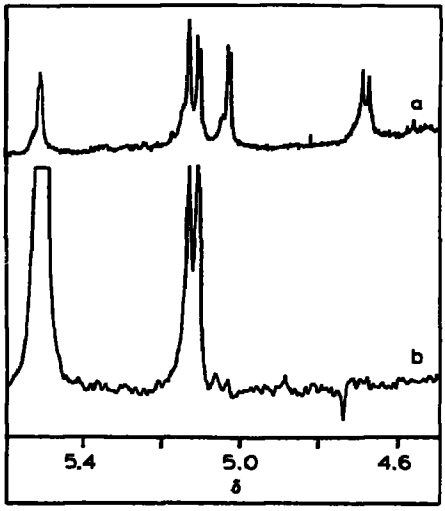

Fig. 4. Nuclear Overhauser effect. (*a*) Normal 500 MHz ^1H-n.m.r. spectrum of native K32 polysaccharide; (*b*) n.O.e. difference spectrum obtained on irradiation of α-Rha H-2 (δ 5.52).

An n.O.e. difference experiment[11,12] was conducted in which the rhamnose H-2 resonance (δ 5.52) was saturated. The signals for H-1 of L-rhamnose and H-1 of D-galactose were both enhanced (Fig. 4b), confirming that the D-galactose is (1→3)-linked to L-rhamnose, which places the D-galactose H-1 in close proximity to the L-rhamnose H-2. This may be the reason for the extremely large downfield shift of the H-2 resonance in the native K32 polysaccharide.

Evidence that the side chain is in an unusual orientation with respect to the L-rhamnose unit is also seen in the ^{13}C-n.m.r. data. The C-1 resonance of α-D-galactose is observed at 93.43 p.p.m. (Fig. 1), which places it in the high-field region usually associated with reducing-end anomeric resonances. In a study of blood-group oligosaccharides, Lemieux *et al.*[13] reported a chemical shift value of 94.11 p.p.m. for C-1 of α-D-galactose in the trisaccharide α-L-Fuc(1→2)-[α-D-Gal(1→3)]-β-D-Gal. This is one of several examples from their study showing that the anomeric resonances of residues situated at branch points in oligosaccharides may be seen at higher fields than usual. As the effect is not always observed it must be due to the particular orientations adopted by side-chain residues in some circumstances.

Sugar configuration. — Analysis of the (*R*)-2-octyl glycosides of the neutral sugars of the K32 polysaccharide showed that D-glucose, D-galactose, and L-rhamnose were present. The optical rotation data for the polysaccharide and its polymeric derivatives confirmed this, and showed that the glucuronic acid is in the D-form (Table V). The optical rotation data also provide further evidence for the structures of **SM1** and **SM2**, as the experimental values for these polymers agree well with the theoretical values.

Cross-reaction of ϕ32 with Klebsiella *K55 polysaccharide.* — The structure proposed for the *E. coli* K32 polysaccharide is identical to that found[14] for *Klebsiella* K55. Comparison of n.m.r. data for the two polysaccharides confirmed their identity in addition, a crude preparation of phage ϕ32 was able to form a zone of

TABLE V

OPTICAL ROTATION DATA FOR K32 POLYSACCHARIDE AND DERIVATIVES

Polysaccharide	$[\alpha]_D^0$	
	Calculated[a]	*Observed*
Native K32 polysaccharide	+77.7°	+80.9° (*c* 0.147)
K32 polysaccharide, carboxyl-reduced	+80.7°	+75.3° (*c* 0.122)
SM1	+37.7°	+23.9° (*c* 0.214)
SM2	−57.8° (−8.62°)[b]	−6.45° (*c* 0.124)

[a]Value calculated by summing contributions from each residue. These were assumed to equal the optical rotations of the corresponding methyl glycosides. [b]Value calculated using the compositional data for this polysaccharide, as determined by ^{1}H-n.m.r. (Table IV).

clearing on a lawn of *Klebsiella* K55 bacteria. This indicates a close similarity between the structures of the capsules of the two organisms.

CONCLUSIONS

The capsular polysaccharide of *E. coli* K32 is another example of the "2 + 2" type. *E. coli* K30 is also of this type.

EXPERIMENTAL

General methods. — All concentrations were carried out at 40°. Solutions were deionized with Amberlite IR-120 (H^+) ion-exchange resin. A Perkin–Elmer model 457 spectrometer was used to record the infrared spectra of methylated polysaccharides dissolved in spectroscopic grade CCl_4. Optical rotations were determined with a Perkin–Elmer model 141 polarimeter. Measurements were made at ambient temperatures (21–23°) on aqueous solutions (~1 mg/mL) in a 1 dm cell.

Analytical g.l.c. separations were performed on a Hewlett–Packard 5890A capillary gas chromatograph fitted with flame ionization detectors. A fused silica capillary column (15 m × 0.256 mm) coated with DB-17 was used with a helium carrier-gas flow rate of 1.1 mL/min. The temperature was held at 180° for 2 min and then raised 5°/min to 220° (program 1) for the separation of fully acetylated alditols, or held at 180° for 1 min and then raised 2°/min to 250° (program 2) for the separation of partially methylated alditol acetates. G.l.c.-m.s. analyses were performed on either a Nermag R10-10 mass spectrometer or a Kratos MS-50 mass spectrometer with capillary columns coated with DB-17. Electron-impact ionization was accomplished with a 70 eV electron beam (current 100 μA, ion source at 200°).

Isolation and purification of K32 polysaccharide. — *E. coli* K32 was cultured on a solid Mueller–Hinton agar supplemented with sucrose (2% w/v) and NaCl (2% w/v) for 4 days at ambient temperature. The bacteria were harvested and suspended in 2% phenol (~1 L). After several hours stirring at room temperature, the cells were removed by centrifugation (10,000 × g, 2 h). The supernatant was poured into three volumes of ethanol, the precipitate was collected and redissolved in 500 mL of distilled water, and the solution was again centrifuged. The second supernatant was adjusted to 0.1M NaCl and poured into three volumes of ethanol to give a crude polysaccharide precipitate. This was redissolved in water (500 mL), the solution was dialyzed for 3 days against running tap water, and the polysaccharide was precipitated with cetyltrimethylammonium bromide. The precipitate was redissolved in 4M NaCl, the solution was dialyzed against distilled water and ultracentrifuged, and the purified polysaccharide was recovered from the supernatant by lyophilization.

Sugar analysis of K32 polysaccharide. — Native K32 polysaccharide (25 mg) was dissolved in 2M trifluoroacetic acid (TFA; 13 mL), the solution was heated to 100°, and periodically 2 mL aliquots of the reaction mixture were taken. After

removal of the acid by coevaporation with water, each aliquot was reduced with $NaBH_4$. The alditol acetates were prepared (1:1 acetic anhydride–pyridine) and analyzed by g.l.c. The molar proportions of the neutral sugars in each aliquot are reported in Table I.

Methanolysis (0.3M methanolic HCl, reflux, 16 h) and reduction ($NaBH_4$ in dry methanol) of the K32 polysaccharide (10 mg) followed by hydrolysis (2M TFA, 100°, 16 h) and preparation of the alditol acetates was used to detect and quantify the uronic acid present (Table I).

Carboxyl reduction of K32 polysaccharide. — Using the method of Taylor and Conrad[15], K32 polysaccharide (250 mg) was reduced with 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (1.65 g) and aqueous $NaBH_4$ (3M, 40 mL). The procedure was repeated once to yield a neutral polysaccharide (230 mg). The polymer was analyzed using ^{1}H-n.m.r., g.l.c.-m.s. of alditol acetates, and methylation as recorded in Tables I, II, and III.

Sequential Smith degradation of K32 polysaccharide. — A portion of the carboxyl-reduced polysaccharide (200 mg) was dissolved in 0.05M $NaIO_4$ (50 mL) and one crystal of $NaClO_4$ was added. The solution was held in the dark at room temperature for 2 days, then ethylene glycol (1.0 mL) was added to stop the reaction, and the solution was dialyzed against tap water for 1 day. Sodium borohydride (100 mg) was added and the mixture was left standing for 1 h. The solution was then neutralized with 50% aqueous acetic acid and dialyzed for 2 days. The product was recovered by lyophilization and the periodate-oxidation procedure was repeated. The polyol was subsequently dissolved in 50 mL of 50% aqueous acetic acid and the solution was held for 1.5 h at 100°, then neutralized and dialyzed. A nondialyzable polymeric product (**SM1**) was recovered by lyophilization.

A second Smith degradation was performed on **SM1** (100 mg) in exactly the same manner to yield a second polymer, **SM2** (52 mg). Polymers **SM1** and **SM2** were analyzed by hydrolysis (Table I), methylation (Table II), and ^{13}C-n.m.r. and ^{1}H-n.m.r. spectroscopy (Tables III and IV). The optical rotation of each was also determined (Table V).

Methylation analysis of K32 polysaccharide. — A procedure based on that of Isogai *et al.*[16] was used for methylation analyses. Dry, deionized K32 polysaccharide (5 mg) was dissolved in dimethyl sulfoxide (2 mL). Dry, powdered NaOH (50 mg) was added and the reaction mixture was stirred under nitrogen for 1 h before the addition of CH_3I (4 mL). After 3 h the excess CH_3I was removed in a stream of nitrogen, the residue was suspended in water (20 mL) and the solution dialyzed (16 h) against running tap water. The methylated polysaccharide was recovered by lyophilization. Completeness of methylation was assessed by i.r. spectroscopy and, if required, further methylation was achieved by the method of Purdie and Irvine[17]. The completely methylated polymer was hydrolyzed (2M TFA, 100°, 16 h), reduced ($NaBH_4$), and converted to the alditol acetates (1:1 pyridine–acetic anhydride) for analysis by g.l.c. and g.l.c.-m.s. The partially methylated alditol acetates were identified by comparison of their retention times and mass spectra with those of authentic standards (Table II).

In order to determine the linkages of the uronic acid in the K32 polysaccharide, a portion (5 mg) was methylated as described above. The methylated polymer was dissolved in dry oxolane (5 mL) and $LiAlH_4$ (30 mg) was added. After proceeding overnight the reaction was quenched with ethanol, and the solvents were evaporated. The residue was dissolved in $CHCl_3$ (10 mL) and the solution washed with 2M HCl (2 × 20 mL) and water (1 × 20 mL). The $CHCl_3$ was removed by evaporation and the residue dried. I.r. spectroscopy showed complete reduction of the uronic acid (absence of C=O stretch). The polymer was remethylated and analyzed as described above (Table II).

N.m.r. analyses. — One-dimensional ^{1}H-n.m.r. spectra were recorded at high temperature (95°) using a Bruker WH400 spectrometer, and chemical shifts were referenced to sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) at δ 0.00. Samples (10–15 mg) were deuterated by lyophilization and dissolved in D_2O (0.5 mL), then acetone (δ 2.23) was added as an internal standard. More viscous samples were autohydrolyzed (100°, 15 min) prior to analysis. The proton homonuclear-shift-correlated (COSY) 2D-n.m.r. experiment and the 1D nuclear-Overhauser-enhancement difference experiment were performed on a Bruker AM500 spectrometer: data were acquired at 77°. ^{13}C-N.m.r. spectra were recorded at ambient temperature using Bruker WH400 and Varian XL300 spectrometers. Samples (~30 mg) were dissolved in D_2O (0.5 mL) and acetone (CH_3 31.07 p.p.m. from DSS) was used as an internal standard. Coupled spectra were obtained using a gated decoupling sequence[18]. ^{1}H-N.m.r. spectra of synthetic rhamnose derivatives were obtained on Varian EM60 and Bruker WH400 spectrometers, using samples dissolved in $CDCl_3$ containing Me_4Si as an internal standard.

Determination of sugar configuration. — The configurations of the sugars of the K32 polysaccharide were determined by the method of Leontein *et al.*[19]. Polysaccharide (20 mg) was hydrolyzed (2M TFA, 100°, 16 h) and the sugar components were separated by preparative paper chromatography (Whatman No. 1 paper, 8:2:1 pyridine–acetic acid–water). The resolved sugars were eluted and converted into glycosides with (*R*)-(−)-2-octanol. The derivatives were analysed by g.l.c.-m.s. and their configurations determined by comparison with g.l.c.-m.s. data for authentic standards.

Cross-reaction of E. coli *K32 bacteriophage and* Klebsiella *K55.* — Bacteriophage ϕ32 was propagated by tube lysis as previously described[20]. The crude ϕ32 preparation and serial dilutions were spotted (20 μL) onto a lawn of *Klebsiella* K55 bacteria which was then incubated overnight at 37° and examined for zone clearing and plaque formation.

Synthesis of partially methylated methyl rhamnosides. — Methyl 2-*O*-benzyl-α-L-rhamnopyranoside (**1**) was prepared from L-rhamnose as described by Lipták *et al.*[21]. Compound **1** (0.6 g) was dissolved in Me_2SO (12 mL), dry powdered KOH (1 g) and CH_3I (1 mL) were added, and the reactants were stirred for 1 h at ambient temperature. Analysis of the reaction mixture by t.l.c. (9:1 $CHCl_3$–acetone) showed one major product (**2**, R_F 0.68), two minor products (R_F 0.41, 0.30) and a

trace of starting material (R_F 0.2). After removal of CH_3I in a stream of N_2 the reaction mixture was poured into 100 mL of water, which was extracted with CH_2Cl_2 (3 × 50 mL). The organic phase was dried with $MgSO_4$ and concentrated to a syrup (0.823 g). Chromatography of the syrup on a column of silica gel G60 (100 g, eluant 9:1 $CHCl_3$–acetone) gave 0.341 g (51%) of the major product (**2**, identified by t.l.c.), δ_H 7.10–7.50 (5 H, Ph–H), 4.55–4.75 (3 H, H-1, CH_2), 3.60, 3.45, 3.35 (3 × 3 H, OCH_3), 3.00–3.80 (sugar C*H*), and 1.33 (3 H, CCH_3).

Compound **2** (340 mg) was dissolved in $CHCl_3$ (5 mL) and palladium–charcoal (50 mg) was added. The mixture was stirred under H_2 at 30° until hydrogenolysis was complete (150 min). The reaction mixture was filtered, the catalyst was washed with 10 mL of $CHCl_3$, and the organic phase was washed with water (2 × 10 mL), dried with $MgSO_4$, and concentrated. T.l.c. (9:1 $CHCl_3$–acetone) showed one product (R_F 0.12, u.v.-inactive), which was methyl 3,4-di-*O*-methyl-α-L-rhamnopyranoside (**3**). After purification by passage through a column of silica gel G60 (100 g, 9:1 $CHCl_3$–acetone) the yield was 96 mg (43%), δ_H 4.68 (1 H, H-1), 4.01 (1 H, H-2), 3.42 (1 H, H-3), 3.08 (1 H, H-4), 3.58 (1 H, H-5), 3.54, 3.49, 3.36 (3 × 3 H, OCH_3), 2.52 (1 H, O*H*), and 1.31 (3 H, CCH_3).

Compound **3** (48 mg) was dissolved in 1:1 pyridine–acetic anhydride and left overnight at ambient temperature. The reaction was quenched by the addition of ethanol (2 mL) and the excess reagents were coevaporated with water. The residue was shown by t.l.c. (9:1 $CHCl_3$–acetone) to consist of one product, namely methyl 2-*O*-acetyl-3,4-di-*O*-methyl-α-L-rhamnopyranoside (**4**, R_F 0.9), which was purified by column chromatography on silica gel G60 (30 g, eluant 9:1 $CHCl_3$–acetone), δ_H 5.25 (1 H, H-2), 4.60 (1 H, H-1), 3.53 (1 H, H-3), 3.06 (1 H, H-4), 3.60 (1 H, H-5), 3.56, 3.41, 3.36 (3 × 3 H, OCH_3), 2.16 (3 H, COCH_3), and 1.32 (3 H, CCH_3).

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