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The capsular antigen of *Escherichia coli* serotype O8:K102:H$^-$

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ABSTRACT

The structure of the capsular antigen of *E. coli* O8:K102:H$^-$ was investigated by methylation analysis, β-elimination of the methylated polysaccharide, lithium–ethylenediamine mediated degradation, and by 1D and 2D ^{1}H and ^{13}C NMR spectroscopy of the lithium-degraded and native polysaccharides. The capsular antigen was shown to have the following branched pentasaccharide repeating unit:

$$\begin{array}{l} \rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow 4)\text{-}\alpha\text{-D-Gal}p\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad 3 \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad \uparrow \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad 1 \\ \qquad\alpha\text{-D-Glc}p\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{A} \end{array}$$

INTRODUCTION

Escherichia coli O8:K102:H$^-$ bacteria were first isolated[1] in a London maternity ward. The capsular polysaccharide is heat stable and belongs to the group[2] of *E. coli* antigens which are characterised by high molecular weights and low electrophoretic mobilities. Many of these capsular antigens resemble those of *Klebsiella* serotypes. The structure of the capsular antigen of *E. coli* O8:K102:H$^-$, which we now report, is devoid of amino sugars and labile substituents.

RESULTS AND DISCUSSION

Isolation, composition, methylation analysis, and 1D NMR studies of the capsular antigen.—*E. coli* O8:K102:H$^-$ bacteria (culture No. 6CB10/1) were grown on Mueller–Hinton agar as described[3], and the capsular material was extracted with aqueous 1% phenol. The crude polysaccharide material, which was obtained by

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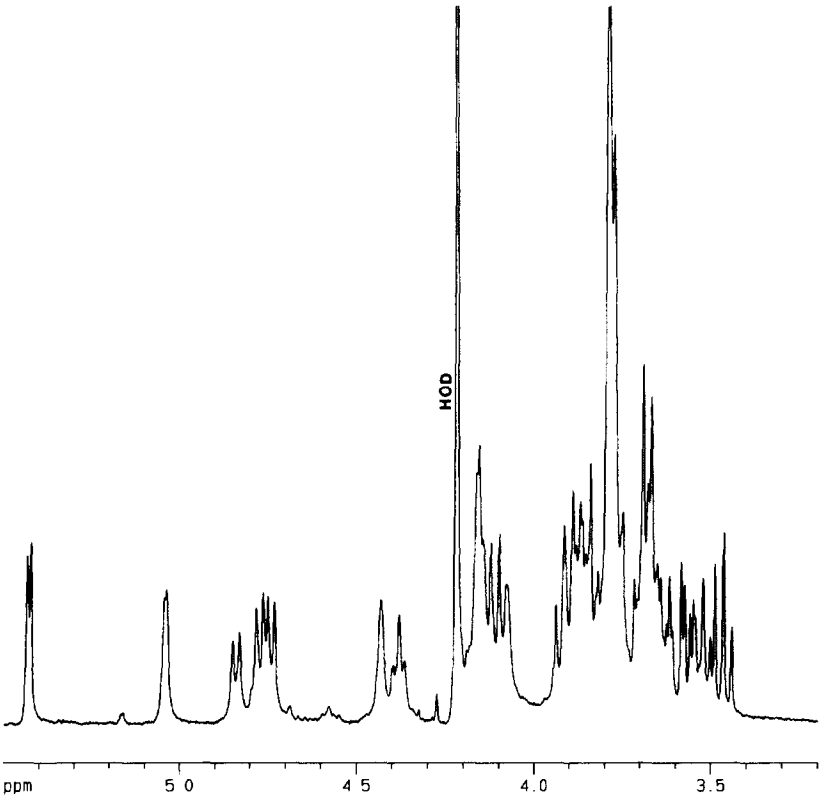


Fig. 1. ^{1}H NMR spectrum of *E. coli* K102 polysaccharide at 80°.

precipitation of the cell-free aqueous extract with ethanol, was associated with lipid. Capsular material free of O-antigen could be prepared via complexation with cetyltrimethylammonium bromide only after the crude polysaccharide had been delipidated with aqueous 1% acetic acid at 100° for 1 h. The purified capsular polysaccharide (**PS**) had $[\alpha]_D$ +106° (water) and was polydisperse in GPC on Sephacryl S500 with peak maxima at M_r 1×10^7 and 3×10^5.

GLC of the acetylated aldononitriles[4], derived from the products of an acid hydrolysate of **PS**, revealed Glc and Gal in the molar ratio 1.0:2.4. The sugars were present in the molar ratio 1.00:1.75 following reduction of the uronic acid in **PS**. These results indicate **PS** to comprise a pentasaccharide repeating unit, consisting of Glc, GlcA, and Gal in the molar ratios 1:1:3. The residues were shown to be D by GLC of their acetylated (−)-2-octyl glycosides[5].

The ^{1}H NMR spectrum of **PS** (Fig. 1), recorded at 80°, contained signals for H-1α at δ 5.43 ($J_{1,2}$ 3.9 Hz) and 5.04 ($J_{1,2}$ 3.4 Hz), and for H-1β at δ 4.85 ($J_{1,2}$ 7.7 Hz), 4.77 ($J_{1,2}$ 7.7 Hz), and 4.74 ($J_{1,2}$ 7.6 Hz). The ^{1}H-decoupled ^{13}C NMR spectrum confirmed a pentasaccharide repeating unit with signals for C-1 at 105.20, 105.07, 103.74, 100.99, and 99.86 ppm, a signal for C=O at 174.20 ppm, and

TABLE I

Methylation analysis of the *E. coli* K102 polysaccharide (**PS**)

Methylated sugars [a] as alditol acetates	T [b]	Molar ratios [c]			
		I	II	III	IV
2,3,4,6-Glc	1.00	0.62	0.57	0.08	
2,4,6-Gal	1.67	0.99	1.00	0.96	0.96
2,3,6-Gal	1.76	1.00	1.00	2.00	2.00
2,6-Gal	2.34	0.73	1.02	0.10	
2,3-Glc	3.04		0.52		

[a] 2,3,4,6-Glc = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol, etc. [b] Retention time relative to that of 2,3,4,6-Glc on DB-225 at 205°. [c] **I**, **PS**; II, carboxyl-reduced **PS**; III, base-catalysed degraded **PS**; IV, lithium-degraded **PS** (**DP**)

signals for CH_2OH at 62.02, 61.34, and 61.21 (2 C) ppm. The latter chemical shifts indicated that none of the residues were 6-linked.

Methylation analysis of **PS**, without and with carboxyl reduction of the methylated products, gave the results shown in Table I (columns I and II, respectively), which indicated terminal Glc, 3,4-linked Gal, 4-linked Gal, 3-linked Gal, and 4-linked GlcA.

Base-catalysed degradation of **PS**.—Methylated **PS** was degraded[6] with methylsulphinyl carbanion and the products were alkylated with trideuteriomethyl iodide. GLC–MS (Table I, column III) of the partially methylated alditol acetates derived from an acid hydrolysate of the product(s) showed almost complete loss of the terminal Glc and 3,4-linked Gal, and the concomitant production of 4-linked Gal. These results are consistent with the presence of the partial structure **1** in the repeating unit of **PS**.

D-Glc-(1 → 4)-D-GlcA-(1 → 3)-D-Gal-(1 →
4
↑

1

Thus, the main chain of **PS** comprises two 4-linked and one 3-linked Gal with the Glc-(1 → 4)-GlcA side chain 3-linked to a 4-linked Gal.

The sequence of the Gal residues in the main chain of **PS** was established by NMR spectroscopy of the degraded polysaccharide (**DP**) produced by treating **PS** with lithium in ethylenediamine[7]. GLC of the aldononitriles derived from an acid hydrolysate of **DP** showed it to be composed almost entirely of Gal. Methylation analysis of **DP** (Table I, column IV) indicated a trisaccharide repeating unit consisting of two 4-linked and one 3-linked Gal.

The ^1H NMR spectrum of **DP** contained resonances for H-1α at δ 4.97 and H-1β at δ 4.71 and 4.67, and the ^1H-decoupled ^{13}C NMR spectrum contained C-1 signals at 105.45, 105.20, and 101.20 ppm. The chemical shifts of most of the ^1H and ^{13}C resonances of **DP** (Table II) were established mainly from COSY[8], RELAY COSY[9], and ^1H–^{13}C correlation (HETCOR) experiments[10]. Fig. 2 shows

TABLE II

^{1}H NMR and ^{13}C NMR data [a] for the trisaccharide repeating unit of the lithium-degraded polymer (**DP**) at 40°

Residue		Proton or carbon						
		1	2	3	4	5	6a	6b
→ 4)-α-Gal (**a**)	H	4.97	3.94	4.03	4.29	4.43	3.84	3.71
	^3J (Hz)	3.8	10.4	2.5				
	C	101.20	69.70	70.60	79.30	71.10		
→ 4)-β-Gal (**b**)	H	4.71	3.65	3.77	4.05	3.78		
	^3J (Hz)	7.6	10.1	3.1				
	C	105.45	71.70	73.20	78.10	75.90		
→ 3)-β-Gal (**c**)	H	4.67	3.78	3.88	4.15	3.73		
	^3J (Hz)	7.5	10.1	3.0				
	C	105.20	71.50	83.05	69.40	75.70		

[a] Chemical shifts in ppm relative to internal acetone at δ 2.23 for ^{1}H and at 31.07 ppm for ^{13}C.

the COSY contour map of **DP**. All the ^{1}H resonances for residue **a** were traced readily via their cross-peaks. The H-4/H-5 cross-peak had a low intensity and is not shown in the contour map. The chemical shifts for H-1/4 of residues **b** and **c** were established from the COSY (Fig. 2) and RELAY COSY (not shown) experiments. The chemical shift of the resonance for H-5 of **c** was determined from the NOE between H-4 and H-5 in a phase-sensitive NOESY experiment[11] (Table III). The ^{13}C resonances for residues **a**–**c** (Table II) were assigned by comparing the ^{1}H assignments with the ^{1}H–^{13}C correlation data obtained from a HETCOR experiment[10] (Fig. 3). Comparison of the NMR data with those for methyl glycosides[12,13] identified residue **a** as 4-linked α-Gal*p*, **b** as 4-linked β-Gal*p*, and **c** as 3-linked β-Gal*p*. These results accord with the methylation data for **DP**.

The sequence of the residues in the trisaccharide repeating unit of **DP** was established from the NOESY experiment[11]. The inter- and intra-residue NOE contacts are listed in Table III. The inter-residue NOEs involving each H-1 and the proton across the linkage permitted the structure **2** to be assigned to the repeating unit of **DP**.

→ 3)-β-D-Gal*p*-(1 → 4)-α-D-Gal*p*-(1 → 4)-β-D-Gal*p*-(1-

c **a** **b**

2

In an attempt to establish which 4-linked Gal in the main polysaccharide chain carried the pendant disaccharide, **PS** was hydrolysed partially with 0.5 M trifluoroacetic acid. However, a low yield of a complex mixture of oligosaccharides resulted and the hoped for aldotriouronic acid was not obtained. **PS** was therefore studied by 2D NMR spectroscopy.

2D NMR study of **PS**.—Assignment of the ^{1}H resonances of residues **a**–**e** in the repeating unit of **PS** was made from COSY[8] and 2D HOHAHA[14] experiments

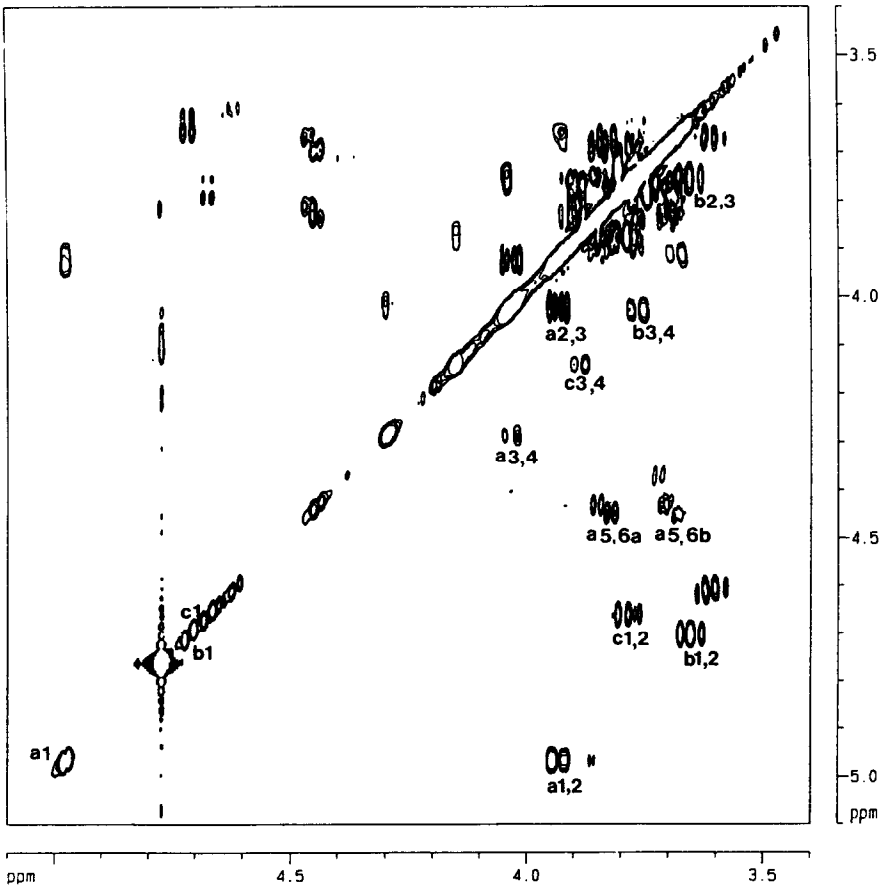


Fig. 2. COSY contour plot of the region δ 5.1–3.4 of the lithium-degraded polysaccharide (**DP**) of *E. coli* K102. The ^{1}H resonances of the sugar residues are labelled **a**–**c**: **a**1 connotes H-1 of residue **a** and **a**1,2 connotes the cross-peak between H-1 and H-2 of residue **a**, etc.

TABLE III

Inter- and intra-residue NOE contacts for the lithium-degraded *E. coli* K102 polysaccharide (**DP**) at 40°

Residue	Proton	NOE contact to
→ 4)-α-Gal (**a**)	H-1	4.05 (**b** H-4), 3.94 (**a**, H-2)
	H-4	4.03 (**a**, H-3)
	H-5	4.29 (**a**, H-4), 4.03 (**a**, H-3)
→ 4)-β-Gal (**b**)	H-1	3.88 (**c**, H-3), 3.77 (**b**, H-3)
	H-4	3.77 (**b**, H-3)
→ 3)-β-Gal (**c**)	H-1	4.29 (**a**, H-4), 3.88 (**c**, H-3)
	H-4	3.88 (**c**, H-3), 3.73 (**c**, H-5)

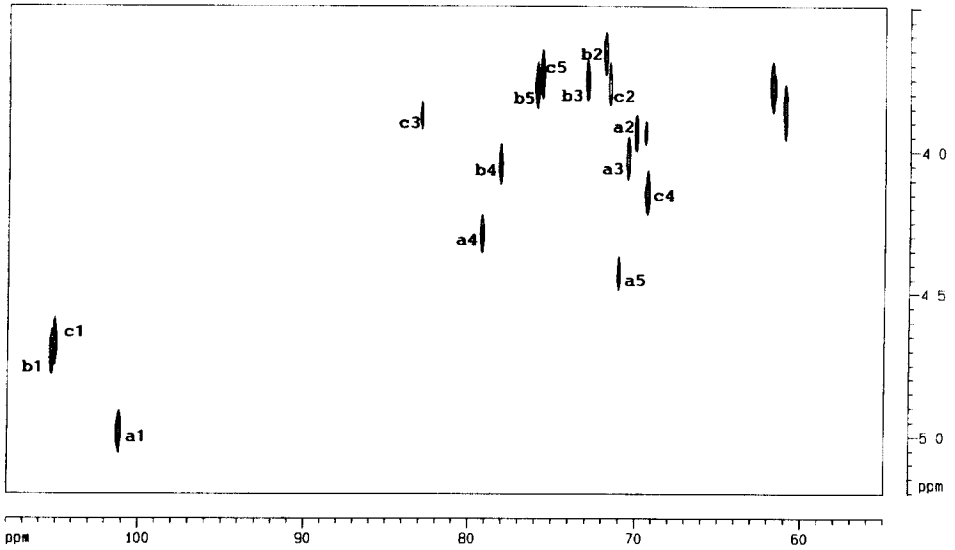


Fig. 3. $^{1}H-^{13}C$ correlation map of the spectral regions f_2 108–55 ppm (^{13}C) and f_1 5.2–3.5 ppm (^{1}H) of the lithium-degraded polysaccharide (**DP**) of *E. coli* K102: **a**1 refers to H-1 and C-1 of residue **a**, etc.

(Table IV); the residues were labelled arbitrarily **a**–**e** in order of decreasing chemical shifts of the H-1 resonances. The corresponding H-2 resonances of residues **a**–**e** and H-3/6 of residue **a** were established readily from the COSY

TABLE IV

^{1}H NMR and ^{13}C NMR data [a] for the *E. coli* K102 polysaccharide (**PS**) at 40°

Residue		Proton or carbon						
		1	2	3	4	5	6a	6b
α-Glc (**a**)	H	5.43	3.55	3.67	3.46	3.60	3.80	3.80
	^{3}J (Hz)	3.9	9.9	9.4	10.0			
	C	99.86	72.36	73.54	69.84	73.04	61.60	
→ 4)-α-Gal (**b**)	H	5.01	4.16	4.13	4.44	4.41	3.78	3.64
	^{3}J (Hz)	3.4	9.9	2.3				
	C	100.99	68.57	80.85	77.17	70.80	60.79	
→ 3)-β-Gal (**c**)	H	4.85	3.75	3.88	4.15	3.68		
	^{3}J (Hz)	7.7	10.4	3.8				
	C	103.74	71.40	82.84	69.27	75.26		
→ 4)-β-GlcA (**d**)	H	4.74	3.52	3.83	3.91	4.11		
	^{3}J (Hz)	7.7	9.0	8.9	9.2			
	C	105.07	73.96	76.47	77.82	75.19		
→ 4)-β-Gal (**e**)	H	4.74	3.67	3.77	4.05	3.78		
	^{3}J (Hz)	7.6	9.7	2.9	0.9			
	C	105.20	71.95	72.97	78.00	75.89		

[a] Chemical shifts in ppm relative to internal acetone at δ 2.23 for ^{1}H and 31.07 ppm for ^{13}C.

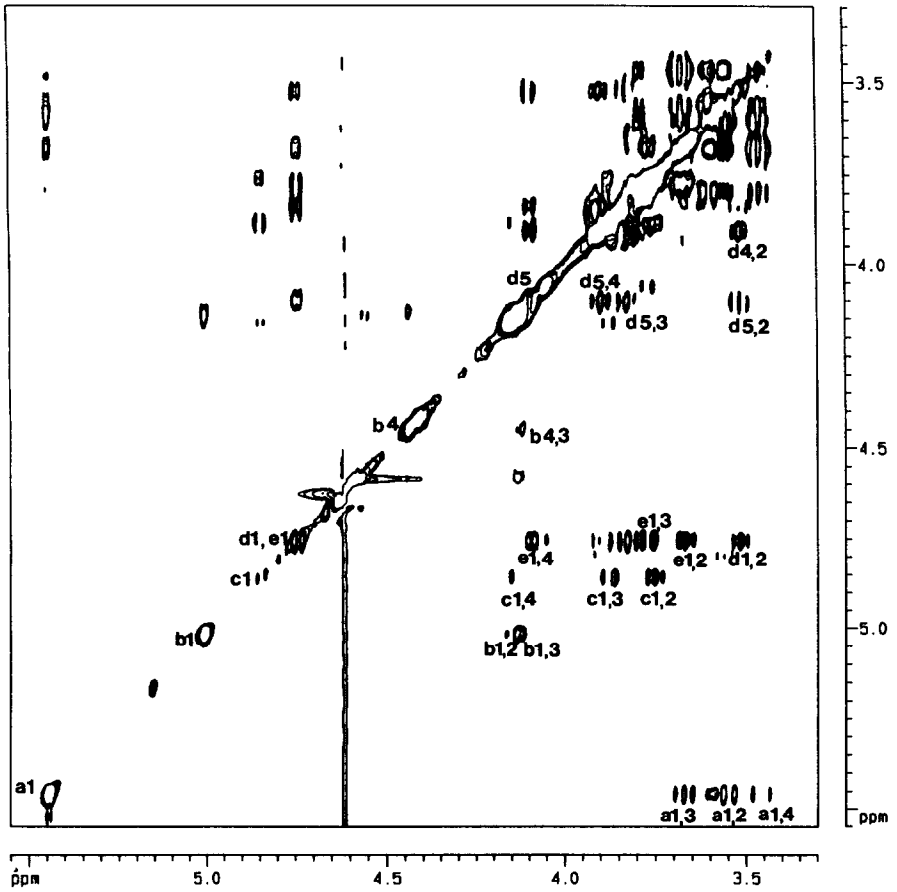


Fig. 4. 2D HOHAHA spectrum of the region δ 5.5–3.3 of the *E. coli* K102 capsular polysaccharide (**PS**): see Fig. 2 for key.

contour map. The chemical shifts for the resonances of H-3 and H-4 of residues **b** and **c** were established from the 2D HOHAHA experiment (Fig. 4). Once the chemical shift of the resonance of H-4 of **b** was known, the resonances for H-5,6a,6b in residue **b** could be traced in the COSY spectrum.

The resonances of H-1 of residues **d** and **e** overlapped at δ 4.74 in the COSY and HOHAHA experiments and they could not be assigned from the cross-peaks in the H-1 track in the HOHAHA contour map. However, since the H-2 resonance for each residue was known, it was possible to trace the chemical shifts for the ^{1}H resonances of residue **d** from its H-2 and H-5 tracks in the HOHAHA spectrum (Fig. 4). The chemical shifts for the resonances of H-2,3,4 of residue **e** could then be assigned by difference. The chemical shift for the resonance of H-5 of residue **e** was established from the H-4/H-5 NOE contact observed in a phase-sensitive NOESY experiment[11] (Table V).

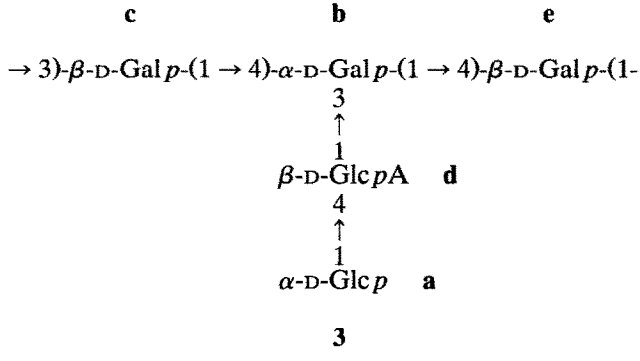
TABLE V

Inter- and intra-residue NOE contacts for the *E. coli* K102 polysaccharide (**PS**) at 40°

Residue	Proton	NOE contact to
α-Glc (**a**)	H-1	3.91 (**d**, H-4), 3.55 (**a**, H-2)
→ 4)-α-Gal (**b**)	H-1	4.16 (**b**, H-2), 4.05 (**e**, H-4)
	H-4	4.13 (**b**, H-3)
	H-5	4.13 (**b**, H-3)
→ 3)-β-Gal (**c**)	H-1	4.44 (**b**, H-4), 3.88 (**c**, H-3), 3.68 (**c**, H-5)
	H-3	3.68 (**c**, H-5)
	H-4	3.68 (**c**, H-5)
→ 4)-β-GlcA (**d**)	H-1	4.13 (**b**, H-3), 4.11 (**d**, H-5), 3.83 (**d**, H-3)
	H-5	3.83 (**d**, H-3)
→ 4)-β-Gal (**e**)	H-1	3.88 (**c**, H-3), 3.78 (**e**, H-5), 3.77 (**e**, H-3)
	H-4	3.78 (**e**, H-5)

The chemical shifts of the ^{13}C resonances of the sugar residues **a**–**e** were determined as before by comparing ^{1}H chemical shift data with the data obtained from a HETCOR experiment (Table IV and Fig. 5). These data, when compared with those for methyl glycosides[12,13], permitted residues **a**–**e** to be identified and their linkage positions to be established. In keeping with the methylation data for **PS**, C-4 of residues **d** and **e**, C-3 of **c** and C-3,4 of **b** experienced significant deshielding.

The sequence of the monosaccharides in the repeating unit was established from the NOESY experiment on **PS**. The observed inter- and intra-residue NOEs are presented in Table V. The strong inter-residue contacts involving each H-1 and the proton across the linkage indicated the pentasaccharide repeating unit of the *E. coli* K102 capsular polysaccharide to have the structure **3**.



3

The NMR data for the β-Gal residues in **PS** were similar to those for these residues in **DP**, but those for the α-Gal residue were quite different and confirmed the side chain to be attached to O-3 of the 4-linked α-Gal in **PS**.

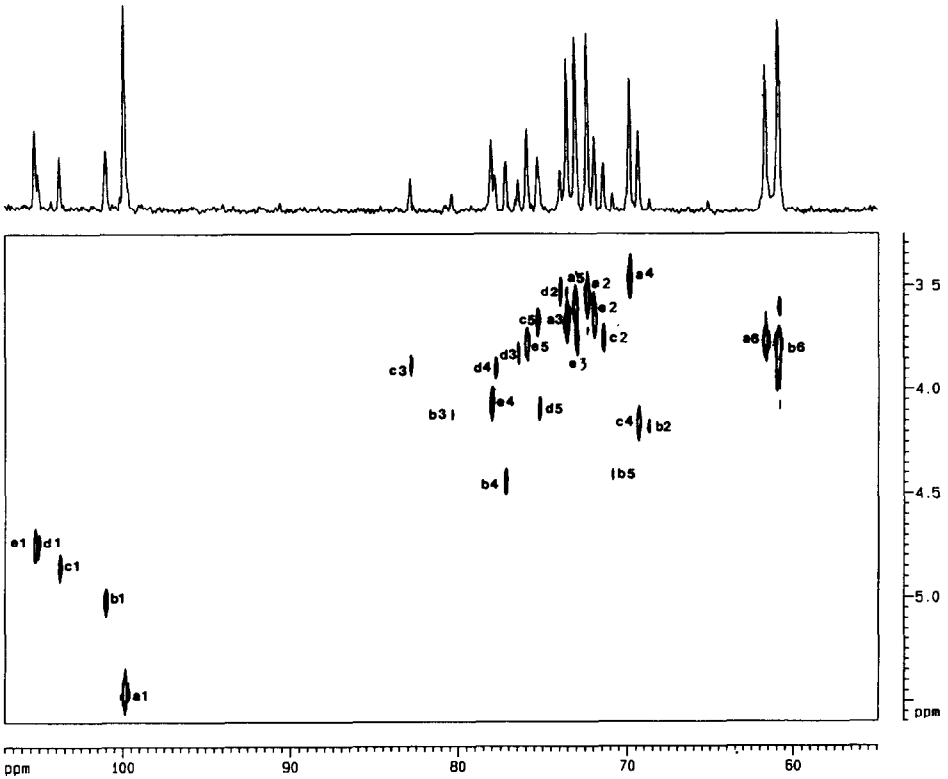


Fig. 5. ^{1}H–^{13}C correlation map of the spectral regions f_2 107–55 ppm (^{13}C) and f_1 5.6–3.3 ppm (^{1}H) of the *E. coli* K102 capsular polysaccharide (**PS**): see Fig. 3 for key.

The structure of the repeating unit of the *E. coli* K102 polysaccharide corresponds to the well established "3 + 2"pattern found in *Klebsiella* serotypes K13[15], K31[16], K74[17], and K80[18]. The repeating unit of *E. coli* K34[19], which has the same monosaccharide composition as K102, is the only other "3 + 2" structure thus far reported in the *E. coli* series. The repeating unit of *E. coli* K102 appears to be unique amongst both the *E. coli* and *Klebsiella* capsular antigens in that it has a main chain of trisaccharide repeating units which are composed entirely of Gal residues. *Klebsiella* K51[20], which has a tetrasaccharide repeating unit with a "2 + 2" pattern, and *Klebsiella* K15, which has a hexasaccharide repeating unit with a "4 + 1 + 1" pattern, are the only *Klebsiella* polysaccharides with a galactan main chain.

EXPERIMENTAL

General methods.—GPC was performed on columns of Biogel P-4 (2.6 × 95 cm) and dextran-calibrated Sephacryl S500 (1.6 × 68 cm), using 0.1 M sodium acetate buffer (pH 5) and a Waters R401 differential refractometer. GLC was performed

with a Hewlett–Packard 5890A gas chromatograph fitted with flame-ionisation detectors and a 3392A recording integrator. A DB-225 (J&W Scientific) fused-silica bonded-phase capillary column (30 m × 0.25 mm, 0.25-μm film) was used with He as the carrier gas, and operated at 205 or 220°, or at 220° for 5 min, then 1°/min to 250°. GLC–MS was conducted with a Hewlett–Packard 5988A system. Spectra were recorded at 70 eV and an ion-source temperature of 200°.

Polysaccharide samples were hydrolysed[21] with 4 M trifluoroacetic acid at 125° for 1 h. Acetylated aldononitriles were prepared as described[4]. Samples were carboxyl reduced by treatment with methanolic 3% HCl (16 h) boiling under reflux, and the methyl esters were reduced with $NaBH_4$ in anhyd MeOH. Methylations were carried out according to the Hakomori method[22] as modified by Phillips and Fraser[23]. Partially methylated alditol acetates were prepared conventionally by reduction of aqueous solutions of hydrolysates with $NaBH_4$ followed by acetylation with 1:1 acetic anhydride–pyridine (100°, 1 h). The methylated polysaccharide was base-degraded[6] in Me_2SO under N_2 with potassium dimsyl, and the products were trideuteriomethylated after 2 h.

Preparation of the E. coli *K102 polysaccharide* (**PS**).—A culture of *E. coli* O8:K102:H$^-$ (No. 6CB10/1) was obtained from Dr. I. Ørskov (Copenhagen) and propagated on Mueller–Hinton agar. The capsular polysaccharide was extracted with aq 1% phenol, separated from the cells by ultracentrifugation, and delipidated with 1% acetic acid at 100° for 1 h prior to purification via complexation with cetyltrimethylammonium bromide.

Treatment of **PS** *with lithium in ethylenediamine.*—**PS** in dry ethylenediamine was treated with lithium as described[7]. The product was isolated by GPC on Biogel P-4 and de-ionised by passage down a column of Amberlite IR-120 (H^+) resin. A portion of the degraded polysaccharide (**DP**) was methylated, and the products of an acid hydrolysate were converted into alditol acetates and subjected to GLC–MS (Table I, column IV).

NMR spectroscopy.—Each sample was deuterium-exchanged by freeze-drying solutions in D_2O, and then dissolved in 99.99% D_2O (~ 0.45 mL); acetone (δ 2.23 for ^1H, and 31.07 ppm for ^{13}C) was used as internal reference. Spectra were recorded at 40 and 80° with a Bruker AMX-400 spectrometer equipped with an X32 computer. The parameters used for 2D experiments were as follows. **DP**: COSY, two-step relay COSY, phase-sensitive NOESY [256 × 2048 data matrix, zero-filled to 1024 data points in t_1; 64 or 144 scans per t_1 value; spectral width, 801 Hz; recycle delay, 0.7 or 1.0 s; mixing delay, 0.3 s (NOESY); fixed delays of 0.036 s (relay COSY); unshifted sine-bell filtering in t_1 and t_2 (COSY and relay COSY); and shifted sine-squared filtering in t_1 and t_2 (NOESY)]; HETCOR, 64 × 2048 data points zero-filled to 256 data points in t_1; spectral width, 1400 Hz in t_1 and 9091 Hz in t_2; 4000 scans per t_1 value; recycle delay, 0.5 s; and a shifted sine-squared filter; **PS**: COSY, phase-sensitive NOESY, HOHAHA [256 × 2048 data matrix zero-filled to 1024 data points in t_1; 128, 160 and 112 scans, respectively, per t_1 value; spectral width, 1805 Hz; recycle delay, 0.6 or 1.0 s, mixing

delay, 0.3 s (NOESY); 50 mlev cycles (HOHAHA); unshifted sine-bell filtering (COSY) and shifted sine-squared filtering in t_1 and t_2 (NOESY and HOHAHA)]; HETCOR, spectral width, 1520 Hz in t_1; the rest of the parameters as for **DP**.

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