Carbohydrate Research, 167 (1987) 279–290
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Klebsiella SEROTYPE K39: STRUCTURE OF AN UNUSUAL CAPSULAR ANTIGEN DEDUCED BY USE OF A VIRAL ENDOGLUCOSIDASE*

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(Received September 8th, 1986; accepted for publication, in revised form, March 4th, 1987)

ABSTRACT

Methylation analysis and graded acid hydrolysis showed that the capsular polysaccharide of *Klebsiella* K39 is of the "5 + 1" type and that the repeating unit contains two nonequivalent residues of D-glucopyranosyluronic acid, one of which constitutes the branch point. A decision between two possible structures, in favor of that shown below, was reached by examination of the hexasaccharide obtained by depolymerization using phage ϕ39, which possessed an endo-β-D-glucosidase. Anomeric configurations were assigned by analysis of ^{1}H- and ^{13}C-n.m.r. spectroscopic data for the polysaccharide and oligosaccharides derived therefrom. The following structure is proposed for the *Klebsiella* K39 antigen:

-3(Glcβ1-4)GlcAβ1-2Manα1-4GlcAβ1-2Manα1-3Glcβ1-

The polysaccharide is the first in the *Klebsiella* genus thus far reported to contain two uronic acid residues per repeating unit.

INTRODUCTION

The capsular polysaccharide from *Klebsiella* serotype K39 was originally[1] thought to be composed of D-glucuronic acid, D-glucose, D-mannose, and D-galactose, but the inclusion of the last named sugar is due to an artifact. The polysaccharide from K39 is one of five strains in the same chemogroup and is the last member to be examined.

*Presented at the XIIIth International Carbohydrate Symposium, Ithaca, August 10–15, 1986.

0008-6215/87/$ 03.50

TABLE I

SUGAR ANALYSIS OF *Klebsiella* K39 POLYSACCHARIDE AND DERIVED PRODUCTS

Sugar[a]	*Molar ratios*[b]					
	I	*II*	*III*[c]	*IV*[c]	*V*	*VI*
Mannose	1.64	2.10	1.56	2.04	1.57	2.11
Glucose	2.00	4.00	1.00	3.00	1.00	3.00
Glucitol[d]					0.70	0.70

[a]Unless otherwise stated, determined as the peracetylated aldononitrile on a capillary DB-225 column at 225°. [b]I, native K39 polysaccharide; II, carboxyl-reduced K39 polysaccharide; III, oligosaccharide **A1**; IV, carboxyl-reduced oligosaccharide **A1**; V, oligoalditol **P1**; VI, carboxyl-reduced oligoalditol **P1**. [c]Determined as the alditol acetates on an SP 2340 column programmed at 195° for 4 min, then increasing by 2 deg.min^{-1} to 265°. [d]Glucitol hexaacetate.

RESULTS AND DISCUSSION

Composition and n.m.r. spectra. — *Klebsiella* K39 bacteria were grown on a sucrose-rich agar medium, and the acidic polysaccharide was isolated and purified by precipitation with cetyltrimethylammonium bromide (CTAB). The product was shown to be polydisperse on gel-permeation chromatography, but, after treatment with a cation-exchange resin (Amberlite IR 120 H^+), a product giving a single broad peak (M_r 3.4 × 10^4) was obtained.

Polysaccharide was isolated from several batches of bacteria, and it was found that some of these contained different proportions of a galactose-rich polymer. This phenomenon has been noted[2] in related studies on *Escherichia coli* but, in the case of the *Klebsiella* strains, has been observed for the first time in our experience with serotype K39. While no systematic study has been made, it appears that the proportion of galactan may be greatly reduced by growing the bacteria at 30°, rather than 37°, and for a shorter time than has been employed with other strains.

G.l.c. analysis of the original polysaccharide and of a carboxyl-reduced sample showed a large increase in the glucose content of the latter, and suggested that K39 polysaccharide contains equimolar amounts of mannose, glucose, and glucuronic acid (Table I, columns I and II).

The ^{1}H-n.m.r. spectrum of a partially autohydrolyzed sample of the polysaccharide indicated the presence of six anomeric protons corresponding to two α-(δ 5.20 and 5.45) and four β-linkages (δ 4.93, $J_{1,2}$ 8 Hz, 1 H, and δ 4.71–4.53, 3 H), see Table II. The assignment of these signals was accomplished by studying the ^{1}H-n.m.r. spectra of the oligosaccharides derived from the polysaccharide (see later). The ^{1}H-n.m.r. spectral data were substantiated by the ^{13}C-n.m.r. data for the polysaccharide (Table II)[3]. The peracetylated (−)-2-octylglycosides[4] prepared from an acid hydrolyzate of a carboxyl-reduced sample of the polysaccharide were all found on analysis by g.l.c. to have the D configuration. From the above results,

TABLE II

N.M.R. DATA FOR *Klebsiella* K39 AND DERIVED PRODUCTS[a]

^{1}H-N.m.r. data				*^{13}C-N.m.r. data*	
δ[b] (p.p.m.)	*$J_{1,2}$[c] (Hz)*	*Integral (no. of H)*	*Assignment[d]*	*δ (p.p.m.)*	*Assignment[f]*
-3(Glcβ1-4)GlcAβ1-2Manα1-4GlcAβ1-2Manα1-3Glcβ1- (K39 polysaccharide)					
(F)	(E)	(D)	(C)	(B)	(A)
5.45	n.o.	1.0	-2Manα1- (B)	102.8	-4GlcAβ1- (C)
5.20	n.o.	1.0	-2Manα1- (D)	102.6	-3,4GlcAβ1- (E)
4.93	8	1.0	Glcβ1- (F)	102.4	Glcβ1- (F)
4.71	—		-3Glcβ1- (A)	101.3	-3Glcβ1- (A)
to		3.0	-4GlcAβ1- (C)	100.9	-2Manα1- (D)
4.53	—		-3,4GlcAβ1- (E)	100.2	-2Manα1- (B)
GlcAβ1-2Manα1-4GlcAβ1-2Manα1-3Glc (**A1**)					
(E)	(D)	(C)	(B)	(A)	
5.47	n.o.	1.0	-2Manα1- (B)	103.0	-4GlcAβ1- (C)
5.23	4	0.45	-3Glcα (A)	102.3	GlcAβ1- (E)
5.15	n.o.	1.0	-2Manα1- (D)	100.9	-2Manα1- (D)
4.66	8	0.55	-3Glcβ (A)	100.2	-2Manα1- (B)
4.62	8	1.0	-4GlcAβ1- (C)	96.7	-3Glcβ (A)
4.57	8	1.0	GlcAβ1- (E)	93.0	-3Glcα (A)
GlcAβ1-2Manα1-4GlcAβ1-2Manα1-3Glucitol (**A1**-alditol)					
(E)	(D)	(C)	(B)	(A)	
5.37	n.o	1.0	-2Manα1- (B)		
5.27	n.o.	1.0	-2Manα1- (D)		
4.58	8	1.0	-4GlcAβ1- (C)		
4.51	8	1.0	GlcAβ1- (E)		
GlcAβ1-2Manα1-4GlcAβ1-2Man (**A2**)					
(E)	(D)	(C)	(B)		
5.35	n.o.	0.9	-2Manα (B)	103.0	-4GlcAβ1- (C)
5.15	n.o.	1.0	-2Manα1- (D)	102.4	GlcAβ1- (E)
4.93	n.o.	0.1	-2Manβ (B)	100.8	-2Manα1- (D)
4.60	8	1.0	-4GlcAβ1- (C)	93.3	-2Manα,β (B)
4.55	8	1.0	GlcAβ1- (E)		
GlcAβ1-2Manα1-4GlcAβ1-2Mannitol (**A2**-alditol)					
(E)	(D)	(C)	(B)		
5.11	n.o.	1.0	-2Manα1- (D)		
4.59	8	1.0	-4GlcAβ1- (C)		
4.55	8	1.0	GlcAβ1- (E)		

TABLE II *(continued)*

^{1}H-N.m.r. data						*^{13}C-N.m.r. data*	
δ^b (p.p.m.)		*$J_{1,2}^c$ (Hz)*	*Integral (no. of H)*	*Assignment^d*		*δ^e (p.p.m.)*	*Assignment^f*
Glcβ1-4GlcAβ1-2Manα1-4GlcAβ1-2Manα1-3Glc^g (**P1**)							
(F)	(E)	(D)	(C)	(B)	(A)		
5.46		n.o.	1.0		-2Manα1-(B) (-3Glcα)	100.14	-2Manα1-(B) (-3Glcα)
5.45		n.o.			-2Manα1-(B) (-3Glcβ)	100.04	-2Manα1-(B) (-3Glcβ)
5.23		4	0.4		-3Glcα(A)	92.95	-3Glcα(A)
5.15		n.o.	1.0		-2Manα1-(D)	100.86	-2Manα1-(D)
4.66		8	0.6		-3Glcβ(A)	96.66	-3Glcβ(A)
4.62		8	1.0		-4GlcAβ1-(C)	102.28	-4GlcAβ1-(C)
4.60		8	1.0		-4GlcAβ1-(E)	102.78	-4GlcAβ1-(E)
4.51		8	1.0		Glcβ1-(F)	102.93	Glcβ1-(F)
Glcβ1-4GlcAβ1-2Manα1-4GlcAβ1-2Manα1-3Glucitol (**P1**-alditol)							
(F)	(E)	(D)	(C)	(B)	(A)		
5.27		n.o.	1.0		-2Manα1-(B)	103.0	-4GlcAβ1-(C)
5.20		n.o.	1.0		-2Manα1-(D)	102.9	-4GlcAβ1-(E)
4.63		8	1.0		-4GlcAβ1-(C)	102.3	Glcβ1-(F)
4.62		8	1.0		-4GlcAβ1-(E)	101.5	-2Manα1-(B)
4.54		8	1.0		Glcβ1-(F)	100.8	-2Manα1-(D)

[a]For the origins of **A1, A2,** and **P1**, see text. [b]Chemical shift measured from internal acetone, δ 2.23. [c]n.o. = not observed. [d]For example, -2Manα1- refers to the anomeric proton of a 2-linked mannopyranosyl unit in the α-anomeric configuration. [e]As in *b* with acetone 31.07 p.p.m. [f]As in *d* but for ^{13}C nuclei. [g]^{1}H- and ^{13}C-signals are correlated (see Fig. 2).

a hexasaccharide repeating unit containing two uronic acid residues may be proposed for the *Klebsiella* K39 polysaccharide.

Methylation analysis. — The analytical values for the original and the carboxyl-reduced (after methylation) polysaccharides[5,6] are given in Table III, columns I and II. The most striking feature of the chromatogram of the reduced polymer was the appearance of two new peaks rather than the more usual single one. These additional peaks were identified as 2-*O*-methyl- and 2,3-di-*O*-methyl-D-glucose, thus confirming the presence of two uronic acid residues, one of which constitutes the branch point. Mild acid hydrolysis of the K39 polysaccharide gave a product which, on methylation and then carboxyl-reduction, showed partial cleavage of the glucopyranosyl side chains with a concomitant change in the ratios of 2-*O*-methylglucose and 2,4-di-*O*-methylglucose (Table III, column III). These results indicate that the side chains in the polysaccharide consist of single glucopyranosyl residues linked to position 4 of the glucopyranosyluronic branch points.

TABLE III

METHYLATION ANALYSES OF *Klebsiella* K39 POLYSACCHARIDE AND DERIVED PRODUCTS

Methylated sugar[a] *(as alditol acetate)*	*Molar ratios*[b,c]								
	I	*II*	*III*[d]	*IV*[d]	*V*[d]	*VI*[d]	*VII*[d]	*VIII*	*IX*
1,2,4,5,6-Glc						0.13		1.15	0.20
1,3,4,5,6-Man							0.36		
2,3,4,6-Glc	0.81	0.60	0.75					1.00	0.24
2,4,6-Glc	1.00	0.93	3.00	3.00	2.64				
3,4,6-Man	1.75	1.95				2.00	1.10	2.39	2.00
2,3,4-Glc					1.10		1.00		
2,3-Glc		1.00	1.63		1.00		0.83		1.76
2,4-Glc[e]									
2-Glc		0.65	0.54						

[a]1,2,4,5,6-Glc = 3-*O*-acetyl-1,2,4,5,6-penta-*O*-methylglucitol, *etc.*; all substitution patterns were confirmed by g.l.c.-m.s. [b]Unless otherwise stated, determined on a DB-225 capillary column at 205°. [c]**I**, native K39 polysaccharide; II, carboxyl-reduced K39 polysaccharide; III, partially hydrolyzed, carboxyl-reduced K39 polysaccharide; IV, **A1** oligosaccharide; V, carboxyl-reduced **A1** oligosaccharide; VI, **A1** oligoalditol; VII, carboxyl-reduced **A2** oligoalditol; VIII, **P1** oligoalditol; IX, carboxyl-reduced **P1** oligoalditol. Carboxyl reduction was performed after methylation in all cases. [d]Determined on an HIEFF-1B column programmed at 165° for 8 min, then increasing by 2 deg.min^{-1} to 200°. [e]Detected on the leading edge of this peak.

Partial acid hydrolysis. — The acidic fraction, obtained by ion-exchange chromatography from the partial acid hydrolyzate of the native polysaccharide, was chromatographed on paper to yield two oligosaccharides, **A1** and **A2**. The results of sugar analyses (Table I, columns III and IV), ^{1}H-n.m.r. spectroscopy (Table II), and methylation analyses (Table III, columns IV, V, and VI) of **A1**

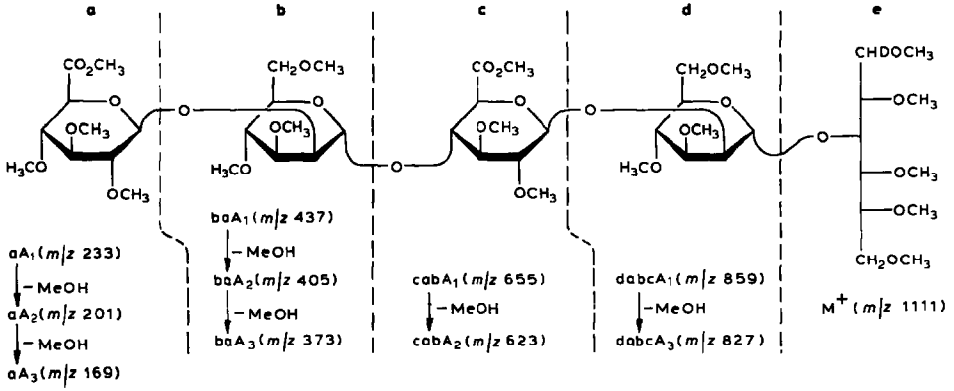

Fig. 1. Fragmentation pattern of oligosaccharide **A1** as the permethylated alditol.

showed it to be a pentasaccharide containing two uronic acid residues, with a non-reducing glucopyranosyluronic acid terminus and a glucose reducing end-group. Sequencing of the sugar residues in **A1** was achieved by e.i. mass spectrometry of the borodeuteride-reduced, permethylated oligosaccharide (Fig. 1), allowing the following structure to be written.

GlcAβ1-2Manα1-4GlcAβ1-2Manα1-3Glc
(E) (D) (C) (B) (A)
A1

The structure of **A2** follows from the comparison of its analytical (not shown), methylation, and ^{1}H-n.m.r. spectroscopic data with those of **A1**.

GlcAβ1-2Manα1-4GlcAβ1-2Man
(E) (D) (C) (B)
A2

The above results permit the proposal of a partial structure for the *Klebsiella* K39 antigen. The two glucuronic acid residues, however, do not occupy identical positions in the chain and the results do not enable the glucuronic acid residue that constitutes the branch point to be unequivocally identified. Bacteriophage depolymerization provides an elegant means for resolving this uncertainty in the structure.

Bacteriophage depolymerization[7]. — *Klebsiella* phage ϕ39 was used for the depolymerization of the K39 polysaccharide. Subsequent dialysis gave a mixture of oligosaccharides from which **P1** and **P2** were obtained by gel-permeation chromatography. ^{1}H- and ^{13}C-n.m.r. spectral analysis (Table II), and sugar analyses (Table I, columns V and VI) indicated that **P1** is a hexasaccharide composed of equimolar amounts of mannose, glucose, and glucuronic acid, with a glucose, rather than a mannose, reducing terminus. The results of the methylation analyses of **P1** (Table III, columns VIII and IX), in conjunction with the information from the partial acid-hydrolysis study, enable the structure for **P1** to be written as follows.

Glcβ1-4GlcAβ1-2Manα1-4GlcAβ1-2Manα1-3Glc
(F) (E) (D) (C) (B) (A)
P1

This structure represents the intact repeating unit of the K39 polysaccharide.

^{1}H-N.m.r. spectroscopy of **P2** indicated that this oligosaccharide was the dimer of the above repeating unit, so it was not further examined.

N.m.r. spectra of derived oligosaccharides. — Data from the ^{1}H- and ^{13}C-n.m.r. spectra of **A1** and **A2**, as well as from the heteronuclear correlation spectrum of **P1** (Fig. 2), are collected in Table II. Analysis of the ^{1}H-n.m.r. data for **A2** allows the signals at δ 5.35 (0.9 H) and 4.93 (0.1 H) to be assigned to H-1 of the α and β anomers, respectively, of the terminal mannose residue (B). It is interesting to note that 90% of this residue exists as the α anomer. The signal at δ 5.15 is assigned to H-1 of an α-linked, in-chain mannopyranosyl unit (D). The β signals at δ 4.55 ($J_{1,2}$ 8 Hz) and 4.60 ($J_{1,2}$ 8 Hz) are due to the glucopyranosyluronic unit (C) interior to B, and the terminal, nonreducing glucopyranosyluronic unit (E). The signal in the ^{13}C-n.m.r. spectrum of **A2** at 93.3 p.p.m. is due to C-1 of the α and β anomers of residue B. The second mannopyranosyl unit shows a signal for C-1 at 100.8 p.p.m., and sugars C and E give signals at 102.4 and 103.0 p.p.m.

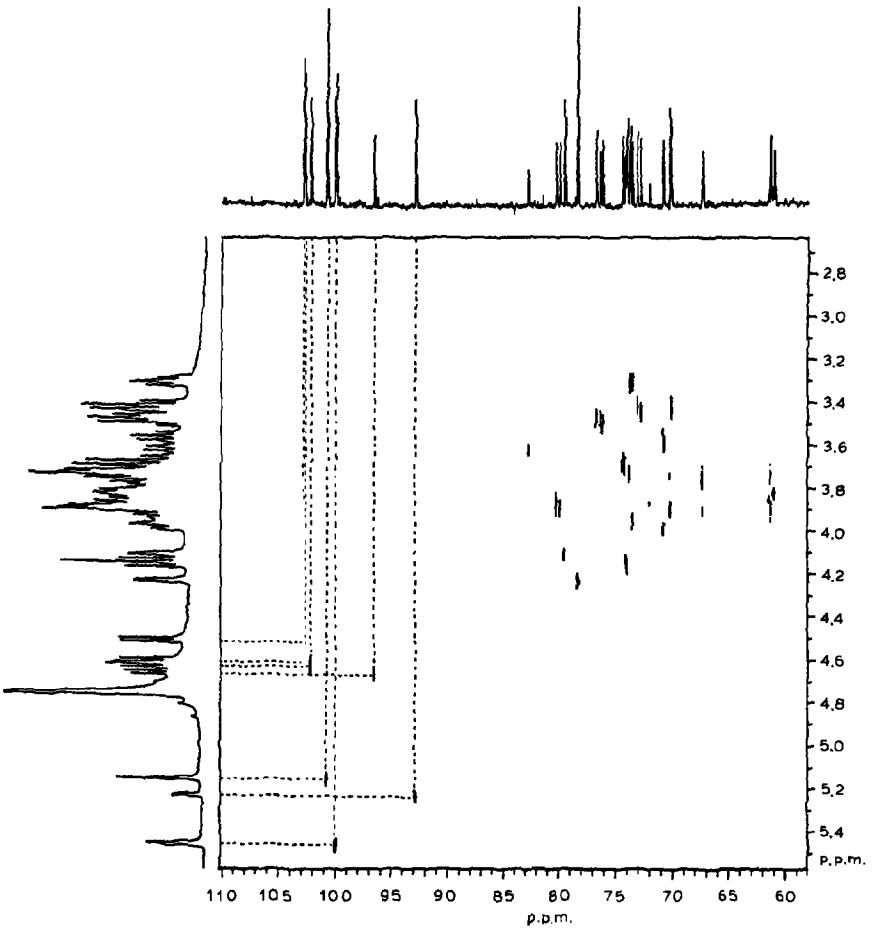

Fig. 2. 2D Heteronuclear correlation spectrum of oligosaccharide **P1**.

Signals in the ^{1}H-n.m.r. spectrum of **A1** (δ 5.23, 0.45 H, $J_{1,2}$ 4 Hz and δ 4.66, 0.55 H) confirm the presence of a glucose residue (A) at the reducing end of the oligosaccharide. The twinned α signal at δ 5.47 is ascribed to B, adjacent to the reducing terminus, and the signal at δ 5.15 to D. The signals at δ 4.62 ($J_{1,2}$ 8 Hz) and 4.57 ($J_{1,2}$ 8 Hz) are due to residues C and E. In the ^{13}C-n.m.r. spectrum of **A1**, the signal at 100.2 p.p.m. is due to B, the remaining signals being assigned by comparison with those of **A2**.

The relationships between the proton and carbon-13 signals of **P1** were established by 2D heteronuclear correlation spectroscopy (see Fig. 2) and assignment of these signals was made by comparison with 1D n.m.r. spectra recorded for **A1** and **A2**. The signals for A, B, and D are readily assigned on the same basis as discussed above. Residues C, E, and F give β signals at δ 4.62 (^{1}H) and 102.28 (^{13}C), δ 4.60 (^{1}H) and 102.78 (^{13}C), and δ 4.51 (^{1}H) and 102.93 (^{13}C). These signals could not be unequivocally assigned.

CONCLUSION

The information acquired during the structural investigation of the *Klebsiella* K39 polysaccharide allows the following structure to be proposed for this antigen:

-3(Glcβ1-4)GlcAβ1-2Manα1-4GlcAβ1-2Manα1-3Glcβ1-.

This is the first structure in the *Klebsiella* K-antigen series shown to have two uronic acid residues per repeating unit. During the analysis, four acidic oligosaccharides (**A1, A2, P1,** and **P2**) were isolated. Immunochemical studies[8] indicate that the *Klebsiella* K39 antigen has structural features in common with the *Klebsiella* K1 (ref. 9) and K31 (ref. 10) antigens. Comparison of the above structures shows that all three contain a 3-linked β-glucopyranosyl unit linked to a substituted glucopyranosyluronic residue. This may constitute the immunodominant region for these antigens; however, the degree and site of substitution, and the anomeric configuration, of the glucopyranosyluronic residue do vary.

EXPERIMENTAL

General methods. — Infrared spectra were recorded on a Beckman IR8 spectrophotometer for solutions in dry chloroform. Optical rotations of aqueous solutions were measured at 21–23° with a Perkin–Elmer model 141 polarimeter, using a 1 cm cell. Unless otherwise stated, g.l.c. analyses were performed on a Hewlett Packard 5890A gas chromatograph fitted with flame ionization detectors and a Hewlett Packard 3392A recording integrator for measuring areas. Separations were achieved on a DB-225 bonded-phase capillary column (30 m × 0.25 mm) having a film thickness of 0.25 μm, using helium as carrier gas, and operating isothermally at 205° or 225°. G.l.c.-m.s. analyses were performed on a VG

Micromass model 16F (40 eV ionization energy) or model 12 (70 eV ionization energy) spectrometer. Mass spectra of a reduced permethylated oligosaccharide from **A1** were recorded on a Kratos MS50 spectrometer. N.m.r. spectra were recorded on a Bruker WP-80, Bruker WH-400, Bruker WM-500 Ft, or a Varian XL-100 spectrometer at ambient temperature (30°) or at 95°. Samples were deuterium-exchanged by freeze-drying from deuterium oxide solutions; acetone (δ 2.23 for ^{1}H- and 31.07 p.p.m. for ^{13}C-spectra) was used as the internal standard.

Solutions were concentrated under reduced pressure at bath temperatures not exceeding 40° and residues from acid hydrolyzates were subjected to several additions and evaporations of water. Peracetylated aldononitrile (PAAN) derivatives were prepared by the method of McGinnis[11]. Alditol acetates were prepared by the reduction of aqueous solutions of hydrolyzates with sodium borohydride or sodium borodeuteride, followed by acetylation with 1:1 acetic anhydride–pyridine for 1 h at 100°. Unless otherwise stated, carboxyl reduction was achieved by methanolyzing the sample in dry 3% methanolic hydrogen chloride under reflux for 16–20 h, neutralizing the mixture with $PbCO_3$, and treating it with sodium borohydride in anhydrous methanol. Methylation of samples was carried out using the Hakomori method[5], as modified by Sandford and Conrad[6].

Preparation and properties of the K39 polysaccharide. — An authentic culture of *Klebsiella* K39 was obtained from Dr. I. Ørskov (Copenhagen), and grown on sucrose-rich agar[12]. After three days at 30°, the acidic capsular polysaccharide was isolated from the bacterial slime and purified by precipitation with CTAB as previously described[13]. The polysaccharide had $[\alpha]_D$ +6.8° (*c* 5.80, water). The native polysaccharide (3.1 mg) was analyzed by gel-permeation chromatography, performed on a column of Sephacryl S500 (1.6 cm × 65 cm), calibrated with dextrans, and using 500:5:2 water–pyridine–acetic acid as eluant at a flow rate of 21 mL.h^{-1}. Fractions of 1 mL were collected and analyzed by the phenol–sulfuric acid method[14]. A sample of the native polysaccharide was passed through a column of Amberlite IR 120 H^+ resin and similarly analyzed by gel-permeation chromatography.

Sugar composition. — The polysaccharide (2.2 mg) was hydrolyzed [2M trifluoroacetic acid (TFA), 16 h, 100°] and the acid was removed. The hydrolyzate was converted into the PAAN derivatives and analyzed by g.l.c. (Table I, column I). Dried polysaccharide (9.8 mg) was carboxyl-reduced and the product was hydrolyzed (2M TFA, 18 h, 100°), the hydrolyzate being analyzed (PAAN) by g.l.c. (Table I, column II).

Methylation analysis. — Polysaccharide (47.9 mg), in the acid form, was methylated and the methylation was shown to be complete by i.r. spectroscopy. A sample of the permethylated polysaccharide was hydrolyzed (2M TFA, 19 h, 100°), derivatized (alditol acetates), and analyzed by g.l.c. and g.l.c.-m.s. (Table III, column I). A second sample was carboxyl-reduced, hydrolyzed, and similarly analyzed (Table III, column II). K39 polysaccharide (254 mg) was hydrolyzed in 0.02M TFA for 3 h on a steam bath. The hydrolyzate was dialyzed against running

tap water and freeze-dried. A sample of this product was methylated and then carboxyl-reduced with lithium aluminum hydride in tetrahydrofuran by refluxing for 3 h followed by 18 h at room temperature. The product was hydrolyzed in 2M TFA for 17 h on a steam bath and the alditol acetates were prepared for g.l.c. analysis. This was performed on a Hewlett Packard 5710A gas chromatograph (HP 5710A) fitted with dual flame ionization detectors; column 3% HIEFF-1B; temperature program 165° for 8 min, then increasing by 2 deg.min $^{-1}$ to 200° (see Table III, column III).

Partial acid hydrolysis. — The native polysaccharide (700 mg) was hydrolyzed in M TFA (90 mL) for 7 h on a steam bath. The hydrolyzate was concentrated, and the neutral and acidic fractions were separated on a column of Bio-Rad AG 1-X2, the former being eluted with water and the latter with 10% formic acid. The acidic fraction was applied to Whatman 3 MM chromatography paper and irrigated with 2:1:1 v/v 1-butanol–acetic acid–water for 4 d. The chromatographic bands were then visualized with silver nitrate and two fractions were isolated, namely **A1** (80 mg) and **A2** (36 mg). Fraction **A2** was further purified, using Whatman No. 1 paper and irrigating with the same solvent for 4 d, to give a final yield of 15 mg.

A sample of **A1** (<1 mg) was hydrolyzed (3M TFA, 20 h, steam bath), and a second portion (3 mg) was carboxyl-reduced and hydrolyzed (2M TFA, 21 h, steam bath). The alditol acetates derived from each hydrolyzate were analyzed by g.l.c.: HP 5710A; column 3% SP 2340; temperature program 195° for 4 min, then increasing by 2 deg.min^{-1}to 265° (see Table I, columns III and IV). Fraction **A1** (12 mg) was methylated, a portion of the product was hydrolyzed (2M TFA, 17 h, steam bath), and the alditol acetates were prepared. A second portion of the permethylated **A1** was carboxyl-reduced with lithium aluminum hydride as described above, hydrolyzed (2M TFA, 12 h) and the alditol acetates were prepared. The permethylated alditol acetates were analyzed by g.l.c.: HP 5710A; column 3% HIEFF-1B; temperature program 165° for 8 min, then increasing by 2 deg.min^{-1} to 200°. The results (Table III, columns IV and V) were confirmed by g.l.c.-m.s. The oligosaccharide alditol was prepared by reducing an aqueous solution of **A1** (4.3 mg) with sodium borodeuteride for 2.5 h). The product was then methylated, hydrolyzed (2M TFA, 14 h, steam bath), and the derived alditol acetates were analyzed by g.l.c.-m.s. (Table III, column VI). A second sample of **A1** (7.7 mg) was similarly converted to the permethylated oligosaccharide alditol and analyzed by e.i.-m.s., with beam energies of 20 eV and 70 eV. The mass spectral data thus obtained are presented in Fig. 1.

A sample of **A2** (7.6 mg) was converted to the permethylated oligosaccharide alditol and carboxyl-reduced with lithium aluminum hydride, as described above. The product was analyzed by g.l.c.-m.s. as the alditol acetates (Table III, column VII).

Bacteriophage depolymerization. — Bacteriophage ϕ39 was isolated from sewage, purified by repicking single plaques, and propagated on its host strain, *Klebsiella* K39, in nutrient broth until a titre of 2.5×10^{13} plaque-forming units

(PFU) was reached. After purification by dialysis and volume reduction under reduced presure at 30°, 145 mL of bacteriophage solution containing 1.3×10^{13} PFU was used to cause depolymerization of K39 polysaccharide (504.5 mg) for 72 h at 30°, as previously described[15]. Dialysis of the reaction mixture yielded a crude degradation product from which **P1** (178.2 mg) and **P2** (171.5 mg) were isolated by gel-permeation chromatography using a column of Bio-Gel P4 (2.6 cm × 70 cm) and 500:5:2 water–pyridine–acetic acid as eluant.

A sample of **P1** (4.9 mg) was reduced (sodium borohydride) and methanolyzed as described above. A portion of the product was hydrolyzed (2M TFA, 18 h, 100°), while the remainder was carboxyl-reduced and then hydrolyzed (2M TFA, 16 h, 100°). The hydrolyzates were analyzed as the PAAN derivatives by g.l.c. (Table I, columns V and VI). The permethylated oligosaccharide alditol was prepared from **P1** (10.0 mg) by reduction in aqueous solution with sodium borohydride followed by methylation. A portion of the product was hydrolyzed (2M TFA, 17 h, 100°), the remainder being carboxyl-reduced and hydrolyzed (2M TFA, 16 h, 100°). The derived alditol acetates from each hydrolyzate were analyzed by g.l.c. and g.l.c.-m.s. (Table III, columns VIII and IX).

Determination of absolute configuration[4]. — K39 polysaccharide (13.7 mg) was carboxyl-reduced, then dissolved in (−)-2-octanol (0.5 mL) with one drop of TFA and heated in a sealed ampoule overnight at 130°. After concentration to dryness under vacuum at 55°, the residue was acetylated with 1:1 acetic anhydride–pyridine (1 h, 100°). The peracetylated octyl glycosides thus prepared were analyzed by g.l.c. (230°).

ACKNOWLEDGMENTS

The authors thank Dr. I. Ørskov (International Escherichia Centre, Copenhagen) for the culture of *Klebsiella* K39, Professor A. M. Stephen (University of Cape Town) for arranging the mass spectrometry, and Mr. I. Antonowitz (N.C.R.L., Pretoria) for recording the 500 MHz n.m.r. spectra. We are grateful to the C.S.I.R. (Pretoria) for financial support of H.P. and for the doctoral bursary granted to A.N.A., and to N.S.E.R.C. (Ottawa) for continued financial assistance.

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