Carbohydrate Research, 92 (1981) 287–298
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

STRUCTURAL INVESTIGATION OF THE CAPSULAR POLYSACCHARIDE OF *Klebsiella* SEROTYPE K26

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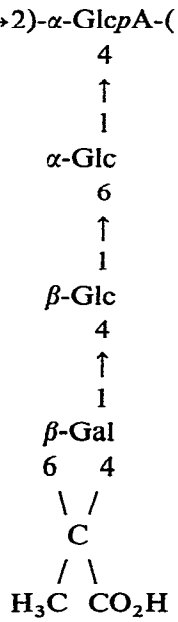
(Received December 27th, 1980; accepted for publication, January 23rd, 1981)

ABSTRACT

The structure of the capsular polysaccharide from *Klebsiella* K26 has been determined by using the techniques of methylation, periodate oxidation, partial hydrolysis, and β-elimination. N.m.r. spectroscopy (^1H and ^{13}C) was used to establish the nature of the anomeric linkages and to identify oligosaccharides obtained by the different degradative techniques employed.

The polysaccharide is comprised of repeating units of the heptasaccharide shown.



INTRODUCTION

Klebsiella serotype K26 is one of 17 strains whose capsular polysaccharides are composed of D-glucuronic acid, D-galactose, D-glucose, and D-mannose. Eight

of these polysaccharides have 1-carboxyethylidene substituents, and, in this subgroup, the structures of *Klebsiella* K7 (ref. 1), K13 (ref. 2), K30 (ref. 3), K31 (ref. 4), and K46 (ref. 5) are known. We now report the structure of the capsular polysaccharide from *Klebsiella* serotype K26, a member of this subgroup; those of serotypes K35 and K69 remain to be examined.

This polymer is shown to be based on a heptasaccharide repeating-unit ("four-plus-three" type) and, in this respect, is similar to the polysaccharide isolated[6] from *Klebsiella* K41. In the latter, however, the D-glucuronic acid residue is in the side chain, and the branch point is a D-galactofuranosyl residue. The structure of the polysaccharide from *Klebsiella* K26 is, therefore, unique in this series.

RESULTS AND DISCUSSION

Composition and n.m.r. spectra. — The isolation and purification of the polysaccharide were achieved as previously described[1-6]. The purified product obtained after Cetavlon precipitation had $[\alpha]_D$ +80°, and was shown to be homogeneous by gel-permeation chromatography ($\overline{M}_w = 1 \times 10^7$). The ^{1}H-n.m.r. spectrum of the polysaccharide indicated the presence of seven anomeric protons, corresponding to four α- and three β-linkages; also, one 1-carboxyethylidene acetal grouping per repeating unit was detected. The ^{13}C-n.m.r. spectrum confirmed these results, and, from the chemical shift (25.7 p.p.m.) of the methyl group of the acetal, it was possible to assign the *R* configuration to the acetal carbon atom[7].

TABLE I

METHYLATION ANALYSES OF K26 POLYSACCHARIDE AND DERIVED PRODUCTS

Methylated sugars[a] *(as alditol acetates)*	T[b]			*Mole %*[c]		
	Column A[d] *(SP 2340)*	*Column B*[e] *(ECNSS-M)*	*Column C*[e] *(OV-225)*	*I*[f]	*II*	*III*
2,3,4,6-Gal	1.14	1.25	1.19	—	—	14.7
3,4,6-Man	1.47	1.95	1.82	12.4	13.7	13.3
2,4,6-Man	1.47	2.08	1.90	17.8	16.0	14.0
2,4,6-Gal	1.51	2.29	2.03	15.2	15.3	12.2
2,3,4-Glc	1.62	2.50	2.26	15.4	17.5	16.8
2,3,6-Glc	1.70	2.50	2.26	16.3	17.9	17.2
3,6-Glc	2.04	4.40	3.70	—	11.2	11.7
2,3-Gal	2.24	5.64	4.70	11.4	8.4	—
3-Glc	—	9.49	7.40	11.5	—	—

[a]2,3,4,6-Gal = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, *etc.* [b]Relative retention-time, referred to 2,3,4,6-Glc as 1.00. [c]Values are corrected by use of the effective, carbon-response factors given by Albersheim *et al.*[19]. [d]Programmed for 4 min at 160°, and then 2°/min to 230°. [e]Isothermal, 170°. [f]I, Original capsular polysaccharide; II, compounds from methylation of carbodiimide-reduced, capsular polysaccharide; III, compounds from methylation of the deacetalated, methylated, reduced, capsular polysaccharide.

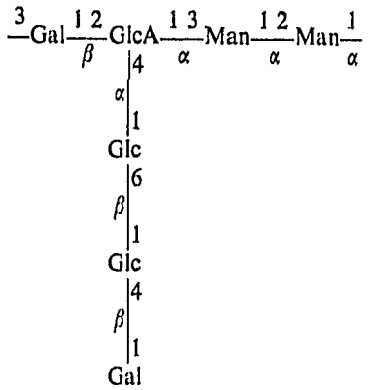
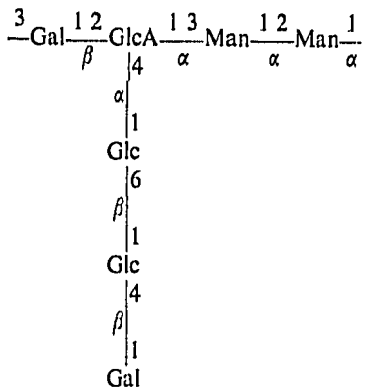
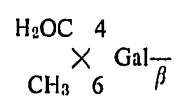
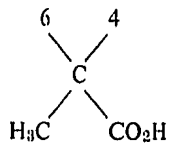
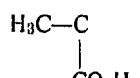
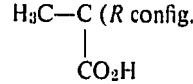
TABLE II

N.M.R. DATA FOR *Klebsiella* K26 POLYSACCHARIDE AND THE DERIVED OLIGOSACCHARIDES

Compound[a]	^{1}*H-N.m.r.data*				^{13}*C-N.m.r. data*	
	δ[b]	$J_{1,2}$ *(Hz)*	*Integral proton*	*Assignment*[c]	*p.p.m.*[d]	*Assignment*[e]
GlcAα(1→3)Man-OH	5.32	2.5	1	GlcAα—	101.42	GlcAα—
A_1	5.20	2	0.6	3-Manα—OH	94.82	3-Manβ—OH
	4.92	2	0.4	3-Manβ—OH	94.33	3-Manα—OH
GlcAα(1→3)Manα(1→2)Man-OH	5.37	2	3	3-Manα—	102.86	3-Manα—
A_2	5.34	2.5		GlcAα—	101.36	GlcAα—
	5.18	2	0.3	unknown		
	5.08	2	0.7	2-Manα—OH	93.56	2-Manβ—OH
	4.94	1	0.3	2-Manβ—OH	93.35	2-Manα—OH
GlcAα(1→3)Manα(1→2)Manα(1→3)Gal-OH	5.34	2.5	2	GlcAα—	103.06	3-Manα—
A_3	5.29	2		3-Manα—	101.33	GlcAα—
	5.17	2	0.3	3-Galα—OH	97.23	3-Galβ—OH
	5.07	2	1	2-Manα—	95.38	2-Manα(1→3)Galβ—OH
	4.64	7	0.7	3-Galβ—OH	95.11	2-Manα(1→3)Galα—OH
					93.10	3-Galα—OH

TABLE II *(continued)*

Compound[a]	^{1}*H-N.m.r. data*				^{13}*C-N.m.r. data*	
	δ[b]	$J_{1,2}$ *(Hz)*	*Integral proton*	*Assignment*[c]	*p.p.m.*[d]	*Assignment*[e]
Glc-$\frac{1\,6}{\beta}$Glc-OH	5.24	3	0.4	6-Glc-OH (α)	103.5	Glc- (β)
N_1	4.65	8	0.6	6-Glc-OH (β)	96.81	6-Glc-OH (β)
	4.52	8	1	Glc- (β)	92.95	6-Glc-OH (α)
Gal-$\frac{1\,4}{\beta}$Glc-$\frac{1\,6}{\beta}$Glc-OH	5.24	3	0.4	6-Glc-OH (α)		
N_2	4.67	7.5	0.6	6-Glc-OH (β)		
	4.55	7.5	0.4	4-Glc-$\frac{1\,6}{\beta}$Glc-OH (α)		
	4.53	7.5	0.6	4-Glc-$\frac{1\,6}{\beta}$Glc-OH (β)		
	4.46	7.5	1	Gal- (β)		
Gal-$\frac{1\,2}{\beta}$GlcA-$\frac{1\,3}{\alpha}$Man-$\frac{1\,2}{\alpha}$Gly	5.51	3	1	2-GlcA- (α)	105.41	Gal- (β)
SH_1	5.10	2	1	3-Man- (α)	100.92	3-Man- (α)
	4.64	7	1	Gal- (β)	100.22	2-GlcA- (α)

	5.49	2	2	4-GlcA—; 6-Glc—		
	5.27	2	1	3-Man—		
	5.01	2	1	2-Man—		
	4.63	7.5	1	Gal—		
	4.51	7.5	1	4-Glc—		
	4.45	7.5	1	3-Gal—		
	5.50		2	4-GlcA—; 6-Glc—	105.41	
	5.28		1	3-Man—	103.70	3-Gal—; 4-Glc—
	5.08		1	2-Man—	103.24	3-Man—
	4.7–4.5		3	Gal— 4-Glc— 3-Gal—	101.00 100.76	6-Glc— 2-GlcA—
	1.65		3		99.87 25.77	2-Man— 

[a]For the source of A_1, A_2, A_3, N_1, N_2, and **SH**, see text. [b]Chemical shift relative to internal acetone; δ 2.23 downfield from sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS). [c]The numerical prefix indicates the position in which the sugar is substituted; the α or β, the configuration of the glycosidic bond, or the anomer in the case of a (terminal) reducing-sugar residue. Thus 3-Gal$\underset{\alpha}{}$— refers to the anomeric proton of a 3-linked galactosyl residue in the α-anomeric configuration. The absence of a numerical prefix indicates a (terminal) nonreducing group. [d]Chemical shift in p.p.m. downfield from Me_4Si, relative to internal acetone; 31.07 p.p.m. downfield from D.S.S. [e]As in *c*, but for ^{13}C nuclei.

Paper chromatography of an acid hydrolyzate of the polysaccharide showed galactose, glucose, glucuronic acid, mannose, and an aldobiouronic acid. Determination, as alditol acetates, of the sugars obtained from the carboxyl-reduced polysaccharide gave mannose, galactose, and glucose in the ratios of 2.0:2.0:3.0. The glucose and mannose were proved to be of the D configuration by circular dichroism (c.d.) measurements made on the alditol acetates. Galactose and glucuronic acid were also assigned the D configuration, from c.d. measurements made on partially methylated derivatives that were isolated subsequently.

Methylation analysis. — Methylation of the K26 polysaccharide, followed by reduction of the uronic ester, hydrolysis, derivatization as alditol acetates, and g.l.c.–m.s. analysis, gave the values shown in Table I, column I. These results indicate that the polysaccharide consists of a heptasaccharide repeating-unit having a branch on the glucosyluronic acid residue; the terminal glycosyl residue is a unit of galactose which has a 1-carboxyethylidene group present as an acetal spanning O-4 and O-6. Methylation of the carboxyl-reduced polysaccharide (see Table I, column II) showed the disappearance of the 3-*O*-methylglucose, and the formation of 3,6-di-*O*-methylglucose, confirming that the glucuronic acid was the branch point. The results of removal of the modified acetal (reduction and methylation) and re-methylation (see Table I, column III) confirmed the location of this group by the formation of 2,3,4,6-tetra-*O*-methylgalactose and the disappearance of the 2,3-di-*O*-methylgalactose.

Partial hydrolysis. — Partial hydrolysis of the native polysaccharide with acid was followed by separation of the acidic and neutral fractions by ion-exchange chromatography.

TABLE III

ANALYSIS OF THE OLIGOSACCHARIDES FROM PARTIAL HYDROLYSIS OF *Klebsiella* K26 POLYSACCHARIDE

Oligosaccharide	$[\alpha]_D$ *(degrees)*[a]	*Sugar analysis (as alditol acetates) (molar proportions)*	*Methylation analysis (as alditol acetates) (molar proportions)*
A_1	+64	Man (1) Glc(GlcA) (1)	2,3,4-Glc (1) 2,4,6-Man (0.9)
A_2	+79	Man (2) Glc(GlcA) (1)	2,3,4-Glc (1) 2,4,6-Man (1) 3,4,6-Man (0.9)
A_3	+106	Gal (1) Man (2) Glc(GlcA) (1)	2,3,4-Glc (1) 2,4,6-Man (1) 3,4,6-Man (0.9) 2,4,6-Gal (0.7)
N_1	+8.8	Glc	2,3,4,6-Glc (1) 2,3,4-Glc (0.9)
N_2	+22	Gal (1) Glc (2)	2,3,4,6-Gal (1) 2,3,6-Glc (1) 2,3,4-Glc (0.9)

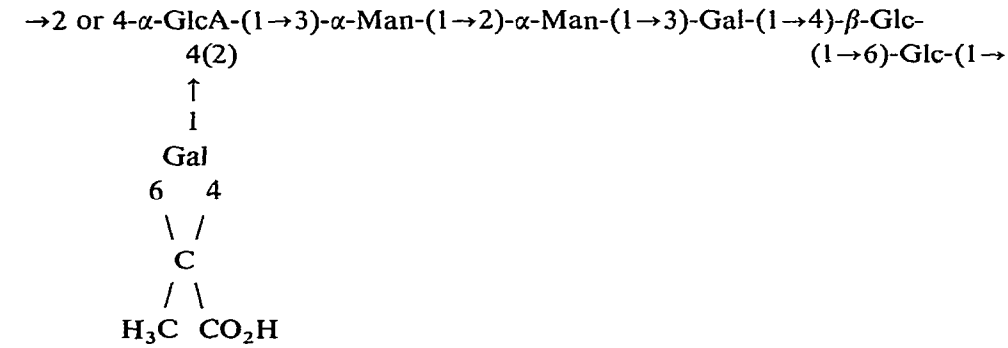
[a]In water.

The neutral fraction contained monosaccharides and a disaccharide (N_1), which were further separated by gel-permeation chromatography. The acidic fraction contained three acidic oligosaccharides (A_1, A_2, and A_3) which were also separated by gel-permeation chromatography. On the basis of their n.m.r.-spectral data (see Table II) and their methylation analysis (see Table III), the structures of these compounds were shown to be as follows.

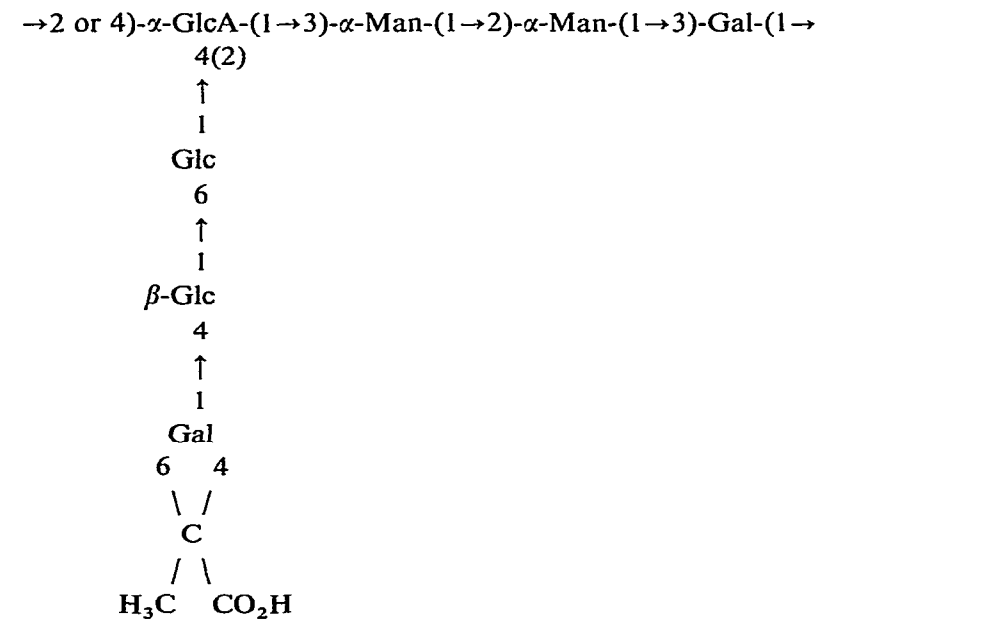
A_1 α-GlcA-(1→3)-Man A_2 α-GlcA-(1→3)-α-Man-(1→2)-Man
A_3 α-GlcA-(1→3)-α-Man-(1→2)-α-Man-(1→3)-Gal N_1 β-Glc-(1→6)-Glc

The aldotetraouronic acid (A_3) obtained from partial hydrolysis had previously been isolated from other *Klebsiella* capsular polysaccharides, K21 (ref. 8) and K74 (ref. 9); gentiobiose (N_1) has been also isolated from K41 (ref. 6) and K61 (ref. 10) by partial hydrolysis.

Two possible structures (A or B) are consistent with the results thus far discussed.



A



B

Periodate oxidation, followed by Smith hydrolysis, and reduction of the product, gave one oligomer which, on the basis of the n.m.r. data and methylation analysis, was shown to be

β-Gal-(1→2)-α-GlcA-(1→3)-α-Man-(1→2)-glycerol.

This result is conclusive evidence in favor of structure B, but still leaves two anomeric linkages unassigned (one α and one β).

Selective hydrolysis with acid. — Treatment of the polysaccharide with 0.01M TFA during 5 h at 95°, and dialysis against distilled water, afforded a nondialyzable, polymeric material and a dialyzate.

The dialyzable material was shown by paper chromatography to be composed of galactose, a disaccharide (identical to $\mathbf{N_1}$) and a trisaccharide ($\mathbf{N_2}$). On the basis of the n.m.r.-spectral data (see Table II) and methylation analysis (see Table III), the structure of $\mathbf{N_2}$ was shown to be as follows.

β-Gal-(1→4)-β-Glc-(1→6)-Glc
$\mathbf{N_2}$

The structure of $\mathbf{N_2}$ demonstrates that the terminal galactose unit has the β configuration, and, thus, the side chain is α-linked to the glucuronic acid. Methylation analysis of the polymeric material gave 3,4-di-*O*-methylglucose, which is derived from the glucuronic acid after removal of the side chain attached to O-4.

The sum of these experiments permitted the complete structure of the polysaccharide to be written, but confirmation was sought by performing a base-catalyzed, uronic acid degradation. On methylation of the degraded product, hydrolysis, conversion into alditol acetates, and g.l.c. analysis, it was possible to observe the formation of 2,3,4,6-tetra-*O*-methylmannose derived from methylation at O-3 of the mannose of the aldobiouronic acid. A decrease was observed in the amount of 2,3,4-tri-*O*-methylglucose, due to the degradation of this sugar on liberation, and exposure to base.

CONCLUSION

The structure of the capsular polysaccharide from *Klebsiella* serotype K26 is thus based on the heptasaccharide repeating-unit shown. This structure is consistent with the analysis reported by Nimmich[11] and with the serological cross-reaction, observed[12] with *Klebsiella* K21, that is due to 4,6-*O*-(1-carboxyethylidene)-D-galactopyranosyl units present in both polymers.

EXPERIMENTAL

General methods. — The instrumentation used for infrared and n.m.r. spectroscopy, g.l.c., g.l.c.–m.s., circular dichroism, and measurements of optical rotation has been described previously[13]. Paper chromatography was conducted by the

descending method, using Whatman No. 1 paper and the following solvent systems (v/v): (*1*) 18:3:1:4 ethyl acetate–acetic acid–formic acid–water, (*2*) 8:2:1 ethyl acetate–pyridine–water, (*3*) 2:1:1 1-butanol–acetic acid–water, and (*4*) 4:1:5 1-butanol–ethanol–water (upper phase). Chromatograms were developed with silver nitrate, or by spraying with *p*-anisidine hydrochloride in aqueous 1-butanol and heating the papers for 5–10 min at 110°. Preparative paper-chromatography was performed by the descending method, using Whatman No. 3 MM and solvent *3*. Analytical-g.l.c. separations were achieved in stainless-steel columns (1.8 m × 3 mm) with nitrogen as the carrier-gas and a flow rate of 20 mL/min. The columns used were: (*A*) 3% of SP-2340 on Supelcoport (100–120 mesh), (*B*) 5% of ECNSS-M and (*C*) 3% of OV-225, each on Gas Chrom Q (100–120 mesh), and (*D*) 5% of SE-52 on Chrom W (60–80 mesh). Preparative g.l.c. was conducted in column *E* (1.8 m × 6.3 mm): 5% of Silar 10C on Gas Chrom Q (100–120 mesh).

Gel-permeation chromatography was performed in a column (2.5 × 100 cm) of Bio-Gel P-2 (<400 mesh). The column was irrigated with 500:5:2 water–pyridine–acetic acid at a flow rate of ~8 mL/h. Fractions were collected, freeze-dried, and weighed, and the elution profile was obtained; the fractions were then chromatographed on paper.

Ion-exchange chromatography, for separation of neutral from acidic oligosaccharides, was performed in a column (2 × 28 cm) of Bio-Rad AG1-X2 (formate) resin (200–400 mesh). The neutral fraction was eluted with water, and the acidic, with 10% formic acid.

Preparation and properties of K26 capsular polysaccharide. — A culture of *Klebsiella* K26 (5884), obtained from Dr. Ida Ørskov, Copenhagen, was grown as previously described[13], and the polysaccharide was purified by one precipitation with Cetavlon. The isolated polysaccharide (12 g) had $[\alpha]_D$ +80° (*c* 0.25, water). The average molecular weight was determined by gel chromatography (courtesy of Dr. S. C. Churms, University of Cape Town, South Africa) to be 1×10^7. N.m.r. spectroscopy (^1H and ^{13}C) was performed on the original K26 polysaccharide, but better spectra were obtained after mild treatment of the polysaccharide with 0.1M trifluoroacetic acid during 30 min at 95°, in order to lower the viscosity. The principal signals and their assignments for both the ^1H- and ^{13}C-n.m.r. spectra are recorded in Table II.

Hydrolysis of the polysaccharide. — Hydrolysis of a sample (20 mg) of K26 polysaccharide with 2M trifluoroacetic acid (TFA) overnight at 95°, removal of the acid by successive evaporations with water, followed by paper chromatography (solvents *1* and *2*), showed D-mannose, D-galactose, D-glucose, D-glucuronic acid, and an aldobiouronic acid. The quantitative sugar analysis of the carboxyl-reduced polysaccharide was performed as previously described[14]. The alditol acetates of mannose, galactose, and glucose were identified by g.l.c. (column *A*, programmed for 4 min at 195°, and then at 2°/min to 260°), and found to be present in the ratios of 2.0:2.0:3.0. Preparative g.l.c. (column *E*, programmed from 210° and then at

4°/min to 250°), followed by measurements of the c.d. spectra, showed[15] both the mannitol and the glucitol hexaacetates to be of the D configuration.

Methylation analysis. — The capsular polysaccharide (290 mg) in the free acid form, obtained by passing the sodium salt through a column of Amberlite IR-120 (H^+) resin, was dissolved in dry dimethyl sulfoxide (40 mL) and methylated by the Hakomori procedure[16]. The product (300 mg), recovered after dialysis against tap water, showed complete methylation (no hydroxyl absorption in the i.r. spectrum). Carboxyl reduction of the fully methylated polysaccharide (90 mg) with $LiAlH_4$ in anhydrous oxolane, hydrolysis with 2M trifluoroacetic acid, reduction with sodium borohydride, and acetylation with 1 : 1 acetic anhydride–pyridine gave a mixture of partially methylated alditol acetates which was analyzed by g.l.c. and g.l.c.–m.s. in columns *B* and *C* (see Table I, column I). A good separation of 2,3,4- from 2,3,6-tri-*O*-methylglucose was obtained with column *A* programmed for 8 min at 160° and then at 2°/min up to 230° for 32 min. The compounds were also characterized, as the trimethylsilyl derivatives of the alditols, by g.l.c. (column *D*, 170°). From preparative g.l.c. (column *E*, 215°), 2,3-di-*O*-methylgalactose and 3-*O*-methylglucose were isolated. The galactitol derivative showed a positive c.d. curve, indicating that the galactose had the D configuration, and the glucitol derivative showed a negative c.d. curve, confirming the D configuration of the 3-*O*-methylglucitol pentaacetate[15].

Carboxyl-reduced, K26 polysaccharide, obtained by using the technique described previously[17], was methylated. G.l.c. analysis of the partially methylated alditol acetate is shown in Table I, column II. The carboxyl-reduced and methylated polysaccharide was treated with 50% acetic acid for 90 min on a steam bath, in order to remove the 1-methoxyisopropylidene group and the product was further re-methylated by the method of Hakomori[16]. The result of the g.l.c. analysis of the derived alditol acetate is shown in Table I, column III.

Partial hydrolysis. — The K26 polysaccharide (1 g) was dissolved in 2M trifluoroacetic acid (75 mL) and the solution was heated for 2 h on a steam bath. After removal of the acid by successive evaporations with water, an acidic and a neutral fraction were separated on a column of Bio-Rad AG1-X2 ion-exchange resin. The acidic fraction (430 mg) was separated on Bio-Gel P-2, to give 82 mg of a pure aldobiouronic acid (A_1), 42 mg of a pure aldotriouronic acid (A_2), and 125 mg of a pure aldotetraouronic acid (A_3). Paper chromatography of the neutral fraction showed glucose, galactose, and mannose, plus a neutral disaccharide (N_1) (which was also separated on Bio-Gel P-2).

The analyses performed on each oligosaccharide were as follows. (*a*) *Sugar analysis.* Acidic oligosaccharides were treated with 3% HCl in anhydrous methanol for 8 h on a steam bath. The methyl ester obtained was reduced with sodium borohydride in anhydrous methanol, followed by hydrolysis with 2M TFA, reduction to the alditols, and acetylation with 1 : 1 acetic anhydride–pyridine. The alditol acetates obtained were analyzed by g.l.c. in column *A* (195° for 4 min, and then 2°/min to 260°). Neutral oligosaccharides were hydrolyzed, and analyzed similarly. (*b*) *Methylation analysis.* All the methylations were conducted by the method of Hakomori[16]

(the acidic oligosaccharides being reduced with lithium aluminum hydride in anhydrous oxolane after methylation), hydrolysis with 2M TFA, and conversion into alditol acetates which were analyzed by g.l.c., and g.l.c.–m.s.. on column *B* or *C*, or both.

The results obtained for each oligosaccharide are given in Table III, and the n.m.r. data in Table II.

Periodate oxidation. — A solution of K26 polysaccharide (1.0 g) in water (150 mL) was mixed with 0.1M $NaIO_4$ and 0.4M $NaClO_4$ (150 mL), and kept in the dark at 4°. After 240 h (consumption of periodate, 5.2 mol per mol of K26), ethylene glycol (10 mL) was added. The polyaldehyde was dialyzed overnight, reduced to the polyalcohol with $NaBH_4$ (1 g), neutralized with 20% acetic acid, dialyzed, and freeze-dried, to yield the polyalcohol (600 mg).

Part of the polyalcohol (300 mg) was treated with 0.5M TFA for 20 h at room temperature, the acid was removed by repeated addition and evaporation of water, and the product was reduced with sodium borohydride. The excess of sodium borohydride was decomposed with IR-120 (H^+) resin, and borate was removed by evaporation with methanol. Paper chromatography (solvent *3*) of the products showed glycerol, erythritol or threitol (or both), and an oligomer having R_{Gal} 0.28. Hydrolysis overnight with 2M TFA, and paper chromatography of the hydrolyzate in solvent *1*, showed glycerol, erythritol or threitol (or both), mannose, galactose, glucuronic acid, and an aldobiouronic acid. Sugar analysis of the carboxyl-reduced product (as the alditol acetates) by g.l.c. showed the presence of glycerol, threitol, erythritol, mannitol, galactitol, and glucitol. The ratios of the respective hexitols were 1.0:1.0:1.1. The rest of the material was separated in a column of Bio-Gel P-2. The elution profile obtained indicated that the Smith hydrolysis had not proceeded to completion, but 25 mg of a pure oligomer ($\mathbf{SH_1}$) was isolated.

$\mathbf{SH_1}$ had $[\alpha]_D$ +67° (*c* 3, water) and R_{Gal} 0.28 (solvent *3*); n.m.r. data are given in Table II. Sugar analysis of the carboxyl-reduced product as alditol acetates, by g.l.c., gave glycerol, mannose, galactose, and glucose in the ratios of 1:1:1:1. Methylation by the method of Hakomori[16], with the processing usual for acidic oligosaccharides, gave the partially methylated alditol acetates corresponding to 2,3,4,6-tetra-*O*-methylgalactose, 2,4,6-tri-*O*-methylmannose, and 3,4-di-*O*-methylglucose.

Selective, partial hydrolysis. — A solution of K26 polysaccharide (500 mg) in 0.01M TFA (70 mL) was heated on a steam bath for 7 h. The acid was removed, and the product was dialyzed against distilled water (1 L), to afford a polymeric material (410 mg) and a dialyzate (80 mg). Paper chromatography of the dialyzable fraction showed pyruvic acid, galactose, a disaccharide (identical to $\mathbf{N_1}$), and a trisaccharide ($\mathbf{N_2}$). The trisaccharide was isolated by preparative paper-chromatography: yield 25 mg, $[\alpha]_D$ +22° (*c* 2.5, water). Sugar analysis indicated glucose and galactose in the ratio of 2:1. On methylation hydrolysis, reduction, and per(trimethylsilyl)ation of the alditols, g.l.c. (column *D*) revealed compounds corresponding to 2,3,4,6-tetra-*O*-methylgalactose, 2,3,6-tri-*O*-methylglucose, and 2,3,4-tri-*O*-methylglucose.

Methylation analysis of the polymeric material by g.l.c. (column *C*) and g.l.c.–m.s. indicated the presence of 3,4-di-*O*-methylglucose, in lieu of the 3-*O*-methylglucose found originally.

Uronic acid degradation[18]. — A sample (60 mg) of methylated K26 polysaccharide was carefully dried and then, together with a trace of *p*-toluenesulfonic acid, was dissolved in 19:1 dimethyl sulfoxide–2,2-dimethoxypropane (20 mL) under N_2 in a flask that was then sealed. Dimethylsulfinyl anion (10 mL) was added, and allowed to react for 16 h at room temperature, when methyl iodide (6 mL) was added. The excess of the base was neutralized with 50% acetic acid, and the methylated, degraded product was isolated by partition between chloroform and water. Hydrolysis, and g.l.c. analysis of the alditol acetate derivatives, showed the presence of 2,3,4,6-tetra-*O*-methylmannose, 3,4,6-tri-*O*-methylmannose, 2,4,6-tri-*O*-methylgalactose, 2,3,4-tri-*O*-methylglucose, 2,3,6-tri-*O*-methylglucose, and 2,3-di-*O*-methylgalactose in the ratios of 1.0:1.0:0.9:0.4:1.0:1.0.

ACKNOWLEDGMENTS

We thank Dr. I. Ørskov for a culture of *Klebsiella* K26 (5884), and Dr. S. C. Churms for the determination of the molecular weight. We are indebted to NSERC for continued financial support, and one of us (J. Di F.) acknowledges with thanks the award of a MacMillam Bloedel Graduate Scholarship and a University Graduate Fellowship.

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