*Carbohydrate Research*, 144 (1985) 263–276
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

# STRUCTURE OF THE CAPSULAR POLYSACCHARIDE OF *Klebsiella* SEROTYPE K79*

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(Received December 11th, 1984; accepted for publication in revised form, June 3rd, 1985)

ABSTRACT

The structure of the capsular polysaccharide from *Klebsiella* K79 was determined by the techniques of methylation, periodate oxidation, β-elimination, chromic acid oxidation, and partial hydrolysis. N.m.r. spectroscopy ($^1H$ and $^{13}C$) was used extensively to establish the nature of the anomeric linkages of the polysaccharide and of oligosaccharides derived through degradative procedures. The polysaccharide was found to have the heptasaccharide, "5 + 2" repeating unit:

3)-β-D-Gal-(1→3)-β-D-GlcA-(1→2)-α-L-Rha-(1→3)-α-L-Rha-(1→3)-α-L-Rha-(1→
4
↑
1
α-D-Glc
6
↑
1
α-D-Glc

## INTRODUCTION

The bacterial genus *Klebsiella* is divided into 77 different serotypes, which are distinguished by the structures of their capsular polysaccharides. Structural analyses of about 73 of these polysaccharides have thus far been described. As part of our continuing effort to explain the serology of this genus on a structural basis, we now report our results on K79.

*Klebsiella* serotype K79 is one of the four strains (the others are K18, K19, and K41)[1] whose capsular polysaccharides are composed of D-galactose, D-glucose, D-glucuronic acid, and L-rhamnose. Four other strains (K12, K36, K55, and K70)[1],

*Presented in part at the XIIth International Carbohydrate Symposium, Utrecht, The Netherlands, 1–7th July, 1984.

0008-6215/85/$ 03.30 

besides having the same sugar composition, have in addition acetate and/or 1-carboxyethylidene substituent groups. Recently, the capsular polysaccharide from *Klebsiella* K50 was reported[2] as the first example of a "5 + 2" type structure wherein the branch point is a D-glucosyl unit. The pattern for K79 is also of the "5 + 2" type, but in this case it is the uronic acid residue that is the branch point. The structure is, therefore, novel in this series. The rhamnotriose unit in this polysaccharide is also noteworthy as similar partial structures are to be found in *Klebsiella* K32 and K34 (ref. 1).

## RESULTS AND DISCUSSION

*Composition and n.m.r. spectra.* — *Klebsiella* K79 bacteria were grown on an agar medium and the acidic polysaccharide was purified by two precipitations with cetyltrimethylammonium bromide[3,4]. The product was monodisperse by gel-permeation chromatography ($M_r = 3 \times 10^6$) and had $[\alpha]_D$ +18°, which compares well with the value of +19° calculated by using Hudson's rules of isorotation[5]. Analysis of the acidic polysaccharide gave galactose, glucose, and rhamnose in the molar ratios 1.20:2.00:2.00 (Table I, column I). When the uronic acid in the polysaccharide was reduced[6], the molar quantities of glucose and rhamnose increased (Table I, column II). This indicated that the glucuronic acid is linked to a rhamnosyl unit, which was later confirmed by a β-elimination reaction. The K79 polysaccharide is thus comprised of galactose, glucose, glucuronic acid, and rhamnose in the ratios 1:2:1:3. Circular dichroism measurements[7] on the alditol acetates demonstrated rhamnose to be of the L configuration, and the glucose and glucuronic acid to be of the D configuration. Galactose was shown to have the D configuration by the circular dichroism curve of the 2,4,6-tri-*O*-methyl derivative[7].

TABLE I

SUGAR ANALYSIS OF *Klebsiella* K79 POLYSACCHARIDE AND DERIVED PRODUCTS

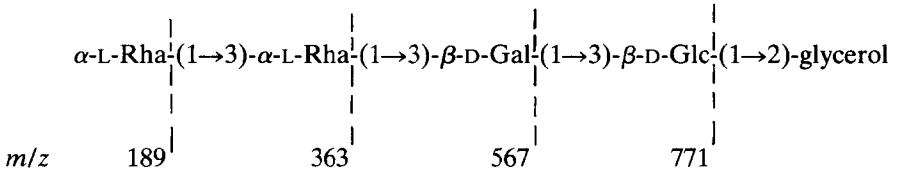
| *Sugar (as alditol acetate)* | *Molar ratios*[a,b] | | | | | | |
|---|---|---|---|---|---|---|---|
| | *I* | *II* | *III* | *IV* | *V* | *VI* | *VII* |
| Galactose | 1.20 | 1.00 | 1.00 | 1.00 | — | 0.26 | 1.00 |
| Glucose | 2.00 | 2.77 | 0.93 | 0.95 | 1.00 | 1.00 | 0.97 |
| Glycerol | — | — | +[c] | 0.2[c] | — | — | — |
| 1,2-Propanediol | — | — | +[c] | — | — | — | — |
| Rhamnose | 2.00 | 2.75 | 1.85 | 1.75 | 1.51 | 1.47 | 0.85 |

[a]Determined on an SP-2340 column programmed at 195° for 4 min, and then at 2°/min to 260°. [b]I, original acidic polysaccharide; II, carbodiimide-reduced polysaccharide; III, polyol from periodate oxidation, IV, tetrasaccharide isolated from Smith degradation; V, chromium trioxide-oxidation product of original polysaccharide; VI, chromium trioxide-oxidation product of carbodiimide-reduced polysaccharide; VII, carboxyl-reduced trisaccharide **5**, obtained from partial hydrolysis. [c]Quantitation inaccurate due to high volatility.

The $^1$H-n.m.r. spectrum of the polysaccharide indicated the presence of seven anomeric protons (Table II). These correspond to four $\alpha$, two $\beta$, and one borderline signal, which was proved by chromic acid degradation[8] to be that of an $\alpha$ linkage. There are thus five $\alpha$ and two $\beta$ linkages in the repeating unit[9]. From the high-field region[9] of the spectrum, it was further deduced that: (a) three 6-deoxysugar units were present, and (b) acetate and pyruvate, which are common features of *Klebsiella* capsular polysaccharides, were absent. These results were substantiated by the $^{13}$C-n.m.r. spectrum[10], which included a signal at 173 p.p.m., attributed to the C=O group of the glucuronic acid.

*Methylation analysis*[11,12]. — Analyses were carried out on the polysaccharide (a) in its original form, (b) after reduction of the uronic acid following methylation, and (c) after remethylation of the product from *b*. The results, presented in Table III, columns I to III, confirm the concept of a heptasaccharide repeating unit. They show that the branch point is a glucosyluronic residue linked at C-3 and C-4, and that one of the glucose units is a terminal, nonreducing residue.

*Periodate oxidation*[13]. — Carboxyl-reduced K79 polysaccharide[6] consumed five moles of periodate per repeating unit, which is in agreement with theory. Sugar analysis of the periodate-oxidized products showed that galactose, glucose, and rhamnose survived in the ratios 1.00:0.93:1.85 (Table I, column III). Methylation analysis of this same product revealed that the glucose unit which survived the degradation is actually the reduced glucuronic acid residue (see Table III, column IV). A preparative Smith degradation[14,15], conducted on the carboxyl-reduced K79 polysaccharide, yielded the oligosaccharide **1**, which on acid hydrolysis gave galactose, glucose, and rhamnose in the ratios 1.00:0.95:1.75 (Table I, column IV). N.m.r. analyses (Table II) on **1** confirmed it to be a tetrasaccharide and indicated the rhamnose units to be $\alpha$-linked[16], whereas the galactose and glucose units are $\beta$-linked. From the methylation data (Table III, column V) a nonreducing terminal rhamnose unit is indicated; the presence of a 2,4,6-tri-*O*-methylglucose derivative indicates that the branch point residue of the K79 polysaccharide, glucuronic acid, has a side chain linked to it at C-4. Chemical-ionization mass spectrometric analysis[17] of the permethylated **1** showed peaks at *m/z* 771, 567, 363, and 189, among others. The structure of **1** is given below and the sources of some pertinent fragments from methylated **1** are indicated.



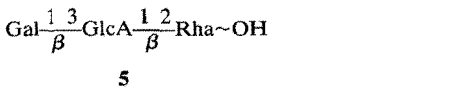

**1**

*β-Elimination*[18]. — A base-catalyzed uronic acid degradation of the permethylated polysaccharide, followed by remethylation, gave the result shown in

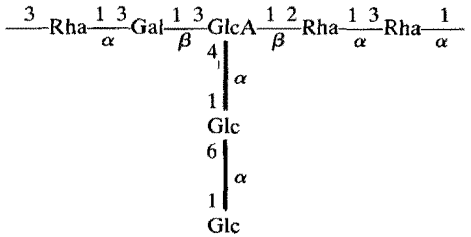
TABLE II

NMR DATA (400 MHz) FOR *Klebsiella* K79 CAPSULAR POLYSACCHARIDE AND DERIVED OLIGOSACCHARIDES

| *Compound*[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*[e] *(p.p.m.)* | *Assignment*[f] |
| Rha$\frac{1\ 3}{\alpha}$Rha$\frac{1\ 3}{\alpha}$Gal$\frac{1\ 3}{\beta}$Glc$\frac{1\ 2}{\beta}$Gro[g] | 5.08 | s | 2 | Rha$\frac{}{\alpha}$ | 103.99 | $\frac{3}{}$Gal$\frac{}{\beta}$ |
| **1** | | | | | | |
| | | | | $\frac{3}{}$Rha$\frac{}{\alpha}$ | 103.02 | Rha$\frac{}{\alpha}$ |
| | 4.69 | b | 2 | $\frac{3}{}$Gal$\frac{}{\beta}$ | | $\frac{3}{}$Rha$\frac{}{\alpha}$ |
| | | | | $\frac{3}{}$Glc$\frac{}{\beta}$ | 102.84 | $\frac{3}{}$Glc$\frac{}{\beta}$ |
| | 1.31 | s | 6 | $CH_3$ of Rha | 61.73 | C-6 of hexoses |
| | | | | | 17.50 | C-6 of Rha |
| Rha$\frac{1\ 3}{\alpha}$Rha$\frac{1\ 3}{\alpha}$Rha$\frac{1\ 3}{\alpha}$Gal~OMe | 5.40 | 3 | 0.5 | $\frac{3}{}$Gal$\frac{}{\alpha}$ | | |
| **2** | | | | | | |
| (permethylated in $CDCl_3$) | 5.10 | s | 2.0 | $\frac{3}{}$Rha$\frac{}{\alpha}$ | | |
| | 5.02 | s | 1.0 | Rha$\frac{}{\alpha}$ | | |
| | 4.80 | 7 | 0.5 | $\frac{3}{}$Gal$\frac{}{\beta}$ | | |
| | 1.31, 1.25 | m | | $CH_3$ of Rha | | |



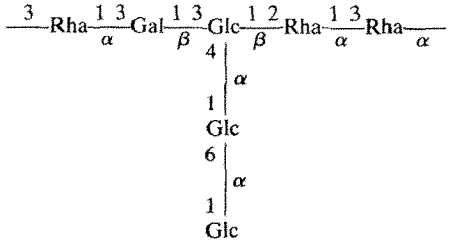
**5**



K79 Polysaccharide

| | | | | | |
|---|---|---|---|---|---|
| 5.23 | s | 0.5 | $\xrightarrow{2}$Rha$\underset{\alpha}{}$OH | 104.01 | Gal$\underset{\beta}{}$ |
| | | | | | $\xrightarrow{3}$GlcA$\underset{\beta}{}$ |
| 4.84 | s | 0.5 | $\xrightarrow{2}$Rha$\underset{\beta}{}$OH | 97.29 | $\xrightarrow{2}$Rha$\underset{\beta}{}$OH |
| 4.65 | 8 | 1.0 | Gal$\underset{\beta}{}$ | 93.38 | $\xrightarrow{2}$Rha$\underset{\alpha}{}$OH |
| 4.50 | t | 1.0 | $\xrightarrow{3}$GlcA$\underset{\beta}{}$ | 17.30 | C-6 of Rha |
| 1.31 | s | 3 | $CH_3$ of Rha | | |
| 5.62 | s | 1.0 | $\xrightarrow{6}$Glc$\underset{\alpha}{}$ | 179.60 | C=O of acetone |
| 5.33 | s | 1.0 | Glc$\underset{\alpha}{}$ | 173.00 | C=O of GlcA |
| 5.05 | s | 2.0 | $\xrightarrow{3}$Rha$\underset{\alpha}{}$ | 104.79 | $\xrightarrow{3}$Gal$\underset{\beta}{}$ |
| 4.95 | s | 1.0 | $\xrightarrow{2}$Rha$\underset{\alpha}{}$ | 103.81 | $\xrightarrow{3,4}$GlcA$\underset{\beta}{}$ |
| 4.65 | 8 | 1.0 | $\xrightarrow{3}$Gal$\underset{\beta}{}$ | 103.57 | $\xrightarrow{3}$Rha$\underset{\alpha}{}$ |
| 4.49 | 8 | 1.0 | $\xrightarrow{3,4}$GlcA$\underset{\beta}{}$ | 102.77 | $\xrightarrow{2}$Rha$\underset{\alpha}{}$ |
| | | | | 101.69 | $\xrightarrow{6}$Glc$\underset{\alpha}{}$ |
| 1.32 | s | 9 | $CH_3$ of Rha | 99.37 | Glc$\underset{\alpha}{}$ |
| | | | | 61.86 | C-6 of hexoses |
| | | | | 17.55 | C-6 of Rha |

TABLE II *(continued)*

| *Compound*[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*[e] *(p.p.m.)* | *Assignment*[f] |
|  | 5.46 | s | 1.0 | $\xrightarrow{6}$Glc$_{\alpha}$ | 179.30 | C=O of acetone |
| | 5.37 | s | 1.0 | Glc$_{\alpha}$ | 104.72 | $\xrightarrow{3}$Gal$_{\beta}$ |
| | 5.07 | s | 2.0 | $\xrightarrow{3}$Rha$_{\alpha}$ | 103.59 | $\xrightarrow{3}$Rha$_{\alpha}$ |
| | 4.97 | s | 1.0 | $\xrightarrow{2}$Rha$_{\alpha}$ | 102.80 | $\xrightarrow{4}$Glc$_{\beta}$ (3 ↓) |
| Reduced K79 Polysaccharide | 4.66 | 8 | 1.0 | $\xrightarrow{3}$Gal$_{\beta}$ | 102.75 | $\xrightarrow{2}$Rha$_{\alpha}$ |
| | 4.49 | 8 | 1.0 | $\xrightarrow{3,4}$Glc$_{\beta}$ | 101.78 | $\xrightarrow{6}$Glc$_{\alpha}$ |
| | 1.32 | s | 9.0 | $CH_3$ of Rha | 99.70 | Glc$_{\alpha}$ |
| | | | | | 61.83 | C-6 of hexoses |
| | | | | | 17.50 | C-6 of Rha |

[a]For the origin of compounds **1**, **2**, and **5**, see text [b]Chemical shift relative to sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS). [c]Key: b = broad, unable to assign accurate coupling constant, s = singlet; t = triplet; m = multiplet. [d]For example $\xrightarrow{3}$Gal$_{\beta}$ refers to the anomeric proton of a 3-linked galactosyl residue in the β-anomeric configuration. The absence of a numerical prefix indicates a (terminal) nonreducing group. [e]Chemical shift relative to $Me_4Si$ [f]As indicated in *d*, but for anomeric $^{13}$C nuclei [g]Gro = glycerol.

TABLE III

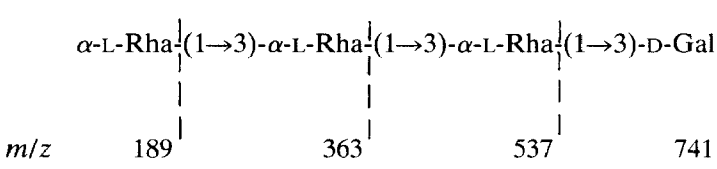
METHYLATION ANALYSES OF *Klebsiella* K79 POLYSACCHARIDE AND DERIVED PRODUCTS

| *Methylated sugar*[a] *(as alditol acetate)* | *Mol %*[b,c] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *I* | *II* | *III* | *IV* | *V* | *VI* | *VII* | *VIII* | *IX* |
| 2,3,4-Rha | — | — | — | — | 20 | 19 | 25 | 17 | — |
| 3,4-Rha | 16 | 15 | 15 | — | — | — | — | — | 30 |
| 2,4-Rha | 34 | 28 | 29 | 50 | 30 | 40 | 55 | 42 | — |
| 2,4,6-Gal | 16 | 14 | 14 | 25 | 28 | 18 | 20 | 5 | — |
| 2,3,4,6-Gal | — | — | — | — | — | — | — | — | 36 |
| 2,3,4,6-Glc | 16 | 14 | 15 | — | — | 18 | — | 21 | — |
| 2,4,6-Glc | — | — | — | — | 22 | — | — | — | — |
| 2,3,4-Glc | 16 | 14 | 13 | — | — | 5 | — | 15 | — |
| 2,4-Glc | — | — | — | — | — | — | — | — | 34 |
| 2,6-Glc | — | — | 14 | 25 | — | — | — | — | — |
| 2-Glc | — | 15 | — | — | — | — | — | — | — |

[a]2,3,4,6-Glc = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol, etc. [b]Values are corrected by use of the effective carbon-response factors given by Albersheim *et al.*[21]. [c]Determined on an OV-225 column programmed at 180° for 4 min, and then at 2°/min to 230°. I, original acidic polysaccharide; II, after reduction of uronic ester; III, product from remethylation after reduction of uronic ester; IV, product from periodate oxidation; V, product from Smith degradation; VI, product from β-elimination and remethylation; VII, product from preparative β-elimination; VIII, product from the chromic acid-degraded reduced polysaccharide; IX, methylated and reduced trisaccharide from partial hydrolysis.

Table III, column VI. The presence of 2,3,4-tri-*O*-methylrhamnose, in place of the previously observed 3,4-di-*O*-methylrhamnose, confirmed that the glucuronic acid is linked to position 2 of a rhamnose unit. A decrease observed in the amount of 2,3,4-tri-*O*-methylglucose was attributed to the degradation of this sugar on liberation and exposure to base[19]. Hence, this result indicates that the repeating unit of K79 polysaccharide has an isomaltose side chain; from the analyses conducted on **1**, this side chain is linked to C-4 of the glucuronic acid residue.

A preparative, base-catalyzed uronic acid degradation conducted on the permethylated polysaccharide with the intention of generating oligosaccharides, followed by methylation, gave only **2** as the oligomeric product. Hydrolysis of **2**, followed by conversion of the products to the partially methylated alditol acetates, indicated the presence of a terminal rhamnose, two 3-linked rhamnoses, and a 3-linked galactose (see Table III, column VII). $^1$H-N.m.r. analysis of the permethylated **2** revealed the presence of four anomeric protons (Table II). The signals at δ 5.40 and 4.80 can be attributed to a reducing galactosyl residue. Analysis of **2** by chemical-ionization mass spectrometry[17] showed peaks at *m/z* 741, 537, 363, and 189 among othes. This confirmed the structure of **2** as shown.




**2** (permethylated)

Attempts to isolate the side-chain isomaltose residue were not successful. It is possible that the permethylated α-Glc-(1→6)-Glc~OH was degraded further under the basic conditions used, as has been noticed previously[19] in the case of *Klebsiella* K26.

*Chromic acid oxidation*[8]. — Analyses were conducted on (a) the peracetylated original polysaccharide, and (b) the peracetylated carbodiimide-reduced polysaccharide. The results, presented in Table I, columns V and VI, show that galactose and glucuronic acid have been degraded and therefore are β-linked. The result from the methylation analysis of the product from *b* (Table III, column VIII) is similar to that from the β-elimination, except that the 3-linked β-galactosyl residue, which has been degraded, is now diminished. The presence of only one molar portion of 2,3,4-tri-*O*-methylrhamnose per repeating unit shows that the galactosyl residue must be linked to the glucuronic acid. This is in agreement with the data previously obtained.

An attempt to generate selectively cleaved oligosaccharides by chromic acid oxidation of the peracetylated carbodiimide-reduced polysaccharide gave two products, **3** and **4**, isolated by paper chromatography after deacetylation of the oxidized residue. Signals at δ 5.03 (1.5 H), 4.92 (1 H), and 1.31 (9 H) in the $^1$H-n.m.r. spectrum of **3** indicated it to be a rhamnotriose, but the spectrum of **4** was not definitive. Poor yields of **3** and **4** precluded further analyses.

*Partial hydrolysis.* — Acid hydrolysis of the native polysaccharide, with the intention of isolating the isomaltose side-chain, followed by separation on paper chromatography, gave four compounds. Compound **5** ($R_{lactose}$ 0.98) is of the most interest because it possesses a mobility comparable to that of isomaltose. However, on the basis of its sugar analysis (Table I, column VII), n.m.r.-spectral data (Table II), and methylation analysis (Table III, column IX), the structure of **5** is deduced to be as follows.

β-Gal-(1→3)-β-GlcA-(1→2)-Rha

**5**

The structure of the capsular polysaccharide from *Klebsiella* K79 is thus based on the heptasaccharide repeating unit shown in the Abstract, and is consistent with the qualitative analysis reported by Nimmich[20].

## EXPERIMENTAL

*General methods.* — Solutions were concentrated at bath temperatures not exceeding 40°. Deionizations were performed on a column of Amberlite IR-120 ($H^+$) resin. Optical rotations were measured on aqueous solutions at 23–25° with a Perkin–Elmer model 141 polarimeter (10 cm cell). Circular dichroism (c.d.) spectra were recorded with a Jasco J-500A automatic recording spectropolarimeter equipped with a quartz cell of 0.3 mL capacity and a path length of 0.1 cm. Compounds were dissolved in spectroscopic grade acetonitrile, and the spectra were recorded in the range 200–290 nm. The infrared (i.r.) spectra of methylated derivatives were recorded with a Perkin–Elmer model 457 spectrophotometer for solutions in spectroscopic grade carbon tetrachloride.

Analytical paper chromatography was performed by the descending method, using Whatman No. 1 paper and the following solvent systems: (1) 18:3:1:4 ethyl acetate–acetic acid–formic acid–water, (2) freshly prepared 2:1:1 1-butanol–acetic acid–water, (3) 8:2:1 ethyl acetate–pyridine–water, and (4) the upper phase of 4:1:5 1-butanol–ethanol–water. Preparative paper chromatography was conducted by using Whatman No. 1 paper and solvent system 1 or 2. Chromatograms were developed either with alkaline silver nitrate or by spraying with a solution of *p*-anisidine hydrochloride in aqueous 1-butanol, then heating for 10 min at 110°.

Analytical g.l.c. separations were performed on alditol acetate derivatives using a Hewlett–Packard 5700 instrument fitted with dual flame-ionization detectors. Stainless steel columns (1.8 m × 3 mm) were used with nitrogen as the carrier gas, flow rate 20 mL/min. The columns employed were: (A) 3% of SP-2340 on Supelcoport (100–120 mesh), programmed at 195° for 4 min, and then at 2°/min to 260°; (B) 3% of OV-225 on Gas Chrom Q (100–120 mesh), isothermal at 170°, or programmed at 180° for 4 min, and then at 2°/min to 230°; and (C) 5% of SP-1000 on Gas Chrom Q (100–120 mesh), isothermal at 220°. Preparative g.l.c. was conducted with an F and M model 720 dual-column instrument fitted with thermal conductivity detectors, and operated with helium as the carrier gas at a flow rate of 40 mL/min. A stainless steel column (1.8 m × 6.3 mm) was used with (D) 3% of SP-2340 on Supelcoport (100–120 mesh), programmed from 190° at 4°/min to 240° (for alditol acetates), or from 160° at 2°/min to 240° (for partially methylated alditol acetates). G.l.c.–m.s. was performed on a KRATOS MS80RFA instrument with helium as the carrier gas, flow rate 25 mL/min. The capillary columns used for separations were: (E) DB-225 (0.25 mm × 15 m), programmed at 180° for 4 min, then at 10°/min to 250°; and (F) SE-30 (0.25 mm × 15 m), programmed at 60° for 1 min, then at 10°/min to 250°. Chemical-ionization mass spectrometric analyses of oligosaccharide derivatives were performed on a Nermag R 10-10 quadrupole mass spectrometer.

$^1$H-N.m.r. specctra were recorded with a Bruker WH-400 instrument. Spectra were recorded either at ambient or elevated temperature (95 ±5°), with acetone ($\delta$ 2.23 p.p.m.) as an internal standard. Samples (10–20 mg) were prepared

by dissolving them in $D_2O$ and freeze drying (three cycles), then dissolving the residue in $D_2O$ for transfer to 5 mm-diameter tubes. $^{13}C$-N.m.r. experiments were conducted on a Bruker WH-400, a Bruker WP-80, or a Varian CFT-20 instrument. All spectra were recorded at ambient temperature, and acetone ($\delta$ 31.07 p.p.m.) was used as the internal standard. Samples were dissolved in the minimum volume of $D_2O$, and examined in tubes of 5 or 10 mm diameter.

*Preparation and properties of K79 capsular polysaccharides.* — A culture of *Klebsiella* K79, obtained from Dr. Ida Ørskov, Copenhagen, was grown as previously described[3,4], and the polysaccharide was purified by two precipitations with cetyltrimethylammonium bromide. The purified polysaccharide (0.95 g per liter of medium) was shown to be homogeneous by gel chromatography. Its molecular mass was estimated as $3 \times 10^6$ daltons, and it had $[\alpha]_D$ +18° (*c* 0.91, water). N.m.r. spectroscopy ($^{13}C$ and $^1H$) was performed on the original material, and the principal signals and their assignments are recorded in Table II.

*Analysis of K79 polysaccharide.* — A sample (10 mg) of K79 polysaccharide was hydrolyzed with 2M trifluoroacetic acid (TFA) for 18 h on a steam bath. After removal of the acid by repeated coevaporation with water, paper chromatographic analyses (solvents 1 and 3) of the hydrolyzate showed D-galactose, D-glucose, L-rhamnose, and an aldobiouronic acid. The results of the g.l.c. analysis, on column A, of the sugars as their alditol acetates are shown in Table I, column I.

*Reduction of K79 polysaccharide.* — Following the published procedure[6], K79 polysaccharide (1.0 g) was reduced with 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate (4 g) and aqueous sodium borohydride (3M, 250 mL). The neutral polysaccharide (960 mg) obtained after two treatments had the composition shown in Table I, column II. Preparative g.l.c. (column D), followed by measurements of the circular dichroism spectra[7], showed the glucitol hexaacetate to be of the D configuration, and the rhamnitol pentaacetate to be of the L configuration.

*Methylation analysis.* — A sample of K79 polysaccharide in the free acid form (96 mg), obtained by passing the aqueous solution through a column of Amberlite IR-120 ($H^+$) resin, was methylated by the Hakomori procedure[11,12]. The product, recovered after dialysis and purified by extraction with dichloromethane and passage through a column of Sephadex LH-20, showed complete methylation (no hydroxyl absorption in the i.r. spectrum). One part (30 mg) was hydrolyzed (2M TFA, 20 h, 95°), after which paper chromatography in solvent 4 revealed five components: 2,3,4,6-tetra-*O*-methylglucose ($R_{TMG}$ 1.00, pink spot)*, 2,4- and 3,4-di-*O*-methylrhamnoses ($R_{TMG}$ 0.85, greenish brown spot), 2,3,4-tri-*O*-methylglucose ($R_{TMG}$ 0.83, brownish pink spot), 2,4,6-tri-*O*-methylgalactose ($R_{TMG}$ 0.71, pink spot), and a methylated aldobiouronic acid ($R_{TMG}$ 0.50, pink spot). Analytical figures for the neutral sugars are presented in Table III, column I. A second portion of the methylated polysaccharide (60 mg) was reduced with lithium aluminum

*$R_{TMG}$ = mobility relative to that of 2,3,4,6-tetra-*O*-methylglucose

hydride in refluxing oxolane. The product was divided into two equal parts, one of which was hydrolyzed and analyzed with the results shown in Table III, column II. The paper chromatograms of this hydrolyzate using solvent 4 showed the disappearance of the spot at $R_{TMG}$ 0.50 and the additional appearance of 2-*O*-methylglucose ($R_{TMG}$ 0.26, pink). The other half of the methylated, reduced polysaccharide was remethylated[11,12] and hydrolyzed, whereupon paper chromatography showed the disappearance of the spot at $R_{TMG}$ 0.26 and the appearance of 2,6-di-*O*-methylglucose ($R_{TMG}$ 0.56). The g.l.c. results are given in Table III, column III. G.l.c.–m.s. analyses were conducted with columns E and F. 1,3,5-Tri-*O*-acetyl-2,4,6-tri-*O*-methylgalactitol was isolated by preparative g.l.c. (column D) and found to give a positive c.d. curve, indicating that the galactose had the D configuration[7].

*Periodate oxidation*[13] *and Smith degradation*[14,15] *of neutral K79 polysaccharide.* — A sample of the carbodiimide-reduced K79 polysaccharide (20 mg) was dissolved in water (5 mL) and sodium metaperiodate solution (0.03M, 5 mL) was added. The reaction was conducted at room temperature and in the dark. Aliquots (0.1 mL) were withdrawn periodically and diluted 250 times with water, and the absorbances[13] of the resulting solutions were measured in a Gilford spectrophotometer, model 240, at 223 nm. The periodate consumption reached a plateau after about 50 h and approximately 0.08 mmoles $IO_4^-$ had been consumed, equivalent to 5 moles of $IO_4^-$ per mole of repeating unit. Ethylene glycol (0.2 mL) was added to destroy the excess periodate, the polyaldehyde was reduced with sodium borohydride, and the solution was neutralized with acetic acid (50%), dialyzed, and lyophilized to yield the polyol (12 mg). Sugar and methylation analyses of the polyol gave the results shown respectively in Table I, column III and Table III, column IV.

A solution of carbodiimide-reduced K79 polysaccharide (520 mg) in water (200 mL) was mixed with sodium metaperiodate solution (60 mL, 0.5M) and kept in the dark at room temperature. After 3 d, the excess periodate was destroyed by the addition of ethylene glycol (10 mL). After dialysis, for 1 d, the lyophilized polyaldehyde, redissolved in water, was reduced to the polyol with sodium borohydride, and the solution was neutralized with acetic acid (50%), dialyzed, and lyophilized. A second treatment was conducted, and after similar workup the polyol was recovered (yield 400 mg).

A sample of the polyol (360 mg) was subjected to selective hydrolysis with 0.5M TFA for 24 h at room temperature. Paper chromatographic analysis of the hydrolyzate in solvent system 1 (41 h) gave a spot at $R_{Glc}$ 0.74. No monosaccharides were observed. Separation by preparative paper chromatography using solvent 1 (2 d) gave a tetrasaccharide, **1** (120 mg). Sugar and methylation analyses on **1** gave the results shown respectively in Table I, column IV and Table III, column V. For n.m.r. analyses, see Table II. A sample of **1** (23 mg) was methylated by the Hakomori procedure[11] and the product was analyzed by chemical-ionization (methane) m.s.[17]. The spectrum showed peaks at *m/z* 189, 363, 567, 771 and 890.

*Uronic acid degradation*[18]. — Methylated K79 polysaccharide (70 mg), dried *in vacuo,* was dissolved in a mixture of 19:1 dimethyl sulfoxide–2,3-dimethoxypropane (20 mL) together with a trace of *p*-toluenesulfonic acid and stirred under nitrogen. Sodium methylsulfinylmethylide (2M, 6 mL) was added and the reaction mixture was left stirring for 18 h at room temperature. Methyl iodide was added to the frozen mixture and it was stirred for another 7 h at room temperature, then the excess base was neutralized by adding 50% acetic acid. The methylated, degraded product was recovered by partition between dichloromethane and water (3 × 20 mL). A portion of this product was hydrolyzed (2M TFA, 6 h, 95°) and the sugars released were analyzed as described previously for the methylation analysis (Table III, column VI).

The fractionation of the rest of the methylated, degraded product on a Sephadex LH-20 column (1.2 × 50 cm), using 1:1 chloroform–methanol as eluant, gave two main fractions. The first fraction was deduced to be polymeric as evidenced by $^1$H-n.m.r., t.l.c., and analysis of the sugars as their partially methylated alditol acetates. The second fraction was considered to be a methylated tetrasaccharide (**2**) on the basis of $^1$H-n.m.r. (Table II) and g.l.c. data (Table III, column VII). Chemical-ionization (methane) m.s.[17] of **2** gave a spectrum showing peaks at *m/z* 189, 363, 537, 741 and 758.

*Chromic acid oxidation*[8]. — To a solution of the original K79 polysaccharide (15 mg) in formamide (5 mL) were added acetic anhydride (4 mL) and pyridine (4 mL), and the mixture was stirred at room temperature for 36 h. Following dialysis for 2 d, the retentate was freeze dried, and the acetylated polysaccharide was subjected to a second acetylation. After similar workup, the peracetylated polysaccharide was dissolved in glacial acetic acid (3.5 mL), $CrO_3$ (100 mg) was added, and the mixture was stirred for 20 h at room temperature. The product, isolated by partition between chloroform and water, was analyzed as the alditol acetates (see Table I, column V).

Some carbodiimide-reduced K79 polysaccharide (95 mg) was acetylated as described above. Complete acetylation was checked by i.r. spectroscopy (absence of hydroxyl absorbance between 3650 and 3000 $cm^{-1}$). After passage through a Sephadex LH-20 column (acetone as solvent), the peracetylated polysaccharide was dissolved in glacial acetic acid (25 mL), $CrO_3$ (280 mg) was added, and the mixture was stirred at room temperature for 27 h. The product, isolated by partition between chloroform and water, was examined as follows: (a) Sugar analysis: A portion of the product (10 mg) was hydrolyzed (2M TFA), reduced with sodium borohydride, and acetylated with 1:1 acetic anhydride–pyridine. See Table I, column VI for g.l.c. data. (b) Methylation analysis: A portion of the product (15 mg) was methylated by the Hakomori procedure[11], hydrolyzed (2M TFA), reduced with sodium borohydride, and acetylated to give partially methylated alditol acetates. For the g.l.c. data, see Table III, column VIII.

The remainder of the chromic acid-oxidized product (35 mg) was deacetylated by stirring in sodium hydroxide solution (0.5M) for 26 h at room

temperature, then reduced with sodium borohydride. Paper chromatography of the product, after passage through Amberlite IR-120 ($H^+$) resin and removal of the borate by coevaporation with methanol, showed two predominant spots ($R_{Glc}$ 1.17 and $R_{Glc}$ 0.24 or $R_{lactose}$ 1.0). Separation by preparative paper chromatography (solvent 2) gave two products, **3** (4 mg) and **4** (5 mg). The characterization of **3** as a rhamnotriose by $^1$H-n.m.r. analysis is described in the Results section.

*Partial hydrolysis.* — A solution of the K79 polysaccharide (630 mg) in 0.025M $H_2SO_4$ was heated for 1.5 h on a steam bath. After neutralization with $PbCO_3$ and centrifugation, the supernatant was dialyzed (mol. wt. cut-off 3400) against distilled water. The nondialyzable material (480 mg) was lyophilized and subjected to a second hydrolysis (0.5M TFA, 1.5 h, 95°), followed by dialysis. The dialyzable material from the hydrolyses (620 mg) was passed through a column of Amberlite IR 120 ($H^+$) resin, after which paper chromatography revealed the presence of monosaccharides (galactose, glucose, and rhamnose) and four oligosaccharides. Preparative paper chromatography (solvent 2) of the hydrolyzate gave four compounds. Compound **5** (12 mg, $R_{lactose}$ 0.98), which was isolated pure, was of the most interest because its mobility was comparable to that of isomaltose. However, **5** was deduced to be an acidic trisaccharide on the basis of its n.m.r. spectra (Table II), sugar analysis on the carboxyl-reduced[22] product (Table I, column VII), and methylation analysis with reduction of the uronic ester (Table III, column IX). $^1$H-N.m.r. analyses of the other three oligosaccharides revealed that the samples were not homogeneous and hence these were not further examined.

ACKNOWLEDGMENTS

We thank Dr. I. Ørskov for the culture of *Klebsiella* K79, Dr. S. C. Churms (University of Cape Town, South Africa) for the determination of the molecular weight, and N.S.E.R.C. (Ottawa) for continued financial support. Some initial experiments on *Klebsiella* K79 were carried out by the late Dr. A. B. Zanlungo during his tenure of a C.I.D.A. Fellowship.

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