Carbohydrate Research, 140 (1985) 263–275
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

A STRUCTURAL INVESTIGATION OF THE CAPSULAR POLYSACCHARIDE OF *Klebsiella* K14

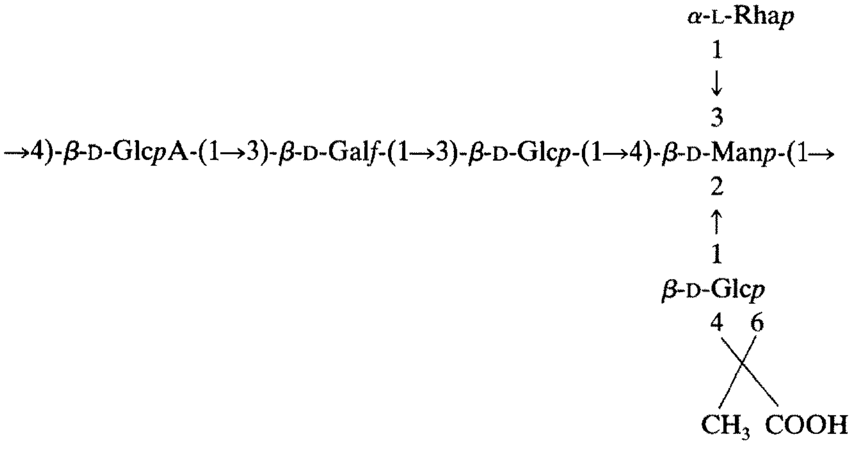
GUY G. S. DUTTON,
Department of Chemistry, University of British Columbia, Vancouver, B.C. V6T 1Y6 (Canada)

HARALAMBOS PAROLIS, AND LESLEY A. S. PAROLIS
School of Pharmaceutical Sciences, Rhodes University, Grahamstown 6140 (South Africa)

(Received June 25th, 1984; accepted for publication, November 5th, 1984)

ABSTRACT

The structure of the capsular polysaccharide isolated from *Klebsiella* serotype K14 has been investigated employing a combination of chemical and spectroscopic methods. The repeating structure is shown to be of the "4 + 1 + 1" type, and it carries a 1-carboxyethylidene acetal substituent at positions 4 and 6 of a terminal glucose residue. The polysaccharide is one of a group of only three *Klebsiella* polysaccharides that have been found to contain a galactofuranose residue in the repeating unit. The repeating unit has the following structure:



$$
\begin{array}{lc}
 & \alpha\text{-L-Rha}p \\
 & 1 \\
 & \downarrow \\
 & 3 \\
\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{A-}(1\rightarrow 3)\text{-}\beta\text{-D-Gal}f\text{-}(1\rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-} & \text{Man}p\text{-}(1\rightarrow \\
 & 2 \\
 & \uparrow \\
 & 1 \\
 & \beta\text{-D-Glc}p \\
 & 4\quad 6 \\
 & \times \\
 & CH_3\quad COOH
\end{array}
$$

INTRODUCTION

The structure of *Klebsiella* K14 capsular polysaccharide has been investigated in our continuing program of structural studies on the *Klebsiella* exopolysaccharides, undertaken in an attempt to relate chemical structure to antigenic character. There are only two serotypes whose capsular polysaccharides have the qualitative composition D-galactose, D-glucose, D-glucuronic acid, D-mannose, and

L-rhamnose[1]. These are K14, which also contains 1-carboxyethylidene groups, and K67, whose structure has been published[2].

RESULTS AND DISCUSSION

Composition and n.m.r. spectra. — The isolation and purification of the polysaccharide were achieved as previously described[3]. The purified product obtained by Cetavlon precipitation had $[\alpha]_D$ −80.9° and was shown to be homogeneous ($\bar{M}_w = 5 \times 10^6$) by gel permeation chromatography, kindly carried out by Dr. S. C. Churms, University of Cape Town, South Africa. Hydrolysis of the polysaccharide and g.l.c. analysis of the derived peracetylated aldononitriles showed the presence of glucose, galactose, mannose, and rhamnose in the molar ratios 1.8:0.7:1.0:1.1. After reduction of the uronic acid present these sugars appeared in the ratios of 2.7:0.9:1.0:1.1. The glucose, galactose, glucuronic acid, and mannose were shown to be of the D-configuration and rhamnose of the L-configuration by measurement of the optical rotations of the liberated sugars after hydrolysis.

The high viscosity of the native polysaccharide resulted in a poorly resolved ^{1}H-n.m.r. spectrum. Despite the lack of detail, it was still possible to compare the integral of the anomeric proton of galactose with that of the protons of the pyruvic acid acetal group. In this way it was found that in the first batch of polysaccharide the substitution by acetal groups was about 80% of the stoichiometric value, and in a second batch >90%. The stability of the acetal subsituent to hydrolysis is known[4] to be dependent upon its mode of attachment. In proposing the structure of *Klebsiella* K14 polysaccharide we have assumed that the acetal group is present in a regular manner in each repeating unit, and that the experimental values represent adventitious losses during the isolation and purification of the polysaccharide.

A much improved ^{1}H-n.m.r. spectrum was obtained after the acid form of the polysaccharide was briefly heated in water (100°). In the spectrum (Table I and Fig. 1a) of this partially depolymerized polysaccharide, twinning of the signals of some of the anomeric protons (δ 4.5–5.5) as well as of the methyl protons of the rhamnose residues (δ 1.24 and 1.26) was noted. This is attributed to the effect, on certain protons, of the presence or absence of pyruvate in the repeating unit of the polymer. Since the spectrum showed that after depolymerization approximately 60% of the pyruvate acetal groups had been removed, resonances integrating for 0.4 H were attributed to those sugar units present in repeating units containing pyruvate, and those integrating for 0.6 H to sugar units present in repeating units devoid of pyruvate. The proton resonances affected by the presence or absence of pyruvate were those of the terminal rhamnose unit, the mannose unit carrying both branches, the glucose unit carrying the pyruvate, and the in-chain 3-linked glucose unit. The resonance most affected was that of the anomeric proton of the α-linked rhamnose, where pyruvate caused an upfield shift of 0.13 p.p.m. The mannose anomeric proton was shifted 0.02 p.p.m. upfield. However, for the glucose residue

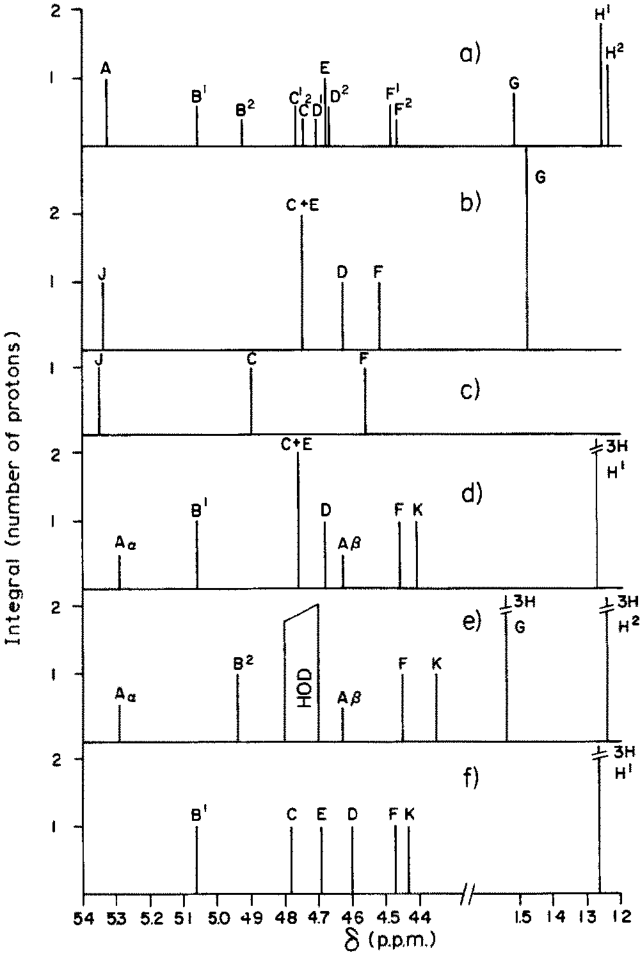


Fig. 1. ^{1}H-N.m.r. spectra (400 MHz) of K14 polysaccharide and derived products. Key: a) autolyzed polysaccharide, b) product of selective periodate oxidation, c) Smith degraded product, d) compound **P1**, e) **P2**, f) compound **P1** reduced. All spectra were obtained at 95° except e), which was recorded at ambient temperature.

carrying pyruvate, the shift was 0.04 p.p.m. to lower field. Examination of a model of the repeating unit did not offer any immediately obvious explanation for this phenomenon. It is, however, clear that the pyruvate exerts a considerable effect on the anomeric protons of the mannose and the three residues linked to it.

In the ^{13}C-n.m.r. spectrum the methyl resonance of the pyruvate acetal group occurs at 25.4 p.p.m., indicating the *S*-configuration for the acetal carbon[5]. A signal at 109.06 p.p.m. clearly indicates the β-anomeric configuration for the D-galactofuranose units[6]. Definitive assignment of the signals in the spectrum of the autolyzed polysaccharide could not be made because of the high degree of twinning caused by partial hydrolysis of the pyruvate acetal substituent.

TABLE I

^{1}H-N.M.R. DATA FOR *Klebsiella* K14 POLYSACCHARIDE AND DERIVED POLY- AND OLIGO-SACCHARIDES

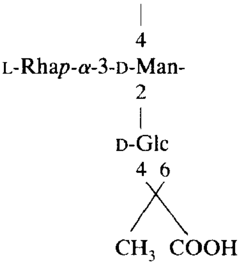
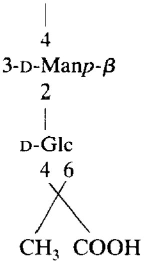
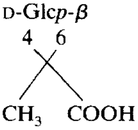
Compound	*δ (p.p.m.)*[a]		$J_{1,2}$ *(Hz)*	*Integral (no. of H)*	*Assignment*[b]	*Symbol*[c]
	ambient	*95°*				
Polysaccharide[d]		5.33	n.o.	1.0	3-D-Gal*f*-β	A
		5.06	n.o.	0.6	| 4 L-Rha*p*-α-3-D-Man- 2 | D-Glc	B^1
		4.93	n.o.	0.4	 | 4 L-Rha*p*-α-3-D-Man- 2 | D-Glc 4 6 CH_3 COOH	B^2
		4.77	n.o.	0.6	| 4 3-D-Man*p*-β 2 | D-Glc	C^1
		4.75	n.o.	0.4	 | 4 3-D-Man*p*-β 2 | D-Glc 4 6 CH_3 COOH	C^2
		4.71	8	0.4	 D-Glc*p*-β 4 6 CH_3 COOH	D^1
		4.67	8	0.6	D-Glc*p*-β	D^2
		4.68	8	1.0	4-D-Glc*p*A-β	E

TABLE I *(continued)*

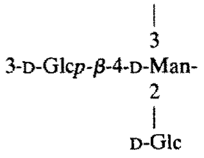
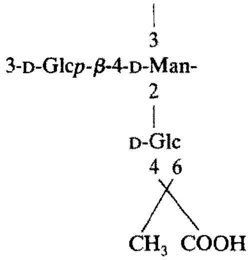
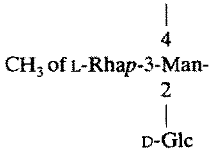
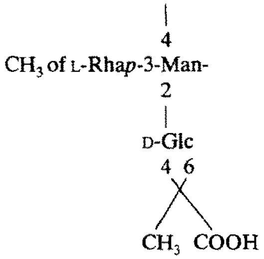
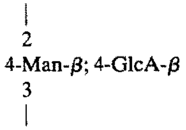
Compound	*δ (p.p.m.)*[a]		$J_{1,2}$ *(Hz)*	*Integral (no. of H)*	*Assignment*[b]	*Symbol*[c]
	ambient	*95°*				
		4.49	7	0.6	 $\text{3-D-Glc}p\text{-}\beta\text{-4-D-}\overset{\overset{\mid}{3}}{\underset{\underset{\underset{\text{D-Glc}}{\mid}}{2}}{\text{Man}}}\text{-}$	F^1
		4.47	7	0.4	 $\text{3-D-Glc}p\text{-}\beta\text{-4-D-}\overset{\overset{\mid}{3}}{\underset{\underset{\underset{\underset{\underset{\text{CH}_3\ \ \text{COOH}}{\overset{4\ 6}{\times}}}{\text{D-Glc}}}{\mid}}{2}}{\text{Man}}}\text{-}$	F^2
		1.52		1.2	CH_3 of pyruvate	G
		1.26	6 ($J_{5,6}$)	1.8	 $\text{CH}_3\text{ of L-Rha}p\text{-3-}\overset{\overset{\mid}{4}}{\underset{\underset{\underset{\text{D-Glc}}{\mid}}{2}}{\text{Man}}}\text{-}$	H^1
		1.24	6 ($J_{5,6}$)	1.2	 $\text{CH}_3\text{ of L-Rha}p\text{-3-}\overset{\overset{\mid}{4}}{\underset{\underset{\underset{\underset{\underset{\text{CH}_3\ \ \text{COOH}}{\overset{4\ 6}{\times}}}{\text{D-Glc}}}{\mid}}{2}}{\text{Man}}}\text{-}$	H^2
P1	5.28	5.29	n.o.	0.5	3-Gal-α	A
	5.07	5.06	n.o.	1.0	Rha-α	B^1
		4.76	n.o.	2.0	 $\overset{\overset{\mid}{2}}{\underset{\underset{\mid}{3}}{4}}\text{-Man-}\beta\text{; 4-GlcA-}\beta$	C + E
	4.67	4.68	8	1.0	Glc-β	D
	4.63	4.63	8	0.5	3-Gal-β	A
	4.45	4.46	8	1.0	Glc-β	F
	4.40	4.41	n.o.	1.0	H-2 of Man	K
	1.25	1.27	6 ($J_{5,6}$)	3.0	CH_3 of Rha	H^1

TABLE I *(continued)*

Compound	*δ (p.p.m.)*[a]		*J*$_{1,2}$ *(Hz)*	*Integral (no. of H)*	*Assignment*[b]	*Symbol*[c]
	ambient	*95°*				
P2	5.29		n.o.	0.5	3-Gal-α	A
	4.94	n.o.		1.0	Rha-α	B^2
	4.7–4 8		obscured by residual HOD peak			
	4.63		8	0.5	3-Gal-β	A
	4.45		8	1.0	Glc-β	F
	4.35		n.o.	1.0	H-2 of Man	K
	1.54			3.0	CH_3 of pyruvate	G
	1.24		6 ($J_{5,6}$)	3.0	CH_3 of Rha	H^2
P1 (reduced)		5.06	n.o	1.0	Rha-α | 2	B^1
		4.78	n o.	1.0	4-Man-β 3 |	C
		4.69	8	1.0	4-GlcA-β	E
		4.60	8	1.0	Glc-β	D
		4.47	8	1.0	Glc-β	F
		4.43		1.0	H-2 of Man	K
		1.26	6 ($J_{5,6}$)	3.0	CH_3 of Rha	H^1
Product of selective periodate oxidation[e]		5.34	n.o.	1.0	3-Ara-α | 2	J
		4.75	n.o.	2.0	4-Man-β and 4-GlcA-β 3 |	C + E
		4.63	8	1.0	Glc-β 4 6  CH_3 COOH	D
		1.48	n.o.	3.0	CH_3 of pyruvate	G
Product of complete periodate oxidation (Smith degradation)		5.35	n.o.	1.0	3-Ara-α	J
		4.90	n.o.	1.0	4-Man-β	C
		4.56	8	1.0	3-Glc-β	F

---

[a] Chemical shift downfield from sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS). [b] The assignment is for the anomeric proton of the first named sugar residue. Adjacent units are given only when they influence the chemical shift of this proton. [c] See the structure of **P1** for an explanation of the symbols A, B, etc. [d] Polysaccharide in H_2O, heated for 15 min at 90°, dialyzed, and freeze dried. [e] Selectively oxidized polysaccharide reduced, hydrolyzed (with 0.5M TFA, 48 h), dialyzed, freeze dried.

TABLE II

METHYLATION ANALYSES OF K14 POLYSACCHARIDE AND DERIVED POLY- AND OLIGO-SACCHARIDES

Methylated sugars[a] *(as alditol acetates)*	T[b]	*Molar ratios*[c]						
		I	*II*	*III*	*IV*	*V*	*VI*	*VII*
1,2,3,4,5-Ara	0.21						0.43	
2,3,5-Ara	0.40				0.21			
2,3,4-Rha	0.49	1.00	1.00	0.34				1.0
2,5-Ara	1.06[d]				0.79	0.26		
2,3,4,6-Glc	1.00	0.28	0.40	0.26		0.22	1.00	1.0
2,3,5,6-Gal				0.93				
2,4,6-Glc	1.98	1.00	1.00	1.00	1.00	1.00		
2,5,6-Gal	2.23	0.46	0.92					0.4
2,3,6-Man	2.36[d]				0.51		0.87	
6-Man	4.40	1.00	1.00	0.23				0.9
2,3-Glc	5.50	0.88	1.52	0.80		0.92		1.6
3,6-Man	3.96[d]				0.47	0.93		
2,4,6-Gal								0.5

[a] 2,3,4-Rha = 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl-L-rhamnitol, etc. [b] G.l.c. retention time relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol on OV-225 (see Experimental). [c] I, Methylated native polysaccharide; II, methylated, reduced native polysaccharide; III, methylated product from uronic acid degradation; IV, methylated Smith-degraded polymer; V, methylated partially oxidized polymer; VI, methylated further hydrolyzed Smith-degraded polymer; VII, methylated, reduced, hydrolyzed **P2**. [d] Retention time relative to 2,3,4,6-Glc on ECNSS-M (3% on Gas Chrom Q), isothermal at 180°.

Methylation analysis. — Methylation of K14 polysaccharide followed by g.c.–m.s. analysis of the derived alditol acetates gave the results shown in Table II, column I, while reduction of the methylated polysaccharide gave the figures shown in column II. These data indicate that the polysaccharide has a hexasaccharide repeating unit containing two terminal groups and a double branch point. The ^{1}H-n.m.r. spectrum of the native polysaccharide had indicated that up to 20% of the pyruvate was lost during isolation of the polysaccharide. The low value obtained in the methylation analysis for 2,3-di-*O*-methylglucose and the presence of a complementary amount of 2,3,4,6-tetra-*O*-methylglucose confirm this and locate the pyruvate acetal at positions four and six of a terminal glucose unit. The results also show that the uronic acid is linked to galactose, which is present in the furanose form in the polymer.

Base-catalyzed degradation. — In a single operation the permethylated polysaccharide was treated with dimethylsulphinyl carbanion and directly alkylated with methyl iodide[7,8]. An oligomeric product was obtained whose analysis is given in Table II, column III. These results establish that the 4-linked uronic acid is located in the main chain, linked to O-3 of the galactofuranose unit. The fact that most of the 6-*O*-methylmannose is destroyed during the base degradation clearly indicates that the mannose is glycosidically linked to the glucuronic acid. The low value for 2,3,4-tri-*O*-methylrhamnose is consistent with the finding that rhamnose is linked to O-3 of the branched mannose, and thus it is now β to the potential aldehyde group of the released mannose and undergoes elimination and degradation. The terminal glucose unit linked to O-2 of the mannose survives elimination as expected.

Periodate oxidation. — When periodate oxidation was performed on the sodium salt of the polysaccharide, the consumption of periodate reached a plateau after 120 h (5.25 moles of periodate per mole of repeating unit). The theoretical value is 5 moles, however the increased consumption is consistent with the loss of some pyruvate acetal groups.

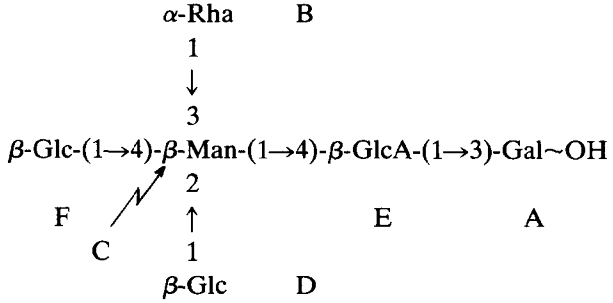
Smith degradation[9]. — Smith degradation yielded a non-dialyzable substance the hydrolysis of which afforded erythritol, arabinose, mannose, and glucose in the molar ratios 0.9:1.2:0.8:1.0. The appearance of arabinose confirms the presence of galactofuranose in the polymer and overall the results are consistent with the conclusions drawn from the methylation analysis of the polysaccharide. Methylation of the Smith-degraded product gave the results shown in Table II, column IV, which indicate incomplete hydrolysis of interresidue acetal linkages due probably to the presence of the carboxyl groups of the uronic acid and the pyruvate acetal substituent. The results show that the mannose residue is linked in-chain through O-4 and thus carries branches at O-2 and O-3. ^{1}H-N.m.r. spectroscopy showed (Fig. 1c) the presence of α-arabinofuranose[10], β-mannopyranose, and β-glucopyranose residues, thus allowing the resonances at δ 4.49 and 4.47 in the spectrum of the autolyzed native polysaccharide to be assigned to the in-chain glucose residue, and confirming the position of resonance of the C-1 protons of galactofuranose and mannopyranose.

The Smith-degraded polymer was subjected to further mild acid hydrolysis. Paper chromatography showed arabinose and a major nonreducing oligomeric product. The mixture was reduced with sodium borohydride and methylated (Table II, column VI). The results indicate the cleavage of the labile arabinofuranose linkage and show that the glucose unit is linked to O-4 of the mannose residue, thus locating the position of the glucose residue in the main chain and establishing that the galactofuranose is linked to O-3 of the in-chain glucose residue.

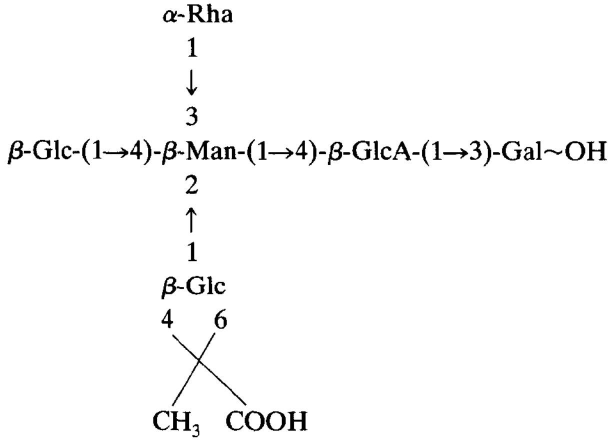
Selective periodate oxidation. — Painter *et al.*[11] have shown that certain sugars are preferentially oxidized by periodate. Advantage has been taken, in this experiment, of the sensitivity of the rhamnose residues to attack by periodate. The native polysaccharide was oxidized with periodate for a period of time sufficient only to degrade the galactofuranose and the terminal rhamnose. Reduction of the aldehydes formed and subsequent treatment with dilute acid yielded a polymer, which on methylation gave the results in Table II, column V. These indicate that the rhamnose residue is linked to O-3 of the branched mannose. Comparison of the data in columns IV and V of Table II shows that the terminal glucose, carrying pyruvate, must be linked to O-2 of the mannose.

The ^{1}H-n.m.r. spectrum of the partially oxidized polysaccharide (Fig. 1b) showed the absence of one α-anomeric resonance, thus allowing the two fractional signals (B^1, B^2, Fig. 1a) at δ 4.93 (0.4 H) and δ 5.06 (0.6 H) in the spectrum of the autolyzed polysaccharide to be assigned to the rhamnose residue. The signal (B^2) at δ 4.93 is attributed to the resonance of the α-rhamnose units present in repeating units containing pyruvate and the resonance (B^1) at δ 5.06 to those α-rhamnose units present in repeating units devoid of pyruvate.

Partial hydrolysis. — Partial hydrolysis of the native polysaccharide with acid and separation by paper electrophoresis and paper chromatography resulted in the isolation of two acidic oligosaccharides.



P1



P2

Product **P1** had $[\alpha]_D$ −39.6° and **P2** had $[\alpha]_D$ −33.2°. Both oligosaccharides had a d.p. of 6 and galactose as the reducing end sugar[12]. The ^{1}H-n.m.r. spectra showed that the only difference between the two saccharides was the presence of pyruvate in **P2** (see Fig. 1d and e). Methylation analysis of **P2** gave the results shown in Table II, column VII; thus cleavage of the polysaccharide occurred at the acid-labile galactofuranose linkage and resulted in the isolation of an intact repeating unit of the polysaccharide. The presence of 2,5,6- and 2,4,6-tri-*O*-methylgalactose is consistent with galactose occupying the reducing terminus of the oligosaccharide. The appearance of 2,3,4,6-tetra-*O*-methylglucose confirms that the galactofuranose is linked to the 3-linked glucose unit in the polysaccharide.

The ^{1}H-n.m.r. spectrum of the autolyzed, original polysaccharide (Fig. 1a) shows certain signals to be twinned due to the presence of some polymer without acetal substituents and some with. These species correspond to the two oligosaccharides **P1** and **P2**. Examination of the spectra of the individual oligomers (Fig. 1d and e) permits the assignment of the signals in the autolyzed polymer (Fig. 1a). Thus in the spectrum of **P1** (Fig. 1d) the resonance of the anomeric proton (B^1) of the α-linked rhamnose occurred at δ 5.06 and in that of **P2** (Fig. 1e) at δ 4.94 (B^2), justifying the assignments of these two α-resonances in the spectrum of the autolyzed polysaccharide to the rhamnose residue in repeating units without and with pyruvate, respectively. In the spectra of **P1** and **P2** the resonances of the reducing galactose unit occurred at δ 5.29 (α) and δ 4.63 (β), and in the spectrum of **P1** reduced (Fig. 1f) these signals disappeared as expected. This confirms the assignment of the resonance (A) at δ 5.33 in the spectrum of the autolyzed native polysaccharide to the 3-linked galactofuranose unit.

In the ^{1}H-n.m.r. spectrum of **P1** (Fig. 1d) the resonances at δ 5.06, 4.76, 4.68, and 4.46, due to the anomeric protons of the L-rhamnose (B^1), D-mannose (C), and two terminal D-glucose units (D + F), coincide with those assigned to these residues in repeating units devoid of pyruvate in the spectrum of the autolyzed polysaccharide. Similarly in the spectrum of **P2** at ambient temperature (Fig. 1e) the resonances at δ 4.94 (B^2) and δ 4.45, due to the L-rhamnose (B^2) and terminal D-glucose residues, coincide with those assigned to these residues in the spectrum of the autolyzed polysaccharide in which the repeating units carry pyruvate. It was not possible to ascertain the positions of resonance of the anomeric protons of the mannose, glucuronic acid, and terminal pyruvylated glucose residues in **P2**, as these were obscured by the residual HOD peak. The spectrum had to be run at ambient temperature in order to avoid hydrolysis of the 1-carboxyethylidene groups.

CONCLUSION

The structure of the capsular polysaccharide of *Klebsiella* serotype K14 is thus as shown in the Abstract. It is interesting to note that this is the third polysaccharide in this series to contain a D-galactofuranose unit. The other two examples are the capsular antigens derived from *Klebsiella* K12 (ref. 13) and K41 (ref. 14) but in each of these two instances the furanose residue represented the branch point.

EXPERIMENTAL

General methods. — Optical rotations were measured in a 1 dm cell at 23–25° using a Perkin–Elmer model 141 polarimeter. Solutions were evaporated under diminished pressure at temperatures not exceeding 40°. Paper chromatography was conducted by the descending method using Whatman No. 1 paper and the following solvent systems (v/v): I, 8:2:1 ethyl acetate–pyridine–water; II, 2:1:1 1-butanol–acetic acid–water; and III, 18:3:1:4 ethyl acetate–acetic acid–formic acid–water.

Chromatograms were developed with silver nitrate[15] (A), *p*-anisidine HCl (ref. 16) (B), and periodate–benzidine[17] (C). Paper electrophoresis was performed in a Savant high-voltage (5 kV) system, with kerosene as the coolant. The buffer used was 5:2:743 (v/v) pyridine–acetic acid–water, pH 5.3. Strips (70 × 20 cm) of Whatman No. 1 paper were used for all runs, with application of 15–25 mA for 1–1.5 h. Analytical g.l.c. separations were performed using a Hewlett–Packard model 5710 A gas chromatograph fitted with dual flame ionization detectors. A stainless steel column (1.8 m × 3 mm) of 3% OV-225 on Gas Chrom Q (100–120 mesh) was used and operated at a temperature of 190° (isothermal) except as otherwise stated. G.c.–m.s. was performed with a Micromass-12 instrument with a Watson–Biemann separator. Spectra were recorded at 70 eV with an ionization current of 100 μA and an ion source temperature of 200°.

^{1}H- And ^{13}C-n.m.r. spectra were recorded on a Bruker WH-400 FT spectrometer at ambient temperature or at 95° as required. Samples in D_2O were hydrogen exchanged by freeze drying three or four times from 99.7% D_2O. In all cases, acetone (δ 2.23 for ^{1}H and 31.07 p.p.m. for ^{13}C, measured against aqueous sodium 4,4-dimethyl-4-silapentane-1-sulfonate) was used as the internal standard.

Isolation and purification of the polysaccharide. — A culture of *Klebsiella* K14 (1193) was obtained from Drs. F. and I. Ørskov, Copenhagen and propagated on a sucrose-rich nutrient agar. The capsular polysaccharide was isolated and purified as previously described[3], to yield 7.42 g.

Analysis of component sugars. — Polysaccharide (15 mg) was hydrolyzed overnight (16 h) with 2M trifluoroacetic acid (TFA) at 100°. After evaporation of the acid, the liberated sugars were transformed into peracetylated aldononitriles, according to the method of McGinnis[18], and analyzed by g.l.c. on a column of OV-225 at 210° (isothermal). To ascertain the identity of the uronic acid, the polysaccharide (25 mg) was methanolyzed with 3% methanolic hydrogen chloride, reduced overnight with sodium borohydride (50 mg) in anhydrous methanol, and hydrolyzed with 2M TFA. G.l.c. of the derived peracetylated aldononitriles gave the identity of the uronic acid.

Methylation analysis. — A portion of the polysaccharide (42 mg) was methylated by the Hakomori method[19]. The product was divided into two portions. One was hydrolyzed with 2M TFA overnight at 100°, the hydrolyzate was reduced with sodium borohydride (1 h) and the alditols were acetylated with 1:1 acetic anhydride–pyridine (1 h, 100°). The other portion was methanolyzed (3% methanolic HCl, 16 h, 80°), reduced (sodium borohydride in dry methanol, 16 h), hydrolyzed with 2M TFA overnight at 100°, and converted into the corresponding alditol acetates as for portion one. Analysis of both by g.l.c. and g.c.–m.s.[20,21] allowed the assignments given in Table II, columns I and II.

Uronic acid degradation. — A sample of the methylated polysaccharide (75 mg) was subjected to a base-catalyzed degradation as previously described[22] and directly re-alkylated with methyl iodide. Hydrolysis of the degraded product with 2M TFA, reduction with sodium borohydride, and acetylation with 1:1 acetic anhydride–pyridine and g.c.–m.s. analysis of the corresponding alditol acetates gave the results listed in Table II, column III.

Smith degradation[9]. — A solution of K14 polysaccharide (14.8 mg) in water (5 mL) was mixed with a 0.03M solution of $NaIO_4$ (5 mL) and the reaction was allowed to proceed at room temperature in the dark. The consumption of periodate was followed spectrophotometrically[9]. A second solution of polysaccharide (502 mg) in water (50 mL) was mixed with a solution (50 mL) of 0.1M $NaIO_4$–0.4M $NaClO_4$, and the reaction was allowed to proceed at room temperature in the dark for 96 h. Ethylene glycol (5 mL) was then added, the solution was dialyzed against running water (3 d), and freeze-dried to yield 430 mg of material. This residue was dissolved in water (50 mL) and sodium borohydride (500 mg) was added. After 16 h the borohydride was neutralized by the dropwise addition of 10% aqueous acetic acid, and the solution was dialyzed (3 d) and freeze dried. The residual material was oxidized with periodate (40 mL, 0.05 M in $NaIO_4$ and 0.20M in $NaClO_4$). After 48 h, reduction afforded the polyalcohol (306 mg). This product was hydrolyzed for 48 h with 0.5M TFA at room temperature. After dialysis and centrifugation to remove insoluble contaminants the material was freeze dried (230 mg). A portion was methanolyzed, reduced, hydrolyzed, and converted to peracetylated aldononitriles. Another portion (50 mg) was methylated by the Hakomori method and analyzed by g.c.–m.s. as partially methylated alditol acetates.

A portion (80 mg) of the polymeric material resulting from the Smith degradation was redissolved in 0.5M TFA (10 mL) and allowed to stand at room temperature for 48 h. The TFA was removed by evaporation and the product was dissolved in water and freeze dried. Paper chromatography (solvent III) was performed on the material. After spraying with reagents A and B, arabinose was observed in the chromatograms. With reagent C arabinose and a nonreducing oligosaccharide were observed. The further hydrolyzed Smith-degraded material (75 mg) in water was then reduced with $NaBH_4$ (1 h), and a portion (10 mg) was methylated by the Hakomori method, hydrolyzed and converted to alditol acetates for g.c.–m.s. analysis.

Selective periodate oxidation. — A solution of polysaccharide (30 mg) in water (10 mL) was mixed with a solution (10 mL) of 0.03M $NaIO_4$ and the reaction was allowed to proceed at room temperature in the dark. The uptake of periodate was monitored every hour, spectrophotometrically[9] and by analysis of sugar composition. After 1 h, most of the rhamnose had been consumed, as had the galactose, which was replaced by a complementary amount of arabinose. The experiment was repeated on 600 mg of polysaccharide, and after 1 h the reaction was terminated by the addition of ethylene glycol (5 mL). The material was worked up as before and a polysaccharide (410 mg) was obtained. A portion of this material (10 mg) was hydrolyzed and converted to peracetylated aldononitriles. A further portion (25 mg) was methylated (Hakomori), hydrolyzed, and converted to alditol acetates.

Partial hydrolysis. — A sample of polysaccharide (502 mg) was dissolved in 0.5M TFA (100 mL) and allowed to stand at room temperature (96 h). Paper chromatography (solvents II and III, reagent A) showed no significant products of hydrolysis. The solution was then heated on a steam bath (90°, 15 min). After evaporation of the acid, paper chromatography (solvent I) showed that very little neutral material was present. However, in solvent II, after 14 d, two distinct oligosaccharides appeared (spray A). The partial hydrolyzate was then applied to large papers (60 × 60 cm) and the oligosaccharides were separated (solvent II). The oligosaccharides were extracted into water, freeze-dried, and purified by high voltage paper electrophoresis, to yield **P1** (11.8 mg) and **P2** (24.0 mg).

Product **P1** was reduced ($NaBH_4$, 1 h) and the reduced product and **P2** (2 mg) were hydrolyzed and transformed into peracetylated aldononitriles. Product **P2** was also methylated (Hakomori), reduced, hydrolyzed, and analyzed by g.l.c. as partially methylated alditol acetates.

ACKNOWLEDGMENTS

The authors thank N.S.E.R.C., Ottawa, for financial support of this work, and the South African Council for Scientific and Industrial Research for an overseas bursary and a research bursary granted to H.P. and L.A.S.P. respectively. We are grateful to Dr. Ida Ørskov for the culture of *Klebsiella* used, and to Dr. S. C. Churms for the molecular weight measurement.

REFERENCES

1 W. NIMMICH, *Z. Med. Mikrobiol. Immunol.*, 154 (1968) 117–131; *Acta Biol. Med. Ger.*, 265 (1971) 397–403.
2 G. G. S. DUTTON AND D. N. KARUNARATNE, *Carbohydr. Res.*, 119 (1983) 157–169.
3 K. OKUTANI AND G. G. S. DUTTON, *Carbohydr. Res.*, 86 (1980) 259–271.
4 G. M. BEBAULT, G. G. S. DUTTON, N. A. FUNNELL, AND K. L. MACKIE, *Carbohydr. Res.*, 63 (1978) 183–192.
5 P. J. GAREGG, P. E. JANSSON, B. LINDBERG, F. LINDH, J. LÖNNGREN, I. KVARNSTRÖM, AND W. NIMMICH, *Carbohydr. Res.*, 78 (1980) 127–132.
6 J. H. BRADBURY AND G. A. JENKINS, *Carbohydr. Res.*, 126 (1984) 125–156.
7 B. LINDBERG AND J. LONNGREN, *Methods Carbohydr. Chem.*, 7 (1976) 142–148.
8 G. O. ASPINALL, T.-N. KRISHNAMURTHY, W. MITURA, AND M. FUNABASHI, *Can. J. Chem.*, 53 (1975) 2182–2188.
9 G. W. HAY, B. A. LEWIS, AND F. SMITH, *Methods Carbohydr. Chem.*, 5 (1965) 361–370.
10 B. CAPON AND D. THACKER, *Proc. Chem. Soc.*, (1964) 369.
11 K. M. AALMO, M. F. ISHAK, AND T. J. PAINTER, *Carbohydr. Res.*, 63 (1978) c3–c7.
12 I. A. MORRISON, *J. Chromatogr.*, 108 (1975) 361–364.
13 G. G. S. DUTTON AND A. V. SAVAGE, *Carbohydr. Res.*, 83 (1980) 351–362.
14 G. CHAMBAT, J.-P. JOSELEAU, M. LAPEYRE, AND A. LEFEBVRE, *Carbohydr. Res.*, 63 (1978) 323–326.
15 W. E. TREVELYAN, D. P. PROCTER, AND J.-S. HARRISON, *Nature*, 166 (1950) 444.
16 L. HOUGH, J. K. N. JONES, AND W. H. WADMAN, *J. Chem. Soc.*, (1950) 1702–1706.
17 H. T. GORDON, W. THORNBURG, AND L. N. WERUM, *Anal. Chem.*, 28 (1956) 849.
18 G. D. MCGINNIS, *Carbohydr. Res.*, 108 (1982) 284–292.
19 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
20 P. E. JANSSON, L. KENNE, H. LIEDGREN, B. LINDBERG, AND J. LÖNNGREN, *Chem. Commun. Univ. Stockholm*, 8 (1976) 1–75.
21 J. LONNGREN AND S. SVENSSON, *Adv. Carbohydr. Chem. Biochem.*, 29 (1974) 41–106.
22 G. O. ASPINALL AND K.-G. ROSELL, *Carbohydr. Res.*, 57 (1977) c23–c26.