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STRUCTURAL STUDIES OF THE *Klebsiella* TYPE 47 CAPSULAR POLYSACCHARIDE

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ABSTRACT

The structure of the capsular polysaccharide from *Klebsiella* Type 47 has been investigated Methylation analysis and characterization of oligosaccharides obtained on acid hydrolysis were the principal methods used The polysaccharide is composed of tetrasaccharide repeating-units, and a structure for these units is proposed

INTRODUCTION

Klebsiella have been differentiated into a large number of serologically distinct types, determined by their capsular polysaccharides or K-antigens Nimmich[1,2] has determined the sugar compositions of the K-antigens elaborated by the types 1–80 Full or partial structures have been reported for only a few of these antigens We recently reported structural studies of the K9 polysaccharide[3], which is composed of L-rhamnose, D-galactose, and D-glucuronic acid residues In the present communication, structural studies of the K47 polysaccharide, which is composed of the same sugar residues, are reported

RESULTS AND DISCUSSION

The polysaccharide, isolated as previously described[1], had $[\alpha]_{578}$ −46° It did not show any significant i r absorption in the 1735 cm^{-1} region, demonstrating the absence of *O*-acetyl or other *O*-acyl groups Glucose, galactose, and rhamnose were obtained from a hydrolysate of the polysaccharide *via* lithium aluminium deuteride reduction of the trimethylsilyl ethers The relative proportions 0 7 1 0 1 7 of the three sugars and the presence of two deuterium atoms at C-6 in the glucose were demonstrated by g l c[4]–m s[5] of the derived alditol acetates D-Galactose and L-rhamnose were isolated from a hydrolysate and their configurations were demonstrated by their optical rotations The D configuration of the glucuronic acid was demonstrated by the action of β-D-glucuronidase on the aldobiouronic acid obtained on hydrolysis of the polysaccharide

The polysaccharide was methylated by the Hakomori procedure[6] and part of the product was reduced with lithium aluminium deuteride The two samples were hydrolysed and the sugars in the hydrolysates were analysed, as their alditol acetates, by g l c–m s [7] The results are summarized in Table I The components were readily identified from their retention times and mass spectra 2,3-Di-*O*-methyl-D-glucose, which was obtained only from the carboxyl-reduced product, was dideuterated at C-6 and thus derived from the D-glucuronic acid residue

TABLE I

METHYL ETHERS FROM THE HYDROLYSATE OF THE METHYLATED (*A*) AND METHYLATED, CARBOXYL-REDUCED (*B*) CAPSULAR POLYSACCHARIDE FROM *Klebsiella* TYPE 47

Sugars[a]	T[b]	*Mole %*	
		A[c]	B[c]
2,3,4-Rha	0 46	21	23
2-Rha	1 52	39	29
2,4,6-Gal	2 28	41	26
2,3-G	5 39	—	22

[a]2,3,4-Rha = 2,3,4-tri-*O*-methyl-L-rhamnose, etc [b]Retention time of the corresponding alditol acetate, relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on an ECNSS-M column at 170° [c]Average values from three determinations

The results of the sugar and methylation analyses suggest that the polysaccharide contains a tetrasaccharide repeating-unit composed of one D-galactose residue, two L-rhamnose residues, and one D-glucuronic acid residue The analysis also gives information on the positions through which the sugar residues are linked The carboxyl reduction is often not quantitative, which accounts for the low estimate of D-glucose and 2,3-di-*O*-methyl-D-glucose in the sugar and methylation analyses, respectively 2,3,4-Tri-*O*-methyl-L-rhamnose and derivatives are volatile, and part of this sugar was probably lost during concentration of solutions

The polysaccharide was subjected to partial, acid hydrolysis and four fractions, A_1, A_2, A_3, and A_4, containing acidic oligosaccharides were isolated and investigated (Table II) Each appeared to be homogeneous on paper chromatography The fractions were reduced with sodium borodeuteride and methylated, and the permethylated oligosaccharide alditol esters of A_1, A_2, and A_3 were investigated by g l c–m s [3,11,12] Systematic studies by Kochetkov[8], Bauer[9 10], and Kärkkäinen[11 12], and their co-workers, facilitated the interpretation of the spectra Portions of the permethylated oligosaccharide alditol esters were reduced with lithium aluminium deuteride and hydrolysed, the products were reduced with borohydride and acetylated, and the mixtures of alditol acetates were analysed by g l c–m s [7] (Table II)

G l c of compounds obtained after reduction and methylation of the fractions A_1 and A_2 showed that each contained a major component (90% or more) There was complete agreement between the structures of these components determined by

TABLE II

ACIDIC OLIGOSACCHARIDES OBTAINED ON PARTIAL HYDROLYSIS WITH ACID OF THE CAPSULAR POLYSACCHARIDE FROM *Klebsiella* TYPE 47

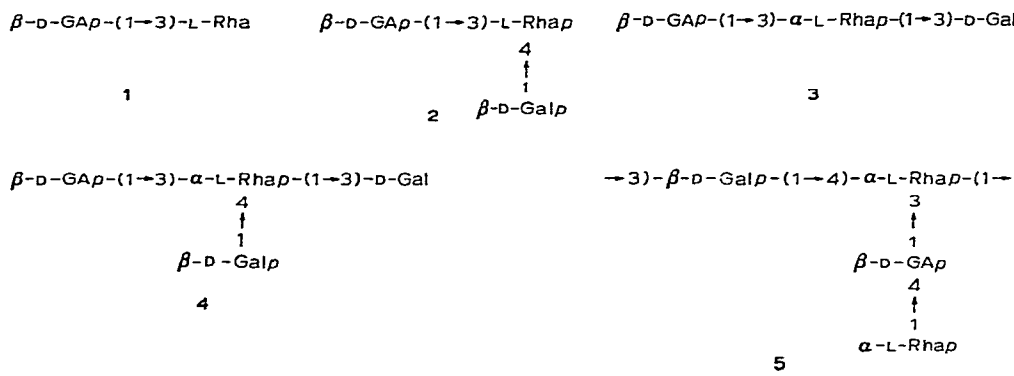
Fraction	*Yield (mg)*	$[\alpha]_{578}^{20}$ *(degrees)*	*Paper-chromatographic mobility*[a]	T_{MEL}[b]	*Methylation analyses*
A_1	33	−25	0 97	0 46 (at 190°)	2,3,4-G[c,e] (2 49)[d] 1,2,4,5-Rha[f] (—)
A_2	7	−21	0 55	7 0 (at 230°)	2,3,4-G[e] (2 49) 2,3,4,6-Gal (1 25) 1,2,5-Rha[f] (0 35)
A_3	38	—	0 55	7 0,7 9 (at 230°)	2,3,4-G[e] (2 49) 2,3,4,6-Gal (1 25) 2,4-Rha (0 99) 1,2,4,5,6-Gal[f] (0 42) 1,2,5-Rha[f] (0 35)
A_4	11	—	0 35	—	2,3,4-G[e] (2 49) 2-Rha (1 52) 2,3,4,6-Gal (1 25) 1,2,4,5,6-Gal[f] (0 42)

[a]Relative to D-glucose [b]Retention time of the permethylated alditols, relative to permethylated melibiitol on the XE-60 column [c]See Table I note *a* [d]See Table I, note *b* [e]Deuterium labelling in the 6-position [f]Deuterium labelling in the 1-position

the two methods As the m s of oligosaccharide derivatives were analogous to those obtained in a previous study[3], there is no need to comment on them Fraction A_1 contained an aldobiouronic acid (**1**), and A_2 an aldotriouronic acid (**2**) The anomeric natures of the glycosidic linkages are evident from the low optical rotations of the products and from the fact that the aldobiouronic acid was hydrolysed by the action of a β-D-glucuronidase

Fraction A_3 contained comparable amounts of two components, as demonstrated by g l c of the reduced and methylated product One of these was chromatographically and mass-spectrometrically indistinguishable from the aldotriouronic acid (**2**) in A_2 The m s of the other component, which has a higher retention time on g l c , proved it to be a linear aldotriouronic acid (**3**) In agreement with this assignment, the alditol acetates derived from 2,4-di-*O*-methyl-L-rhamnose and 1,2,4,5,6-penta-*O*-methyl-D-galactitol, monodeuterated at C-1, were obtained in addition to those obtained from A_2 on methylation analysis of borodeuteride-reduced A_3 Finally, methylation analysis of borodeuteride-reduced A_4 proved it to contain the aldotetraouronic acid **4** as the main component

All sugar residues in the polysaccharide are pyranosidic as demonstrated by methylation analyses of the polysaccharide (2,3,4-tri-*O*-Me-L-Rha, 2,4,6-tri-*O*-Me-D-Gal) and the acidic oligosaccharides (2,3,4-tri-*O*-Me-D-G, 1,2,5-tri-*O*-Me-L-Rha)



The β-linkages of the D-glucuronic acid and the D-galactose residues are discussed above From the low optical rotation of the polysaccharide, it is inferred that the L-rhamnosidic residues are α-linked The change in optical rotation of the polysaccharide during acid hydrolysis was followed polarimetrically A steady increase from $[\alpha]_{578}$ $-55 \rightarrow -4°$ was noticed during 5 h The main contribution to this increase can be attributed to cleavage of the L-rhamnosidic linkages, since these should be the most readily hydrolysed links in the polysaccharide The observed increase in optical rotation therefore supports the assumption that the L-rhamnose residues in the polysaccharide are α-linked

Only a single structure (5) of a tetrasaccharide repeating-unit is compatible with the combined evidence presented above

EXPERIMENTAL

General methods. — Concentrations were carried out under diminished pressure at bath temperatures which did not exceed 40° For g l c , Perkin–Elmer 900 or 990 instruments fitted with flame-ionisation detectors were used Separations were performed on glass columns (180 × 0 15 cm) containing (*a*) 3% ECNSS-M on Gas Chrom Q (100/120 mesh) at 170° (for partially methylated alditol acetates) or 190° (for alditol acetates) and (*b*) 3% XE-60 on the same support (for permethylated oligosaccharide alditol derivatives) For quantitative evaluation of the g l c , a Hewlett–Packard 3370B integrator was used For mass spectrometry, a Perkin–Elmer 270 g l c –m s instrument fitted with OV-225 S C O T columns (for alditol acetates and partially methylated alditol acetates) or XE-60 columns was used Mass spectra were recorded at an ionisation potential of 70 eV, an ionisation current of 80 μamp, and an ion-source temperature of 80° Paper chromatography was performed on Whatman No 1 paper in the solvent system ethyl acetate–acetic acid–water (3 1 1) The compounds were detected with 3% *p*-anisidine hydrochloride in ethanol at 120° Optical rotations were recorded using a 10-cm micro-cell with a Perkin–Elmer 141 instrument, and i r spectra were recorded with a Perkin–Elmer 257 instrument

Isolation of the polysaccharide from Klebsiella *K-Type 47 (strain 9682)* — This was performed as previously described[1] The polysaccharide showed $[\alpha]_{578}^{20}$ −46° (*c* 0 3, water) In the i r spectrum (KBr), no significant absorptions around 1735 cm^{-1} (*O*-acyl region) were observed The percentages of nitrogen (0 35%) and phosphorus (0 12%) in the material were insignificant

Sugar and methylation analyses — These were performed as previously described[3 6] Rhamnose and galactose were isolated by preparative paper chromatography of a hydrolysate of the polysaccharide (50 mg) The samples showed $[\alpha]_{578}^{20}$ +20° and +52° (*c* 0 2, water), respectively

Configuration of glucuronic acid — The aldobiouronic acid **1** (2 mg) was incubated for 24 h with β-D-glucuronidase (Sigma Chemical Company) in 4mM phosphate buffer (pH 6 8, 1 ml) Paper chromatography showed that the oligosaccharide had been hydrolysed to glucuronic acid and rhamnose

Isolation of oligosaccharides — The polysaccharide (400 mg) was hydrolysed in 0 13M sulphuric acid (40 ml) at 100° for 1 h The hydrolysate was neutralised with barium carbonate The filtered solution was added to the top of a column (0 9 × 15 cm) of Dowex-1 x8 (AcO^-) resin which was eluted first with water, yielding galactose and rhamnose, and then with acetic acid (6%), yielding acidic oligosaccharides

Characterization of oligosaccharides — All the fractions, which were later characterized, gave single spots on paper chromatography (the mobilities are given in Table II) The oligosaccharides (2-mg samples) were transformed into alditols by reduction with sodium borodeuteride (20 mg) in water (5 ml) After treatment with Dowex-50(H^+) resin and concentration, boric acid was removed from the residue by codistillation with methanol The oligosaccharide alditols were then methylated by Hakomori's procedure, as previously described[3 6] The methylated oligosaccharide alditol esters of A_1, A_2, and A_3 were analysed by g l c–m s [11,12], using an XE-60 column The m s of the components showed, *inter alia*, the following peaks (relative intensities in brackets) A_1 46 (11), 59 (36), 60 (15), 88 (31), 90 (20), 101 (94), 103 (19), 169 (10), 201 (100), 206 (6), 233 (11), 266 (8), A_2 46 (10), 59 (28), 88 (100), 90 (8), 101 (70), 169 (10), 187 (35), 201 (70), 219 (42), 233 (7), 424 (0 2), 484 (0 2), A_3 (peak with T_{MEL} 7 9) 45 (72), 46 (20), 88 (100), 89 (27), 90 (24), 101 (95), 133 (12), 169 (8), 201 (100), 233 (20), 236 (28) In order to transform the methylated acidic oligosaccharides into alditol acetates, the preparations were dissolved in dry ethyl ether (15 ml) and lithium aluminium deuteride (50 mg) was added The solutions were refluxed for 3 h After processing, the products were hydrolysed with 0 13M sulphuric acid for 12 h at 100°, and the methylated sugars were converted into alditol acetates and analysed by g l c–m s [7] The *T*-value for the tetra-*O*-methyl-L-rhamnitol derivative from fraction A_1 was too low to be determined accurately

Acid hydrolysis of the polysaccharide — A solution of the polysaccharide in 0 25M sulphuric acid was kept at 80° and the optical rotation was monitored A steady increase of $[\alpha]_{578}^{80}$ −55→−4° (*c* 0 2, 0 25M sulphuric acid) was observed during 5 h

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