Preliminary communication

Capsular polysaccharide from *Klebsiella* type 21

G. G. S. DUTTON and Y. M. CHOY
Department of Chemistry, University of British Columbia, Vancouver (Canada)
(Received September 25th, 1971; accepted in revised form, November 4th, 1971)

Bacteria of the genus *Klebsiella* belong to the family *Enterobacteriaceae*, the chemistry of which has been reviewed[1]. Approximately eighty types of *Klebsiella* are recognised on the basis of immunochemical tests. One of the characteristics of *Klebsiella* bacteria is the formation of a capsular polysaccharide that is antigenic and the composition of which has been stated to be the same as that of the slime polysaccharide excreted into the medium[2]. Different species of *Klebsiella* are thus classified according to their capsular antigens into K-types.

The great majority of K-types 1 to 72 contain[3] D-glucuronic acid in combination with such hexoses as D-glucose, D-galactose, and D-mannose. Many strains contain, in addition, L-rhamnose and a few L-fucose residues. The presence of bound pyruvic acid in *Klebsiella* was first reported[4] in the case of *K. rhinoscleromatis* (type 3), and pyruvic acid has subsequently been found[5] in several different species. The pyruvic acid moiety has been shown to be base stable and acid labile, thus suggesting an acetal rather than an ester linkage. In certain cases, pyruvic acid acetal appears to be an immunodominant unit and accounts, at least in part, for cross reactions between certain species of *Klebsiella*, *Pneumococci*, and *Rhizobia*[6].

Detailed structures have been given for the capsular polysaccharides of *Klebsiella* K-types 2 (ref. 7), 8 (ref. 8), and 54 (refs. 9, 10). A pentasaccharide repeating unit has been proposed[11] for *K. aerogenes* (NCTC 9644) based on a computer-assisted microanalysis. We report here the main features of the capsular material from K-type 21, as shown by methylation studies on the original capsular polysaccharide, the residual polysaccharide recovered after autohydrolysis, and the carboxyl-reduced polysaccharide. Fragments released on autohydrolysis were also examined.

In addition to K-type antigens, *Klebsiella* also possess somatic O antigens, and the carbohydrate composition of these O antigens of nineteen K-types belonging to the O groups 1 to 12 has been determined[12]. The O antigenic polysaccharides appear to consist of a common core in association with characteristic sugars such as D-ribose, D-galactose, D-mannose, and L-rhamnose. In certain cases 3-*O*-methyl-(?)D-mannose is found[13], a sugar that is hard to differentiate by paper chromatography from rhamnose, and *Klebsiella* K73:010 has been shown to contain 3-*O*-methyl-L-rhamnose[14]. In the only structural studies on O antigens so far reported, *Klebsiella* K66:01 and K64:06 have both been shown

to contain O specific side chains of α-(1→3)-linked D-galactopyranose residues[15], and the heterogeneity of the lipopolysaccharide of *Aerobacter aerogenes* (NCTC 243) has been investigated[16,17].

A culture of *Klebsiella* K21 (1702/49) was obtained from Dr. Ørskov, Copenhagen, as an agar slant and was grown on sucrose–yeast-extract agar. Cells were harvested after 3 days, diluted with water containing 1% phenol, and centrifuged. Capsular polysaccharide was obtained by concentrating the aqueous solution, pouring it into ethanol, and purifying the crude material by Cetavlon precipitation. The freeze-dried product had $[\alpha]_D^{20}$ +130° (*c* 0.38, H_2O). Acid hydrolysis of the polysaccharide (0.5M H_2SO_4, 8 h at 100°) gave, after ion-exchange separation, a neutral fraction containing D-mannose and D-galactose in approximately equal amounts (1:1.2), as judged by g.l.c. of the trimethylsilyl derivatives or of the alditol acetates. G.l.c. of the acetates permitted recovery of the acetates and identification of D-mannitol hexaacetate, m.p. and mixed m.p. 118–121°, and galactitol hexaacetate, m.p. 162°. The acidic fraction was separated into two parts by paper chromatography. The slower-migrating, major portion had the mobility of an aldobiouronic acid and was converted into the ester glycoside which, on reduction and hydrolysis, gave approximately equimolecular amounts of D-glucose and D-mannose.

Separate portions of the polysaccharide were dissolved in water (initial pH 2–2.3) and heated for 8 h at 95°. Paper chromatography of the concentrated solution showed a fast-moving spot, chromatographically identical with pyruvic acid and having the same characteristic fluorescence when sprayed with *o*-phenylenediamine and examined by u.v.[18]. The chromatogram also showed D-galactose and slow-moving oligomers. In one experiment, the aqueous solution was extracted with ether and the ether extract was treated with 2:4-dinitrophenylhydrazine to yield the derivative of pyruvic acid m.p. 214–216°, undepressed by an authentic sample[19].

Fully methylated capsular polysaccharide, obtained by the method of Hakomori[9,20] on acid hydrolysis gave neutral and acidic fractions (*A* and *B*, Table I). The former showed, by paper chromatography (butanone–water), four major components that were separated on a cellulose column and, as their alditol acetates, by g.l.c.[21]. The alditol acetates of 2,4,6- and 3,4,6-tri-*O*-methyl-D-mannose were not separated on a column of ECNSS-M but were resolved on a column of 5% butanediol succinate programmed from 150° at 2°/min; retention times 17.2 and 19.6 min, respectively. The positions of the methoxyl groups in these two compounds, and in all other methylated alditol acetates isolated in this study, were assigned by examination of their mass spectra[22]. Each of the foregoing methylated mannitol acetates was demethylated with boron trichloride[23] and acetylated to give D-mannitol hexaacetate (g.l.c., m.p., and mixed m.p. 118–121°). The 2,4,6-isomer was further characterised as the crystalline alditol acetate, m.p. and mixed m.p.[24] 65–67°. 2,4,6-Tri-*O*-methyl-D-galactose was identified as the crystalline sugar m.p. 103–105° (from ether) (lit.[25] m.p. 102–105°), by the mass spectrum of its alditol acetate (T 21.4 min, ECNSS-M), and by demethylation to D-galactose. 2,3-Di-*O*-methyl-D-galactose was identified as the alditol acetate (T 29.4 min, ECNSS-M) and by the fact that demethylation of the alditol and acetylation gave galactitol hexaacetate, m.p. and mixed m.p. 162°. Hydrolysis of the reduced acid-fraction (*B*, Table I) gave 2,4,6-tri-*O*-methyl-D-mannose and 2-*O*-methyl-D-glucose, characterized as alditol acetates.

TABLE I

METHYL ETHERS FROM THE HYDROLYSATES OF METHYLATED *KLEBSIELLA* K21 POLYSACCHARIDES

Sugars	*Sample* [a]				
	A	B	C [b]	D	E
2,3,4,6-Tetra-*O*-methyl-D-galactose				+	
2,3,4,6-Tetra-*O*-methyl-D-mannose				+	
2,4,6-Tri-*O*-methyl-D-galactose	+		0.8	+	
2,4,6-Tri-*O*-methyl-D-mannose	+	+	1.0	+	+
3,4,6-Tri-*O*-methyl-D-mannose	+		1.1	+	
2,3-Di-*O*-methyl-D-galactose	+		1.0	+	
2,4-Di-*O*-methyl-D-glucose					+
2,6-Di-*O*-methyl-D-glucose			1.0		
2-*O*-Methyl-D-glucose		+			

[a] *A*, neutral sugars from methylated original K21 polysaccharide; *B*, reduced aldobiouronic acid fraction from methylated original K21 polysaccharide; *C*, neutral sugars from methylated reduced K21 polysaccharide; *D*, neutral sugars from methylated residual polysaccharide after autohydrolysis; *E*, reduced aldobiouronic acid from methylated residual polysaccharide after autohydrolysis.
[b] Approximate molar ratios; (+) signifies present and has no quantitative significance.

Capsular polysaccharide (0.4 g) was converted into the methyl ester propionate and reduced with lithium borohydride[26,27] to give a neutral product (0.3 g). Hydrolysis of this material, followed by reduction and acetylation, gave (g.l.c.) D-mannitol, galactitol, and D-glucitol hexaacetates in the ratio 1:1:0.6. D-Glucitol hexaacetate had m.p. and mixed m.p. 92–95°.

Methylation and hydrolysis of the lithium borohydride-reduced polysaccharide gave, in addition to the compounds previously obtained, 2,6-di-*O*-methyl-D-glucose (*C*, Table I) identified as the alditol acetate.

Acid hydrolysis of the methylated degraded polysaccharide obtained from the autohydrolysis gave, after separation on ion-exchange resins, a neutral and an acidic fraction. The neutral fraction (*D*, Table I) showed the presence of 2,3,4,6-tetra-*O*-methyl-D-galactose and a significant decrease in 2,3-di-*O*-methyl-D-galactose. This suggests that pyruvic acid is attached to D-galactose as a 4,6-acetal[28]. A small proportion of 2,3,4,6-tetra-*O*-methyl-D-mannose was also present.

The acidic fraction obtained from the methylated degraded gum was reduced and hydrolysed (*E*, Table I). The two main components were identified by g.l.c.—m.s. of their alditol acetates as 2,4,6-tri-*O*-methyl-D-mannose and 2,4-di-*O*-methyl-D-glucose. The isolation of the latter compound shows that D-glucuronic acid is linked through position 3 in the chain and has a side chain (probably D-galactose pyruvate acetal) attached to position 4.

These results show that the aldobiouronic acid is 3-*O*-(D-glucopyranosyluronic acid)-D-mannose, and demonstrate the main structural features of *Klebsiella* K21 capsular polysaccharide. This aldobiouronic acid, as the α-D anomer, has been found in the acidic glucomannan isolated from *Serratia marcescens*[29] and[7] in *Klebsiella* K2. The pyruvic acid may be responsible for the cross reactions reported previously[30,31] between *Klebsiella* K21 and K-types 8, 11, 26, and 35, and other *Enterobacteriaceae*[32,33].

ACKNOWLEDGMENTS

We are indebted to Dr. I. Ørskov, Copenhagen, for a culture (1702/49) of *Klebsiella* K21 and to Dr. P. J. Salisbury of this department for growing the bacteria. The financial support of the National Research Council, Ottawa, is gratefully acknowledged.

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