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Note

Structural studies of the capsular polysaccharide of *Klebsiella* type 30

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The *Klebsiella* type 30 capsular polysaccharide (K 30) is one of several *Klebsiella* extracellular polysaccharides which contain D-glucose, D-galactose, D-mannose, and D-glucuronic acid as the component sugars[1,2]. We now report structural studies of this polysaccharide.

The polysaccharide had $[\alpha]_{578}^{23}$ +16° and, on acid hydrolysis, yielded D-mannose, D-galactose, and D-glucose in the proportions 1.2:1:1. A hydrolysate of the carboxyl-reduced[3] polysaccharide contained the same sugars in the proportions 2:1:2, indicating that K 30 is composed of D-mannose, D-galactose, D-glucose, and D-glucuronic acid residues in the proportions 2:1:1:1. In the ^{1}H- and ^{13}C-n.m.r. spectra, the regions for anomeric protons and carbons, respectively, were not well-resolved. Signals in the ^{1}H-n.m.r. spectrum at δ 1.59 and 2.14, with the relative intensities 1:0.33, and in the ^{13}C-n.m.r. spectrum at 24.6 and 21.6 p.p.m. (relative to external tetramethylsilane), with the relative intensities 1:0.32, indicated that K 30 contains pyruvic acid residues and *O*-acetyl groups in this ratio.

Methylation analyses of original, carboxyl-reduced, and depyruvylated K 30 (Table I, columns *A*, *B*, and *C*) gave the same sugars and essentially the same proportions as the corresponding analyses of the *Klebsiella* type 33 capsular polysaccharide[4] (K 33). K 33 is composed of pentasaccharide repeating-units with the structure **1**, and it seemed possible that K 30 had a closely similar or even identical structure; the only major difference noted was that K 33 contained one *O*-acetyl group per repeating-unit, and K 30 only about one *O*-acetyl group per 3 repeating-units.

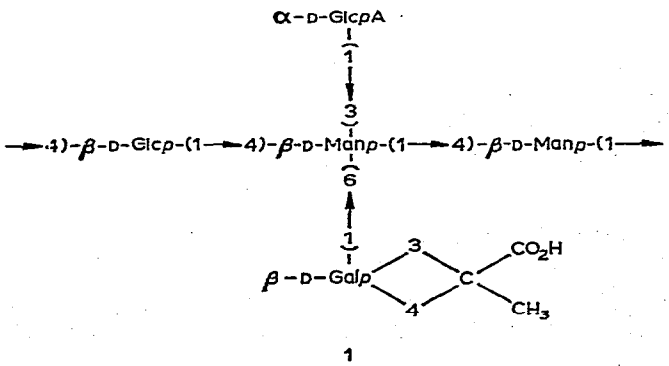
As already mentioned, the n.m.r. spectra of K 30 were not well-resolved. The *O*-deacetylated K 30, however, gave a good ^{1}H-n.m.r. spectrum, which could be superposed on the corresponding spectrum from *O*-deacetylated K 33. The optical rotations of the two *O*-deacetylated polysaccharides were also the same, namely, $[\alpha]_{578}^{24}$ +13°. For ^{13}C-n.m.r. studies, it was advantageous to remove the *O*-acetyl

TABLE I

METHYLATION ANALYSES OF ORIGINAL AND MODIFIED *Klebsiella* TYPE 30 CAPSULAR POLYSACCHARIDES

Methylated sugar[a]	T[b]	*Mole %*[c]			
		A	B	C	D
1,2,3,5,6-Man	0.45				14[d]
2,3,4,6-Glc	1.00		17		29
2,3,4,6-Gal	1.14			16	33
2,3,6-Man	1.79	28	20	31	
2,3,6-Glc	1.94	27	19	27	
2,6-Man	2.65			2	
2,6-Gal	2.77	24	23		
2-Man	4.85	21	21	23	25

[a]1,2,3,5,6-Man = 1,2,3,5,6-penta-*O*-methyl-D-mannitol; 2,3,4,6-Glc = 2,3,4,6-tetra-*O*-methyl-D-glucose, *etc.* [b]Retention time of the corresponding alditol acetate relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on an SP-1000 column at 220°. [c]Polysaccharide: *A*, original; *B*, carboxyl-reduced; *C*, partially depyruvylated by mild, acid hydrolysis; *D*, acidic pentasaccharide alditol, isolated after partial, acid hydrolysis. [d]Monodeuterated at C-1.



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groups and pyruvic acid residues (by mild, acid hydrolysis); the resulting material showed improved solubility. The ^{13}C-n.m.r. spectra of the two polysaccharides after this treatment could also be superposed.

The n.m.r. evidence therefore strongly supports the conclusion that *O*-deacetylated K 33 and K 30 are identical. Minor differences in structure, *e.g.*, that the 4-substituted β-D-mannopyranosyl and β-D-glucopyranosyl residues had changed places, did not seem to be absolutely excluded. Therefore, K 30 was subjected to partial hydrolysis with acid, and a pentasaccharide, $[\alpha]_{578}^{23}$ +24°, was isolated and reduced with sodium borodeuteride to the alditol. Methylation analysis of this pentasaccharide alditol (Table I, column *D*) showed that it contained terminal D-glucopyranosyl and D-galactopyranosyl groups, a D-mannopyranosyl residue linked through O-3, O-4, and O-6, and a D-mannitol residue, linked through O-4. The

sequence of sugar residues is therefore the same as in K 33. The uronic acid residue was not accounted for in this analysis. In the ^{1}H-n.m.r. spectrum, however, its anomeric proton appeared at δ 5.20 ($J_{1,2}$ ~3 Hz), compared to δ 5.17 ($J_{1,2}$ ~2 Hz) in the spectra of deacetylated K 33 and K 30.

The *O*-acetyl groups in K 30 were located by reaction with methyl vinyl ether and an acid catalyst, followed by methylation analysis[5]. D-Glucose, D-galactose, D-mannose, 6-*O*-methyl-D-mannose, and 3-*O*-methyl-D-mannose were obtained in the proportions 25:21:36:12:6. The formation of 3-*O*-methyl-D-mannose is due to uronic acid degradation[6], and was also observed when *O*-deacetylated K 30 was subjected to the same treatment. No 6-*O*-methyl-D-mannose was observed in the latter analysis. The *O*-acetyl groups in K 30 are therefore linked to position 6 of D-mannopyranosyl residues. All these positions are acetylated in K 33, but only about one-third of them in K 30; this seems to be the only detectable difference between the two polysaccharides.

Further confirmation of the similarity of the polysaccharide structure to that of K 33 was obtained by immune precipitation tests. Attempts to prepare high-titre K-30 antisera were not successful, but results were obtained by using a K-33 antiserum. Reaction of the native polysaccharide with K-33 antiserum was much less than the reaction between this antiserum and the homologous polysaccharide. However, when deacetylated K 33 and K 30 were tested, both precipitated the same amount of antibody nitrogen.

A number of lectins tested failed to precipitate the K 30 polysaccharide from solution. The carboxyl-reduced K 30 was strongly precipitated with concanavalin A, indicative of the presence of the terminal α-D-glucopyranosyl residues derived from glucuronic acid in the original polymer.

EXPERIMENTAL

The experimental methods used were essentially the same as in the study of the *Klebsiella* type 33 capsular polysaccharide[4]. The strain used was NCTC 7824, obtained from Dr. F. Ørskov, State Serum Institute, Copenhagen. The polysaccharide was prepared as described earlier for *Klebsiella* type 1 (K 1) polysaccharide[7]. Quantitative estimation of cross-reaction antibody nitrogen was performed by methods similar to those used by Heidelberger and Tyler[8]. The same techniques were used for concanavalin A precipitation.

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REFERENCES

1 W. NIMMICH, *Z. Med. Mikrobiol. Immunol.*, 154 (1968) 117–131.
2 W. NIMMICH, *Acta Biol. Med. Ger.*, 26 (1971) 397.
3 R. L. TAYLOR AND H. E. CONRAD, *Biochemistry*, 11 (1972) 1383–1388.
4 B. LINDBERG, F. LINDH, J. LÖNNGREN, AND W. NIMMICH, *Carbohydr. Res.*, 70 (1979) 135–144.
5 A. N. DE BELDER AND B. NORRMAN, *Carbohydr. Res.*, 8 (1968) 1–6.
6 M. CURVALL, B. LINDBERG, AND J. LÖNNGREN, *Carbohydr. Res.*, 41 (1975) 235–239.
7 C. ERBING, L. KENNE, B. LINDBERG, J. LÖNNGREN, AND I. W. SUTHERLAND, *Carbohydr. Res.*, 50 (1976) 115–120.
8 M. HEIDELBERGER AND J. M. TYLER, *J. Exp. Med.*, 120 (1964) 711–717.