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ISOLATION AND CHARACTERIZATION OF A 3,4-PYRUVIC ACETALATED-D-GALACTOSE-CONTAINING OLIGOSACCHARIDE OBTAINED FROM THE CAPSULAR POLYSACCHARIDE OF *Klebsiella* K33 BY SMITH DEGRADATION

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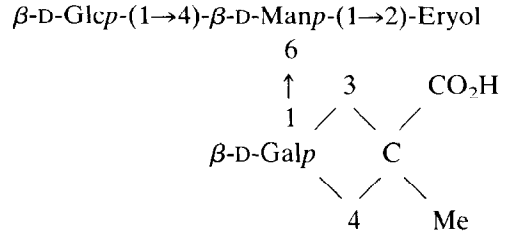
ABSTRACT

The capsular polysaccharide of *Klebsiella* K33 was subjected to Smith degradation, and an oligosaccharide-alditol, R_L 0.8, was isolated. The purified oligosaccharide-alditol was characterized by sugar and methylation analyses, and by mass spectrometry as the permethylated derivative, and found to have the following structure.



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INTRODUCTION

In the course of our studies on the specificity of monoclonal, human, IgM antibodies reacting with *Klebsiella* polysaccharides, it was noted that, at pH 5.0 to 7.0, *Klebsiella* K30 and K33, containing a 3,4-pyruvylated D-galactosyl group (3,4-pyDGal), gave a typical, quantitative, precipitin curve with one monoclonal antibody, IgM^{WEA}, whereas K21 and K11, having a 4,6-pyDGal unit, did not give a precipitate under these conditions[1,2]. At pH 4.0, however, all four polysaccharides reacted identically per unit weight. With another monoclonal antibody, IgM^{MAY}, all four polysaccharides gave an identical precipitin curve at all values of pH from 4.0 to 7.0. To characterize the combining sites of these IgM antibodies, oligosaccharide inhibition-studies were necessary; these were conducted with the methyl glycosides of 4,6-(*R*)- and -(*S*)-pyruvylated D-galactoses[3], but the 3,4-substituted compounds were not available. We now report the isolation and structure of an oligosaccharide containing 3,4-pyruvic acetalated D-galactose, prepared by Smith degradation of *Klebsiella* polysaccharide K33.

EXPERIMENTAL

General. — The *Klebsiella* K33 polysaccharide was prepared[4] by W. N. at the Institute of Medical Microbiology and Epidemiology, University of Rostock, Rostock, German Democratic Republic, and sent to Columbia University. Structural studies on K33 polysaccharide have been described[5].

Solvents were of analytical grade, and all were distilled before use. Optical rotations were measured with a Perkin–Elmer spectropolarimeter Model 141. Infrared spectra were recorded with a Perkin–Elmer Model 521 instrument, for KBr pellets. Descending paper-chromatography was conducted at room temperature, using Schleicher and Schuell (Keene, N. H.) 589 green-ribbon C paper. Sugars were detected with alkaline silver nitrate[6]. Alditol acetates were prepared in the usual way[7]. Gas–liquid chromatography (g.l.c.) of alditol acetates and partially methylated alditol acetates was performed with a Finnegan 9500 instrument equipped with a flame-ionization detector. Separations were performed on a glass column packed with 3% of SP-2340 on Gas Chrom Q (100–120 mesh). Gas–liquid chromatography–mass spectrometry (g.l.c.–m.s.) of alditol acetates and partially methylated alditol acetates was performed on a V.G. microman 7070F mass spectrometer connected to a Perkin–Elmer Sigma-3 gas–liquid chromatograph having the same column as for g.l.c. G.l.c.–m.s. of permethylated oligosaccharide-alditols was conducted with a Hewlett–Packard 5985B instrument equipped with a column (12 m × 0.20 mm i.d.) of OV-1 W.C.O.T. on fused silica. All mass spectra were recorded at 70 eV.

Oxidation of K33 with sodium metaperiodate, and Smith degradation[8]. — To a solution of *Klebsiella* K33 capsular polysaccharide (1 g) in water (650 mL) was added 0.2M sodium metaperiodate (160 mL), and the solution was stirred in the

dark for 120 h at 4°. The excess of periodate was decomposed with ethylene glycol (10 mL), and the solution was dialyzed against distilled water, and lyophilized. To the polyaldehyde in water (400 mL) was added sodium borohydride (10 g). After 10 h at room temperature, the pH was adjusted to 7.0 with 50% acetic acid, and the solution was dialyzed against distilled water, and lyophilized, to yield the polyol (0.8 g). To a solution of the isolated polyol in water (50 mL) was added M HCl (50 mL), hydrolysis was allowed to proceed for 24 h at room temperature, and the hydrolyzate was made neutral with sodium carbonate, and lyophilized, and the material was desalted by passage through a column of Biogel P-2. The Smith-hydrolyzed material was subjected to preparative paper-chromatography using 9:2:2 ethyl acetate–acetic acid–water, and the isolated oligosaccharide (R_L 0.8) also gave a single spot in paper chromatography using 10:1:3 1-butanol–ethanol–water; R_L 0.8 had $[\alpha]_{365}$ −17°, $[\alpha]_{436}$ −9°, $[\alpha]_{546}$ −4°, $[\alpha]_{578}$ −3°, and $[\alpha]_{589}$ −3°(*c* 0.3, water).

Hydrolysis of R_L *0.8* — R_L 0.8 (1 mg) was hydrolyzed for 20 h at 100° with 0.5M sulfuric acid, the acid neutralized, and the alditol acetates prepared. Pyruvate was estimated according to Sloneker and Orentas[9].

Methylation studies on R_L *0.8.* — Methylation of R_L 0.8 was effected by both the Kuhn[10,11] and Hakomori[12] methods. To a solution of oligosaccharide R_L 0.8 (2 mg) in *N*,*N*-dimethylformamide (0.5 mL) were added silver oxide (300 mg), methyl iodide (100 μL), and Drierite (0.5 g), and the mixture was shaken in a sealed tube for 16 h at room temperature, centrifuged, the residue washed with chloroform, and the supernatant liquor evaporated to dryness. A suspension of the residue in chloroform was washed several times with water, and the organic layer was evaporated to dryness. The methylation procedure was carried out three times, after which, no hydroxyl groups were detected by i.r. spectroscopy.

In another experiment, to R_L 0.8 (2 mg) dissolved in dimethyl sulfoxide (2 mL) was added (externally prepared) 2M dimsylsodium (1 mL), and the mixture was kept overnight at room temperature, and cooled; methyl iodide (1 mL) was added, and the mixture was stirred for 3 h, suspended in chloroform, washed with water, and the organic layer evaporated to dryness.

RESULTS AND DISCUSSION

Analysis of the oligosaccharide-alditol (R_L 0.8), isolated after Smith degradation of K33 capsular polysaccharide, gave a monosaccharide composition of D-glucose, D-galactose, D-mannose, and erythritol in the ratios of 1.0:1.1:1.0:0.7 (see Fig. 1); the content of pyruvate was 12.7% (theoretical value, 13.6%).

R_L 0.8 was methylated by both the Kuhn[10,11] and the Hakomori[12] methods, and the product, as the partially methylated alditol acetates and as the permethylated oligosaccharide-alditol, was analyzed by g.l.c.–m.s. The partially methylated alditol acetates of R_L 0.8 corresponded to 2,3,4,6-tetra-*O*-methylglucose, 2,6-di-*O*-methylgalactose, and 2,3-di-*O*-methylmannose in approximately equimolar

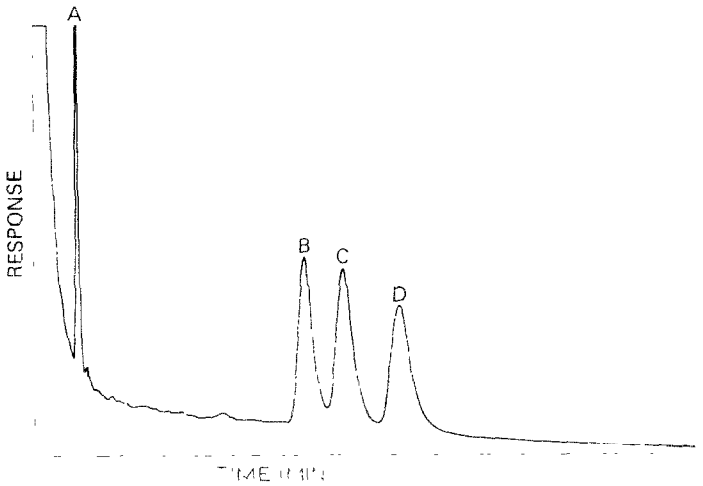


Fig. 1. G.l.c. pattern, of alditol acetates prepared from R_1 0.8, on 3% of SP2340. (Key: A, erythritol, 7.7 min; B, mannitol, 9.0 min; C, galactitol, 10.1 min; and D, glucitol, 11.6 min.)

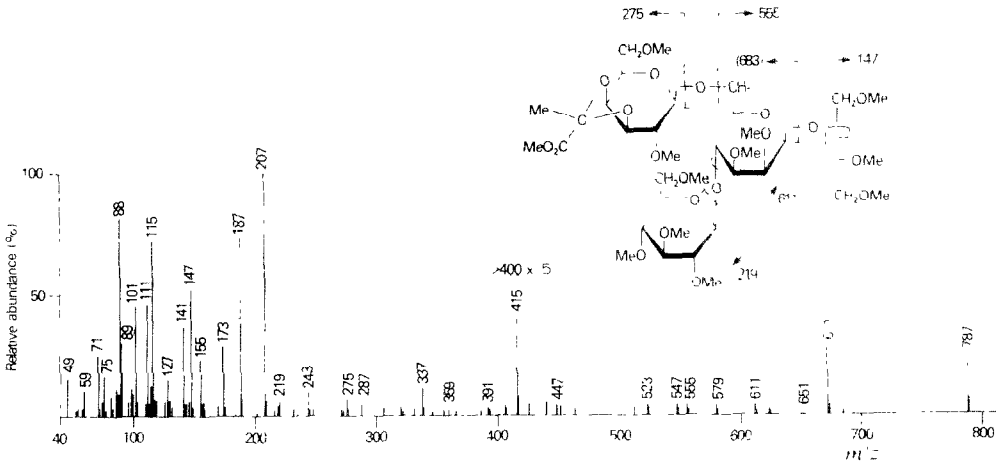


Fig. 2. Mass spectrum of permethylated R_1 0.8 (primary-fragmentation pattern).

amounts; 1,3,4-tri-*O*-methylerythritol was seen, but was not quantitated owing to its volatility.

No significant difference could be seen between the two methods as determined by g.l.c.–m.s. The mass spectrum of permethylated R_1 0.8 is shown in Fig. 2. The largest ion observed, originating from the molecular ion, is m/z 787, formed by elimination of a methoxycarbonyl radical (·COOMe) from the pyruvic acetal. Two different A-series of fragments[13] are present, indicating two different, non-reducing termini. The primary and secondary A-series of fragments m/z 219, 187, and 155 show a nonreducing hexose. The other A-series ions, m/z 275 and 243, indicate a nonreducing, pyruvic acetalated hexose. Secondary fragments are formed

from *m/z* 275 and 243 by elimination of methyl pyruvate, to give *m/z* 173 and 141, respectively. The fragment *m/z* 651, originating from *m/z* 683 (not seen), combined with the previous A-series of ions show a di-*O*-substituted hexose. Further elimination from *m/z* 683 gives secondary fragments of *m/z* 391, 359, 447, and 415. The alditol-containing ions, the J-series[13], *m/z* 147 and 207, show a tetritol. The next J-series, *m/z* 611, 671 contain the alditol, the di-*O*-substituted hexose, and the pyruvic acetalated hexose. Secondary fragments, formed by elimination of methanol from *m/z* 611, are *m/z* 579 and 547. Another J-fragment, *m/z* 555, and a secondary fragment, *m/z* 523, include 2 hexose residues and the tetritol residue. The absence of the J-fragment *m/z* 615 supports the inference that O-3 of the terminal D-galactosyl group is blocked by engagement in the 3,4-pyruvic acetal.

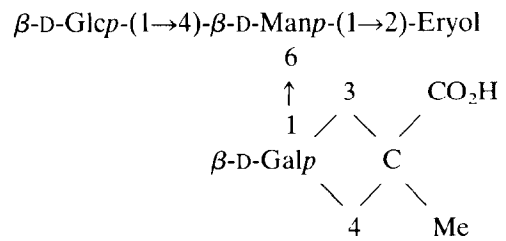
The mass spectrum gives no information concerning the relative positions of substitution of the branched D-mannosyl residue. However, in other studies[5], it was found that the D-glucosyl and the pyruvic acetalated D-galactosyl group are respectively (1→4)- and (1→6)-linked to the D-mannosyl residue.

The anomeric configuration was shown to be β, in agreement with the negative value of the optical rotation ($[\alpha]_D^{20}$ −3°). It was demonstrated by Garegg *et al.*[3] that the pyruvic acetal has the (*S*) configuration.

Treatment with strong base during Hakomori methylation did not degrade the 3,4-pyruvic acetalated D-galactosyl unit. This was confirmed both by methylation analysis and by g.l.c.–m.s. of the permethylated oligosaccharide-alditol. Lindberg *et al.*[5] also found this to be so, when intact K33 polysaccharide was subjected to methylation analysis.

Periodate oxidation of the K33 polysaccharide unexpectedly did not decompose the 4-*O*-substituted D-glucosyl residue, and the subsequent, mild hydrolysis with acid did not hydrolyze the pyruvic acetal group. The reason why the D-glucosyl residue is resistant to periodate oxidation could be steric hindrance, or interaction with the carboxylic acid group of the acetal. Lindberg *et al.*[5] found that this D-glucosyl residue is completely lost when the K33 polysaccharide is carboxyl-reduced prior to the Smith degradation. Mild hydrolysis with 0.5M HCl for 24 h at room temperature did not hydrolyze the pyruvic acetal. Previous structural studies[5] had shown that the acetal is hydrolyzed by 0.13M sulfuric acid during 1.5 h at 100°.

The present data strongly suggest that the oligosaccharide-alditol obtained after Smith degradation of the capsular polysaccharide from *Klebsiella* K33 has the following structure.



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