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ISOLATION AND CHARACTERIZATION OF 4-*O*-[3,4-*O*-(1-CARBOXYETHYLIDENE)-β-D-GALACTOPYRANOSYL]ERYTHRITOL FROM *Klebsiella* K33 POLYSACCHARIDE

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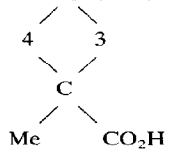
ABSTRACT

The tetrasaccharide-alditol, R_L 0.8, from one stage of Smith degradation of *Klebsiella* K33, was subjected to Smith degradation to yield a disaccharide-alditol. The purified disaccharide-alditol was characterized by sugar analysis, methylation analysis, and mass spectrometry. The following structure was found.

β-D-Gal*p*-(1→4)-erythritol



INTRODUCTION

For studies[1,2] on the specificity of monoclonal, human-IgM antibodies reacting with *Klebsiella* polysaccharides, it is necessary to prepare compounds containing 3,4-pyruvic acetalated and 4,6-pyruvic acetalated D-galactose. The isolation

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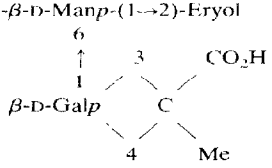
and characterization of a 3,4-pyruvic acetalated D-galactose-containing tetrasaccharide-alditol (R_L 0.8), namely,



obtained by Smith degradation of *Klebsiella* K33 polysaccharide, has been described[3]. We now report the isolation and characterization of 4-*O*-[3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranosyl]erythritol obtained by Smith degradation of tetrasaccharide R_L 0.8.

EXPERIMENTAL

General. — The tetrasaccharide-alditol R_L 0.8 was available[3]. Solvents were of analytical grade, and all were distilled before use. Alditol acetates were prepared by reduction with $NaBH_4$, and acetylation with pyridine and acetic anhydride[4]. Optical rotations were measured with a Perkin–Elmer polarimeter Model 141. Infrared spectra were recorded with a Perkin-Elmer Model 521 for KBr pellets. Gas–liquid chromatography (g.l.c.) of alditol acetates was performed with a Perkin–Elmer 990 gas chromatograph equipped with a flame-ionization detector and a glass column packed with 3% of SP-2340 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 conducted with a Hewlett–Packard 5992A instrument, using the same column as for g.l.c. G.l.c.–m.s. of per-*O*-methylated oligosaccharide-alditol was achieved with a Hewlett–Packard 5985B instrument equipped with a column (12 m × 0.20 mm) of OV-1 W.C.O.T. fused silica. All mass spectra were recorded at 70 e.V.

Oxidation of R_L 0.8 with sodium metaperiodate[5], *and Smith degradation*[6]. — The oligosaccharide-alditol (100 mg) was dissolved in 15mM sodium metaperiodate solution (80 mL), and the reaction allowed to proceed in the dark at 37°. An aliquot (10 μL) was removed at intervals, diluted to 2.5 mL with water, and the $A_{222.5}$ recorded. When uptake of periodate ceased, the excess was decomposed by adding the calculated amount of ethylene glycol (50 μL), the material reduced with sodium borohydride, the solution de-ionized with Dowex 50 (H^+) resin, and boric acid removed by repeated evaporation with methanol. The resulting material was hydrolyzed with 0.5M HCl (5 mL) for 24 h at room temperature, and the solution made neutral with sodium carbonate, and desalted on a column of Bio-Gel P-2, to yield the disaccharide-alditol (43 mg). The specific optical rotations at 589, 578, 546, 436, and 365 nm were 30, 31, 37, 63, and 96°, respectively.

Sugar analysis. — The oligosaccharide-alditol (0.5 mg) was hydrolyzed with 0.5M sulfuric acid for 20 h at 100°, the acid neutralized, and the alditol acetates prepared. Pyruvate was estimated according to Sloneker and Orentas[7].

Methylation analysis. — Methylation of the oligosaccharide was conducted by the Kuhn method[8,9]. To a solution of the oligosaccharide-alditol (1 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 kept for 5 h at 37°, and shaken in the dark for 20 h at room temperature. After centrifugation, the residue was washed with chloroform, and the supernatant liquors were combined, and evaporated to dryness. The residue was extracted with chloroform, and the extract was washed several times with water and evaporated to dryness. After three methylations, no hydroxyl groups were detectable by i.r. spectroscopy.

A portion of the per-*O*-methylated oligosaccharide-alditol was hydrolyzed with 0.5M sulfuric acid, and the alditol acetates were prepared as before.

RESULTS AND DISCUSSION

Sugar analysis after Smith degradation of R_L 0.8 showed galactose and erythritol in the ratio of 2:1.0. Pyruvate was 21.5% (theoretical, 23.4%). Traces (<0.1%) of mannose and glucose were also detected. Partially methylated alditol acetates prepared after methylation according to Kuhn indicated 1,2,3-tri-*O*-methylerythritol and 2,6-di-*O*-methylgalactose, but the relative proportions could not be obtained owing to the volatility of the erythritol derivative. Analysis of the per-*O*-methylated derivative by g.l.c.–m.s. gave the mass spectrum shown in Fig. 1. The molecular ion (M^+) is not seen, but $M^+ - 59$ (*m/z* 379) is formed by loss of a methoxycarbonyl radical ($\cdot CO_2Me$). The A-series[10] of primary and secondary fragments, *m/z* 275 and 243, indicate a hexose substituted with a methyl pyruvic

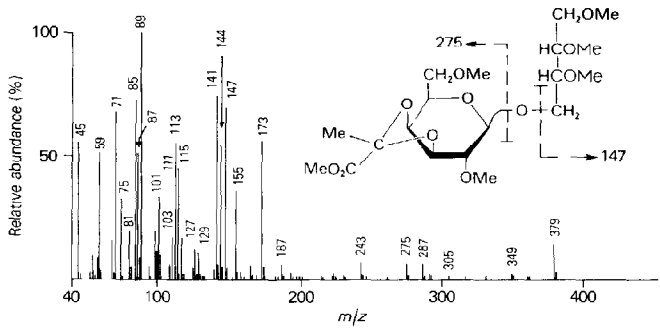


Fig. 1. Mass spectrum of the per-*O*-methylated oligosaccharide-alditol obtained after Smith degradation of R_L 0.8.

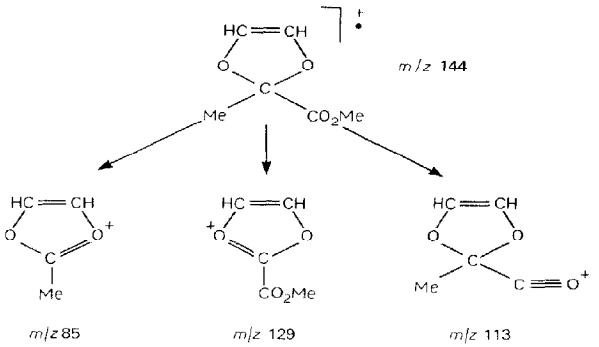
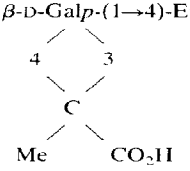


Fig. 2. Structures of secondary fragments formed by eliminations from the H_1^3 ion, *m/z* 144.

acetal group. Further eliminations of methyl pyruvate from *m/z* 275 and 243 give the secondary fragments, *m/z* 173 and 141, respectively, in agreement with previous observations[3]. The J_2 fragment, *m/z* 147, shows a tetritol. The ions produced by cleavage of carbon–carbon bonds of the alditol, *m/z* 45, 89, 349 ($M^+ - 89$), and 305 ($M^+ - 133$), indicate that the tetritol is substituted on O-4 by the pyruvic acetalated hexose. The mass spectrum also gives some information about the positions of the acetal group. The absence of the J_1 fragment, *m/z* 207, shows that O-3 of the hexose is one of the atoms substituted by the acetal, and vicinal substitution of the hexose by the acetal group is confirmed by the H_1^5 fragment, *m/z* 144. The H_1^3 radical-ion gives rise to further eliminations, to give *m/z* 85, 113, and 129, as shown in Fig. 2. The exact positions of the acetal group were obtained from the methylation analysis.

Smith degradation of R_L 0.8 did not produce any β-D-Gal*p*-(1→4)-erythritol, which demonstrates, as previously observed[3], that the 3,4-pyruvic acetal group is resistant to acid hydrolysis in 0.5M HCl during 24 h at room temperature.

The structure of the disaccharide-alditol obtained after two consecutive, Smith degradations of *Klebsiella* K33 polysaccharide must, therefore, be as follows.



The anomeric configuration[11], and the configuration of the acetal[12], had previously been determined.

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REFERENCES

1 E. A. KABAT, J. LIAO, H. BRETTING, E. C. FRANKLIN, D. GELTNER, B. FRANGIONE, M. E. KOSHLAND, J. SHYONG, AND E. F. OSSERMAN, *J. Exp. Med.*, 152 (1980) 979–995.
2 A. S. RAO, E. A. KABAT, W. NIMMICH, AND E. F. OSSERMAN, *Mol. Immunol.*, 19 (1982) 609–618.
3 A. S. RAO, E. A. KABAT, N. F. WHITTAKER, B. NILSSON, D. A. ZOPF, AND W. NIMMICH, *Carbohydr. Res.*, 116 (1983) 271–276.
4 A. S. RAO, N. ROY, AND W. NIMMICH, *Carbohydr. Res.*, 76 (1979) 215–224.
5 G. O. ASPINALL AND R. J. FERRIER, *Chem. Ind. (London)*, (1957) 1216.
6 I. J. GOLDSTEIN, G. W. HAY, B. A. LEWIS, AND F. SMITH, *Methods Carbohydr. Chem.*, 5 (1965) 361–370.
7 J. H. SLONEKER AND D. G. ORENTAS, *Nature (London)*, 194 (1962) 478–479.
8 R. KUHN, H. TRISCHMANN, AND I. LOW, *Angew. Chem.*, 67 (1955) 32–37.
9 H. G. WALKER, JR., M. GEE, AND R. M. MCCREADY, *J. Org. Chem.*, 27 (1962) 2100–2102.
10 N. K. KOCHETKOV AND O. S. CHIZHOV, *Adv. Carbohydr. Chem.*, 21 (1966) 39–93.
11 B. LINDBERG, F. LINDH, AND J. LONNGREN, *Carbohydr. Res.*, 70 (1979) 135–144.
12 P. J. GAREGG, P.-E. JANSSON, B. LINDBERG, F. LINDH, J. LONNGREN, I. KVARNSTROM, AND W. NIMMICH, *Carbohydr. Res.*, 78 (1980) 127–132.