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Preliminary communication

D-Allose-containing polysaccharide synthesized from methanol by *Pseudomonas* sp.

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Currently, microbial utilization of methanol has attracted much attention in connection with the production of single-cell protein. Among micro-organisms, certain species are known to elaborate polysaccharides during their growth in methanol-containing media[1–3]; however, no information has been available concerning the chemical nature of these products. In the course of a search for methanol-utilizing micro-organisms, we became aware that a newly isolated, soil bacterium, designated *Pseudomonas viscogena* TS-1004, is capable of producing an abundant amount of an acidic polysaccharide that contains D-allose, in addition to D-galactose, D-glucose, D-mannose, and D-glucuronic acid. In this Communication, we report the isolation of D-allose, and the results of a preliminary investigation of the structure of the polysaccharide. This acidic polysaccharide, which appears to have a branched structure, exhibits unique properties, such as the formation of a water-insoluble, brittle gel in the presence of calcium ion.

The micro-organism was grown by submerged culture under aerobic conditions, in a medium containing 1% of methanol, 0.02% of yeast extract, and certain inorganic compounds, pH 7.0, for 48 h at 30°. During the cultivation, the pH of the medium was maintained at 7.0 by addition of methanol–ammonia solution (C/N = 10). After clarification of the culture broth by centrifugation, the polysaccharide was precipitated from the supernatant liquor by addition of acetone, and purified through the formation of an insoluble complex with cetylpyridinium chloride; yield, 21% on the basis of the methanol furnished. The purified, water-soluble polysaccharide had $[\alpha]_D$ +12.9° (*c* 0.9, water) and +36.9° (*c* 0.8, M sodium hydroxide). Its homogeneity was assessed by ultracentrifugal analysis ($s_{20,w}$ 3.78×10^{-13}). High-performance, liquid chromatography (h.p.l.c.) on a column of Toyo Soda G-3000 SW indicated that its molecular weight is $\sim 1 \times 10^5$.

To examine the sugar composition, the polysaccharide (7 g) was hydrolyzed by heating with M sulfuric acid for 8 h at 100°, and the hydrolyzate was extracted with ether. No organic acid was detected in the ether extract. The aqueous layer was made neutral ($BaCO_3$), and the suspension filtered, and the filtrate was decationized by passing it through a column of Amberlite IR-120B (H^+) ion-exchange resin. The hydrolysis products were fractionated by chromatography on a column of cellulose, using 10:3:3 (v/v) 1-butanol–

pyridine–water and then 2:1 (v/v) ethanol–water. The monosaccharides eluted from the column were separated by preparative, paper chromatography using Whatman 3MM paper; D-galactose, D-mannose, D-glucuronic acid, and an unknown component that showed behavior similar to that of D-glucose on a paper chromatogram, were isolated. The first three sugars were identified by paper chromatography, paper electrophoresis, gas–liquid chromatography (g.l.c.), and specific rotation; after borohydride reduction, methyl esterification, carboxyl reduction, and acetylation[4], the glucuronic acid was also identified (as the glucitol derivative) by g.l.c.

The unknown component, which appeared to be in admixture with D-glucose and the other monosaccharide, was treated with D-glucose oxidase (type V, Sigma Chemical Co.), and the unreacted monosaccharide was purified by preparative, paper chromatography. The sugar, $[\alpha]_D$ +15° (*c* 2.0, water), showed the same behavior as authentic D-allose in paper chromatography, paper electrophoresis, g.l.c., and h.p.l.c. using JEOL resin LCR-3 (borate form, Japan Electronic Optical Lab., Tokyo)[5]. Its per-*O*-benzoyl derivative[6], having m.p. and mixed m.p. 220–221°, $[\alpha]_D$ −7.8° (*c* 2.4, chloroform), C:H = 70.28:4.55 (calc., 70.28:4.60), was identical with 1,2,3,4,6-penta-*O*-benzoyl-β-D-allopyranose. Thus, this sugar was identified as D-allose.

The molar proportions of the sugar components in the hydrolyzate of the polysaccharide were estimated to be D-galactose (55.4%), D-mannose (13.1%), D-glucose (10.7%), D-allose (9.8%), and D-glucuronic acid (11.0%), as analyzed by g.l.c. as the corresponding alditol acetates (of the neutral sugars) (see Fig. 1), and by the Dische carbazole–sulfuric acid method (for D-glucuronic acid)[7].

The mode of the glycosidic linkages of the sugar residues in the polysaccharide was examined by periodate oxidation, and by the Smith degradation technique. The polysaccharide (3.5 g) was oxidized with 0.05M sodium metaperiodate for 6 days at 4° in the dark. The production of formic acid and the consumption of periodate were, respectively, 0.12 and 0.95 molecule per hexosyl residue. After reduction of the oxidized polysaccharide with sodium borohydride, a portion of the glycan polyalcohol was completely hydrolyzed

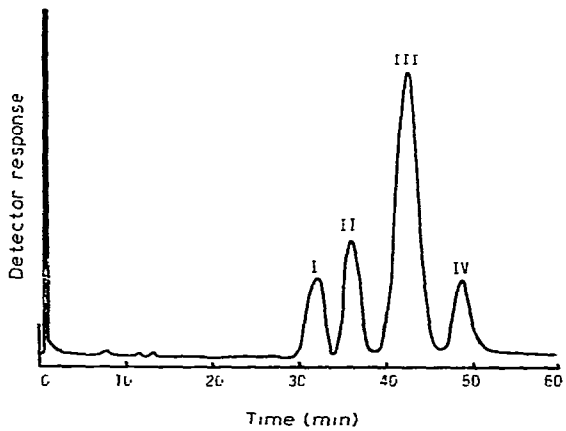

Fig. 1. G.l.c. profile of the neutral-sugar components of the polysaccharide produced by *P. viscogena* TS-1004. (The sugars, as their corresponding alditol acetates, were separated on a column of 3% of ECNSS-M on Gaschrom Q at 180°. I, D-Allose; II, D-mannose; III, D-galactose; and IV, D-glucose.)

by heating with M sulfuric acid for 3 h at 100°. The hydrolysis products were examined by paper chromatography and, as the alditol acetates, by g.l.c., which revealed the presence of glycerol, erythritol, D-mannose, and D-galactose, in the molar ratios of 0.36:0.71:0.03:1.0(and a trace of threitol.

Another portion of the glycan polyalcohol was hydrolyzed with 0.05M sulfuric acid for 13 h at 50° (mild, Smith degradation[8]). Glycerol, erythritol, and an oligosaccharide were detected by paper chromatography. This oligosaccharide (yield, 42% of the glycan polyalcohol; 31% of the original polysaccharide) was purified by chromatography on a column of Bio-Gel P-2. The purified oligosaccharide, which had $[\alpha]_D$ +86° (*c* 0.6, water) and an absorption at 880 cm^{-1} in its i.r. spectrum, was composed of D-galactose and erythritol (molar ratio, 4:1). G.l.c. revealed that the hydrolysis products of the methylated oligosaccharide[9] gave 2,3,4,6-tetra- and 2,4,6-tri-*O*-methylgalactose in the molar ratio of 1.0:2.9, together with a peak corresponding to a tri-*O*-methylerythritol, as analyzed after conversion into the corresponding polyalcohol acetates. The action of β-D-galactosidase (Seikagaku Kogyo Co., Tokyo) on the oligosaccharide yielded D-galactose and erythritol as the hydrolysis products. From these results, the oligosaccharide may have the sequence β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→2)-D-erythritol. The high yield of this oligosaccharide suggests that, in the main chain of the polysaccharide, a large proportion of the D-galactosyl residues joined by β-(1→3)-linkages is most probably located, as tetrasaccharide units.

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