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Note

Structural studies of the capsular polysaccharide of *Diplococcus pneumoniae* type 31

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In a previous publication[1], it was reported that the capsular polysaccharide from *Diplococcus pneumoniae* Type 31 (S31) contains residues of D-galactose, L-rhamnose, and D-glucuronic acid in the approximate molar proportions of 2:2:1. It was observed that S31 is very susceptible to hydrolytic cleavage, indicating the presence of furanosidic linkages in the polysaccharide. Hydrolysis of S31 with 45% formic acid for 45 min at 100° gave D-galactose, L-rhamnose, and an aldobiouronic acid, namely, a (glucosyluronic acid)-galactose. The aldobiouronic acid had a low, positive, specific rotation (+15°), suggesting that the glycosidic linkage in the compound had the β configuration. The results of sedimentation studies[1] on S31 suggested that it is a linear polymer. The present Note describes a further structural study of the polysaccharide.

RESULTS AND DISCUSSION

Polysaccharide S31 was first methylated by the Hakomari methylation procedure[2], and the product was remethylated with methyl iodide and silver oxide in *N,N*-dimethylformamide[3]. The permethylated S31 was purified by passing it through a column of Sephadex LH 20; the i.r. spectrum of the product did not have a recognizable peak for hydroxyl group. The permethylated S31 thus obtained was reduced with lithium aluminum hydride, and the mixture of alditol acetates prepared[4] from the reduction product was analyzed by g.l.c. (columns *A* and *B*); it showed peaks for 3,4-di-*O*-methylrhamnose, 2,4-di-*O*-methylrhamnose, 2,5,6-tri-*O*-methylgalactose, and 2,3-di-*O*-methylglucose in the approximate ratios of 5:5:10:4. The results are summarized in Table I.

As no tetra-*O*-methylgalactose, tri-*O*-methylglucose, or tri-*O*-methylrhamnose was obtained in the methylation analysis, the polysaccharide is most probably linear, supporting the results of the sedimentation studies[1]. Moreover, it is obvious that all of the galactose residues in S31 are present as (1→3)-linked galactofuranosides. All

TABLE I

ALDITOL ACETATES OF PARTIALLY METHYLATED SUGARS FROM PERMETHYLATED S31 AFTER REDUCTION WITH LITHIUM ALUMINUM HYDRIDE

Sugars	T*		*Molar proportion*
	ECNSS-M	*OV-225*	
3,4-Di-*O*-methylrhamnose	0.91	0.87	1
2,4-Di-*O*-methylrhamnose	0.98	0.94	1
2,5,6-Tri-*O*-methylgalactose	2.27	1.95	2
2,3-Di-*O*-methylglucose	5.39	4.50	0.8

**T* is the retention time compared to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol as unity.

TABLE II

OXIDATION OF PERACETYLATED, CARBOXYL-REDUCED S31 WITH CHROMIUM TRIOXIDE AT 50°

Time of oxidation (h)	*Sugar analysis*			
	Rhamnose	*Galactose*	*Glucose*	*myo*-Inositol
0	1.36	1.37	0.71	1
1	0.20	0.29	0.17	1
2	0.17	0.23	0.08	1

of the peaks were identified by direct comparison with the peaks of authentic samples; that the trimethylgalactose was a 2,5,6- and not a 3,5,6-tri-*O*-methylgalactose was proved by comparison with the alditol acetate of the latter (R_t 2.21, column *A*), and also by comparing the methyl glycosides of these two tri-*O*-methylgalactoses[5] in g.l.c. (column *C*) with the product from the methanolysis of permethylated and reduced S31. The chromatogram showed (besides the peaks of methyl 3,4-di-*O*-methylrhamnoside, methyl 2,4-di-*O*-methylrhamnoside, and methyl 2,3-di-*O*-methylglucoside) the peaks of methyl 2,5,6-tri-*O*-methyl-α,β-galactofuranoside (retention times[5] 4.25 and 7.4 in column *C*, compared to methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucoside as unity).

Carboxyl-reduced S31 was prepared as before[1]. The product was acetylated, and the peracetate subjected to oxidation with chromium trioxide[6]. The proportions of different sugars, as determined by g.l.c. while the oxidation proceeded for different periods of time, are summarized in Table II. The results (see Table II) show that both the rhamnose and the glucuronic acid residues in S31 are β-linked. It is, however, not feasible to decide, from the results of chromium trioxide oxidation, the configuration of the galactose residues, because of their presence as furanosides[6] in S31. However, the low, negative, rotation of the polysaccharide, $[\alpha]_D$ −19° (water)[7], suggests that S31 has both α- and β-linkages in its chain. It is, therefore, probable that the galactofuranoside units are α-linked, and it is concluded that S31 may have any

one of six structures possible for its pentasaccharide repeating unit. One of these structures is shown in **1**.

→4)-β-GlcA-(1→3)-α-Gal*f*-(1→3)-β-Rha*p*-(1→3)-α-Gal*f*-(1→2)-β-Rha*p*-(1→

1

Five other structures may be written by changing the positions of the last three sugar residues in **1**.

EXPERIMENTAL

Materials and methods. — Polysaccharide S31 was kindly supplied by Dr. David Axelrod, Division of Laboratories and Research, State of New York, Albany, N.Y.

The polysaccharide was purified as previously described[7]. Samples of 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-glucose were obtained from Professor T. E. Timell, College of Environmental Science and Forestry, State University of New York, Syracuse, N.Y.

G.l.c. was performed with a Hewlett–Packard Model 5731A Gas Chromatograph. Column *A* (1.83 m × 6 mm) was glass, packed with 3% of CNSS-M on Gas Chrom Q (100–120 mesh); column *B* (1.83 m × 3 mm) was glass, packed with 3% of OV-225 on Gas Chrom Q (100–120 mesh), and column *C* (1.83 m × 3 mm) was stainless steel, packed with 15% of diethyleneglycol succinate on Chromosorb W (80–100 mesh). These were operated at 190°.

Methylation analysis. — The polysaccharide (5.5 mg), dissolved in dimethyl sulfoxide (3 mL) was methylated[2] with 2M methylsulfinylcarbinyl sodium (1.5 mL) and methyl iodide (1.5 mL). The reaction mixture was poured into ice water (25 mL), and extracted with four 15-ml portions of dichloromethane. The extract was washed three times with water, dried (anhydrous sodium sulfate), and evaporated to dryness. The product was dried over phosphorus pentaoxide under vacuum, and remethylated[3,5] with methyl iodide (1 mL) and silver oxide (1.5 g) in *N*,*N*-dimethylformamide (3 mL). The permethylated S31 was then passed through a column (20 × 1.5 cm) of Sephadex LH-20, using 2:1 chloroform–acetone as the eluant. The void volume contained the permethylated polysaccharide (3.5 mg). The i.r. spectrum of the product had no band for hydroxyl stretching-vibration. The product was dissolved in dry tetrahydrofuran (4 mL), refluxed with lithium aluminum hydride (100 mg) for 5 h, and kept overnight at room temperature. The excess of reductant was decomposed by dropwise addition of ethyl acetate and aqueous tetrahydrofuran, and the inorganic materials were filtered off. The filtrate was evaporated to dryness, giving the carboxyl-reduced, permethylated S31 (3 mg). A portion of this product (1.5 mg) was hydrolyzed with 90% formic acid for 1 h. The formic acid was evaporated off, and the product was rehydrolyzed, with 0.5M sulfuric acid, for 16 h. The mixture was processed in the usual way, and the alditol acetates were prepared, and analyzed by g.l.c. (columns *A* and *B* at 170°). The results are given in Table I.

In another experiment, the carboxyl-reduced, permethylated S31 (1 mg) was methanolyzed with 3% hydrogen chloride in methanol, and the mixture was analyzed by g.l.c. (column *C*).

Chromium trioxide oxidation. — A mixture of polysaccharide S31 (2 mg) and *myo*-inositol (0.4 mg) was acetylated[6] with acetic anhydride (0.5 mL), formamide (0.3 mL), and pyridine (0.5 mL) by stirring overnight at room temperature. The product was reduced[1] with a solution (1 mL) of diborane in tetrahydrofuran. The partially acetylated, carboxyl-reduced S31 thus obtained was reacetylated with acetic anhydride (1 mL) and pyridine (1 mL) for 3 h at 60°. The material was then subjected to chromium trioxide oxidation[6]. Analysis of the sugar components that survived the oxidation at periods of 0, 1, and 2 h was conducted by g.l.c. (column *A* at 190°). The results are summarized in Table II.

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