Carbohydrate Research, 119 (1983) 300–302
Elsevier Science Publishers B. V., Amsterdam – Printed in The Netherlands

Note

Structure of the capsular polysaccharide of *Diplococcus pneumoniae* type 31

LAKSHMI BATAVYAL AND NIRMOLENDU ROY

Department of Macromolecules, Indian Association for the Cultivation of Science, Calcutta 700032 (India)

(Received March 5th, 1983; accepted for publication, March 26th, 1983)

The polysaccharide from *Diplococcus pneumoniae* type 31 (S-31) contains[1] D-galactosyl, L-rhamnosyl, and D-glucosyluronic acid residues in the molar ratios of ~2:2:1. Methylation analysis of S-31 provided information about the linkages of the different sugar units[2]. Oxidation of carboxyl-reduced S-31 with chromium trioxide also gave valuable information. On the basis of these results, six possible structures were suggested[2] for the repeating unit of the polysaccharide. Although chromium trioxide oxidation gave a clear indication that the D-glucosyluronic acid and the two L-rhamnosyl residues have the β-anomeric configuration, it provided no information about the configuration of the D-galactofuranosyl residues.

Polysaccharide S-31 has[3] a specific rotation of $-19°$. The two β-L-rhamnopyranosyl residues contribute to the rotation in the positive direction, and the β-D-glucosyluronic acid residue, in the negative direction. Therefore, the two D-galactofuranosyl units are most probably present in the β-anomeric configuration, and it was an obvious mistake to suggest[2] that the two D-galactosyl residues have the α-anomeric configuration because such a polysaccharide would have a high, positive specific rotation. The same conclusion was drawn by Tyler[4], who calculated the specific rotation of S-31 for all possible anomeric configurations of the D-galactosyl and L-rhamnosyl residues. Moreover, S-10 and S-31 have been known to exhibit a minor, usually one-way, cross-reaction[5]. It was, therefore, to be expected[6] that β-D-galactofuranoside linkages are present in S-31, as found in S-10. Consequently, all of the sugar residues in S-31 are β-linked. The present work was undertaken in order to decide the sequence of the different sugar residues in the pentasaccharide repeating-unit of S-31.

The S-31 polysaccharide was permethylated, and the product was degraded with sodium methoxide in boiling methanol for 0.5 h and for 2.5 h, respectively. During this treatment, the uronic acid residue and the sugar residue immediately preceding it were degraded to a considerable extent. The degradation product was then hydrolyzed, and the hydrolyzate analyzed by g.l.c. It was observed that the

0008-6215/83/$03.00

TABLE I

DEGRADATION OF PER-*O*-METHYLATED S-31 WITH BASE

Methylated sugars	*Mole %*[a]		
	A	*B*	*C*
3,4-Di-*O*-methylrhamnose	20.8	28.4	31.2
2,4-Di-*O*-methylrhamnose	20.8	17.1	6.3
2,5,6-Tri-*O*-methylgalactose	41.6	54.5	62.5
2,3-Di-*O*-methylglucose	16.7		

[a]Key: A, alditol acetates[2] from per-*O*-methylated and reduced S-31; B, alditol acetates from per-*O*-methylated S-31 after degradation with sodium methoxide for 0.5 h; C, alditol acetates obtained from permethylated S-31 after degradation with sodium methoxide for 2.5 h.

proportion of 2,4-di-*O*-methylrhamnose had decreased considerably in 2.5 h, in comparison with the 3,4-di-*O*-methylrhamnose and 2,5,6-tri-*O*-methylgalactose found in the mixture of alditol acetates from permethylated and reduced S-31. The results, shown in Table I, clearly indicated that the 3-*O*-substituted L-rhamnosyl residue immediately precedes the D-glucosyluronic acid residue in the chain (reading from left to right).

The S-31 polysaccharide was *O*-deacetylated. Oxidation of the product with sodium metaperiodate, followed by treatment with sodium borohydride, gave a polyol, complete hydrolysis of a portion of which gave arabinose and rhamnose in the ratio of 2:1. The arabinose must have been formed from the 3-*O*-substituted D-galactofuranosyl residue, and the presence of rhamnose showed that the 2-*O*-substituted rhamnosyl residue was degraded by periodate, and that the 3-*O*-substituted one survived the oxidation. The rest of the polyol was hydrolyzed at room temperature, the hydrolyzate reduced with sodium borohydride, and the alditol product divided into two parts. One part was acetylated; analysis of the acetate by g.l.c. showed the presence of arabinose only. The other part was hydrolyzed with 0.5M sulfuric acid for 20 h at 100°, and the hydrolyzate acetylated. Examination by g.l.c. showed the presence of arabinose and rhamnose in the ratio of 1:1. These results clearly showed that periodate oxidation of S-31 produced arabinose and a disaccharide having the structure Ara(1→3)Rha*p*, the arabinose and the arabinosyl group arising from the oxidation of *O*-3-linked D-galactofuranosyl units. All these results can be explained only if S-31 has structure **1**.

→2)*β*-L-Rha*p*(1→3)*β*-D-Gal*f*(1→3)*β*-L-Rha*p*(1→4)*β*-D-Glc*p*A(1→3)*β*-D-Gal*f*(1→

1

EXPERIMENTAL

Materials and methods. — Polysaccharide S-31 was kindly supplied by Dr. K. Amiraian, Division of Laboratories and Research, State of New York, Albany, N.Y. Alditol acetates were prepared as already described[7].

G.l.c. was performed with a Hewlett–Packard Model 5731A Gas Chromatograph. Column *A* (1.83 × 6 mm) was glass, packed with 3% of EGNSS-M on Gas-Chrom Q (100–120 mesh); column *B* (1.83 m × 6 mm) was glass, packed with 3% of OV-225 on Gas-Chrom Q (100–120 mesh).

Base degradation of per-*O*-methylated S-31 was performed by using a known method[7]. The product was analyzed as the alditol acetates in columns *A* and *B* at 170°.

Oxidation of S-31 with periodate. — Polysaccharide S-31 was *O*-deacetylated with 0.01M sodium hydroxide solution for 2 h. To a 0.05% solution (20 mL) of the deacetylated S-31 was added 0.2M sodium metaperiodate solution (5 mL), and the mixture was kept in the dark for 48 h at 5°. The excess of periodate was decomposed by adding ethylene glycol (0.5 mL), and after 3 h, the mixture was dialyzed against distilled water. Sodium borohydride (50 mg) was added, and the solution was kept for 4 h at room temperature, decationized with Dowex-50W X8 (H^+) ion-exchange resin, and evaporated to dryness. Boric acid was removed by repeated addition and evaporation of methanol. Alditol acetates prepared from a portion of the polyol thus obtained were analyzed by g.l.c. (column *A* at 190°). The rest of the polyol was hydrolyzed with 0.5M sulfuric acid for 8 h at room temperature. The acid was neutralized with barium carbonate, the suspension filtered through a Celite bed, and the filtrate decationized with Amberlite IR-120 (H^+) ion-exchange resin, the suspension filtered, and the filtrate concentrated to a small volume. The material was then reduced with sodium borohydride in the usual way, and the solution was divided into two parts. One part was acetylated with acetic anhydride–pyridine, and the product examined by g.l.c. (column *A* at 190°). The other part was hydrolyzed with 0.5M sulfuric acid for 20 h at 100°, and, after neutralization of the acid, the solution was evaporated to dryness. The residue was acetylated with acetic anhydride–pyridine and the products examined by g.l.c. (column *A* at 190°).

REFERENCES

1 N. Roy, W. R. Carroll and C. P. J. Glaudemans, *Carbohydr. Res.*, 12 (1970) 89–96
2 N. Roy, *Carbohydr. Res.*, 63 (1978) 333–336
3 C. P. J. Glaudemans and H. P. Trefers, *Carbohydr. Res.*, 4 (1967) 181–184
4 J. M. Tyler, *Carbohydr. Res.*, 99 (1982) 75–77
5 W. A. Walter, V. H. Gulvin, M. W. Beattie, H. Y. Collier and H. B. Bucca, *J. Immunol.*, 41 (1941) 279–294
6 M. Heidelberger, *Fortschr. Chem. Org. Naturst.*, 18 (1960) 503–536
7 A. S. Rao and N. Roy, *Carbohydr. Res.*, 76 (1979) 215–224