*Carbohydrate Research*, 48 (1976) 81–84


# STRUCTURAL STUDIES ON THE CAPSULAR POLYSACCHARIDE OF *Klebsiella* STRAIN 6412

BENGT LINDBERG

*Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-104 05 Stockholm (Sweden)*

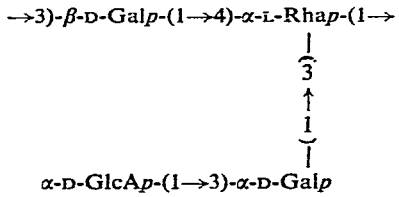
AND WOLFGANG NIMMICH

*Institut für Medizinische Mikrobiologie und Epidemiologie der Universität Rostock, DDR-25 Rostock 1 (German Democratic Republic)*

(Received November 10th, 1975; accepted for publication, November 25th, 1975)

## ABSTRACT

The structure of the capsular polysaccharide from *Klebsiella* strain 6412 has been investigated by methylation analysis and Smith degradation. The anomeric natures of the glycosidic linkages were determined from the optical rotations and n.m.r. spectra of original and degraded materials. The polysaccharide contained the tetrasaccharide repeating-unit shown below.




## INTRODUCTION

A *Klebsiella* strain isolated from a patient having a urinary-tract infection produces a capsular polysaccharide which is composed of glucuronic acid, galactose, and rhamnose[1]. We now report structural studies of this polysaccharide.

## RESULTS AND DISCUSSION

The polysaccharide (PS) was isolated as previously described for other *Klebsiella* K-antigens[2]. It did not contain pyruvic acid residues, *O*-acetyl groups, or other *O*-acyl groups, as is evident from its n.m.r. and i.r. spectra. An acid hydrolysate of the PS contained L-rhamnose and D-galactose in the proportion 1:1.3, and an acid hydrolysate of carboxyl-reduced[3] PS contained L-rhamnose, D-galactose, and D-glucose in the proportions 1.2:2:1. These sugars accounted for 70% of the polysaccharide material. The sugars were isolated and proved to have the configurations

given above. The proportions of the three sugars, indicating a tetrasaccharide repeating-unit, were confirmed by n.m.r. data for the PS, which showed signals for the methyl protons ($\delta$ 1.2–1.4), from the L-rhamnose residues, and for the anomeric protons ($\delta$ 4.6–5.3) in the ratio 3:4.

Methylation analyses[4] on the original and carboxyl-reduced PS (Table I, columns *A* and *B*) afforded convincing evidence that the tetrasaccharide repeating-unit consisted of a terminal D-glucopyranosyluronic acid group, two D-galactopyranosyl residues linked at O-3, and an L-rhamnosyl residue, most probably pyranosidic and linked at O-3 and O-4. The pyranosidic nature of this residue was demonstrated by the resistance of the PS to acid hydrolysis under conditions where furanosidic linkages should be cleaved.

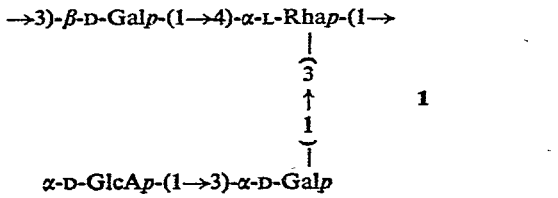
TABLE I

METHYLATION ANALYSES OF ORIGINAL AND MODIFIED PS

| *Methylated sugar*[a] | T[b] | *Mole %*[c] | | | |
|---|---|---|---|---|---|
| | | *A* | *B* | *C* | *D* |
| 2,3-Rha | 0.98 | — | — | — | 33 |
| 2,3,4,6-Glc | 1.00 | — | 21 | — | — |
| 2,3,4,6-Gal | 1.25 | — | — | 24 | 6 |
| 2-Rha | 1.52 | 41 | 30 | 37 | 18 |
| 2,4,6-Gal | 2.28 | 59 | 49 | 39 | 43 |

[a]2,3-Rha = 2,3-di-*O*-methyl-L-rhamnose, *etc.* [b]Retention time of the corresponding alditol acetate relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on an ECNSS-M column at 170°. [c]Polysaccharide: *A*, original; *B*, carboxyl-reduced; *C*, carboxyl-reduced, one Smith-degradation; *D*, carboxyl-reduced, two Smith-degradations.

The sequence of the sugar residues was determined by subjecting carboxyl-reduced PS to two consecutive Smith-degradations[5], that is periodate oxidation, borohydride reduction, and hydrolysis with acid under mild conditions. The result of each degradation was followed by methylation analysis (Table I, columns *C* and *D*). The first degradation resulted in elimination of the terminal D-glucopyranosyl group and creation of a terminal D-galactopyranosyl group. The latter was eliminated in the second degradation, leaving a polymeric product containing D-galactopyranosyl residues linked at O-3 and L-rhamnopyranosyl residues linked at O-4. The sequence of the sugar residues given in structure **1** for the tetrasaccharide repeating-unit is consequently established.




**1**

The PS had $[\alpha]_{578}$ +88°, and showed signals in the region for anomeric protons in the n.m.r. spectrum at $\delta$ 5.22 ($J_{1,2}$ low, 1 H), 5.09 ($J_{1,2}$ low, 2 H), and 4.68 ($J_{1,2} \simeq 7$ Hz, 1 H). The chemical shifts indicate that three of the sugar residues in the repeating unit are $\alpha$-linked and one is $\beta$-linked. The latter could not be the L-rhamnopyranosyl residue, because of the high value observed for the coupling constant. The product from the first Smith-degradation had $[\alpha]_{573}$ +36°, and showed signals for three anomeric protons at $\delta$ 5.23 (1 H), 5.10 (1 H), and 4.66 (1 H), respectively. The terminal D-glucopyranosyluronic acid group in the PS, removed after carboxyl-reduction and one Smith-degradation, is consequently $\alpha$-linked. The product from the second Smith-degradation had $[\alpha]_{578}$ −3°, and showed signals for two anomeric protons at $\delta$ 5.10 and 4.67, respectively, demonstrating that the D-galactopyranosyl residue in the side chain is $\alpha$-linked. The result also demonstrates that the D-galactopyranosyl residue and the L-rhamnopyranosyl residue in the polymeric product obtained after two degradations are $\beta$- and $\alpha$-linked, respectively. From the combined evidence, structure **1** is proposed for the tetrasaccharide repeating-unit of the capsular polysaccharide from *Klebsiella* strain 6412.

It is evident from Table I, columns *C* and *D*, that the Smith-degradations were not complete, probably because the conditions for the hydrolysis of the polyalcohols were too mild. A weak signal at $\delta$ 5.22 in the n.m.r. spectrum of the twice-degraded product was also observed, and could be assigned to terminal $\alpha$-D-galactopyranosyl residues which had not been degraded.

## EXPERIMENTAL

*General methods.* — These were the same as in the investigation of the *Klebsiella* type 81 capsular polysaccharide[6].

The isolation of the polysaccharide from *Klebsiella* strain 6412 was performed as described earlier[2]. The polysaccharide, $[\alpha]_{578}$ +89° (*c* 1.0, water), showed no significant i.r. absorption at $\sim 1735\ cm^{-1}$ (*O*-acyl region).

*Sugar and methylation[4] analyses.* — These were performed as previously described. The identifications of the methylated sugars by g.l.c.–m.s. of their alditol acetates were unambiguous and will not be discussed.

*Treatment of the PS with dilute acid.* — The PS (33 mg) was dissolved in 0.01M sulfuric acid (5 ml). The initial optical rotation, $[\alpha]_{578}^{22}$ +89°, was unchanged after 2 h at 100°, and the product could be recovered by dialysis, indicating that the PS does not contain furanosidic linkages.

*Carboxyl-reduction of the PS.* — This was performed by the method devised by Taylor and Conrad[3]. L-Rhamnose, $[\alpha]_{578}^{22}$ +13° (*c* 0.6, water); D-galactose, $[\alpha]_{578}^{22}$ +77° (*c* 0.4, water); and D-glucose, $[\alpha]_{578}^{22}$ +45° (*c* 0.3, water); were isolated from a hydrolysate of the carboxyl-reduced polysaccharide.

*Smith degradation.* — Carboxyl-reduced PS (29 mg) in 0.05M sodium metaperiodate (20 ml) was kept in the dark at 4° for 100 h. Excess periodate was then

reduced with ethylene glycol (100 mg), sodium borohydride (100 mg) was added, and excess of reagent was decomposed with acetic acid after 6 h at room temperature. The solution was dialysed for 24 h, 2M sulfuric acid added to a concentration of 0.25M, and the solution then kept for 30 h at room temperature. It was then dialysed and freeze-dried. The product (19 mg), $[\alpha]_{578}^{22}$ +36° (*c* 1.0, water), was characterized by n.m.r. and methylation analysis.

The product from the first degradation (17 mg) was subjected to a second degradation and recovered as described above. This product (10 mg), $[\alpha]_{578}^{22}$ −3° (*c* 1.0, water), was characterized by n.m.r. and methylation analysis.

ACKNOWLEDGMENTS

The skilled technical assistance of Miss Birthe Abrahamsson and Mrs. Lena Liedgren is gratefully acknowledged. This work was supported by the Swedish Medical Research Council (B76-03X-02522-08), by Knut och Alice Wallenbergs Stiftelse, by Harald Jeanssons Stiftelse, and by Stiftelsen Sigurd och Elsa Goljes Minne.

REFERENCES

1 W. NIMMICH, unpublished results.
2 W. NIMMICH, *Z. Med. Mikrobiol. Immunol.*, 154 (1968) 117–131.
3 R. L. TAYLOR AND H. E. CONRAD, *Biochemistry*, 11 (1972) 1383–1388.
4 B. LINDBERG, *Methods Enzymol.*, 28B (1972) 178–195.
5 I. J. GOLDSTEIN, G. W. HAY, B. A. LEWIS, AND F. SMITH, *Methods Carbohyd. Chem.*, 5 (1965) 361–370.
6 M. CURVALL, B. LINDBERG, J. LÖNNGREN, AND W. NIMMICH, *Carbohyd. Res.*, 42 (1975) 73–82.

*Added in proof.* Serological studies, performed by Dr I. Ørskov, Statens Seruminstitut, Copenhagen, indicate that strain 6412 is closely related to *Klebsiella* type 55. The latter, however, contains D-glucose[1] in addition to the other sugars found in the strain 6412 polysaccharide, which has now been confirmed. Strain 6412 should therefore represent a separate type, for which we propose the number K 83. The immunological determinant of K 83 should be similar to that of K 55.