Carbohydrate Research, 51 (1976) 287–290

Note

4-*O*-[(*S*)-1-Carboxyethyl]-D-glucose: a component of the extracellular polysaccharide material from *Aerococcus viridans* var. *homari*

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(Received February 18th, 1976; accepted for publication, March 10th, 1976)

The Gram-positive bacterium *Aerococcus viridans* var. *homari* (formerly *Gaffkya homari*) is highly pathogenic to lobsters. Recent studies have shown that virulent strains are heavily encapsulated, whereas avirulent strains have minimal capsular material[1].

Polysaccharide material was extracted from formalin-killed cells with hot, aqueous sodium chloride and then precipitated with cold ethanol. The precipitate was dissolved in distilled water, protein was removed with chloroform, and then the polysaccharide was purified by gel chromatography.

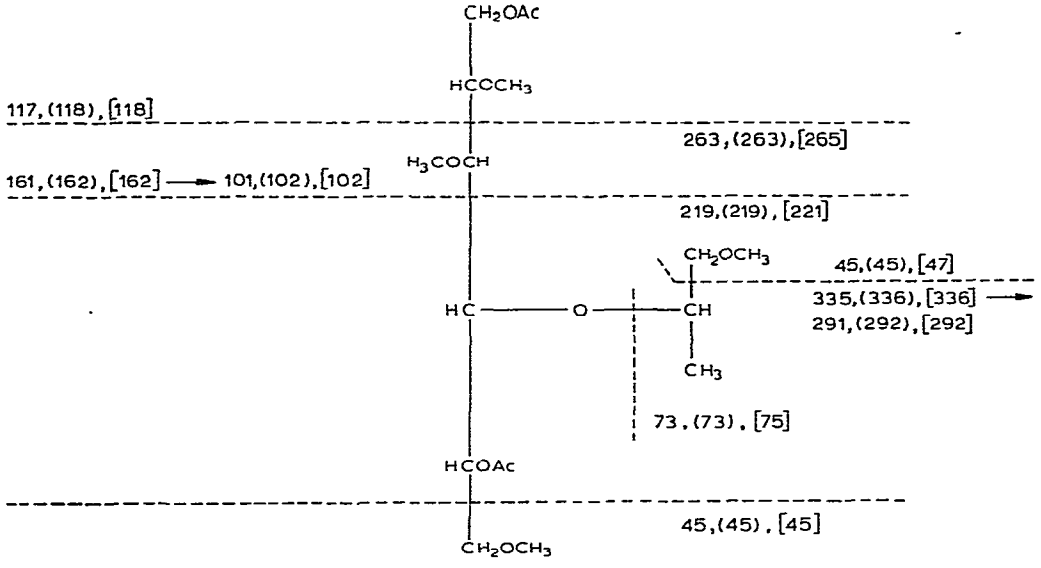
Preliminary chemical investigations suggested that the material was not homogeneous. Acid hydrolysis yielded, *inter alia*, an acidic sugar (**1**) which was not identical with those commonly observed. Carboxyl-reduction[2] and acid hydrolysis of the polysaccharide material yielded the corresponding neutral sugar (**2**), which was isolated by p.c.

The alditol acetate of **2** was indistinguishable on g.l.c. and m.s. from that of 4-*O*-[(*S*)-2-(1-hydroxy)propyl]-D-glucose. This substance has been synthesized and was obtained from 4-*O*-[(*S*)-1-carboxyethyl]-D-glucuronic acid, a sugar found as a component of the *Klebsiella* type 37 capsular polysaccharide[3] (K 37). Inasmuch as the only pertinent fragments in the mass spectrum of the alditol acetate were *m/e* 43 and 101, the spectrum furnished only limited structural information.

Treatment of **2** with boron tribromide[4] yielded D-glucose identified by its reaction with D-glucose oxidase and by g.l.c.[5]–m.s.[6] of its alditol acetate. The sugar (**2**) showed $[\alpha]_{578}$ +59° in good agreement with the value (+57°) for synthetic 4-*O*-[(*S*)-2-(1-hydroxy)propyl]-D-glucose[3].

Methylation[7] of the carboxyl-reduced polysaccharide material followed by acid hydrolysis, reduction with sodium borohydride, and acetylation produced a

mixture containing a partially methylated alditol acetate (**3**) indistinguishable by g.l.c. and m.s. from that obtained[3] from the terminal 4-*O*-[(*S*)-1-carboxyethyl]-D-glucuronic acid residue on similar treatment of K 37. The mixture of methylated sugars was also reduced with sodium borodeuteride. In a third, analogous experiment, both the carboxyl-reduction of the polysaccharide and the reduction to alditols were performed with deuterated reagents. Some pertinent fragments in the mass spectrum of **3** and its deuterated analogues are depicted in the formula. The mass numbers in brackets and in square brackets refer to the fragments obtained after reduction of the sugars using borodeuteride and after reduction of both carboxyl groups and sugars using borodeuteride, respectively.



3

The pairs of peaks *m/e* 117–263 and 161–219 indicate that the molecular weight of **3** is 380. Application of the principles for the fragmentation of partially methylated alditol acetates[8], as well as those found for the corresponding compound derived from K 37, strongly support the structure of **3** given in the formula. The shift of fragments containing the substituent at O-4 by two mass units on carboxyl-reduction using sodium borodeuteride demonstrates the presence of a carboxyl group at this position in the orginal sugar. However, the parent sugar is obviously a hexose (D-glucose) rather than a hexuronic acid (D-glucuronic acid) as in K 37, since the presence of C-6 in a fragment does not involve a shift of two mass units on carboxyl-reduction with deuterated reagent.

From the experiments reported above, it is concluded that **1** is 4-*O*-[(*S*)-1-carboxyethyl]-D-glucose. It is noteworthy that, in addition to 4-*O*-[(*S*)-1-carboxyethyl]-D-glucuronic acid found in K 37, ethers of lactic acid and D-glucose, and L-

rhamnose, although with unspecified configuration of the lactic acid moiety, have recently been reported as constituents of *Shigella dysenteriae* type 3 and 5 lipopolysaccharides[9], respectively. Therefore, sugars etherified with lactic acid appear to be more common than earlier anticipated. The biosynthetically related pyruvic acid acetals have been found as constituents in numerous bacterial polysaccharides.

EXPERIMENTAL

General methods. — These were the same as those reported in Ref. 3.

Isolation of the polysaccharide material. — *A. viridans* var. *homari* (Rabin's strain) was grown for 24 h in Trypticase Soy broth (BBL, 116 litres) at 24–28° in stationary culture using Fernbach flasks (2 litres of medium per flask). Cells were killed with formaldehyde (final concentration, 0.5%) to prevent leakage of cell constituents collected by centrifugation, washed twice with 3% aqueous sodium chloride, and suspended in 3% aqueous sodium chloride (fluid:cells, 3:1 v/v). The suspension was heated (100°, 6 h) and then centrifuged. The supernatant fluid was added to ethanol (−40°) that contained minimal amounts of sodium acetate to aid precipitation. After standing overnight, the precipitate was collected, and dissolved in distilled water, and insoluble material was removed by centrifugation. The supernatant fluid was deproteinized with chloroform[10], and then freeze-dried (yield, 1.8 g). The extracted material (in 200-mg batches) was chromatographed using a column (2.5 × 50 cm) of Sephadex G-200 eluted with 3% aqueous sodium chloride at a flow rate of 4 ml/h. Fractions (4 ml) were collected, and monitored with anthrone[11] and absorption at 260 and 280 nm. Material which was eluted before contaminating protein (absorption at 280 nm) was collected and freeze-dried (yield, 400 mg).

Isolation and characterization of 4-O-[(*S*)-2-(*1-hydroxy*)*propyl*]-D-*glucose* (**2**). — Carboxyl-reduced[2] polysaccharide (50 mg) was hydrolysed with 0.25M sulfuric acid (5 ml) for 16 h at 100°. The solution was neutralised ($BaCO_3$) and filtered. The title compound (6 mg, amorphous powder), $[\alpha]_{578}^{24}$ +59° (*c* 0.5, water), was isolated by preparative paper chromatography (Whatman 3MM; ethyl acetate–acetic acid–water, 3:1:1), followed by gel filtration (Sephadex G-15) and freeze-drying.

Compound **2** was indistinguishable from an authentic specimen[3] on p.c. (R_{Glc} 1.60, above solvent system) as well as on g.l.c. [*T* (relative to glucitol hexaacetate) = 2.44, SP-1000 glass-capillary column, 240°] of the derived alditol acetate. The diastereoisomer derived from the corresponding ether of D-lactic acid showed *T* = 2.52.

A sample of **2** (1 mg) was treated[4] with boron tribromide (1 ml) in dichloromethane (1 ml). The reaction mixture was analysed by D-glucose oxidase (Glox-reagent, KABI, Stockholm, Sweden), and by g.l.c.[5]–m.s.[6] after reduction and acetylation.

Methylation analysis[7,12] of the carboxyl-reduced[2] polysaccharide material yielded, *inter alia*, a partially methylated alditol acetate (**3**) indistinguishable on g.l.c. [*T* = 1.79 (retention time relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-

glucitol), SP-1000, 220°] from the corresponding derivative obtained from K 37 after similar treatment. The mass spectrum of **3** contained, *inter alia*, the following fragments (relative intensities in brackets): 43(100), 45(25), 73(66), 87(12), 101(16), 117(19), 131(4), 161(3), 187(4), 219(3), 263(0.5), and 291(1).

ACKNOWLEDGMENTS

The skilful technical assistance of Miss Birthe Abrahamsson is gratefully acknowledged. This work was supported by grants from the Swedish Medical Research Council (B75-03X-2522-07C), from Knut och Alice Wallenbergs Stiftelse, from Harald Jeanssons Stiftelse, and from Sigurd och Elsa Goljes Minne.

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