Carbohydrate Research, 79 (1980) 313–315

Note

Structure of the extracellular polysaccharide from *Corynebacterium insidiosum*

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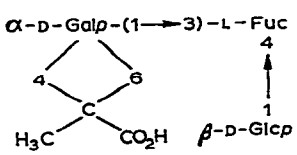
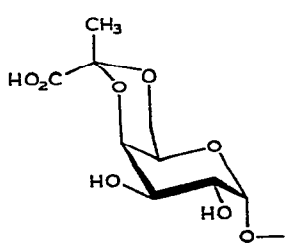
(Received June 19th, 1979; accepted for publication, July 6th, 1979)

The extracellular polysaccharide from *Corynebacterium insidiosum* has been investigated by Gorin and Spencer[1,2]. It is composed of D-glucose, D-galactose, L-fucose, and pyruvic acid residues in the proportions 1:1:2:1, suggesting that the polysaccharide is composed of tetrasaccharide repeating-units. The acidic oligosaccharide **1** was obtained by graded hydrolysis with acid. The pyruvic acid acetal was proved[3] to have the *R* configuration, as in **2**. The same configuration was found in three other bacterial polysaccharides, in which pyruvic acid is linked to O-4 and O-6 of D-galactopyranosyl residues[4]. We now report complementary studies of the *C. insidiosum* polysaccharide.



1



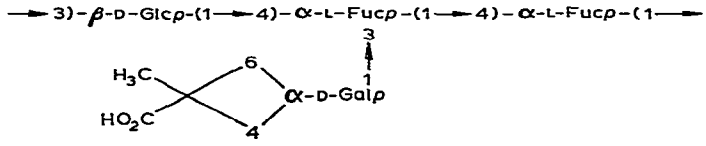
2

In the ^{13}C-n.m.r. spectrum of the polysaccharide, signals for anomeric carbons were observed at 104.7 and 102.1–101.3 p.p.m. (3 C), indicating that one sugar residue, known to be the D-glucopyranosyl residue in **1**, is β-linked and that three residues are α-linked. In the high-field region, signals were observed at 27.1 and 17.3 p.p.m. (2 C), assigned to the methyl carbons of the pyruvic acid acetal and the L-fucosyl residues, respectively. The former signal appears at this field when the acetal has the *R* configuration. The corresponding signal for an acetal having the *S* configuration appears[4] at ~17 p.p.m.

In the ^{1}H-n.m.r. spectrum, signals at about δ 1.24 (6 H) and 1.41 (3 H) were assigned to the methyl protons of the L-fucosyl residues and the pyruvic acid acetal, respectively. Signals for anomeric protons were observed at about δ 5.32 (2 H), 4.98 (1 H), and 4.54 (1 H). The spectrum was not well-resolved and no accurate values for the coupling constants could be obtained. It was evident, however, that the coupling constant for the signal at δ 4.54 was high, as expected for a β-D-glucopyranoside, and that the other coupling constants were low, as expected for α-D-galactopyranosides and α-L-fucopyranosides. The $[\alpha]_D$ value (—98°) for the polysaccharide is also in agreement with these results.

Methylation analysis of the polysaccharide gave approximately equimolecular amounts of 2,3-di-*O*-methyl-L-fucose, 2-*O*-methyl-L-fucose, 2,4,6-tri-*O*-methyl-D-glucose, and 2,3-di-*O*-methyl-D-galactose, which were analysed by g.l.c.–m.s. of their alditol acetates. From these analyses, it was concluded that the L-fucosyl residues were pyranosidic and linked through O-4, or furanosidic and linked through O-5. The low rate of hydrolysis of the polysaccharide in 10mM trifluoroacetic acid at 85° indicated that the former alternative is correct.

From these results, and those previously published[1,2], it is concluded that the *C. insidiosum* extracellular polysaccharide is composed of tetrasaccharide repeating-units having the structure 3.



3

EXPERIMENTAL

The polysaccharide was prepared as previously described[1], with the exception that a synthetic medium, composed of D-glucose (1.5%), yeast-nitrogen bas (Difco, 0.13%), casamino acids (Difco, 0.1%), and calcium carbonate (0.3%), was used.

General methods and methods for methylation analysis were the same as in a previous publication[5].

In partial, acid hydrolysis of the polysaccharide, the material (2 mg) was dissolved in 0.01M trifluoroacetic acid (2 ml) and kept at 85° for 2.5 h. The product was recovered by evaporation of the solvent. Methylation analysis of this material demonstrated that some of the pyruvic acid acetals had been removed, but that the cleavage of glycosidic linkages was insignificant.

ACKNOWLEDGMENTS

The skilled technical assistance of Miss Viveka Eriksson is gratefully acknowl-

edged. This work was supported by the Swedish Medical Research Council (B79-03X-02522-11C) and Stiftelsen Sigurd och Elsa Goljes Minne.

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