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Note

Welan gum (S-130) contains repeating units with randomly distributed L-mannosyl and L-rhamnosyl terminal groups, as determined by FABMS

Per-Erik Jansson *, Göran Widmalm

Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm (Sweden)

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Most bacterial polysaccharides are composed of oligosaccharide repeating units. There are, however, several examples of polysaccharides in which only part of the "repeating" units contain a certain substituent, e.g., an *O*-acetyl group, an α-D-glucopyranosyl group, or an acetalically linked pyruvic acid residue [1]. In a small group of polysaccharides, L-rhamnose in the "repeating unit" is replaced by L-mannose [2]. These structural variations may be random, or regular as, for example, in a block co-polymer, or the bacterium may produce two populations of polysaccharides, each with its own repeating unit, but which are difficult to separate. This structural problem has been solved for some polysaccharides. Thus, only a fraction of the *Salmonella thompson* O-antigen polysaccharide which did not contain α-D-glucopyranosyl groups was hydrolysed by the phage ϕ14, demonstrating the presence of two populations, one without and one with such groups in the repeating unit [3]. Hydrolysis of the O-antigen polysaccharide from *Salmonella typhi* $253T_4$ with phage P22 on the other hand gave dimers and trimers of the repeating unit containing 0, 1, and 2 or 0, 1, 2, or 3 α-D-glucopyranosyl groups, respectively, demonstrating a random distribution of these groups [4]. A related method of studying this problem would be to subject the polysaccharide to a partial chemical degradation and characterise the fragments derived from two or more repeating units.

We now report such studies of the polysaccharide welan gum elaborated by *Alcaligenes* ATCC 315 55. It is composed of "repeating units" with the structure **1**, in which the terminal α-L-rhamnopyranosyl groups are partially replaced by α-L-mannopyranosyl groups [5].

* Corresponding author.

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$$\rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{-}(1 \rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{A-}(1 \rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{-}(1 \rightarrow 4)\text{-}\alpha\text{-L-Rha}p\text{-}(1 \rightarrow$$
$$\begin{array}{c} 3 \\ \uparrow \\ 1 \\ \alpha\text{-L-Rha}p \text{ or } \alpha\text{-L-Man}p \end{array}$$

1

It further contains *O*-acetyl groups, linked to O-2 of the 3-linked β-D-glucopyranosyl residues[2].

Welan gum was treated with 0.1 M trifluoroacetic acid at 100°C for 30 min followed by reduction with sodium borohydride. It was expected that mainly α-L-rhamnopyranosidic, and possibly α-L-mannopyranosidic but few β-D-glucopyranosidic, linkages should be hydrolysed under these conditions. The product was fractionated on a column of Sephadex G-50 and several oligosaccharides were obtained. The fraction containing octa- to deca-saccharides, on sugar analysis as alditol acetates, gave L-Man and L-Rha in the ratio 1:2.5, compared to 1:5 in the polysaccharide. In another sugar analysis, in which the reducing sugar was converted into an alditol acetate and the remaining sugars were converted into acetylated aldononitriles, the following components were obtained: Rha, Man, Glc, rhamnitol, and glucitol in the relative proportions 6:4:19:3:1. This experiment demonstrated, as expected, that the cleavage of β-D-glucopyranosyl linkages was low.

In the ^{1}H NMR spectrum of this fraction, signals for α-anomeric protons were observed, *inter alia*, at δ 5.32, 5.21, and 5.13, in the approximate relative proportions 1:0.8:2.2. These chemical shifts are the same as those observed for the polysaccharide, and indicate that both side-chain L-Rha and L-Man are still present.

An FAB-mass spectrum in the negative mode (Fig. 1) showed, *inter alia*, three ions at *m/z* 1635.6, 1619.6, and 1603.6, with the relative intensities 1:2:1. The

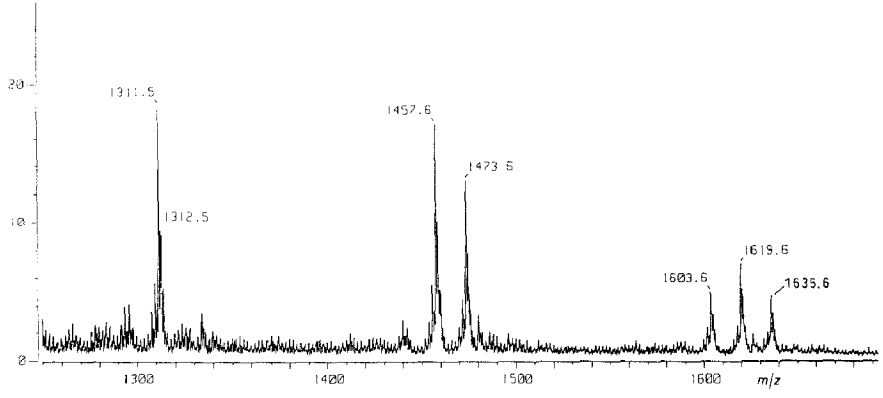

Fig. 1. FAB-mass spectrum, obtained in the negative mode, of octa- to deca-saccharides from graded hydrolysis of *O*-deacetylated welan gum.

mass numbers are those expected for decasaccharides derived from two "repeating units" with two side-chain α-L-Man, one side-chain α-L-Man and one side-chain α-L-Rha, or two side-chain α-L-Rha, respectively, formed by cleavage of two α-L-rhamnopyranosyl linkages in the polysaccharide chain. Ions with these mass numbers could, however, also be formed by hydrolysis of other linkages, one of which has to be a β-D-glucopyranosidic linkage in the chain. As discussed above, however, hydrolysis of these linkages is not very important. Peaks at 1457.6 and 1473.6 originate from nonasaccharides with either a side-chain L-Rha or L-Man group, and that at 1311.5 from the octasaccharide. None of these fragments is diagnostic.

The formation of the decasaccharide containing one α-L-Rha and one α-L-Man group therefore demonstrates that these groups are scrambled in welan gum. The enzymes responsible for the linking of these groups to the "repeating unit" and the polymerisation of the "repeating units" therefore do not discriminate between these sugars. The two sugars most probably also have a common biosynthetic origin, possibly dTDP-D-*xylo*-hexos-4-ulose [7].

1. Experimental

General.—FAB-mass spectra in the negative mode were recorded on a Jeol SX 102 instrument, using Xe atoms (6 kV) and a matrix of glycerol, at a resolution of 1000.

Isolation of oligosaccharides after mild acid hydrolysis.—*O*-Deacetylated welan gum (68 mg) was hydrolysed with 0.1 M CF_3CO_2H at 100°C for 30 min. The hydrolysate was neutralised with aq NaOH, then reduced with $NaBH_4$, and the boric acid removed as its methyl ester, and lyophilised. The hydrolysate was fractionated on a Sephadex G-50 column (2.5 × 90 cm) yielding, *inter alia*, an octa- to deca-saccharide fraction (3.4 mg).

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