An Inulin-like Fructan Produced by *Streptococcus mutans*, Strain JC2

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In studies of polysaccharides elaborated by bacteria, isolated from human dental plaque, *Streptococcus mutans*, strain JC2,¹ was one of the organisms investigated. An enzyme preparation with hexosyltransferase activity was isolated from the supernatant fluid of a glucose broth culture by chromatography on hydroxylapatite.² When the enzyme preparation was incubated with sucrose, alcohol-precipitable polysaccharide material was formed. This, on acid hydrolysis, yielded comparable amounts of D-glucose and D-fructose. Judging from previous results,^{3,4} the mixture most probably contained a glucan and a fructan. Indeed, a fructan, $[\alpha]_D^{22} - 33^\circ$ (c 0.3, 0.1 M NaOH), was isolated by precipitation with barium hydroxide as was a glucan, $[\alpha]_D + 192^\circ$ (c 0.3, 0.1 M NaOH) by precipitation with ethanol. Gel filtration chromatography on Sepharose 2B, as devised by Arturson and Granath,⁵ showed that both polysaccharides had high molecular weights, $\bar{M}_w \approx 2 \times 10^7$.

The two polysaccharides were subjected to methylation analyses as previously described for dextrans⁶ and fructans⁷ and the methylated sugars obtained were analysed by GLC-MS.⁸ The results, given in Table 1, show that the glucan is of the type generally elaborated by *Streptococcus* species, e.g. an α -glucan with 3-, 6-, and 3,6-linked residues. The fructan, however, was not of the levan type with (2 $\rightarrow$ 6)-linkages, known to be produced by several bacteria, but of the inulin type, with (2 $\rightarrow$ 1)-linked β -fructofuranose residues. Some branching through the 6-position was observed. The sugars obtained in the methylation analysis were reduced with sodium borodeuteride. The deuterium labelling observed on MS showed that the tri-*O*-methyl-hexitol came from 3,4,6-tri-*O*-methyl-D-fructose and not from 1,3,4-tri-*O*-methyl-D-fructose. The alditol acetate derivatives of these sugars cannot be separated by GLC; however, the corresponding acetylated sugars are readily separated,⁹ and not even traces of the 1,3,4-tri-*O*-methyl derivative were observed.

Until recently, only fructans of the levan type were known to be formed by bacterial fructosyltransferases. After the completion of the present work, another report⁹ on a bacterial (2 $\rightarrow$ 1)-linked fructan, from *Streptococcus mutans*, strain Ingbritt, has come to our knowledge.

Table 1. Methyl ethers from the hydrolysate of the methylated fructan (A) and the methylated glucan (B).

Sugars	T ^a	Mol %	
		A	B
1,3,4,6-Tetra- <i>O</i> -methyl-D-fructose	0.82	5	
2,3,4,6-Tetra- <i>O</i> -methyl-D-glucose	1.00		22
3,4,6-Tri- <i>O</i> -methyl-D-fructose	1.82	89	
2,4,6-Tri- <i>O</i> -methyl-D-glucose	1.82		10
2,3,4-Tri- <i>O</i> -methyl-D-glucose	2.22		46
2,4-Di- <i>O</i> -methyl-D-glucose	4.21		22
3,4-Di- <i>O</i> -methyl-D-fructose	4.31	6	

^a Retention times of the corresponding alditol acetates on the OV-225 column relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

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