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Note

Further evidence for the structure of a mannofucogalactan from the fruit bodies of *Flammulina velutipes* (Fr.) Sing.: Smith degradation of oligosaccharides obtained by partial acid hydrolysis†

TAKAFUMI MUKUMOTO AND HARUKI YAMAGUCHI*

Department of Agricultural Chemistry, College of Agriculture, University of Osaka Prefecture, Sakai, Osaka 591 (Japan)

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In our previous studies[1], methylation analysis and Smith degradation revealed that a mannofucogalactan isolated from the fruit bodies of *Flammulina velutipes* (Fr.) Sing. has a backbone of (1→6)-linked α-D-galactopyranose residues, and approximately every third of these is substituted at O-2 by either 3-*O*-α-D-mannopyranosyl-L-fucopyranosyl residues or by single L-fucopyranosyl groups. In the course of further studies on the fine structure of the heterogalactan, partial acid-hydrolysis afforded oligosaccharides having various degrees of polymerization. The investigations reported herein were undertaken to analyze the linkage positions of these oligosaccharides, and to obtain further corroborating evidence for the structure of the mannofucogalactan.

Structural studies on reducing oligosaccharides produced by partial hydrolysis of polysaccharides and complex carbohydrates have usually been performed by methylation followed by g.l.c.–mass spectrometry. To analyze the linkage position of reducing oligosaccharides readily on microquantities, we recently proposed a simple method[2] whereby oligosaccharides are oxidized with periodate under conditions minimizing overoxidation, after which step the hydroxy and oxo compounds produced by subsequent borohydride reduction and acid hydrolysis are determined by g.l.c. This method has been applied for linkage-analysis of the reducing oligosaccharides obtained by partial acid-hydrolysis of the mannofucogalactan.

The polysaccharide was subjected to stepwise hydrolysis with acid, and the fragments of low molecular-weight were isolated after each treatment. The main disaccharide component separated by chromatography on thick paper gave equimolar amounts of mannose and fucose on acid hydrolysis, and was indistinguishable, by optical rotation and p.c., from the 3-*O*-α-D-mannopyranosyl-L-fucose previously

*To whom correspondence should be sent.
†Abbreviation: p.c., paper chromatography.

obtained by controlled acetolysis of the polysaccharide[1]. Support for these results was furnished by g.l.c. analysis of a methanolyzate of the disaccharide, before and after borohydride reduction. Other oligosaccharides gave only galactose on acid

TABLE I

OLIGOSACCHARIDES FROM PARTIAL ACID-HYDROLYSIS OF THE HETEROGALACTAN[a] (100 mg)

Oligosaccharide	*Yield (mg)*	$[\alpha]_D$	R_{Gal}[b]
Mannosylfucose	17.8	−3.0°	1.16
Galactobiose	3.4	123°	0.76
Galactotriose	4.0	152°	0.50
Galactotetraose	4.6	174°	0.31
Galactopentaose	4.4	167°	0.17
Galactohexaose	4.6	183°	0.08

[a]Sample of 100 mg. [b]Relative to galactose on a t.l.c. plate.

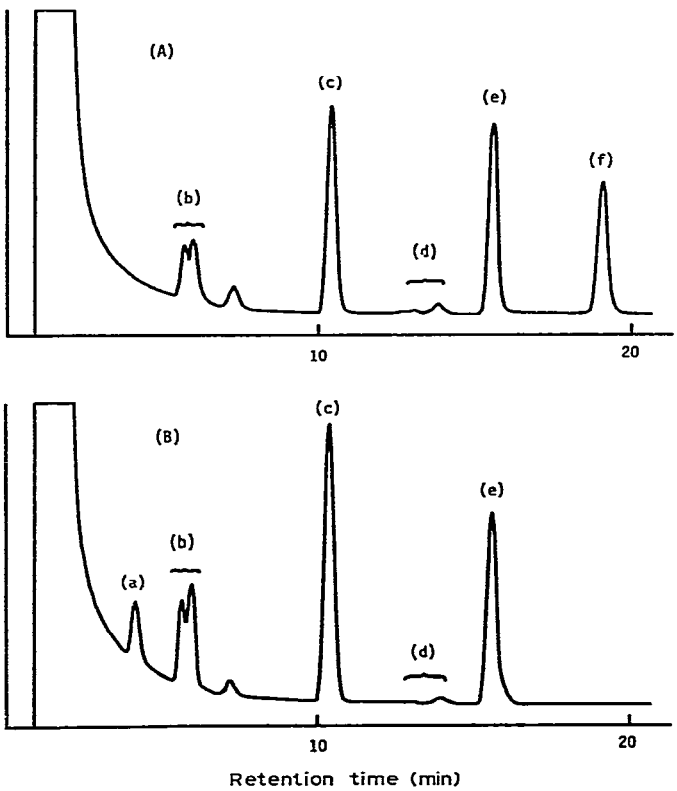

Fig. 1. G.l.c. separation of hydroxy and oxo compounds obtained by Smith degradation from mannosylfucose (A) and galactopentaose (B): (*a*), 1,2-ethanediol; (*b*), glycolaldehyde (oximes); (*c*), glycerol; (*d*), glyceraldehyde (oximes); (*e*), 2-ethyl-2-(hydroxymethyl)-1,3-propanediol; (*f*), 1-deoxy-L-arabinitol.

hydrolysis and, on partial hydrolysis, the higher members of the series yielded the lower ones. These results suggested that the galacto-oligosaccharides belong to a homologous series. A list of the oligosaccharides obtained is given in Table I, together with some of their properties.

To elucidate the structures of these reducing oligosaccharides, they were subjected to Smith degradation, followed by g.l.c. analysis of the products. The gas chromatogram obtained for the main disaccharide, a mannosylfucose, indicated that 1 mol of it gave nearly 1 mol each of glycolaldehyde and glycerol, with an additional product having a retention time intermediate between that of erythritol and of arabinitol (Fig. 1A). Mass spectrometry of the unidentified peak (*f*) showed ions at *m/e* 103, 117, 205, 219, and 307, as expected for a 2,3,4,5-tetra-*O*-trimethylsilyl-1-deoxypentitol[3,4]. The primary fragments (*m/e* 117 and 219) are known to be characteristic of deoxy groups in *O*-trimethylsilylated alditols[3]. A reference sample of a 1-deoxypentitol was not available, but, as L-fucose is the only deoxy sugar in the disaccharide, the detection of this series of fragments clearly shows substitution at O-3 of the reducing fucopyranose residue. These results are in accord with the previous finding that (1→3)-linked disaccharides of D-hexopyranoses, on Smith degradation, give 1 mol each of glycolaldehyde, glycerol, and D-arabinitol[2]. The formation of 3-*O*-α-D-mannopyranosyl-L-fucopyranose in high yield on partial acid hydrolysis is consistent with the findings previously obtained on the polysaccharide[1], where a large proportion of the fucose residues was found resistant to periodate oxidation, and 2,4-di-*O*-methyl-L-fucose was a major component of a hydrolyzate of the permethylated polysaccharide. On the other hand, all of the galacto-oligosaccharides gave equimolar amounts of glycolaldehyde and glycerol, together with 1,2-ethanediol, regardless of their degrees of polymerization (Fig. 1B). The analytical values found for these oligosaccharides are summarized in Table II, together with those for the mannosylfucose. As shown previously, 1,2-ethanediol is the product derived from C-5 and C-6 of a reducing-end hexopyranose residue substituted at O-6, and equi-

TABLE II

HYDROXY AND OXO COMPOUNDS FROM SMITH DEGRADATION OF THE OLIGOSACCHARIDES

Oligosaccharide	*1,2-Ethanediol*	*Glycolaldehyde*	*Glycerol*	*Glyceraldehyde*	*Threitol*	*1-Deoxy-L-arabinitol*
	(moles/mole of oligosaccharide)					
Mannosylfucose	0	0.88	1.10	(+)[a]	0	+
Galactobiose	+[a]	0.98	1.00	0	0	0
Galactotriose	+	1.81	2.03	0	0	0
Galactotetraose	+	2.81	2.88	0	0	0
Galactopentaose	+	3.81	3.73	(+)	0	0
Galactohexaose	+	4.60	4.66	(+)	(+)	0

[a] +, Present; (+), traces.

molar amounts of glycolaldehyde and glycerol are formed from a hexopyranosyl residue linked through C-1 and C-6, or from a non-reducing end[2]. The detection of traces of glyceraldehyde and threitol suggests that the periodate oxidation of higher members of this series is retarded by steric hindrance between sugar residues. Prolongation of the time of periodate oxidation led to the disappearance of these minor compounds produced from the higher galacto-oligosaccharides. Galactotriose and galactotetraose also yielded small amounts of glyceraldehyde and threitol when periodate-oxidation times were shorter than 3 h. These features of degradation of the galacto-oligosaccharides are similar to those previously found from isomaltose, gentiobiose, and isomaltotriose[2]. The results again show, therefore, that the galacto-oligosaccharides form a homologous series and are derived from the backbone of (1→6)-linked α-D-galactopyranosyl residues.

These findings on the oligosaccharides obtained by partial acid-hydrolysis confirm the proposed structure of the mannofucogalactan. In addition, it is demonstrated that the present method, used for the linkage analysis of reducing oligosaccharides, offers much saving in time and sample as compared with methylation analysis.

EXPERIMENTAL

Materials and general methods. — The mannofucogalactan was prepared as previously described[1]. Methyl α-D-glucopyranoside was of commercial origin.

P.c. was performed with Whatman No. 1 paper (ascending method) with the solvent system 40 : 11 : 19 (v/v) butanol–ethanol–water. Washed Whatman 3MM paper was used for preparative p.c. Components were detected with alkaline silver nitrate. T.l.c. was performed on silica gel plates (Merck) with the solvent system 6 : 1 : 3 (v/v) 2-propanol–ethyl acetate–water, and carbohydrates were detected with 5% methanolic sulfuric acid by heating at 110° for 10 min. G.l.c. was performed with a Shimadzu gas chromatograph, model 4APF, equipped with a hydrogen flame-ionization detector, and the following columns: (*a*) a glass column (200 × 0.4 cm, i.d.) of 4% of SE-52 on Chromosorb W (80–100 mesh) at 175°, and (*b*) a glass column (200 × 0.3 cm, i.d.) of 5% of SE-30 on Chromosorb W (60–80 mesh) at 100–190° (4°/min). G.l.c.–mass spectrometry was performed with chromatographic column (*b*) coupled to a Hitachi RMU-6MG mass spectrometer. The mass spectra were recorded at an ionizing potential of 20 eV.

Optical rotations were measured at 25° with a Union Giken PM-70 Polarimeter. Other analytical procedures used in this work were described in the previous paper[1].

Partial acid hydrolysis of the mannofucogalactan. — The polysaccharide (50 mg) was dissolved in 0.05M sulfuric acid (5 mL) and kept for 3 h at 90°. Sulfuric acid was removed by neutralization with barium carbonate, followed by centrifugation. The neutral, supernatant solution was evaporated to low volume and the partially hydrolyzed polysaccharide was fractionated by chromatography on a column (180 × 2 cm) of Bio-gel P-2 (100–200 mesh). The column was eluted with water at a flow rate of

6 mL/h. Carbohydrates in the fractions were detected by the orcinol–sulfuric acid reaction. The polysaccharide fraction excluded from the gel was evaporated to a syrup and hydrolyzed again exactly as just described. A new polysaccharide fraction obtained from the same column of Bio-gel was further submitted to a similar, third hydrolysis and fractionation. Oligosaccharide fractions obtained from the gel column were analyzed by t.l.c., and those showing the same t.l.c. behavior were combined and then rechromatographed on Bio-gel P-2 as just described. T.l.c. and p.c. showed the disaccharide fraction to be a mixture, and two disaccharides were separated by using thick paper. The oligosaccharides thus obtained were characterized as described next. Each oligosaccharide (~20–30 μg) was reduced with aqueous sodium borohydride and desalted by passing the solution through a small column of Dowex-50 X8 (H^+) resin, and then evaporating it with repeated addition and evaporation of methanol. The reduced oligosaccharide was treated with 0.5 mL of M methanolic hydrogen chloride for 6 h at 80° in a sealed tube, isolated by evaporation of the solution, and then converted into the trimethylsilyl ethers in 50 μL of anhydrous pyridine containing 10 μg of methyl α-D-glucopyranoside as an internal standard. G.l.c. analysis was performed with chromatographic column (*a*).

Smith degradation of oligosaccharides and g.l.c. analysis of the products. — Each galacto-oligosaccharide (~0.1 μmol) or mannosylfucose (~0.2 μmol) was dissolved in 0.2 mL of 0.5M acetate buffer (pH 3.2) containing 2.14 mg of sodium metaperiodate, and the solution was maintained at 30°. Disaccharides were oxidized for 5 h and the higher oligosaccharides for 7 h. The oxidized oligosaccharides were reduced with sodium borohydride, desalted, and subjected to procedures for g.l.c. analysis of the degradation products exactly as described previously[2].

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