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Polysaccharides from *Polyporus giganteus*

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Two water soluble polysaccharides have been isolated from fruit bodies of *Polyporus giganteus*. One is an α-glucan of the glycogen type with an average total chain length of 8–9 glucose residues and an average interior chain length of 2–3 residues. The other polysaccharide consists of chains of (1→6)-β-linked D-galactopyranose residues, every second or third of which is substituted in the 2-position by a 3-*O*-β-D-mannopyranosyl-β-L-fucopyranosyl residue.

The information available on fungal polysaccharides is mainly restricted to the presence and properties of these substances in moulds and yeast-like fungi.[1] Few members of the group Basidiomycetes have been investigated in these respects and then only with reference to the skeletal polysaccharide. In the *Poria* [2] and *Polyporus* [3,4] species examined this appears to be a linear glucan, with linkages mainly of the (1→3)-β-type, although some (1→3)-α-linkages might also be present.[3,4]

The present communication deals with the isolation and structural examination of the water soluble polysaccharides present in the fruit bodies of the annual fungus *Polyporus giganteus*, collected from the trunk of an old oak *(Quercus robur)*. The water soluble polysaccharides were extracted with hot water after distintegration of the material and removal of acetone soluble extractives. Further extraction with 4 M sodium hydroxide, followed by acidification of the extract yielded a precipitate with $[\alpha]_D$ + 22° (1 M sodium hydroxide) which gave glucose as the main product of total hydrolysis. Partial formolysis gave glucose, traces of other monosaccharides and a series of oligosaccharides, indistinguishable from the laminaridextrins by chromatography. This material therefore seems to consist mainly of the (1→3)-β-linked glucan referred to above.

The polysaccharide part of the aqueous extract gave on hydrolysis galactose, glucose, mannose, fucose and traces of xylose. When treated with cetyl trimethylammonium hydroxide (CTA-OH) [5] in order to precipitate any acidic polymers present, a neutral glucan, in all probability identical to that present in the sodium hydroxide extract, was precipitated. In an analogous way a neutral, linear (1→3)-β-glucan from *Pullularia pullullans* with single-unit branches at the 6-positions was precipitated by CTA-OH.[6]

Further addition of CTA-OH to strongly alkaline reaction precipitated a second, water soluble glucan. Treatment of the residue in boric acid solution with CTA-OH precipitated a heteropolysaccharide composed of D-galactose, D-mannose and L-fucose leaving in solution a further quantity of the second glucan.

An aqueous solution of the second glucan gave a reddish brown colour reaction with iodine. Redissolving samples of it in water left small insoluble residues, consisting essentially of (1→3)-β-glucan, which could be removed by centrifugation at 34 000 × *g*. A sample purified thus three times had $[\alpha]_D$ + 191° in water and gave glucose only on hydrolysis. When it was treated with a (1→3)-β-glucanase from Basidiomycete QM 806,[8] kindly provided by Dr. E. T. Reese, a significant amount of glucose was released, however, showing a persisting contamination of (1→3)-β-glucan.

Partial hydrolysis of the α-glucan yielded, besides glucose, maltose and isomaltose, which were isolated and characterised. A methylated sample (OCH_3 43.2 %, $[\alpha]_D$ + 182° in chloroform) yielded upon hydrolysis a mixture of methylated sugars which was resolved by carbon column chromatography to give as fully characterised components 2,3,4,6-tetra-*O*-methyl-D-glucose (13 mole %), 2,3,6-tri-*O*-methyl-D-glucose (73 %) and 2,3-di-*O*-methyl-D-glucose (11 %) together with small amounts of 2,6-di-*O*-methyl-D-glucose (1 %) and 2-*O*-methyl-D-glucose (2 %) as identified by paper chromatography and electrophoresis. The presence of the last two ethers is probably due to undermethylation resulting from the resistance of the 3-position towards methylation.[9] 2,4,6-Tri-*O*-methyl-D-glucose was not detected indicating that the methylated α-glucan was free from contaminating β-glucan.

A sample of the α-glucan consumed 0.98 mole of periodate per 162 g with liberation of 0.10 moles of formic acid. Considering the types of linkage known to be present from the results presented above, this is equivalent to 10 % terminal, non-reducing residues, and 78 % 4-mono- and 4,6-disubstituted residues. It further shows the presence of 12 % non-oxidisable material. This consists at least partly of β-glucan as indicated by the formation of some glucose on hydrolysis of the oxidised polysaccharide after treating it with sodium hydridoborate.[10] The periodate oxidation data give an average chain length of 8.8 residues. This agrees well with the 8—9 residues indicated by the methylation analysis.

Treatment with crystalline β-amylase converted 36.5 % of the sample, corresponding to 42 % of α-glucan, to maltose which is equivalent to 3.7 glucose residues in a chain of 8.8 residues. As β-amylases are considered to leave 2—3 residues projecting from a branching point[11] there would be 5—6 residues exterior and 2—3 interior to the branching points in the polysaccharide. The residual polysaccharide consumed 0.93 mole of periodate with liberation of 0.14 mole of formic acid per 162 g. Assuming that the low degree of oxidation (79 %) is due to the non-oxidisable contaminants apparently present in the original sample these data give an average chain length of 5.6 glucose residues in satisfactory agreement with the 5.1 residues calculated from the original chain length and the maltose liberated.

The mode of branching in the α-glucan has not been examined in detail as has been done for some glycogens.[12] Furthermore, the fact that the varying

degree of contamination of different samples, presumably by mainly (1→3)-β-glucan, was not first fully realised, obscured the results somewhat. Still, the results permit classifying the *Polyporus giganteus* α-glucan as a glycogen with chains a few glucose residues shorter than commonly occurring in that group of polysaccharides.[11,12]

The heteropolysaccharide had $[\alpha]_D$ + 65° in water and gave on hydrolysis D-galactose, D-mannose and L-fucose in the relative proportions 2.6:1:1 as estimated after chromatographic separation by hypoiodite oxidation. It gave viscous solutions with strongly film-forming properties, indicative of an essentially linear structure.[13]

Table 1. Methyl ethers from hydrolysed, methylated *Polyporus giganteus* heteropolysaccharide.

Methyl ether of	*O*-Methyl groups in position	Mole %
D-Galactose	—	1.2
»	3	5.0
»	2,3	1.2
»	3,4	23.7
»	2,3,4	} 36.5
L-Fucose	2,4	}
»	2 or 4	0.8
D-Mannose	2,3,4,6	24.5
D-Glucose	2,3,6	5.8
»	2,3,4,6	0.4

The methylated heteropolysaccharide (OCH_3 39.6 %, $[\alpha]_D$ + 56° in chloroform) yielded on hydrolysis a mixture of methylated sugars which were separated (Table 1) and characterised. As appears from Table 1 the component sugars are all present in the pyranosidic form. D-Mannose is present exclusively as terminal, non-reducing residues and L-fucose as 3-substituted ones. The isolation of 2,3,4-tri- and 3,4-di-*O*-methyl-D-galactose shows D-galactose to occur both 6-mono- and 2,6-disubstituted. Of the minor components isolated the tri- and tetra-*O*-methyl-D-glucoses obviously originate from contaminating α-glucan; the others result from undermethylation or demethylation and bear no structural significance.

For further information on the mode of combination of the different monosaccharides in the original polymer this was subjected to a step-wise partial hydrolysis. In the first steps, D-mannose, L-fucose and, as major product, a disaccharide composed of these monosaccharides were split off. D-Galactose appeared together with a galactobiose in traces only. After several steps the high molecular weight part of the hydrolysate, which formed very viscous aqueous solutions with low optical rotations, contained D-galactose and traces of D-mannose and L-fucose residues. It was partially hydrolysed to give D-galactose and a series of oligosaccharides (Table 2). The disaccharide of that series, also formed in small amounts during the preceding hydrolysis steps, was identified as 6-*O*-β-D-galactopyranosyl-D-galactose. From the optical rotations of the different oligosaccharides, from their R_M/n values and from the fact that each of them gave galactose and the corresponding lower members

Table 2. Properties of the oligosaccharides from partially hydrolysed *Polyporus giganteus* heteropolysaccharide.

	$[\alpha]_D$	M_G[a]	R_{Gal}[b]	R_M/n
Mannosylfucose	± 0	0.55	0.90	—
Galactobiose	+ 28.3	0.79	0.47	0.35
Galactotriose	+ 18.6	0.75	0.24	0.34
Galactotetraose	+ 13.2	0.73	0.12	0.33
Galactopentaose	+ 8.4	0.71	0.06	0.33

[a] In 0.1 M borate buffer of pH 10; [b] R_F for galactose 0.36 (solvent D).

of the series on partial hydrolysis, these oligosaccharides evidently constitute a homologous, (1→6)-β-linked series. No oligosaccharides other than those described above were observed in the series of hydrolysates.

The disaccharide released during the first stage of the hydrolysis had $[\alpha]_D$ ± 0° in water and showed no tendency to crystallise. Mannose was the only reducing sugar found after hydrolysis of a sample previously reduced with sodium hydridoborate. The disaccharide had higher mobility than fucose itself on paper electrophoresis in sulphonated phenylboronic acid buffer of pH 6.6.[14] These observations combined with the isolation from the hydrolysed, methylated polysaccharide of 2,4-di-*O*-methyl-L-fucose show the disaccharide to possess a 3-*O*-β-D-mannopyranosyl-L-fucose structure. The high positive rotation of the original polysaccharide (+ 65°) and the low contributions from the β-D-mannosidic and β-D-galactosidic residues indicates that the L-fucosyl residues also possess β-configuration.

The above results are in accordance with a polysaccharide structure consisting of a linear backbone of (1→6)-β-linked D-galactopyranose residues, every second or third of which is substituted in the 2-position by a 3-*O*-β-D-mannopyranosyl-β-L-fucopyranosyl unit (Fig. 1). Further confirmation of the

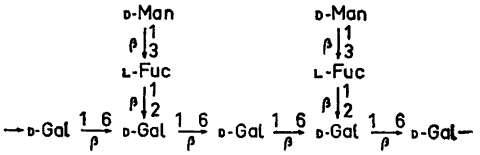

Fig. 1. Constitution of *Polyporus giganteus* heteropolysaccharide.

structure was given by the periodate uptake (1.34 moles per anhydrosugar residue) and formic acid liberation (0.51 moles), in good agreement with the values 1.34 and 0.57 moles, respectively, expected for a polysaccharide of the assumed structure with Gal:Man:Fuc = 2.6:1:1. Finally, fucose was the only detectable sugar in a hydrolysate of the periodate-oxidised and subsequently reduced polysaccharide.

EXPERIMENTAL

Paper chromatograms were run on Whatman No. 1 and 3 MM papers in the following solvents:

A. Butanol, ethanol, water 10:3:5,
B. Ethyl acetate, pyridine, water 8:2:1,
C. Ethyl acetate, acetic acid, water 3:1:1,
D. Ethyl acetate, pyridine, water 2:1:2, upper phase,
E. Water-saturated butan-2-one,
F. Toluene, ethanol, water, 10:7:1, upper phase.

Paper electrophoresis was carried out on Whatman No. 3 papers in 0.1 M borate buffer of pH 10 and 0.1 M sulphonated phenylboronic acid of pH 6.6.[14] Anisidine hydrochloride was used as spray reagent. Optical rotations were measured in water at 22° unless otherwise stated.

Extraction and separation of polysaccharides.

Extraction of the polysaccharides. The fungus in ethanol was disintegrated in a Turmix blender, air-dried and then continuously extracted for 30 h with acetone. The residue (600 g as air-dried) was extracted twice with water at 80° and once with boiling water. Extracted polysaccharides were isolated by precipitation from the concentrated extracts with ethanol. The different extracts showed similar composition (optical rotations, products of hydrolysis) and were combined and reprecipitated from aqueous solution with ethanol to give a light-coloured powder (39 g). Paper chromatographic examination of a hydrolysed sample showed the presence of galactose, glucose, mannose, fucose and xylose in the approximate proportions 2:3:1:1:trace.

By ultrasonic treatment of part of the residue (260 g) in water (3 l) at 40—50° for 1 h in a Philips L364 ultrasonic generator a further 2.4 g of material was extracted. Another part (220 g) was extracted twice with 4 M sodium hydroxide (2.5 l) under nitrogen at room temperature for 1 h. The latter extracts were combined and neutralised with acetic acid and the slowly coagulating precipitate recovered by centrifuging, washed with water and ethanol and dried to give a cream-coloured powder (6.0 g) with $[\alpha]_D$ + 22° (*c*, 0.9 in 1 M sodium hydroxide). A sample was treated at 100° with 90 % formic acid for 30 min and then, by adding water, in 25 % formic acid for a further 2 h. The product was found by paper chromatography to contain glucose, a series of oligosaccharides indistinguishable from the laminaridextrins and traces of other sugars.

Fractionation of the water soluble polysaccharides. The water soluble polysaccharide fraction (31 g) was dissolved in water (2.5 l) and 0.23 M CTA—OH (700 ml) was added until red reaction with phenolphtalein. (The high buffer capacity of the solution indicates the presence of considerable amounts of material other than neutral polysaccharides.) The precipitate formed was collected by filtration, washed with water and treated with dilute acetic acid. The resulting gelatinous mass was washed with ethanol to give a water insoluble powder (A, 1.51 g). Further CTA—OH (300 ml) yielded a second precipitate which gave, after treatment with acetic acid and precipitation with ethanol, a water soluble fraction (B, 4.26 g). The supernatant gave on deionisation, concentration and precipitation by ethanol a third fraction (C, 2.84 g).

Fraction A gave glucose only on hydrolysis. Partial hydrolysis, conducted as described above for the polysaccharide extracted by sodium hydroxide, yielded on examination by paper chromatography as principal disaccharides maltose and laminaribiose.

Fraction B, also giving glucose only on hydrolysis, was dissolved in water and passed through a DEAE cellulose column, the main part (3.42 g) not being absorbed. After precipitation with ethanol, it was dissolved in water and freed from water-insoluble material by centrifugation at 34 000 × g and then again precipitated with ethanol. The residue after three such treatments (2.27 g) had $[\alpha]_D$ + 191° (*c*, 0.75) and gave opalescent aqueous solutions which turned reddish brown upon addition of iodine solution.

Fraction C gave galactose, glucose, mannose and fucose on hydrolysis. A glucose-free polysaccharide was obtained by gradual precipitation with 0.23 M CTA-OH from 0.06 M

Table 3. Purification of crude *P. giganteus* heteropolysaccharide (2.76 g dissolved in 220 ml 0.06 M boric acid).

Fract.	0.23 M CTA-OH added (ml)	Weight (g)	Constituent sugars Gal	Gl	Man	Fuc
C_1	15	0.50	+	tr	+	+
C_2	15	0.52	+	−	+	+
C_3	50 + 5 ml 1 M NaOH	0.22	+	tr	+	+
C_4	Residue	0.77	+	+	+	+

boric acid solution,[7] as summarised in Table 3. A further quantity of this polysaccharide (1.1 g) was obtained in similar manner from fraction C_4 combined with the fraction obtained by the ultrasonic treatment of the residual fungus described above.

The α-glucan

Partial hydrolysis. Slightly impure α-glucan (6.0 g) was treated with 0.1 M sulphuric acid (600 ml) for 1 h at 100°. The solution was neutralised, concentrated and poured into ethanol. The precipitate was then subjected to a similar treatment. The combined non-precipitated fractions (2.07 g) were absorbed on a carbon column and eluted with an aqueous ethanol gradient. The following substances were obtained: D-Glucose (1.21 g) $[\alpha]_D$ + 50° (*c*, 0.8); β-pentaacetate, m.p. and mixed m.p. 128−130°.

Isomaltose (30 mg), $[\alpha]_D$ + 106° (*c*, 0.42); β-octaacetate, m.p. and mixed m.p. 138−140°.

Maltose (213 mg), $[\alpha]_D$ + 122° (*c*, 1.0); β-octaacetate, $[\alpha]_D$ + 63° (*c*, 0.5 in chloroform), m.p. and mixed m.p. 157−159°.

Methylation analysis. The polysaccharide (1.52 g) was dissolved in hot dimethylformamide (30 ml) and the solution, after cooling, treated with acetic anhydride (10 ml) and pyridine (15 ml) first at room temperature overnight and then at 45° for 2 h. The product was precipitated by adding ice-water, collected by filtering and dried. It was then precipitated from chloroform solution by light petroleum to give a white powder (2.50 g) with $[\alpha]_D$ + 166° (*c*, 0.4 in chloroform). This was methylated as described for the acetylated α-glucan from *Pullularia pullullans* [6] to give a final product (1.14 g) with $[\alpha]_D$ + 182° (*c*, 4.3 in chloroform) and OCH_3 43.2 %.

The methylated polysaccharide was dissolved in 90 % formic acid (50 ml) and kept at 100° for 45 min. After evaporating the formic acid under reduced pressure the hydrolysis was continued in 0.25 M sulphuric acid (50 ml) at 100° overnight. After neutralisation (barium carbonate) the resulting mixture of methylated sugars was resolved by chromatography on a carbon-Celite column using as irrigants a gradients of water → 50 % aqueous ethanol → 50 % aqueous acetone.

The following ethers were obtained, the first two being tentatively identified by comparing their chromatographic and electrophoretic mobilities with those of authentic specimens:

2-O-Methyl-D-glucose (17 mg), $[\alpha]_D$ + 16°;
2,6-Di-O-methyl-D-glucose (9 mg):
2,3-Di-O-methyl-D-glucose (97 mg), $[\alpha]_D$ + 42°, m.p. and mixed m.p. 100−105°, 1,4,5,6-tetra-*O*-azobenzoyl-2,3-di-*O*-methyl-D-glucitol,[15] m.p. 179−180°, undepressed on admixture with an authentic specimen;
2,3,6-Tri-O-methyl-D-glucose (685 mg), $[\alpha]_D$ + 63°, m.p. and mixed m.p. 115−116°;
2,3,4,6-Tetra-O-methyl-D-glucose (131 mg), $[\alpha]_D$ + 75°, m.p. 75−85°; m.p. and mixed m.p. of aniline derivative 136−138°.

Action of β-amylase. The glucan (98 mg) was incubated with β-amylase (2 mg; Nutritional Biochemicals Corp., crystalline grade) at 28° as described by Bell and Manners.[16] The maltose liberated corresponded to 36.5 % of the molecule as estimated by the Somogyi method.[17] The digest was then poured into ethanol and the precipitated dextrins collected. The supernatant was found by paper chromatography to contain maltose and

a trace of glucose. The dextrins had after treatment with ion exchange resins $[\alpha]_D$ + 126°. Treatment with 0.25 M sulphuric acid at 100° for 1 h gave on paper chromatographic examination, in order of their relative quantities, glucose, isomaltose, maltose and maltotriose.

Periodate oxidations. Periodate oxidations were carried out with sodium metaperiodate at room temperature. The periodate consumption was estimated by the arsenite method [18] and the liberated formic acid by iodometric titration. Analytical data were extrapolated to zero time. The α-glucan consumed 0.98 mole of periodate with liberation of 0.10 mole of formic acid per 162 g. The corresponding data for the β-dextrins were 0.93 and 0.14 mole, respectively.

Action of (1→3)-β-glucanase on α-glucan. A solution of α-glucan (30 mg) and (1→3)-β-glucanase [8] (0.5 mg) in 0.025 M citrate buffer of pH 4.2 (10 ml) was kept at 55° for 20 h. The solution was treated with ion-exchange resins, concentrated and poured into ethanol. The supernatant was concentrated to a syrup (5 mg) containing glucose together with some citric acid. A control experiment using soluble starch gave only a trace of glucose.

The heteropolysaccharide

The heteropolysaccharide fractions were combined and precipitated from aqueous solution with ethanol. After one reprecipitation the polysaccharide had $[\alpha]_D$ + 65° (*c*, 0.7) and dissolved readily to give viscous solutions. It gave on hydrolysis D-galactose, D-mannose and L-fucose in the relative proportions 2.6:1:1 as determined by hypoiodite oxidation after paper chromatographic separation. The isolated sugars were not crystallised but characterised thus:

D-galactose, $[\alpha]_D$ + 70°, as galactitol, m.p. 185—187°, undepressed on admixture with an authentic sample;
D-mannose, $[\alpha]_D$ + 19°, as methyl *α*-D-mannopyranoside, m.p. and mixed m.p. 192—194°;
L-fucose, $[\alpha]_D$ −54°, as the 1-methyl-1-phenylhydrazone, m.p. and mixed m.p. 182—184°.

In addition the hydrolysate contained small amounts of glucose.

Methylation analysis. The polysaccharide (860 mg) was acetylated and methylated as described for the α-glucan to yield a product (570 mg) with $[\alpha]_D$ + 56° (*c*, 0.34 in chloro-

Table 4. Fractionation of hydrolysed methylated *P. giganteus* heteropolysaccharide.

Fract.	Weight (mg)	Components	R_G[a]	% of fraction
1	158	Gal	0.23	5
		3-Me-Gal [b]	0.36	15
		3,4-Me_2-Gal	0.55	80
2	13	3,4-Me_2-Gal	0.53	31
		2,3-Me_2-Gal	0.60	23
		2,3,4-Me_3-Gal	0.78	15
		2- or 4-Me-Fuc	0.67	31
3	195	2,3-Me_2-Gal	0.61	3
		2,3,4-Me_3-Gal	0.76	97
		2,4-Me_2-Fuc	0.76	
4	37	2,3,6-Me_3-Glu	0.87	91
		unknown	0.93	9
5	141	2,3,4,6-Me_4-Man	0.95	100
6	11	2,3,4,6-Me_4-Man	0.95	72
		2,3,4,6-Me_4-Glu	1.00	28

[a] Mobility in solvent A relative to 2,3,4,6-tetra-*O*-methyl-*d*-glucose.
[b] 3-*O*-Methyl-D-galactose, *etc.*

form) and OCH_3 39.6 %. The low methoxyl content (theroretical value 44 %) seemed to be only partly due to undermethylation. The methylated polysaccharide was hydrolysed and the resulting monomer mixture fractionated on a carbon-Celite column as described above. Mixed fractions were subjected to further fractionation on thick filter papers and the relative proportions of the components estimated by hypoiodite oxidation of aliquots. The results are summarised in Tables 1 and 4. The different ethers were characterised by demethylation with boron trichloride,[19] by paper chromatographic and electrophoretic comparison with authentic samples and, for the major components, by preparation of crystalline samples as follows:

3,4-Di-O-*methyl*-D-*galactose* crystallised from ethyl acetate and had m.p. 116—118° and $[\alpha]_D$ + 99 → 111° (*c*, 0.5). When a solution of the ether was seeded with 3,4-di-*O*-methyl-D-galactose of m.p. 164—166°,[20] kindly provided by Dr. D. J. Bell, it crystallised with m.p. and mixed m.p. 164—166°. It gave an aniline derivative with m.p. 147—149°,

2,3,4-Tri-O-*methyl*-D-*galactose* was obtained pure on refractionation of fraction 4 on a carbon-Celite column. It had $[\alpha]_D$ + 96° (*c*, 0.6) and gave an aniline derivative, m.p. 162—164°, undepressed on admixture with authentic 2,3,4-tri-*O*-methyl-D-galactosyl-*N*-phenylamine.

2,4-Di-O-*methyl*-L-*fucose*. This ether crystallised partly from a syrup containing also 2,3,4-tri-*O*-methyl-D-galactose. The syrup was dissolved in ethyl acetate and the crystals recrystallised twice from that solvent. No reference sample was available but the m.p. 135—136° and $[\alpha]_D$ −92° (*c*, 0.7) agree with values reported for 2,4-di-*O*-methyl-L-fucose.[21] The ether was not oxidised by periodate.

2,3,4,6-Tetra-O-*methyl*-D-*mannose* had $[\alpha]_D$ + 7° (*c*, 2.0). It was treated with a slight excess of aniline in ethanol at room temperature for 2 days. The product was recrystallised from light petrol to give needles which rearranged around 140° and melted at 156—158°. When the ether was refluxed with a large excess of aniline in ethanol, the major part of the product had after one recrystallisation m.p. 146—148° undepressed on admixture with a specimen of 2,3,4,6-tetra-*O*-methyl-D-mannosyl-*N*-phenylamine prepared in a similar manner and recrystallised several times to a sharp melting point of 146—147°. The minor part rearranged around 140° and melted gradually between 149 and 155°. 2,3,4,6-Tetra-*O*-methyl-D-mannose thus appears to give two isomeric aniline derivatives.

Periodate oxidation. The polysaccharide was oxidised as described for the *a*-glucan. Periodate consumption was 1.34 moles and formic acid liberation 0.57 mole per anhydrohexose residue.

Partial hydrolysis. The polysaccharide (890 mg) was given successive hydrolytic treatments at 100° for 1 h, first three times in 0.05 M hydrochloric acid, then twice in 0.10 M hydrochloric acid. The hydrolysis mixture was neutralised (Dowex 3) after each treatment and the part to be hydrolysed further precipitated with ethanol. The first two treatments released almost exclusively mannose, fucose and a mannosyl fucose, the latter also galactose and galactose-containing oligosaccharides. The residue (216 mg) gave a viscous solution that required too high dilution for an accurate determination of its low optical rotation. It was hydrolysed with 0.25 M sulphuric acid at 100° for 1 h to give further amounts of the last-named products. The hydrolysates were fractionated by carbon column and thick filter paper chromatography. D-Galactose, D-mannose and L-fucose were characterised as described above.

3-O-D-*Mannopyranosyl*-L-*fucose* (100 mg) had $[\alpha]_D \pm 0°$ (*c*, 1.4) and gave equimolecular amounts of mannose and fucose on hydrolysis. Its M_G-value on paper electrophoresis at 40° and 10 V/cm in 0.1 M sulphonated phenylboronic acid of pH 6.6 was 0.9 as compared with 0.4, 1.1, and 1.9 for L-fucose, D-galactose, and 3-*O*-methyl-D-galactose, respectively. The disaccharide was readily soluble in methanol, poorly so in ethanol. The alditol and its octaacetate were prepared but failed to crystallise.

The galactose containing oligosaccharides formed an homologous series and were obtained in yields of 32, 20, 6, and 4 mg, respectively. Their properties are summarised in Table 2. The first member of the series 6-O-*β*-D-*galactopyranosyl*-D-*galactose* crystallised from methanol on seeding with an authentic specimen. The crystals gave off solvent of crystallisation above 80°, giving a tough glass in a manner similar to that of the authentic specimen.

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