

## A Mannan Elaborated by a *Scarcina* species

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A species of *Scarcina* (either *flava* or *aurantica*) synthesises an extracellular mannan, the methylated derivative of which gave on hydrolysis 3,4-di-, 2,3,4-tri-, 2,4,6-tri-, 3,4,6-tri- and 2,3,4,6-tetra-*O*-methyl-*D*-mannose in the approximate relative proportions 3:1:1:3. As products of partial hydrolysis the following oligosaccharides were isolated and characterised: 2-*O*- $\alpha$ -*D*-mannopyranosyl-*D*-mannose, 3-*O*- $\alpha$ -*D*-mannopyranosyl-*D*-mannose, 6-*O*- $\alpha$ -*D*-mannopyranosyl-*D*-mannose, *O*- $\alpha$ -*D*-mannopyranosyl-(1 $\rightarrow$ 6)-*O*- $\alpha$ -*D*-mannopyranosyl-(1 $\rightarrow$ 6)-*D*-mannose and *O*- $\alpha$ -*D*-mannopyranosyl-(1 $\rightarrow$ 2)-*O*- $\alpha$ -*D*-mannopyranosyl-(1 $\rightarrow$ 6)- $\alpha$ -*D*-mannose. The significance of these findings is discussed.

A coccoid, gram-negative bacterium, identified as *Scarcina flava* or *aurantica*, was found to be responsible for the extensive slime-growth in a paper mill. When grown on a synthetic medium with potassium acetate as carbon source<sup>1</sup> it synthesised an extracellular mannan. This was freed from accompanying acidic non-carbohydrate material by chromatography on a diethylaminoethyl (DEAE) cellulose column. Small amounts of material giving glucose and rhamnose on hydrolysis were separated by precipitating the mannan, as its borate complex, with cetyltrimethylammonium hydroxide.<sup>2</sup> The presence (in this non-precipitable fraction) of a glucan and a rhamnoglucon was indicated (see Experimental).

The mannan had  $[\alpha]_D + 76^\circ$ , changing to  $+ 9^\circ$  on acid hydrolysis, indicating the presence of  $\alpha$ -glycosidic linkages. It contained 1.11 % N and its IR spectrum indicated the presence of protein. On hydrolysis only 81 % of reducing sugars (mannose only) were formed, as estimated by hypiodite oxidation. The mannan was electrophoretically homogeneous in borate buffer of pH 10 and had  $M_G$  0.68. On columns of Sephadex G-100 or G-200 it travelled as a broad band, but examination of the head and tail fractions (optical rotations and electrophoretic mobilities) did not reveal any significant differences. Furthermore, the mannan could not be subfractionated on DEAE-cellulose using gradient elution with borate buffer. From this the mannan appears to be essentially homogeneous but to have a broad molecular weight distribution.

Hydrolysis of the fully methylated mannan afforded a mixture of the methyl ethers listed in Table 1. Their separation was effected by a combination

Table 1. Methyl ethers from the hydrolysate of the methylated mannan.

Methyl ether	Molar ratio	Chromatographic mobility (solvent A) **	Retention time (GLC) ** column a column b		$R_F$ *** (TLC)
3,4-Me <sub>2</sub> -Man *	1.00	0.76	—	1.84	—
2,3,4-Me <sub>3</sub> -Man	0.30	0.90	2.32	1.34	0.45
2,4,6-Me <sub>3</sub> -Man	0.39	0.90	2.72	1.56	0.62
3,4,6-Me <sub>3</sub> -Man	0.27	0.90	2.32	1.34	0.54
2,3,4,6-Me <sub>4</sub> -Man	1.00	1	1	1	—

\* 3,4-Di-*O*-methyl-D-mannose, etc.

\*\* Relative to 2,3,4,6-tetra-*O*-methyl-D-mannose.

\*\*\* Kieselgel G, butanone.

Table 2. Oligosaccharides from partially hydrolysed mannan (3 g).

Oligosaccharide	Yield mg	$[\alpha]_D$	$R_{Man}$ Solv A Solv B		$M_G$ (Germanate)
I Man(1→6)Man	137	+ 62°	0.43	0.58	1.54
II Man(1→3)Man	67	+ 46°	0.52	0.52	1.18
III Man(1→2)Man	35	+ 36°	0.52	0.52	0.46
IV Man(1→2)Man(1→6)Man	87	+ 66°	0.22	0.29	1.22
V Man(1→6)Man(1→6)Man	30	+ 67°	0.19	0.29	1.52

of carbon column, thick filter paper and thin layer chromatography, and paper electrophoresis in borate buffer. Their relative proportions were estimated by a combination of quantitative paper chromatography, gas liquid chromatography and paper electrophoresis. Owing to the complex separation procedure, the relative proportions of the three trimethyl ethers are somewhat uncertain.

Stepwise partial hydrolysis of the mannan was undertaken with intermediate isolation of mannose and lower oligosaccharides (up to the tetramers) by chromatography on Sephadex G-25. The mixture was fractionated by chromatography on Sephadex G-25 and thick filter paper and by paper electrophoresis in germanate buffer.<sup>3</sup> The oligosaccharides and some of their properties are listed in Table 2. The disaccharides were characterised by the melting point of either the sugars or suitable derivatives. No crystalline derivatives of the trisaccharides could be prepared. Their structures were determined by their chromatographic and electrophoretic behaviour, optical rotations and from the results of partial hydrolysis, periodate oxidation and micromethylation studies.

The polysaccharide consumed 1.35 moles of periodate per mannose residue with the liberation of 0.45 moles of formic acid. These values, which are corrected for 20 % non-carbohydrate content in the polysaccharide, are in good agreement with the values 1.31 and 0.44, respectively, estimated from the results of the methylation analysis. Similarly, the production of glycerol

and mannose in the molar ratio 9:1 on application of the Smith degradation procedure<sup>4</sup> is in agreement with the estimated ratio of 8:1.

The mannan produced by the *Scarcina* species has a complex structure and contains  $\alpha$ -(1 $\rightarrow$ 2),  $\alpha$ -(1 $\rightarrow$ 3) and  $\alpha$ -(1 $\rightarrow$ 6) mannosidic linkages. The isolation from the hydrolysate of the methylated polysaccharide of large and equimolar amounts of 2,3,4,6-tetra-*O*-methyl-D-mannose and 3,4-di-*O*-methyl-D-mannose shows that the mannan contains a high proportion of branching points, in all of which a mannose residue is substituted in the 2- and 6-positions. The presence of the three tri-*O*-methyl-D-mannoses shows that mannose residues, only substituted in the 2-, 3- or 6-position, are also present.

Unfortunately, no definite conclusions can be drawn as to the location of the three different types of linkages in the polysaccharide. It is significant, however, that no tetrasaccharide was detected that had an electrophoretic mobility in germanate buffer as high as that of the  $\alpha$ -(1 $\rightarrow$ 6) linked disaccharide and trisaccharide; this suggests that the tetrasaccharide of that homologous series was absent. As the (1 $\rightarrow$ 6)-mannosidic linkage was found to be more resistant to acid hydrolysis than the (1 $\rightarrow$ 2) and (1 $\rightarrow$ 3) linkages, it seems that the main chain of the polysaccharide does not contain exclusively  $\alpha$ -(1 $\rightarrow$ 6) linkages. It was also found that the production of the  $\alpha$ -(1 $\rightarrow$ 2) and  $\alpha$ -(1 $\rightarrow$ 3) linked disaccharides occurred mainly in the early stages of the partial hydrolysis, suggesting that a large proportion of the non-(1 $\rightarrow$ 6)-linkages are situated in side-chains. The repeating unit given in Fig. 1 accommodates the observed structural details but is of course only one of several possible alternatives.

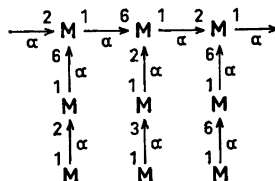


Fig. 1. Hypothetical repeating unit of the mannan.

The mannan from the *Scarcina* species appears to be similar to the mannans from the yeasts *Saccharomyces cerevisiae*<sup>5,6</sup> and *S. rouxii*<sup>7</sup> and from *Bacillus polymyxa*<sup>8</sup> in that it contains  $\alpha$ -(1 $\rightarrow$ 2),  $\alpha$ -(1 $\rightarrow$ 3) and  $\alpha$ -(1 $\rightarrow$ 6) linked mannose residues and a high proportion of branching points involving the 2- and 6-positions. It yields three tri-*O*-methyl-D-mannoses in methylation analysis; this has previously been reported only for the mannan from baker's yeast.<sup>5,6</sup>

The present investigation shows, however, that the simultaneous existence of these ethers might be overlooked unless a combination of several fractionation methods is employed.

#### EXPERIMENTAL

Paper chromatograms were run on Whatman No. 1 and (for preparative purposes) Munktell No. 333 filter paper, in the following systems:

- A. Butanol, ethanol, water, 10:3:5
- B. Ethyl acetate, acetic acid, water, 3:1:1
- C. Ethyl acetate, pyridine, water, 8:2:1

D. Ethyl acetate, pyridine, water, 2:1:2 (upper phase)

E. Water-saturated butanone

Paper electrophoresis was carried out on Whatman No. 3 filter paper and on glass fibre sheets manufactured by Grycksbo Pappersbruk AB in the following buffer systems: 0.1 M borate buffer of pH 10, molybdate buffer<sup>9</sup> of pH 5.0, 0.05 M germanate buffer<sup>3</sup> of pH 10.7.

Thin layer chromatography was carried out on Kieselgel G. Spray reagents used were anisidine hydrochloride, silver nitrate-sodium ethoxide, 2,3,5-triphenyltetrazolium chloride and  $\alpha$ -naphthol-sulphuric acid.

Gas-liquid chromatography<sup>10</sup> was carried out on 180  $\times$  0.65 cm columns in a Perkin-Elmer Model 800 Gas Chromatograph using (a) 15% w/w of butane-1,4-diol succinate on Chromosorb W at 175° and (b) 15% w/w of polyphenyl ether (OS138) on Chromosorb W at 200°.

Optical rotations were measured at 20° in water unless otherwise stated.

*Cultivation of the organism.* A medium of the following composition was used (g/l): D-Glucuronic acid 58, KOH 16.5, KNO<sub>3</sub> 10, KH<sub>2</sub>PO<sub>4</sub> 6.8, MgSO<sub>4</sub> · 7H<sub>2</sub>O 0.123, MnSO<sub>4</sub> · 4H<sub>2</sub>O 0.002, K<sub>2</sub>SO<sub>4</sub> 0.174, Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> 0.020, ZnSO<sub>4</sub> · 7H<sub>2</sub>O 0.014, CaCl<sub>2</sub> · 4H<sub>2</sub>O 0.183, yeast extract 4. The pH was adjusted to 7. The cultivation was done at 37° in 2 l conical flasks containing 500 ml of culture medium. The time allowed for growth was 10 days and the average yield of crude polysaccharide was 0.8 g per litre.

*Isolation of polysaccharides.* The culture solution (6 l) was poured into ethanol (20 l). The precipitate formed was allowed to settle and was collected by centrifugation. It was then extracted with water (dissolution was aided by a mild ultrasonic treatment of the mixture) and insoluble material, consisting mainly of cells, was removed by centrifugation. The centrifugate was dialysed against running tap-water and the polysaccharides were recovered by precipitation with ethanol.

The crude polysaccharide was added to the top of a DEAE-cellulose column (60  $\times$  10 cm). Elution, first with water, then with 0.1 M and 1 M potassium acetate solution gave fractions A (1.53 g), B (3.08 g), and C (1.22 g), respectively. On acid hydrolysis both fractions A and B yielded mannose as the main sugar, together with small amounts of glucose and rhamnose. Electrophoresis in borate buffer on glass fibre sheets showed for both one major component ( $M_G$  0.68) and one minor ( $M_G$  0.2–0.3). Fraction A had  $[\alpha]_D + 70^\circ$ , fraction B  $[\alpha]_D + 62^\circ$ . Fraction C was of non-carbohydrate nature.

Fractions A and B were combined and dissolved in 0.06 M boric acid (400 ml). Slow addition of 0.2 M CTA-OH (30 ml) gave a colloidal precipitate which was coagulated with M sodium hydroxide (1 ml). The precipitate was collected by centrifugation, washed with water and dissolved in dilute acetic acid. Precipitation with ethanol yielded fraction I (3.3 g).

The CTA-borate solution was deionised and the polysaccharide II (440 mg) was recovered by precipitation with ethanol.

Fraction I had  $[\alpha]_D + 76^\circ$  (c, 0.4) and gave only mannose on hydrolysis. It was electrophoretically homogeneous in borate buffer ( $M_G$  0.68). Nitrogen content (1.11%) and IR spectrum indicated the presence of protein. It could not be subfractionated by chromatography on Sephadex G-100 or G-200 or by chromatography on DEAE-cellulose using gradient elution with borate solution.

Fraction II gave mannose, glucose and rhamnose on hydrolysis. Refractionation with CTA-OH–boric acid gave an essentially mannose-free fraction with  $[\alpha]_D + 130^\circ$ . Preparative electrophoresis on glass fibre sheets gave as major component a glucan ( $M_G$  0.15). The minor component ( $M_G$  0.30) gave on hydrolysis glucose and rhamnose in the approximate proportion 2:1. The quantity obtained was insufficient for structural studies.

*Methylation analysis.* The polysaccharide (2.0 g) was acetylated and then exhaustively methylated as described for the  $\alpha$ -glucan from *Pullularia pullulans*.<sup>1</sup> The final product (950 mg) had  $[\alpha]_D + 86^\circ$  (c, 0.9, in chloroform) and 42.6% methoxyl content. Unmethylated and monomethylated monomers were absent in a hydrolysed sample, indicating the low methoxyl content to be owing to non-carbohydrate impurities rather than to incomplete methylation.

The methylated mannan (730 mg) was hydrolysed by treatment with 90% formic acid (50 ml) at 100° for 45 min, followed by 0.25 M sulphuric acid (75 ml) at 100° for 18 h. After neutralisation with barium carbonate and deionisation, the hydrolysate was

Table 3. Carbon column fractionation of the methyl ethers from the methylated mannan (730 mg).

Fraction	Weight, mg *	Components
1	23.2	3,4-Me <sub>2</sub> -Man
2	120.6	3,4-Me <sub>2</sub> -Man
3	51.4	3,4-Me <sub>2</sub> -Man
4	86.0	2,4,6-Me <sub>3</sub> -Man 2,3,4-Me <sub>3</sub> -Man
5	26.0	2,3,4-Me <sub>3</sub> -Man
6	105.4	3,4,6-Me <sub>3</sub> -Man
7	38.0	3,4,6-Me <sub>3</sub> -Man
8	263.0	2,3,4,6-Me <sub>4</sub> -Man 2,3,4,6-Me <sub>4</sub> -Man

\* The weights are not representative for the actual composition of the mixture, as some of the fractions, especially the later, also contained non-carbohydrate material.

fractionated on a carbon-Celite column, irrigated with a gradient of 5–35 % aqueous ethanol. The results are given in Table 3. Fractions containing ethers of different degree of methylation were further fractionated by chromatography on thick filter paper (solvent A). The 2,3,4- and 3,4,6-tri-*O*-methyl-*D*-mannoses were separated by preparative thin layer chromatography using butanone as solvent and a minimum exposure to iodine vapour for detection. The bands were scraped off the plates and the sugars recovered by extraction with ethanol. Some properties of the ethers are summarised in Table 1. They were characterised as follows:

*3,4-Di-O-methyl-D-mannose* crystallised from ethyl acetate. M.p. and mixed m.p. 70–72°,  $[\alpha]_D + 33^\circ$  (c, 0.13). It had  $M_G$  0.53 in borate buffer.

*3,4,6-Tri-O-methyl-D-mannose* crystallised on seeding and was recrystallised from ethyl ether-light petroleum. M.p. and mixed m.p. 102–104°,  $[\alpha]_D + 9.5^\circ$  (c, 0.6). It had  $M_G$  0.32 in borate buffer.

*2,4,6-Tri-O-methyl-D-mannose* crystallised from isopropyl ether. M.p. 62°,  $[\alpha]_D + 16.5^\circ$ . The crystals contained solvent of crystallisation. A sample was converted to the amide which had m.p. 143°. The values reported for the 2,4,6- and 2,3,4-tri-*O*-methyl-*D*-mannonoamides<sup>5,12</sup> are similar. The m.p. was, however, depressed on admixture with the latter amide and the IR-spectrum was also different from that given by the 2,3,4-tri-*O*-methyl-*D*-mannonoamide.

*2,3,4-Tri-O-methyl-D-mannose* was indistinguishable from an authentic sample by paper, thin layer and gas-liquid chromatography. On reduction with sodium hydridoborate and subsequent periodate oxidation a sugar was obtained which was chromatographically indistinguishable from 2,3,4-tri-*O*-methyl-*L*-arabinose.

*2,3,4,6-Tetra-O-methyl-D-mannose* had  $[\alpha]_D + 31^\circ$  (c, 2.4 in methanol). It was indistinguishable from an authentic sample by paper, thin layer and gas-liquid chromatography. It was converted to the aniline derivative with m.p. and mixed m.p. 143–144°.

*Quantitative analysis of the methylated mannan.* A sample was hydrolysed and fractionated on thick filter paper (solvent A). The di-, tri-, and tetra-methyl ethers were eluted and their molar ratios determined by hypiodite oxidation to be 1.0:0.96:1.0.

A sample was hydrolysed and the tri-*O*-methyl fraction isolated as above. Part of it was converted to the methyl glycosides and fractionated by gas-liquid chromatography (column b) to give the ratio 2,3,4- plus 3,4,6-tri-*O*-methyl-*D*-mannose to 2,4,6-tri-*O*-methyl-*D*-mannose as 1.48:1.00. Another sample was fractionated by paper electrophoresis in borate buffer and the fraction with  $M_G = 0$  was recovered and analysed as above. The ratio of the electrophoretically immobile 2,3,4- and 2,4,6-tri-*O*-methyl-*D*-mannoses was found to be 0.77:1.00. The molar ratios of 2,3,4-, 3,4,6-, and 2,4,6-tri-*O*-methyl-*D*-mannoses were thus calculated to be 0.77:1.0:0.71.

*Partial hydrolysis of the mannan.* A sample (3.0 g) of the mannan was hydrolysed by treatment with 90 % formic acid (100 ml) at 100° for 2 h. The solution was concentrated to 20 ml, water (100 ml) was added and the heating continued for 1 h. After concentration the product was fractionated on a Sephadex G-25 column (60 × 5 cm). The lower oligosaccharides (DP ≤ 4) were collected and the higher rehydrolysed by the same technique in 5 stages for a total of 12 h with removal of the lower fragments after each stage. The hydrolysates were combined and fractionated on a column of Sephadex G-25 (110 × 3.7 cm) into mono-, di-, tri-, and tetrasaccharide fractions.

*The disaccharide fraction* on chromatographic and electrophoretic examination revealed the presence of three compounds (I–III). The mixture could not be fractionated by carbon column chromatography. Repeated preparative paper chromatography (solvent A) afforded component I ( $R_{\text{Man}} 0.43$ ) separated from II and III ( $R_{\text{Man}} 0.52$ ). The latter mixture was separated by preparative paper electrophoresis in germanate buffer (1.5 kV, 2 h, 40°). The bands were eluted with water and the eluates were immediately treated with excess Dowex 50W (H<sup>+</sup>). After removal of the resin the solutions were concentrated and filtered to remove insoluble material. The disaccharides were finally purified from inorganic material by chromatography on thick filter paper. The yields and some properties of the disaccharides are given in Table 2.

*6-O- $\alpha$ -D-Mannopyranosyl-D-mannose*<sup>13</sup> (I). The disaccharide crystallised on seeding with an authentic sample. After recrystallisation from ethanol it had m.p. and mixed m.p. 187.5° and  $[\alpha]_{\text{D}} + 61.5^{\circ}$  (c, 1.0).

*3-O- $\alpha$ -D-Mannopyranosyl-D-mannose* (II). The disaccharide,  $[\alpha]_{\text{D}} + 46^{\circ}$  (c, 0.62), gave only mannose on hydrolysis. On treatment with 0.02 M calcium hydroxide for 24 h at room temperature it was extensively degraded at a rate comparable to that for laminaribiose. Reduction of the disaccharide with sodium hydridoborate gave an alditol which was immobile on electrophoresis in molybdate buffer. The alditol gave a crystalline acetate, which was recrystallised from isopropyl ether. It melted at 115–116° and had  $[\alpha]_{\text{D}} + 57^{\circ}$  in good agreement with the values reported for 3-O- $\alpha$ -D-mannopyranosyl-D-mannitol.<sup>13</sup> The rapid alkaline degradation of the disaccharide and its high  $M_{\text{G}}$ -value on electrophoresis in germanate buffer shows that it is (1→3)- and not (1→4)-linked.

*2-O- $\alpha$ -D-Mannopyranosyl-D-mannose*<sup>7</sup> (III). The disaccharide was obtained as a syrup and gave only mannose on hydrolysis. It gave no coloration with 2,3,5-triphenyltetrazolium chloride, indicating the presence of a (1→2)-linkage. The alditol had  $M_{\text{Mannitol}} 0.86$  on electrophoresis in molybdate buffer. The alditol crystallised on seeding with an authentic sample and, after recrystallisation from methanol-ethanol, melted at 137.5–138° (Ref. 7: 136–137°).

*The trisaccharide fraction.* Chromatographic and electrophoretic examination indicated the presence of at least four components. By a combination of chromatography on thick filter paper (solvent A) and preparative electrophoresis in germanate buffer the two main components were isolated. Their properties are given in Table 2.

*O- $\alpha$ -D-Mannopyranosyl-(1→2)-O- $\alpha$ -D-mannopyranosyl-(1→6)-D-mannose* (IV). The trisaccharide was obtained as a syrup with  $[\alpha]_{\text{D}} + 66^{\circ}$  (c, 0.3) and gave only mannose on hydrolysis. It gave a positive reaction with 2,3,5-triphenyltetrazolium chloride, indicating that the 2-position of the reducing endgroup was unsubstituted. The derived alditol was homogeneous on electrophoresis in molybdate buffer with  $M_{\text{Mannitol}} 0.56$ . Partial hydrolysis of the trisaccharide with 1 % oxalic acid at 100° for 2 h yielded two disaccharides which were chromatographically indistinguishable from 6-O- $\alpha$ -D-mannopyranosyl-D-mannose and 2-(or 3-)-O- $\alpha$ -D-mannopyranosyl-D-mannose. Partial hydrolysis of the derived alditol gave only the (1→2)- or (1→3)-linked disaccharide.

The trisaccharide was methylated with iodomethane and silver oxide in dimethylformamide and the methanolysate of the product was examined by gas-liquid chromatography, using columns a and b. Components with retention times similar to those of 2,3,4,6-tetra-O-methyl-D-mannose and 2,3,4- and/or 3,4,6-tri-O-methyl-D-mannose were detected. Examination of the hydrolysate of the methylated trisaccharide by paper electrophoresis in borate buffer and by thin layer chromatography showed the presence of the same three methylated mannoses.

On periodate oxidation the derived alditol consumed 6.5 moles of periodate (estimated spectrophotometrically<sup>14</sup>) with the liberation of 3.8 moles of formic acid (estimated by iodometric titration) and 0.93 moles of formaldehyde (estimated with chromotropic acid). The calculated values are 7, 4, and 1 moles, respectively.

O- $\alpha$ -Mannopyranosyl-(1 $\rightarrow$ 6)-O- $\alpha$ -D-mannopyranosyl-(1 $\rightarrow$ 6)-D-mannose (V). The trisaccharide gave only mannose on hydrolysis and gave a positive reaction with the tetrazolium reagent. The derived alditol on electrophoresis in molybdate gave only one spot with  $M_{\text{Mannitol}}$  0.56. Partial hydrolysis of either the trisaccharide or the alditol gave only one disaccharide, chromatographically indistinguishable from 6-O- $\alpha$ -D-mannopyranosyl-D-mannose. A sample of the trisaccharide was methylated and investigated as above, when the presence of 2,3,4-tri- and 2,3,4,6-tetra-O-methyl-D-mannose was demonstrated by gas-liquid and thin layer chromatography.

*The tetrasaccharide fraction.* This fraction was examined by paper electrophoresis in germanate buffer. In the complicated mixture there was virtually no component present with the high  $M_G$ -value (about 1.5) expected for a tetrasaccharide with three contiguous (1 $\rightarrow$ 6)-linkages.

*Periodate oxidation of the mannan.* The periodate consumption, 1.35 moles per hexose residue, was estimated by the arsenite method and the formic acid liberated (0.45 moles) by iodometric titration. (Values were obtained by extrapolation to zero time). Correction was made for the true mannan content as determined by hypiodite oxidation of a hydrolysate.

A sample of the mannan was oxidised at room temperature (for 8 days) with an excess of 0.05 M sodium metaperiodate. Acetic acid and excess potassium iodate were added and the liberated iodine extracted with chloroform. The resulting polyaldehyde was then precipitated with ethanol, redissolved in water, reduced with sodium hydridoborate and hydrolysed.<sup>4</sup> Chromatographic examination of the hydrolysate revealed the presence of glycerol and mannose; erythritol was absent. Estimation by the periodate-chromotropic acid and Somogyi methods respectively gave the molar ratio of glycerol to mannose as 9:1.

*Acknowledgement.* The authors are indebted to Fil. kand. Rolf Eriksson for indentifying the organism, to Dr. C. T. Bishop, Professor J. K. N. Jones, and Dr. A. S. Perlin for several reference samples, and to *Cellulosaindustriens stiftelse för teknisk och skoglig forskning samt utbildning* for financial support.

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Received February 8, 1965.