SOLUBLE POLYSACCHARIDES OF Rhodotorula flava

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One of us has shown previously [1] that some yellow forms of yeast organisms of the genus Rhodotorula such as Rh. gelatinosa, Rh. luteola, and others contain in their cells and liberate into the nutrient medium heteropolysaccharides of similar composition and structure – xyloglucuronomannans.

The cellular and extracellular heteropolymers of Rh. flava differ from the polysaccharides of other yellow species of Rhodotorula by the absence of the uronic component. The present paper gives the results of a study of the structure of the xylomannans of Rh. flava.

In an investigation of the composition of the polysaccharide preparations obtained from the cells of Rh. flava and from a filtrate of the culture liquid after the growth of the microorganism in beakers, we found that they were similar with respect to their contents of mannose (30-36%) and xylose (20%), but differed substantially with respect to their glucose content [2, 3].

The polysaccharides isolated from them with the aid of Fehling's reagent consisted only of D-mannose and D-xylose (according to paper chromatography and the characteristic indices for the phenylhydrazones of D-mannose and D-xylose) in a ratio of 3:2. They did not contain nitrogen and phosphorus, and each gave a single band on electrophoresis in phosphate buffer (pH 3). In each case, gel chromatography on Sephadex G-200 in phosphate buffer (pH 6.6) gave a single symmetrical peak with $V_e = 21$ and 24 ml for the extracellular and cellular polysaccharides, respectively. The chromatographic separation of dialyzates of these fractions after hydrolysis showed the presence of mannose and xylose in them (Table 1). The oxidation of the xylomannans in 0.05 M $NaIO_4$ solution was complete after 48 h. The two polymers absorbed approximately equal amounts of $NaIO_4$ and liberated approximately equal amounts of HCOOH (moles per mole of anhydroaldose with mean mol. wt. 147; see Table 1). A chromatographic investigation in system I of the hydrolyzates of the polyaldehydes showed the presence of mannose in them. As a result of the hydrolysis of the products of tetrahydroborate reduction of the oxidized xylomannans, mannose and glycerol were formed.

Thus, in the xylomannans 50-55% of the sugar units are attacked by periodate, and 45-50% of the residues are resistant to oxidation. The intact units consist of mannose.

On Smith degradation and subsequent mild hydrolysis [4], the cellular xylomannose of Rh. flava yielded a mannan with $[\alpha]_D^{+20}$ +45.5° (0.5%; H_2O), which, after hydrolysis, gave only mannose, absorbed 0.20 and 0.21 mole of sodium periodate per mole of anhydromannose and did not liberate formic acid after oxidation for 24 h and 48 h, respectively. Similar results were obtained in a study of the products of the dehydration of the extracellular xylomannan.

Figure 1 shows the distribution of the spots of the methyl derivatives in hydrolyzates of the methylated xylomannan and the mannan in comparison with tetramethylglucose on FN1 chromatographic paper in system 1. When the chromatogram was treated with p-anisidine hydrochloride the hydrolyzate of the methylated mannan (II) showed an intense spot b' at the level of the trimethyl derivative of mannose, a weak spot *a'* corresponding to 2,3,4,6-tetra-O-methyl-D-mannose, and a weak spot d' of a dimethylated mannose giving a coloration with dimethylaniline. The homogeneity of the trimethylmannose fraction was shown by gas-liquid chromatography. The peak obtained was identical with that of a standard sample of methyl 2,4,6-tri-O-methylmannoside [3].

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TABLE 1. Characteristics of the Xylomannans

Xylomannan	$[\alpha]_D^{20}$ (0.25%, H_2O), deg	Reducible substances after hydrolysis (according to Hagedorn – Jensen), %	Comp. of the monosaccharides, %		Periodate oxidation			
			mannose	xylose	HCOOH	$NaIO_4$	units attacked by the periodate	units resistant to oxidation
					moles/mole of aldose residue		%	
Cellular	+20,7	98,0	60,0	40,0	0,55	1,05	55	45
Extracellular	+18,0	95,0	60,0	40,0	0,50	0,99	50	50

TABLE 2

Oligosaccharide	$R_{lactose}$ in system 2	Hydrolysis products of the oligosaccharide	Oligosaccharide	$R_{lactose}$ in system 3	Hydrolysis products of the oligosaccharide
K_1	1,79	Xylose, mannose	M_1	2,61	Xylose, mannose (traces)
K_2	1,69	Xylose	M_2	1,99	
K_3	1,50		M_3	1,62	
K_4	1,32		M_4	1,24	Mannose
K_5	0,88		M_5	0,50	

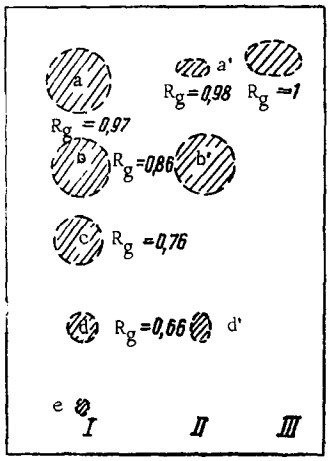


Fig. 1. Chromatogram of hydrolyzates of methylated xylomannan and of mannan: I) hydrolyzate of methylated xylomannan; II) hydrolyzate of methylated mannan; III) tetramethylglucose.

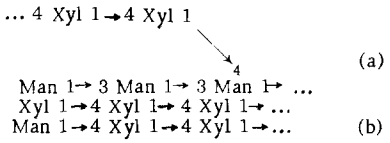
The results of the chromatographic analysis of the hydrolyzates of the methylated xylomannan (I) showed the presence of four main spots, of which the most intense was spot *a*. On a number of slow types of chromatographic paper, its heterogeneity was clearly seen, and it may be assumed that it contains completely methylated mannose and xylose. Spots b and d correspond to the position of the methyl derivatives b' and d' of the methylated mannan. Spot c, from its position and color, may be ascribed to a dimethylxylose [5]. A gas-liquid chromatogram of a methanolyzate of the methylated xylomannan showed the presence of methyl glycosides of the following sugar derivatives: 2,3,4,6-tetra-O-methylmannose, 2,3,4-tri-O-methylxylose, 2,3-di-O-methylxylose, and 2,4,6-tri-O-methylmannose. No dimethyl derivative of mannose was isolated under these conditions. However, taking into account the existing set of methyl derivatives and the information obtained about spot d with the aid of paper chromatography, it may apparently be assumed that it is due to 2,6-di-O-methylmannose. The weak spot e on a chromatogram of a hydrolyzate of the methylated xylomannan in the region of monomethyl derivatives may be the result of a possible incomplete methylation.

In preliminary experiments on the partial hydrolysis of the xylomannans (0.1 N H_2SO_4, 100°C) we observed that after only 15 minutes boiling, a considerable amount of xylose began to be split out. An appreciable amount of mannose appeared on the chromatograms 2 h after the beginning of hydrolysis. Table 2 gives the results of a study of the two series of oligosaccharides obtained by the three-hour hydrolysis of the xylomannan from the cells of Rh. flava in 1 N H_2SO_4 at 80°C (series K) and by further hydrolysis in 1 N H_2SO_4 at 100°C for 2 h (series M).

On three-hour hydrolysis at 80°C, the chromatograms showed the presence of a large amount of xylose, traces of mannose, and the oligosaccharides K_1-K_5. The complete hydrolysis of the majority of these fragments gave only xylose. The further hydrolysis of the partially hydrolyzed polymer at 100°C led to the isolation of – in addition to considerable amounts of mannose and xylose – the oligosaccharides M_1-M_5, of which only M_4 and M_5 consisted solely of mannose.

The IR spectra of the cellular and extracellular xylomannans had a similar form: strong absorption bands at 715 and 770 cm^{-1}, extremely weak bands at 815, 880, and 915 cm^{-1}, and bands of medium intensity at 890 and 935 cm^{-1} which were more sharply defined in the spectra of the extracellular xylomannan.

Thus, on the basis of the experimental results obtained it is possible to put forward some basic features of the structure of the polysaccharides studied. The xylomannan from Rh. flava is a branched polymer, which is confirmed by an analysis of the hydrolyzates and methanolyzates of the methylated polymer. A study of dehydrated xylomannan shows that its basic link consists of D-mannose residues with an α-1→3 linkage. According to the results of partial hydrolysis, the side chains of the substance apparently consist of fairly long segments composed of xylose units. The majority of them are terminated by xylose residues. However, as a gas-liquid chromatography of the methylated product and partial hydrolysis showed (the disaccharide K_1, see Table 2), in some side chains the terminal units may possibly be mannose. A representation of the structure of individual sections of the xylomannan of Rh. flava is given below:



where (a) is a fragment of the main chain with a branching point and b represents parts of the side chain.

EXPERIMENTAL

The polysaccharide preparations were obtained from Rh. flava (VKM* 331) by treating the dry defatted cells with 0.1 N HCl (1:20 w/v) in an autoclave at 0.5 atm for 30 min, and from a filtrate of the culture liquid after the growth of the microorganism on a synthetic nutrient medium containing 5% of glucose and a complex of vitamins of group B [6].

The xylomannans were isolated from the initial preparations in the form of the insoluble copper complex formed with Fehling's reagent [4], and were analyzed by methods described previously [7, 8].

Paper chromatography was performed in the following solvent systems: 1) butanol–ethanol–water–ammonia (40:10:49:1), 2) butanol–ethanol–water (40:11:19), and 3) ethyl acetate–acetic acid–water (9:2:2). Spots on the chromatograms were revealed with ammoniacal silver nitrate solution, aniline hydrogen phthalate, p-anisidine hydrochloride, and dimethylaniline.

Electrophoresis in a borate buffer and gel chromatography on Sephadex G-200 were performed as described previously [3]. A sample (0.15 g) of xylomannan was hydrolyzed in 1 N H_2SO_4 (4 ml) at 100°C for 4 h. The solution was neutralized with $BaCO_3$, filtered, and evaporated at 45°C. The evaporated solution was treated with 0.5 ml of water and a mixture of 0.1 ml of phenylhydrazine with 0.5 ml of 25% acetic acid. The resulting product was separated by filtration and was crystallized twice from methanol–ethanol. It proved to be identical with the phenylhydrazone of D-mannose. The filtrate was decolorized with activated carbon and concentrated, and the product was twice crystallized from ethanol. The product so obtained was identical with a standard sample of D-xylose.

The periodate oxidation of the xylomannans was performed in 0.05 M $NaIO_4$ solution at 20°C, and the products of oxidation and of borohydride reduction were studied by the methods described in a previous paper [8].

Methylation of the Xylomannan. The xylomannan (1 g) from the cells of Rh. flava was methylated five times by Haworth's method [9]. Beginning with the third addition of methylating agent to the reaction mixture, 20 ml of acetone was added each time to prevent the separation of the partially methylated product from it and foaming. This gave 1.1 g of partially methylated product, of which 0.5 g was then methylated by the method of Falconer and Adams [10] and, finally, by Purdie's method [11]. Yield 0.51 g (OCH_3 38%; the IR spectrum showed the presence of OH groups).

The methylated product (20 mg) was hydrolyzed in 72% H_2SO_4 (0.5 ml) with cooling for 1 h and then, after dilution of the mixture to a concentration of 8%, by boiling for 5 h [12]. The hydrolysis products were studied chromatographically in system 1. Part of the methylated xylomannan (100 mg) was subjected to methanolysis in a 4% solution of hydrogen chloride in methanol (5 ml) for 24 h. The methanolysis products were separated by the GLC method in a Tswett-1 chromatograph with a flame-ionization detector using a column (100×0.4 cm) containing 5% of neopentyl glycol succinate on Celite G-22 at 165°C with nitrogen as carrier gas (V=30/ml min).

*All-Union Collection of Microorganisms.

Preparation of Mannan from the Xylomannan. The xylomannan (1 g) was oxidized in a solution of $NaIO_4$ (2.9 g of $NaIO_4$ in 160 ml of H_2O) for 72 h. The solution was evaporated at 45°C to a volume of 30 ml. The oxidized polysaccharide was precipitated by the addition of 300 ml of glacial acetic acid. The product was filtered off, washed with acetic acid and acetone, and dried. The polyaldehyde was dissolved with trituration in water (30 ml) containing sodium tetrahydroborate (130 mg). After the mixture had stood for 3 h, the excess of the reagent was destroyed with acetic acid. The solution was treated with Amberlite IR-120 (H^+) and evaporated. The residue was evaporated with methanol three times and was then dissolved in water (15 ml). The polyalcohol was precipitated with 200 ml of ethanol. The resulting product (0.4 g) was hydrolyzed in 70 ml of water acidified to pH 2 with sulfuric acid at 100°C for 1 h. The hydrolyzate was neutralized with barium carbonate, deionized with MV-3 ion-exchange resin, and evaporated to 7 ml. Glycerol was detected in this solution by paper chromatography. The solution was treated with acetone (140 ml), which precipitated the mannan, and this was washed with ethanol and ether and was dried. The yield of the substance was 0.3 g. Gorin et al. [4] used a similar method to obtain a 1→3-bound mannan from the pentose-containing mannan of Trichosporon cutaneum. The mannan was characterized by subjecting it to periodate oxidation and was methylated in a similar way to the xylomannan.

Partial Hydrolysis. The xylomannan (200 mg) was hydrolyzed in 3.3 ml of 1 N H_2SO_4 in a sealed tube in a water bath with automatic temperature regulation at 80±1°C for 3 h. The solution, which still retained some viscosity, was diluted with water, neutralized with $BaCO_3$, filtered, and evaporated to its initial volume. Then 20 ml of ethanol was added to precipitate the polysaccharide, which was separated by centrifuging. The centrifugate was evaporated at 45°C and investigated chromatographically in system 2 (oligosaccharides of series K). The partially hydrolyzed polysaccharide (90 mg) was rehydrolyzed in 1 N H_2SO_4 (2 ml) at 100°C for 2 h. The neutralized hydrolyzate was evaporated and studied chromatographically in system 3 (oligosaccharides of series M). The positions of the oligosaccharides on the chromatograms were determined in relation to that of lactose. The oligosaccharides were accumulated by paper chromatography and were eluted with acetone–water (1:1), the extract being evaporated to dryness at 45°C. The substances obtained were hydrolyzed in 1 N H_2SO_4 at 100°C for 2 h. The neutralized hydrolyzates were studied in system 1.

SUMMARY

1. The cellular and extracellular xylomannans of the yeast organism Rh. flava are polymers of similar structure.

2. The xylomannan of Rh. flava is a branched polysaccharide, the main chain of which consists of D-mannose residues bound by α-1→3 linkages, and the side chains consist predominantly of xylose units.

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