Isolation and identification of 4-*O*-β-D-xylopyranosyl--L-arabinopyranose from the peach gum polysaccharide. I.

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Three neutral oligosaccharides were isolated from the hydrolyzate of peach gum polysaccharide. Their separation was accomplished on a cross-linked starch by elution with distilled water. We investigated only the disaccharide giving on hydrolysis D-xylose and L-arabinose and after reduction with sodium tetrahydroborate D-xylose and L-arabitol. 2,3,4-Tri-*O*-methyl--D-xylose and 2,3-di-*O*-methyl-L-arabinose were identified by paper chromatography in the hydrolyzate of the methylated disaccharide. Mass spectrometry showed that both D-xylose and L-arabinose were in pyranose form linked together by 1 → 4 glycosidic bond. On the basis of optical rotation and infrared spectroscopy the glycosidic bond was assumed to be β. Thus the studied disaccharide had the structure 4-*O*-β-D-xylopyranosyl-L-arabinopyranose.

An acidic polysaccharide with a backbone composed of D-galactose was prepared from the gum of peach trees (*Prunus persica* [L.] BATSCH.) [1, 2]. The exact composition of side chains however, has not been identified as yet but they are most probably formed by L-arabinose and D-xylose units.

On the basis of the published data it is assumed that the majority of arabinose units present in plant gum polysaccharides appears in the labile furanose form. *White* [3] proved the presence of L-arabinopyranose end units in the sapote gum polysaccharide and *Jones* [4] isolated 3-*O*-β-L-arabinopyranosyl-L-arabinose from ε galactan of red spruce. This disaccharide was also isolated by *Andrews* and co-workers [5] from peach gum polysaccharide.

Experimental

Paper chromatography was performed on Whatman No 1 paper in the systems S_1: ethyl acetate—pyridine—water (8 : 2 : 1) and S_2: *n*-butanol—ethanol—water (4 : 1 : 5). Saccharides were detected by anilinium hydrogen phthalate [6] and alkali silver nitrate [7].

Optical rotation was measured on a Bendix—Ericsson 143 A polarimeter. Infrared spectra were taken on a Zeiss UR-10 spectrometer using KBr pellets. Mass spectrum of the permethylated disaccharide was obtained with an MCh 1306 mass spectrometer at an ionizing potential of 70 eV. The temperature in the inlet system was 30°C and that of the ionizing chamber 120°C.

Preparation of disaccharide

The peach gum (900 g) was dissolved in distilled water (25 l) and hydrolyzed in a reaction kettle at 100°C for 36 hours. Then the polysaccharide was precipitated with acidified

ethanol according to [1]. The obtained polysaccharide (517 g) was filtered off on a sintered glass G3, washed with diluted ethanol (3 portions of ethanol : 1 portion of water), ethanol and air dried. In such a way prepared polysaccharide (500 g) was suspended in 0.1 N sulfuric acid (25 l) and mixed at room temperature for 5 days. The polysaccharide was precipitated as above (yield 92.5 g). The supernatant containing neutral and acidic mono- and oligosaccharides was neutralized with barium carbonate and deionized on a Dowex 50WX2 (H^+ form). Neutral saccharides were separated from the acidic ones on a column of Dowex 1X8 (acetate form, 100—200 mesh) by elution with water. Neutral oligosaccharides (R_{Xyl} 0.67, 0.29, and 0.09) and traces of D-xylose and L-arabinose were detected by paper chromatography. Monosaccharides were separated on a charcoal (Darco G 60) column (50 × 4 cm) by elution with distilled water and oligosaccharides were eluted from the column by 10% ethanol. The mixture of oligosaccharides was separated on a starch column (200 3 cm) [8, 9]. The flow rate of the column was 0.3 ml min^{-1}. Distilled water was used as eluent, 9-ml fractions were collected and estimated by paper chromatography in the system S_1. Three oligosaccharides of R_{Xyl} 0.09 (fractions 23—30; yield 40 mg), R_{Xyl} 0.29 (fractions 36—42; yield 60 mg), and R_{Xyl} 0.67 (fractions 48—56; yield 350 mg) were obtained. The last one was used for further studies.

Hydrolysis of disaccharide

The disaccharide (R_{Xyl} 0.67, 20 mg) of $[\alpha]_D^{20}$ −32° (*c* 1 in water) was dissolved in 1 N sulfuric acid (1 ml) and hydrolyzed in a sealed tube at 105°C for 2 hours. The precipitate was filtered after neutralization with barium carbonate and washed with water. The filtrate was evaporated at reduced pressure. Paper chromatography in the system S_1 showed the presence of L-arabinose and D-xylose detected with anilinium hydrogen phthalate in the filtrate.

Reduction of disaccharide

The disaccharide (20 mg) was dissolved in distilled water (2 ml) and sodium tetrahydroborate (40 mg) dissolved in distilled water (5 ml) was added and stirred for 12 hours. The excess sodium tetrahydroborate was removed by Dowex 50WX2 (H^+ form). The formed boric acid was removed by manifold evaporation with methanol. A compound of R_{Xyl} 0.70 was detected with alkaline silver nitrate after chromatography in the system S_1. Detection with anilinium hydrogen phthalate gave negative results.

Hydrolysis of the reduced disaccharide

The reduced disaccharide (10 mg) was dissolved in 1 N sulfuric acid (1 ml) and hydrolyzed in a sealed tube at 105°C for 2 hours. Further treatments were as described at hydrolysis of the disaccharide. D-Xylose was detected with anilinium hydrogen phthalate and D-xylose and L-arabitol with alkaline silver nitrate by paper chromatography in the system S_1.

Methylation analysis

The disaccharide (320 mg) was methylated with 30% sodium hydroxide (30 ml) and dimethyl sulfate (20 ml) according to *Haworth* [10] during 16 hours. The partially methylated product (300 mg in 1 ml of dimethyl sulfoxide and 5 ml of (methylsulfinyl)-methylsodium) was further methylated with methyl iodide (20 ml) added dropwise to the solution according to *Hakomori* [11]. The methylated product was extracted with

chloroform and after concentration, the ether—petroleum ether solution was washed with water till dimethyl sulfoxide was present.

Hydrolysis of the methylated disaccharide

The methylated disaccharide (20 mg) was hydrolyzed with 70% sulfuric acid (0.5 ml) [12] at room temperature for 45 minutes. Then distilled water (3.5 ml) was added and the hydrolysis was prolonged for 6 hours at 105°C in the sealed tube. After neutralization with barium carbonate and paper chromatography in the system S_2, 2,3,4-tri-*O*-methyl--D-xylose (R_{MG} 0.99) and 2,3-di-*O*-methyl-L-arabinose (R_{MG} 0.77) were identified. The R_{MG} values are related to those of 2,3,4,6-tetra-*O*-methyl-D-glucose. The methylated disaccharide was used also for mass spectrometry.

Results and discussion

The studied disaccharide was prepared by hydrolysis of the peach gum polysaccharide. Autohydrolysis of the polysaccharide gave a degraded polysaccharide and the following monosaccharides: D-galactose, L-arabinose, and D-xylose. The degraded polysaccharide was further hydrolyzed with sulfuric acid giving three neutral oligosaccharides and traces of D-xylose and L-arabinose.

The monosaccharides were removed on the column of charcoal by elution with distilled water and the oligosaccharides were eluted with 10% ethanol. The mixture of oligosaccharides was fractionated on the cross-linked starch column by distilled water. Three oligosaccharides were obtained giving separate spots on paper chromatography. In this work we describe only the structure of the disaccharide (R_{Xyl} 0.67) which gave D-xylose and L-arabinose on hydrolysis. In the hydrolyzate of the reduced disaccharide, D-xylose and L-arabitol were identified by paper chromatography. Thus L-arabinose formed the reducing end in the disaccharide.

The hydrolyzate of the methylated disaccharide contained 2,3,4-tri-*O*-methyl-D--xylose and 2,3-di-*O*-methyl-L-arabinose. The mass spectrum of the permethylated oligosaccharide is in Fig. 1 and was interpreted according to [13, 14] (Scheme 1).

The values of *m/e* peaks for the baB_1 ions (*m/e* 336), abJ_1 ions (*m/e* 235) and the identity of aA_1 and aA_2 ions with the bA_1 (*m/e* 175) and bA_2 (*m/e* 143) ions, respectively, prove the presence of two pentapyranose units in the studied disaccharide. The molecular weight was determined according to [14] following the equation: $M = aA_1 + bA_1 +$

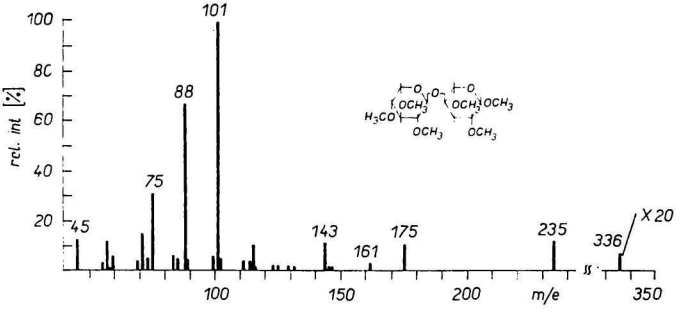
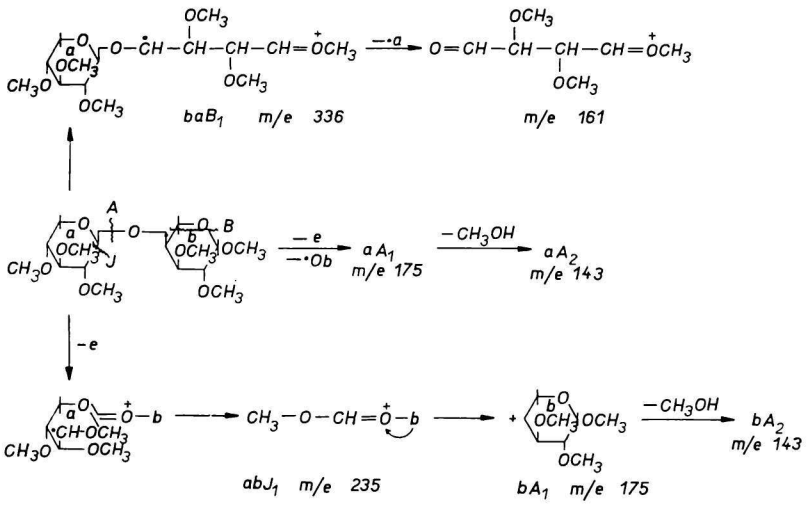


Fig. 1. Mass spectrum of the methylated disaccharide.



Scheme 1

16 = 175 + 175 16 = 366. The presence of the baB_1 ions (m/e 336) and ions at m/e 161 proves that the pentapyranose units in the disaccharide are linked together by 1 → 4 bond. The negative value of optical rotation and the presence of absorption band at 890 cm^{-1} in the infrared spectrum of the disaccharide points to β anomeric character of this bond.

The obtained results show that the studied disaccharide is 4-O-β-D-xylopyranosyl-L-arabinopyranose. The other two oligosaccharides will be described in our further work.

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