NEUTRAL OLIGOSACCHARIDES FROM THE ENZYME HYDROLYSATE OF THE POLYSACCHARIDE OF PEACH-TREE GUM (*Prunus persica* (L.) BATSCH)

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Using enzyme catalyzed hydrolysis of partially degraded polysaccharide of peach-tree gum 6-O-β-D-galactopyranosyl-D-galactose and O-β-D-galactopyranosyl-(1 → 3)-O-β-D-galactopyranosyl-(1→6)-D-galactose were obtained the structures of which were determined by gas chromatography and mass spectrometry of partially methylated alditol acetates.

In structural studies of the gum from *Virgilia oroboides* SMITH and coworkers[1] isolated 6-O-β-D-galactopyranosyl-D-galactose, 3-O-β-D-galactopyranosyl-D-galactose, and O-β-D-galactopyranosyl-(1 → 6)-O-β-D-galactopyranosyl-(1 → 3)-D-galactose. Hirst and coworkers[2] isolated crystalline 3-O-β-D-galactopyranosyl-D-galactose from *Acacia pycnantha* gum. In structural studies of polysaccharides of gums from the trees of the *Prunus* genus it was found that the main polysaccharide chain of peach-tree gum[3] is formed of D-galactose units, bound by β(1 → 6) glycoside bonds. Using multistep hydrolysis with hydrochloric acid Kubala and Rosík isolated oligosaccharides composed of D-galactose units bound by β(1 → 6) glycoside bonds. So far we have not isolated from the polysaccharide of peach-tree gum any oligosaccharide in which the D-galactose units are bound by (1 → 3) and also (1 → 6) glycoside bonds. During the further investigation of the structure of polysaccharide of peach-tree gum we made use of the enzyme preparations from *Aspergillus flavus*, isolated in our laboratory[5]. The enzyme-catalyzed hydrolysis of partially degraded polysaccharide of peach-tree gum[6] afforded 4 neutral oligosaccharides in addition to monosaccharides. In this paper we discuss the oligosaccharides *I* and *II*.

EXPERIMENTAL

Paper chromatography was carried out on Whatman paper No 1 in the systems S_1: ethyl acetate–pyridine–water (8 : 2 : 1) and S_2: ethyl acetate–pyridine–acetic acid–water (5 : 5 : 1 : 3). The saccharides were detected with aniline hydrogen phthalate[7] and alkaline solution of silver nitrate[8]. The fractions from the column were detected with phenol–sulfuric acid reagent[9]. Optical rotation was measured on a Perkin–Elmer 141 polarimeter. The mass spectra were measured on a Jeol JMS-D 100 spectrometer with double focussing, at 23 eV energy of the ionizing electrons, the

emission current was 100 μA and the temperature of the ionization cell 150–180°C. Gas chromatography was carried out on a JGC-20K instrument at inlet pressure of 1 atm, temperature 170–210°C (4°C/min), stationary phase was 3% of OV-225. The solutions were concentrated on a vacuum rotatory evaporator at 40°C. The partially degraded polysaccharide of the peach-tree gum was prepared and characterized as described before[6].

Enzymatic Hydrolysis of Partially Degraded Polysaccharide

The polysaccharide (5 g) was dissolved in 0·1M acetate buffer (250 ml) of pH 4·7, containing 0·1M-NaCl, the solution was overlayered with toluene and incubated with the extracellular enzyme preparation from *Aspergillus flavus*[5] at 30°C for 5 days. During the incubation the increase in the number of reducing groups was followed using the method by Somogyi[10] and the product of hydrolysis by paper chromatography in system S_1. The hydrolysis was terminated by inactivating the enzyme by 10 min boiling of the solution. The remaining polysaccharide was precipitated from the solution concentrated to 50 ml by addition of acidified (1% HCl) ethanol, in a 1 : 3 ratio. The precipitate was centrifuged off, washed with dilute ethanol (60%), dissolved in water, and the solution was percolated through a column of Dowex 50WX4 (H^+) and Dowex 1X8 (CH_3COO^-). Freeze-drying of the filtrate afforded 1·55 g of polysaccharide. The supernatant was percolated on a column of Dowex 50WX4 (H^+) and the mixture of saccharides was then separated to neutral and acid components on a Dowex 1X8 (CH_3COO^-) column by elution with water and 4M acetic acid, respectively. Evaporation of the solutions gave 2·05 g of neutral and 0·065 g of acid components.

Separation of the Mixture of Neutral Mono- and Oligosaccharides

The mixture of saccharides (100 mg) was put on a column of Sephadex G-25 medium (2·6 × 200 cm) and eluted with water at 8 ml/h flow rate. The fraction that contained monosaccharides and 5 oligosaccharides was further separated on a column with charcoal Darco G-60 (4 × 50 cm) using 5, 10, 15 and 20% aqueous ethanol solutions at 15 ml/g flow rate. The mixed fractions of oligosaccharides obtained on elution with 15 and 20% solutions were rechromatographed on a column of charcoal Darco G-60 and Whatman cellulose powder in a 1·5 : 1 ratio (column dimension 4 × 40 cm) using gradient elution with ethanol (5 → 30%), at a 20 ml/h flow rate. In this manner 4 chromatographically pure fractions of oligosaccharides were obtained: *I* (2·2 mg; R_{Gal} 0·53), *II* (2·8 mg; R_{Gal} 0·33), *III* (2 mg; R_{Gal} 0·21) and *IV* (1·4 mg; R_{Gal} 0·71).

Total Hydrolysis of Oligosaccharides

The oligosaccharide (10 mg) was heated with 0·5M sulfuric acid (2 ml) in a sealed test tube at 100°C for 8 h. The solution was neutralized with barium carbonate and deionized on a Dowex 50WX4 (H^+) column.

Reduction of Trisaccharide and the Hydrolysis of the Product

Sodium borohydride (100 mg) dissolved in water (5 ml) was added to the trisaccharide (20 mg) and the solution was stirred at room temperature for 12 h. The pH of the solution was adjusted to 3·5 by addition of Dowex 50WX4 (H^+) which also led to the decomposition of excess borohydride. The solution was filtered, evaporated to dryness and the boric acid was eliminated by evaporation with methanol (3 × 20 ml). The reduced saccharide (15 mg) was hydrolyzed

with 0·5M sulfuric acid (3 ml) in a sealed test tube at 100°C for 1 h and the hydrolysate was worked up as in the case of total hydrolysis of unreduced oligosaccharides.

Methylation, Hydrolysis, Reduction and Acetylation of Oligosaccharides

The oligosaccharide (15 mg) was methylated with dimethyl sulfate and sodium hydroxide according to Haworth[11] and methyl iodide and silver oxide according to Purdie[12]. The methylated product was hydrolyzed with 90% formic acid (10 ml) at 100°C for 1 h. Sulfuric acid (0·13M; 9 ml) was added to the concentrated solution and the hydrolysis was continued for 10 h. The solution was neutralized with $BaCO_3$, filtered and concentrated to 4 ml. The methylated saccharides were reduced with $NaBH_4$ (150 mg) under stirring for 2 h. The solution was acidified with Dowex 50WX4 (H^+) to pH 3·5, filtered, evaporated to dryness, and the boric acid was eliminated by evaporation with methanol (3 × 25 ml). The reduced product was acetylated with acetic anhydride and pyridine (1 : 1; 4 ml) at 100°C for 1 h. The excess reagent was eliminated by co-distillation with toluene, the product was dissolved in dichloromethane or chloroform, and evaporated.

RESULTS AND DISCUSSION

The degraded polysaccharide from the peach-tree gum was hydrolyzed using an extracellular enzyme preparation from *Aspergillus flavus*. From the hydrolysate containing mono- and oligosaccharides the latter were isolated by column chromatography on Sephadex G-25 with water, then on active charcoal or a mixture of charcoal and cellulose powder by gradient elution with aqueous ethanol. Two oligosaccharides were obtained, the homogeneity of which was proved by paper chromatography in the systems S_1 and S_2, and their structures were determined.

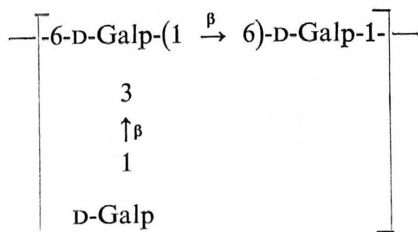
The oligosaccharide *I* gave D-galactose only on total hydrolysis. The alditol acetates of the partially methylated monosaccharides obtained after hydrolysis of the methylated disaccharide gave the following peaks of ions in their mass spectra (m/e): 101, 117, 129, 145, 161 and 205, characteristic of 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-hexitol, as well as the peaks of the ions m/e 87, 99, 101, 117, 129, 159, 161, 173, 189 and 233, characteristic of 1,5,6-tri-O-acetyl-2,3,4-tri-O-methylhexitol. These derivatives were also detected by gas chromatography. The optical rotation of the disaccharide (+25·7°; *c* 0·5 in water) indicated a β-glycosidic bond between the D-galactose units. From these results it follows that the structure of this compound is 6-O-β-D-galactopyranosyl-D-galactose and that it is identical with the disaccharide isolated from the acid hydrolysate[4], with which it has identical chromatographic mobility.

The oligosaccharide *II* gave only D-galactose on total hydrolysis. Alditol acetates of the partially methylated monosaccharides obtained on hydrolysis of the methylated oligosaccharide gave the following peaks of ions in mass spectrometry (m/e): 101, 117, 129, 145, 161 and 205, characteristic of 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylhexitol, 87, 99, 101, 117, 129, 159, 161, 173, 189 and 233, characteristic of 1,5,6-tri-O-acetyl-2,3,4-tri-O-methylhexitol, and also 87, 99, 101, 117, 129, 143, 159, 161, 173,

189, 201 and 233, characteristic of 1,3,5-tri-O-acetyl-2,4,6-tri-O-methylhexitol. The presence of these derivatives was also proved by gas chromatography. The identified derivatives demonstrated that the oligosaccharide is linear and that it contains the (1 →3) and (1 → 6) glycosidic bonds. The optical rotation value of the oligosacharide (+27·9°; *c* 0·5 in water) indicates β-glycosidic bonds between the D-galactose units. The sequence of the glycosidic bonds was determined after partial hydrolysis of the reduced saccharide, in which D-galactose and a disaccharide were identified. According to its chromatographic mobility (R_{Gal} 0·72; in S_2) the disaccharide was identical with 3-O-β-D-galactopyranosyl-D-galactose[1]. The presence of this disaccharide in the hydrolysate of the reduced trisaccharide indicates that the (1 → 3) bond is first from the non-reducing end of the trisaccharide and the (1 → 6) bond is at the reducing end of the trisaccharide. According to these results the investigated trisaccharide has the structure of O-*β*-D-galactopyranosyl-(1 → 3)-O-β-D-galactopyranosyl-(1 → 6)-D-galactose.

As we have already published[3], the D-galactose units in the main chain of the polysaccharide from peach-tree gum are bound by β(1 → 6) glycosidic bonds. This is also indicated by the results of partial hydrolysis of the degraded polysaccharide during which a number of acid oligosaccharides was obtained that are composed of D-glucuronic acid or its 4-O-methyl derivative and D-galactose units (up to 3) bound by the mentioned glycosidic bonds. In these acid oligosaccharides that undoubtedly originate from the main chain of the polysaccharides in no case could a (1 → 3) glycosidic bond be found.

From the results of the methylation analysis and periodate oxidation of the polysaccharide from peach-tree gum it was judged[3] that the D-galactose units are also present in the side chains and that they are bound to D-galactose of the main chain in the position $C_{(3)}$. The isolation of the mentioned trisaccharide supports the earlier assumption. We consider that the investigated trisaccharide has been formed by simultaneous action of exo- and endoglycanohydrolases, while the galactose units were split off up to the branching point under the effect of the β-galactosidase acting from the non-reducing end of the saccharide chain (by an exo-mechanism). The isolated trisaccharide is characterized by the following sequence in the polysaccharide:



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