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

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Structures of two neutral oligosaccharides from the enzyme hydrolysate of the polysaccharide of peach gum were determined by mass spectrometry and gas chromatography. Tetrasaccharide *III* was proved to be O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→6)-D-galactose and the disaccharide *IV* 3-O-α-D-mannopyranosyl-D-galactose.

Four neutral oligosaccharides were obtained by hydrolysis of the degraded polysaccharide of peach gum[1] under catalysis of a mixture of extracellular enzymes of *Aspergillus flavus*[2]. Our preceding paper[1] dealt with the structure of two oligosaccharides, namely 6-O-β-D-galactopyranosyl-D-galactose and O-β-D-galactopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→6)-D-galactose, this paper concerns the structure elucidation of a neutral oligosaccharide *IV* from the enzyme hydrolysate, in which a D-mannose unit was found, and a higher oligosaccharide *III* consisting of D-galactose units. So far, no neutral oligosaccharide has been isolated from the peach gum polysaccharide in which D-mannose was embodied.

EXPERIMENTAL

Systems used for paper chromatography on paper Whatman No 1 were S_1: ethyl acetate–pyridine–water (8 : 2 : 1), S_2: ethyl acetate–acetic acid–water (18 : 7 : 8) and S_3: ethyl acetate–pyridine–acetic acid–water (5 : 5 : 1 : 3). Saccharides were visualized with anilinium hydrogen phthalate[3] and alkaline solution of silver nitrate[4]. The purity of methylated saccharides was checked by thin layer chromatography on silica gel 60F-254 in the system benzene–acetone (3 : 1), detection with sulfuric acid. Optical rotation was measured with a Perkin–Elmer, model 141, polarimeter. Solutions were concentrated in a vacuum rotary evaporator at 40°C.

Mass spectra of both methylated saccharides and methylated alditol acetates were recorded with a JMS-D 100 (JEOL) spectrometer at an ionizing electron energy 23 eV, trap current 100 μA and ionization chamber temperature 150—180°C. Gas chromatography combined with mass spectrometry was effected with a JGC-20K apparatus at the He inlet pressure 0·1 MPa, injection port temperature 220°C, column temperature 170—210°C (4°C/min), stationary phase 3% OV-225. Hewlett–Packard, model 5711 A, gas chromatograph (N_2 carrier gas, 30 ml/min) equipped with a flame-ionizing detector was employed for gas chromatography.

Total Hydrolysis of Oligosaccharides

The oligosaccharide (10 mg) was hydrolyzed 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 the excess of Ba^{2+} ions was removed on a Dowex 50W 4 (H^+) column.

Reduction of the Disaccharide and Hydrolysis of the Product

Sodium tetrahydroborate (100 mg) dissolved in water (5 ml) was added to the disaccharide (20 mg). The solution was stirred at room temperature for 12 h, pH was then adjusted with Dowex 50W 4 (H^+) to 3·5 in order to decompose the excess of sodium tetrahydroborate. The solution was filtered and evaporated to dryness; boric acid was removed by evaporation with methanol (3 × × 20 ml). The reduced disaccharide (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 described with the total hydrolysis of nonreduced oligosaccharides.

Methylation, Hydrolysis, Reduction and Acetylation of the Tetrasaccharide

The oligosaccharide (15 mg) was methylated with dimethyl sulfate and sodium hydroxide according to Haworth[5] and with methyl iodide and silver oxide according to Purdie[6]. 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 at 100°C for additional 10 h; after this time the solution was neutralized with barium carbonate, filtered and concentrated to 4 ml. Methylated saccharides were reduced under stirring with sodium tetrahydroborate (150 mg) for 2 h, the solution was acidified with Dowex 50W 4 (H^+) to pH 3·5, filtered and evaporated to dryness. Boric acid was removed by evaporation with methanol (3×25 ml). The reduced product was acetylated with acetic anhydride–pyridine (1 : 1, 4 ml) at 100°C for 1 h. The excess of reagents was distilled off with toluene and the product being dissolved in dichloroethane was concentrated.

Methanolysis of Methylated Oligosaccharides

The methylated disaccharide (5 mg), prepared as before, was methanolyzed with 4% methanolic hydrogen chloride (5 ml) in a sealed glass capillary tube at 105°C for 7 h, neutralized with silver carbonate, centrifuged and the supernatant vaccum dried. The methylated tetrasaccharide was partially methanolyzed by the same procedure for 1 h.

RESULTS AND DISCUSSION

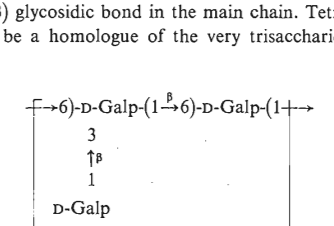
In addition to already described two neutral oligosaccharides[1] further two ones (*III* and *IV*) were isolated from the enzyme hydrolysate of peach gum polysaccharide. They were found to be homogeneous, as checked by paper chromatography in three various systems, and their mobilities in S_3 were R_{Gal} 0·21 (*III*) and 0·71 (*IV*).

The oligosaccharide *III* gave upon total hydrolysis D-galactose only. Its mobility and R_F value of its methylated derivative on paper chromatography and thin layer chromatography, respectively, indicated, when contrasted with the mobilities of reference substances (methylated di- and trisaccharides), that a tetrasaccharide is in-

volved. Mass spectrometry of methylated alditol acetates, obtained by hydrolysis of the methylated oligosaccharide followed by reduction and acetylation, displayed peaks at *m/e* 101, 117, 129, 145, 161 and 205 which are characteristic of 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylhexitol; those at *m/e* 87, 99, 101, 117, 129, 159, 161, 173, 189 and 233 are indicative of 1,5,6-tri-O-acetyl-2,3,4-tri-O-methylhexitol and those at *m/e* 87, 99, 101, 117, 129, 143, 159, 161, 173, 189, 201 and 233 are diagnostic of 1, 3, 5-tri-O-acetyl-2,4,6-tri-O-methylhexitol[7]. The identified derivatives provided evidence that the oligosaccharide is linear and contains (1→6) and (1→3) glycosidic bonds. The value of specific rotation +25·2° (*c* 0·5, water) indicated the β-anomeric character of glycosidic bonds between D-galactose units[8].

The sequence of glycosidic bonds was estimated after a partial methanolysis of the methylated oligosaccharide, which afforded a signle product, homogeneous on thin layer chromatography; its R_F value was identic with that of methylated 6-O-β-D-galactopyranosyl-D-galactose. Mass spectrum of this product corroborated the (1→6) glycosidic bond[9,10] on the basis of characteristic baD_1 ions at *m/e* 353. The *m/e* values of ions aA_1 and bA_1 served for calculation of the molecular weight of the dimer (M = 219 + 219 + 16 = 454). The fact that no monosaccharide, neither di- or trisaccharides containing a (1→3) glycosidic bond result from this partial methanolysis indicated that the (1→3) glycosidic bond found with partially methylated alditol acetates is located between the second and third units of galactose, in other words in the middle of the tetrasaccharide chain; the first and the second, as well as the third and the fourth D-galactose units were (1→6) glycosidically bound. Hence the structure of the tetrasaccharide under study is O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→6)-D-galactose.

The result of our preceding paper[1], dealing with the structure of a trisaccharide, was that D-galactose present in the side chain was (1→3) glycosidically bound to D-galactose of the backbone. This was stated on the basis of the fact that we have not yet identified a (1→3) glycosidic bond in the main chain. Tetrasaccharide identified in this paper might be a homologue of the very trisaccharide with a longer side chain:



or alternatively, a segment of the main chain:

$$\rightarrow[\rightarrow 6)\text{-D-Gal}p\text{-}(1 \xrightarrow{\beta} 6)\text{-D-Gal}p\text{-}(1 \xrightarrow{\beta} 3)\text{-D-Gal}p\text{-}(1 \xrightarrow{\beta} 6)\text{-D-Gal}p\text{-}(1 \rightarrow] \rightarrow$$

So far, we did not find a (1→3) glycosidic bond in the backbone after chemical degradation. This fact can be rationalized by a greater lability of the (1→3) than the (1→6) glycosidic bonds under conditions of acid hydrolysis[8].

Total hydrolysis of oligosaccharide *IV* afforded D-galactose and D-mannose in an equimolar ratio. The reducing saccharide in the hydrolysate of the reduced disaccharide was found to be D-mannose showing thus that the reducing end of the disaccharide was D-galactose. This sequence of monosaccharides was also proved by gas chromatography: the methanolyzed methylated disaccharide consisted of methyl 2,3,4,6-tetra-O-methyl-D-mannopyranoside and methyl 2,4,6-tri-O-methyl-D-galactopyranoside. The molecular weight of rings was determined from the mass spectrum of the methylated saccharide, which showed the presence of ions $a(b)\,A_1$ and $a(b)A_2$ at m/e 219 and 187 characteristic of two hexose units in the dimer[9]. Molecular weight of the dimer (M = 219 + 219 + 16 = 454) was calculated from the m/e values of ions aA_1 and bA_1. The presence of peaks[9] of great abundance at m/e 159 and 71 and the absence of those characteristic of other bonds evidenced the (1→3) glycosidic bond in the disaccharide under investigation. Optical rotation of the disaccharide +42·1° (*c* 1·0, water) indicated the α-anomeric character of the glycosidic bond between D-galactose and D-mannose. Basing upon results presented, the structure of the disaccharide is 3-O-α-D-mannopyranosyl-D-galactose.

The aldobiouronic acid, reported in our paper[11] had D-glucuronic acid bound by a glycosidic bond to $C_{(2)}$ of D-mannose. This was the first information concerning the position of D-mannose in polysaccharides of gums from trees belonging to genus *Prunus*. Since the content of mannose is, in regard to other constituents of the polysaccharide, very low (*c.* 2%), we were unable to obtain a neutral oligosaccharide by acid hydrolysis containing also D-mannose (glycosidic bonds are more labile than with acid oligosaccharides). Isolation of such a disaccharide after enzyme-catalyzed hydrolysis of degraded polysaccharide of peach gum is important for determination of the polysaccharide fine structure. The new finding that D-mannose is bound in the polysaccharide chain to $C_{(3)}$ of D-galactose together with known facts (low content of D-mannose in the polysaccharide, its binding in aldobiouronic acid and inability to isolate an oligosaccharide containing D-mannose from the acid hydrolysate) might indicate D-mannose to form the centre of branching. Consequently, it is poorly accessible for degradation in native undegraded polysaccharide and therefore, the content of D-mannose in the polysaccharide remaining after gradual acid degradation increases[12].

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