CHARACTERIZATION OF POLYSACCHARIDES FROM VARIOUS VARIETIES OF PEACH TREES (*Prunus persica* (L.) BATSCH.)

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Methylation analysis of 6 polysaccharides of gums from various varieties of peach trees was carried out. Different methylation methods were used. The results are correlated with the presently known structural characteristics of the peach gum polysaccharide.

In our study[1] we analysed 6 samples of gum polysaccharides from peach trees of the *Prunus* genus. We determined their equivalent weights, the content of methyl groups and the molar ratio of acid and neutral components. From the results it was evident that these polysaccharides differed little in their physico-chemical properties. In order to obtain more data on their chemical structure, we methylated and then oxidized these polysaccharides.

The polysaccharides were methylated using the methods according to Haworth[2], Purdie[3], Hakomori[4] and Kuhn[5]. Since polysaccharides are poorly soluble in organic solvents, we endeavoured to achieve complete methylation by applying these methods in various combinations. Björndal and coworkers[6] described the methylation of polysaccharides by the above-mentioned methods and found that methylations according to Haworth and Purdie were more advantageous than in the strong polar N,N-dimethylformamide. The methylation according to Hakomori was rapid and complete and could be achieved in one step. However, polysaccharides which were insoluble in dimethyl sulfoxide had to be first methylated using Haworth's method. Methylation can be complicated by the presence of uronic acids in the polysaccharide. Esterified uronic acids can undergo β-elimination in strongly alkaline medium, especially when the unit of the acid is substituted[7] in the position $C_{(4)}$. Ovodov and Evtushenko[8] found that during partial methylation of methyl β-D-xylopyranoside according to Haworth, Purdie and Kuhn the reactivity of the —OH groups decreased in the order 2-OH > 4-OH > 3-OH. When Hakomori's method was applied, 4-O-methyl derivative was obtained in the first step and the reactivity of the —OH groups was the following: 4-OH > 2-OH > 3-OH. In contrast to other methods 2,3,4-tri-O--methyl derivative was obtained in a very short time.

We methylated the investigated polysaccharides (samples 1–6) in two ways: *a*) The polysaccharide samples were first methylated by the methods elaborated by Haworth and Purdie. After hydrolysis the partially methylated saccharides were reduced, acetylated and determined by gas chromatography. Samples 2 and 4 were also analysed by mass spectrometry. The results obtained are collected in Table I. *b*) The polysaccharide samples (1–6) were first methylated using Haworth's method. Then the partially methylated polysaccharides were dialysed and lyophilized. Samples 1–3 were further methylated by Purdie's method, samples 4–6 by Hakomori's method and sample 6 also by Kuhn's method. The samples were further worked up as in the preceding case. The results are presented in Table II. Very weak peaks on gas chromatograms were not indentified and therefore we have not included them in the tables.

The following structurally more important derivatives were determined by gas chromatography: 2,3,5-tri-O-methyl-L-arabinose, 2,3,4-tri-O-methyl-L-rhamnose, 2,3,4-tri-O-methyl-D-xylose, 2,5-di-O-methyl-L-arabinose, 2,3-di-O-methyl-L-arabinose, 2,3,4-tri-O-methyl-D-galactose, 2,6-di-O-methyl-D-galactose, 2,4-di-O-methyl-D-galactose and 2-O-methyl-D-galactose. The molar ratios of these methyl derivatives, calculated from the area under the peaks on gas chromatograms, are given in Table I. The results obtained agree with those presented in paper[1] and they do not indicate differences in the structures of the individual polysaccharides.

We expected that on application of various methods of repeated methylations similar results would be obtained. The results of individual methods (Haworth--extraction-Purdie-hydrolysis; Haworth-dialysis-Hakomori-hydrolysis and Haworth--dialysis-Kuhn-hydrolysis) differ very much. The considerable difference is especially apparent in the case of sample 6 which was methylated using the Hakomori as well as the Kuhn methods. With other samples similar differences were also found. The difference can arise from different reactivity of the —OH groups as indicated in papers[6,8], where methylation was carried out in different solvents, leading to different products. It is also difficult to discern whether metylation is complete. The methylated polysaccharides are hygroscopic, dimethyl sulfoxide or other organic solvents are difficult to remove, which can affect the results. In the methylation of polysaccharide samples the partially methylated polysaccharide (after Haworth's methylation) was extracted with chloroform in the first case, the combined extracts were concentrated and further methylated according to Purdie. In the second case the partially methylated polysaccharides were first dialysed, lyophilized and further methylated using the methods given in Table II. Methylated polysaccharides were also hydrolysed in two ways. Samples 1–3 were prehydrolysed with 72% sulfuric acid and samples 4–6 with 90% formic acid.

In all the methylated polysaccharides we measured a negative optical rotation value, which shows that in polysaccharides β-glycosidic bonds predominate. The values measured are listed in Table III from which it is evident that with the decreasing equivalent weights the values of specific optical rotation also decrease.

TABLE I

Partially methylated alditol acetates identified by gas chromatography

Product	R_T[a]	Polysaccharide[b]				
		2	3	4	5	6
1,4-Di-O-acetyl-2,3,5-tri-O-methyl-L-arabinitol + traces of 1,5-di-O-acetyl-2,3,4-tri-O-methyl-L-rhamnitol	0·432	1·11	1·04	1·08	1·12	1·29
1,5-Di-O-acetyl-2,3,4-tri-O-methyl-D-xylitol	0·565	1·00	1·00	1·00	1·00	1·00
1,3,4-Tri-O-acetyl-2,5-di-O-methyl-L-arabinitol	0·880	0·38	0·28	0·24	0·28	0·28
1,4,5-Tri-O-acetyl-2,3-di-O-methyl-L-arabinitol	1·052	1·81	1·56	1·41	1·66	1·47
1,5,6-Tri-O-acetyl-2,3,4-tri-O-methyl-D-galactitol	2·848	0·32	0·23	0·21	0·31	0·27
1,3,4,5-Tetra-O-acetyl-2,6-di-O-methyl-D-galactitol	3·073	0·25	0·32	0·27	0·32	0·24
1,3,5,6-Tetra-O-acetyl-2,4-di-O-methyl-D-galactitol	4·921	0·79	0·91	1·05	1·25	1·16
1,3,4,5,6-Penta-O-acetyl-2-O-methyl-D-galactitol	6·123	0·84	0·71	0·87	0·99	0·87

[a] Retention times, relative to 1,5-di-O-2,3,4,6-tetra-O-methyl-D-glucitol; [b] relative molar proportion of the components in % of the total peak area.

TABLE II

Partially methylated alditol acetates identified by gas chromatography

Product	R_T[a]	Polysaccharide[b]						
		1 P	2 P	3 P	4 H	5 H	6 H	6 K
1,4-Di-O-acetyl-2,3,5-tri-O-methyl-L-arabinitol + traces of 1,5-di-O-acetyl-2,3,4-tri-O-methyl-L-rhamnitol	0·436	1·26	0·41	0·99	1·28	0·90	0·78	1·52
1,5-Di-O-acetyl-2,3,4-tri-O-methyl-D-xylitol	0·571	1·00	1·00	1·00	1·00	1·00	1·00	1·00
1,3,4-Tri-O-acetyl-2,5-di-O-methyl-L-arabinitol	0·883	0·62	0·02	0·50	0·35	0·22	0·10	0·61
1,4,5-Tri-O-acetyl-2,3-di-O-methyl-L-arabinitol	1·064	1·77	0·49	2·02	1·97	1·30	1·16	1·74
1,5,6-Tri-O-acetyl-2,3,4-tri-O-methyl-D-galactitol	2·882							
1,3,4,5-Tetra-O-acetyl-2,6-di-O-methyl-D-galactitol	3·036	0·75	0·44	0·62	0·12	0·17	0·05	0·69
1,3,5,6-Tetra-O-acetyl-2,4-di-O-methyl-D-galactitol	5·038	1·11	0·80	1·45	0·34	0·47	0·22	1·17
1,3,4,5,6-Penta-O-acetyl-2-O-methyl-D-galactitol	6·252	0·72	0·30	1·52	0·50	0·13	—	0·35

[a] Retention times, relative to 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol; [b] relative molar proportion of the components in % of the total peak area.

The samples were oxidized with sodium periodate. The observed values of the periodate consumption and the formation of formic acid are given in Table III. The oxidation with sodium periodate did not indicate substantial differences in the composition of polysaccharides either. The oxidation products were evaluated by paper chromatography only, they were not determined quantitatively. According to our paper[9] L-rhamnose, a part of D-galactose and of L-arabinose are attacked during the oxidation. 4-O-Methyl-D-glucuronic acid could not be detected in the oxidation products either.

In the samples of polysaccharides we identified the following D-galactose derivatives: 2,3,4-tri-O-methyl-D-galactose, 2,6-di-O-methyl-D-galactose, 2,4-di-O-methyl-D-galactose and 2-O-methyl-D-galactose. Rosík and coworkers[9] described the structural properties of the degraded polysaccharide from the peach gum of *Prunus persica* (L.) BATSCH and found that D-galactose formed predominantly the main chain by the structural units→ 6-O-β-D-galactopyranosyl-1→ 6-O-β-D-galactopyranosyl-1→. Simultaneously they found that D-galactose split off only partially during the autohydrolysis and that it was also present in the side chains bound by β(1 → 3) glycosidic bonds. Some units of D-galactose from the main chain formed the branching points at carbon $C_{(3)}$. In this study the identified derivatives, 2,6-di-O-methyl-D-galactose and 2-O-methyl-D-galactose, show that further variations of the D-galactose bonds are in polysaccharides of gums from various sorts of peach trees also possible. We have not detected these derivatives in degraded polysaccharides.

In our study[1] we determined in sample 6 the molar ratio of D-galactose: D-mannose as 4·27 : 0·05. After autohydrolysis of this sample the molar ratio was 1·97 : 2·40, which shows that D-mannose does not split from the polysaccharide under the conditions of autohydrolysis. Since the content of D-mannose in the samples of polysacharides is very low, we could not determine the methyl derivatives in the investigated samples of polysaccharides either.

TABLE III
Characterization of polysaccharides

Polysaccharide	1	2	3	4	5	6
$[\alpha]_D$	—86·3	—60·2	—56·2	—68·3	—74·3	—78·3
Equivalent weight	1 955	2 170	2 185	2 050	1 970	2 025
Molecular mass	76 000	94 000	161 000	73 000	51 000	128 000
$IO_4^{(-)}$[a] consumption	10·8	11·0	11·6	11·0	9·0	11·8
HCOOH[a] formation	1·0	0·9	1·2	0·95	1·2	1·2

[a] Mol per 1 equivalent of polysaccharide.

In the case of pentoses we determined the following methyl derivatives: 2,3,5-tri-O-methyl-L-arabinose, 2,5-di-O-methyl-L-arabinose, 2,3-di-O-methyl-L-arabinose and 2,3,4-tri-O-methyl-D-xylose. L-Arabinose and D-xylose are split off during autohydrolysis and the identified derivatives of 2,3,5-tri-O-methyl-L-arabinose and 2,3,4-tri-O-methyl-D-xylose show that a part of the L-arabinose and D-xylose units form the terminal non-reducing units. Some units of L-arabinose also occur in pyranose form and are mutually bound by the β(1 → 4)-glycosidic bonds, as evidenced by the identified derivative of 2,3-di-O-methyl-L-arabinose. Rosík and co-workers isolated and identified 4-O-β-D-arabinopyranosyl-L-arabinopyranose[10] and 4-O-β-D-xylopyranosyl-L-arabinopyranose[11] from the polysaccharide of peach tree gum. The remaining L-arabinose units are then bound by β(1 → 3)-glycosidic bonds.

L-Rhamnose forms the non-reducing terminal units, as shown by the identified 2,3,4-tri-O-methyl-L-rhamnose. On the gas chromatogram this derivative overlaps with 2,3,5-tri-O-methyl-L-arabinose, but we proved its presence by mass spectrometry.

Methylation analysis of the samples of polysaccharides from various varieties of peach trees indicated that the most suitable method of methylation is that according to Haworth and Purdie. The authors mentioned above[6] also came to a similar conclusion. The results achieved are realistic and it may be concluded from them that the samples of polysaccharides mentioned have a similar structure. However, they differ in equivalent weights and molecular masses given in Table III. The molecular masses were determined by ultracentrifuging and they are given in our earlier paper[14]. Other methylation methods did not afford results that correspond to the effort invested. In our opinion they are not suitable for the structural studies of such complex polysacharides as those from the gums of fruit trees.

EXPERIMENTAL

General Methods

The samples of gums 1—5 were from the cultured varieties of peach trees, botanically characterized, while the sample 6 was a mixture of gums from various varieties of peach trees. The preparation of polysaccharide samples, the determination of equivalent weights and the method of gas chromatography were described in a previous paper[1]. The mass spectra of peaks from gas chromatography were measured on a Perkin–Elmer 270 instrument at ionizing potential of 70 eV, 65 μA. As stationary phase OV 225 SCOT was used, at 210°C. Specific rotation was measured on a Bendix NLP Automatic Polarimeter 143 C.

Methylation by Haworth's Method

a) Sample weight was about 100 mg of polysaccharide. For the reaction 13·7 ml of dimethyl sulfate and 21·6 ml of 30% NaOH were used. The reagents were added in parts over three days at room temperature. After termination of the methylation the solution was heated at 60°C

for half-an-hour, neutralized first with 1M-H_2SO_4, then with conc. H_2SO_4, and finally extracted three times with chloroform. The extract was dried over anhydrous sodium sulfate and filtered. The extract was evaporated *in vacuo* (Table I).

b) Sample weight was about 200 mg of polysaccharide. The amount of dimethyl sulfate used was 29·4 ml in 50 ml of 30% NaOH. The reagents were added in parts over 3 days under cooling with ice and under nitrogen. When methylation was over the solution was heated at 60°C for 30 min, then cooled, neutralized, filtered, and diluted with water (50 ml). The solution was dialysed for three days in water, then evaporated to a smaller volume and lyophilized (Table II).

Methylation by Purdie's Method

a) The sample weighted about 55 mg and consisted of partially methylated polysaccharides. 10 ml CH_3I and 600 mg Ag_2O were used for methylation. This took 3 days under reflux. The mixture was cooled and filtered. The precipitate on the filter was washed with chloroform and the solutions were combined and evaporated *in vacuo* (Table I).

b) The sample weight was about 100 mg of partially methylated polysaccharides 1, 2, and 3. 15 ml of CH_3I and 1 g of Ag_2O were used and methylation was carried out as under *a*).

Methylation by Hakomori's Method

The sample weight was about 30 mg of partially methylated samples 4, 5, and 6. The polysacharides were dissolved in 3 ml of dimethyl sulfoxide for 2 h at 52°C. After cooling, 0·5 ml of methylsulfinylmethyl sodium solution (prepared according to ref.[15]) was added at a temperature not exceeding 25°C and the solution was stirred for 30 min. Then 2 ml of CH_3I were added dropwise, keeping the temperature below 25°C, and the mixture was stirred for another 30 min. The solution was then dialysed overnight. The methylated polysaccharide was extracted 3 times with chloroform, the extracts were combined and evaporated.

Methylation by Kuhn's Method

Partially methylated polysaccharide (sample 6, 40 mg) was dissolved in 10 ml of N,N-dimethylformamide, 8 ml of CH_3I and 500 mg of Ag_2O were added and the solution was stirred for 20 h at room temperature. After filtration it was evaporated *in vacuo*. The residue was dissolved in chloroform, filtered, and evaporated. The polysaccharide methylated in this manner was dissolved in 10 ml of CH_3I and 400 mg of Ag_2O were added to it. Methylation proceeded under reflux for 3 days. During the next two days another 5 ml of CH_3I and 400 mg of Ag_2O were added. The mixture was then filtered and evaporated, the residue was redissolved in boiling CH_3I, filtered and evaporated. The sample was then repeatedly dissolved in chloroform (3 times) and dried.

Hydrolysis of Methylated Polysaccharides

Methylated polysaccharides (samples 2—6, methylated as described under *a*) and samples 4—6 as described under *b*)) were dissolved in 90% formic acid (3 ml) and hydrolysed on a boiling water bath for 1 h. After evaporation *in vacuo* and dissolution in 0·25M-H_2SO_4 (3 ml) the mixture was transferred into a capillary and sealed. Hydrolysis took place on a boiling water bath for 16 h. After neutralization (with $BaCO_3$) the hydrolysate was filtered and deionized on a column of Zerolite 325 (H^+). The mixture of partially methylated monosaccharides was

reduced with $NaBH_4$ (150 mg) under stirring overnight (16 h). The excess $NaBH_4$ was decomposed with the cation exchanger Zerolite 325 (H^+) until the mixture was neutral. It was filtered off and the solution concentrated. The boric acid formed was eliminated by evaporation with methanol (4 times with 5 ml). Acetic anhydride (2 ml) and pyridine (2 ml) were added to the samples and acetylation was allowed to proceed for 20 min on a boiling water bath. Acetic anhydride was evaporated in the presence of toluene in a vacuum. The samples were dissolved repeatedly in chloroform and evaporated under reduced pressure and submitted to gas chromatographic analysis. Samples 2 and 4 (methylated according to procedure *a*)) were also analysed by mass spectrometry. Methylated polysaccharides (samples 1, 2, and 3, methylated according to procedure *b*)) were dissolved in 72% H_2SO_4 (0·5 ml) and allowed to stand at room temperature for 45 min. Water (3·5 ml) was then added and hydrolysis was continued on a boiling water bath for 6 h. After cooling, H_2SO_4 was neutralized with $BaCO_3$, the precipitate formed was filtered off and the solution was deionized on a column of Zerolite 325 (H^+). Reduction and acetylation took place in the above-mentioned manner.

Oxidation with Periodate

Polysaccharides (samples 1—6, 1 g each) were oxidized with 250 ml of 0·02M sodium metaperiodate in dark at +5°C for 9 days. The consumption of periodate and the formation of formic acid were determined using the thiosulfate method[12] with amperometric indication[12]. The results were calculated per one equivalent of polysaccharide.

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