Note

Infrared spectroscopy of peach-gum polysaccharides of *Prunus persica* (L.) Batsch

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Plant-gum polysaccharides show strong i r. absorption for the carboxyl group in the range 1620–1800 cm^{-1}. Two different carboxyl-group absorptions can be detected under high resolution[1].

In the present work, we have tried to interpret the range 1600–1800 cm^{-1} of the i r. spectra of 18 samples of a peach-gum polysaccharide (*Prunus persica*) prepared in different ways. Their molecular weights established by sedimentation in an ultracentrifuge are $\sim 1.0 \times 10^6$ and their equivalent weights are in the range of 2100 to 2500 The structural features of this peach-gum polysaccharide have been published[2]

RESULTS AND DISCUSSION

Peach-gum polysaccharide contains D-glucuronic acid, 4-*O*-methyl-D-glucuronic acid, D-galactose, D-mannose, L-arabinose, and D-xylose. Correlation of such a complicated structure with the frequencies observed in its i.r. spectrum can only have empirical significance. It is assumed that the vibrations of $-CO_2H$ groups are fairly independent of the rest of the molecule. Valence frequencies of the bonds in the carboxyl group itself are very sensitive to structural changes and grouping of atoms. These are reflected over the range 1600–1800 cm^{-1} in the spectra of acidic polysaccharides.

Comparative studies on spectra of gum polysaccharides reveal some anomalies in the range mentioned. Data on band positions are tabulated (Table II).

Several reports in the literature, mainly in the field of cellulose, indicate similar problems. Jayme and Tio[3] compared the spectrum of *O*-(carboxymethyl)cellulose with the spectrum of its sodium salt. The spectrum of *O*-(carboxymethyl)cellulose shows two bands at 1730 and 1600 cm^{-1}, whereas the sodium salt shows bands at 1600 and 1320 cm^{-1}. In the same work, the authors compared the spectra of alginic acid and the sodium salt of alginic acid. In the spectrum of alginic acid, there is only one band at 1730 cm^{-1}, attributed to the vibration of the carboxylic carbonyl group. Sodium alginate shows a band at 1600 cm^{-1} and a new band occurs at 1408 cm^{-1}.

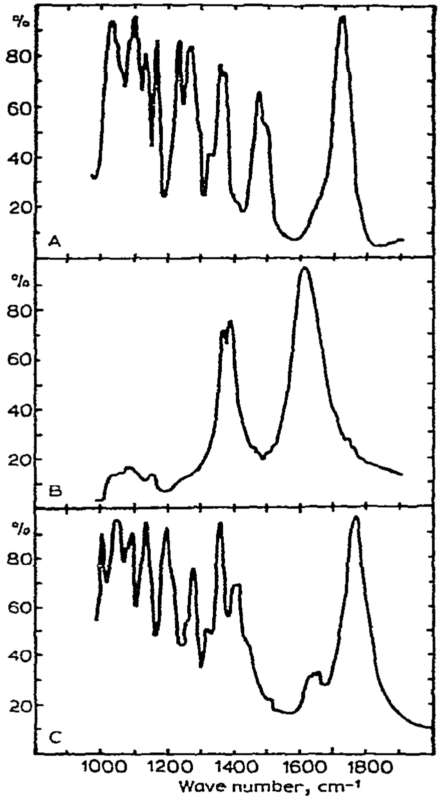
Similar results were published by Schwartz and Kienzl[4] on the sodium salt of *O*-(carboxymethyl)cellulose. The authors attributed the bands at 1600 and 1320 cm^{-1} to the asymmetric and symmetric vibration of CO_2^- groups. Zhbankov[5] found that the frequencies in the region 1650–1550 cm^{-1} of the spectra of salts of oxidized celluloses depended on the mass of the metallic cation. Bands in this region could be attributed either to binding with different cations (bonds with one or several carboxyl groups, respectively) or to ionization and/or lack of ionization of $-CO_2Me$ groups. Cerezo *et al*[6] have found in the i.r. spectrum of brea-gum polysaccharide a very strong band at 1765–1760 cm^{-1} attributed to the lactone group. The authors assumed that a lactone is formed during precipitation of the polysaccharide. The original gum shows only very weak absorption in the range 1800–1725 cm^{-1}.

For better interpretation of the i.r. spectra of peach-gum polysaccharides, we have recorded the spectra of D-glucuronolactone (Koch-Light Laboratories, Ltd.), D-glucuronic acid (Fluka AG, Switzerland), and its calcium salt (prepared in our laboratory). These spectra are shown in Fig. 1. D-Glucuronolactone is most probably the 6,3-lactone[7] and it shows a band at 1770 cm^{-1}. The spectrum shows three additional, weak, absorption bands at 1655–1630 cm^{-1} that could be due to absorption by water or some impurities. D-Glucuronic acid shows an absorption band at 1720 cm^{-1} and its calcium salt at 1615 cm^{-1}. A recent paper[8] gives the i.r. absorption spectrum of barium D-glucuronate

Spectra of the peach-gum polysaccharides could be divided into two groups. Spectra of samples 1, 11, and 12 (Table II) belong to the first group. They show strong absorption at 1625 cm^{-1} and a very weak band at 1740 cm^{-1} (Fig. 2). The strong band is due to the salt of D-glucuronic acid, which is in the dissociated form, and bands arise from asymmetric vibrations of the CO_2^- group. It is also clear from these spectra that the native polysaccharide, and that precipitated without the use of acid, contains no free carboxyl groups. The presence of such metal ions as those of Mn, Si, Fe, Al, Ni, Pb, Mg, Sn, Cu, K, and Na in the polysaccharides was proved by qualitative and quantitative spectral analysis. Cations are still present after multiple deionization of the polysaccharides on Dowex-50 (H^+). It is to be noted that the amides of uronic acids also show the Amide I absorption in this region. This possibility is discounted for these polysaccharide samples because sample 1 contains no nitrogen Very weak shoulders at 1740 cm^{-1} indicate that the polysaccharide molecule contains traces of free carboxyl groups. Ethanol, methanol, and acetone have no effect on the i.r. spectrum of the "native" polysaccharide (samples 1, 11, and 12).

The remaining samples form the second group (Table II). Their i.r. spectra show two characteristic, strong absorption-bands, the first in the region 1655–1645 cm^{-1} and the second in the region 1755–1745 cm^{-1}. Also, other very weak bands occur at 1730–1660 cm^{-1} (sample 16 in Fig. 2).

The band at 1755–1745 cm^{-1} is due to the valence vibration of $C=O$ groups from un-ionized carboxylic acid groups. This change of spectrum could be asscribed to the process of ion-exchange $-CO_2^-M^+ \rightarrow -CO_2H$ occurring on precipitating the polysaccharide with ethanol in a weakly acidic medium. The second band, occurring



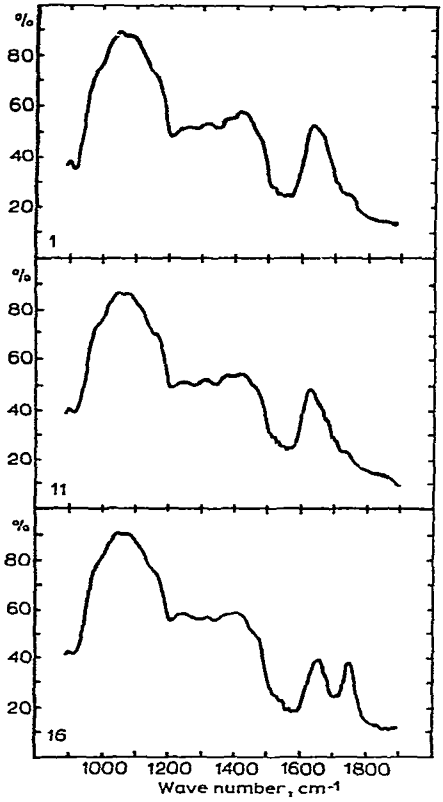


Fig 1. I r. spectra of D-glucuronic acid (*A*), calcium D-glucuronate (*B*), and D-glucuronolactone (*C*).

Fig 2. I r. spectra of polysaccharide samples 1, 11, and 16. The ordinate gives % transmittance

at 1655–1645 cm^{-1}, is attributed to the ionized carboxyl groups, as in the first group of polysaccharides; it is thus concluded that the polysaccharide molecule contains a certain proportion of carboxyl groups that do not exchange ions. So far, we have no exact explanation for this observation. In this spectral region, esters and lactones of acids show their characteristic absorption bands, and therefore sample 1 has been analyzed for $-OCH_3$ groups, and these were found present to the extent of 4.53%. It may be assumed, however, that these methoxyl groups arise from 4-*O*-methyl-D-glucuronic acid, which is present in the molecule of the polysaccharide. To see whether esterification takes place during precipitation and further processing the samples 13–18 were kept, after precipitation, at laboratory temperature with continual stirring for 7–28 days (Table I). The intensity of bands at 1755–1745 cm^{-1} in the i.r. spectra would increase if esterification were to be taking place. However, no difference in the intensities of the bands was observed. Lactonization could be excluded because γ-lactones absorb mainly at higher wave-numbers. Very weak absorption bands in the region 1730–1720 cm^{-1} could arise from esters of acids or from valence vibration of C=O groups (different from that of the carbonyl group of the carboxylic acid).

TABLE I

PREPARATION OF THE POLYSACCHARIDE SAMPLES

Sample No.	*Proportion of*		
	Stock solution (ml)	*Precipitant (ml)*	% H^+ (*v/v*)
1[a]	200	None	None
Ethanol			
2	200	400	1
3	200	600	1
4	200	800	1
5	200	1000	1
6	200	600	0,5
7	200	600	2
8	200	600	4
10[b]	200	600	None
11	200	600	None
13[c]	200	600	1
14[d]	200	600	1
15[e]	200	600	1
16[f]	200	600	1
Methanol			
17[d]	200	600	2
18[d,g]	200	600	2
Acetone			
9	200	600	1
12	200	600	None

[a] Freeze-dried. [b] To the solution 8 g of NaOH was added and after 2 h the polysaccharide was precipitated. [c] After precipitation, the suspension of polysaccharide was kept with stirring at laboratory temperature for 7 days. [d] As for *c*, but for 14 days. [e] As for *c* but for 11 days [f] As for *c* but for 28 days [g] For sample 18, sulfuric acid was used, in other cases hydrochloric acid was used.

Equivalent weights of each sample were measured, and these are listed in Table II. An attempt was made to correlate these values with the intensities of absorption bands at 1755–1745 cm^{-1}. The equivalent weights fell in the range 2,200–2,500, except for those of samples 17 and 18, which were higher. On the other hand, the equivalent weights of the samples 9 and 10 were substantially lower. From these facts, it is assumed that the polarity of the reagent used for precipitation has an essential effect on the physicochemical properties of the polysaccharides prepared. The low equivalent weight of the sample 10 could be explained by the following ion-exchange: $-CO_2^-M^+ + Na^+ \rightarrow -CO_2^-Na^+ + M^+$, because this polysaccharide had been first treated with sodium hydroxide and then precipitated. The Na^+ ion is easily replaceable by H^+ ion. The variations in equivalent weights have no appreciable effect on the intensity of absorption bands in the region studied.

TABLE II

ABSORPTION BANDS OF POLYSACCHARIDES

Sample	*Bands at (cm^{-1})*[a]						*Equivalent wt.*
1	1625				1740 (sh)	—	—
2		1650		1670 (sh)		1745	2225
3		1650		1670 (sh)		1745	2330
4		1655		1670 (sh)		1745	2285
5		1650				1745	2275
6		1650		1670 (sh)		1748	2370
7		1650				1745	2375
8		1650		1670 (sh)		1745	2475
10		1650		1680 (sh)		1745	1680
11	1625				1740 (sh)	—	—
13		1650				1745	2515
14		1655		1670 (sh)	1720 (sh)	1748	2450
15		1645	1660 (sh)	1670 (sh)	1720 (sh)	1750	2245
16		1645	1660 (sh)	1670 (sh)	1720 (sh)	1745	2445
17		1650	1660 (sh)	1670 (sh)	1730 (sh)	1755	3640
18		1645			1730 (sh)	1750	3075
9		1652				1745	1380
12	1625					—	—

[a] sh = shoulder.

EXPERIMENTAL

Preparation of polysaccharide samples. — The crude gum (300 g), collected in the orchard at Jahodná (South Slovakia), was dissolved in 6 l of distilled water and kept for 24 h at room temperature. The dense, viscous solution was blended in a Turmix blender and filtered through fine, sintered glass (G-3). The solution was made up with water to 3% of polysaccharide. From this stock solution, 200 ml was taken for precipitation of each sample (numbers 1–18), as listed in Table I. Precipitants used were ethanol, methanol, and acetone.

The precipitated samples were washed first with the diluted, acidified precipitant (except for samples 1, 11, and 12), and then with diluted, neutral precipitant until free from chloride ions. The ratio of water–ethanol used was the same as for the precipitation. Further washing was performed with 96% ethanol and finally with ether, to give a fine powder. Each sample was dried at 25°/10 mtorr.

Equivalent weights were determined by titration of the polysaccharides with 0.1M sodium hydroxide with an automatic titrator TTT 1C to an endpoint at pH 7.5.

For recording the infrared spectra, KBr pellets were prepared from 3 mg of polysaccharide. Spectra were recorded with a Zeiss UR-10 spectrometer.

Analysis of sample 1 gave the following data: OMe 4.53% (Zeisel); N absent

(Dumas and Kjeldahl methods); sedimentation constant (determined with an ultracentrifuge model MOM G110) $s = 7.4 \times 10^{-13}$, mol. wt. $= 7.1 \times 10^5$.

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