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INFRARED SPECTROSCOPY STUDY OF *Gleditsia macracantha* SEEDS

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Seeds of Gleditsia macracantha *were shown to contain a protein—carbohydrate complex using IR spectroscopy. It was found that the carbohydrate composition was heterogeneous and included a neutral polysaccharide and a carboxypolysaccharide containing acetyl, ester, and ionized carboxylic groups.*

Keywords: water-soluble polysaccharide, potential energy distribution (PED), stretching and bending vibrations, esters, carboxylic groups, symmetric and asymmetric vibrations.

We studied previously the water-soluble polysaccharides (WSPS) from *Gleditsia macracantha* Desf. seed coating and found that they were galactomannans [1, 2]. Chemical and spectral methods showed that the principal chain of the galactomannan consisted of β-1,4-bound mannopyranose units where several mannose units were substituted in the 6-position by α-D-galactopyranose [3, 4].

The goal of the present work was to analyze directly using IR spectroscopy the fractional carbohydrate composition of the seed coating of *G. macracantha* and to compare the results with those from previous basic research. Considering the composition of the cell walls of the plant seeds and fruit, bands associated with vibrations of polysaccharides, protein, and lignin should appear in their IR spectra [5, 6]. Spectra were recorded in the range 700–1900 cm^{-1}. The range 400–700 cm^{-1} was not examined because it does not contain useful information owing to the presence of highly overlapping weak bands on a background of absorbed water vibrations. Characteristic bands for CH (2650, 2915 cm^{-1}) and OH (broad band at 3360 cm^{-1}), which are not a characteristic signature of the studied compounds and are observed in all organic compounds containing these groups, appeared in the region above 1900 cm^{-1}.

Up to 21 bands (Fig. 1a, 1) could be distinguished in the most informative region of the seed coating spectrum (fingerprint) at 700–1900 cm^{-1}. An overlapping group of very strong bands (1–3) at 766, 775, and 812 cm^{-1} was assigned to hydroxyl torsional vibrations τ(OH) and CCO and COC bending vibrations of pyranose rings [8–11]. Two groups of very weak bands (4–6) at 876, 900, and 915 cm^{-1} and very strong bands (7–11) at 965, 1020, 1070, 1100, and 1150 cm^{-1} were observed in the range 850–1200 cm^{-1}. Both groups were assigned to CC and CO vibrations of pyranose rings in polymer chains. The band shape of this group was characteristic of each polysaccharide and could be used as a signature [7]. Band (11) at 1150 cm^{-1} made the principal contribution to the potential energy distribution (PED) of coordinates C(1)O(5) and C(5)O and was observed in spectra of all polysaccharides [8–11]. It could be used as an internal standard for quantitative measurements. Bands for COH, OCH, and CCH bending vibrations of the polysaccharides (bands 12–18) and complex vibrations of hydroxyl, carboxylic ester, and acetyl groups appeared in the range 1200–1500 cm^{-1}. Let us examine separately these bands and their contributions to the PED because the state of these groups has a substantial effect on the polysaccharide properties.

Carboxylic group COOH [12–14]. Complex vibrations: 1250 cm^{-1} C(6)OH, CO, CC, C=O; 1399 C(6)OH, CO, CC=O, CC; 1740 C(6)=O, CO, COH.

Vibrations of C(6)OH and C(6)=O groups make the principal contribution to the PED according to calculations [12]. The other groups contribute less.

Ester COOCH$_3$ [13, 14]: 1225 cm^{-1} $\delta(COC)_E + \nu(CO)_E$; 1375 $\delta_s(CH_3)_E$; 1460 $\delta_{as}(CH_3)_E$; 1735 $\nu(C{=}O)_E$.

Acetyl OCOCH$_3$ [15]: 1240 cm^{-1} $\nu(CC,CO)_a + \delta(OCO,CCO)_a$; 1735 $\nu(C{=}O)_a$.

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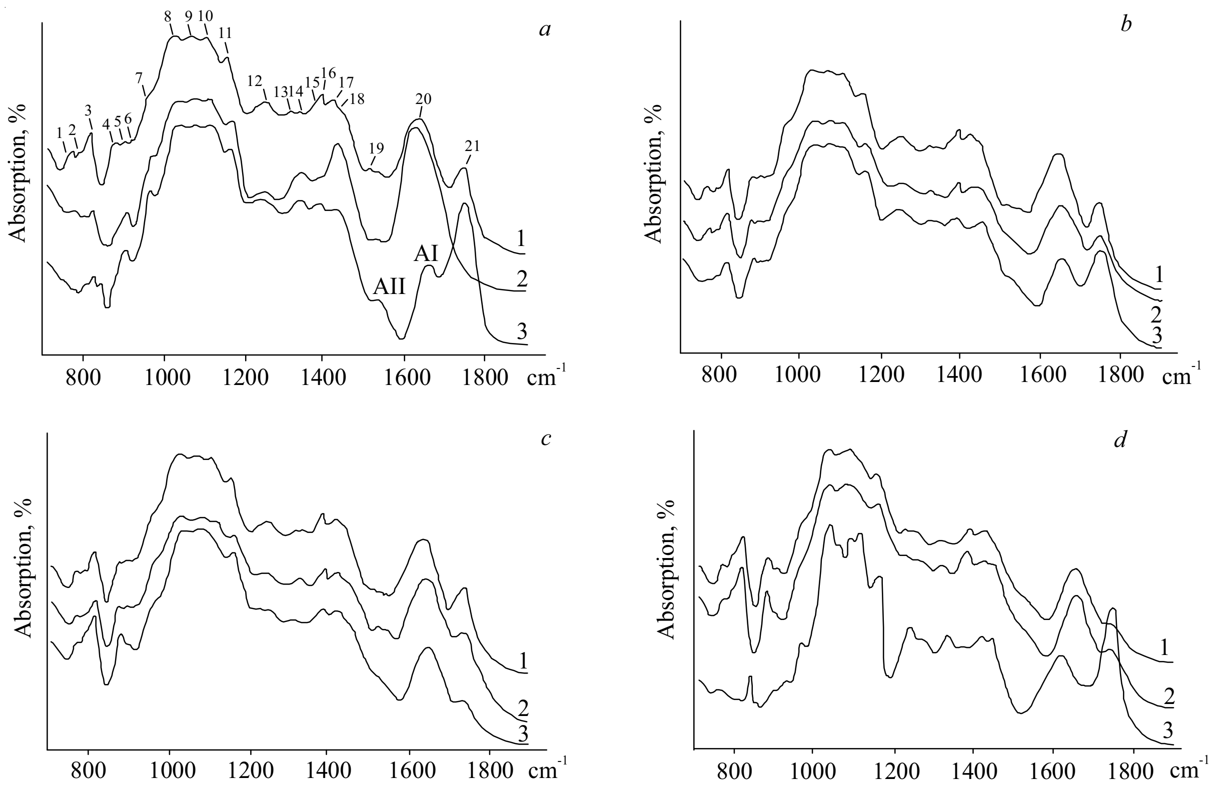

Fig. 1. IR spectra of *G. macracantha* seed coating: seed coating (1) (a, b, c, d); after $Ca(OH)_2$ treatment (2), after subsequent HCl treatment (3) (a); after $CaCl_2$ treatment (2), after subsequent HCl treatment (3) (b); fraction insoluble in water (2), fractions forming colloidal solution (3) (c); colloidal fraction after HCl treatment (2), apple pectin (3) (d).

Ionized carboxylic group COO^- [16], the carboxylic group H atom is replaced by a metal cation: 1430 cm^{-1} $\nu_s(COO^-)$; 1620 $\nu_{as}(COO^-)$.

A weak band for water bending vibrations $\delta(HOH)$ appeared near 1630 cm^{-1}.

Protein polypeptide bond vibrations [17, 18]: 1560 cm^{-1} $\delta(NH) + (CN)$ – amide II (A II); 1650 $\nu(C{=}O) + \nu(CN) + \delta(NH)$ – amide I (A I).

A group of characteristic bands in the range 1400–1600 cm^{-1} for vibrations of lignin benzene rings and conjugated double bonds did not appear in the spectrum because of their low content [19].

The state of the functional groups of seed coating polysaccharides was studied using IR spectroscopy. Band 1.21, which was a superposition of $\nu(C{=}O)_H$, $\nu(C{=}O)_E$, and $\nu(C{=}O)_a$, disappeared upon treatment of seed coating powder with aqueous $Ca(OH)_2$. Two strong bands for $\nu_{as}(COO^-)$ at 1620 cm^{-1} and $\nu_s(COO^-)$ at 1430 appeared in the spectrum (Fig. 1a, 1 and 2). Thus, the alkaline solution hydrolyzed acetyl and ester groups. The group of strong bands (7–11) did not change because the structure of the polymer chain was not changed (Fig. 1a, 1 and 2). The supernatant liquid became viscous because the galactomannan was extracted. Subsequent treatment with HCl in EtOH (70%, pH 2) of the insoluble part caused bands associated with vibrations of COO^- groups to disappear (Fig. 1a, 3) and a band at 1750 cm^{-1} for $\nu(C{=}O)_H$ to appear. Two bands of the polypeptide bond A I at 1650 and A II at 1540 were clearly visible.

Seed coating powder in $CaCl_2$ solution formed a colloidal solution. Therefore, the powder was treated with $CaCl_2$ in EtOH (70%). The action of $CaCl_2$ did not change the spectrum of seed coating (Fig. 1b, 2). Consequently, the seed coating did not contain free carboxylic groups and band (21) was a superposition only of $\nu(C{=}O)_E$ and $\nu C{=}O)_a$ groups.

Treatment of seed coating with HCl in EtOH (70%) caused band 21 to grow stronger upon substitution of the metal by H in the carboxylic groups (Fig. 1b, 3). This meant that the carboxylic groups of the seed coating carboxypolysaccharides were present in the salt form.

A precipitate of insoluble particles formed upon prolonged standing of the colloidal aqueous solution. Figure 1 shows that spectra of the seed coating and the insoluble precipitate were similar (1c, 1 and 2) and that they differed from the spectrum of the gel obtained by adding EtOH to the dissolved part. Band 10 was missing and band 21 was very weak in the spectrum (1c, 3). Therefore, the fractions had different structures for the polysaccharide chains. The colloidal fraction, in contrast with the insoluble part, was a group of neutral polysaccharides. This was confirmed by the fact that the spectrum of gel treated with HCl in EtOH (70%) did not change substantially (Fig. 1d, 1 and 2). The spectra (1d, 1 and 2) differed considerably from that of apple pectin (1d, 3).

Thus, the results led to the conclusion that seed coating of *G. macracantha* contained a protein–polysaccharide complex. The carbohydrate composition was heterogeneous and contained a neutral polysaccharide and carboxypolysaccharide with acetyl, ester, and ionized carboxylic groups.

EXPERIMENTAL

Raw Material Preparation. *G. macracantha* seeds (10 g) were separated from the germ. Seed coating was ground in a ball mill to particle size $\varnothing \leq 0.125$ mm. Samples for IR spectroscopy were mulled to produce an even distribution in KBr pellets.

IR Spectra of *G. macracantha* Seed Coating. Spectra were recorded on a Specord M-80 dual-beam spectrometer (Carl Zeiss, Jena) in the range 700–1900 cm^{-1}. Seed coating powder was immersed for 1 h in $Ca(OH)_2$ solution (0.5 M) in EtOH (70%). The spectrum was recorded after the alkaline hydrolysis.

A sample was treated analogously with HCl (0.1 M) in EtOH (70%), after which the spectrum was recorded. Treatment with $CaCl_2$ (0.5 M) in EtOH (70%) was carried out in the same manner.

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