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POLYSACCHARIDES OF SAPONIN-BEARING PLANTS. XII. POLYSACCHARIDES OF *Acantophyllum knorringianum* AND THEIR BIOLOGICAL ACTIVITY

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The physicochemical properties and qualitative and quantitative monosaccharide composition of Acantophyllum knorringianum *pectin were determined. It has been found using periodate-nitric-acid oxidation and IR spectroscopy that galacturonan is a polymer consisting of* α*-1→4-bonded D-galacturonic acid units. The isolated polysaccharide from roots had immunostimulating activity.*

Key words: *Acantophyllum knorringianum*, pectin, carbohydrates, galacturonan, immunostimulating activity.

We previously reported on the investigation of carbohydrates of *Acantophyllum knorringianum* [Knorring spinyleaf, Caryophyllaceae (Pink)] [1]. In continuation of the study of polysaccharides of this plant, we analyzed the pectin and determined the biological activity of water-soluble polysaccharide from the roots.

Pectin isolated from the aerial part by the literature method [1] is a grayish-white flocculent powder that is insoluble in most organic solvents and dissolves in DMSO, formamide, and water to give viscous solutions. The yield of pectin is 5.4%, mp 250-300°C (dec.), $[\alpha]_D^{30}$ +140° (*c* 0.1, H_2O), η_{rel} = 1.3 (*c* 0.2, H_2O). The molecular weight according to sedimentation is 38,000. The galacturonic anhydride content is 41% [2]. Comparison of the IR spectrum of the pectin (see Experimental) with that in the literature [3, 4] suggests that α-glycoside bonds dominate in the pectin.

The quantitative properties (%) obtained by titration [5] were: free carboxylic groups K_f = 7.2, methoxylated carboxylic groups K_e = 15.3, degree of esterification λ = 68. Therefore, the pectin is considered to be highly esterified.

Total acid hydrolysis of the pectin produces D-galacturonic acid and the monosaccharides rhamnose, arabinose, xylose, glucose, and galactose in the ratio 1.2:1.4:1.3:1.2:1.0 according to paper and gas chromatography.

Partial acid hydrolysis of the pectin produced galacturonan consisting of only D-galacturonic acid units. The galacturonan, like the starting pectin, has a high positive specific rotation $[\alpha]_D$ +170° (*c* 1.0, 10% NH_4OH). Therefore, it can be assumed that the glycoside bond between galacturonic acid units in the pyranose form has the α-configuration.

The IR spectra of the starting pectin and galacturonan, which contain absorption bands at 857 and 831 cm^{-1}, are consistent with this.

Absorption bands at 1642 and 1638 cm^{-1} are assigned to stretching vibrations of the carboxylic acid carbonyls.

Furthermore, the starting pectin exhibits absorption bands at 1233 and 1270 cm^{-1}, corresponding to ester CO groups.

The frequencies of the most characteristic vibrations in the IR spectra of the pectin and galacturonan were assigned by consulting the literature.

Successive oxidation by periodic and nitric acids of methoxylated galacturonan revealed the type of bonding between the galacturonic acid units [6]. The production of tartaric acid as the main oxidation product and IR spectroscopy (triplet for pyranose rings) are consistent with an α-1→4 bond between the galacturonic acid units. The side chains consist of rhamnose, arabinose, xylose, glucose, and galactose units.

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The results have shown that pectin of *A. knorringianum* is a highly esterified polysaccharide that differs from pectin isolated from the aerial part of other representatives of the Caryophyllaceae family, in particular, *Allochruza gypsophyloides*, with respect to the composition and ratio of monosaccharides, molecular weight, and degree of esterification.

Polysaccharide from the plant root was isolated by the literature method [1]. The resulting polysaccharide was a white or yellowish-white amorphous powder that was odorless and soluble in water. It gave a negative reaction with iodine—starch. The monosaccharide composition includes galactose and arabinose according to paper and gas chromatography. The polysaccharide has a high positive specific rotation $[\alpha]_D$ +150° (*c* 1%, H_2O). The IR spectrum contains absorption bands at 3470, 3350, 2935, 1655, 1350, 1079, 875, and 799 cm^{-1}.

The immunomodulating properties of the polysaccharide from *A. knorringianum* were studied as before [6]. This enabled a quantitative estimate of the complex process of forming antibodies to B-lymphocytes and a quantitative determination of antibody-forming cells (AFC) in spleens of immunized animals.

The experiments were performed on generic white mice of mass 18-20 g.

Sheep erythrocytes (SE) were used as antigens. Mice were immunized i.p. with a 30% suspension of SE in 0.2 mL to set up the Erne reaction. The AFC in the spleen were determined four days after immunization at the peak of the immune response.

The results showed that 16,480 ± 741.9 AFC accumulated in the spleen of control mice. Calculated per 1×10^6 nucleus-containing spleen cells, its quantity was 282.0 ± 10.11.

The studied preparation in doses of 50 and 100 mg/kg caused the AFC to increase to 29,600 ± 4793.2 and 58,480 ± 298.4 (stimulation of 1.80 and 3.55 times, respectively).

Calculated per 1×10^6 nucleus-containing spleen cells, the AFC counts at the corresponding doses were 573 ± 19.57 and 964 ± 15.11, respectively (stimulation by 2.03 and 3.42 times).

Thus, the studied preparations are low-toxicity compounds that act at both tested doses as immunostimulants for antibody formation in mice.

EXPERIMENTAL

IR spectra were recorded on a Perkin—Elmer Model 2000 IR-Fourier spectrometer in KBr pellets (5 mg pectin per 200 mg KBr).

Specific rotation was measured on a Golw polarimeter for l = 1 dm at 22°C.

Viscosity was determined using the flow time of 0.2% solutions in water through a 0.73-mm capillary in a VPZh viscometer at 23 ± 5°C.

Sedimentation analysis was performed in a MOM-3170 ultracentrifuge. Sedimentation curves were obtained for c = 10 mg/mL in water, 50,000 rpm, 20°C, and 5 min scan times. The results were $S = 3.35\times10^{-13}$ and $D = 9.07\times10^{-7}$.

The quantitative properties of the pectin were determined by titration [5]; chromatographic analysis and paper and gas chromatography, by the published methods [1].

Solutions were evaporated in a rotary evaporator at 40°C.

Pectin Isolation. Air-dried raw material (100 g) was treated with water at room temperature. Pectin was extracted by buffer at pH 7.5 [0.5% $(NH_4)_2C_2O_4$ in 0.5% $H_2C_2O_4$] at 70°C for 6-8 h at 1:15-1:10 ratios. The extract was concentrated. The pectin was precipitated by alcohol. Yield, 5.4 g.

IR spectrum (ν, cm^{-1}): 3600-2500, 1642, 1399, 1270, 1233, 1106, 857, 721, 679, 503, 494.

Total acid hydrolysis of the pectin and galacturonan was performed in H_2SO_4 (2 N) at 100°C for 12 h.

Partial Hydrolysis of Pectin. A solution of pectin (1 g) in H_2SO_4 (150 mL, 0.5 N) was heated to 100°C for 4 h. The precipitate was separated by centrifugation, washed with aqueous alcohol and alcohol, and dried. Yield of galacturonan, 0.3 g.

IR spectrum (ν, cm^{-1}): 3600-2500, 1742, 1638, 1327, 1147, 1101, 1014, 948, 831, 586, 459.

Preparation of Partially Methoxylated Galacturonan. A suspension of galacturonan (1 g) in dry HCl in absolute MeOH (20 mL, 5%) was boiled on a water bath for 7 h. The methoxylated galacturonan was separated by centrifugation, washed with alcohol and acetone, and dried. Yield, 0.38 g.

Oxidation of partially methoxylated galacturonan after acid hydrolysis produced oxalic and tartaric acids [7].

The acute toxicity of the pure polysaccharides was determined in experiments on white mice of both sexes of mass 21 g. The compounds were administered as aqueous solutions in doses of 1000, 2500, and 5000 mg/kg. The results showed that the behavior and mortality of the mice did not change noticeably 14 days after administration of the preparations.

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