

On the basis of the results of methylation, Smith degradation, partial hydrolysis, GLC-MS, and ^{13}C NMR spectroscopy a partial structure has been suggested for the repeating unit of the O-specific polysaccharide of the lipopolysaccharides of the serovars investigated.

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CHEMICAL INVESTIGATION OF BIOMASS OF A CULTURE OF GINSENG CELLS

III. POLYSACCHARIDES OF A CALLUS CULTURE OF GINSENG

T. F. Solov'eva, V. A. Khomenko,
N. I. Uvarova, N. A. Konstantinova,
V. S. Faustov, and G. B. Elyakov

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A water-soluble polysaccharide fraction has been isolated from a callus culture of ginseng with a yield of 16-20%. It has been shown that it included starch and acidic polysaccharides - arabinogalactans and a xyloglucan.

Chemical investigations of the polysaccharides of ginseng that began in the 1960s [1, 2] have been taken up again after a long interval [3, 4]. The renewal of interest in this subject is apparently due in part to advances in the field of ginseng tissue culture which permit the growth of the cell mass of ginseng by an industrial method.

We have isolated a polysaccharide fraction from a culture of ginseng tissue with the aim of the further study of its biological properties. The water-soluble polysaccharides of the cell were isolated by successive extraction with water, ammonium oxalate, and sodium carbonate. The yields and characteristics of the high-molecular-mass fractions obtained are given below (%):

Solvent	Yield	Monosaccharides	Uronic acids	Protein	Ash
Water	16.8	43.4	10.7	2.5	3.2
Oxalate	5.9	31.8	18.9	1.7	2.9
Sodium carbonate	4.9	24.2	8.2	59.5	5.1

Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Branch, USSR Academy of Sciences, Vladivostok, All-Union Scientific Research Institute of Biochemical Machinery Design, USSR Ministry of the Medical Industry, Moscow. Translated from *Khimiya Prirodnkh Soedinenii*, No. 6, pp. 771-772, November-December, 1989. Original article submitted January 11, 1989; revision submitted June 8, 1989.

The high-molecular-mass fractions from the aqueous and oxalate extracts consisted of polysaccharides, while the alkali, somewhat unexpectedly, extracted mainly proteins.

The qualitative monosaccharide composition of the water-soluble polysaccharides was determined. Among neutral monosaccharides, galactose and arabinose predominated and, in addition to these, there were glucose, xylose, and rhamnose. A similar monosaccharide composition of the polysaccharides of a ginseng tissue culture has been reported recently [5].

It must be mentioned that on repeated successive extraction of the cells with water it was possible to observe an increase in the amount of arabinose in the extracted polysaccharide fractions. On the subsequent treatment of the cells with ammonium oxalate, an acid polysaccharide was extracted in which arabinose predominated considerably over other neutral monosaccharides. This gives grounds for assuming the presence in the polysaccharide fraction of ginseng of several acidic arabinogalactans differing by their ratios of arabinose and galactose residues. So far as concerns glucose, it was present, at least partially, as a component of starch. In actual fact, the water-soluble polysaccharide gave a coloration with iodine. When a solution of iodine in potassium iodide was added to a solution of the polysaccharide an iodine-glucan complex precipitated. The polysaccharides included uronic acid residues, but their amount was comparatively low even in the polysaccharide extracted by ammonium oxalate. This distinguishes the pectins of a callus ginseng culture from classical pectins.

To investigate the composition of the water-soluble polysaccharides they were fractionated on DEAE-cellulose. When they were separated on an ion-exchanger three fractions were obtained the analysis of which permitted the conclusion that the polysaccharides extracted by water from the biomass of ginseng cells of the strain under investigation included starch and at least two acidic polysaccharides - an arabinogalactan and a xyloglucan. As is known, acidic arabinogalactans are widely represented in ginseng roots [2-4]. However, only structural investigations will permit the determination of the extent to which the polysaccharide detected in the roots and a tissue culture of ginseng are related. So far as concerns the xyloglucan, no such polysaccharides have hitherto been detected in ginseng roots.

EXPERIMENTAL

The biomass of a commercial strain of ginseng cultivated by the surface method in the Kirov biochemical factory was investigated.

The carbohydrates were determined by the method of Dubois et al., [6], protein by Lowry's method [7], and the amount of uronic acid by that of Anderson [8]. Monosaccharides were identified with the aid of GLC in the form of polyol acetates on a Pye-Unicam chromatograph with glass columns filled with: a) 3% of ECNSS-M on the support Gas-Chrom Q (100-200 mesh) at 190°C; and b) 3% of QF-1 on the same support at 160-210°C, 5 deg/min. Chromatography on DEAE-cellulose was carried out as described in [2].

Isolation of the Polysaccharide Fraction. The biomass, after treatment with methanol was extracted successively on the boiling water bath with water, 0.5% ammonium oxalate solution, and 10% sodium carbonate solution. The high-molecular-mass fractions were precipitated from the extracts with ethanol and were dissolved in water and lyophilized.

SUMMARY

1. Fractions of high-molecular-mass compounds have been isolated from a callus culture of ginseng by extraction with water, ammonium oxalate, and sodium carbonate and have been analyzed.
2. The yield of water-soluble polysaccharides amounted to 16-20% of the weight of the tissue that had previously been extracted with methanol.
3. It was found that the polysaccharides extracted by water from the ginseng biomass included starch and acidic polysaccharides - arabinogalactans and a xyloglucan.

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PECTIN SUBSTANCES OF THE MULBERRY

K. K. Kasymalieva, A. A. Khidoyatov,
and Z. Dzh. Ashubaeva

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Several variants of the isolation of pectin substances from mulberry boughs are considered.

In the present paper we consider several variants of the isolation of pectin substances from mulberry (*Morus*) boughs - wastes from silkworm rearing - which can be used as a source of gel-forming polymers.

In order to ensure the maximum yield of acidic polysaccharides, the raw material was hydrolyzed by the freezing method described in [3]. Such factors as the pH of the medium, the time and temperature of hydrolysis were varied.

In the first stage, two-hour hydrolysis of the plant raw material was carried out by the method generally adopted [1, 2] at 90°C. The yield of pectin material amounted to 1.38%. Analysis of its functional composition showed (Table 1) that the product obtained consisted of a weakly methoxylated pectin (16.2% of -COOH groups and 1.1% of -OCH₃ groups) containing 64.8% of D-galacturonic acid (GA) according to the carbazole method [6].

Experiments on the isolation of the pectin by the freezing and hydrolysis of the plant raw material showed that the temperature parameters exerted an influence on the yield of pectin, which reached its maximum values at 80-90°C and pH 1.2 (see Table 1).

The pectin obtained by precipitation with acetone contained 3.5-3.8% of ash, which affected the absorption band of the stretching vibrations of the carboxylic C=O groups. The amount of D-galacturonic acid determined by a titrimetric method [5, 6] was between 57 and 58% (see Table 1).

When the pH of the reaction mixture was changed to 1.2, the temperature dependence of the yields of pectin likewise changed and its maximum was found at a temperature of about 90°C (see Table 1). Functional-group analysis showed that the pectin obtained was a weakly methoxylated product. With a rise in the temperature of hydrolysis the amount of D-galacturonic acid increased, which was possibly due to the saponification of the L-arabinofuranosyl residues of the main chain of the rhamnogalacturonan. With a further rise in the temperature of hydrolysis the degradation of the polygalacturonan macromolecule itself increased still more.

An investigation of the influence of the pH of the hydrolyzing reagent on the yield and functional composition of the pectin from mulberry boughs showed that the highest yield pectin (4.2-4.7%) was observed under the following experimental parameters: time of freezing at -20°C 30 min; hydrolysis-extraction of the pectin at 80-90°C for 60 min; pH of the medium 1.2. The amount of polyuronide fraction and the level of D-galacturonic acid in the preparation fell, in the main, with a rise in the pH of the medium.

In view of the absence of literature information on the carbohydrate composition of the pectin isolated from mulberry boughs, we first investigated the carbohydrate composition of

Institute of the Chemistry of Plant Substances, Uzbek Academy of Sciences, Tashkent.
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