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POLYSACCHARIDES OF CULTURES OF PLANT TISSUES.

I. PROPERTIES AND PARTIAL STRUCTURE

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UDC 581.143.6:547.917

Information is given on the structure and properties of the polysacharides (PSCs) isolated from the biomass of cultures of rose, mint, poppy, tobacco, ginseng, rose-root stonecrop, and yam using titrimetric, chromatographic, and IR and ^{13}C NMR spectroscopic methods. The monocarbohydrate compositions of the hydrolysis products have been established and methods for the practical utilization of these PSCs have been planned.

The method of obtaining biologically active compounds by cultivating plant tissues and cells is finding ever-increasing practical use. Aqueous alcoholic extracts of the biomass of cultures of ginseng and rose-root stonecrop, which possess physiological activity, are being obtained under factory conditions (Kirov, Volgograd, Kiev, etc.). A number of articles of national consumption (the "Lesnaya nimfa" ["wood nymph"] series of creams and the foaming and wetting agents "Diona" and "Iya") based on preparations from ginseng biomass have been developed and are being mass-produced.

The creation of waste-free technologies is impossible without a complex investigation of the biomass of plant cultures which, together with the desired products — glycosides, alkaloids, and essential oils— also contain other valuable substances. These are represented primarily by the carbohydrate fractions of such cultures as ginseng, rose-root stonecrop, yam, poppy, tobacco, rose, and mint. In our opinion, the wastes of these cultures can serve as a raw material for obtaining fodder proteins, nutrient substances for the microbiological industry, (after suitable treatments), etc. A comparison of the structure and properties of the polysaccharides (PSCs) of fragments of cultures of tissues and of native plants will permit a more accurate idea of the nature of carbohydrate metabolism in both cases.

We have previously described the PSCs of a whole series of essential-oil plants [1-4], and in the present communication we give the results of a study of the carbohydrate fragments of cultures of the tissues of these plants.

The PSCs of the fractions from the biomasses investigated, after the elimination of the biologically active substances and lipids, were extracted with water, oxalate buffer, and aqueous HCl solution. The PSCs were precipitated by various methods: with salt solutions, by electrolysis [5], and with alcohols. The best results were obtained in the last case

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M. V. Frunze Simferopol' State University. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 343-346, May-June, 1988. Original article submitted July 21, 1987; revision submitted November 11, 1987.

TABLE 1. PSC—OSC—MSC Ratios and Titrimetric Analyses of the Main Functional Groups of the PSCs

Biomass	Extractant	PSC—OSC—MSC ratio	Results of analysis, %				
			K_c	K_e	K_o	λ	CH_3O
Rose	Oxalate buffer	7,3:1,9:1,0	16,67	14,06	30,73	45,7[illegible]	9,70
Mint	"	8,9:4,5:1,0	12,98	54,00	66,98	80,62	37,26
Poppy	"	1,5:1,2:1,0	6,75	16,20	22,95	70,88	11,16
Tobacco	"	1,4:1,0:1,1	18,14	18,45	36,59	50,42	12,71
Yam	"	2,9:1,0:1,1	1,58	63,00	64,56	97,56	43,40
Ginseng	Water	1,5:1,0:1,4	2,25	19,19	21,44	89,50	13,22
Ginseng	Oxalate buffer	2,6:1,0:2,2	2,25	23,69	25,94	91,32	16,32
Stonecrop	Water	1,7:1,0:1,2	2,70	12,50	14,85	84,91	8,37
Stonecrop	Oxalate buffer	2,4:1,0:1,5	2,99	42,14	46,13	93,37	29,02

PSC — polysaccharides; OSC — oligosaccharides; MSC — monosaccharides.

(preliminary acidification of the alcohols to pH 3.0-3.5 with concentrated HCl). The precipitates were separated off by centrifugation and the precipitation operation was repeated three times, after which the material was dialyzed and freeze-dried.

The compositions of the extracts and the products of the subsequent hydrolysis of the PSCs were studied with the aid of a Technikon automatic carbohydrate analyzer. Information on the amounts of mono-, oligo-, and polysaccharides is given in Table 1.

In the fruit of the yam, the water-soluble fraction was distinguished by a low PSC content, while for ginseng the amounts of PSCs and monosaccharides (MSCs) were fairly close. The maximum amount of PSCs and the absence of [sic; cf. Table 1] MSCs was characteristic for mint. Oxalate extracts retained approximately the same ratio of PSCs to MSCs for ginseng; in the case of the yam, stonecrop, and mint the relative amount of PSCs rose considerably. We subsequently studied only the PSCs of the fragments of the biomass of the plant cultures.

The PSCs consisted of pulverulent light-colored fibrous substances practically insoluble in organic solvents and soluble to limited degree in water (even 1% solutions gave stable gels), melting with decomposition at 260-320°C. The molecular weights determined by the viscometric method [5] ranged between 18,000 and 25,000 c.u.

The results of determinations of the main functional groups of the PSCs by a modified titrimetric method [6, 7] are given in Table 1.

The PSCs of the yam, ginseng, and rose-root stonecrop are characterized by low levels of free carboxy groups (K_c) but fairly high degrees of esterification (λ = 91-97%). In the case of poppy, mint, rose, and tobacco tissues K_c was considerably higher (17-18%) and λ was in the range of 46-81%.

A comparison of the IR spectra of the PSCs of the biomass of tissue cultures that were being studied with the spectra of apple pectin showed that they were fairly close, differences being expressed only in the intensities of the main characteristic bands (free and esterified carboxy groups), and our results agree well with those from the IR spectroscopy of pectins by M. P. Filippov and other authors [8-10] (Table 2).

The presence of pyranose rings was confirmed by vibrations in the 1000-1100 cm^{-1} region, while at 756-930 cm^{-1} there was a superposition of a number of weak bands and it was impossible to determine configurations unambiguously from the spectra. In the case of the yam PSCs, certain differences are observed which are obviously due to the presence of considerable amounts of accompanying substances.

The monocarbohydrate compositions of the PSCs were studied by partial (2-5 h) and complete (16-20 h) acid hydrolysis (2 N HCl, 100°C). Analysis was carried out with the aid of the paper, ion-exchange, and gas-liquid varieties of chromatography [11-13].

The results obtained (Table 3) show that the PSCs of tissue cultures differ from one another both in the nature of their monocarbohydrate inclusions and with respect to their ratios.

TABLE 2. Frequencies of the Vibrations in the IR Spectra of the PSCs of plant Tissue Cultures (cm^{-1})

PSC	1*	2	3	4	5	6	7	8	9	10
Rose	840, 900	1015, 1040, 1060		1230	1320	1420	1610	1740	2900	3400
Mint	820, 870	1000, 1010, 1070	1150	1230	1320	1410	1610	1720	2850	33
Tobacco	840	1040—1060		1230	1320	1370, 1450	1650	1760	3100	3400
Poppy	840, 880	1020—1070		1250	1320	1370, 1450	1640	1730	2800	3350
Ginseng	820, 880	1000, 1030, 1050, 1070		1250	1320	1370, 1420	1620	1740	2850	3350
Stonecrop	810, 870	1010, 1040, 1080	1140	1230	1320	1380, 1415, 1530	1620	1740	2850	3350
Yam	820—840, 890	1000 broad	1140	—	1310	—	1620	—	2800	3300
Apple pectin	830, 910	1000, 1030, 1060, 1085		1230, 1260	1320	1370, 1410, 1430	1610	1740	2900	3400
Literature	840±5 880±10	1070—1140	1113, 1145	1222, 1272	1331	1378, 1425	1612, 1650	1735, 1750	2600 2937	3200, 3600

*1) α- and β-glycosidic bonds, $\gamma(OH)_a$; 2) $\nu(C-C)$ $(C-O)_r$ and ν, $\delta(C-OH)_a$; 3) $\nu(C-O-O)$; 4) $\delta(OH)_a$, $\delta(CH)_r$, $\delta(OH)_c$, $\nu(C-O-C)_e$; 5) $\delta(CH)_r$; 6) $\nu_s(COO^-)$, ν, $\delta(C-OH)_c$, $\delta_{as}(CH_3)_e$ $\delta_s(CH_3)_e$; 7) $\delta(H_2O)$, $\nu_{as}(COO^-)$; 8) $\nu(C=O)_e$, $\nu(C=O)_c$; 9) $\nu(CH)_r$, $\nu(CH)_a$, $\nu(OH)_a$, $\nu(OH)_c$; 10) $\nu(OH)_a$, $\nu(H_2O)$. Here: ν — stretching vibrations; δ — deformation planar and γ — nonplanar vibrations: as — asymmetric; s — symmetric; c — carboxy groups; a — alcohol groups; e — ester groups; r — pyranose rings.

The ^{13}C spectra gave a clearer idea of the structure of the PSCs of ginseng and yam [14]:

1) the presence of a signal at 99.1 ppm (g) and 101.1 ppm (y), corresponding to C_1, and to one of a substituted carbon atom (78.3 and 79.0 ppm, respectively) indicated that the main chains of the PSCs were homopolumeric and had the α-D-configuration;

2) the presence of uronic acids was confirmed by signals in the 175.1 and 167.9 ppm regions, corresponding to carboxy groups;

3) the absence of signals in the 16.0-18.0 and 54.0-56.0 ppm regions permitted the assumption that the PSCs investigated had no 6-deoxysugar and methoxy groups, respectively; and

4) shifts in the 72.0-75.0 ppm region (comparison with the CH_3 groups of gluco- and galactopyranosides) in the case of the yam permitted us to consider that the main chain of the PSC consists of α-D-glucuronic acid residues, while the presence of hexapyranoses or pentoses was shown by a peak in the 62.0 ppm region. Consequently, yam PSCs have fairly appreciable inclusions in the chain, or persistent impurities, consisting of neutral sugars.

EXPERIMENTAL

Isolation of the PSCs. After preliminary extraction of the biologically active substances with 40-70% EtOH, the air-dry biomass was first treated with H_2O at 60-65°C (6 h) and was then extracted with a buffer of 0.25% $(NH_4)_2C_2O_4$ in 0.25% $H_2C_2O_4$ solution, pH 7.5, under the same conditions at a liquor ratio of 1:5-1:10. The extracts were concentrated in a rotary evaporator.

Purification. The PSCs of the fractions were precipitated with acetone, with calcium chloride, by electrophoresis, and with alcohols (MeOH, EtOH, iso-PrOH) previously acidified with concentrated HCl to pH 3.0-3.5. The last method gave the best results. The precipitates were separated off by centrifugation (3500-4000 rpm, 40-45 min), and the precipitation operation was repeated three times. Dialysis was carried out on Diaflo PM-10 and PM-30 membranes.

TABLE 3. Monocarbohydrate Compostitons and Ratios of the MSCs of Hydrolysates of the PCs of Plant Tissue Cultures

PSCs	Extraction conditions	Hydrolysis (2 N HCl 100°C) h	Ratio of MSCs					
			Rha	Ara	Xyl	Man	Gal	Glc
Rose	Oxalate buffer, 60°C	2	1,3	3,3	2,0	1,0	8,0	2,2
"	"	3	1,5	4,7	2,6	1,0	10,0	3,4
"	"	5	1,6	2,9	1,4	1,0	5,5	1,6
Mint	"	3	3 9	1,5	1,5	—	—	1,0
Yam	"	3	1,0	1,3	2,2	2,6	2,3	9,6
Ginseng	H_2O, 20°	3	3,0	10,8	1,0	—	—	1,0
"	H_2O, 60°	3	5,0	9,6	1,0	4,9	—	1,6
"	Oxalate buffer, 60°C	3	1,2	1,3	1,0	—	—	1,3

The carbohydrate fractions were analyzed with the aid of a Technikon automatic analyzer using DA-X4 ion-exchange resin (USA) with elution by sodium borate buffer (pH 9.0), 55°C, at a rate of 90 ml/h. The substances were detected with orcinol–H_2SO_4 (3% solution of orcinol in 90% H_2SO_4). The peaks were recorded under flow conditions at λ 420 nm.

Fractionation. The mono- and oligosaccharides were separated on columns (Sephadex G-50, Pharmacia) with elution by a buffer (10 ml of CH_3COOH, 4 ml of pyridine, 1 liter of H_2O; pH 4.5). The fractions were analyzed on the carbohydrate analyzer.

Molecular Weight [5]. The absolute viscosities of the PSCs were determined in a Winston viscometer, and the densities were determined pycnometrically.

Titrimetric Analysis [7]. The main functional groups were determined by a modified procedure [6].

Monocarbohydrate Composition [4]. Hydrolysis was carried out with 2 N HCl at 100°C in sealed tubes for 2-5 h (partial) and 16-20 h (total). The hydrolysates were neutralized, the precipitates were separated off, and the mother liquors were evaporated at 45°C in a rotary evaporator.

Chromatographic analysis was performed in strict accordance with the procedure described previously [4, A, B, C].

IR Spectroscopy. The spectra were recorded on an IR-75 instrument (GDR) in the 400-4000 cm^{-1} interval and on an IKS-22 instrument (USSR) in the 700-4000 cm^{-1} interval in the form of tablets (1 mg of PSC per 100 mg of KBr). The characteristic frequencies were assigned in accordance with the literature [8].

^{13}C NMR Spectroscopy. The lyophilized PSC fraction of a tissue culture (120-150 mg) was dissolved with stirring and heating (60-65°C) in 10 ml of H_2O and was freed from sparingly soluble inclusions in an ultracentrifuge (Beckman LS-65, V = 55,000 rpm; t = 5-10°C; vacuum of 100 mm Hg; time 60 min). The supernatant after purification was transferred to a flask, evaporated, and lyophilized. The dried PSC was transferred to a sagittal flask with a volume of 15-20 ml and was dissolved with heating (60-80°C) in 15 ml of D_2O, the mixture being left for 48 h for complete dissolution and then being stirred again with heating for 2-3 h. The viscous colloidal solution was transferred to an ampul. The spectrum was recorded on a Bruker Physik WP-60 spectrometer at 15.08 MHz with Et_3N (2-3%) as internal standard, at a temperature of 60°C.

SUMMARY

1. The PSCs from the biomasses of cultures of isolated mint, rose, poppy, tobacco, yam, rose-root stonecrop and ginseng tissues have been isolated and partially characterized.

2. The physical and physicochemical properties of these PSCs have been studied.

3. The monocarbohydrate compositions of the products of the acid hydrolysis of the PSCs have been established by chromatographic methods.

4. Pycnometric analysis, and IR and ^{13}C spectroscopy have permitted the assumption that the polymeric chains of the PSCs are constructed mainly of galacturonic acid residues with a predominance of α-1,4-glycosidic bonds.

5. In the case of the yam PSCs, the chain of the biopolymers obviously consists of glucuronic acid residues and is characterized by an appreciable (more than 5%) inclusion of neutral carbohydrates.

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POLYSCCHARIDES OF PLANT TISSUE CULTURES.

II. ACID HYDROLYSIS OF WASTES FROM A GINSENG TISSUE CULTURE

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UDC 581.143.6:547.917

The conditions of the acid hydrolysis of industrial wastes from a culture of ginseng tissue have been investigated and the optimum conditions — liquor ratio, concentration of acid (HCl and H_2SO_4) and the temperature have been determined. The ratio of monosaccharides has been established with the aid of gas-liquid chromatography. These hydrolysates can be used as nutrient media or additives for them in the microbiological industry.

We have previously given information on the properties and structure of the PSCs of some plant cultures [1]. In the present communication methods for the practical utilization of production wastes from a ginseng tiussue culture (biomass) are dissussed. One of the possible technological approaches to their processing is acid hydrolysis. PSCs are subdivided according to their rates of hydrolysis to readily and difficulty hydrolyzable and from the sequence arabinan > galactan > xylan > mannan > cellulose > polyuronides, which is due to the different stabilities of the corresponding glycosidic bonds in an acid medium.

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Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp 346-350, May-June, 1988. Original article submitted July 21, 1987; revision submitted November 11, 1987.