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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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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TABLE 1. Dependence of the Degree of Hydrolysis (%) on the Liquor Ratio and on the Time of Hydrolysis

Acid	Concentration	Liquor ratio					
		1:50	3:50	1:50	3:50	1:50	3:50
		time of hydrolysis, h					
		12		24		48	
HCl	0,5	14	16	39	41	40	41
	1,0	18	22	41	44	44	44
	3,0	26	26	51	52	53	55
	6,0	33	34	61	64	64	65
H_2SO_4	0,5	15	17	34	35	63	64
	2,0	25	24	46	46	47	48
	6,0	52	53	66	68	70	70

TABLE 2. Dependence of the Amount of MSCs on the Time of Hydrolysis and on the Temperature

Temperature, °C	Concentration, N	Amounts of monosaccharides (%) at the following times of hydrolysis, h					
		6	9	24	6	9	24
		HCl			H_2SO_4		
40	4,0	0,320	0,345	0,360	0,320	0,365	0,390
	6,0	0,460	0,520	0,580	0,450	0,570	0,610
60	2,0	0,310	0,405	0,530	0,395	0,500	0,580
	3,0	0,370	0,490	0,600	0,520	0,585	0,620
	4,0	0,515	0,595	0,610	0,535	0,610	0,660
	6,0	0,475	0,650	0,680	0,565	0,675	0,700
80	1,0	0,370	0,435	0,480	0,345	0,465	0,510
	2,0	0,480	0,550	0,550	0,435	0,545	0,680
	3,0	0,560	0,540	0,530	0,485	0,515	0,530
	4,0	0,585	0,540	0,500	0,540	0,535	0,480
	6,0	0,560	0,505	0,470	0,555	0,530	0,480

The choice of the conditions of hydrolysis is also affected by the stability with respect to acids of the monosaccharides separated on hydrolysis. Thus, on the hydrolysis of arabinans, heating with 0.01 N acid is sufficient for the cleavage of the arabinofuranoside bonds [2], while cellulose must be hydrolyzed by dissolving it in 72% H_2SO_4 and then diluting the solution with H_2O and heating for a long time at 100°C [3]. The acid hydrolysis of polyuronides is possible only under more severe conditions [4, 5], and then the uronic acids undergo decarboxylation. The majority of PSCs are exhaustively hydrolyzed by 2 N H_2SO_4 at 100°C in 4 h. The monosaccharides formed in the process of hydrolysis may react with substances of noncarbohydrate nature present in the plant raw material and also be cleaved with conversion into a furfural derivative, different MSCs taking part in such reactions to different degrees. The hydrolysis of the new raw material in order to obtain MSCs must therefore be carried out under the mildest possible conditions, which are selected for each concrete case. In the choice of the optimum conditions for the acid hydrolysis of PSCs of ginseng biomass, a detailed study was made of the influence of the liquor ratio of the reaction mixture, the temperature, and the concentration of the acids on the rate and completeness of hydrolysis. Analysis of the monocarbohydrate composition of the hydrolysates was performed by gas-liquid chromatography, which is the most informative method in this case [6-8],

It was established that the degree of hydrolysis does not depend appreciably on the liquor ratio of the reaction mixture within the interval from 1:50 to 3:50. Under the given conditions hydrolysis is almost complete in less than 24 h. An increase in the time of incubation of the reaction mixture did not lead to an appreciable rise in the degree of hydrolysis (Table 1). In view of this, further investigations were performed at a liquor ratio of the reaction mixture of 1:50 with incubation for 24 h.

TABLE 3. Amounts of MSCs in the PSCs Isolated from the Wastes of a Ginseng Tissue Culture on Hydrolysis by HCl and by H_2SO_4.

Temperature	Concentration, N	Acid	Amount of MSCs in the hydrolysate, %							
			degree of hydrolysis	total amount of MSCs, %	Rha	Rib	Ara	Xyl	Man	Glc	Gal
80	0.5	HCl	38.5	0.42	2,77	4,07	68,18	2,07	—	5.14	17,78
	1,0		41 5	0,48	2.89	2,49	49,90	4,80	3,83	7,53	28,56
	2,0		46,0	0,55	1.70	2,81	48,18	5,21	2.98	5,46	33,66
	0.5	H_2SO_4	41,5	0,51	2,42	5.65	59,55	1,51	—	7,24	23,62
	1,0		46,4	0,68	2,26	2,41	53,69	3.90	2.00	3,58	27,17
	2,0		54,0	0,53	3,41	3,05	44,38	6,91	1,50	10.41	30,34
115	0,5	HCl	54.1	0,65	6 52	3,31	33,08	4,53	3,81	19,46	28,83
	1,0		59,3	0,49	14,30	2,18	34,86	3,62	4,34	12,16	28,54
	0,5	H_2SO_4	69,0	0.71	8,22	2,52	35,23	9,56	4 40	13,21	26,85
	1,0		72,0	0,80	8,56	24,58	29,82	3,59	3 59	6,62	22,47

The temperature interval (40-115°C) and concentrations of acids (0.5-6.0 N) that were selected permit the general laws of the dependence of the efficacy of the hydrolytic process on these factors to be revealed. Table 2 gives information on the dependence of the degree of hydrolysis and amounts of MSCs on the temperature and the concentration of the acids.

Analysis of the results obtained shows that exhaustive hydrolysis was not achieved even under severe conditions (115°C, 6 N H_2SO_4, 24 h) and a further rise in the temperature led to an intensification of the degradation of the carbohydrates. Lowering the concentration of the acids to 0.5 N requires a considerable increase in the energy input (a temperature of 160-180°C) and will cause a more intense corrosion of the technological equipment. The use of concentrated acids (3-6 N) with subsequent neutralization of the hydrolysates requires a large consumption of alkalis and complicates the process of filtration in the final stages of the operation.

In light of what has been said above, the optimum regimes for hydrolyzing the PSCs of industrial wastes from ginseng tissue culture are the following: 1) 60°C, 4 N HCl, 24 h, and 3 N H_2SO_4, 24h; 2) 80°C, 2-3 N HCl, 9-24 h, and 2 N H_2SO_4, 9-24 h; 3) 115°C, 0.5 N HCl, 24 h , and 0.5-1.0 N H_2SO_4, 24 h.

Another important aspect of investigations with the aim of the further use of the hydrolysates as nutrient media in the microbiological industry is the qualitative and quantitative evaluation of the MSCs of the composition by gas-liquid chromatography. A comparison of the results of fractionation on Sephadex G-50 of the aqueous oxalate extract of the ginseng biomass shows that in the case of the oxalate buffer the extract consists mainly of PSCs together with a small amount of MSCs, while in an aqueous extract the MSCs predominate.

A preliminary study of the hydrolysates with the aid of paper chromatography and a Technicon carbohydrate analyzer does not give a complete idea of the composition of the MSCs.

Table 3 gives the results of determinations of the ratio of MSCs in the hydrolysates with the aid of GLC. It can be seen from an analysis of chromatograms that the optimum conditions for acid hydrolysis with respect to the yield of MSCs are: for HCl — 0.5 N, 115°C, and 2.0 N, 80°C; for H_2SO_4 — 0.5 N, 115°C, and 1.0 N, 80°C.

EXPERIMENTAL

1. Acid Hydrolysis. To 1 g of dry sample (wastes from the biomass of a ginseng tissue culture from the Kirov Pharmaceutical Chemical factory) was added 50 ml of a solution of acid of the appropriate concentration (0.5-6.0 N). Hydrolysis was performed with periodic stirring at temperatures of 40, 60, 80, and 115°C. The liquor ratios were 1:50 and 3:50, and the times of hydrolysis 6, 9, 12 24, and 48 h.

During the experiment, 0.5-ml samples of the hydrolysate were taken (in triplicate) after predetermined intervals of time, and their carbohydrate contents were determined.

After the end of the experiment, the reaction mixture was neutralized, the carbohydrate content was determined, and the residue, after washing and drying, was weighed.

2. The concentration of carbohydrates in a hydrolysate was determined by the orcinol–sulfuric acid method [7]. To 0.5 ml of a 15-fold diluted sample was added 0.5 ml of a 0.2% aqueous solution of orcinol and 3.5 ml of a 60% (by volume) solution of H_2SO_4. The resulting mixture was heated at 100-110°C for 60 min. The intensity of coloration was determined with the aid of a photoelectric colorimeter at λ 520 nm. The optical densities of three parallel determinations were averaged. The concentration of carbohydrates in the solution was determined from a calibration curve plotted on the basis of known dilutions of glucose.

3. Completeness of the Hydrolysis of the Biomass. The unhydrolyzed part of the biomass (para. 1) washed free from soluble compounds and salts was dried on the filter, which had been weighed previously on an analytical balance, in a thermostat at 55-60°C to constant weight (20-24 h). The weight was determined with allowance for the "loss" in the weight by the filter itself. The completeness of hydrolysis was calculated as the ratio of the weight of the hydrolyzed fraction to the initial weight of the sample of biomass and was expressed as a percentage.

4. Chromatographic Methods of Analysis. A. The hydrolysates (filtrates after neutralization) were fractionated by column chromatography on Sephadex G-50. Pyridine–CH_3COOH–H_2O (4:10:986) was used as buffer. The carbohydrate components in the fractions were revealed by the orcinol–sulfuric acid method (para. 2) without dilution of the eluates on the Technicon or on the photoelectric colorimeter at λ 520 nm.

B. The preliminary detection of the MSCs was performed by paper chromatography (FN-8 paper) in the ethyl acetate–pyridine–H_2O (8:2:1) system. The amount of hydrolysate deposited in each case was 0.04 ml. Chromatography (using markers) was carried out twice. The chromatogram after drying was stained with a solution of aniline phthalate and was dried at 105-111°C.

C. Gas-liquid chromatography (GLC). Trimethylsilyl derivatives, the complete acetates of polyols, or the sugar derivatives formed on the acetylation of their oximes are used for the analysis of carbohydrates [7, 6]. We used the last two methods.

D. Conditions of chromatography. The following stationary phases were first tested: I) 3% of OV-17 on Chromaton N (0.125-0.180 mm); II) 3% of XE-60 on Chromation N-super (0.250-0.315 mm); III) 5% of XE-60 on Chromaton N-AW (0.125-0.160 mm); and IV) 5% of XE-60 on Chromaton N-AW-HMDS (0.125-0.160 mm). Phases III and IV gave the best results.

Chromatography was performed in the following instruments: LKhM-8MD (USSR), model 5, with an FID detector using nitrogen as the carrier gas at the rate of 30 ml/min. Glass columns (3 x 1500 mm); phase III. Temperature of the evaporator 275°C; programming of the temperature of the column thermostat between 190 and 250°C at the rate of 3°C/min. Time of analysis ≈20 min.

Chrom-5 (Czechoslovakia), FID, carrier gas nitrogen at 30 ml/min; hydrogen, 30 ml/min; air 300 ml/min; glass column (2.5 × 2500 mm); phase IV. Temperature of the evaporator and the detector 260°C; temperature raised in the interval of 190-250°C at the rate of 3°C/min. Time of analysis 20-25 min. The hydrocarbon $C_{22}H_{46}$ was used as internal standard (retention time 2'10"). The concentrations of MSCs in the hydrolysates were calculated from the formula

$$C_i = \frac{S_i \cdot m_{st} \cdot k}{S_{st} \cdot m_{mix}} \cdot 100\ \%,$$

where C_i is the concentration of the i-th component in the mixture;
S_i is the area of the peak of the component on the chromatogram;
S_{st} is the area of the peak of the internal standard;
m_{st} is the weight of the standard;
m_{mix} is the weight of the mixture under investigation; and
k is a correction factor for the degradation of the MSCs in the synthesis of derivatives according to C_1 and C_2 for the GLC (under our conditions, taken as 1).

SUMMARY

1. The conditions for the acid hydrolysis of industrial wastes from a ginseng tissue culture have been studied. The temperatures, the liquor ratios, and the concentrations of acids permitting the maximum yield of MSCs at the optimum degree of hydrolysis to be obtained have been selected.

2. The qualitative and quantitative compositions of the MSCs of hydrolysates have been studied with the aid of gas-liquid chromatography; they can find use as nutrient media or additives for such media.

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DETECTION OF PROSTAGLANDIN $F_{2\alpha}$ IN TISSUES OF *Populus Balsamifera* AND *Larix Sibirica*

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The identification of prostaglandin $F_{2\alpha}$ isolated from the buds and cambial zone of *Populus balsamifera* and the buds of *Larix sibirica* Ledeb, is described. Identification was performed with the aid of TLC, GLC, HPLC, chromatomass fragmentography, and PMR spectroscopy. This is the first time that the presence of prostaglandin $F_{2\alpha}$ in the living tissues of higher plants has been demonstrated.

In papers published previously, the identification of prostaglandins (PGs) $F_{1\alpha}$, E_1, and E_2 isolated from the living tissues of *Populus balsamifera* and *Larix sibirica* Ledeb. was described [1, 2]. In the present paper we describe the identification of $PGF_{2\alpha}$, also isolated from these tissues. The living tissues are the buds and cambial zone of the trumk of *Populus balsamifera* and the buds and cambial zone of the shoots of *Larix sibirica*. The PGs were separated and identified by TLC, GLC, high-performance liquid chromatography (HPLC), chromatomass fragmentography, and PMR spectroscopy.

On TLC using system 1, the R_f value of the PG isolated and of standard $PGF_{2\alpha}$ were identical at 0.18, and in system 2 they both had R_f 0.50.

Two different instruments and different phases were used for the GLC analysis of the PGs. The PGs were converted into volatile derivatives: the carboxy groups were methylated with diazomethane, and the alcoholic hydroxy groups were converted into trimethylsilyl ether groups. The corresponding derivatives of standard $PGF_{2\alpha}$ were used as markers. The $PGF_{2\alpha}$ was estimated quantitatively with the aid of an internal standard, as which the marker was used. A conformation of the correctness of the identification was obtained by means of a mixed melting point (the same marker was used). On chromatograms, the reten-

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