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PRELIMINARY INVESTIGATION OF THE WATER-SOLUBLE POLYSACCHARIDES FROM THE LEAVES OF THE BAMBOO *Sasamorpha chiisanensis*

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It has been shown that the water-soluble polysaccharides of the leaves of the bamboo *Sasamorpha chiisanensis* Nak., after the removal of starch, contain residues of D-galactose, D-glucose, mannose, arabinose, D-xylose, rhamnose, and glucuronic and galacturonic acids and form an involved polysaccharide complex. Eight polysaccharide fractions differing in their uronic acid contents and in the ratio of neutral sugars have been isolated with the aid of chromatography on DEAE-cellulose.

Extracts of many higher plants possess an antitumoral action [1] which is frequently connected with the presence of polysaccharides [2]. The latter are usually free of cytotoxic properties but are powerful biological stimulators ensuring the antitumoral protection of the organism by increasing its nonspecific powers of resistance.

It has been shown in a number of studies that the antitumoral activity of extracts from the leaves of several species of bamboo (*Sasa albomarginata* [3-5], *S. kurilensis* [6], *S. senanensis* [7], *and S. nipponica* [8]) is due to the polysaccharides present in them. An aqueous extract from the bamboo *Sasamorpha chiisanensis* Nak. is used in Korean medicine. The aim of the present work was to isolate and study the composition of the polysaccharide fraction of this extract.

The total aqueous extract (TAE) is a dark resinous substance that is obtained by the treatment of leaves of *S. chiisanensis* with hot water followed by evaporation and lyophilization. To isolate the water-soluble polysaccharides, the TAE was first treated with chloroform–methanol (2:1) to eliminate lipids, pigments, and substances of low molecular weight soluble in organic solvents (about half the initial material was eliminated) and the residue was extracted with water at room temperature, after which the polysaccharides were precipitated from the aqueous solution by the addition of four volumes of ethanol. The yield of combined water-soluble polysaccharides (CWPs) amounted to about 6% on the TAE; the products of

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TABLE 1. Fractionation of the Polysaccharides of *S. chiisanensis* Nak. on DE-52 (CO_3^{2-}) and the Compositions of the Fractions in Terms of Total Sugars and Uronic Acids

Eluent	Fraction	Weight, mg	Yield, %	Amount, %, of	
				total sugars	uronic acids
H_2O	I	293	17	58	1.6
$(NH_4)_2CO_3$, M					
0.1	II	429	20,6	48	24,8
0,18	III	212	10	47	38.6
0,3	IV	225	7	31	19.6
0.5	V	145	3	23	-
NaOH, M					
0,1	VI	369	19,4	52,5	—
0,3	VII	43	1,6	37.5	—
0.5	VIII	24	0.4	19.0	—

TABLE 2. Molar Ratios of Neutral Sugars in Fractions I-VII

Fraction	Rhamnose	Arabinose	Xylose	Mannose	Galactose	Glucose
I	0,04	0,34	0,18	0,46	1	0,53
II	—	0,35	0,20	0,28	1	0,48
III	0,24	0.32	0,13	0,34	1	0,61
IV	0,09	0,73	0,13	0,36	1	0,86
V	0,06	0,24	0,19	0,27	1	0,81
VI	—	0,5	0,23	0,23	0.38	1
VII	—	0.25	0.20	0.24	0 35	1

complete acid hydrolysis were found by chromatographic methods to contain glucose, galactose, mannose, arabinose, xylose, rhamnose, galacturonic acid, and glucuronolactone. After preparative separation with the aid of paper chromatography and a determination of optical activity, it was established that the glucose, galactose, and xylose belonged to the D-series.

It might be assumed that the main component of the mixture of monosaccharides in the hydrolysate — D-glucose — arose at least partially from starch. In actual fact, when the CWPs were treated with amyloglucosidase [9] glucose was found in the products of enzymatic hydrolysis while in the polymer fraction resistant to the action of the enzyme the amount of this sugar had decreased and it did not change on repeated treatments with amyloglucosidase. The amount of starch in the CWPs calculated on the basis of these results was 22.6%.

The mixture of polysaccharides obtained after amylolysis was fractionated by ion-exchange chromatography on DEAE-cellulose in the carbonate form [10]; the column was washed with water and with solutions having increasing concentrations of ammonium carbonate and NaOH. The fractions were freed from salts by dialysis and were lyophilized, and the total amount of sugars and the amount of uronic acids in the residue were determined (Table 1), and, after complete acid hydrolysis, the molar ratios of the neutral monosaccharides were found (Table 2). The results of fractionation indicated the highly involved nature of the polysaccharide complex of the leaves of *S. chiisanensis*. Fractions (II-IV) eluted from the column with ammonium carbonate, contained uronic acids in addition to a variegated set of neutral monosaccharides and were probably pectin materials. The main neutral monosaccharide in this fraction was galactose. Elution under more severe conditions gave fractions (V-VII), in which the amount of glucose had increased while uronic acids were absent. These fractions can be assigned to the hemicelluloses and, obviously their retention on the DEAE-cellulose is not connected with ion exchange but is due to strong adsorption on the surface of the cellulose fibers.

The results obtained show that, as in the case of many other grasses [11], the fraction of water-soluble polysaccharides from the leaves of *S. chiisanensis* consists of a complex mixture of polysaccharides. Further investigations will be directed to a study of the biological activity of the CWPs and their components.

EXPERIMENTAL

PC was performed by the descending method on FN-11 paper in the following systems: 1) butan-1-ol—pyridine—water (6:4:3); 2) methyl ethyl ketone—acetic acid—water saturated with H_3BO_3 (9:1:11); and 3) ethyl acetate—acetic acid—formic acid—water (18:3:1:4). The sugars were revealed with aniline phthalate. Preparative TLC was performed on 6 × 9 cm plates with an unfixed layer of silica gel L 5/40 μm (Czechoslovakia); the sugars were revealed with H_2SO_4 and heating. The GLC of polyol acetates was performed on a Pye-Unicam 104 chromatograph with a flame ionization detector (0.6 × 120 cm column with 3% of ECNSS-M, Gas-Chrom Q, 185°C). Spectroscopic determinations were carried out on a SF-4A spectrophotometer. Specific rotations were measured on a AI-EPN photoelectronic polarimeter (VNIIEKIPRODMASh [All-Union Scientific Research and Experimental Planning Institute of Food Engineering]. The total sugars were determined from the reaction with phenol—H_2SO_4 and were calculated as glucose [12], while uronic acids were determined with the m-hydroxybiphenyl reagent and were calculated as galacturonic acid [13].

Hydrolysis was carried out with 2 N H_2SO_4 at 100°C for 5 h. The hydrolysates were neutralized with $BaCO_3$, centrifuged, partially evaporated in vacuum at 40°C, and investigated by PC. The residue from the hydrolysate was evaporated to dryness and dissolved in aqueous ethanol, and the solution was centrifuged, evaporated, treated with a 1% solution of trimethylamine, and evaporated again, and the residue was evaporated in water, treated with $NaBH_4$, and left overnight. On quantitative analysis by the GLC method, before the treatment with $NaBH_4$ a known amount of inositol was added [14]. The mixture was neutralized with an excess of Dowex 50 W × 8 (H^+) and was evaporated to dryness and the residue was then evaporated several times with methanol.

Acetylation was carried out with a 1:1 mixture of pyridine and acetic anhydride at 20°C for a day. After the end of acetylation, the mixture was evaporated in vacuum to dryness and the residue was then evaporated successively several times with toluene and heptane. The zone of polyol acetates was isolated by preparative TLC in the chloroform—acetone (49:1) system, and this was then analyzed by GLC.

Isolation of the Polysaccharides. The leaves of *S. chiisanensis* (about 300 kg, gathered in September, 1983) were sorted and were dried in the air in a place protected from sunlight. Portions of 30 kg each were extracted with 30-50 liters of water at 96-98°C (heating by bubbling superheated steam for 5 h). The extraction of each portion was repeated three times. The dark green solution was evaporated to ~10 liters and was lyophilized under factory conditions to a dark brown resinous mass (TAE).

This product (136 g) was ground to a fine powder and extracted with 1.5 liters of chloroform—methanol (2:1). The residue was separated off by centrifugation and the extraction was repeated five times after which the extracts were of a constant light coloration. The yield was 55.3%. The residue from the extraction by organic solvents (75.16 g) was extracted with 750 ml of water at 20°C for 24 h. The undissolved matter was eliminated by centrifugation and the extraction was repeated twice. The aqueous extracts were combined, evaporated at 40°C to 500 ml, and dialyzed against distilled water for 4 days. The undialyzable fraction was treated with 4 volumes of ethanol. The precipitate that deposited was separated off by centrifugation and was washed with water—ethanol (1:4), with ethanol, and with acetone and was dried in vacuum over P_2O_5. The yield of combined water-soluble product (CWP) was 4.6 g. The gray-colored CWP contained 37.6% of carbohydrates according to the phenol—H_2SO_4 reaction. After hydrolysis, PC in system 1 showed the presence of uronic acids (R_g 0.13), galactose (R_g 0.8), glucose, mannose and arabinose (R_g 1.18), xylose (R_g 1.46), rhamnose (R_g 1.6), and glucuronolactone (R_g 1.93). The mannose and arabinose were eluted and were separated by rechromatography in system 2. The zone with R_g 0.13 was eluted, treated with Dowex 50 W×8 (H^+), and rechromatographed in system 3. Galacturonic acid was detected (R_g 0.42). The same neutral sugars were detected by the GLC of the corresponding polyol acetates as by PC.

The product was then subjected to amylolysis in order to separate the starch.

Amylolysis of the CWP [9]. Portions of the CWP weighing 0.6 g were each dissolved in 150 ml of water, a solution of 0.180 g of amyloglucosidase in 150 ml of 0.2 M acetate buffer, pH 4.5 was added, and the mixture was kept in a thermostat at 60°C for 24 h. After the end of the reaction, the mixture was kept in a boiling water bath for 15 min, filtered, and evaporated to 70-100 ml, and the polysaccharides were precipitated with 10 volumes of methanol. The precipitate was separated off by centrifugation, and the solution was treated with Dowex 50W×8 (H^+) and was analyzed for its glucose content. The precipitate was washed with ethanol

and with acetone and was dried over P_2O_5 in vacuum. The total yield from several portions was 3.57 g. The amount of carbohydrates was 30% according to the phenol–H_2SO_4 reaction. After hydrolysis the same monosaccharide composition was found by PC and GLC as before amylolysis, but the glucose content had fallen. On repeated amylolysis of part of the product from the first treatment with amyloglucosidase, glucose could not be detected by the PC method in the products of enzymatic hydrolysis, and its amount in the polysaccharide had not decreased. Without further purification, the product was separated on a column of DE-52 in the carbonate form.

Fractionation of the Polysaccharides on DE-52 (Whatman) [10]. A solution of 3 g of the WSPs after amylolysis in 50 ml of water was deposited on a 2.5 × 20 cm column of DE-52 in the carbonate form. Elution was performed successively with water (1 liter), with solutions of $(NH_4)_2CO_3$ – 0.1 M (1 liter), 0.18 M (0.5 liter), 0.3 M (1.1 liter), and 0.5 M (0.9 liter) – and then with solutions of NaOH – 0.1 M (1.7 liter), 0.3 M (1.6 liter), and 0.5 M (0.5 liter). Fractions with a volume of 30 ml were collected and were analyzed for total sugars by the phenol–H_2SO_4 method. The corresponding fractions with the peak concentration of carbohydrates were combined, neutralized with acetic acid, partially evaporated, and dialyzed against distilled water for 2-4 days. Then they were lyophilized and dried in vacuum over P_2O_5. A total of 79% of the carbohydrates deposited on the column was eluted (without taking into account the carbohydrates in the intermediate eluates between the fractions). The yields, amounts of total sugars and of uronic acids, and the ratios of the neutral monosaccharides in polysaccharides (I-VIII) are given in Tables 1 and 2. Polysaccharide I was weakly colored; (II) and (III) cream-colored; (IV) beige; (V) black; (VI) brown; and (VII) dark beige.

In repeat experiments, the yields and the compositions of the WSP and the fractions were reproduced.

SUMMARY

The water-soluble polysaccharides of the leaves of the bamboo *Sasamorpha chiisanensis* Nak. have been isolated by precipitation with ethanol from the undialyzable fraction of aqueous extracts and have been freed from starch and investigated for their mono- and polysaccharide compositions.

It has been shown that they contain D-galactose, D-glucose, mannose, arabinose, D-xylose, rhamnose, and glucuronic and galacturonic acid residues. Eight fractions of polysaccharides differing in their uronic acid contents and ratios of neutral sugar residues have been isolated with the aid of chromatography on DEAE-cellulose.

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