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POLYSACCHARIDES OF IRIDACEAE

IV. A XYLOGLUCOGALACTAN OF THE BULBS OF *Juno drepanophylla*

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Polysaccharides (WSPSs, PSs, and HMCs) have been isolated from various organs of *Juno Drepanophylla* and their qualitative compositions have been characterized. ESPS-I from the bulbs has been studied and has proved to be a polydisperse high-molecular-weight and branched amyloid. In its physicochemical properties and composition, WSPS-I is close to amyloids of the first class known from the literature. On the basis of the results of methylation, oxidation with chromium trioxide, and IR and ^{13}C NMR spectra, it has been established that the glucopyranose residues are linked with one another by β-(1 → 4) bonds, and the xylo and galactopyranose residues by α(1 → 4) bonds with the C-6 position of the glucose residue.

Plants from the family Iridaceae of the genus *Juno* Tratt, are rich in polysaccharides [1]. In the present paper we give the results of investigations of the polysaccharides of the *Juno drepanophylla* (Aitch. et Baker) Rodion. From various organs of the plant the ethanol-soluble (ES) sugars, the water-soluble polysaccharides (WSPSs), the pectin substances (PSs), and the alkali-soluble polysaccharides (hemicelluloses – HMCs) were extracted successively. The amounts of the polysaccharides and their monosaccharide compositions are given in Table 1. The amounts of ES sugars were (%): in the bulbs with roots – 5.5; leaves – 1.2; and seeds – 2.2. The presence of monosaccharides (glucose, fructose), sucrose, and unidentified fructooligosaccharides was detected with the aid of paper chromatography.

As can be seen from Table 1, the amount of WSPSs in the bulbs with roots was the greatest, which served as an argument for their further investigation.

WSPS-I was obtained by precipitation from aqueous solution with methanol (1:4), and WSPS-II was isolated additionally from the mother solution with a mixture of methanol and acetone (3:2).

The ash content of WSPS-I was 0.23-0.3% and they contained no nitrogen or methoxy groups; $[\alpha]_{546}$ +66.7° (c 0.25; water), +56.4° (c 0.25; 0.5% KOH).

Analysis in the ultracentrifuge, gel chromatography on Sepharose-4B, and the high-pressure liquid chromatography (LC) of WSPS-I showed their inhomogeneity. For a 1% solution of WSPS-I in 0.3% sodium chloride in the ultracentrifuge, S was found to be $10.3 \cdot 10^{-13}$ sec, and D $4.6 \cdot 10^{-7}$ cm^2/sec, giving a discrete peak on the sedimentogram.

On a column containing Sephadex G-100 and on Sepharose-4B, WSPS-I emerged earlier than dextran with a molecular weight of 2 million.

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TABLE 1. Amounts of Polysaccharides and Monosaccharide Composition of Juno drepanophylla

Plant organ	Substance	Yield, % of the abs. dry weight of the raw material	Monosaccharides, mole					
			Gal	Glc	Man	Xyl	Ara	Rha
Bulbs with roots	WSPS-I	16.0	5,2	2,5	Tr	1 0	—	Tr
	WSPS-II	8 2	30.3	3 0	1.2	1,0	—	Tr
	PSs	5 2	7.3	3,0	1,0	3 5	Tr	Tr
	HMCs	12,0	1,5	17,0	—	1,0	—	—
Leaves	WSPSs	3,5	10 0	11,3	Tr	5,0	3,0	1,0
	PSs	7,7	4,0	4 0	—	1 1	1 0	2.5
Unripe seeds (without pods)	WSPS-A	6,4	20,6	9.0	17,0	2,0	Tr	1.0
	WSPS-B	1 9	37,5	16.0	22,0	3.5	Tr	1,0
	PSs	3.5	2,3	5,6	1 7	2,0	Tr	1,0
	HMC-I	12.0	33,0	18,0	18,0	6.6	1.0	1.0
	HMC-II	30,0	6,8	3,7	1.7	Tr	1.0	Tr

TABLE 2. Relative Viscosity of WSPS-I from Juno drepanophylla at 20-23°C

Concentration, %	Water	% NaOH	0.9% NaCl	0.2 M sodium acetate (pH 7.4)	0,2M Na_2SO_4
0.5	33,4	5,6	3,7	—	—
0,4	17,2	4.6	2,8	4,1	4,4
0,3	8.6	3,0	2,1	2.1	3,1
0,2	4,8	2,1	1,7	2,1	2,0
0,1	2,4	1,3	1,3	—	—
0,05	1,6	1,2	1,2	—	—

TABLE 3. Relative Retention Times of Methylated WSPS-I and of Polystyrenes (PSts), min

Sample	Column 3 (μ-Styrogel 10^4 – 10^3)
OMe-WSPS-I	130
Pst $104 \cdot 10^4$	97
$305 \cdot 10^3$	108
$128 \cdot 10^3$	129
$30 \cdot 10^3$	137

The relative viscosity indices of the polysaccharides in water and salt solutions (Table 2) were used to select the concentration under the conditions of analysis by high-pressure LC.

WSPS-I and standard samples of dextrans and amylopectin with molecular weights of 2-4 million were analyzed on columns 1 and 2. It was found that under the conditions of analysis WSPS-I issued at the same time as the amylopectin and earlier than dextrans T-250 and T-500.

The results of LC of the polysaccharides and of gel filtration showed that WSPS-I was of high molecular weight and polydisperse, and it formed a viscous solution in water at concentrations of 0.4% and above.

In nonaqueous conditions of analysis on columns 3 for high-pressure LC the methylated ESPS-I polysaccharide was close to a polystyrene standard with mol. wt. $128 \cdot 10^3$ (Table 3) and from the results of analysis by the light-scattering method the polysaccharide had a mol. wt. of 170,000.

The WSPS-I fully methylated by Hakomori's method [2] had $[\alpha]_D$ +42° (c 0.52; chloroform). Analyses of the partially methylated acetates of the polyols derived from the monosaccharides

TABLE 4. Monosaccharide Composition of Methylated WSPS-I and Mass Spectra of the Monosaccharide Residues

Monosaccharide residues	R^*_{rel}	Ratio molar	m/z
Xyl-(1→	1,0	2,0	87, 101, 117, 129, 145, 161
Gal-(1→	1,52	1,5—2,0	87, 89, 101, 117, 129, 131, 145, 161, 233
→4)-Gal-(1→	1,7	2,2	87, 101, 117. 129, 161, 145, 189
→4)-Glc-(1→	2,67	1,8	87, 89, 101, 117, 129, 131, 161, 173, 233
→4,6-Glc-(1→	4,57	4,0	87, 89, 101, 111, 117, 127, 129, 141, 142, 159, 161, 189, 201, 215, 229, 261

*R_{rel} – relative retention time in relation to 1,5-OAc_2-2,3,4-OMe_3-xylitol.

TABLE 5. High-Molecular-Weight Fractions of WSPS-I from Enzymatic Hydrolysis and the Composition of the Monosaccharides in the Form of the Corresponding Polyol Acetates (mole)

Enzyme	Yield of the fraction, % on the weight of the WSPSs-1	(c 0,5; water)		Xyl	Glc	Gal
		$[\alpha]_D$	$[\alpha]_{546}$			
Pectinase	33,3	+58	+68	1,4	1,0	2,8
Diastase	84,0	+58	+68	1,5	1,0	1,9
Cellulase	81,3	+68	+86	1,5	1,0	3,3

of a hydrolysate of WSPS-I were carried out under conditions described in the literature [3, 4] (Table 4).

It can be seen from Table 4 that the terminal monosaccharide residues of the nonreducing ends of the chains of the polysaccharides proved to be xylose and galactose. Some of the galactose residues were substituted at C-4. The main chain of the polymer consists of (1 → 4)-bound glucopyranose residues having branchings at the C-6 atom. Xylose and galactose are linked by (1 → 4)-bonds to the main chain of the polymer. The degree of polymerization of the polysaccharide is 69-70.

The β and α configurations of the glucosidic bonds were confirmed by IR spectroscopy (900-950, 800-810, and 770 cm^{-1} [5-7]) by oxidation of the acetylated polysaccharides their chromium trioxide by a known method and by analysis of the ^{13}C NMR spectrum of the WSPS-I.

The ^{13}C NMR spectrum contains the following signals that are characteristic for the C-1 and C-6 carbon atoms (the other signals are not given because of the complexity of the spectrum and the absence of model compounds for comparison): for C-1 (ppm): 105.5, 102.9, 99.8, 99.3, 98.65, 97.25, and 95.5; and for C-6 (ppm): 61.8, 62.3, 67.75, and 69.6. The characteristics of the ^{13}C NMR spectrum that were found are close to those given in the literature [6] for known polysaccharides. It is sufficient to point that of the signals of the anomeric carbon atoms that at 102.9 may be assigned to β-D-glucose; those at 99.8, 99.3, and 98.65 to an α-D-xylopyranose residue present at the nonreducing end of the chain of the polymer; and those at 97.25 and 95.55 to a β-D-glucopyranoside unit present at the reducing end of the polymer. These assignments may be considered preliminary results for answering the question of the configurations of the glycosidic bonds.

The treatment of WSPS-I with the enzymes diastase, pectinase, and cellulase in acetate buffer (pH 4.7) gave high-molecular-weight (HMW) fractions and low-molecular-weight products from dialysis. In the dialysates from the enzymatic treatment, together with glucose, galactose, and xylose, oligosaccharides close in mobility on paper to maltose, isomaltose, lactose, and cellobiose and a number on paper to maltose, isomaltose, lactose, and cellobiose and a number of higher oligosaccharides containing pentose residues were detected. The characteristics of the high-molecular-weight fractions of polysaccharides are given in Table 5. If we compare the ratio of the monosaccharides of WSPS-I and their fractions containing high-molecular-weight products, it can be seen that the amounts of glucose residues have decreased but the optical rotations have remained similar.

With the exception of the fraction obtained by the use of the enzyme diastase, the fractions proved to be nonhomogeneous on gel filtration and LC with molecular weights of between $40 \cdot 10^3$ and $70 \cdot 10^3$. The molecular weight of the fraction produced by the enzyme diastase was close to that of the WSPSs-I. It follows from this that diastase hydrolyzes the WSPS-I by 16%.

Thus, the WSPS-I isolated from the bulbs with roots of Juno drepanophylla represents a nonhomogeneous highly branched amyloid of the first class containing galactose residues in the molecule [8] and close in its physicochemical properties and composition to amyloids known from the literature from the seeds of tamarind [9, 10], nasturium [11], balsam [12], turnip [13], shoots of white mustard seeds [14], quince seed wastes [10], soursop seeds [15], peduncles of Indian figs [10, 16], the cell wall of potatoes [17], tobacco leaves [6, 18], beans [19], and bamboo shoots [20]. In the species of plants mentioned, the polysaccharides consist of xylogalacto-, arabinogalactoxylo-, and xyloglucans and are included in the first class of the classification of amyloids [8]. In the structural respect, they have a main polysaccharide chain consisting of β(1→4)-bound D-glucose residues in which there are side chains at C-6 consisting of arabinose, xylose, galactose, and rhamnose residues linked by α-(1→4)-, α-(1→3)-, and α-(1→2)-bonds.

EXPERIMENTAL

Paper chromatography (PC) was performed on FN-1, -3, and -11 papers (Filtrak) by the descending method using (ratios given by volume) butan-1-ol—pyridine—water (6 : 4: 3)) (system 1), and thin-layer chromatography (TLC) on Silufol UV-254 plates (Kavalier) and on type L 5/40 silica gel (Chemapol) fixed with gypsum in the methyl ethyl ketone—1% ammonia (30:4) and (30:1) systems (system 2). Aniline hydrogen phthalate was used to identify the spots (10-15 min at 105-110°C). The viscosities of the solutions were measured at 20-23°C in an Ostwald viscometer. Specific rotations were obtained on a EPN automatic polarimeter at a wavelength of 546 nm.

Gel filtration was performed on Sephadex G-200 and Sepharose-4D (Pharmacia). The standards used were dextrans T-2000, -500, -250, -110, and -80 (Pharmacia), amylopectin with mol. wt. 2-4 million (Serva), and polystyrenes with mol. wts. of $104 \cdot 10^4$, $305 \cdot 10^3$, $128 \cdot 10^3$, and $30 \cdot 10^3$ (Altex). The enzymes used were diastase, 2800 U/g (Merck), standardized pectinase (Sweden), and cellulase of fungal origin supplied by the Institute of Microbiology of the UzSSR Academy of Sciences. Sedimentation analysis was performed on a MOM-3170 instrument (Hungary) at 5000 rpm and a temperature of 20°C using 1.0% solutions in 0.3% NaCl with a recording interval of 5 min. High-pressure LC was carried out on a Dupont 830 instrument (USA) fitted with SE-100 and SE-500 columns (6.2 × 250 mm) connected in series and filled with silica gels having a mean particle size of 8 μ and pore diameters of 10 and 50 nm, respectively (column 1), and on Zorbax PSM-60 and -100 columns (column 2). The detector was a LDC-1107 differential refractometer (USA), RIX8, pressure P = 68-70 bar; rate of feed of eluent V_{flow} = 0.9 ml/min. The mobile phase was 0.2 M sodium acetate, pH 4.78, for aqueous solutions of the samples, while for the analysis of the methylated polysaccharide it was tetrahydrofuran (Serva) with μ-Styrogel 10^4 and 10^5 (column 3) (7.8 × 300 mm, Waters), UV detector at 254 nm with a LDC-1107 differential refractometer connected in parallel.

The amounts of sugars were determined by GLC using 5% of the stationary phase Silicone XE-60 and 5% of SE-30 according to [21]. Aldononitrile and polyol acetates were obtained as described by Ovodov [22]. The mass spectra of partially methylated polyol acetates and of polyol acetates were obtained on a Varian MAT 111 Gnom chromato-mass spectrometer using a column (0.3 × 120 cm) containing 15% of OV-1 on Chromosorb-W.

Isolation of the EP Sugars, the WSPSs, the PSs, and the HMCs. Samples of powdered bulbs with roots (72 g of air-dry raw material), leaves (70 g of air-dry raw material), and unripe seeds without hulls (11.5 g of air-dry raw material) were defatted in a Soxhlet apparatus (chloroform—acetone (4:1)) and they were boiled with anhydrous methanol and extracted by being heated with 82% ethanol for 5 h; the ethanolic extracts were concentrated and from the residues, after their dissolution in water, the noncarbohydrate components were removed by successive treatments with 10% lead acetate and saturated sodium sulfate solution. The yields of the ES sugars were (g) from the bulbs — 3.3; from the leaves — 0.5; and from the seeds — 0.25 (dried powders). The free mono- and oligosaccharides were analyzed under PC conditions (system 1). The WSPSs, PSs, and HMCs were extracted successively from the remains of the raw material in accordance with [21]. The yields of the products and their qualitative and quantitative compositions are given in Table 1.

Acid Hydrolysis and Quantitative Determination of the Monosaccharides. Samples of the polysaccharides (50 mg) were hydrolyzed with 1 N H_2SO_4 at 100°C for 40 h in sealed tubes, and the reaction mixtures were then neutralized with $BaCO_3$ and filtered, and the filtrates were treated with KU-2 resin (H^+) and were concentrated to a syrup in a rotary evaporator. The qualtitative compositions of the products were analyzed by PC in system 1. The amounts of monosaccharides in the form of aldononitrile and polyol acetates were determined by the GLC method [21]. The presence of trace amounts of uronic acid in the WSPSs was found by PC (system 1).

Gel Filtration. A solution of the WSPSs-I (20 mg) in 0.1 M phosphate buffer (pH 6.8) was passed through a column (1.5 × 109 cm) of Sephadex G-200 that had been calibrated with standard dextrans.

Liquid Chromatography. Solutions of the WSPSs-I and of dextran and amylopectin standards in 0.5% concentrations in 0.2 M acetate buffer (pH 4.78) were analyzed on columns 1 and 2. Before analysis, the solutions were carefully filtered through a Millipore membrane filter of type GS0.22 μm.(Waters). The analyses of the methylated WSPS-I and the polystyrenes on column 3 from tetrafuran as solvent in a concentration of 1% are given in Table 3.

Methylation of WSPS-I. The Hakomori methylation [2] of 1 g of WSPS-1 gave 820 mg of fully methylated WSPS-I. On TLC in the chloroform–acetone (1:1) system, the methylated WSPS-I showed a single spot. $[\alpha]_{546}$ +33.3° (c 1.1; chloroform and $[\alpha]_D^{23}$ +42°C (c 0.52; chloroform). The amount of methoxy groups was 42.13%. No absorption bands of OH groups were observed in the IR spectrum. A hydrolysate of the permethylated WSPS-I was analyzed by PC (system 1), and by TLC (system 2), and the corresponding polyol acetates were analyzed by chromato-mass spectrometry (Table 4).

Enzymatic Hydrolysis of WSPS-I with Diastase, Cellulase, and Pectinase. In three flat-bottomed flasks, 0.7 solutions of WSPS-I in acetate buffer (200 ml [2; no units given]) were prepared. To the solution in the first flask was added 100 mg of diastase, to the second 100 mg of cellulase, and to the third 100 mg of pectinase. After they had been uniformly suspended by means of a magnetic stirrer all the solutions with the enzymes were thermostated at 37°C. To each flask was added 0.5 ml of toluene and its neck was closed with a wad of adsorbent cotton. The time of hydrolysis was 72 h. Each flask was then placed in a boiling water bath for 10 min to inactivate the enzyme, and the contents were then treated with KU-2 (H^+) and were dialyzed against distilled water for 3 days. After three changes of water the dialysate was evaporated to a viscous syrup and was analyzed by TLC in system 1.

The mono- and oligosaccharides detected on paper have been discussed above. The undialyzable fractions were removed from the Cellophane, concentrated to half their volume, and poured into methanol taken in a ratio of 1:4 to the solution. The precipitate that deposited was separated off, dewatered with ethanol, acetone, and ether, and dried over P_2O_5. The yields of precipitates were 1.26, 1.22, and 0.500 g, respectively. The mother methanolic solutions were combined with the syrups from the dialyzed fractions.

Reaction with Iodine. In a concenration of 0.0001 mg/ml in the presence of a 20% solution of sodium sulfate, the WSPSs-I gave a greenish-blue coloration with 1 ml of a 0.5% solution of iodine in a 1% solution of potassium iodide [9].

Oxidation by Chromium Trioxide. A solution of 50 mg of acetylated WSPS-I in acetic acid was oxidized with chromium trioxide according to [4]. Xylose, galactose, and traces of glucose were detected in a hydrolysate of the oxidized WSPS-I by PC in system 1.

SUMMARY

1. The water-soluble polysaccharide WSPS-I isolated from the roots of the Juno drepanophylla is a high-molecular-weight polydisperse and highly branched amyloid xyloglucogalactan. By its physicochemical properties WSPS-I has been assigned to the first class of amyloids, containing galactopyranose residues in the molecule.

2. The main polysaccharide chain consists of β(1→4)-bound galacto- and glucopyranose residues. At the points of branching of the glucose residues, xylo- and galactopyranose residues with α(1→4)-bonds form side chains at C-6.

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