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BIOLOGICALLY ACTIVE POLYSACCHARIDES FROM MEDICINAL PLANTS OF THE FAR EAST

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In a search for biologically active polysaccharides, we have investigated ten species of plants from six families of the flora of the Far East. By successive extractions with water and solutions of ammonium oxalate and of sodium hydroxide we have isolated polysaccharides from the roots and epigeal parts and have determined their qualitative and quantitative monosaccharide compositions. The polysaccharides obtained have been studied for immunostimulating, antitumoral, and antiviral activities.

Plant polysaccharides are attracting ever-increasing attention as an important class of biopolymers possessing a broad spectrum of biological action. Antitumoral, immunostimulating, hypoglycemic, anticomplement, antiinflammatory, anticoagulant, and other types of activities have been found in these compounds [1-4].

The rich and unique flora of the Far East may serve as a source of biologically active polysaccharides. To select the most interesting and promising sources, we have made a comparative study of ten species of plants from six families of the Far Eastern flora: *Polgonatum odoratum* (Mill.) Druce and *Polygonatum stenophyllum* Maxim. (Liliaceae), *Ampelopsis japonica* (Thunb.) Makino (Vitaceae), *Angelica dahurica* (Fisch. ex Hoffm.) Benth. et Hook, f., *Glehnia littoralis* F. Schmidt, *Heracleum moellendorffii* Hance and *Saposhnikovia divaricata* (Turcz.) Schischk. (Umbelliferae), *Aralia continentalis* Kitagawa (Araliaceae), *Paeonia lactiflora* Pall. (Paeoniaceae), and *Arctium lappa* L. (Compositae). All the plants mentioned grow in Maritime Territory and in the Amur region and are used in folk medicine [5].

The roots and epigeal parts of the plants were investigated separately. The raw material was comminuted and treated with methanol to eliminate low-molecular-mass compounds. The polysaccharides were isolated by successive extraction with water and solutions of ammonium oxalate and sodium hydroxide, with heating. The total yield (7-30% on the dry weight of the raw material) and the distribution of the polysaccharides over the fractions depended primarily on the source (Table 1).

The qualitative and quantitative monosaccharide compositions of the polysaccharides isolated were established after acid hydrolysis by means of PC and GLC in the form of polyol acetates. Uronic acids were determined by a known procedure [6]. The monosaccharide compositions of the polysaccharides are given in Table 1.

All the polysaccharides isolated had practically the same qualitative monosaccharide composition and contained residues of arabinose, galactose, glucose, uronic acids, and, more rarely, rhamnose and mannose; xylose was mainly present in trace amounts. An exception was formed by the monosaccharides of *Arctium lappa*, which, together with the monosaccharides common for all the plants, contained a considerable amount of fructose. The quantitative monosaccharide compositions depended on the method of extraction and the source of the polysaccharides.

The polysaccharides of the aqueous extracts were characterized by a higher level of glucose than the oxalate and alkaline extracts. It is most probable that the higher level of glucose in the water-soluble polymers is explained by the presence of a glucan – a reserve polysaccharide of plants. This hypothesis was confirmed by an increase in the amount of glucose in polysaccharides from raw material gathered in the autumn, when there is an accumulation of reserve polysaccharides. It must be mentioned that the biopolymers of the oxalate extracts both of the roots and of the epigeal parts contained a larger amount of uronic acids than the other extracts, with the exception of the polysaccharides from the stems of *Saposhnikovia divaricata*.

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TABLE 1. General Characteristics of the Polysaccharides Isolated

Plant, organ	Extract	Yield,* %	Monosaccharide composition, † mol. %						Uronic acids,‡ %
			Rha	Ara	Xyl	Gal	Man	Glc	
Ampelopsis japonica, roots	Aqueous	3.2	Tr.	24	–		29	47	25
	Oxalate	5.6	Tr.	33	–		27	40	50
	Alkaline	2.2	Tr.	64	–		18	18	29
Angelica dahurica, stems	Aqueous	8.1	5	16	–		44	35	20
	Oxalate	4.9	21	23	–		46	10	26
roots	Aqueous	9.4	3	14	–		16	67	34
	Oxalate	1.1	11	32	–		37	20	44
	Alkaline	4.5	27	23	–		43	17	52
Aralia continentalis, roots	Aqueous	5.3	Tr.	11	–		21	68	26
	Oxalate	4.2	19	35	–		27	19	67
	Alkaline	4.1	8	16	–		29	47	5
Arctium lappa L., roots	Aqueous	9.3	–	22	–		34	44	4
	Oxalate	5.3	–	27	–		33	40	82
	Alkaline	1.2	–	73	–		21	6	11
Glehnia littoralis, stems	Aqueous	5.0	12	10	–		53	25	22
	Oxalate	2.7	40	12	–		38	10	24
	Alkaline	0.3	16	11	–		62	11	n.d.
roots	Aqueous	6.0	Tr.	Tr.	–		13	87	18
	Oxalate	3.6	Tr.	Tr.	–		17	83	37
	Alkaline	1.5	5	12	–		40	43	9
Heracleum moellendorffii, stems	Aqueous	13.3	7	13	–		35	45	n.d.
	Oxalate	8.0	8	17	Tr.		32	43	58
	Alkaline	10.0	10	14	5		53	18	n.d.
roots	Aqueous	7.3	13	28	Tr.		41	19	45
	Oxalate	10.7	14	22	5		30	29	57
	Alkaline	3.0	15	28	Tr.		17	40	14
Paeonia lactiflora, roots	Aqueous	2.8	Tr.	8	–		20	72	5
	Oxalate	0.6	Tr.	9	–		30	61	54
	Alkaline	12.4	10	44	–		28	18	34
Polygonatum odoratum, stems	Aqueous	10.8	6	12	13		55	14	17
	Alkaline	5.0	18	11	8		63	Tr.	11
roots	Aqueous	6.2	Tr.	Tr.	Tr.		81	19	16
	Oxalate	3.4	8	15	Tr.		65	12	51
	Alkaline	3.4	13	44	Tr.		43	Tr.	18
Polygonatum stenophyllum, stems	Aqueous	4.9	Tr.	7	–		27	66	29
	Oxalate	1.0	4	9	–		40	47	32
	Alkaline	6.2	9	17	–		45	29	6
roots	Aqueous	7.0	Tr.	8	–		26	66	13
	Oxalate	1.4	Tr.	14	–		57	29	n.d.
	Alkaline	3.3	9	21	–		70	Tr.	0
Saposhnikovia divaricata, stems	Aqueous	7.5	Tr.	20	–		50	30	6
	Oxalate	1.9	9	30	–		35	36	0
	Alkaline	7.4	12	33	–		42	13	53
roots	Aqueous	2.4	16	46	–		23	15	25
	Oxalate	5.4	14	60	–		17	9	71
	Alkaline	6.5	Tr.	30	–		40	30	21

*Yield calculated on the dry weight of the raw material.

†Molar percentages calculated from GLC results.

‡Uronic acids were determined by the method of [6].

TABLE 2. Immunostimulating and Antitumoral Activities of Plant Polysaccharides

Plant (extract, organ)	Immunostimulating activity	Antitumoral activity
Ampelopsis japonica		
1. Aq. extr. roots	70	43
Angelica dahurica		
2. Aq. extr. stems	53	41
3. Aq. extr. roots	–	n.d.
Glehnia littoralis		
4. Aq. extr. stems	71	39
5. Ox. extr. stems	–	n.d.
Paeonia lactiflora		
6. Aq. extr. roots	–	n.d.
Polygonatum odoratum		
7. Aq. extr. stems	80	25
8. Aq. extr. roots	23	47
Polygonatum stenophyllum		
9. Aq. extr. stems	25	n.d.
10. Aq. extr. roots	32	n.d.
11. Ox. extr. roots	30	n.d.
Saposhnikovia divaricata		
12. Aq. extr. roots	–	n.d.
13. Aq. extr. stems	–	n.d.
14. Ox. extr. roots	–	n.d.

On the basis of the results that we obtained, it may be assumed that the polysaccharides isolated were acidic arabinogalactans and, more rarely, rhamnogalacturonans. At the same time, many plants contained accompanying reserve glucans in addition to acidic polysaccharides.

As a preliminary, approximately one third of the polysaccharides isolated were tested for immunostimulating activity. The index of immunostimulation that we used was the change in weight of the spleen and liver of experimental animals (mice) [7]. The results are given in Table 2.

All the preparations investigated can be divided into three groups. To the first group belong extracts showing no activity whatever. These are the oxalate extract of the stems of *Glehnia littoralis*, the aqueous extracts of the roots of *Paeonia lactiflora* and *Angelica dahurica*, and also all the extracts of *Saposhnikova divaricata* that were investigated. In the second group come the polysaccharides of *Polygonatum stenophyllum* and *Polygonatum odoratum*, with a weak (25-40%) immunostimulating activity. To the third group belong extracts with a high immunostimulating activity. Thus, the aqueous extract of the root of *Ampelopsis japonica* increased the weight of the spleen by 70%; that of the stems of *Polygonatum odoratum*, by 80%; that of the stems of *Glehnia littoralis*, by 71%; and that of the stems of *Angelic dahurica*, by 53%. The weight of the liver did not change after the administration of any of the extracts investigated. It must be mentioned that immunostimulating activity was shown mainly by polysaccharides from the epigeal parts of the plants.

The polysaccharides exhibiting the greatest immunostimulating activity were tested for antitumoral activity [8]. The results on the antitumoral activities of the polysaccharides are given in Table 2.

Polysaccharides (**1**), (**2**), (**4**), (**7**), and (**8**), when administered before the transplantation of the tumor, exhibited weak antitumoral activity, reducing the weight of a tumor by 43, 41, 39, 25, and 47%, respectively. On the administration of solutions of the polysaccharides after the transplantation of the tumor, no healing effect was observed either in the ascitic or in the solid variants.

The testing of the extracts with the aim of finding substances possessing antiviral activity in relation to the antigen of the virus of the Aleutian disease of mink [9] showed that the highest activity (more than 40%) was possessed mainly by the polysaccharides of the roots; *Ampelopsis japonica* (alkaline extract, 100%; aqueous extract, 70%). *Heracleum moellendorffii* (alkaline extract, 60%), *Saposhnikovia divaricata* (alkaline extract, 70%), *Aralia continentalis* (oxalate extract, 90%; alkaline, 40%), *Paeonia lactiflora* (oxalate extract, 50%) and *Arctium lappa* (alkaline extract, 70%). The only polysaccharides from the epigeal parts of the plants that exhibited activity were those of *Glehnia littoralis* (oxalate extract, 44%; alkaline, 42%) and *Saposhnikovia divaricata* (alkaline extract, 50%).

The results obtained permit the conclusion that in the majority of cases immunostimulating activity is shown by the polysaccharides of the epigeal parts of the plants investigated, and antiviral activity by those of the roots.

EXPERIMENTAL

Descending paper chromatography of the monosaccharides was conducted in the solvent system *n*-butanol–pyridine–water (6:4:3 by volume) on Filtrak FN-12, FN-15 paper. The monosaccharides were detected with an alkaline solution of silver nitrate and with aniline hydrogen phthalate. GLC analysis was conducted on a Pye-Unicam 104 instrument in glass columns (0.4 × 150 cm) with 3% of QF-1 and 3% of OV-225 on Gas Chrom Q (100-102 mesh). The monosaccharides were analyzed in the form of polyol acetates and aldononitriles in the temperature interval from 175 to 225°C at 5°C/min. Chromato-mass spectrometry was performed on a LKB 9000s instrument.

Isolation of the Polysaccharides. The plant raw material was separated into epigeal parts and roots, and these were washed and comminuted. The low-molecular-mass components were eliminated by extraction with methanol at room temperature for two days. The residual raw material was dried in the air and used for extraction of the polysaccharides. The dry residue was covered with water (1/10 by weight), and the mixture was heated in the boiling water bath for 3 h. The solution was separated by filtration, and was concentrated and evaporated in a rotary evaporator, after which the polysaccharides were precipitated with ethanol (acetone) (1/4, by volume).

The precipitate was separated off by centrifugation and a solution of it in water was freeze-dried. This gave the polysaccharide of the aqueous extract. The residue after aqueous extraction was covered with a 0.25 M solution of ammonium oxalate (1/10), and the mixture was heated in the boiling water bath for 2 h. The solution was separated by filtration, it was dialyzed against mains water for two days and then against distilled water, and it was evaporated and precipitated with ethanol (acetone). A solution of the precipitate in water was freeze-dried (oxalate polysaccharide). The residue after oxalate extraction was covered with a 3% solution of caustic soda, and the mixture was heated in the boiling water bath for 2 h. The procedure for the oxalate extraction was repeated, giving the alkali-extracted polysaccharide.

Acid Hydrolysis. A polysaccharide (10 mg) was hydrolyzed with 1 N trifluoroacetic acid at 100°C for 4-5 h. The acid was eliminated by evaporation with methanol. The monosaccharides were investigated by paper chromatography and GLC in the form of polyol acetates.

REFERENCES

1. G. Franz, Planta Med., **55**, 493 (1989).
2. R. Srivatava and D. K. Kulshreshtha, Phytochemistry, **28**, 2877 (1989).
3. H. Wagner, Pure Appl. Chem., **62**, 1217 (1990).
4. C. K. Wong, K. L. Leung, K. P. Fung, and Y. M. Choy, J. Inter. Med. Res., **22**, 299 (1984).
5. N. K. Fruentov, Medicinal Plants of the Far East [in Russian], Khab. Kn. Izd. (1974).
6. A. I. Usov, M. I. Bilan, and N. G. Klochkova, Bot. Mar., **38**, 43 (1995).
7. H. L. Ko, W. Rozkowski, J. Jiljaszwiez, and G. Pulverer, Med. Microbiol. Immunol., **170**, 1 (1981).
8. Z. P. Sof'ina, Vopr. Onkol., **22**, 82 (1976).
9. V. M. Mahnir, Yu. N. Shumilov, A. M. Kovalevskaya, L. A. Romanenko, and S. D. Gebelny, Comp. Biochem. Physiol., **106C**, 661 (1993).