Galactomannanes from Seeds of Legumes (*Leguminosae* Juss.) Growing in Siberia

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Absract

The results of studies on seeds of leguminous species growing under the conditions of temperate climatic zone concerning the presence of storage water-soluble polysaccharides galactomannanes are presented. A quantitative estimation is made for the galactomannane content in seeds of 45 species of 13 genuses from 7 tribes belonging to the leguminous family. For 23 species a chemical characteristic has been determined such as the ratio between mannose and galactose monomers in the molecule of galactomannane. The results obtained can be used in searching for domestic sources of phytogenous hydrocolloids being of interest in various scientific and practical fields.

Key words: storage polysaccharides of seeds, galactomannanes, phytogenous hydrocolloids, leguminous species

INTRODUCTION

The world market of hydrocolloids, which amounts to 2.8 billion US dollars in the beginning of 21st century, is actively developing [1]. Notwithstanding to all the variety of the materials with hydrocolloid properties more and more actual the search for novel plant sources of this material group grows more and more urgent. One of leading positions in the world after cellulose and starch is occupied by the manufacture of galactomannanes (GM). Nowadays on a global industrial scale (without taking Russia into account) there are three GM produced such as guaran, carubin and tara gum [2].

A wide practical application of GM is caused by their unique rheological properties and biological activity. Moreover, the GM could serve as a source for the obtaining of crystalline mannose. The scope of GM application in various fields of the industry is extremely wide. Galactomannanes are applied in the food, pharmaceutical, medical, cosmetic, paper, textile, geological industries as thickeners, emulsifying agents, stabilizers of disperse systems, gelling and structure-forming agents [3–9].

Extensive GM studies have begun abroad in the second half of the last century. A beginning of research concerning this problem in Russia was made with the works of the researchers from the Laboratory of carbohydrates of the Bach Institute of Biochemistry, RAS (Moscow) [10].

Chemically, the GM represent storage water-soluble heteropolysaccharides. The macromolecule of the majority of GM consists of the main chain wherein the residues of *D*-mannose are covalently bound to each other by means of 1,4-β-glycosidic linkage. From the main chain lateral chains in the form of individual links of *D*-galactose covalently attached by 1,6-α-linkage. The number of lateral branches corresponds to the number of the galactose residues.

Galactomannanes are widely distributed in the nature; and are found out in seeds of plants species belonging to 11 botanical families. For the species of leguminous family *(Leguminosae* Juss.) the GM are not only the most widespread carbohydrate reserve of seeds, but also they carry out vitally important physiological functions for plants. Nowadays the GM are found out in seeds of more than 200 species the Leguminosae family, which amounts a little more than 1.0 % of the total number of world flora species belonging to this family [2, 11–15].

Despite of a significant theoretical and practical interest to this group of storage phytogenous heteropolysaccharides, the studies on GM from the seeds of the species belonging to Leguminosae family those grow under the conditions of temperate climatic zone, are much less often available in the literature as compared to the works devoted to the GM of tropical and subtropical species of this plant family.

The purpose of the present work consisted in searching for new GM sources from the seeds of species belonging to the Leguminosae family those grow in climatic conditions of Siberia.

EXPERIMENTAL

The subject of inquiry was presented by seeds of 45 wild and introduced species of Leguminosae family (Faboideae subfamily) growing in Siberia. Moreover, nine leguminous species of the Kazakhstan flora (the East Kazakhstan Region) were investigated. The seeds of wild species were given by our colleagues from the Central Siberian Botanical Garden (CSBG), SB RAS (Novosibirsk). The seeds of introduced species were harvested at the experimental plot of the Laboratory of Rare and Endangered Plants of the CSBG, SB RAS.

Preparations of total water-soluble polysaccharides (WP) were isolated according to a unified modified pattern which included the grinding of vegetative material (3–5 g) up to size of 50–100 mesh, its extraction with hot water at a ratio of 1 : 20 ($T \geq 80$ °C under permanent stirring for 4 h), centrifuging the extract at 10000*g* during 1 h, adding to the supernatant an equal volume of 96 % ethyl alcohol and holding for 14–15 h at 4 °C to obtain a complete of sedimentation. A fibrous sediment of total WP obtained was separated by means of centrifugation at 1500*g* during 15 min; then it was dehydrated with successive washing with ethyl alcohol at increasing concentration (70, 85 and 96 %) and then dried up to constant mass reached. The isolation of GM from total WP preparations (1 g of a total WP preparation was dissolved in 100 mL of water or NaOH) was carried out with the help of Feling's reagent adding it dropwise till the formation of a sediment finished (in 30 min the completeness of sedimentation was examined in an aliquot of the supernatant). In 4 h a water-insoluble polysaccharide-copper complex formed was washed with water and centrifuged, and then the sediment was destroyed in a mortar pounding the sediment in the cold with a cooled 5 % ethanolic solution of hydrochloric acid. A purified GM preparation was washed with ethyl alcohol at growing concentration (70, 85 and 96 %) and then dried up to constant mass reached [16].

The complete acid hydrolysis of polysaccharide was carried out with 1 M H_2SO_4 solution during 4 h using a boiling water bath. The hydrolyzate obtained was neutralized with a Dowex 1 ion exchange resin preliminary charged to produce the HCO_3^- form. The reduction of monosaccharides was carried out using sodium borohydride at a room temperature during 10 h. The sugar alcohols obtained (mannitol and galactite) were acetylated in a dehydrated mixture of fresh distilled acetic anhydride and pyridine (1 : 1) whose excess was removed after carrying out of reaction by means of distilling off under vacuum using a rotary evaporator at 40 °C. The gas-liquid chromatographing of the monosaccharide derivatives obtained was carried out with Chromosorb as a stationary phase using a Chrom-5 chromatograph (Czech Republic), with a column 3 m length, 5 % XE-60 being used as a liquid phase. Nitrogen was used as a carrier gas; the separation temperature amounted to 230 °C [17].

RESULTS AND DISCUSSION

The total content for WP and GM has been determined in seeds of 45 wild and introduced species of 13 genuses belonging to seven tribes

TABLE 1

Total content of water-soluble polysaccharides (WP) and galactomanines (GM) contained therein for seeds of wild and introduced leguminous species

Tribe	Species	Content, %		Mannose : galactose ratio
		WP	GM	
Sophoreae	*Vexibia alopecuroides* (L.) Yakovlev (w)	2.9	1.0	n/d
Thermopsideae	*Thermopsis lanceolata.* R. Br. (w)	4.3	2.6	n/d.
	T. mongolica Czefr. (w)	1.7	n/d	n/d
Galegeae	*Astragalus tibetanus* Benth. ex Bunge (w)	12.3	5.3	1.10
	A. danicus Retz. (w)	8.9	7.3	1.34
	A. glycyphyllos L. (w)	17.8	14.0	1.27
	A. ellypsoides Ledeb. (w)	11.1	7.9	n/d
	A. testiculatus Pall. (w)	8.4	4.9	1.39
	A. petropyllensis Bunge (w)	12.7	9.9	n/d
	A. sabuletorum Ledeb. (w)	6.6	5.3	n/d
	A. falcatus Lam. (i)	18.1	16.2	1.30
	A. cicer L. (i)	13.1	10.5	1.33
	Galega officinalis L. (i)	8.7	5.9	n/d
	G. orientalis Lam. (i)	10.5	7.4	1.24
	Oxytropis camppanulata Vass. (w)	8.3	2.7	1.51
	O. alpina Bunge (w)	11.4	3.6	1.50
	O. strobilaceae Bunge (w)	7.9	2.5	1.55
	O. pilosa (L.) DC. (w)	10.4	8.5	n/d
	O. sulphurea (Fisch. ex DC.) Bunge (w)	7.2	4.2	n/d
	O. sylvatica (Pall.) DC. (w)	10.7	8.1	n/d
	O. sylvatica (Pall.) DC. (w)	6.2	5.1	n/d
	O. leucotricha Turcz. (w)	10.7	8.6	n/d
	O. myryophylla (Pall.) DC. (w)	6.0	2.4	n/d
	O. songorica (Pall.) DC. (w)	8.4	6.9	n/d
Hedysareae	*Hedysarum alpinum* L. (w)	4.8	0.8	n/d
	H. theinum Krasnob. (w)	3.9	0.3	1.98
	H. turczaninovii Peschkova (w)	4.8	0.7	2.70
	H. alpinum L. (i)	3.0	0.4	2.50
	H. gmelinii Ledeb. (i)	2.1	0.4	2.60
	H. neglectum Ledeb. (i)	4.2	0.5	1.90
	H. flavescens Regel & Schmalh. (i)	2.5	0.5	2.13
	Onobrychis arenaria (Kit.) DC. (i)	3.5	n/r	n/d
Loteae	*Lotus corniculatus* L. (i)	10.3	7.5	1.25 [2]
Vicieae	*Vicia unjuga* A. Br. (w)	11.7	2.8	n/d
	V. unjuga A. Br. (w)	12.4	1.7	n/d
	V. amoena Fisch. (w)	12.2	1.0	n/d
	V. costata Ledeb. (w)	4.5	0.3	n/d
	Lathyrus sylvestris L. (i)	1.2	n/r	n/d
Trifolieae	*Melilotus albus* Medik. (w)	5.0	3.1	1.13 [2]
	Trigonella orthoceras Kar.& Kir. (w)	9.7	8.0	n/d
	Trifolium ambiquum Bieb. (i)	9.5	6.6	1.09
	T. hybridum L. (i)	10.8	7.9	1.07
	T. lupinaster L. (i)	10.2	7.8	1.07
	T. medium L. (i)	7.4	4.2	1.18
	T. pannonicum Jacq. (i)	9.3	5.5	1.28
	T. pratense L. (i)	8.7	6.1	1.27
	T. rubens L. (i)	10.7	6.9	1.30
	T. trychocephalum Bieb. (i)	5.3	2.4	1.18

Note. w − wild species, i − introduced species; n/r − not revealed, n/d − not determined of Faboideae subfamily. The mannose : galactose ratio (M/G) within the GM molecule in seeds of 23 species from four genuses is presented in Table 1.

The content of GM in seeds of the species under investigation ranged from insignificant value (0–4.0 %) for the species Sophoreae, Thermopsideae, Hedysareae, Vicieae tribes, to intermediae (5.0–10.0 %) and high value (>10.0 %) for the species of Loteae, Trifolieae, Galegeae tribes.

The Sophoreae, Thermopsideae and Loteae tribes are presented by seed samples of one species for each tribe. It has been revealed that the seeds of *Vexibia alopecuroides* (Sophoreae) and *Thermopsis lanceolata* (Thermopsideae) species are deficient in GM, whereas the content of GM in seeds of *Lotus corniculatus* (Loteae) is close to intermediate value (7.5 %) (see Table 1).

In seeds of the species belonging to Hedysareae tribe the content of GM varies within the range of 0.3–0.8 % [18], whereas in seeds of *Onobrychis arenaria* species no GM were found out, though the total WP therein exceeds 3.0 %.

In seeds of *Lathyrus sylvestris* species (Vicieae tribe) no GM were revealed, too, but in minor amounts there are WP present (1.2 %). The content of GM in seeds of species of *Vicia* genus amounts to 0.2–2.8 %, whereas the total WP ranges within 3.7–12.4 %.

The content of GM for the species of Trifolieae tribe varies within the range of 2.4–8.0 %, which is in a good agreement with the data from literature data concerning GM content in legumes of temperate latitudes [19, 20].

The species of Galegeae tribe dominate in the leguminous flora of Siberia; thus they are most comprehensively presented in this research. The content of GM in seeds of the species from this tribe ranges from insignificant value (2.4–2.5 % for *Astragalus alopecurus, Oxytropis strobilaceae*, *O. campanulata)* to high value (14.0 and 16.2 % for *A. falcatus* and *A. glycyphyllos*, respectively) (see Table 1). For seeds of species belonging to related *Astragalus* and *Oxytropis* genuses the content of GM also varies over a wide range being in a good agreement with known data for species of Galegeae tribe [2]. The highest content of GM is revealed in seeds of wild plant species *Astragalus glycyphyllos* (14.0 %) and an introduced species *A. falcatus* (16.2 %).

The ratio between monomers M/G in the molecule of GM determines physicochemical properties (density, solubility, viscosity of aqueous solutions) and represents one of the major chemical characteristics. We have determined this parameter for 23 species belonging to four genuses. For six species of genus *Astragalus* the M : G ratio varies within the range of 1.10–1.39, for three species of *Oxytropis* genus this value amounts to 1.50–1.55, for six species of genus *Hedysarum* it is equal to 1.90–2.70. The M : G ratio for eight species of *Trifolium* genus ranges from 1.07 to 1.30. For the species of *Oxytropis* and *Hedysarum* genuses we first have determined the M/G ratio (see Table 1). The data obtained indicate that seeds of the species under investigation growing in climatic conditions of middle latitudes are characterized by the presence of mainly high-substituted GM with an increased galactose-containing fraction. This fact points out a higher solubility of these GM and coincides with the generally accepted opinion that a lower M/G ratio, as a rule, is inherent in GM from species of Faboideae subfamily, whereas for more than a half of studied taxons of this subfamily this value amounts to 1.0–1.5 [13, 21].

CONCLUSION

Using chemical screening the GM content was studied for 45 species of 13 genuses belonging to 7 tribes of Faboideae subfamily from Leguminosae family, growing in Siberia and in the East Kazakhstan Region. The content of GM in seeds of the species under investigation ranges from 0.3 to 16.2 %. No GM have been revealed in seeds of *Onobrychis arenaria* species (Hedysareae tribe) and *Lathyrus sylvestris* species (Vicieae tribe), in spite of the presence of water-soluble polysaccharides therein.

For GM isolated from seeds of 23 species of four genuses (*Astragalus*, *Oxytropis*, *Hedysarum*, *Trifolium*) belonging to three tribes (Galegeae, Hedysareae, Trifolieae), the mannose-to-galactose ratio within the GM molecule have been determined. For the species of *Oxytropis* and *Hedysarum* genuses this ratio has been determined for the first time. The data obtained data are in a good agreement with the data from the literature for GM species of Faboideae subfamily from temperate climatic zones. As a whole, the leguminous species under investigation presented in Siberia by the only taxons of Faboideae subfamily are characterized by the presence of high-substituted GM (with an increased galactose-containing fraction) in seeds, whose content is lower as compared to that for tropical leguminous species.

Seeds of *Astragalus glycyphyllos* and *A. falcatus* species could represent possible sources of legumes' GM for climatic conditions of Siberia since they are characterized by the maximal content of GM among the species under investigation, amounting to 14.0 and 16.2 %, respectively. In order to obtain more comprehensive information, a full-scale search is required.

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