Chemistry of Natural Compounds, Vol. 36, No. 3, 2000

GALACTANS AND GALACTAN-CONTAINING POLYSACCHARIDES OF HIGHER PLANTS

A. O. Arifkhodzhaev

UDC 547.917

Research on the chemical structure and physiological activity of galactan and galactan-containing polysaccharides of higher plants is reviewed. The principal chain in galactan-containing polysaccharides consists of 1→3, 1→4, 1→6, and α- and β-bonded D-galactopyranoses. The side chains contain separate or bonded chains of galactose, arabinose, glucose, rhamnose, and uronic acids. The relationship of chemical structure and physiological activity of the polysaccharides of higher plants is discussed.

Key words: polysaccharides, arabino-4-galactans, arabino-3,6-galactans, galactans, immunomodulators, mutagenic activity.

Extensive investigations of polysaccharides from higher plants have demonstrated that they possess valuable properties and physiological activity. Thus, they are widely used in commerce. Their immunomodulating activity, including anticomplementary, has been experimentally documented. It was shown that the physiological activity of polysaccharides is mainly due to their chemical structure [1-17].

Galactans and galactan-containing polysaccharides (galactans consist only of galactose units whereas other monosaccharides are bonded to them in galactan-containing polysaccharides) in addition to other carbohydrates are widely distributed in plants. They have been found in plants belonging to 30 families [18-93], mainly in gum, pectinic substances, hemicellulose, and other complicated polymers. They are encountered in almost all plant organs in the free state (Table 1). Arabinogalactans are common galactan-containing polysaccharides. Aspinall [94] separated arabinogalactans by the structure of the main polysaccharide chain into two types: arabino-4-galactans (I) and arabino-3,6-galactans (II). The structures of these compounds are discussed below. Polysaccharides with arabinogalactan side chains are also known.

Table 1 gives the properties of galactans and galactan-containing polysaccharides of higher plants. These polysaccharides contain both neutral and acidic sugars.

ISOLATION, PURIFICATION, AND STRUCTURAL METHODS

Polysaccharides are isolated from plants by traditional methods of extraction by cold [22, 24, 39, 43, 47, 48, 49, 58, 63] and hot water [27, 30, 37, 50, 59], phosphate buffers [44], ammonium oxalate [64], and aqueous alkali. Polysaccharides from gum are also isolated by extraction with cold [34, 36, 53, 54, 62] and hot water [23]. They are precipitated from the extract by alcohol [19, 25, 27, 34, 40, 41, 51, 54, 58, 68]. Because the isolated polysaccharides are a heterogeneous mixture, homogeneous compounds are purified and prepared by various methods: purification on ion-exchange resins [44, 46], treatment with Fehling solution [25], dialysis [64], separation on DEAE cellulose [19, 20, 22, 40, 43, 48, 59], fractional precipitation with alcohol [24, 44, 63] and $CaCl_2$ solution [20], and gel chromatography on Sephadex [23, 27, 38, 41, 49, 50, 56, 59, 68], Sepharose [46], and Sephacryl [46, 50].

In certain instances degraded polysaccharides are prepared using boiling water [23], autohydrolysis [34, 54, 56, 70], gentle hydrolysis [33, 34, 62, 66, 67], basic degradation [48], Smith degradation [53, 55], and enzymic hydrolysis [44, 71]. The homogeneity of the polysaccharides is monitored by gel filtration, electrophoresis [95, 96], and ultracentrifugation [44, 53].

S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (99871) 120 64 75. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 185-197, May-June, 2000. Original article submitted April 5, 1999.

0009-3130/00/3603-0229$25.00

TABLE 1. Properties of Galactans and Galactan-containing Polysaccharides of Higher Plants

Family, species, and plant organ	Monosaccharide composition, ratio, %	Mol. mass, arb. units	$[\alpha]_D$, deg	Bond type	Reference
Galactans					
Alliaceae, *Allium cepa*, onion	Gal (91)	-	-	(1→4), (1→6)	[18]
Allium sativum, garlic	Gal:Ara (97.3:1.6)	-	+70	(1→4), (1→6)	[19]
Leguminosae, *Dolichos lablab*, seed casing	Gal:GalUA (95.6:tr.)	120000	+51.3	(1→2), (1→4), (1→6)	[20]
Solanaceae, *Nicotiana tabacum*, leaf fiber	Gal (100)	80000	+66	(1→4)	[21]
	Gal (100)	60000	+55	(1→4)	
Salicaceae, *Salix alba* L., bark	Gal (100)	5350	+49.5	(1→4), (1→6)	[22]
Arabino-4-galactans					
Araucoriaceae, *Araucoria cookii*, gum,	Gal:Ara:Rha:GlcUA (66.2:8.9:7.6:11.5)	-		(1→2), (1→3)	[23]
degrad. gum	Gal:Ara:GlcUA (66.2:12.2:21.0)	-	-26	(1→4), (1→6)	
Cactaceae, *Opuntia dillanii*, rind	Gal:Ara (75:25)	-	+266.8	(1→3), (1→4)	[24]
Leguminosae, *Centrosema plumari*, seeds	Gal:Ara (92.5:7.0)	-	+13	(1→4)-(1→6)*	[25]
Loganiaceae, *Strychnos nux-vomica*, seeds	Gal:Man:Xyl:Ara (55:22:11:11)		+69	(1→3), (1→4), (1→6)	[26]
Rutaceae, *Aegle marmelos*, cambium wood layer	Gal:Ara:Rha:Xyl:Glc (43.0:36.0:5.0:7.0:4.5)	-	+33.8	(1→2)-(1→5)*	[27]
Solanaceae, *Lycopersicon esculentum*, fruit	Gal:Ara:Rha:GalUA (58.0:14.5:3.5:22.0)			(1→4), (1→5)	[28]
Solanum tuberosum, tuber	Gal:Ara (96:4)	-	+87	(1→3), (1→4), (1→6)	[29]
Symplocaceae, *Sympolcos spicata*, leaves	Gal:Ara (37.5:62.5)	-	+71	(1→3), (1→4), (1→6)	[30]

TABLE 1. (continued)

Family, species, and plant organ	Monosaccharide composition, ratio, %	Mol. mass, arb. units	$[\alpha]_D$, deg	Bond type	Reference
Arabino-3,6-galactans					
Aceraceae, *Acer saccharum*, maple sap	Gal:Ara:Rha (51:44:5) Gal:Ara:GalUA (85.5:2.3:8.1)	-	-41	(1→3), (1→6)	[31]
Anacardiaceae, *Anacardium occidental*, nut shell	Gal:Ara:Rha (85:10:5) (70.5:25.3:4.3)	-	-	(1→3), (1→6)	[32, 33]
Anacardiaceae, *Odina wodier*, gum	Gal:Ara (67.3:19.5)	-	-44	(1→3), (1→4), (1→6)	
	Gal:Ara:Rha:UA (28.6:50.0:1.4:20.0)		+55.3	(1→3), (1→6)	[34, 35]
Anacardiaceae, *Rhus vernicifera*, gum	Gal:Ara:Rha:GlcUA (65.1:4.8:2.0:27.8)	84000	-0.8	(1→3), (1→6)	[36]
	(67.0:5.2:4.1:26.7)	2700	+8.7	(1→3), (1→6)	
Araliaceae, *Panax notoginseng*, roots	Gal:Ara (85.7:13.6)	1500000	-20.7	(1→3), (1→6)	[37]
Asteraceae, *Calendula officinalis*, flowers	Gal:Ara:Rha (41.0:34.2:24.8)	15000	+1.3	(1→3), (1→6)	
	Gal:Ara (72.4:27.6)	25000	-22.7	(1→3), (1→6)	[38]
	Gal:Ara (51.4:48.7)	35000	-38.6	(1→3), (1→6)	
Caryophyllaceae, *Allochrusa gypsophiloides*, underground parts, roots	Ara:Glc:Gal (65.8:21.0:13.2) Glc:Gal (16.6:83.4)	2000	+176	(1→2), (1→6)	[39–42]
Cruciferae, *Brassica campestris*, seeds	Gal:Ara:GalUA (48.0:46.0:6.0)	-	-49.2	(1→2)-(1→6)*	[43]
Raphanus sativus, seeds	Gal:Ara (16:83)	40000	-109	(1→3), (1→6)	[44]
Fabaceae, *Acacea senegal*, gum	Gal:Ara:Rha:GlcUA (32:57:0.4:10)	$2.3 \cdot 10^6$	+112	(1→2)-(1→6)*	[45]
Gramineae, *Saccharum spontaneum*, cane	Gal:Ara	70000	-56	(1→3), (1→4), (1→6)	[46]
Triticum aestivum, seeds	Gal:Ara (58.3:41.6)	-	-43.5	(1→3), (1→6)	[47]

TABLE 1. (continued)

Family, species, and plant organ	Monosaccharide composition, ratio, %	Mol. mass, arb. units	$[\alpha]_D$, deg	Bond type	Reference
Gymnospermae,					
Larix sibirica, wood	Gal:Ara (88:12)	40000	+10	(1→3), (1→6)	[48]
	Gal:Ara (90.9:9.1)	29000	+10	(1→3), (1→6)	[49]
Leguminosae,					
Glycyrrhiza uralensis, roots	Gal:Ara:Rha:GalUA (37.8:54.0:2.7:5.1)	69000	-56.3	(1→3), (1→5), (1→6)	[50]
	Gal:Glc:Ara:Rha:GalUA (19:1.9:22.8:19:38)	10700	+61	(1→2)-(1→6)	
	Gal:Glc:Ara:Rha (43.5:39.2:14.5:14.5)	69000	+9.5	(1→2)-(1→6)	[51]
Lannea coromandelica, gum	Gal:Ara (75:25)	-	+45	(1→4)-(1→6)	[52]
	Gal:Ara:Rha:GalUA(GlcUA) (70:11:2:17)	257000	+27	(1→3)-(1→6)	[53]
Lannea grandis, gum	Gal:Ara:GalUA (63.9:21.3:14.2)	980000	-37	(1→3)-(1→6)	[54]
Lannea humilis, gum	Gal:Ara:Rha:GalUA(GlcUA) (75:11:2:12)	25700	+43	(1→3)-(1→6)	[55]
Pangamia glabra, gum	Gal:Ara:GlcUA (23.1:43.9:23.5)	-	+73	(1→2), (1→3), (1→4), (1→6)	[56]
Liliaceae,					
Aloe arborescens, leaves	Gal:Ara (40:60)	30000	-66.7	(1→2), (1→6)	[57]
Chlorophytum arundinaceum, fruit	Gal:Glc (18.2:81.8)	-	+62.5	(1→4), (1→6)	[58]
Malvaceae,					
Malva verticillata, seeds	Gal:Glc:Ara (37.5:43.75:18.75)	77000	-13.9	(1→3)-(1→6)	[59, 60]
Azadirachta indica, fruit	Gal:Ara:Rha:GlcUA (33.5:51.5:6.1:8.9)	220000	20.2	(1→2), (1→3), (1→4), (1→6)	[61]
Meliaceae,					
Swietenia mahogony, gum	Gal:Ara:Rha:GalUA (66.6:9:7.2:18)	-	+10	(1→2), (1→3), (1→4), (1→6)	[62]
Pinaceae,					
Pinus banksiana, wood	Gal:Ara (92.3:7.1)	8500		(1→3), (1→4), (1→6)	[63]
Rubiaceae,					
Coffea arabica, beans	Gal:Ara (71.0:29.0)	-	+27	(1→3), (1→6)	[64]

TABLE 1. (continued)

Family, species, and plant organ	Monosaccharide composition, ratio, %	Mol. mass, arb. units	$[\alpha]_D$, deg	Bond type	Reference
Rutaceae,					
Aegle marmelos,	Gal:Ara:Rha:GalUA				
gum,	(71.0:12.5:6.5:7.0)	-	+84	(1→2)-(1→6)	[65, 66]
degrad. gum	(79.5:3.5:4.2:8.6)	-	+44	(1→2)-(1→6)	[67]
Sapotaceae,					
Madhuca indica,	Gal:Ara:Rha:Xyl:GlcUA	-	-9.3	(1→2), (1→3), (1→4),	[68, 69]
flowers	(71.7:21.3:3.8:2.5:20.0)			(1→6)	[70]
	Gal:Glc:Ara:GlcUA	-	-19.5	(1→3), (1→4), (1→6)	
	(44.9:26.5:15.0:11.5)				
Umbelliferae,					
Angelica acutiloba	Gal:Ara:Rha:GalUA	128000	-57.3	(1→2)-(1→6)	
Kitagawa,	(25:55:7.5:10)				
roots	Gal:Ara:GalUA:GlcUA	7700	-14.8	(1→3)-(1→6)	[71-73]
	(34.5:34.5:27.6:3.4)				
	Gal:Ara:Rha	$2.5 \cdot 10^4$	-	(1→3), (1→5), (1→6)	
	(43.5:52.1:4.35)	$3.2 \cdot 10^3$			
Nondetermined polysaccharides					
Araliaceae,					
Cussonia spicata,	Gal:Ara:Rha:GlcUA	125000	-18	-	
gum	(23:58:7:12)				
	Gal:Ara	102000	-	(1→3), (1→4), (1→6)	[74]
		92000			
Araucoriaceae,					
Araucoria bidwilli,	Gal:Ara:Rha:GlcUA	-	-	(1→3), (1→4), (1→6)	[75]
gum	(65.5:15.0:5.0:15.0)				
Bombacaceae,					
Salmalia malabarica,	Gal:Ara	-	+18	(1→2), (1→3), (1→4),	[76]
seeds	(70:30)			(1→6)	
Bambuseae,					
Phyllostachys edulis,	Gal:Ara:Xyl	-	-26.5	(1→3), (1→6)	[77, 78]
runners	(50:50:3.4)				
Cactaceae,					
Opuntia ficus,	Gal:Ara:Rha:Xyl	-	-170	(1→2), (1→4)	[79]
berries	(35.7:37.5:11.5:15.5)				
Cruciferae,					
Brassica napis,	Gal:Ara	$7 \cdot 10^6$	-111	-	[80]
seeds	(10:90)				
Iridaceae,					
Gladiolis gandavensis,	Gal:Ara:Glc	-	-	(1→3), (1→6)	[81]
stigma	(64.3:23.8:11.9)				
Leguminosae,					
Glycin max,	Gal:Ara	330000	-	(1→3)-(1→6)	[82, 83]
	(66:34)				
Lupinus albus,	Gal:UA	-	38	(1→4)	[84]
seeds					
Lupinus luteus,	Gal:Ara:Rha:Xyl:GalUA	480000	+38	-	[85]
seeds	(42.7:28.5:5.7:5.7:17.1)	210000	+56	-	
	(40.0:26.6:6.6:6.6:20.0)				

TABLE 1. (continued)

Family, species, and plant organ	Monosaccharide composition, ratio, %	Mol. mass, arb. units	$[\alpha]_D$, deg	Bond type	Reference
Phaseolus atropurpureus, leaves	Gal:Ara:GlcUA (53.0:33.2:13.8)	-	+9	(1→3), (1→6)	[86]
Phaseolus lunatus, seeds	Gal:Ara:GlcUA (50.0:39.6:8.1)	-	-	-	[87]
Phaseolus vulgaris, roots	Gal:Ara (73.3:26.7)	25000	-50	(1→3), (1→6)	[88]
Prosopsis juliflota, gum	Gal:Ara:Rha:4-OMe-GlcUa (36:36:9:18)	-	+60	(1→3), (1→4), (1→6)	[89]
Liliaceae,					
Asparagus officinalis, seeds	Gal:Ara:Man:Rha (53.1:38.4:2.2:6.3)	-	-	-	[90]
Lilium longiflorum, runners	Gal:Ara:Rha:GlcUA (57:26:6:11)	-	-	(1→3), (1→4), (1→6)	[91]
Rubiaceae,					
Coffea indica, beans	Gal:Ara (20:80)	-	-103	(1→3), (1→5), (1→6)	[92]
Theaceae,					
Camellia sinensis, leaves	Gal:Ara (66.0:34.0)	19400	-38.2	-	[93]

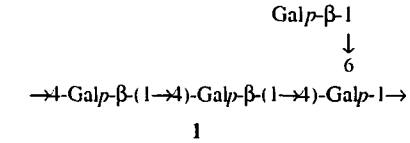
*(1→2)-(1→5) = (1→2), (1→3), (1→4), (1→5); (1→2)-(1→6) = (1→2), (1→3), (1→4), (1→5), (1→6).

Structures of galactans and galactan-containing polysaccharides are established by chemical and spectral methods. The use of these methods is illustrated below for several examples.

GALACTANS

The properties of galactans found in plants and obtained from partial cleavage of galactan-containing polysaccharides are listed in Table 1.

Indian scientists isolated galactan B_6 with $[\alpha]_D$ +70 from garlic (*Allium sativum*) [19]. The methylation products contained 2,3,4,6-tetra-OMe-, 2,3,6-tri-OMe-, and 2,3-di-OMe-D-Gal*p* in the ratio 1:2:1, respectively. The products of periodate oxidation are erythritol and glycerine, the formation of which is consistent with 1→4 and 1→6 bonds. Chemical transformations of galactan suggested the following structure for the repeating unit (**1**):



1

The galactan polysaccharide Ps-N1 with molecular weight 17,500 and $[\alpha]_D$ +51.3° was isolated from the seed casing of *Dolichos lablab* Linn. [20]. The methylation products of Ps-N1 include 2,3,4,6-tetra-OMe-, 2,3,6-tri-OMe-, and 2,3-di-OMe-D-Gal*p* in the molar ratio 1:28:0.88. Periodate oxidation, Smith degradation, enzymatic hydrolysis, and spectroscopy suggest structure **2** for the repeating unit of Ps-N1:

$x + y = 28$ **2**

The neutral fraction of pectinic substances from tobacco (*Nicotiana tabacum*) yielded two galactans with molecular weights 80,000 ($[\alpha]_D$ +66°) and 60,000 ($[\alpha]_D$ +55°) [21]. Methylation of the polysaccharides formed only 2,3,6-tri-OMe-D-Gal*p*, which is consistent with a linear polysaccharide structure. This was confirmed by the preparation of the di-, tri-, tetra-, and pentasaccharides by partial hydrolysis of the polysaccharides, which consist of β-(1→4)-bonded galactopyranosides. The structures of both polysaccharides are identical and consist of β-(1→4)-bonded galactopyranosides.

An analogous galactan was isolated from pectinic substances of white willow bark (*Salix alba* L.) [22]. It has a linear structure and consists of 33 galactose residues bonded (1→4) and (1→6) to each other.

The methylation products contain 2,3,4,6-tetra-OMe-, 2,3,6-tri-OMe-, 2,3,4-tri-OMe-, and 2,6-di-OMe-Gal*p* in the ratio 1.0:31.2:1.1:1.4. Partial hydrolysis produces oligosaccharides consisting of only (1→4)-bonded galactopyranosides.

Thus, the examples presented show that galactans consist mainly of β-(1→4)-bonded galactopyranose residues and have some branching at the galactose C-6 atom.

ARABINO-4-GALACTANS

Type I arabino-4-galactans [45] are found in plants (Table 1). The cambium layer of the marmelade tree (*Aegle marmelos*) yielded an arabinogalactan with $[\alpha]_D$ +33.8° [27].

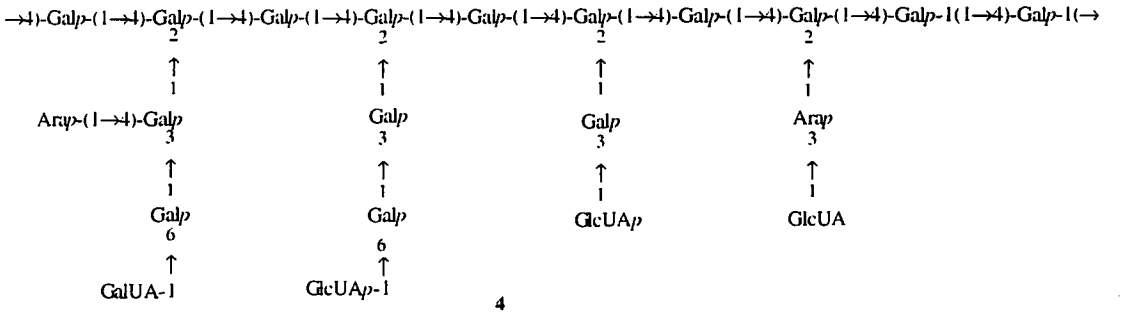
Methylation, Smith degradation, partial hydrolysis, and oxidation of the acetylated polysaccharide with chromic anhydride demonstrated that it is highly branched arabinogalactan. The sequence of β-(1→4)-bonded galactopyranose predominates in the principal chain of the polysaccharide. The side chains have galactopyranose and arabinofuranose residues. Partial hydrolysis gave three oligosaccharides, the structures of which are (**3**):

I. β-Gal*p*-(1→4)-β-Gal*p*-(→4)-Gal*p*

II. β-Gal*p*-(1→4)-Gal*p*
2
↑
β-Gal*p*-1

III. β-Gal*p*-(1→4)-Gal*p* **3**

The degraded polysaccharide from *Araucoria cookii* gum contains 66.2% galactose, 12.5% arabinose, and 21% galacturonic acid [23]. Data of methylation, periodate and chromic anhydride oxidation, and partial hydrolysis showed that the principal chain of the polysaccharide consists of the monosaccharide unit β-(1→4)-Gal*p* in which each second D-Gal*p* unit has a side chain at the C-2 position. The side chain is a disaccharide [GlcUA*p*-β-(1→6)-Gal*p* or GlcUA*p*-β-(1→3)-Ara*p*] or a tetrasaccharide {GlcUA*p*-β-(1→6)-Gal*p*-β-(1→3)-[Ara*p*-(1→4)]-Gal*p*}. The following polysaccharide structure is proposed on this basis (**4**):



4

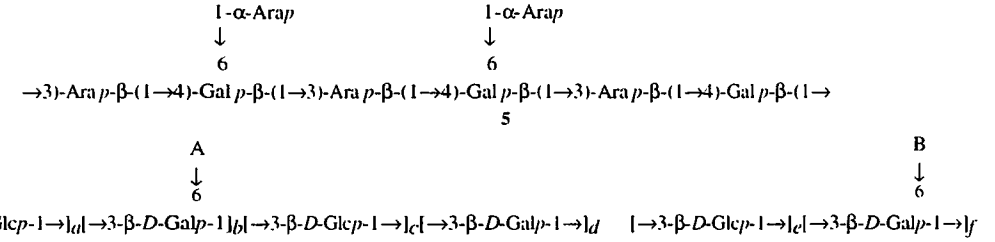
An arabinogalactan from seeds of *Centrosema plumati* consists of 92.5% D-Gal*p* and 7% L-Ara*f* with $[\alpha]_D$ +13° [25]. Partial hydrolysis, periodate oxidation, Smith degradation, and methylation suggest that the principal chain consists of (1→4)-bonded Gal*p* residues. Furthermore, (1→6)-bonded Gal*p* and (1→5)-bonded Ara*f* residues were found.

The rind of *Opuntia dillenii* yielded an arabinogalactan consisting of arabinose and galactose in the ratio 1:3. The methylation products contained 2,6-di-OMe-D-Gal*p*, 2,3,6-tri-OMe-D-Gal*p*, 2,3,4,6-tetra-OMe-D-Gal*p*, and 2,3,5-tri-OMe-L-Ara*f* in the ratio 13:10:1:12. Oxidation and other properties suggest the following fragments:

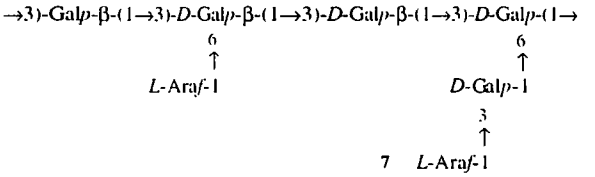
$$\left.\begin{array}{l}\rightarrow 4\text{-Gal}p\text{-}1\rightarrow 4\text{-Gal}p\text{-}1\rightarrow \text{.....23 residues}\\ 12 \text{ side chains - Ara}f\text{ 1 residues}\rightarrow\\ 1 \text{ side chain– Gal}p\text{ 1 residue}\rightarrow\end{array}\right] \text{ at Gal}p\text{ C-3}$$

An arabinogalactan from potato (*Solanum tuberosum*) consists mainly of β-(1→4)-bonded Gal*p* residues with branching at C-3 and C-6. This was established by methylation, periodate oxidation, and IR spectroscopy [29]. A polysaccharide from seeds of *Strychnos nux-vomica* has an analogous structure [26].

Structures in which the principal chain consists of (1→4)-bonded Gal*p* and (1→3)-bonded Ara*p* residues are also known. The polysaccharide isolated from leaves of *Symplocos spicata* is one of these (**5**) [30].



A: α-*L*-Ara*f*-1→5-Ara*f*-1→4-*D*-Gal *p*-1→
B: β-*D*-Glc*p*-1→[4-*D*-Gal*p*-1]$_3$[3-*D*-Glc*p*-1→]$_9$ **6**
(*a* + *c* + *e*): *a* : *d* : *f* = 4:3:2:1



The examined examples indicate that arabino-4-galactans consist of β-(1→4)-bonded Gal*p* residues in the principal chain with branching at C-2, C-3, and C-6 of the Gal*p*. The arabinose residues are situated mainly in the polysaccharide side chains.

ARABINO-3,6-GALACTANS

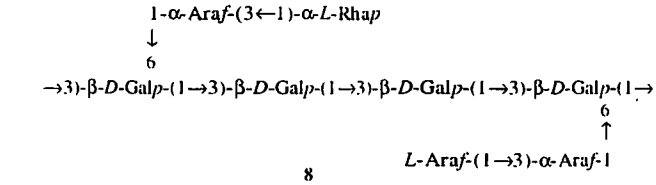
Arabino-3,6-galactans belong to type II [45]. These widely distributed polysaccharides were found in plants of various families (Table 1). Their structures are rather complicated. We include polysaccharides for which the structures were most studied.

Japanese researchers isolated from seeds of *Malva verticillata* the neutral polysaccharide MVS-I, which consists of L-arabinose, D-galactose, and D-glucose in the ratio 3:6:7 and has $[\alpha]_D^{24}$ -13.9° [60]. The structure of MVS-I was established by periodate oxidation, Smith degradation, methylation, partial acidic and enzymatic hydrolysis, and chromatography-mass spectrometry (**6**).

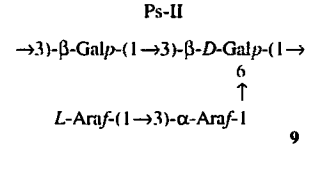
The coffee beans (*Coffea arabica*) yielded an arabinogalactan consisting of Ara*f* (28.6%) and Gal*p* (71.4%) with $[\alpha]_D$ +27° [64]. The hydrolysis products of the permethylate contained 2,3,5-tri-OMe-L-Ara*f*, 2,4-di-OMe-D-Gal*p*, and 2,4,6-tri-OMe-D-Gal*p*. The ratio of 2,4,6-tri-OMe-D-Gal*p* and 2,4-di-OMe-D-Gal*p* was 3:2. The structure of the repeating unit is shown (**7**).

Three polysaccharides were isolated from flowers of *Calendula officinalis* [38]. The monosaccharide composition and analysis of the methylation products demonstrated that they all consist mainly of β-(1→3)-bonded D-galactose with branching at C-6. The side chains consist of disaccharide chains α-L-Ara*f*-(1→3)-Ara*f* and α-L-Rha*p*-(1→3)-Ara*f* or α-L-Ara*f* units. The structural characteristics of the polysaccharides were supported by periodate oxidation, Smith degradation, acid hydrolysis, and ^{13}C NMR spectroscopy.

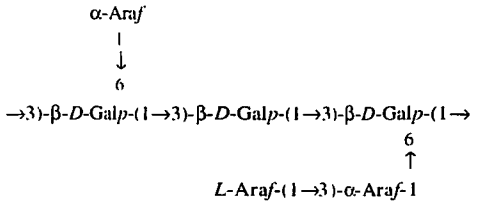
The following repeating unit in these polysaccharides (**8-10**) was proposed:



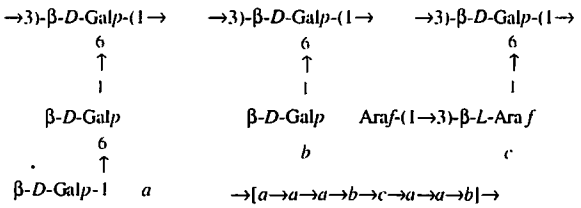
8



9

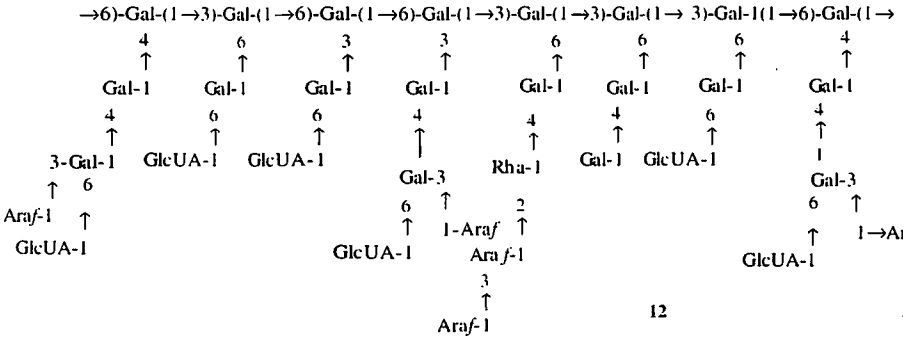


10



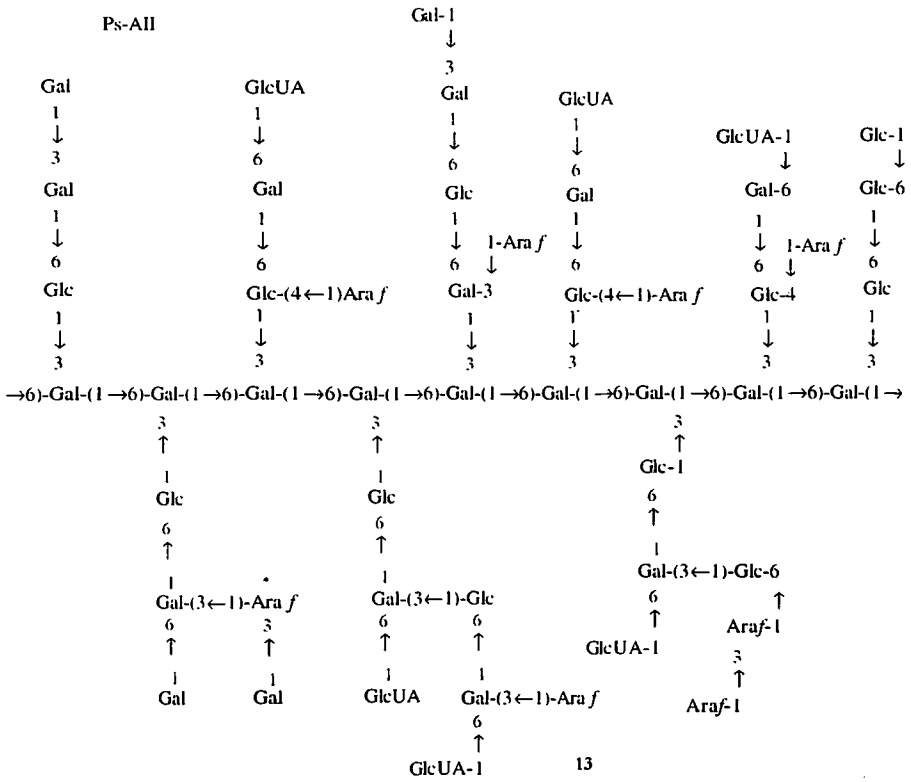
11

Ps-AI



12

Ps-AII

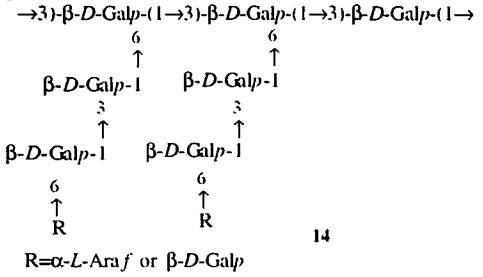


13

Wood of *Larix sibirica* yielded an arabinogalactan of molecular weight 29,000 that contained 9.1% Ara*f* and 90.9% Gal*p* and had $[\alpha]_D$ +10° [49]. Methylation, enzymatic hydrolysis, structural studies of the oligosaccharides from partial hydrolysis, and ^{13}C NMR spectroscopy suggested that the repeating unit of the polysaccharide and its fragments had structure **11**.

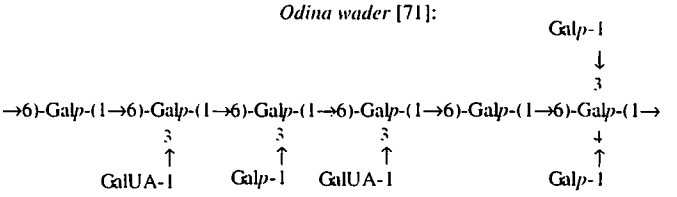
Two polysaccharides were isolated from flowers of *Madhuca indica* (Mahya). Their structures were studied by methylation, periodate oxidation, Smith degradation, chromic oxidation, autohydrolysis, and structural investigations of the oligosaccharides and degraded polysaccharides. Structures **12** and **13** were proposed for the repeating unit.

An arabinogalactan named sanchinan A of molecular weight 1,500,000 was isolated from roots of Sanchi-Ginseng (*Panax notoginseng*) [37]. Its structure was proved by the methods mentioned above. Repeating unit of structure **14** was proposed.



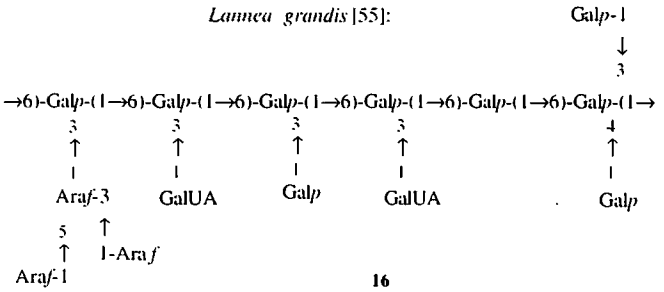
14

Odina wader [71]:



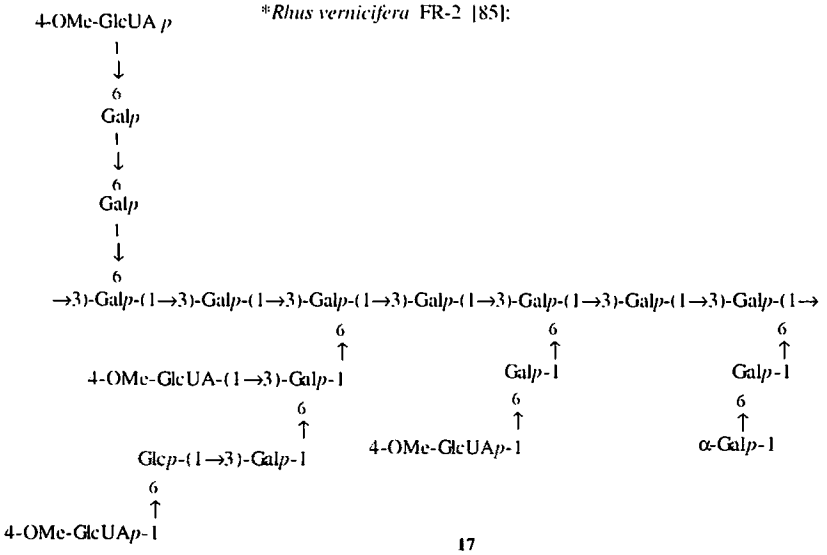
15

Lannea grandis [55]:



16

**Rhus vernicifera* FR-2 [85]:



17

*All the monosaccharides are bonded between themselves through a β-linkage.

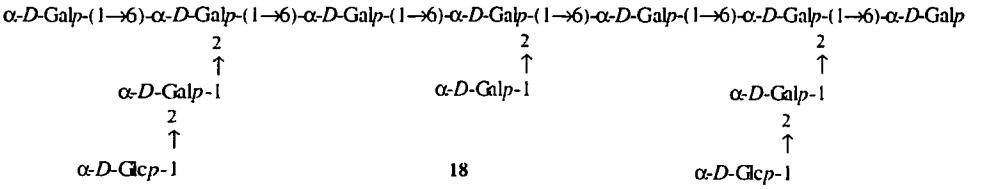
The same methods were used to establish the structure of complicated polysaccharides from plant gum (**15-17**).

In contrast with the above polysaccharides, those isolated from the subterranean organs of *Allochrusa gypsophiloides* Rgl [39, 40] have Glc*p* residues bonded to the principal Gal*p* chain. A glucogalactan isolated from roots of *A. gypsophiloides*

[41] has molecular weight 2,000, $[\alpha]_D$ +176°, and contains 16.6% Glc*p* and 83.4% Gal*p*. Only glycerine was observed after periodate oxidation and subsequent Smith degradation. This is consistent with the presence of 1→2 and 1→6 bonds between the hexose units.

Methylation of the glucogalactan by the method of Hakomori produced 2,3,4,6-tetra-OMe-Gal*p*; 2,3,4,6-tetra-OMe-Glc*p*, 3,4,6-tri-OMe-Gal*p*, 2,3,4-tri-OMe-Gal*p*, and 3,4-di-OMe-Gal*p* in the ratio 2:2:2:3:3, respectively. Methylation indicated that the principal chain consists of 1→6-bonded D-Gal*p* residues. The presence of 3,4-di-OMe-Gal*p* is consistent with branching at C-2 of the Gal*p*.

Structure **18** was assigned to the glucogalactan and the oligosaccharides resulting from partial hydrolysis on the basis of chemical and spectral methods (chromatography-mass spectrometry, ^{13}C NMR, and IR) [42].

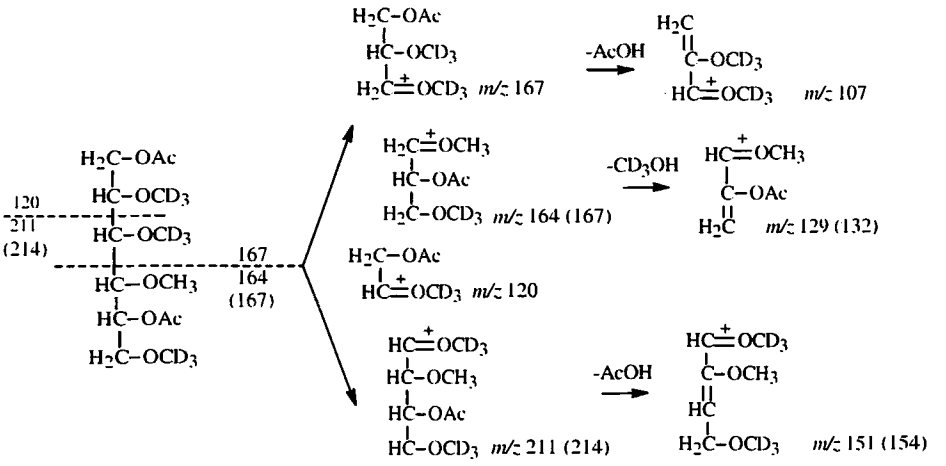


Thus, the principal chains of arabino-3,6-galactans consist mainly of β-(1→3)- and β-(1→6)-bonded galactopyranose units. Side chains are bonded to the principal one at C-2, C-3, C-4 and C-6 of the galactopyranose.

SPECTRAL METHODS

Spectral methods in addition to chemical ones are commonly used to study the structure of galactans and galactan-containing polysaccharides. The main spectral methods of the modern chemistry of carbohydrates are mass spectrometry and ^{13}C NMR spectroscopy. Their application can be illustrated using several examples.

Mass Spectrometry. Much information can be gained by using this technique to study the structure of galactans and galactan-containing polysaccharides. Like for other polysaccharides, mass spectrometric studies of galactans and galactan-containing polysaccharides are performed on their various derivatives [97-99]. Toman et al. [22] used this method to study the structure of the galactan from white willow bark (*Salix alba* L.). They characterized the relative intensities of 1,5,6-tri-OAc-2,3,4-tri-OMe-Gal, 1,4,5-tri-OAc-2,3,6-tri-OMe-Gal, and 1,3,4,5-tetra-OAc-2,6-di-OMe-Gal in the *m/z* range 45-205. The strongest signals for the first compound are *m/z* (%): 88 (100), 101 (67.9), 75 (52.4), 73 (22.7), and 45 (32.0); for the second, 101 (100), 88 (33.3), 75 (45.8), 71 (21.9), 45 (80.1); for the third, 87 (100), 88 (17.8), 74 (63.2), 71 (17.8), 45 (57.2).



Scheme 1

TABLE 2. Relative GLC Retention Times and Principal Fragments in Mass Spectra of Partially Methylated Polyol Acetates

Components	Relative retention time	Principal fragments	Bonds
1,4-Ac-2,3,5-Me-*L*-arabite	0.69	43, 45, 71, 87, 101, 117, 129, 161	*L*-Ara*f*-(1-
1,5-Ac-2,3,4-Me-*L*-arabite	0.79	43, 101, 117, 161	*L*-Ara*p*-(1-
1,3,5-Ac-2,4-Me-*L*-arabite	1.04	43, 87, 113, 117, 233	-3,5)-*L*-Ara*f*-(1-
1,4,4-Ac-2,3-Me-*L*-arabite	1.13	43, 87, 101, 117, 129, 189	-4,5)-*L*-Ara*p*-(1-
1,2,5-Ac-3,4-Me-*L*-rhamnite	0.95	43, 89, 129, 131, 189	-2)-*L*-Rha*p*-(1-
1,2,4,5-Ac-3-Me-*L*-rhamnite	1.28	43, 87, 101, 129, 143, 189, 203	*L*-Rha*p*-(1-
1,5-Ac-2,3,4,6-Me-*D*-sorbite	1.00	43, 45, 71, 87, 101, 117, 129, 145, 161, 205	*D*-Glc*p*-(1-
1,5-Ac-2,3,4,6-Me-*D*-dulcite	1.09	43, 45, 71, 87, 101, 117, 129, 145, 161, 205	*D*-Gal*p*-(1-
1,3,5-Ac-2,4,6-Me-*D*-dulcite	1.36	43, 45, 87, 101, 117, 129, 161	-3)-*D*-Gal*p*-(1-
1,4,5-Ac-2,3,6-Me-*D*-dulcite	1.44	43, 45, 87, 99, 101, 113, 117, 233	-4)-*D*-Gal*p*-(1-
1,5,6-Ac-2,3,4-Me-*D*-dulcite	1.58	43, 87, 99, 101, 117, 129, 161, 189	-6)-*D*-Gal*p*-(1-
1,2,4,5-Ac-3,6-Me-*D*-dulcite	1.75	43, 45, 87, 99, 113, 129, 189, 233	-2,4)-*D*-Gal*p*-(1-
1,3,5,6-Ac-2,4-Me-*D*-dulcite	2.01	43, 87, 117, 129, 189	-3,6)-*D*-Gal*p*-(1-

The arabinogalactan from the lac tree (*Rhus vernicifera*) contains 4-OMe-D-glucuronic acid on the unreducing end [36]. The deuteromethyl derivative was obtained from reduced 4-OMe-D-GlcUA and studied by mass spectrometry (Scheme 1). Strong peaks occurred at *m/z* 214, 154, and 132 (8.0, 6.1, and 17.8%). Chromatography-mass spectrometry was used to study partially methylated acetates of the polyols from the galactan from *Dolichos lablab* [20], the arabinogalactan from *Malva verticillata* [59] and *Glycyrrhiza uralensis* [50], and the glucogalactan from *Allochrusa gypsophiloides* [41].

Table 2 presents certain chromatography-mass spectrometric data of partially methylated acetates of sugar polyols.

^{13}C NMR Spectroscopy. This is one of the main methods for establishing the structure of natural compounds. It is successfully applied to the modern chemistry of carbohydrates. The structure of the arabinogalactan of *Larix sibirica* was studied by this method [49]. The structure of the polysaccharide was established by comparing spectra of six oligosaccharides obtained from this arabinogalactan by partial hydrolysis. The spectral data confirm the results from chemical studies (Table 3).

Signals of the methyl and acetyl groups in natural arabinogalactans are observed at 57.01 (methyl) and 21.75 and 178.26 ppm (acetyl). Anomeric C atoms in β-Gal*p*, α-Ara*p*, and α-Ara*f* are observed near 105.31 (105.85), 110.13, and 111.94 ppm, respectively.

A study of β-(1→4)-bonded arabinogalactans by ^{13}C NMR showed that peaks at 105.6 and 78.9 ppm and at 108.9 and 68.3 ppm are due to C-1 and C-4 of β-(1→4)-bonded galactopyranose residues and C-1 and C-5 of α-(1→5)-bonded arabinofuranose residues, respectively. The ratio of peak intensities at 105.6 (C-1 Gal*p*) and 108.9 ppm (C-1 Ara*f*) is 4:1 [28].

The spectrum of the *Allochrusa gypsophiloides* glucogalactan [41] contains strong signals at 100.2 (C-1), 69.4 (C-2), 70.4 (C-3), 70.5 (C-4), 72.2 (C-5), and 62.4 ppm (C-6) from C atoms of the galactopyranose residues and weaker resonances at 99.9 (C-1), 72.5 (C-2), 74.3 (C-3), 70.8 (C-4), 73.3 (C-5), and 61.8 ppm (C-6) that are typical of C atoms of the glucopyranose residues. Peaks at 62.4 and 63.2 ppm belong to C-6 of the unsubstituted hexapyranoses. Chemical shifts of 100.2 and 99.9 ppm indicate that the galactopyranose and glucopyranose residues have the α-configuration. Substituted C-6 of galactopyranoses resonate at 66.7 and 68.2 ppm. A chemical shift of 92.1 ppm corresponds to the resonance of C-1 in the reducing part of an α-galactopyranose. The resonance range from 102.3 to 103.0 ppm is typical of galactopyranose C-1 in a branching position. Signals of substituted galactopyranose C-2 appear at 81.9 and 86.6 ppm [41].

The galactan-containing polysaccharide of *Rhus vernicifera* was also studied by ^{13}C NMR [36]. The polysaccharide consists mainly of β-(1→3)-bonded Gal*p* residues and has a side chain of C-6 of Gal*p* residues. This was confirmed by strong signals for C-1 (104.1 and 103.2 ppm), C-3 (82.3 ppm), and C-6 (60.9, 61.5, and 62.0 ppm).

^{13}C NMR was used to establish the structure of arabinogalactans from *Angelica acutiloba* [72], *Calendula officinalis* [38], *Panax notoginseng* [37], *Saccharum spontaneum* [46], and others.

TABLE 3. ^{13}C NMR Chemical Shifts of Arabinogalactan Oligosaccharides

Compound	Residue	C-1	C-2	C-3	C-4	C-5	C-6
β	*A* β	96.6	69.0	69.3	69.7	63.8	-
L-Ara*p*-(1 ·3)-*L*-Ara	*B*α	97.4	71.0	78.0	69.7	66.7	-
A B	*B* β	93.4	69.3	74.5	69.7	62.8	-
β	*A* β	103.7	72.3	73.8	70.5	74.8	62.4
D-Gal*p*-(1 ·3)-*D*-Gal*p*	*B*α	93.5	69.6	79.5	70.0	71.9	62.4
A B	*B* β	97.7	73.8	80.1	70.0	76.4	62.4
D-Gal*p*-(1-6)-*D*-Gal*p*	*A* β	104.0	72.6	73.4	70.1	76.0	61.8
A B	*B*α	93.2	69.6	69.9	70.1	71.6	69.6
	B β	97.3	73.4	74.6	70.1	74.6	69.1
D-Gal*p*-(1→6)-*D*-Gal*p*- A C B	*A*(*D*)	104.0 (103.6)	73.0	73.9	71.0	75.2	61.3
-(1→6)-Gal*p*-(1- 3	*B*	104.0	73.0	75.4	71.0	75.4	68.9
↑ *D*-Gal*p*-1 D	*C*	103.6	73.9	81.7	70.4	72.9	68.9

Thus, ^{13}C NMR can not only determine the configuration, type of bonds, and size of the carbocycle but also establish the structure of galactans and galactan-containing polysaccharides, assign them to one or another type of polysaccharide, and locate the site of attachment of the sugars to the principal chain.

PHYSIOLOGICAL ACTIVITY OF POLYSACCHARIDES

Physiologically active plant polysaccharides are widely used to cure ulcers and eliminate salts of heavy metals and radionuclides from the body. Furthermore, most galactan-containing polysaccharides of higher plants are immunomodulators that activate the reticulo-endothelial system (RES) and increase the phagocytotic index. Polysaccharides-glycyrrhizans UA, UB, and UC from *Glycyrrhiza uralensis* [50, 100] noticeably increase the phagocytotic index compared with a control, for which the known phagocytosis activator zymozan was used [100]. Glycyrrhizan UA possesses high anticomplementary activity.

Chemical modification of arabinogalactans noticeably decreases or destroys the activity. Mild acid hydrolysis of glycyrrhizan UA to remove the side chains containing arabinose noticeably decreases its activity. This indicates that the complicated side chains are important to the activity [100].

A study of the interdependence of structure and anticomplementary activity of the arabinogalactan from roots of *Angelica acutiloba* [71] showed that its activity decreases in cleaved polysaccharides and oligosaccharides compared with that of arabinogalactan AGIIb-I.

The main neutral polysaccharide MVS-I that is isolated from seeds of *Malva verticillata* exhibited high RES activity [60]. The effects of MVS-I and its periodate oxidation product (POP), the Smith degradation product (SDP), and the enzymatic degradation product (EDP) on RES activity were studied *in vivo* in a test of carbon clearance compared with the positive control zymozan. The cleavage products of the polysaccharide (POP, SDP, EDP) have a lower activity than for MVS-I itself. MVS-I exhibits high anticomplementary activity. In contrast with typical arabino-3,6-galactans, the principal chain of MVS-I consists of alternating β-(1 ·3)-bonded D-galactopyranose and D-glucopyranose residues and has no hexouronic acids. The side chain contains L-arabinofuranose residues α-(1 ·5)-bonded, β-(1 ·4)-bonded D-galactopyranoses, β-(1 ·4)-bonded D-glucopyranoses, and D-galactopyranoses, and β-(1 ·3)-bonded D-glucopyranoses attached to the principal chain through C-6 of D-galactopyranose. Removal of the side chains destroys the RES activity [60].

Glycyrrhizan UA exhibits significant mutagenic activity. It is interesting that removal of the side chains consisting of α-(1 ·5)-bonded L-arabinose residues significantly decreases the mutagenic acitivty. This indicates that the side chains of

arabinose carbohydrates and the 3,6-galactan chain are important to the appearance of this activity [100].

Arabinogalactans (Ps-I, Ps-II, and Ps-III) from *Calendula officinalis* possess immunostimulating activity in several tests of the immunological system *in vitro* [38]. The immunological specificity of the macromolecules is directly related to the size of the branched galactan core because the groups determining the immunological activity are located in the branched region and make an important contribution to the physiological activity.

It was established that 1,3- and 1,5-bonded L-arabinose residues in the side chain of arabino-3,6-galactan determine the RES-stimulating, phagocytotic, and mutagenic activity of these polysaccharides. Obviously the physiological activity depends mostly on structural details, i.e., on the structure of the side chains, their position along the principal chain, the macromolecular conformation, and the mechanism of forming dimers and aggregates. The localization of the polysaccharides in the plant cell, the biosynthesis and nature of the interaction of the polysaccharides with receptors of other cells, and their metabolic path in the organism play determining roles in the appearance of physiological activity.

A generalization of the above data suggests that the physiological activity is directly related to structural features of polysaccharides from higher plants.

REFERENCES

1. H. Yamada, Y. Ohshima, and T. Miyazaki, *Chem. Pharm. Bull.*, **30**, 1784 (1982).
2. H. Yamada, H. Kiyohara, J. C. Cyong, Y. Kojima, Y. Kumazawa, and Y. Otsuka, *Planta Med.*, **50**, No. 2, 163 (1984).
3. H. Yamada, K. Ohtani, H. Kiyohara, J. C. Cyong, Y. Otsuka, Y. Ueno, and S. Omura, *Planta Med.*, No. 2, 121 (1985).
4. H. Yamada, Y. Otsuka, and S. Omura, *Planta Med.*, No. 4, 311 (1986).
5. H. Yamada, T. Nagai, J. C. Cyong, Y. Otsuka, M. Tomoda, N. Shimizu, and K. Shimada, *Carbohydr. Res.*, **141**, 101 (1985).
6. M. Tomoda, M. Takahashi, and S. Nakatsuka, *Chem. Pharm. Bull.*, **21**, 707 (1973).
7. M. Tomoda, M. Yokoi, and K. Ishihawa, *Chem. Pharm. Bull.*, **29**, 2887 (1981).
8. M. Tomoda, N. Shimizu, K. Shimada, K. Gonda, and H. Sakabe, *Chem. Pharm. Bull.*, **32**, 2182 (1984).
9. M. Tomoda, S. Kaneko, M. Eboshi, and T. Naganura, *Chem. Pharm. Bull.*, **25**, 1357 (1977).
10. M. Tomoda, N. Satoh, and K. Shimada, *Chem. Pharm. Bull.*, **28**, 824 (1980).
11. H. Wagner and A. Proksch, *Int. Conf. Chem. Biotechnol. Biol. Acta Nat. Prod.*, **3**, 200 (1981).
12. M. Tomoda and Y. Suzuki, *Chem. Pharm. Bull.*, **25**, 3061 (1977).
13. M. Tomoda, Y. Suzuki, and N. Satoh, *Chem. Pharm. Bull.*, **27**, 1651 (1979).
14. M. Tomoda, N. Shimizu, H. Suzuki, and T. Takasu, *Chem. Pharm. Bull.*, **29**, 2777 (1981).
15. M. Tomoda, K. Shimada, and N. Shimizu, *Chem. Pharm. Bull.*, **31**, 2677 (1983).
16. G. Yu. Lavrenova and I. P. Chernov, *Pharmakol. Toksikol.*, **46**, 85 (1983).
17. T. Fujiiwara, E. Sugishita, T. Takeda, Y. Ogihara, M. Shimizu, T. Nomura, and Y. Tomita, *Chem. Pharm. Bull.*, **32**, 1385 (1984).
18. S. K. Sen, B. P. Chatterjee, and C. V. N. Rao, *J. Chem. Soc. C*, No. 10, 1788 (1971).
19. N. N. Das, A. Das, and A. K. Mukherjee, *Carbohydr. Res.*, **56**, No. 2, 337 (1977).
20. K. Ghosh and A. Das, *Carbohydr. Res.*, **126**, 287 (1984).
21. S. Eda and K. Kato, *Agric. Biol. Chem.*, **42**, No. 12, 2253 (1978).
22. R. Toman, S. Karacsonyi, and V. Kovacik, *Carbohydr. Res.*, **25**, No. 2, 371 (1972).
23. G. T. Kanti and C. V. N. Rao, *Indian J. Chem., Sect. B*, **23**, No. 1, 52 (1984).
24. B. K. Srivastava and C. S. Pande, *Planta Med.*, **25**, 92 (1974).
25. A. M. Unrau, *Can. J. Chem.*, **42**, No. 4, 916 (1964).
26. P. Andrews, L. Hough, and J. K. N. Jones, *J. Chem. Soc.*, 806 (1954).
27. R. K. Basak and A. K. Mukherjee, *Carbohydr. Res.*, **110**, No. 1, 145 (1982).
28. R. Pressey and D. S. Himmelsbach, *Carbohydr. Res.*, **127**, No. 2, 356 (1984).
29. P. J. Wood and I. R. Siddiqui, *Carbohydr. Res.*, **22**, No. 1, 212 (1972).

30. R. D. Tiwari and H. L. Tripathi, *Planta Med.*, **29**, 376 (1976).
31. G. A. Adams and C. T. Bishop, *Can. J. Chem.*, **38**, No. 12, 2380 (1960).
32. S. Bose and P. L. Soni, *J. Indian Chem. Soc.*, **49**, No. 6, 593 (1972).
33. S. Bose and P. L. Soni, *Indian J. Chem.*, **11**, 996 (1973).
34. A. K. Bhattacharyya and C. V. N. Rao, *Can J. Chem.*, **42**, No. 1, 107 (1964).
35. A. K. Gupta and S. Mukherjee, *Indian J. Chem.*, **11**, No. 7, 648 (1973).
36. R. Oshima and J. Kumanotoni, *Carbohydr. Res.*, **127**, No. 1, 43 (1984).
37. K. Ohtani, K. Mizutani, S. Hatono, R. Kasai, R. Sumino, T. Shiota, M. Ushijima, J. Zhou, T. Fuwa, and O. Tanaka, *Planta Med.*, **53**, No. 2, 166 (1987).
38. J. Varlien, A. Liptak, and H. Wagner, *Phytochemistry*, **28**, No. 9, 2379 (1989).
39. A. O. Arifkhodzhaev and E. S. Kondratenko, *Khim. Prir. Soedin.*, 230 (1983).
40. A. O. Arifkhodzhaev and D. A. Rakhimov, *Khim. Prir. Soedin.*, 434 (1992).
41. A. O. Arifkhodzhaev, *Khim. Prir. Soedin.*, 530 (1996).
42. A. O. Arifkhodzhaev, *Khim. Prir. Soedin.*, 534 (1996).
43. R. Siddiqui and P. J. Wood, *Carbohydr. Res.*, **24**, No. 1, 1 (1972).
44. Y. Tsumuraya, Y. Hashimoto, and S. Yamamoto, *Carbohydr. Res.*, **161**, No. 1, 113 (1987).
45. A. E. Clarke, R. L. Anderson, and B. A. Stone, *Phytochemistry*, **18**, No. 4, 521 (1979).
46. J. D. Blake, M. L. Clarke, and P. L. Jansson, *Carbohydr. Res.*, **115**, 265 (1983).
47. H. Neukom and H. Markwalder, *Carbohydr. Res.*, **39**, No. 2, 387 (1975).
48. G. F. Antonova and A. I. Usov, *Bioorg. Khim.*, **10**, No. 12, 1664 (1984).
49. S. Karacsonyi, V. Kovacik, J. Alfold, and M. Kubackova, *Carbohydr. Res.*, **134**, 265 (1984).
50. M. Tomoda, N. Shimizu, M. Kanari, R. Gonda, S. Arai, and Y. Okuda, *Chem. Pharm. Bull.*, **38**, No. 6, 1667 (1990).
51. N. Shimizu, M. Tomoda, M. Kanari, R. Gonda, A. Satoh, and N. Satoh, *Chem. Pharm. Bull.*, **38**, No. 11, 3069 (1990).
52. R. Ramachandran and B. C. Joshi, *Phytochemistry*, **7**, No. 11, 2057 (1968).
53. D. M. W. Anderson and A. Hendrie, *Carbohydr. Res.*, **26**, No. 1, 105 (1973).
54. S. R. Chaudhuri and S. K. Mukherjee, *J. Indian Chem. Soc.*, **46**, 109 (1969).
55. D. M. W. Anderson and A. Hendrie, *Carbohydr. Res.*, **22**, No. 2, 265 (1972).
56. S. Guha, S. Basu, and C. V. N. Rao, *Indian J. Chem., Sect. B*, **24**, No. 2, 171 (1985).
57. A. Yagi, H. Nishimura, T. Shida, and I. Nishioka, *Planta Med.*, **3**, 213 (1986).
58. R. Gupta, O. C. D. Gupta, P. C. Gupta, and C. S. Pande, *Planta Med.*, **37**, No. 1, 94 (1979).
59. N. Shimizu and M. Tomoda, *Chem. Pharm. Bull.*, **35**, No. 12, 4981 (1987).
60. N. Shimizu, H. Asahara, M. Tomoda, R. Gonda, and N. Ohara, *Chem. Pharm. Bull.*, **39**, No. 10, 2630 (1991).
61. A. K. Sen, A. K. Das, and N. Banerji, *Indian J. Chem., Sect. B*, **32**, 862 (1993).
62. P. K. Gosal and S. Thakur, *Carbohydr. Res.*, **112**, No. 2, 253 (1983).
63. C. T. Bishop, *Can. J. Chem.*, **35**, No. 9, 1010 (1957).
64. M. L. Wolfrom and D. L. Patin, *J. Org. Chem.*, **30**, No. 12, 4060 (1965).
65. A. Roy, A. K. Mukherjee, and C. V. N. Rao, *Carbohydr. Res.*, **41**, 219 (1975).
66. A. Roy, A. K. Mukherjee, and C. V. N. Rao, *Carbohydr. Res.*, **54**, 115 (1977).
67. A. Roy, S. B. Bhattacharya, A. K. Mukherjee, and C. V. N. Rao, *Carbohydr. Res.*, **50**, 87 (1976).
68. N. Sarkar and B. P. Chatterjee, *Carbohydr. Res.*, **112**, 113 (1983).
69. N. Sarkar and B. P. Chatterjee, *Carbohydr. Res.*, **125**, 145 (1984).
70. N. Sarkar and B. P. Chatterjee, *Carbohydr. Res.*, **127**, No. 2, 283 (1984).
71. H. Kiyohara, H. Yamada, J.-C. Cyong, and Y. Otsuka, *J. Pharmacobio.-Dyn.*, **9**, No. 4, 339 (1986).
72. H. Kiyohara, H. Yamada, and Y. Otsuka, *Carbohydr. Res.*, **167**, 221 (1987).
73. H. Kiyohara and H. Yamada, *Carbohydr. Res.*, **193**, 173 (1989).
74. S. C. Churms and A. M. Stephen, *Carbohydr. Res.*, **19**, No. 2, 211 (1971).
75. G. O. Aspinall, J. A. Molloy, and C. C. Whitehead, *Carbohydr. Res.*, **12**, 143 (1970).
76. S. Das, P. K. Ghosal, and B. Ray, *Carbohydr. Res.*, **207**, No. 2, 336 (1990).

77. E. Mackawa and K. Kitao, *Agric. Biol. Chem.*, **37**, 2445 (1973).
78. E. Mackawa, *Agric. Biol. Chem.*, **39**, No. 12, 2291 (1975).
79. E. S. Amin, O. M. Awad, and M. M. El-Sayad, *Carbohydr. Res.*, **15**, 159 (1970).
80. O. Larm, O. Theander, and P. Aman, *Acta Chem. Scand. B*, **29**, 1011 (1975).
81. P. A. Gleeson and A. E. Clarke, *Carbohydr. Res.*, **83**, 187 (1980).
82. M. Morita, *Agric. Biol. Chem.*, **29**, No. 6, 562 (1965).
83. M. Morita, *Agric. Biol. Chem.*, **29**, No. 7, 626 (1965).
84. E. L. Hirst, J. K. N. Jones, and W. O. Walder, *J. Chem. Soc.*, 1225 (1947); *Chem. Abstr.*, **42**, 1203d (1947).
85. M. Tomoda and M. Kitamura, *Chem. Pharm. Bull.*, **15**, No. 7, 1021 (1967); *Ref. Zh. Biokhim.*, **5**, No. 11, 5F1015 (1968).
86. C. W. Ford, *Phytochemistry*, **11**, 2559 (1972).
87. M. A. Jermyn and Y. M. Yeow, *Aust. J. Plant Physiol.*, **2**, No. 4, 501 (1975).
88. G. B. Hawes and G. A. Adams, *Phytochemistry*, **11**, 1461 (1972).
89. G. O. Aspinall and C. C. Whitehead, *Can. J. Chem.*, **48**, 3840 (1970).
90. M. A. Jermyn and Y. M. Yeow, *Aust. J. Plant Physiol.*, **2**, No. 4, 501 (1975).
91. G. O. Aspinall and K. G. Rosell, *Phytochemistry*, **17**, 919 (1978).
92. J. B. C. Correa and J. D. Fontana, *An. Acad. Bras. Cienc.*, **43**, 803 (1971); *Chem. Abstr.*, **77**, 137362d (1972).
93. T. Mizuno and E. Harghima, *J. Agric. Chem. Soc.*, **44**, 202 (1970).
94. G. O. Aspinall, *Advan. Carbohyd. Chem. Biochem.*, **24**, 333 (1969).
95. D. H. Northcote, *Methods Carbohydr. Chem.*, New York and London, Vol. 5, p. 49 (1965).
96. H. Determann, *Gelchromatographie: Gelfiltration, Gelpermeation, Molekuelsiebe*, Springer-Verlag, Berlin (1967).
97. O. S. Chizhov, L. A. Polyakov, and N. K. Kochetkov, *Dokl. Akad. Nauk SSSR*, **158**, No. 3, 685 (1964).
98. G. Petersson, *Tetrahedron*, **25**, No. 18, 4437 (1969).
99. L. S. Golovkina, O. S. Chizhov, and N. S. Vul'fson, *Izv. Akad. Nauk SSSR, Ser. Khim.*, No. 11, 1915 (1966).
100. N. Shimizu, M. Tomoda, K. Takada, and R. Gonda, *Chem. Pharm. Bull.*, **40**, 2125 (1992).