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Note

Structural and molecular properties of the arabinogalactan isolated from Mongolian larchwood (*Larix dahurica* L.)

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Arabinogalactans (AGs) are widely spread throughout the plant kingdom. They are most abundant in the genus *Larix*[1], often covalently linked to pectin and protein[2]. In view of their physiological role as a dietary fiber as well as their peculiar solution properties, they represent an important class of polysaccharides. Recently, various biological activities were reported for AGs isolated mainly from medical plants[3–8], and some also for larchwood AGs[9]. There are indications[5,8,10] that some side chains, such as arabinosyl residues in particular, play a decisive role in the expression of the biological activity. Also, the solution behaviour of AGs may be influenced by the character of the side chains. All AGs isolated so far from *Larix* species are of the 3,6-β-D-galactan type[2]. However, differences and discrepancies were reported regarding the amount and the character of side chains, even for AGs of the same botanic origin[11,12]. The present paper deals with the structural analysis of the AG isolated from the wood of Mongolian larch (*Larix dahurica* L.) which represents one of the main wood resources of the domestic industry. Except for only a few analytical data[13], no structural features of this AG have been reported.

The water-soluble, nitrogen-free polysaccharide isolated from larchwood meal was found to be a fairly pure AG with arabinose and galactose in the molar ratio 1:6.9 (Table I). The polysaccharide appeared to be homogeneous in free-boundary electrophoresis and had a very narrow molecular weight distribution (Fig. 1) with $\overline{M}_w$ 16000. In contrast, the AG isolated from Western larch (Stractan) was reported[14] to contain also ca. 20% of a high molecular weight fraction.

Partial acid hydrolysis of AG (Table II) yielded, depending on temperature and reaction time, arabinose, galactose, and three oligosaccharides identified by ^{13}C NMR spectroscopy[15–17] as 3-*O*-β-L-arabinopyranosyl-L-arabinose (**3**), 3-*O*-β-D-

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TABLE I

General characterisation of AG, AG-AR, and AG-PA

	AG	AG-AR	AG-PA
Neutral sugars [a] (mol%)			
Arabinose	12.5	6.9	3.5
Galactose	85.9	92.7	94.9
Rhamnose	0.2	0.4	0.5
Fucose	0.2	tr [d]	0.2
Xylose	1.2	tr	0.9
Uronic acid (%) [b]	0.05	0	0
$[\alpha]_D$ (degrees)	+10	+25	n.d.
$\overline{M}_w$ [c]	16000	n.d. [d]	13000
$\overline{M}_n$ [c]	13000	n.d.	10000 11900 [e]
$\overline{M}_w / \overline{M}_n$	1.23	n.d.	1.30

[a] Determined as alditol trifluoroacetates on OV-225. [b] Determined by the carbazole method. [c] Data from HPGPC analysis. [d] tr, Traces. n.d., Not determined. [e] Data from membrane osmometry[24].

galactopyranosyl-D-galactose (**4**), and 6-*O*-β-D-galactopyranosyl-D-galactose (**5**) (Table III). D-Glucuronic acid was present in a very small amount.

Methylation analysis of AG (Table IV) indicated that it has a highly branched 3,6-galactan backbone. Of the nonterminal galactopyranosyl residues, ~ 51% were 3,6-linked, ~ 44% were 6-linked, and only ~ 5% were linked at position 3. The number of branching units agreed well with the number of terminal units, repre-

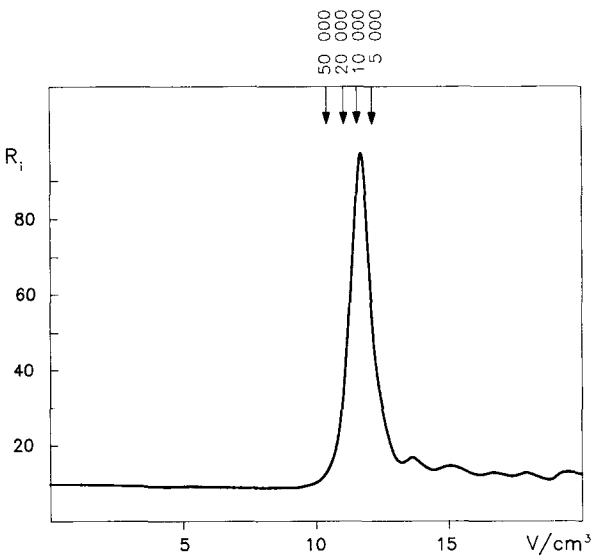

Fig. 1. HPGPC chromatogram of AG on Separon HEMA BIO-100. The arrows indicate the pullulan standards P-5, P-10, P-20, and P-50.

TABLE II

Partial acid hydrolysis of AG

Fraction	R_{Gal} [a]	Hydrolytic conditions [b]			Proposed structure
		80°C-2 h	90°C-4 h	100°C-1 h	
1	1.33	+ + + +	+ + +	+ +	Ara
2	1.00	+ +	+ + +	+ + + +	Gal
3	0.87	+ +	+ +	−	Ara*p*-(1 → 3)-Ara
4	0.57	−	−	+	Gal*p*-(1 → 3)-Gal
5	0.42	−	+ +	+	Gal*p*(1 → 6)-Gal

[a] In solvent *B*. [b] 0.01 M CF_3CO_2H.

TABLE III

^{13}C NMR data for oligosaccharides **3**, **4** and **5**

Oligosaccharide			Chemical shifts (δ/ppm)					
			C-1	C-2	C-3	C-4	C-5	C-6
3 β-Ara*p*-(1 → 3)-Ara	*a*	β	97.68	69.22	69.68	69.95	64.08	
a *b*	*b*	α	96.89	71.29	78.27	69.98	65.96	
		β	93.59	67.92	74.97	69.30	63.11	
4 β-Gal*p*-(1 → 3)-Gal	*a*	β	105.27	72.03	73.50	69.54	76.00 [a]	61.90
a *b*	*b*	α	93.12	68.37	80.31	70.04	71.07	62.08
		β	97.12	71.93	83.39	69.48	75.70 [a]	61.90
5 β-Gal*p*-(1 → 6)-Gal	*a*	β	104.26	71.90	73.75	69.72	76.28	62.10
a *b*	*b*	α	93.44	69.39	70.21	70.44	70.44	70.21 [b]
		β	97.52	72.89	73.75	69.72	74.89	69.90 [b]

[a,b] May be interchanged.

TABLE IV

Methylation analysis of AG, AG-AR, and AG-PA

Sugar derivative	Mode of linkage	AG	AG-AR	AG-PA
		(mol %) [c]		
2,3,5-Me_3-Ara [a,b]	Ara*f*-(1 →	4.8	6.1	4.8
2,3,4-Me_3-Ara	Ara*p*-(1 →	3.6	0	0
2,5-Me_2-Ara	→ 3)-Ara*f*-(1 →	4.7	0	0
2,3,4,6-Me_4-Gal	Gal*p*-(1 →	23.3	21.9	12.5
2,4,6-Me_3-Gal	→ 3)-Gal*p*-(1 →	2.9	16.5	63.6
2,3,4-Me_2-Gal	→ 6)-Gal*p*-(1 →	28.2	26.6	1.6
2,4-Me_2-Gal	→ 3,6)-Gal*p*-(1 →	32.5	28.9	17.5
Terminal/branching		0.98	0.97	0.98

[a] 2,3,5-Me_3-Ara = 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl-L-arabinitol, etc. [b] Determined as alditol acetates on SP-2340. [c] Values were corrected by use of the effective carbon-response factor[18].

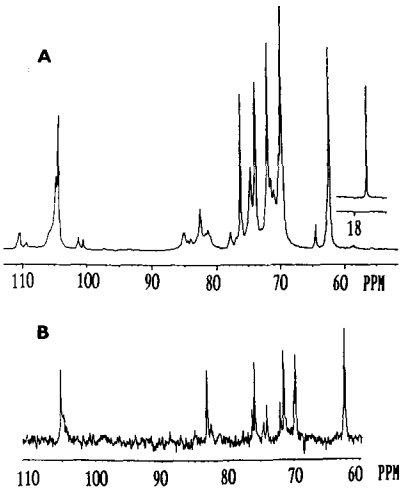

Fig. 2. ^{13}C NMR spectra (D_2O) of A, AG; and B, AG-PA.

sented by galactosyl and arabinosyl groups. Methylation analysis of the acid-stable portion AG-AR (Table IV) showed an accumulation of the 3-linked galactopyranose residues and a concomitant loss of both terminal and 6-linked ones, constituting a further proof of the $(1 \rightarrow 3)$ linkages in the main galactan chain. Mild hydrolysis of AG followed by Smith degradation gave the nondialysable polyalcohol fraction AG-PA with reduced molecular weight ($\overline{M}_w = 13000$).

The ^{13}C NMR spectra of AG and AG-PA are shown in Fig. 2. The assignment of the signals (Table V) was made using the spectra of oligosaccharides **3–5** and published data[5,12,19]. From these results, it is obvious that the C-1 resonances of the 3,6-linked and terminal β-Gal*p* residues are shifted upfield compared to C-1 of the 3- and 6-linked Gal*p* units, respectively. The multiple signals at low field (δ 109.5, 110.4–110.5) were unequivocally assigned to C-1 of α-Ara*f* residues attached to different sites of the galactan backbone. It has been reported[5,20–22] that the C-1 signals of terminal Ara*f* groups linked to other Ara residues usually appear at higher field.

Partial acid hydrolysis and methylation analysis of AG showed that ca. 50% of the Ara residues occur as 3-*O*-β-L-Ara*p*-L-Ara*f* side chains linked probably by α-glycosidic linkages to the galactan core, and easily cleaved by hydrolysis. The signals at δ 101.20 and 64.48 may be related to C-1 and C-5, respectively, of the β-Ara*p* terminal groups[17], which are also present in AG from *Larix sibirica*[11,12]. The proposed attachment of the disaccharide chains by β linkage in the last-mentioned AGs was deduced only from the absence of the corresponding chemical shift of C-1 of α-Ara*f* expected to appear at low field. Terminal α-Ara*f* groups were detected only by methylation analysis in some larch AGs[2]. The ^{13}C NMR spectra of AG and the commercial AG isolated from western larch[14] were

TABLE V

^{13}C NMR data for AG, AG-AR, and AG-PA

Compound	Structure	Chemical shifts (δ/ppm)					
		C-1	C-2	C-3	C-4	C-5	C-6
AG	β-Gal*p*-(1 →	104.52	72.00	73.99	69.89	76.33	62.31
	→ 6)-β-Gal*p*-(1 →	104.88	72.00	73.99	69.89	74.77	70.20
	→ 3,6)-β-Gal*p*-(1 →	104.52	71.50	82.62	69.89	74.77	69.89
			71.03				
	α-Ara*f*-(1 →	110.45	81.80	77.84	85.02	62.31	
		110.42					
	→ 3)-α-Ara*f*-(1 →	109.50	81.45	85.22	84.10	62.31	
	β-Ara*p*-(1 →	101.20				64.48	
	Rha*p*						18.05
AG-AR	β-Gal*p*-(1 → 3)-	105.51	71.99	74.04	69.85	76.30	62.22
	β-Gal*p*-(1 → 6)-	104.50					
	→ 3)-β-Gal*p*-(1 →	105.14	71.41	83.10	69.62	75.89	62.22
	→ 6)-β-Gal*p*-(1 →	104.93	71.99	74.04	69.85	74.78	70.20
	→ 3,6)-β-Gal*p*-(1 →	104.70	71.50	82.70	69.85	74.78	70.20
	β-Gal*p*	97.67					
	α-Gal*p*	93.49					
AG-PA	β-Gal*p*-(1 →	104.62	71.99	74.00	69.86	76.28	62.15
	→ 3)-β-Gal*p*-(1 →	105.11	71.43	83.12	69.63	75.87	62.15
	→ 3,6)-β-Gal*p*-(1 →	104.40	71.43	82.62	69.86	74.51	69.86

qualitatively identical, thus confirming the presence of terminal α-Ara*f* groups in both polysaccharides.

Alkaline degradation of AG under oxygen-free conditions resulted in β-eliminative cleavage of the main 3-linked galactan chain, thus liberating the side

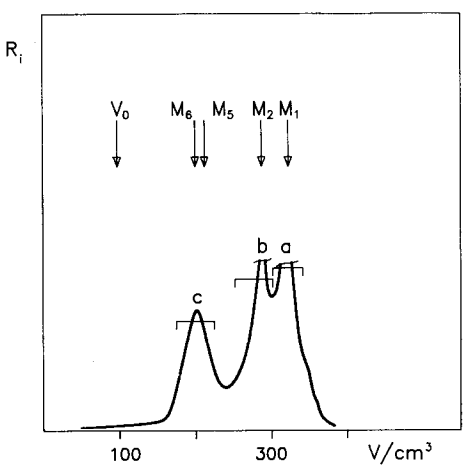

Fig. 3. Gel-filtration profile on Bio-gel P2 of the alkaline degradation product of AG. M_1–M_6: D-Mannose and manno-oligosaccharides. a, b, c: Collected fractions.

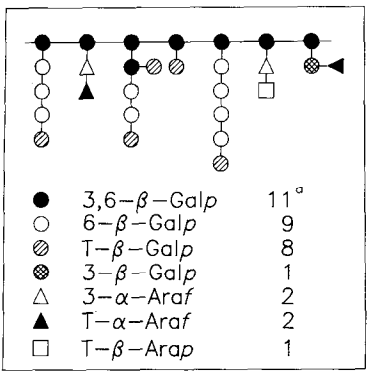

Fig. 4. One of the possible structures of the minimal repeating unit of AG. [a] Number of residues.

chains terminated by metasaccharinic acids. Gel chromatography of the degradation product (Fig. 3) yielded two oligosaccharide fractions **b** and **c**, the DP of which were estimated, by comparison with mannose and manno-oligosaccharides, to be 2 or 3 for **b** and 5 or 6 for **c**.

Based on the results described above, it is concluded that the 3-linked galactan core is substituted, at position 6, with single Gal*p* units and longer side-chains composed of 3 or 4 6-linked β-Gal*p* units as well as with β-Ara*p*-(1 → 3)-α-L-Ara*f* branches. One of the possible structures of the minimal repeating unit of AG is presented in Fig. 4. In accordance with the relatively high content of terminal and 3,6-linked Gal*p* in AG-PA, methylation analysis of fraction **c** indicated that some of the Ara*f* and/or Gal*p* units must be linked to the galactan chain near the backbone. However, single β-Gal*p* and/or β-Gal*p*-(1 → 6)-β-Gal*p* groups were more frequently reported as side chains in larchwood AGs than longer galactan chains[2].

In conclusion, the data suggest that, because of the structural and molecular similarity of AG with that of the commercial polysaccharide, the Mongolian larchwood polysaccharide could find potential industrial applications.

EXPERIMENTAL

General methods.—Wood meal, particle size 2.4–5.6 mm, was prepared from bark-free trunks of Mongolian larch. The commercial AG, Stractan, was purchased from Fluka. Paper chromatography (PC) was performed by the descending method on Whatman No. 1 paper with *A*, 8:2:1 EtOAc–pyridine–water; *B*, 10:4:3 EtoAc–pyridine–water; *C*, 19:3:1:4 EtOAc–AcOH–formic acid–water; and detection with aniline hydrogen phthalate. The procedures for total hydrolysis, quantitative GLC analysis of sugars, free-boundary electrophoresis, determination of uronic acid content, and optical rotation have been described[23,24]. ^{13}C NMR spectra of the samples (3% in D_2O) were recorded with a Bruker AM-300 (75

MHz) spectrometer at 35°C in the inverse gated decoupling mode. Chemical shifts are reported relative to internal MeOH ($\delta_{Me_4Si} = 50.15$).

Gel filtration.—A column (2 × 200 cm) of Bio-gel P2, calibrated with manno-oligosaccharides (DP 2–6)[25], was used. An aqueous solution of the sample (50 mg in 3 mL) was loaded onto the column. The components were eluted with water (0.8 $mL.min^{-1}$) and fractions of 3 mL were assayed for carbohydrates by refractometry. High performance gel permeation chromatography (HPGPC) of AG was performed using a commercial instrument (Laboratorni pristroje, Prague, Czechoslovakia) equipped with two Tessek Separon HEMA BIO-100 exclusion columns (8 × 200 mm) and 0.1 M aq $NaNO_3$ as the eluent. The eluate was monitored by refractometry. A set of pullulans P-5, P-10, P-20, and P-50 (Shodex Standard P-82 Macherey-Nagel) was used for the calibration of the column. Therefore, the molecular weight parameters presented are relative to the pullulan reference material. A computing procedure[26] based on the linear effective calibration curve was applied to obtain the molecular weight distribution.

Isolation of the polysacharide.—The crude polysaccharide was obtained by cold water extraction of the wood meal, and isolated by EtOH precipitation (1:3, v/v). After filtration, the product was redissolved in water (3%) and precipitated with 3 vol of EtOH. The precipitated AG was treated with acidified aq 80% EtOH, washed with aq 80% EtOH to remove inorganics and coloured matter, and finally dried by solvent exchange using EtOH and acetone.

Partial acid hydrolysis.—AG (2 g) was dissolved in 0.01 M CF_3CO_2H (200 mL) and hydrolysed for 2 h at 80°C. After evaporation of the acid, the precipitate formed by the addition of EtOH (4 vol) was filtered off. The precipitate was further hydrolysed with a new batch of the acid for 4 h at 90°C. The hydrolysate was worked up in the same way, and the fraction AG-2 (1.61 g, 85.6% of AG) was obtained. This fraction was hydrolysed in a third step for 1 h at 100°c, yielding the resistant portion AG-AR (0.11 g, 6.2% of AG). The filtrates from the three hydrolysates were combined, evaporated in vacuo, and analysed by PC using solvents *A–C*. Preparative PC (solvent *B*) yielded oligosaccharide fractions **3** (R_{Gal} 0.87), **4** (R_{Gal} 0.57), and **5** (R_{Gal} 0.42), which were identified by ^{13}C NMR spectroscopy[16,17] (Tables II and III).

Methylation analysis.—The polysaccharide (200 mg) was dissolved in Me_2SO (8 mL) and methylated with the Me_2SO–solid NaOH–CH_3I reagent[27] as previously described[23]. The methylated polysaccharide (203 mg), which was free of IR absorption for hydroxyl groups, was hydrolysed with aq 90% formic acid under reflux for 1 h. After evaporation of the acid, the hydrolysis was completed by treatment with 2 M CF_3CO_2H for 3 h at 100°C. The product was reduced with $NaBD_4$ and converted into alditol acetates which were analysed by GLC on SP-2340 and GLC–MS[28].

Smith degradation.—The fraction AG-2 (1 g) isolated in a large-scale experiment, which was carried out under the partial acid hydrolysis conditions described above, was oxidised with 0.05 M sodium metaperiodate (100 mL) for 48 h at 20°C.

Oxidation was stopped by the addition of ethylene glycol, and the solution was dialysed against distilled water. The nondialysable material was reduced with $NaBH_4$ for 16 h at room temperature and kept in 0.5 M CF_3CO_2H for 24 h at room temperature. The product was dialysed against distilled water and freeze-dried, yielding fraction AG-PA (0.33 g).

Alkaline degradation.—AG (100 mg) was dissolved in 0.1 M NaOH (50 mL), deoxygenated, and kept under N_2 for 24 h at 90°C. The mixture was dialysed against distilled water to pH 7, and concentrated in vacuo. The degradation product (78 mg) was further analysed by GPC. Fractions **b** and **c** were methylated as described[27]. Fraction **b** yielded 2,3,4,6-tetra-*O*-methylgalactose, 2,3,4-tri-*O*-methylarabinose, 2,3,5-tri-*O*-methylarabinose, and 2,5-di-*O*-methylarabinose in the molar ratios 10:0.8:0.5:1.1. Traces of 2,4,6-tri-*O*-methylgalactose were also present. Fraction **c** yielded 2,3,4,6-tetra-*O*-methylgalactose, 2,3,4-tri-*O*-methylgalactose, 2,4-di-*O*-methylgalactose, and 2,3,5-tri-O-methylarabinose in the molar ratios 2.7:10:1.2:1.8.

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