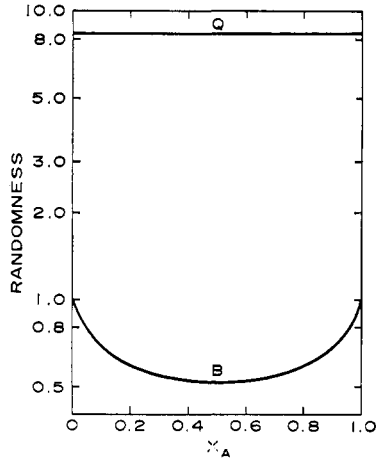

Figure 7. Randomness parameters Q and B *vs.* composition for copolymerizations at 200° for which $\Delta\epsilon_{AB} = 1.0$ kcal/mol.

eq 20 and in Figure 7, Q does not depend upon composition but is only a function of $\Delta\epsilon_{AB}/RT$.

The other parameter was defined by Yamadera and Murano[19] as

$$B = P_{AB} + P_{BA} \quad (27)$$

B, like Q, is equal to one for a random copolymer, but, unlike Q, it is equal to two for a completely alternating copolymer and approaches zero for a block copolymer. It is affected by both changes in composition (Figure 7) and $\Delta\epsilon_{AB}/RT$ (Figure 8). Q is then the simpler of the two parameters, dependent on only one condition, besides temperature, which affects the sequence length distribution of the copolymer. The more complex parameter B reflects the composition dependent residual entropy of copolymerization as shown by curves 1–3 of Figure 8 not approaching two at low temperatures. It is suggested that the concurrent listing of both B and Q would more adequately describe the sequence length distribution of a copolymer than either used alone.

(19) R. Yamadera and M. Murano, *J. Polym. Sci., Part A-1*, **5**, 2259 (1967).

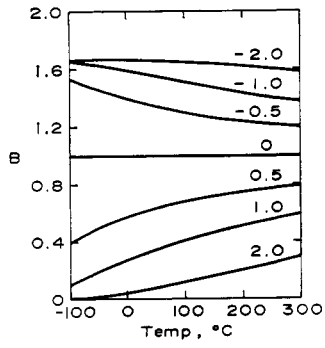

Figure 8. Randomness parameter B *vs.* copolymerization temperature for which $X_A = 0.4$ at ΔH_{AB} values designated by numbers associated with each curve, $\Delta S_{AB} = 0.0$ eu.

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Fractionation and Characterization of Larchwood Arabinogalactan Polymers A and B

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ABSTRACT: Arabinogalactan, the extracellular water-soluble polysaccharide of larchwood, was separated by gpc to obtain fractions of the two polymers A (mol wt 37,000) and B (mol wt 7500) of which it is composed. The fractions were characterized by viscometry, osmometry, and sedimentation equilibrium analyses. The apparent Staudinger constant of polymer B is five times that of polymer A, evidence for its greater linearity. Plots of $[\eta]$ *vs.* M also show that polymer B has fewer or shorter branches than does polymer A. The superiority of gpc over earlier fractionation methods was shown by the apparent monomolecularity of the fractions and by the ability of the method to separate cleanly the component polymers A and B.

Arabinogalactan is an extracellular, water-soluble polysaccharide which is particularly abundant in larchwood (genus *Larix*). It consists of a 1–3 linked backbone of galactose units to whose C-6 position side branches of arabinose and galactose are attached. The polysaccharide is similar to the gum exudates of which gum arabic from acacia is an example, although it has few acidic groups and little or no polyelectrolyte behavior. That it is a highly branched, approximately spherical molecule was first reported by Owens,[1] who found that the polymer had a constant partial specific volume in concentrations as high as 6.0% in water. Mosiman and Svedberg[2] later reported that the polysaccharide is made up of two polymers of approximately 100,000 and 16,000 molecular weight which were labelled A and B. The presence of two polymers was confirmed by Bouveng and Lindberg[3,4] and by Simson, Cote and Timell,[5] who showed that the two polymers were similar in composition and by Ettling and Adams,[6] who separated them by gel permeation chromatography.

In the present study the polysaccharide was obtained by successive extractions of larchwood with water.

(1) H. S. Owens, *J. Amer. Chem. Soc.*, **62**, 930 (1940).
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(6) B. V. Ettling and M. F. Adams, *ibid.*, **51**, 116 (1968).

TABLE I
THE YIELD AND SUGAR ANALYSES OF WATER EXTRACTS OF LARCHWOOD

Fraction	Yield, g	Extraction time, days	Arabinose,[c] %	Galactose,[c] %	G/A ratio[d]
1	45.7	7	19.5	85.1	4.4
2	8.6	14	19.2	85.8	4.5
3[a]	9.9		14.9	77.5	5.2
4[b]	12.9		14.7	78.1	5.3
5	1.8	28	12.5	77.6	6.2

[a] Sticky residue in precipitates of fractions 1 and 2. [b] From extraction of filter pad from fraction 1 with 0.01 *N* NaOH. [c] Limit of detection 0.5%. [d] G, galactose; A, arabinose.

TABLE II
THE WEIGHT, GALACTOSE AND ARABINOSE CONTENTS AND GALACTOSE–ARABINOSE RATIO OF THE GPC FRACTIONS

Fraction	Volume of eluent, ml	Wt of fraction	Arabinose,[a] %	Galactose,[a] %	G/A ratio
1	86	0.0269			
2	69	0.0258	10.4	73.8	7.1
3	52	0.3953	11.2	80.6	7.2
4	52	0.7064	12.6	83.3	6.6
5	52	1.3240	14.8	84.5	5.7
6	52	1.3872	15.5	87.0	5.6
7	69	0.6723	16.7	83.6	5.0
8	69	0.1975	17.4	79.8	4.6
9	69	0.1856	16.9	76.1	4.5
10	140	0.6709	18.4	79.7	4.3
11	410	0.1542			

[a] Limit of detection 0.5%.

The largest extract was then fractionated by gel permeation chromatography to obtain preparative fractions of both polymers A and B. These were then characterized by viscosity and molecular weight analyses in order to compare the hydrodynamic radius of polymer A with polymer B and with other branched polymers. The rapid sedimentation equilibrium method of Yphantis[7] was used to obtain a measure of the polydispersity of the fractions and to measure their weight average molecular weight.

Extraction and Isolation of the Polymer. Air-dried Western larchwood, 250 years old, was used in the investigation. Pie-shaped disks 0.25 in. thick (1600 g) were covered with 10 l. of distilled water and allowed to stand at room temperature for 7 days. After removal of the first extract, two more extractions followed for 7 and 14 days. The three extracts were then concentrated to about 1 l. in volume and precipitated in methanol. The three precipitates plus a sticky residue found in the precipitate from the first two extracts were then redissolved, shaken overnight with 7 g of a 50:50 mixture of Celite and exchange cellulose to remove brown color and let stand overnight with 7 g of IR-120 resin to remove remaining salts. The four solutions were then evaoprated to a volume of about 100 ml and freeze dried.

The yield and sugar analysis of the extracts are shown in Table I. The osmotic molecular weight of the five samples, 26,000, was identical within the error of the analysis.

It is seen that the total yield is about 5% of the air-dried disks and that the ratio of galactose to arabinose increases as the polymer becomes more difficult to extract. No glucose, mannose, xylose, or rhamnose were detectable in the samples.

Fractionation. To obtain fractions of narrower distribution and varying molecular weight, a portion of fraction 1 (Table I) was separated on a G-75 Sephadex column 72 cm long and 5 cm in diameter. A void volume of 500 ml was found with a blue dextran marker which advanced evenly down the column. Water (50 ml) containing 6 g of the arabinogalactan polymer was introduced and the fractions present in 1120 ml of eluent were collected in 75 test tubes. The flow rate was about 0.75 ml/min. The anthrone color reaction[8] was used qualitatively to detect the polymer concentration and to obtain fractions of adequate size for further analysis. Isolation was done by freeze drying. The amount of eluent, the weight and percentage of each fraction and the amount of galactose and arabinose in each fraction are given in Table II. Uronic acid detectable only by titration was present in too small a quantity to be measured by CO_2 evolution. Ultraviolet spectroscopy showed that the small percentage of nonsugars in fractions 3 and 4 (Table I) was nonphenolic.

The ratio of galactose to arabinose in Table II is seen to decrease steadily with decreasing molecular weight. This is the reverse of the increase in the ratio found with decreasing molecular weight and increasing age of the tree attributed to preferential hydrolysis of arabinose units by Timell and coworkers.[9]

Intrinsic Viscosity Determination. The viscosities were measured at 30 ± 0.003° in no. 50 Cannon semimicro Ubbelohde viscometers which gave an efflux time of about 200 sec for water. A solvent mixture of 1% NaCl and 0.25% of the sodium salt of ethylenediamine tetraacetic acid (EDTA) was used routinely in the determinations to counter possible effects of the few acidic groups known to be present. Residual ash is not found in the gpc fractions.

Osmotic Pressure Determinations. Osmotic measurements were made with a Mechrolab 501 instrument. A dense Schleicher and Schuell no. 08 membrane was used which requires about 20 min to equilibrate with solvent alone. The solvent was water, containing 2 or 3 drops of detergent (Wyandotte Pluronic L62) per liter. A solution of the detergent in a concentration of about 5% was also drawn through the instrument capillary before use. The instrument was held at 25° and the air bubble held above rather than below the light beam.

Rapid Sedimentation Equilibrium Analysis. The meniscus depletion method of Yphantis[7] was used to obtain the weight average molecular weight of the

(7) D. A. Yphantis, *Biochem.*, 3, 297 (1964).

(8) T. A. Scott and E. H. Melvin, *Anal. Chem.*, 25, 1656 (1953).

(9) W. A. Cote, Jr., B. W. Simson, and T. E. Timell, *Holzforschung*, 21, 85 (1967).

TABLE III
THE RAPID SEDIMENTATION EQUILIBRIUM MOLECULAR WEIGHT AVERAGES OF THE GPC FRACTIONS AND OF THE UNFRACTIONATED POLYMER

Sample fraction	Concn, g/dl	Rapid sedimentation equilibrium molecular weight: Whole cell M_w	Point cell averages: M_n	M_w	M_z
Unfractionated arabinogalactan	0.10	26,158	14,293	18,685	83,320
	0.05	30,537	17,885	25,189	53,862
	0.01	33,211	23,437	28,167	23,252
3	0.10	45,756	37,749	42,689	48,700
	0.05	45,799	27,059	39,780	52,538
	0.01	47,225	34,423	38,513	54,604
8	0.10	9,760	6,682	8,059	14,442
	0.05	10,512	9,116	10,778	6,425
	0.01	9,948	9,345	7,898	12,374

fractions and to estimate their degree of polymolecularity. In this method the centrifuge is run about three times as fast as in the usual equilibrium method and the sample concentrations used are much lower. By these means the meniscus concentration becomes essentially zero and measurements can be made between the meniscus and any point in the cell. It is also necessary that the samples have a rather narrow molecular weight distribution if the method is to be used successfully. As will be seen, the gpc fractions met this requirement at least in part.

A six-channel equilibrium cell makes it possible to run three solvent–polymer pairs at one time. This allows dependence on concentration to be easily followed. The concentration gradient in the cell was obtained by interferometry. The fringe displacements were measured with an X–Y coordinate comparator provided with digital readout directly onto punch cards.[10] They were then used directly in a FORTRAN program of the Yphantis calculations[7] by Small and Resnik[11] which was modified to suit our needs.

To deter convection effects, 1% aqueous NaCl was the solvent in all centrifuge runs. The velocity required for different molecular weight levels was found from the relation[7] $\sigma = \omega^2 m(1 - \bar{\nu}\rho)/RT$ where the partial specific volume $\bar{\nu}$ and density ρ are known and σ is given the value of 5. The molecular weight M in the equation was estimated from osmotic pressure measurement. Velocities of 29,500 rpm were used for fractions 1–6, 42,400 rpm for fraction 7 and 50,740 rpm for fractions 8, 9 and 10.

The whole cell weight average molecular weight at a number of points in the cell was obtained from a plot of the net fringe displacements *vs.* cell position. Reduced number, weight and Z average molecular weights were also calculated from Yphantis equations.[7]

The Effect of Polymolecularity on the Rapid Equilibrium Method. If the molecular weight distribution of the analyzed sample is too broad, depletion of the polymer at the meniscus is apparently incomplete, and concentration dependence is evident. As seen in Table III this is true of the unfractionated sample both for the whole cell weight average and for the estimates of M_n, M_w, and M_z. The whole cell M_w of the narrower fractions, on the other hand, is quite reproducible and, although the estimates of M_n, M_w, and M_z are not sufficiently reliable to estimate the polymolecularity of the fractions, they are considerably better than those calculated for the unfractionated polymer. This is apparently because the equations used for calculating the three averages[7] assume ideal conditions not found in the gpc fractions.

The Intrinsic Viscosity and Electrophoretic Mobility of Polymers A and B. As can be seen in Table IV the intrinsic viscosity of the three fractions 8, 9 and 10 of lowest molecular weight are similar in magnitude to those of fractions 2–7 although the molecular weight of the latter fractions are about five times as great. The reduced viscosity curves of fractions 8, 9 and 10 also have much higher slopes than do the balance of the fractions, which suggests that they represent polymer B.

To confirm this the electrophoretic mobility of the unfractionated polymer was followed by similar analysis of fractions 4 and 9 believed to be representative of

TABLE IV
THE INTRINSIC VISCOSITY, NUMBER AND WEIGHT AVERAGE MOLECULAR WEIGHT AND APPARENT STAUDINGER CONSTANT OF THE GPC FRACTIONS

Sample fraction	M_n[a]	M_w[b]	$[\eta]$, ml/g	$[\eta]/M_w \times 10^5$
Original	23,400	36,000[c]	4.80	13.4
2	61,200	51,590	5.00	9.7
3	55,000	47,400	4.65	9.8
4	45,500	43,190	4.58	10.6
5	42,000	38,390	4.22	11.0
		(39,200)[c]		
6	34,900	35,410	3.98	11.3
		(34,700)[c]		
7	21,700	26,350	3.92	14.8
8	11,200	10,510	4.88	46.5
		(9,060)[c]		
9	9,500	8,120	3.96	48.8
10	6,700	6,350	3.76	59.0

[a] By osmotic pressure. [b] By rapid sedimentation equilibrium. [c] By slow sedimentation equilibrium.

(10) K. A. Hardacker, unpublished work.
(11) P. A. Small, Jr., and R. A. Resnik, Fortran Program Pas 0010. For the Yphantis Meniscus Depletion Method of Molecular Weight Determination, National Institutes of Health, Bethesda, Md.

TABLE V
CONFIRMATION OF PRESENCE OF POLYMERS A AND B BY ELECTROPHORETIC MOBILITY MEASUREMENT

Sample fraction	Peak	Electrophoretic mobility, μ, $cm^2\ sec^{-1}\ V^{-1} \times 10^5$ Ascending	Descending
Unfractionated arabinogalactan	A	2.4×10^{-3}	2.0×10^{-3}
Unfractionated	B	0.84×10^{-3}	0.76×10^{-3}
4		2.4×10^{-3}	2.1×10^{-3}
9		0.95×10^{-3}	0.88×10^{-3}

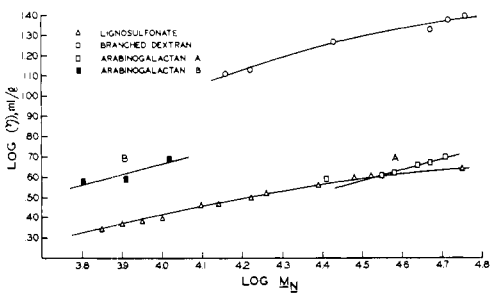

Figure 1. The intrinsic viscosity–molecular weight relation of arabinogalactan gpc fractions compared to other branched polymers.

polymers A and B, respectively. The samples were run in a Spinco Model H instrument in a borate buffer at pH 9.0 at a concentration of 0.3%. An 11-ml cell was used with a 20-mA current at a temperature of 2°. As shown in Table V, analysis of the unfractionated sample resulted in two peaks at $\mu = 2.4 \times 10^{-3}$ and $\mu = 0.84 \times 10^{-3}$, which were then nearly duplicated by single peaks for fractions 4 and 9 representing polymers A and B.

From calculations based on the amount and number average molecular weight of the fractions in Table IV, 15% of the arabinogalactan is polymer B with an average molecular weight of 7500, while the balance, polymer A, fractions 2–7, has an average molecular weight of 37,000.

The more expanded configuration of the smaller polymer B as compared to polymer A is evident from the apparent Staudinger constants, $[\eta]/M$, of fractions 8, 9 and 10 given in Table IV which are four or five times as large as for fractions 2–6. Fraction 7 appears to contain a small amount of polymer B as evidenced by a slight increase in the ratio.

A log–log plot of $[\eta]$ *vs.* M in Figure 1 also illustrates the difference between the two polymers. Polymer A, the more branched of the two, is comparable to lignosulfonate[12] an essentially spherical molecule[13] at a similar molecular weight level and appears to be considerably more branched than a dextran found to contain 5% of trifunctional branch units[14] whose curve lies above. The curve of polymer B, on the other hand, is quite separate and lies well above the lignosulfonate curve. Meaningful estimates of the hydrodynamic radii[15] of the two polymers could not be made from the curves of the two polymers because of the limited number and narrow range of the fractions.

It is seen in Table IV that the apparent osmotic molecular weight of many of the fractions is greater than the weight average by sedimentation. This might occur if either Staverman membrane reflection effects[16] or diffusion is present.[17] Diffusion effects were minimized by taking solvent readings between each concentration and the reflection effects are expected to be present only in the three fractions of lowest molecular weight because of the density of the membrane. Osmotic measurements in water are less reliable than in organic solvents, however, and were less reproducible than those obtained by sedimentation.

Conclusions

Fractions of both polymer A and B were obtainable by gel permeation. Polymer B, present at about 15%, of the composite polymer, was easily identified from its intrinsic viscosity which was similar to fractions of polymer A whose molecular weight was five times as great. As a result, the apparent Staudinger constant $([\eta]/M)$ of polymer B is about five times that of polymer A which emphasizes that polymer B is much less branched or that its branches are very short as compared to polymer A.

The presence of two polymers of unlike molecular weight is largely responsible for the M_w/M_n ratio, 1.54, found for the composite polymer. The molecular weight distribution of the independent polymers A and B are quite narrow as found before.[6]

The relative amount of arabinose as compared to galactose increased with decreasing molecular weight of the fractions and was greatest in the fraction of lowest molecular weight which consists of polymer B. This is in conformity with the work of Bouveng,[4] who also found that the disposition of arabinose was different in the two polymers. Because of the pronounced difference in configuration reflected by the viscosity, however, it does not appear that polymer B is a breakdown product of polymer A. The relative increase in arabinose with decreasing molecular weight of the fractions is in apparent contrast to the view that degradation is accompanied by greater loss of the more labile arabinose units.

Conjectures of this kind may be fruitless in view of the variable composition of the arabinogalactan with position in the tree as well as with species and age of the tree. That the molecular weight of the composite polymer as well as of polymers A and B found in this investigation is considerably lower than originally reported by Mosiman and Svedberg[2] and since reported by other workers[5] is unexplained. The superiority of gpc preparative fractionation compared to older fractionation methods was shown by the monomolecularity of the fractions and by the ability to separate cleanly the component polymers A and B.

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