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CHARACTERIZATION OF A 3-LINKED GALACTAN FROM *PINUS RADIATA* CALLUS CELLS

D. R. FENEMOR

Christchurch College, Canterbury University, New Zealand

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Key Word Index—*Pinus radiata*; Pinaceae; polysaccharides; galactan; arabinogalactan; arabinan; pectin.

Abstract—A polysaccharide was obtained in the neutral fraction of a hot water extract (100°) of cell walls, by DEAE ion exchange chromatography, from callus of *Pinus radiata* cells. The polysaccharide was found by sugar analysis to be composed predominantly of galactose, and methylation analysis showed it to be 3-linked. The anomeric configuration was not determined.

INTRODUCTION

As part of a study on the composition of the cell wall from callus cells of *Pinus radiata*, a chromatographic fractionation was attempted on the largest cell-wall fraction, the 100° extracted fraction. Analytical data from previous work suggested that this fraction contained pectic polymers (rhamnogalacturonan, arabinan and galactan), presumably liberated by a β-elimination mechanism [1]. In an attempt to separate some of the components by ion exchange chromatography with DEAE-cellulose, unusual polysaccharide was detected in the neutral fraction from the column. A similar polysaccharide has only seldom been found [2, 3], and the authors postulate that it was a peripheral wall polysaccharide and possibly had a role in the protection of the wall or cell.

RESULTS AND DISCUSSION

The results of the ion exchange are plotted as an elution profile for the 100° water fraction in Fig. 1. A large neutral carbohydrate fraction was eluted first, followed by a heterogeneous series of acidic fractions containing varying amounts of uronic acid and/or protein. Fractions were pooled (see Fig. 1), each corresponding then to a peak in the elution profile of carbohydrate and protein. The neutral fraction was divided in the ratio 4:1 and the larger portion was methylation analysed. The smaller portion and other pooled fractions were analysed for monosaccharide composition. The monosaccharide analytical data are shown in Table 1. Separation of distinct polysaccharide components occurred. The major components were tentatively identified from the sugar composition in the table.

In fraction 1 there appeared a neutral galactan, or arabinogalactan (from the high galactose content), perhaps contaminated with a small amount of glucan. The identification was confirmed by methylation analysis. Fraction 2 may be an arabinogalactan or an arabinogalactan-protein, while fraction 3 appeared to be a distinct polysaccharide, perhaps an acidic arabinan. Fractions 5, 6 and 7 apparently represent three distinct

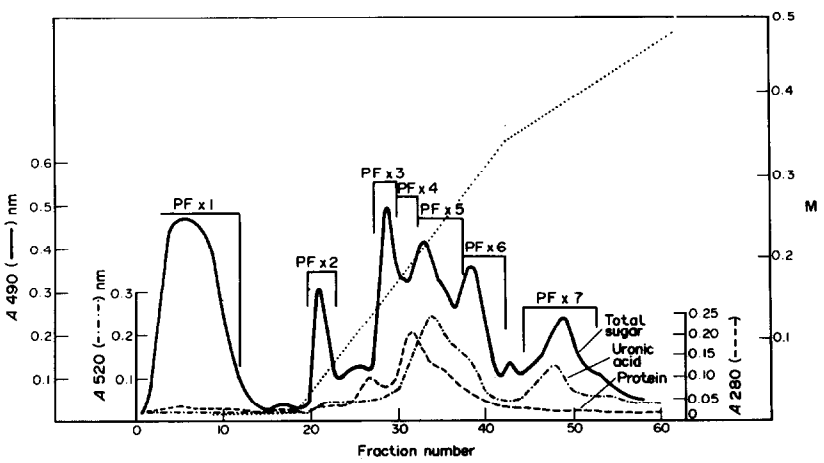

Fig. 1.

Table 1. Neutral sugar percentage composition of fractions of DEAE-cellulose chromatography

Fraction	Sugars Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Polysaccharide
1			6.3			86.0	7.9	Galactan
2		1.3	43.0	2.0		54.0		Arabinogalactan (– protein)
3	0.4	0.3	85.4			14.0		Acidic arabinan
4	5.4	0.8	63.0		0.6	29.0	1.1	Arabinogalactan (– protein)
5								Pectin 1 (sample damaged)
6	11.7	2.5	58.0	1.0	0.4	26.0	0.9	Pectin 2
7	2.7	2.1	71.0			24.1		Pectin 3

Unfortunately fraction 5 containing much of the uronic acid was damaged during the hydrolysis.

pectin fractions. Fraction 5 (the major uronic acid fraction) was eluted at a lower salt concentration than fraction 6, which has a relatively higher neutral sugar content. The order of elution of these two fractions may be governed by the degree of esterification of the uronic acid COOH groups [4], or by covalent association with pectin.

The association of arabinose and galactose in all the acidic fractions may indicate covalent linkage to either pectin or protein (see Table 1) but no association between protein and polysaccharide can be inferred (see profile).

Methylation of the neutral fraction

The neutral fraction was permethylated, hydrolysed and converted to partially methylated alditol acetates. GC/MS of the resulting mixture gave the composition shown in Table 2. The proportion of glucose derivatives was consistent with an origin from starch, which had possibly resisted α-amylase digestion during wall purification.

Remaining residues were mainly arabinose and galactose. The 2,4,6-tri-*O*-methylgalactose represented *ca* 80 mol %. Thus the polysaccharide material of the fraction was largely a linear (1 → 3)-galactan. A low degree of branching at position 6 of the galactose was indicated by the 2,4-di-*O*-methylgalactose. The arabinose residues may or may not be an integral part of the galactan molecule. The (1 → 3)-galactan is probably a β-D-galactan related to the 3,6-linked arabinogalactan type II of Aspinall [5]. This galactan is relatively insoluble in cold water but sufficiently soluble to chromatograph successfully at room temperature on DEAE-cellulose.

Table 2. Methylation data for DEAE-fraction 1. Relative mol % composition of methylated polysaccharide sugar residues (examined as their glycitol acetates)

Monosaccharide	*O*-Methyl ether	Mol %
Arabinose	2,3,5-Tri*	0.8
	2,3,4-Tri	0.1
	3,5-Di	0.1
	2,5-Di	0.9
	2,3-Di	2.5
	2-Mono	4.6
	Nil	2.8
Xylose	2,3,4-Tri*	0.1
	2,3-/3,4-Di	0.3
Galactose	2,3,4,6-Tetra*	1.9
	2,4,6-Tri	72.4
	2,6-Di	1.0
	2,3-Di	0.6
	2,4-Di	0.8
	2-Mono	1.1
Glucose	2,3,4,6-Tetra*	0.6
	2,3,6-Tri	6.9
	2,3-Di	1.3
Rhamnose	3,4-Di	0.1
	3-Mono	0.2

*Derivatives subject to small loss through volatility.

Conclusion

A linear β-(1–3)-D-galactan has been found in hot water extracts from *Rosa glauca* callus tissue by Mollard *et al.* [2, 3]. This galactan appears to be analogous to the galactan found here. It may be that both galactans have a function as a protective covering to the wall, and may not be tightly bonded structural entities, although they are both found in the water-soluble pectin fraction.

EXPERIMENTAL

Preparation of extract. Callus walls, prepared by French pressing washed callus tissue at 2000 psi, were washed with 0.1 M KPi buffer, and several brief H_2O rinses, centrifuging between washes, until the supernatant was free of starch. The walls were then extracted with 4° H_2O, 70° H_2O (each 3 × 1 hr), and treated with α-amylase. The supernatant from the α-amylase treatment was added to the 70° H_2O extract, and the pooled extract was dialysed and lyophilized. These extracts were used in part of a separate study. The walls were then extracted with 100° H_2O to give the largest wall fraction, as under investigation here, and after separation of the residue by centrifugation, the 100° supernatant was lyophilized.

Hydrolysis. Hydrolysis of lyophilized fractions was performed with 0.5 M HNO_3–urea at 100° for 4 hr [6] for monosaccharide analysis.

Ion exchange chromatography. A column of Whatman DE-32 was used, pre-equilibrated with 0.01 M NaPi buffer, pH 7. Elution was by 0.01 M NaPi buffer, pH 7, followed by a linear gradient of NaCl (0–0.5 M) in 0.01 M NaPi buffer, pH 7.

Methylation. This was carried out by the method of ref. [7], and permethylated sugars were reduced and derivatized to alditol acetates and analysed by GC/MS according to the methods of refs. [8, 9].

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