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Carbohydrate Research 337 (2002) 1503–1506

CARBOHYDRATE RESEARCH

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Note

Fungal cell wall galactomannan isolated from *Apodus deciduus*

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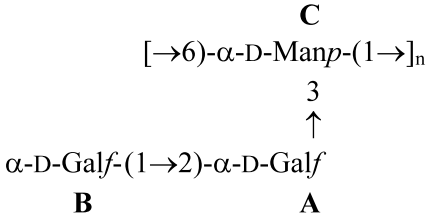
Received 25 March 2002; accepted 1 July 2002

Abstract

The alkali-extractable water-soluble polysaccharide (F1SS) isolated from the cell wall of *Apodus deciduus* has been studied by methylation analysis and NMR spectroscopy, and its structure established as:



where $n \approx 130 \pm 10$. © 2002 Published by Elsevier Science Ltd.

Keywords: Fungi; *Apodus deciduus*; Polysaccharides; NMR spectroscopy

Continuing the search for new alkali-extractable water-soluble cell wall polysaccharides (F1SS) which may be used as immunogenic, taxonomic and evolutive characters,[1] we report on the structure of a galactomannan obtained from the fungal cell wall of *Apodus deciduus*, the type species of a genus described by Malloch and Cain in 1971,[2] which was included in the family Sordariaceae.

The polysaccharide F1SS amounted to around 4% of the dry cell wall material. Monosaccharide analysis revealed the presence of galactose and mannose in a molar ratio 2:1, respectively. The absolute configuration analysis showed that both sugars had the D configuration. Methylation analysis yielded 1,3,5,6-tetra-*O*-acetyl-2,4-di-*O*-methyl-mannitol, 1,2,4-tri-*O*-acetyl-3,5,6-tri-*O*-methyl-galactitol, and 1,4-di-*O*-acetyl-2,3,5,6-tetra-*O*-methyl-galactitol, in the ratio 1:1:1, which indicated the presence of 3,6-di-*O*-substituted mannopyranose, 2-*O*-substituted galactofuranose and terminal galactofuranose, respectively.

The ^{1}H NMR spectrum contained signals for three anomeric protons at δ 5.24 (broad singlet), 5.13 (doublet, J 4.0 Hz), and 4.91 (broad singlet), with integrated areas 1:1:1, which were labeled **A**–**C** from low to high field (Fig. 1(a)) and suggested that the polysaccharide had a trisaccharide repeating unit. The ^{13}C NMR spectrum (Fig. 1(b)) confirmed the proton finding, since it showed signals for three anomeric carbons and fifteen additional singlets in the region 60–90 ppm.

The assignments of the spin system for each sugar unit were achieved by using 2D homo- (DQF-COSY and TOCSY) and heteronuclear (HMQC) techniques. The HMQC allowed to assign most of the carbon signals. An HSQC-TOCSY experiment (Fig. 2) led to the complete assignment of all the protons and carbons of the three repeating residues (**A**–**C**) (see Table 1). On comparison of the values obtained with that of model compounds,[3] it was clear that unit **A** was 2-*O*-substituted Gal*f*; **B**, terminal Gal*f*, and **C**, 3,6-di-*O*-substituted Man*p*.

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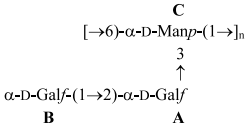
0008-6215/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0008-6215(02)00184-2

Concerning the anomeric configuration, the coupling constant of unit **B** ($J_{1,2}$ 4 Hz) is demonstrative of α configuration of a Gal*f* (compare with $J < 2$ Hz for a β-Gal*f*).[4] In addition, a coupled HMQC experiment revealed that $^{1}J_{\text{H-1,C-1}}$ of units **A**–**C**, were 173.1, 175.9, and 173.0 Hz, respectively, which, together with the values of the anomeric carbon chemical shifts, demonstrated α configuration for all the units.[3,4]

With respect to the connection of the units, a 2D-NOESY experiment (mixing time = 300 ms) contained, inter alia, crosspeaks between the anomeric proton of residue **A** and H-1 of unit **B** and H-2 and H-3 of unit **C**, along with others connecting H-1 of **B** with H-2 of unit **A**, and H-1 of unit **C** with H-6a and H-6b of a second unit of **C**. Since NOE crosspeaks depend on the conformation around the glycosidic bonds, the existence of the crosspeaks does not guarantee knowledge of the exact position of the linkage although, from the methylation analysis and the result of the NOESY, unit **B** must be linked to C-2 of unit **A** and this to C-3 of unit **C**. In addition, an HMBC experiment showed crosspeaks H-1**A**/C-3**C**, H-1**B**/C-2**A**, and H-1**C**/C-6**C** (Fig. 3), which unequivocally demonstrated the sequence **B** → 2**A** → 3**C** → 6**C**′, where **C**′ represents a second residue of 3,6-di-*O*-substituted Man*p*.

From all the combined data, the structure of the polysaccharide F1SS from *A. deciduus* was deduced to be:



The average molecular mass of the polydisperse polysaccharide is in the range of 60–70 kDa, as calculated by gel-permeation chromatography on a Sepharose CL-6B column, previously calibrated with different dextrans. Therefore, the value of *n* above is around 130 ± 10.

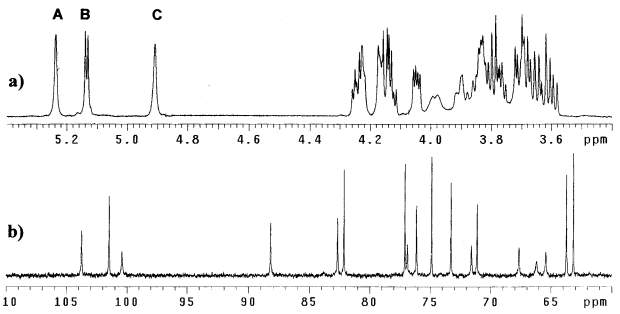


Fig. 1. (a) ^{1}H NMR and (b) ^{13}C NMR spectra of the F1SS polysaccharide isolated from the cell wall of *A. deciduus*. The anomeric protons have been labeled **A**–**C**.

To the best of our knowledge, this is the first example of a structure containing two consecutive α-Gal*f* residues in the chain of a fungal cell wall polysaccharide. Chains of β-Gal*f* linked to mannan cores have been described for a large number of fungal structures.[1,5–9] One of the most simple structures of this type is the galactomannan found in *Neurospora*,[10] with a single β-Gal*f* residue linked at C-2 of each Man*p* of a linear (1 → 6)-α-mannan. It seems that all these structures may have evolved from a linear mannan to different heteropolysaccharides,[1] in parallel to fungal evolution.[11] Therefore, the determination of the structure of polysaccharides F1SS is of potential interest in fungal systematics.

1. Experimental

Microorganisms and growth conditions.—The isolate of *A. deciduus* (CBS 506.70), was maintained in slants of Bacto potato dextrose agar supplemented with Bacto yeast extract (Difco) 1 g L^{-1}. The culture medium and growth conditions were as previously described.[12]

Wall material preparation and fractionation.—Wall material was obtained as reported elsewhere.[13] Polysac-

Table 1
^{1}H and ^{13}C NMR chemical shifts (δ) for the alkali-extractable water-soluble cell-wall polysaccharide F1SS isolated from *A. deciduus*

Residue		Proton or carbon [a]						
		1	2	3	4	5	6a	6b
A	H	5.24	4.23	4.25	4.05	3.84	3.68	3.64
	C	103.8	**88.2**	76.2	82.7	71.2	63.8	
B	H	5.13	4.14	4.15	3.80	3.77	3.71	3.60
	C	101.5	77.1	74.9	82.2	73.3	63.2	
C	H	4.91	4.17	3.90	3.86	~3.84	3.98	3.72
	C	100.5	67.7	**76.9**	65.5	71.6	**66.2**	

[a] Underlined bold numbers represent glycosylation sites.

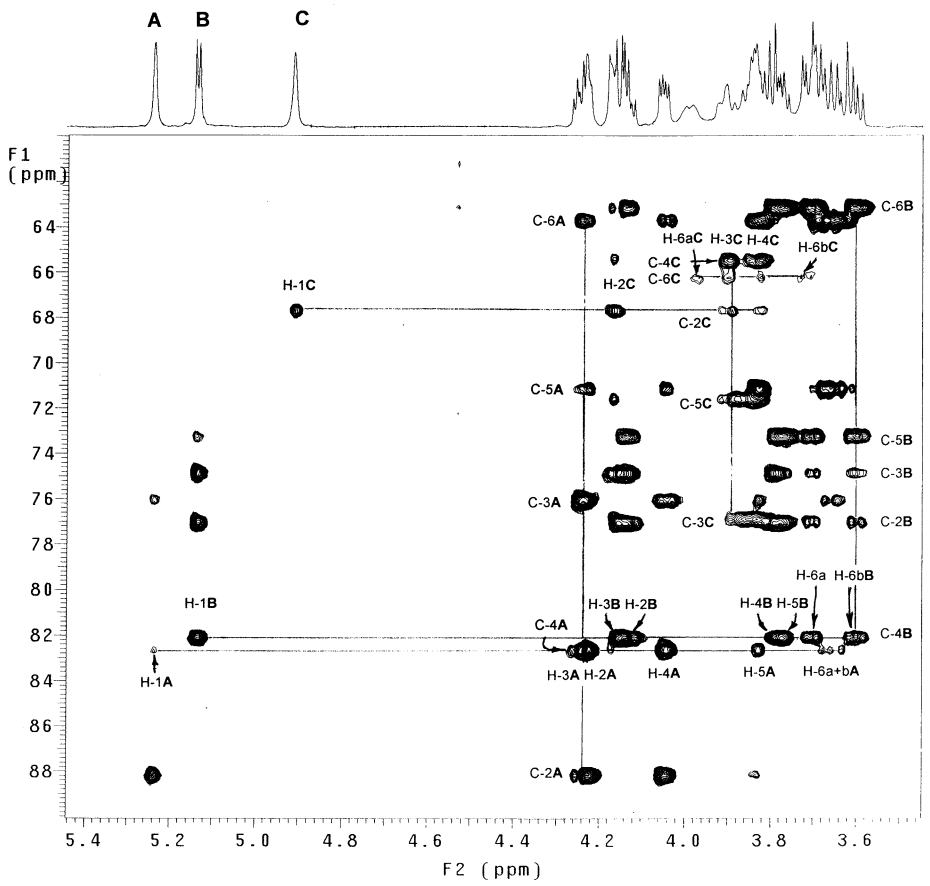


Fig. 2. Key region of the 2D HSQC-TOCSY spectrum of the F1SS polysaccharide isolated from *A. deciduus*. Proton (horizontal) and carbon (vertical) rows of crosspeaks for the three residues of the repeating trisaccharide have been labeled.

charide F1SS was obtained according to Ahrazem et al.[14]

Chemical analysis.—For analysis of neutral sugars the polysaccharide was hydrolyzed with 3 M TFA (1 h at 121 °C). The resulting monosaccharides were converted into their corresponding alditol acetates[14] and identified and quantified by gas–liquid chromatography (GLC) using an SP-2380 fused silica column (30 m × 0.25 mm I.D. × 0.2 μm film thickness) with a temperature program (210–240 °C, initial time 3 min, ramp rate 15 °C min^{-1}, final time 7 min) and a flame-ionization detector.

The monosaccharides released after hydrolysis were derivatized as devised by Gerwig et al.[15] and their absolute configuration was determined by GC–MS of the tetra-*O*-TMSi-(+)-2-butylglycosides obtained.

Methylation analyses.—The polysaccharide (1–5 mg) was methylated according to the method of Ciucanu and Kerek.[16] The methylated material was treated and processed according to Ahrazem et al.[17]

NMR analysis.—1D and 2D-^{1}H and ^{13}C NMR experiments were carried out at 40 °C on a Varian Unity 500 spectrometer with a reverse probe and a gradient unit. Proton chemical shifts refer to residual HDO at δ 4.61 ppm. Carbon chemical shifts refer to internal acetone at δ 31.07 ppm. The polysaccharide F1SS (ca. 20 mg) was dissolved in D_2O (1 mL) followed by

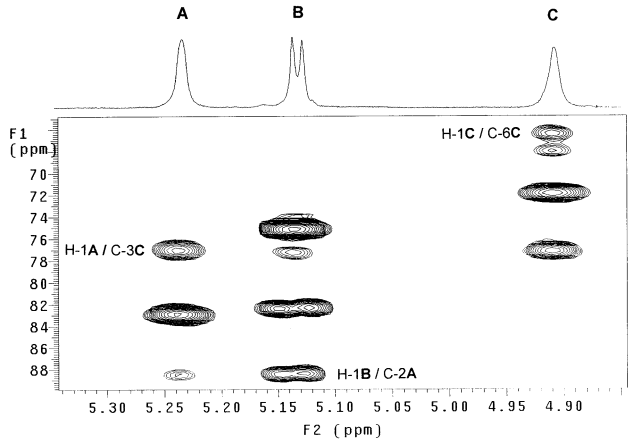


Fig. 3. Anomeric region of the 2D HMBC spectrum of the F1SS polysaccharide isolated from *A. deciduus*. The crosspeaks showing the interglycosidic connections have been labeled.

centrifugation (10,000*g*, 20 min) and lyophilization. The process was repeated twice and the final sample was dissolved in D_2O (0.7 mL, 99.98% D).

2D NMR experiments (DQF-COSY, TOCSY, NOESY, HMQC, HSQC-TOCSY and HMBC) were performed by using the standard Varian software, as described.[18]

Acknowledgements

The authors thank Mr J. López for technical assistance. This work was supported by Grant BQU-2000-1501-C02-01 from Dirección General de Investigación Científica y Técnica.

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