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Structural studies by NMR spectroscopy of the major oligomers from alkali-degraded arabinogalactan from *Larix occidentalis*

Merilyn Manley-Harris[1]

Shafizadeh Rocky Mountain Center for Wood and Carbohydrate Chemistry, University of Montana, Missoula, MT 59812, USA

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Oligomers with terminal metasaccharinic acid residues have been derived from branches on the main chain of arabinogalactan by alkaline degradation. The major oligomers present have been studied by NMR. Individual oligomers existed as epimeric pairs in the approximate ratio 1.5:1. This study confirmed the presence of branches consisting of a single β-D-Gal*p* residue, of two or three β-D-Gal*p* residues linked (1→6) or of two β-D-Gal*p* residues linked (1→6) with the proximate residue further substituted at O-3 by an α-L-arabinofuranosyl residue.

INTRODUCTION

Arabinogalactan (AG) from *Larix occidentalis* has been shown to consist of a (1→3)-linked galactopyranosyl (Gal*p*) backbone bearing extensive numbers of branches mainly at O-6 of the Gal*p* residues (Ponder and Richards, 1997). These branches vary in length and composition. The (1→3)-linked backbone is particularly susceptible to alkaline degradation (Kenner and Richards, 1957), which proceeds from the reducing end with facile elimination of the O-3 substituent and results in 3-deoxyhexonic ('metasaccharinic') acid (msa) residues bearing the former branches (Ponder and Richards, 1997). These msa oligomers have been fractionated by SEC and their structures elucidated. The results of this study have been described previously in this journal (Ponder and Richards, 1997), and that account alluded to the results of NMR spectroscopic studies of the sodium salts of the msa residues of the dimer, trimer and tetramer fractions. This article is a detailed report of those NMR studies.

EXPERIMENTAL

The sodium salts of the msa oligomers were prepared and fractionated as described previously (Ponder and Richards, 1997). Samples were lyophilized from D_2O and then dissolved in D_2O for NMR. *t*BuOH was used as an internal standard having a ^1H shift of 1.203 ppm and a ^{13}C shift of 30.695 ppm relative to external Me_4Si. NMR spectra were acquired using a Varian Unity 400 *plus* spectrometer with temperature controlled at 25°C. ^1H, ^{13}C, APT, H,H-COSY, TOCSY, HMQC and HMBC spectra were acquired using standard software applications packages. For HMQC, a *J* value of 140 Hz was used, and for HMBC, a delay of 50 ms was used for evolution of long-range couplings.

RESULTS AND DISCUSSION

About 90% of the residues in the main chain of arabinogalactan have been shown to be either unbranched or to bear branches composed of one, two or three residues (Ponder and Richards, 1997). Upon alkaline degradation, these give rise, respectively, to monomeric msa residues or to dimers, trimers and tetramers terminating in msa residues. Methylation analysis of the dimer fraction revealed only msa substituted at O-6 and terminal Gal*p*. A similar analysis of the trimer fraction gave msa and Gal*p* both substituted at O-6 and terminal Gal*p*. The putative structures of the dimer and trimer are illustrated in Fig. 1, which also shows the numbering

[1]Permanent address: Chemistry Department, University of Waikato, Hamilton, New Zealand.

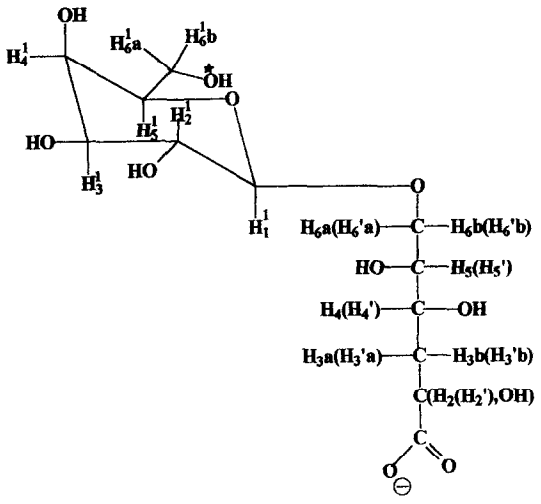

Fig. 1. Putative structure of the dimer from alkaline degradation of arabinogalactan showing numbering system used in text. The position of a β-D-galactopyranosyl residue in the trimer is indicated by '*' and the atoms of this residue are denoted by H_1^2, H_2^2, etc.

system used in this account. Because of the mechanism of its formation, the metasaccharinic acid residue occurs in two epimeric forms (Kenner and Richards, 1957). These epimers are distinguishable by NMR spectroscopy and the effect of the epimeric center extends to C_1^1 and H_1^1 of the attached Gal*p* residue.

Dimer fraction

Figure 2 shows the ^1H spectrum of the dimer fraction. The upfield group of signals between 1.60 and 2.10 ppm are the H_3 and $H_{3'}$ signals of the msa residue. This portion of the spectrum was amenable to first-order analysis and showed similar splitting patterns, albeit with some line-broadening, in the trimer and tetramer fractions. This similarity combined with the fact that the shifts of the other nuclei of the msa residue are similar in dimer, trimer and tetramer spectra indicates that the msa residue is little affected by increasing branch size. The coupling constants indicate that conformations of the C_2–C_3 and C_3–C_4 bonds differ in the two epimers. H_3a and H_3b are both in a *trans* relationship, respectively, to H_2 and H_4, whereas the $C_{2'}$–$H_{2'}$ and $C_{4'}$–$H_{4'}$ bonds bisect the angle between the $C_{3'}$–$H_{3'}a$ and $C_{3'}$–$H_{3'}b$ bonds. Integration of the signals for H_3 and $H_{3'}$ gave the ratio for the two epimers of 1.6:1. The signal for H_1^1, which is expected to be a doublet, consists of two doublets of unequal size. These two doublets integrate in the ratio 1.5:1, indicating that they arise from Gal*p* residues attached to different msa epimers. The chemical shift and the

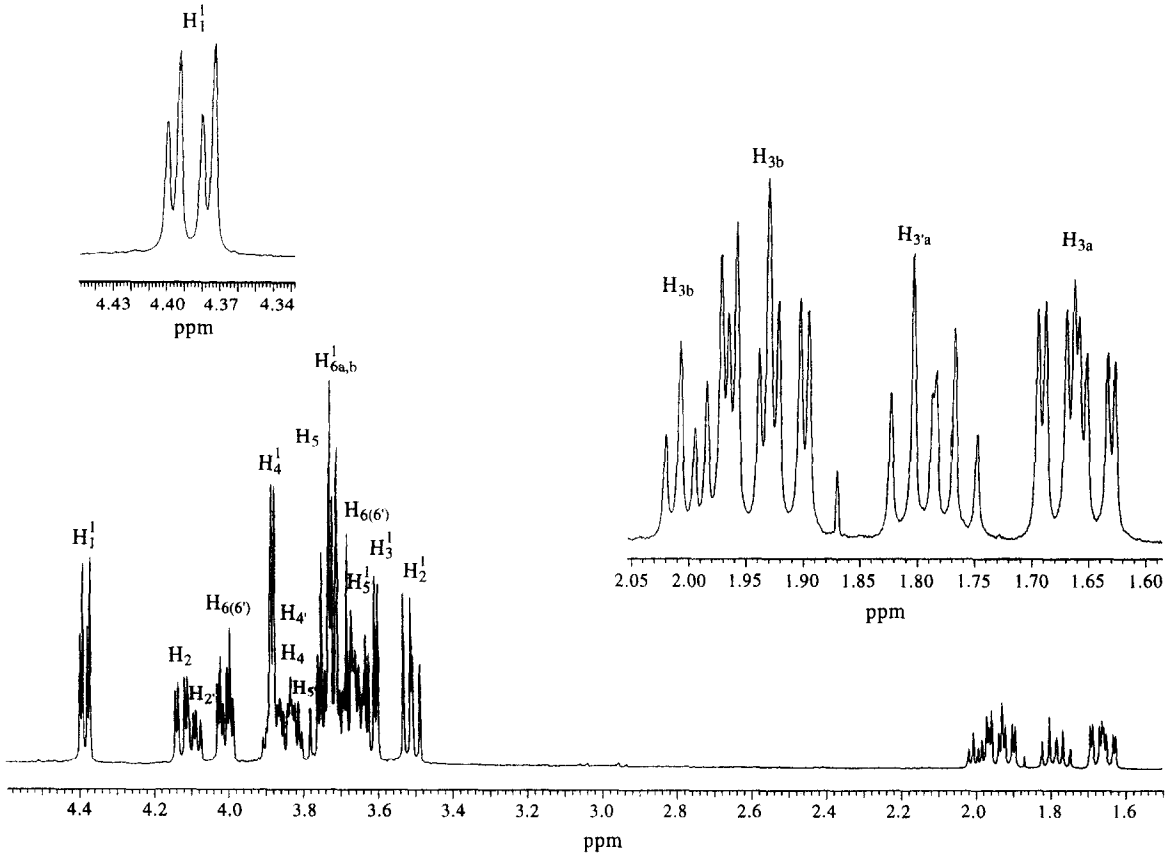

Fig. 2. ^1H spectrum of the dimer fraction from alkaline degradation of arabinogalactan.

Table 1. ^{13}C NMR spectra of metasaccharinic acid dimer and trimer and tetramer

Residue	Carbon chemical shift (ppm)					
	C-1	C-2	C-3	C-4	C-5	C-6
Gal*p* in dimer G^1	104.4	71.9	73.7	69.7	76.2	62.0
Gal*p* in trimer internal G^1	104.3	71.8	73.7	69.7	73.6	70.1
Gal*p* in trimer terminal G^2	104.4	71.8	73.7	69.7	76.2	62.1
Gal*p* in tetramer G^1	104.4	71.8	73.8	69.7	74.2	69.9
Gal*p* in tetramer G^2	104.4	71.8	73.8	69.7	74.2	69.9
Gal*p* in tetramer G^3	104.1	71.0	81.1	69.6	73.5[a]	70.3
Gal*p* in tetramer G^4	104.4	71.8	73.8	69.7	76.2	62.1
Ara*f* in tetramer A	110.4	82.4	77.6	84.9	62.3	
Metasaccharinic acid residues						
Dimer msa	182.7	70.4	38.7	69.3	74.2	72.3
Dimer msa′	182.1	71.4	38.4	70.1	73.0	72.5
Trimer msa	182.8	70.4	38.7	69.2	74.2	72.5
Trimer msa′	182.2	71.3	38.4	70.1[a]	73.0	72.6
Tetramer msa	182.8	70.4	38.7	69.2	73.0[a]	72.5
Tetramer msa′	182.2	71.4	38.4	70.1[a]	73.6[a]	72.6

[a]Some ambiguities may exist.

values of $J_{1,2}$ confirm that these are β-D-Gal*p* residues in the $^4C_1$ conformation. The signal for C_1^1 was also split into two unequal peaks by the effect of the epimers. COSY and HMQC experiments permitted the complete assignment of the ^{13}C (Table 1) and ^1H (Table 2) spectra of the dimer fraction and confirmed that this fraction consisted of two epimers of 6-*O*-(β-D-galactopyranosyl) galactometasaccharinic acid. Confirmation of the linkage was obtained from a cross-peak between C_1^1 and $H_6(H_{6'})$ in the HMBC spectrum.

Trimer fraction

The ^{13}C spectra of the trimer and dimer fractions were very similar, the signals for most carbons in both Gal*p* residues being almost coincident. Exceptions to this were the signals for C_5^1 and C_6^1 which were upfield and downfield-shifted, respectively, compared with C_5^2 and C_6^2. This is the expected effect of substitution at O-6 (Bock *et al.*, 1984). In the ^1H spectrum of the trimer, a new doublet corresponding to H_1^2 was observed in the anomeric region. Apart from H_6^1a and b, which were downfield-shifted, the remaining proton signals in the two Gal*p* residues were almost co-incident. As indicated above, there was no change in the signals for the msa residues. Integration of the signals for H_3 and $H_{3'}$ gave a ratio of 1.4:1 for the two epimeric msa residues. The assignments for the ^{13}C and ^1H spectra of the trimer fraction are detailed in Tables 1 and 2 and confirm the identity of the trimer fraction as a mixture of two epimers of 6-*O*-(6-*O*-β-D-galactopyranosyl-β-D-galactopyranosyl) galactometasaccharinic acid. The only signals that could not be assigned with confidence were those for $H_{4'}$ and $C_{4'}$, which were completely obscured by the downfield-shifted signals of H_6^{1b} and C_6^1. The assignment of these signals is based upon the lack of change of the other signals of the msa residue in passing from the dimer to the trimer.

Tetramer fraction

Methylation analysis of the tetramer fraction gave primarily terminal Gal*p*, terminal arabinofuranose (Ara*f*), 1,3,6-linked Gal*p* and 1,6-linked Gal*p*, in the approximate ratio 14:14:10:10, in addition to the msa residues. A single structure cannot explain these results. The tetramer structures must differ from the trimer structure by the addition of another residue either Ara*f* or Gal*p* at either O-6 or O-3 (Fig. 3). The anomeric region of the ^1H spectrum (Fig. 4) showed a doublet ($J_{1,2}$ 1.2 Hz) at 5.19 ppm representing H_1^A of the Ara*f* residue and confirming the α-L configuration for this residue (Kawabata *et al.*, 1995). The signals for the anomeric protons of the Gal*p* residues are considerably more complex since the signals for H_1^1 and H_1^3 both consist of two unequal doublets due to the presence of epimeric msa residues. In addition, doublets for H_1^2 and H_1^4 were also observed. All doublets had similar values of $J_{1,2}$, indicating that all Gal*p* residues are β-linked and in the $^4C_1$ conformation. Integration of the H_3 and $H_{3'}$ signals gave a ratio of 1.5:1 for the two epimeric msa residues. Both ^1H and ^{13}C spectra are somewhat crowded, but it was possible to assign all the signals with a reasonable degree of confidence (Tables 1 and 2).

An HMBC spectrum of the tetramer fraction (Fig. 5) showed cross peaks relating H_1^A to C_3^3 but not to C_6^3 or $C_6^{1,2}$ (Fig. 5a) and also relating C_1^A to H_3^3 but not to $H_{6a,b}^{1,2}$ or $H_{6a,b}^3$ (Fig. 5b). A cross peak was also observed relating $H_1^{2,4}$ to C_6^3 and to $C_6^{1,2}$ but not

Table 2. ^{1}HNMR spectra of metasaccharinic acid dimer and trimer and tetramer

Residue	^{1}H chemical shift (ppm) coupling constant (Hz)							
	H-1 $J_{1,2}$	H-2 $J_{2,3}$	H-3 $J_{3,4}$	H-4 $J_{4,5}$	H-5	H-6a		H-6b
Gal*p* in dimer G^1	4.38 d 7.6	3.51 dd 10.0	3.62 ddd 3.6	3.88 d 3.6	~3.65 m		3.65–3.75	
Gal*p* in trimer internal G^1	4.40 d 7.6	3.50 dd ~9.8	~3.61 m	~3.90 dd	~3.65 m	4.02 m		3.87 m
Gal*p* in trimer terminal G^2	4.43 d8.0	3.50 dd~9.8	~3.61 m	~3.90 dd	~3.66 m		3.67–3.75	
Gal*p* in tetramer G^1	~4.41 d8.0	~3.50	~3.62	~3.88	~3.72	~4.00		~3.89
Gal*p* in tetramer G^2	~4.44 d6.8	~3.50	~3.67	~4.06	~3.72	~4.00		~3.89
Gal*p* in tetramer G^3	~4.47 d7.8	~3.61	~3.62	~3.88	~3.66	~4.00		~3.89
Gal*p* in tetramer G^4	~4.42 d7.6	~3.50	~3.62	~3.88	~3.64		~3.65–3.75	
Ara*f* in tetramer A	5.19 d1.2	4.17 dd3.6	~3.91	~4.07			A_{5A}~3.79, A_{5B} ~3.64	
Metasaccharinic acid residues	H-2 $J_{2,3a}$ $J_{2,3b}$	H-3a $J_{3a,3b}$	H-3b $J_{3a,4}J_{3b,4}$	H-4	H-5	H-6a		H-6b
Dimer msa	4.13 dd 10.0, 3.2	1.66 ddd 14.4	1.93 ddd 2.4, 10.8	~3.85	~3.72		4.02, 4.00, 3.65-3.70	
Dimer msa′	4.09 dd 7.8, 5.2	1.78 ddd 14.4	1.99 ddd 7.8, 5.2	~3.87	~3.82			
Trimer msa	4.13 dd 10.2, 3.2	1.65 ddd 14.4	1.93 ddd 2.6, 11.0	~3.84	~3.73		4.2 bd, 3.73	
Trimer msa′	4.10 dd 7.9, 5.2	1.79 ddd 14.4	1.99 ddd 7.9, 5.2	~3.86	~3.83			
Tetramer msa	~4.13 10.4, 2.8	1.65 ddd 14.4	1.93 ddd 2.4, 11.0	~3.83	~3.81		~4.00, ~3.65	
Tetramer msa′	~4.08 7.8, 5.0	1.79 ddd 14.4	1.99 ddd 7.8, 5.0	~3.87				

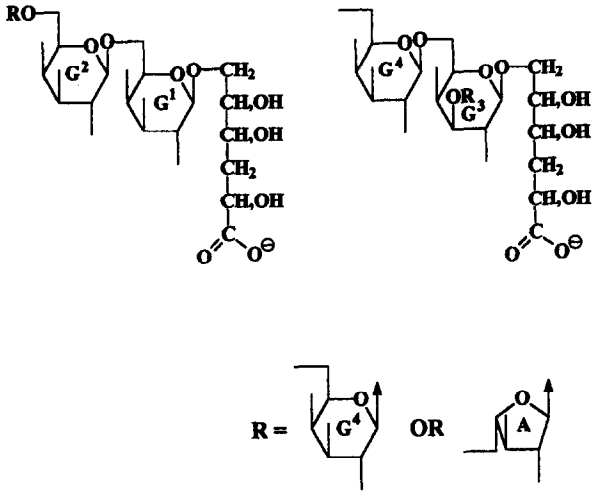

Fig. 3. Putative structures of the major tetramers from alkaline degradation of arabinogalactan showing numbering system used in the text; C-3 from G^4 would be denoted C_3^4 and C-3 from the arabinofuranose residue would be denoted C_3^A, etc.

to C_3^3 (Fig. 5c). This, taken together with the evidence from methylation analysis (Ponder and Richards, 1997), indicated that the tetramer fraction comprised two types of structure each terminating in a msa residue. One type consisted of only 1,6-β-linked Gal*p* residues plus the msa residue and the other type having O-3 of the Gal*p* residue immediately adjacent to the msa residue substituted by an α-linked Ara*f* residue. These two types represent about 83% of the fraction (Ponder and Richards, 1997) and occur in the ratio 1:2. Each type is further divided into two epimers as a result of the epimeric forms of the msa residues.

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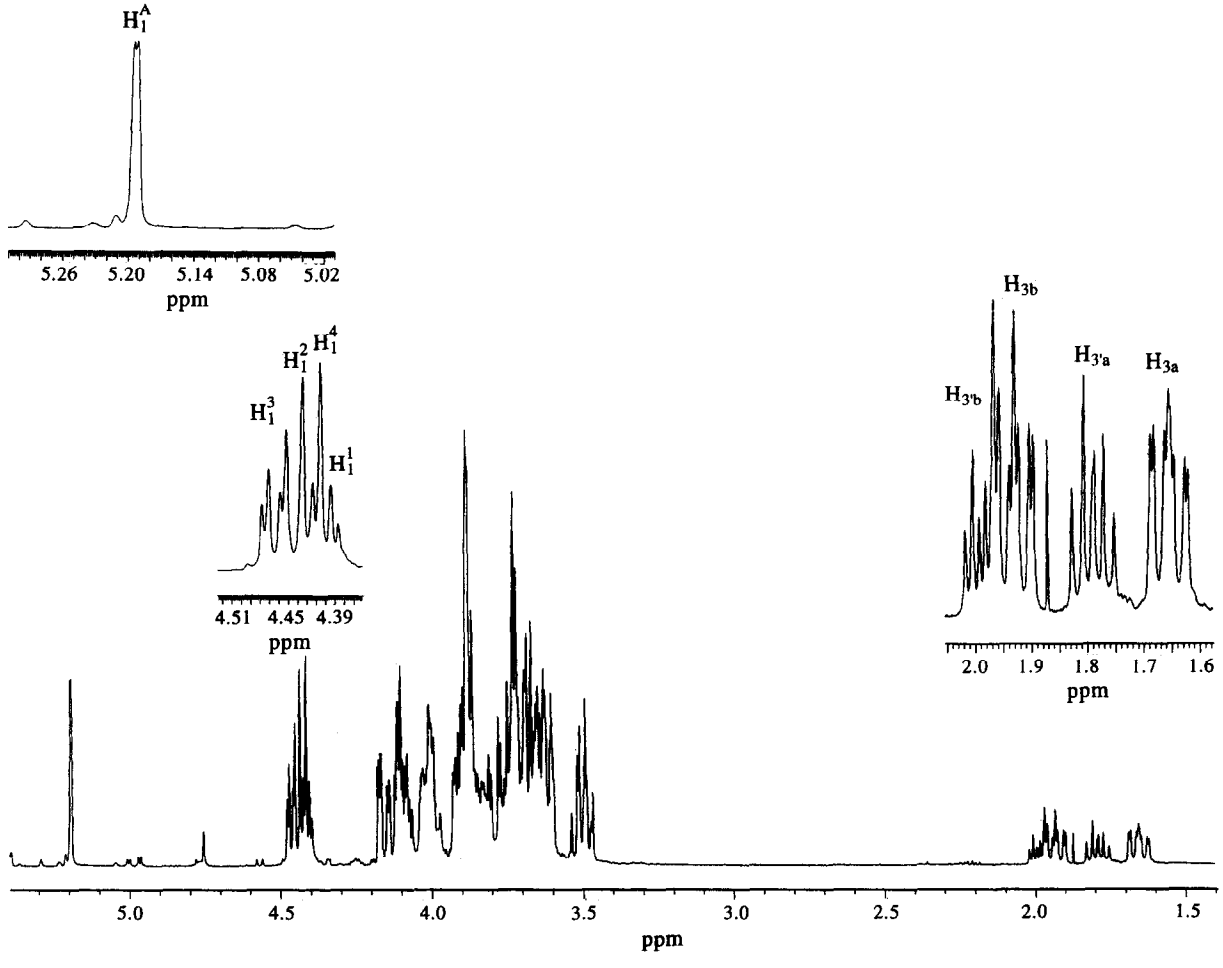

Fig. 4. ^{1}H spectrum of the tetramer fraction from alkaline degradation of arabinogalactan.

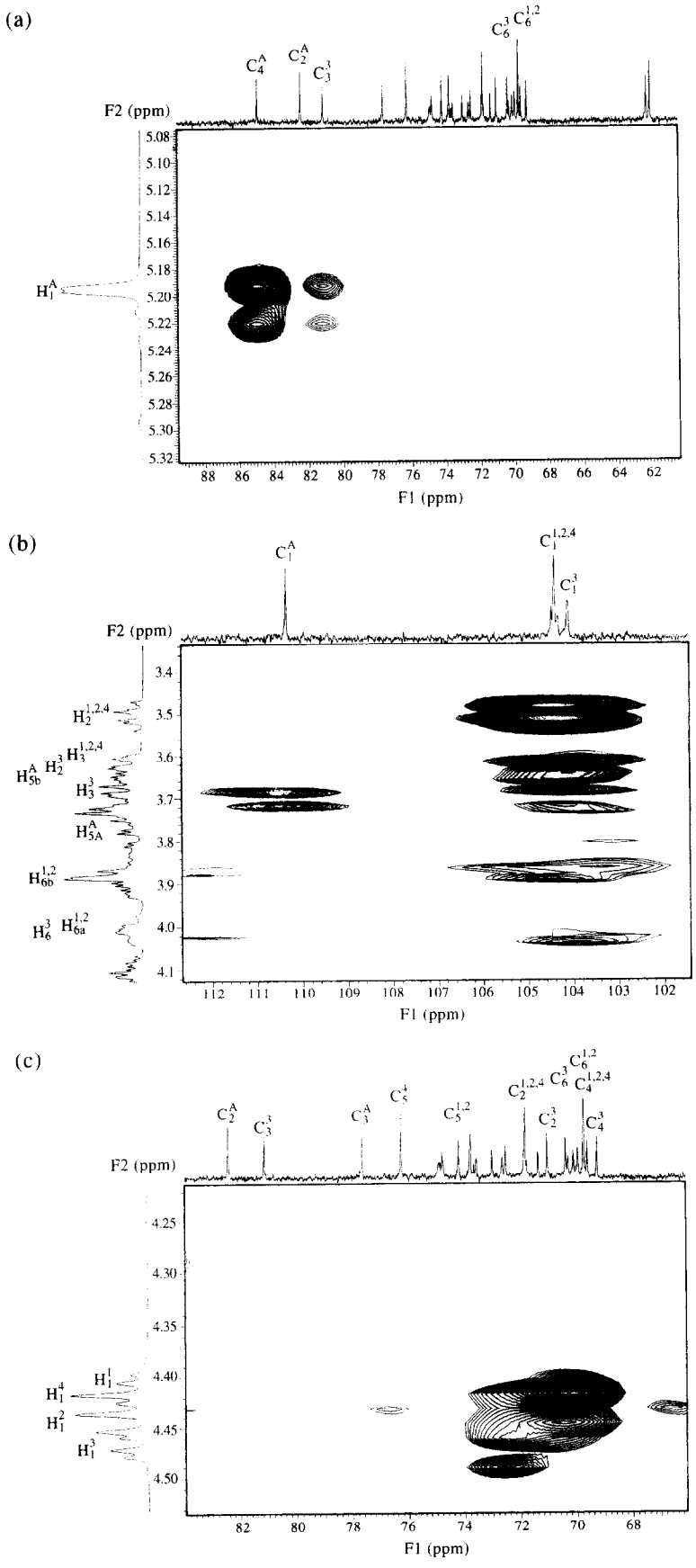

Fig. 5. Portions of the HMBC spectrum of the tetramer fraction from alkaline degradation of arabinogalactan. Cross-peaks are shown that indicate inter-residue linkages.

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