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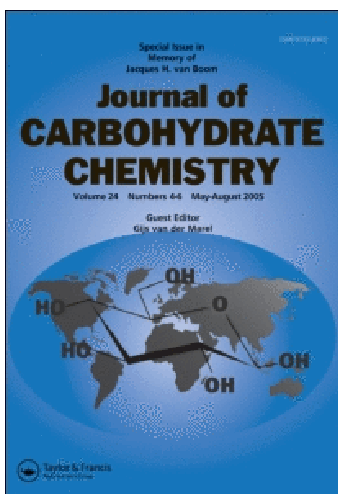

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Carbon-13 NMR Spectra of Peracetylated Derivatives of Methyl L-Arabinofuranoside and Oligosaccharides Related to Arabinoxylan

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CARBON-13 NMR SPECTRA OF PERACETYLATED DERIVATIVES OF METHYL L-ARABINOFURANOSIDE AND OLIGOSACCHARIDES RELATED TO ARABINOXYLAN

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ABSTRACT

The ^{13}C NMR signals of methyl tri-*O*-acetyl-L-arabinofuranosides were assigned on the basis of heteronuclear shift correlated NMR spectra. From the enzymic digest of barley-hull arabinoxylan two oligosaccharides, *i.e.*, α-L-Ara*f*-(1→3)-β-D-xyl*p*-(1→4)-D-xyl*p* and α-L-Ara*f*-(1→3)-β-D-xyl*p*-(1→4)-β-D-xyl*p*-(1→4)-D-xyl*p* were obtained. The NMR spectra of their per-*O*-acetylated derivatives were assigned, and discussed.

INTRODUCTION

Arabinoxylans, which are components of cereal plants, are composed of chains of β-1,4-linked D-xylopyranosyl residues containing L-arabinofuranose as side chains.[1] The structures of oligosaccharides, which are obtained from arabinoxylan by enzymic digestion, have recently been determined by ^{1}H and ^{13}C NMR spectra in D_2O solution.[2-4] Assignments of ^{1}H NMR signals to protons of free oligosaccharides, other than anomeric protons, are not easy, because many peaks overlap in the range of δ 3.0-4.2. The ^{13}C NMR chemical shifts for methyl tri-*O*-acetyl-β-D- xylopyranosides in $CDCl_3$ solution have been reported by

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Petráková and Shraml.[5] We have undertaken a study of ^{13}C NMR spectra of methyl tri-*O*-acetyl-L- arabinofuranosides in order to assign the NMR signals of per-*O*-acetyl derivatives of arabinoxylo-oligosaccharides.

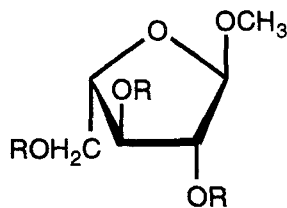
RESULTS AND DISCUSSION

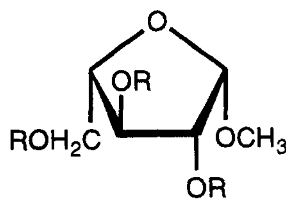
Methyl 2, 3, 5-tri-*O*-acetyl-α-L-arabinofuranoside **1** and methyl 2, 3, 5-tri-*O*-acetyl-β-L-arabinofranoside **2** were synthesized and their NMR spectra were measured. The ^1H NMR signals of **1** and **2** have been assigned by Izumi [6] by addition of an europium shift-reagent. After his assignments were confirmed by 2D ^1H-^1H COSY spectra, the δ values of ^{13}C nuclei of two compounds were determined by heteronuclear shift correlated NMR spectra. The assignments of chemical shifts are summarized in Table 1. The data may be compared with those of respective nuclei in methyl α-L-arabinofuranoside **3** and methyl β-L-arabinofuranoside **4**. The following chemical shifts of **3** and **4** in D_2O solution have been reported by Ritchie *et al.*[7] and Gorin *et al.*[8]

compound **3** C-1, 109.2 ; C-2, 81.8 ; C-3, 77.5 ; C-4, 84.9 ; C-5, 62.4 ; OCH_3, 56.0.

compound **4** C-1, 103.1 ; C-2, 77.4 ; C-3, 75.7 ; C-4, 82.9 ; C-5, 62.4 ; OCH_3, 56.3.

Comparison of the data between compounds **1** and **3** shows that the δ values for C-2, C-3 and C-5 in **1** are very close to those in **3**, while the signal due to C-4 in **1** appears at higher field (- 4.6 ppm) than the C-4 signal in **3**. Similarly, the upfield field shift (- 4.2 ppm) of C-4 signal in **2** is observed, when its δ value is compared with the corresponding C-4 shift in **4**. In the 2D ^1H-^{13}C COLOC spectra of **1**, the cross peaks are observed between C-4 and H-1, and between C-3 and H-5, 5'.





1 R = Ac **2** R = Ac

3 R = H **4** R = H

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Table 1. ^{13}C NMR Chemical Shifts for Peracetylated Methyl L-arabinofuranosides (**1** and **2**)

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*OCH_3*	*CH_3*	*C=O*
1	106.77	81.29	77.21	80.33	63.30	54.96	20.74	169.64
								170.17
								170.57
2	101.13	76.79	75.74	78.69	65.46	55.54	20.79	170.24
								170.24
								170.65

a. Chemical shifts in ppm for solutions in chloroform-*d*.

b. Assignments for C-2 and C-4 of compound **1** shown above are the reverse of those cited in ref. 9.

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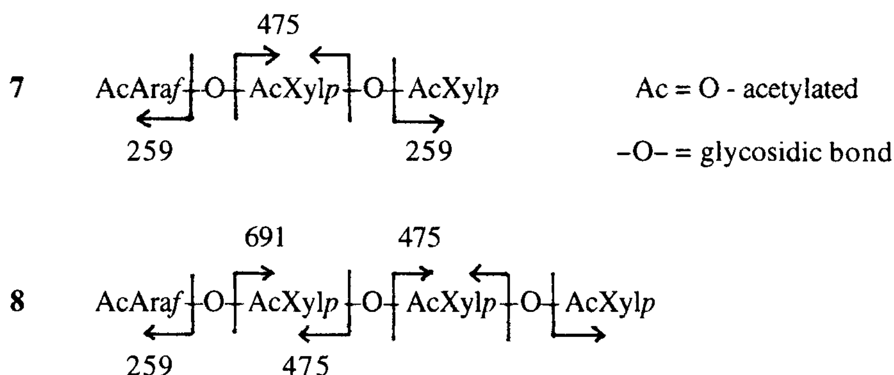
O-α-L-Arabinofuranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose **5** and *O*-α-L-arabinofuranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose **6** were obtained by enzymic digestion of arabinoxylan of barley-hulls. Methylation analysis of **5**, with analysis of the partially methylated sugars as alditol acetates, yielded 2,3,5,-tri-*O*-methylarabinose, 2,3-di-, and 2,4-di-*O*-methylxylose in the ratio of 1 : 1 : 1. Methylation analysis of **6** yielded the same sugars as **5**, and the ratio of 2,3-di- and 2,4-di-*O*-methylxylose was 2.1 : 1. After partial hydrolysis of **6** with 0.05 N hydrochloric acid, arabinose and β-1,4-xylotriose were identified by thin-layer chromatography.

The acetylation of **5** and **6** with acetic anhydride and pyridine gave their peracetylated derivatives; **7** and **8**. The main products **7** and **8** were proved to be β-octaacetate and β-decaacetate, respectively. The mass spectrum (positive-ion FAB) of **7** shows $[M+Na]^+$ signal at m/z 773, and the intense fragment peaks at m/z 475 and 259. In the FAB spectrum of **8**, $[M+Na]^+$ signal at m/z 989, and the fragment peaks at m/z 691, 475 and 259 are observed. Those fragment

peaks can be accounted for by cleavage of the glycosidic linkage. The straight-chain tetrasaccharide structure of **8** was confirmed from the prominent peak at m/z 475 corresponding to $[C_5H_6O(OAc)_3\text{-}O\text{-}C_5H_6O(OAc)_2]^+$.



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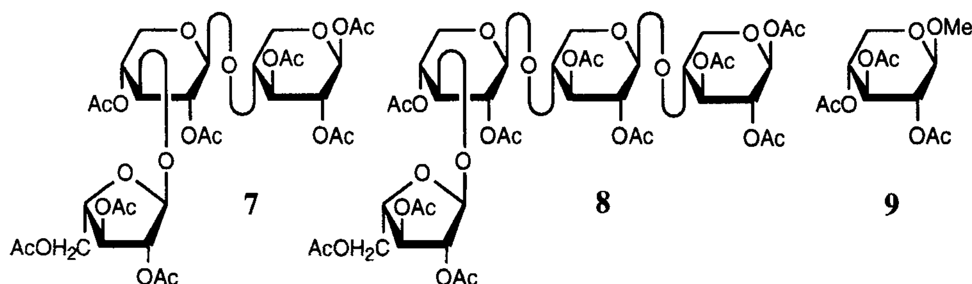
In the ^{13}C NMR of **7** (AcAra*f*→ AcXyl*p*-2→ AcXyl*p*-1), three signals at δ 92.24, 100.25 and 106.52 are assigned to the anomeric carbons of AcXyl*p*-1, AcXyl*p*-2 and AcAra*f*. In the ^{13}C-^{1}H 2D spectrum , the above signals correlate with ^{1}H signals at 5.64 (d, J=7.3 Hz) , 4.47 (d, J=6.7 Hz) and 5.08 (s), respectively. From ^{1}H-^{1}H COSY and HOHAHA spectra of **7**, ^{1}H NMR signals are assigned as shown in Table 2. Comparison of ^{1}H NMR data of compound **7** and methyl 2, 3, 4,-tri-*O*-acetyl-β-D-xylopyranoside **9** (Table 2) shows significant upfield shifts of H-4 of AcXyl*p*-1 and H-3 signal of AcXyl*p*-2.



In the 2D HOHAHA spectrum of **8** (AcAra*f*→ AcXyl*p*-3→ AcXyl*p*-2→ AcXyl*p*-1), the anomeric proton peak at δ 4.61 shows cross peaks at 3.39, 4.12, 4.83 and 5.06; and the anomeric proton peak at δ 4.43 shows cross peaks at δ 3.32, 3.80, 4.04, 4.88 and 4.92. A cross peak is observed between the signals at δ 4.83 and 3.80, respectively. The assignments of ^{1}H NMR signals in **8** (Table 2)

Table 2. ^{1}H NMR Chemical Shifts for *O*-Acetyl Derivatives of Oligosaccharides (**7** and **8**) and Methyl Tri-*O*-acetyl-β-D-xylopyranoside **9**

Compound		*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-5'*
7	AcXyl*p*-1	5.64	4.97	5.15	3.82	3.49	4.00
	AcXyl*p*-2	4.47	4.88	3.78	4.93	3.32	4.04
	AcAra*f*	5.08	4.89	4.91	4.19	4.20	4.38
8	AcXyl*p*-1	5.64	4.97	5.15	3.82	3.47	3.97
	AcXyl*p*-2	4.61	4.83	5.06	3.80	3.39	4.12
	AcXyl*p*-3	4.43	4.88	3.80	4.92	3.32	4.04
	AcAra*f*	5.13	4.87	4.90	4.51	4.27	4.40
9[b]		4.40	4.91	5.17	4.95	3.37	4.13

a. Chemical shifts in ppm for solutions in chloroform-*d*.
b. Assignment taken from ref. 5.

Table 3. ^{13}C NMR Chemical Shifts for *O*-Acetyl Derivatives of Oligosaccharides (**7** and **8**) and Methyl Tri-*O*-acetyl-β-D-xylopyranoside **9**

Compound		*C-1*	*C-2*	*C-3*	*C-4*	*C-5*
7	AcXyl*p*-1	92.24	69.54	72.04	74.51	63.49
	AcXyl*p*-2	101.25	71.04	76.63	69.88	62.05
	AcAra*f*	106.52	81.33	77.22	80.99	63.27
8	AcXyl*p*-1	92.10	69.42	71.90	74.40	63.35
	AcXyl*p*-2	100.59	71.52	69.00	73.65	61.45
	AcXyl*p*-3	100.16	71.13	77.08	69.83	62.01
	AcAra*f*	105.48	81.44	77.76	80.22	62.39
9[b]		101.58	70.74	71.46	68.94	61.98

a. Chemical shifts in ppm for solutions in chloroform-*d*.
b. Assignment taken from ref. 5.

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indicate that L-arabinofuranose links to C-3 of AcXyl*p*-3. The ^{13}C signals from **7** and **8** were assigned on the basis of ^{13}C-^{1}H 2D and INEPT spectra (Table 3). The ^{13}C signals of C-4 of AcXyl*p*-1 and C-3 of AcXyl*p*-2 in compound **7** are observed at lower field than the corresponding C-4 or C-3 peaks in the spectrum of **9**. It was confirmed that the glycosidic linkage of the L-arabinofuranosyl group in two oligosaccharides is α. The δ values of carbons of AcXyl*p*-3 residue in **8** are similar to their respective chemical shifts of AcXyl*p*-2 in **7**. The data described in Table 1 and 2 support the structure of **8**.

The oligosaccharides, which were obtained by enzymic digestion of barley-hull arabinoxylan, were β-D-Xyl*p*-(1→4)-D-Xyl*p*, β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-D-Xyl*p*, trisaccharide **5** and the new tetrasaccharide **6**. The yield of **6** from arabinoxylan was about one third of that of **5**. Kusakabe and his coworkers isolated oligosaccharide **5** and *O*-β-D-xylopranosyl-(1→4)-*O*-[α-L-arabinofuranosyl-(1→3)]-*O*-β-D-xylopranosyl-(1→4)-D-xylopyranose from the enzymic digests of corncobs[10] and rice-straw.[11] Comparison of the oligosaccharides obtained in this and the previous studies[10, 11] suggests that the branching pattern of arabinoxylan of barley-hull is different from that of corncob or rice-straw.

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EXPERIMENTAL

General Procedures. The ^{1}H and ^{13}C NMR were recorded at 500 MHz and 125.65 MHz, respectively, with a JEOL α-500 spectrometer. The mass spectra were obtained with JEOL JMS SX-102A spectrometer. Positive-ion FAB mass spectra were measured, after each sample was dispersed in a *m*-nitrobenzyl alcohol matrix.

Methyl 2, 3, 5-Tri-*O*-acetyl-α-L-arabinofuranoside (1) and Methyl 2, 3, 5-Tri-*O*-acetyl-β-L-arabinofuranoside (2). Methyl α- and β-L-arabinofuranoside (**3** and **4**) were prepared by the method of Mizutani *et al.*[12] and methyl glycosides were acetylated with acetic anhydride-pyridine.[6]

Preparation of Arabinoxylan Oligosaccharides ; *O*-α-L-Arabinofuranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose (5) and *O*-α-L-Arabinofuranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose (6). The barley hulls were extracted with 8 % NaOH aq. solution and, after insoluble material was removed, the filtrate

was neutralized with sulfuric acid. The precipitated polysaccharide was separated by centrifugation and the supernatant was mixed with 2 volumes of MeOH. The precipitated polysaccharide was collected, dialyzed and was mixed with the above polysaccharide. From 3 kg of barley hulls, arabinoxylan (400 g) was obtained.

β-Xylanase was produced by *Streptomyces olivaceoviridis* E. 86, which was isolated by Kusakabe and his co-workers.[10,13] A solution of the enzyme and arabinoxylan (200 g) in McIlvaine buffer (pH 5.7) was incubated for 32 h at 30 ℃. Arabinoxylan oligosaccharides were isolated according to the procedure reported by Yoshida *et al.*[11] After inactivation of the enzyme, the solution, which contained 95 g of sugar, was concentrated and applied to a carbon column. The column was washed with water and then eluted with 40 % aqueous ethanol to give the mixture of oligosaccharides (38 g). β-1,4-Xylobiose and β-1,4-xylotriose were removed by incubation with *Canadia guilliermondii*,[10] which metabolizes monosaccharides, xylobiose and xylotriose. The solution was applied to a carbon column, which was eluted by a linear gradient of 10~40 % aqueous ethanol. The fractions containing oligosaccharides **5** and **6** were collected and concentrated to a white powder (6.6 g). The compounds **5** and **6** were separated and purified by gel chromatography and preparative paper chromatography. The ratio of the amount of **5** and **6** was about 3 : 1.

Methylation Analysis. The oligosaccharide **5** (or **6**) was methylated by the method of Ciucanu and Kerek,[14] and methylated oligosaccharide was hydrolyzed in 10 % trifluoracetic acid for 2 h at 100 ℃. The hydolyzate was reduced with sodium borohydride, peracetylated and analyzed by GLC.[10,11]

Acetylation of Oligosaccharides. A mixture of acetic anhydride and pyridine was cooled to 0 ℃, and oligosaccharide **5** (or **6**) was added. The suspention was stirred at 0 ℃ until **5** (or **6**) was all dissolved. The solution was stirred for 18 h at room temperature, and the peracetate **7** (or **8**) isolated in the usual way, was purified by column chromatography on silica gel (ethyl acetate-hexane, 3 : 1). Compound **7** : white powder, $[\alpha]_D^{28}$ - 59.9° (c 1, chloroform). MAS (FAB) ; m/z 773 (M + Na), 691 (M - OAc), 475, 259, 199. Compound **8** : white powder, $[\alpha]_D^{28}$ - 54.9° (c 1, chloroform). MAS (FAB) ; m/z 989 (M + Na), 907 (M - OAc), 691, 475, 259, 199.

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