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Characterisation by ^{1}H NMR spectroscopy of oligosaccharides derived from alkali-extractable wheat-flour arabinoxylan by digestion with endo-(1 → 4)-β-D-xylanase III from *Aspergillus awamori*

Felix J.M. Kormelink [a], Rainer A. Hoffmann [b], Harry Gruppen [a], Alphons G.J. Voragen [a,*], Johannis P. Kamerling [b] and Johannes F.G. Vliegenthart [b]

[a] *Department of Food Science, Agricultural University, Biotechnion, P.O. Box 8129, 6700 EV Wageningen (Netherlands)*
[b] *Bijvoet Center, Department of Bio-Organic Chemistry, Utrecht University, P.O. Box 80075, 3508 TB Utrecht (Netherlands)*

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ABSTRACT

Alkali-extractable wheat-flour arabinoxylan, treated with endo-(1 → 4)-β-D-xylanase III from *Aspergillus awamori* CMI 142717, was fractionated by Bio-Gel P-2 size exclusion chromatography at 60°C. Column fractions, corresponding to oligosaccharides with degrees of polymerisation from 5 to 10, were collected, and subfractionated by high performance anion-exchange chromatography on CarboPac PA-1. The structures of the oligosaccharides thus obtained were elucidated by ^{1}H NMR spectroscopy, showing chains of (1 → 4)-linked β-D-xylopyranosyl residues differently substituted at O-3 and/or O-2,3 with α-L-arabinofuranosyl groups. The structures were different from those obtained with endo-(1 → 4)-β-D-xylanase I of the same xylanolytic enzyme system.

INTRODUCTION

In recent years, several groups have aimed at isolating arabinoxylan-derived oligosaccharides from various sources by chemical or enzymic degradation for structure elucidation[1–7]. Chemical methods are less specific and may result in removal of side groups, giving modified oligosaccharides which are not representative for the arabinoxylan. With enzymes, specific glycosidic linkages can be split, resulting in unmodified oligosaccharides. Characterisation of these oligosaccharides allows conclusions about the structure of the parent arabinoxylans, and about the pattern of action of the enzymes used.

* Corresponding author.

In a previous study[8], we described the structures of arabinoxylan-derived oligosaccharides obtained with endo-(1 → 4)-β-D-xylanase I from *Aspergillus awamori* CMI 142717. We now report on the structures of arabinoxylan-derived oligosaccharides obtained by treatment of alkali-extractable wheat-flour arabinoxylan with endo-(1 → 4)-β-D-xylanase III from the same organism.

EXPERIMENTAL

Materials.—Chemicals used were of analytical grade. Wheat alkali-extractable arabinoxylan (BE1-U) was prepared according to Gruppen et al.[9]. Endo-(1 → 4)-β-D-xylanase III was purified[10] from *A. awamori* CMI 142717.

Preparation of arabinoxylan oligosaccharides.—A solution of wheat alkali-extractable arabinoxylan (80 mg) in 50 mM sodium acetate buffer (80 mL, pH 5.0) was incubated with endo-(1 → 4)-β-D-xylanase III (0.4 μg/mL) for 24 h at 30°C. After inactivation of the enzyme at 100°C for 10 min, the solution was concentrated to 3 mL by vacuum rotary evaporation, and applied to a Bio-Gel P-2 column (100 × 2.8 cm, 200–400 mesh, Bio-Rad) at 60°C[11]. The column was eluted with distilled water (17 mL/h), and fractions (2.4 mL) were collected and monitored for total neutral sugar content by the automated sulfuric acid assay[12]. Appropriate fractions were pooled, designated ***1*** to ***14***, and concentrated by vacuum rotary evaporation to 2.0 mL. The Bio-Gel P-2 column was calibrated using a mixture of xylose, maltose, raffinose, stachyose, and Dextran T150 (Pharmacia).

Fractions ***5–10*** were subjected to high-performance anion-exchange chromatography (HPAEC), using a Dionex Bio-LC GPM-II quaternary gradient module equipped with a semi-preparative Dionex CarboPac PA-1 column (250 × 9 mm) and a Dionex PED detector in the pulsed amperometric detection (PAD) mode[8]. Samples of 300 μL (5–7 runs) were injected onto the column, and elutions (5 mL/min) involved a linear gradient of sodium acetate in 0.1 M NaOH from 50–250 mM during 40 min. The eluate was neutralised with M acetic acid. Corresponding fractions (1.2 mL) of each run were pooled, desalted using columns (30 × 80 mm) of Dowex 50W-X8 (H^+) and AG3 X4A (HO^-) resins in series, and concentrated by vacuum rotary evaporation, and the residues dried by a constant stream of air.

Neutral monosaccharide composition.—Bio-Gel P-2 fractions (100 μg) were hydrolysed with 2 M CF_3CO_2H for 1 h at 121°C. The samples were cooled to room temperature and CF_3CO_2H was evaporated in a stream of dried air at 40°C. The residues were reduced in 1.5 M NH_3 (0.2 mL), containing 50 mg $NaBH_4$/mL, and the products converted into their alditol acetates[13]. The monosaccharide composition was determined by GLC[10] using inosital as internal standard.

Dionex fractions (10 μg) were also hydrolysed with 2 M CF_3CO_2H as described above. The residues were dissolved in water (0.2 mL) and analysed on a CarboPac PA-1 column (250 × 4 mm). Elution (1 mL/min) involved linear gradients of

sodium acetate in 0.1 M NaOH from 0–100 mM during 5 min, then 100–400 mM during 35 min.

^{1}H NMR spectroscopy.—Samples were repeatedly treated with D_2O (99.9 atom% D, MSD isotopes), finally using 99.96 atom% D at pD ≥ 7. Resolution-enhanced 600-MHz ^{1}H NMR spectra were recorded with a Bruker AM-600 spectrometer (SON-hf-NMR facility, NSR-Center, Nijmegen University), operating at a probe temperature of 25°C. Chemical shifts (δ) are expressed in ppm relative to the signal of internal acetone at δ 2.225 (ref 14), with an accuracy of 0.002 ppm. Full details of the HOHAHA and ROESY experiments have been reported[6].

RESULTS AND DISCUSSION

Alkali-extractable wheat arabinoxylan was digested by endo-(1 → 4)-β-D-xylanase III from *A. awamori*, and the mixture of oligosaccharides was fractionated by Bio-Gel P-2 size exclusion chromatography (Fig. 1). The monosaccharide composition and yield of the fractions obtained are given in Table I. The elution pattern showed a large peak eluting at the void of the column. This arabinoxylan fraction was resistant to further enzymic digestion, probably because of the large amount of arabinosyl substituents (Table I). The eluate between 150 and 250 mL did not show significant peaks corresponding to a degree of polymerisation (dp) higher than 10. Between 250 and 350 mL, one small and five large peaks could be observed, corresponding to dp's of 5 up to 10 (fractions ***5–10***). No large amounts of oligosaccharides corresponding to dp's of 1 up to 4 (fractions ***1–4***) were released by endo-(1 → 4)-β-D-xylanase III. The monosaccharide composition of fractions ***1–3*** (Table I) and the presence of a single peak when run in HPAEC (results not shown) suggest the presence of xylose, xylobiose, and xylotriose, respectively. Fraction ***4*** also gave a single peak in HPAEC, and monosaccharide

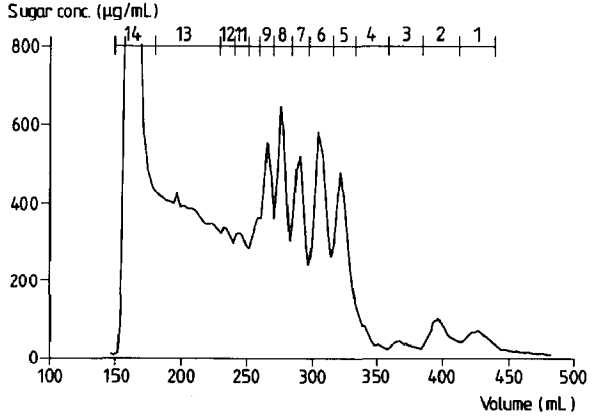


Fig. 1. Elution profile of the arabinoxylan digest on Bio-Gel P-2.

TABLE I

Data on the fractions obtained by chromatography on Bio-Gel P-2 and CarboPac PA-1 of the products formed by the enzymic degradation of wheat arabinoxylan

Fraction		Yield (%)		Monosaccharide composition [c]	
Bio-Gel	CarboPac	Bio-Gel [a]	CarboPac [b]	Ara (%)	Xyl (%)
1				0.0	100.0
2				0.0	100.0
3				0.0	100.0
4				25.0	75.0
5		5.7		19.7	80.3
	5.4		85.4	19.2 [d]	80.8 [d]
6		7.6		27.8	72.2
	6.1		41.9	31.9 [d]	68.1 [d]
	6.4		37.5	15.1 [d]	84.9 [d]
7		6.2		29.0	71.0
	7.2		38.9	25.4 [d]	74.6 [d]
	7.3		42.7	27.0 [d]	73.0 [d]
8		6.3		33.1	66.9
	8.1		40.5	37.3 [d]	62.7 [d]
	8.3		30.6	25.7 [d]	74.3 [d]
	8.4		7.6	36.4 [d]	63.6 [d]
9		4.9		33.4	66.6
	9.1		25.9	39.8 [d]	60.2 [d]
	9.3		18.0	22.0 [d]	78.0 [d]
	9.4		32.8	31.7 [d]	68.3 [d]
10		3.7		35.7	64.3
	10.1		21.0	38.3 [d]	61.7 [d]
	10.2		19.5	30.7 [d]	69.3 [d]
	10.3		32.4	36.6 [d]	63.4 [d]
11		4.1		36.0	64.0
12		4.2		35.4	64.6
13		20.2		37.9	62.1
14		29.4		44.4	52.1

[a] Yield in % of the total amount of neutral sugars present, determined spectrophotometrically. [b] Yield in % of the total PAD response from each Bio-Gel P-2 fraction. [c] Neutral sugar composition of the material in each fraction obtained by GLC analysis. [d] Neutral sugar composition of the material in each fraction obtained by HPAEC.

analysis showed the presence of 25% of arabinose and 75% of xylose. Fractions ***1–4***, which represented 7.7% of the total amount of neutral sugars, were not studied further.

Fractions ***5–10*** were subfractionated by HPAEC (Fig. 2); the monosaccharide composition and yield of each of the subfractions are given in Table I (the numbering of the subfractions obtained links up with that in previous studies[8,15,16]). Fraction ***5*** gave one major component, representing 85% of the PAD response. Fractions ***6*** and ***7*** both afforded two major components, together representing 79 and 82% of the total PAD response, respectively. Fractions ***8–10*** yielded three or more components. The accumulated peak areas of the collected fractions represented 79, 77, and 73% of the total PAD response of ***8–10***, respectively.

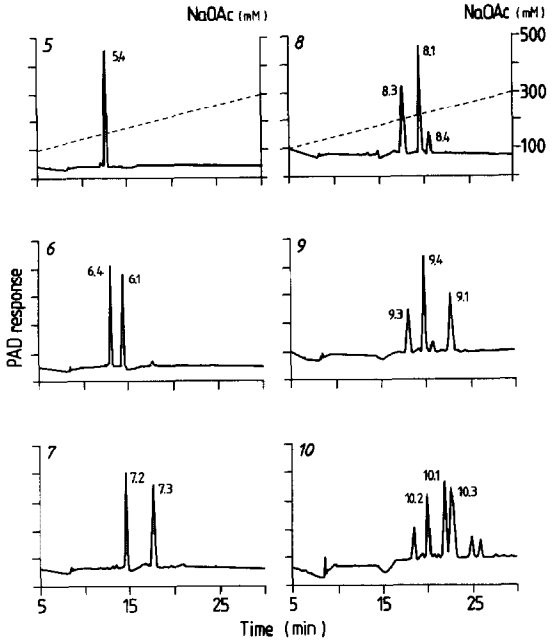


Fig. 2. HPAEC elution profile of Bio-Gel P-2 fractions *5–10* in Fig. 1.

For the identification of the primary structure of the arabinoxylan oligosaccharides isolated by HPAEC (Fig. 2), ^{1}H NMR spectroscopy was applied. The relevant ^{1}H NMR data and the corresponding structures of **5.4, 6.1, 6.4, 7.2, 7.3, 8.1, 8.4, 9.1, 9.4^{I}, 9.4^{II}**, and **10.1** are presented in Tables II and III, respectively. The chemical shift data of these structures matched exactly those of **AX-31**, **AX-33**, **AX-32b**, **AX-34b**, **AX-41**, **AX-54c**, **AX-56**, **AX-59**, **AX-54b**, **AX-54a**, and **AX-57a**, respectively, which were reported previously[6,7]. Fractions **10.2** and **10.3** have complex ^{1}H NMR spectra and will not be discussed further. Fractions **8.3^{I}**, **8.3^{II}**, **9.3^{I}**, and **9.3^{II}** represent new oligosaccharides whose structures are now discussed.

Fraction **8.3**.—The Bio-Gel P-2 elution volume of fraction *8*, in conjunction with the Ara–Xyl ratio of 25.7:74.3 for **8.3**, indicated that **8.3** has to contain one or more diarabinosylxylohexaoses. In the region for the anomeric protons in the ^{1}H NMR spectrum of **8.3** (Fig. 3), the presence of α-Ara*f* H-1 signals at δ 5.39 and β-Xyl*p* H-1 signals at δ 4.49–4.52, in combination with the absence of α-Ara*f* H-1 signals at δ 5.22–5.33 and β-Xyl*p* H-1 signals at δ 4.59–4.64 (refs 6–8 and 17), respectively, showed that single 3-substituted, internal β-Xyl*p* residues are the sole branching points. In the region for β-Xyl*p* H-1 signals, clearly more than six resonances can be observed, indicating the presence of a mixture of structures. Comparison of the H-1 ^{1}H NMR data of **8.3** with those of **7.3** showed that the same reducing diarabinosylxylotetraose unit, denoted (α-Ara*f*-**A^{3X4}**)β-Xyl*p*-**4^{II}**-(α-Ara*f*-**A^{3X3}**)β-Xyl*p*-**3^{II}**-β-Xylp-**2**-Xyl*p*-**1**, was present in one of the diarabinosylxylohexaoses, **8.3^{I}**, of **8.3**. The absence of the nonreducing terminal β-Xyl*p*-**5**

TABLE II

Chemical shifts [a] of the H-1 resonances of compounds **5.4**, **6.1**, **6.4**, **7.2**, **7.3**, **8.1**, **8.4**, **9.1**, **9.3^{I}**, **9.3^{II}**, **9.4^{I}**, and **10.1**

Residue [b]	Compound											
	5.4	**6.1**	**6.4**	**7.2**	**7.3**	**8.1**	**8.4**	**9.1**	**9.3^{I}**	**9.3^{II}**	**9.4^{I}**	**10.1**
α-Xyl*p*-**1**	5.184	5.183	5.184	5.183	5.184	5.184	5.184	5.184	5.184	5.184	5.184	5.184
β-Xyl*p*-**1**	4.584	4.584	4.584	4.584	4.584	4.584	4.584	4.584	4.584	4.584	4.584	4.584
β-Xyl*p*-**2**$_{\alpha}$	4.474	4.465	4.474	4.464	4.474	4.464	4.474	4.464	4.474	4.474	4.464	4.465
β-Xyl*p*-**2**$_{\beta}$	4.477	4.468	4.477	4.467	4.477	4.467	4.477	4.467	4.477	4.477	4.467	4.468
β-Xyl*p*-**3^{II}**	4.514		4.514		4.514		4.501		4.514	4.514		
β-Xyl*p*-**3^{III}**		4.639		4.638		4.637		4.628			4.637	4.639
β-Xyl*p*-**4**	4.442	4.436	4.460	4.454					4.459	4.459		4.443
β-Xyl*p*-**4^{II}**					4.488	4.480					4.480	
β-Xyl*p*-**4^{III}**							4.595	4.579				
β-Xyl*p*-**5**			4.448	4.451	4.431	4.435	4.425	4.428		4.468	4.453	
β-Xyl*p*-**5^{II}**									4.501			
β-Xyl*p*-**5^{III}**												4.628
β-Xyl*p*-**6**									4.459		4.447	4.436
β-Xyl*p*-**6^{II}**										4.514		
β-Xyl*p*-**7**									4.435 [c]	4.440 [c]		
α-Ara*f*-**A^{2X3}**		5.224		5.224		5.224		5.221			5.224	5.224
α-Ara*f*-**A^{3X3}**	5.397	5.274	5.391	5.272	5.388	5.270	5.419	5.293	5.391	5.391	5.270	5.271
α-Ara*f*-**A^{2X4}**							5.231	5.241				
α-Ara*f*-**A^{3X4}**					5.398	5.402	5.274	5.281			5.396	
α-Ara*f*-**A^{2X5}**												5.221
α-Ara*f*-**A^{3X5}**									5.391			5.271
α-Ara*f*-**A^{3X6}**										5.396		

[a] Measured at 600 MHz on solutions in D_2O at 25°C (internal acetone δ 2.225). [b] The Xyl*p* residue in the reducing position is denoted **1**, etc.; **2**$_{\alpha/\beta}$ means that the reducing Xyl*p*-**1** residue is α/β (anomerisation effect). Ara*f*-**A^{2X3}** means arabinofuranose linked to O-2 of Xyl*p*-**3**, etc.; Xyl*p*-**3^{I}** means Xyl*p*-**3** branched at O-2; Xyl*p*-**3^{II}** means Xyl*p*-**3** branched at O-3; Xyl*p*-**3^{III}** means Xyl*p*-**3** branched at O-2,3. [c] Assignments may have to be interchanged.

TABLE III

Structures of oligosaccharides **5.4**, **6.1**, **6.4**, **7.2**, **7.3**, **8.1**, **8.4**, **9.1**, **9.4^{I}**, **9.4^{II}**, and **10.1**

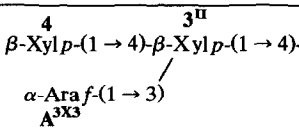
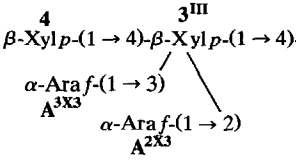
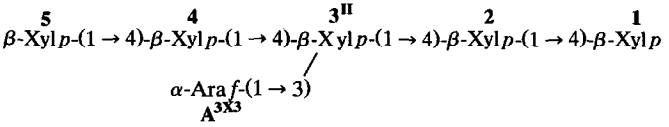
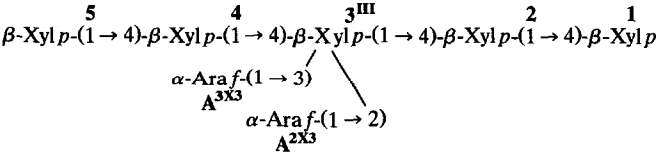
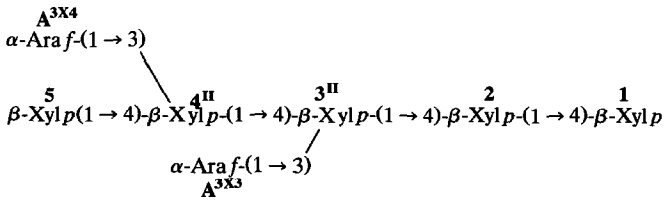
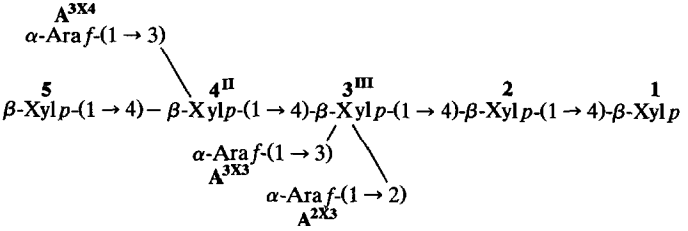
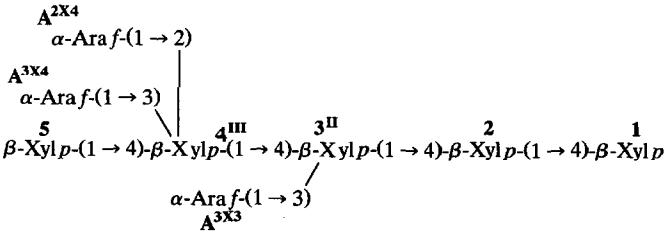
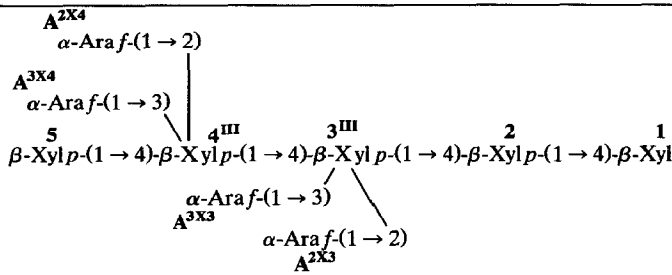
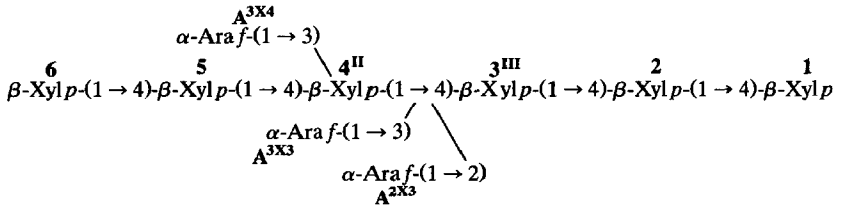
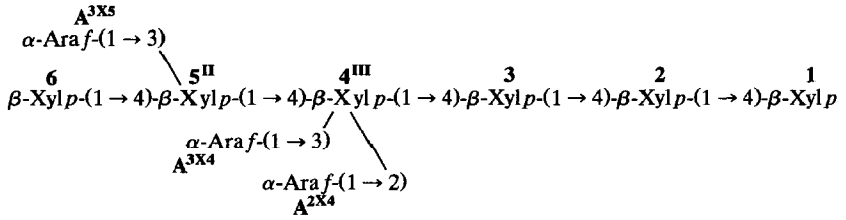
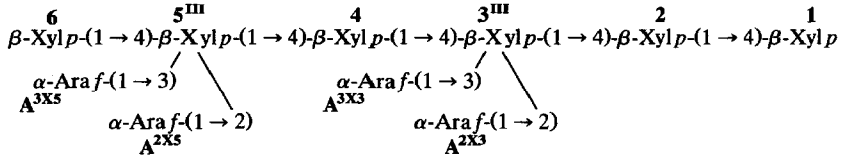
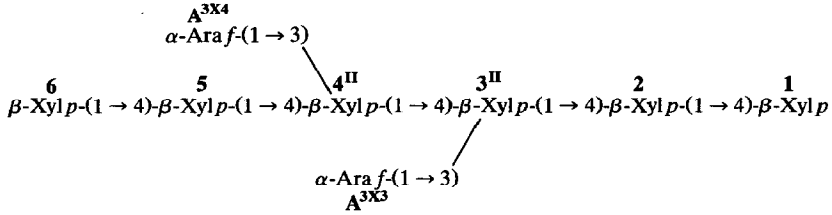
Code	Structure
5.4	
6.1	
6.4	
7.2	
7.3	
8.1	
8.4	

TABLE III (continued)

Code	Structure
9.1	
9.4^{I}	
9.4^{II}	
10.1	

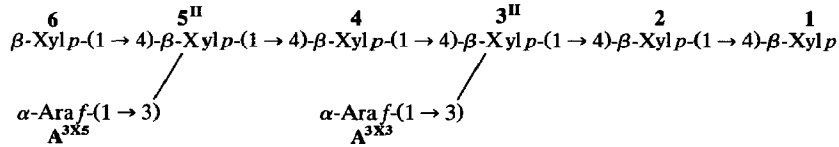
H-1 signal at δ 4.431 and the α-Ara*f*-**A**^{3X4} H-1 signal at δ 5.398 of **7.3** indicated a different nonreducing terminal sequence in **8.3**^{I}. In the 2D HOHAHA spectrum (not shown), the total scalar-coupled network for each residue was observed and the data are summarised in Table IV. Two nonreducing terminal β-Xyl*p* residues were distinguishable in the HOHAHA spectrum, reflected by the characteristic high-field shifts of their H-3,4,5 resonances[6]. From the ROEs between H-1 of β-Xyl*p*-(**n**) and H-4,5*eq* of β-Xylp-(**n** − **1**), observed along the H-1 tracks in the ROESY spectrum (not shown) (Table V), it was concluded that **8.3**^{I} contained an unbranched xylobiosyl group at the nonreducing end, characterised by the H-1 signals at δ 4.446 and 4.449 for the terminal and penultimate residues, respectively (Table IV). The ROESY data show that the H-1 signal at δ 4.449 has a cross-peak with H-4,5*eq* of the 3-branched β-Xyl*p*-**4**^{II} residue of **8.3**^{I}. Thus, this nonreducing xylobiosyl group is 4-linked to β-Xylp-**4**^{II}. The presence of the α-Ara*f*-**A**^{3X3},β-Xyl*p*-**3**^{II} and α-Ara*f*-**A**^{3X4},β-Xyl*p*-**4**^{II} connectivities in the ROESY spectrum com-

pleted the structure of **8.3**^{I}. Because of this nonreducing xylobiosyl extension, the H-1 signal of α-Ara*f*-**A**^{3X4} shifts to higher field as compared to H-1 of the same residue in **7.3**.



8.3^{I}

Identification of a second nonreducing terminal β-Xyl*p* residue in the HOHAHA spectrum of **8.3** pointed to the presence of a second diarabinosylxylohexaose, **8.3**^{II}. Combined HOHAHA and ROESY data (Tables IV and V) identified a nonreducing terminal sequence: β-Xyl*p*-**6**-(α-Ara*f*-**A**^{3X5})β-Xyl*p*-**5**^{II}-β-Xyl*p*-**4**. The characteristic upfield shift of β-Xyl*p*-**5**^{II} H-1 from δ 4.514 to 4.501, which is comparable with the upfield shift observed for β-Xyl*p*-**5**^{III} H-1 in **10.1** (**AX-57a**)[7] from δ 4.639 to 4.628, indicated that this nonreducing terminal sequence had to be linked to a branched β-Xyl*p*. The ROESY data (Table V) show that the β-Xyl*p*-**4** H-1 signal at δ 4.459 has a cross-peak with H-4,5*eq* of the 3-branched β-Xyl*p*-**3**^{II} in **8.3**^{II} (see below). Thus, the arabinosylxylotriose unit was 4-linked to the reducing arabinosylxylotriose unit, (α-Ara*f*-**A**^{3X3})β-Xyl*p*-**3**^{II}-β-Xyl*p*-**2**-Xyl*p*-**1**, of which all ^{1}H NMR signals resonate at chemical shift values identical to those of the corresponding residues in **6.4**. Based on the combined ^{1}H NMR data, the structure of **8.3**^{II} is



8.3^{II}

Fraction **9.3**.—The Bio-Gel P-2 elution volume of fraction *9*, in conjunction with the Ara–Xyl ratio of 22.0:78.0 for **9.3**, indicated that **9.3** has to contain one or more diarabinosylxyloheptaoses. In the region for the anomeric protons in the ^{1}H NMR spectrum of **9.3** (Fig. 4), the presence of α-Ara*f* H-1 signals at δ 5.39 and β-Xyl*p* H-1 signals at δ 4.49–4.52, in combination with the absence of α-Ara*f* H-1 signals at δ 5.22–5.33 and β-Xyl*p* H-1 signals at δ 4.59–4.64 (refs. 6–8), respectively, showed that single 3-substituted, internal β-Xyl*p* residues are the sole branching points. In the region for β-Xyl*p* H-1 signals, more than seven resonances, varying in signal intensities, can be observed, proving the presence of a mixture of structures. Comparison of the H-1 ^{1}H NMR data of **9.3** with those of **8.3**^{II} showed that the same reducing diarabinosylxylopentaose unit, (α-Ara*f*-**A**^{3X5})β-Xyl*p*-**5**^{II}-β-Xyl*p*-**4**-(α-Ara*f*-**A**^{3X3})β-Xyl*p*-**3**^{II}-β-Xyl*p*-**2**-Xyl*p*-**1**, was present in one of the diarabinosylxyloheptaoses, **9.3**^{I}, of **9.3**. The only structure fitting the

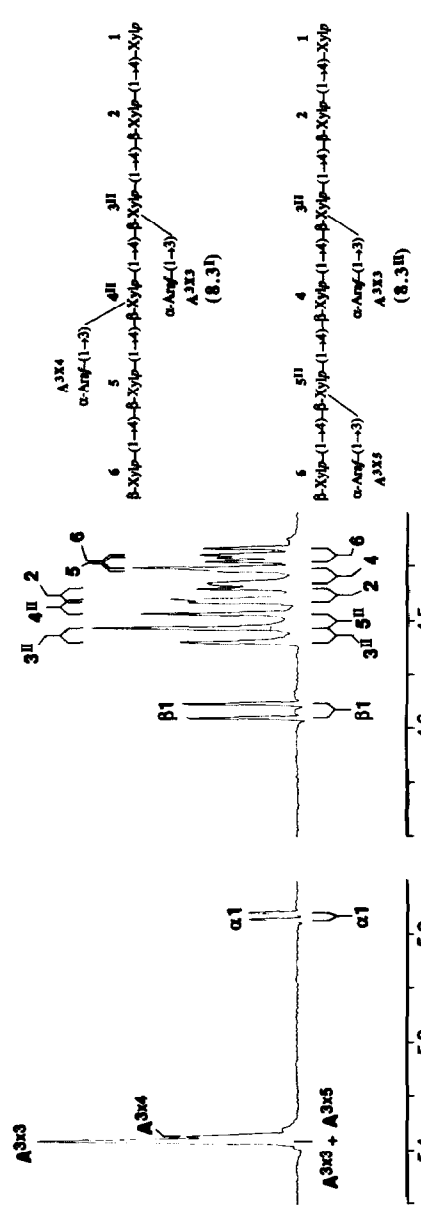
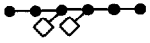


Fig. 3. Resolution-enhanced 600-MHz ^{1}H NMR spectrum of fraction 8.3. The numbers and letters in the spectrum refer to the corresponding residues in the structures.

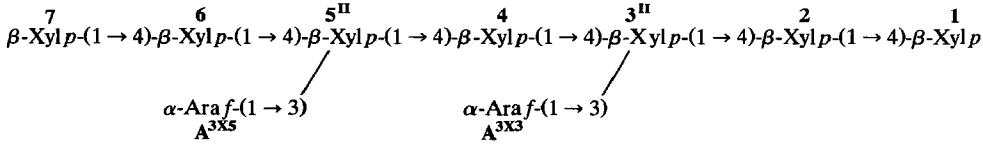
TABLE IV

^{1}H NMR data for the diarabinosylxylohexaoses **8.3^{I}** and **8.3^{II}** derived from enzymically degraded wheat arabinoxylan

Compound [a]	Residue [b]	Chemical shift [c]					
		H-1	H-2	H-3	H-4	H-5*eq*/H-5*proR*	H-5*ax*/H-5*proS*
							
	α-Xyl*p*-**1**	5.184	3.544	———	———	3.73–3.82	———
8.3^{I}	β-Xyl*p*-**1**	4.584	3.248	3.546	3.778	4.054	3.377
	β-Xyl*p*-**2**$_{\alpha}$	4.474	3.298	3.554	3.792	4.105	3.374
	β-Xyl*p*-**2**$_{\beta}$	4.477	3.290	3.551	3.792	4.105	3.374
	β-Xyl*p*-**3^{II}**	4.514	3.440	3.740	3.833	4.121	3.400
	β-Xyl*p*-**4^{II}**	4.489	3.428	3.729	3.792	4.066	3.364
	β-Xyl*p*-**5**	4.449	3.268	3.532	3.748	4.044	3.332
	β-Xyl*p*-**6**	4.446	3.245	3.420	3.619	3.964	3.296
	α-Ara*f*-**A^{3X3}** [d]	5.391	4.155	3.900	4.270	3.794	3.713
	α-Ara*f*-**A^{3X4}**	5.387	4.160	3.909	4.273	3.798	3.719
							
	α-Xyl*p*-**1**	5.184	3.544	———	———	3.73–3.82	———
8.3^{II}	β-Xyl*p*-**1**	4.584	3.248	3.546	3.778	4.054	3.377
	β-Xyl*p*-**2**$_{\alpha}$	4.474	3.298	3.554	3.792	4.105	3.374
	β-Xyl*p*-**2**$_{\beta}$	4.477	3.290	3.551	3.792	4.105	3.374
	β-Xyl*p*-**3^{II}**	4.514	3.440	3.744	3.833	4.121	3.400
	β-Xyl*p*-**4**	4.459	3.278	3.537	3.755	4.049	3.340
	β-Xyl*p*-**5^{II}**	4.501	3.433	3.744	3.817	4.117	3.390
	β-Xyl*p*-**6**	4.440	3.243	3.413	3.594	3.911	3.276
	α-Ara*f*-**A^{3X3}** [d]	5.391	4.160	3.909	4.273	3.798	3.719
	α-Ara*f*-**A^{3X5}**	5.391	4.160	3.909	4.273	3.798	3.719

[a] Compounds are represented by short-hand symbolic notation: ●, Xyl; ◇, α-Ara*f*; ●—●, β-Xyl*p*-(1 → 4)-Xyl*p*;  , α-Ara*f*-(1 → 3)-β-Xyl*p*. [b] See Table II for the key. [c] Measured at 600 MHz on solutions in D_2O at 25°C (internal acetone δ 2.225). [d] Assignments may have to be interchanged.

foregoing requirements and consistent with the observed enzyme mode-of-action (see also refs 6 and 7) is **8.3^{II}** extended at the nonreducing end by one unsubstituted β-Xyl*p* residue, giving structure **9.3^{I}**. This nonreducing terminal xylobiosyl unit is characterised by the H-1 resonances at δ 4.459 (see **6.4**) and 4.435 of the penultimate and terminal β-Xyl*p* residue, respectively.



9.3^{I}

The intensities of the β-Xyl*p* H-1 resonances at δ 5.184/4.584, 4.474/4.477, 4.514, and 4.459, and the intensity of the α-Ara*f* H-1 resonance at δ 5.391

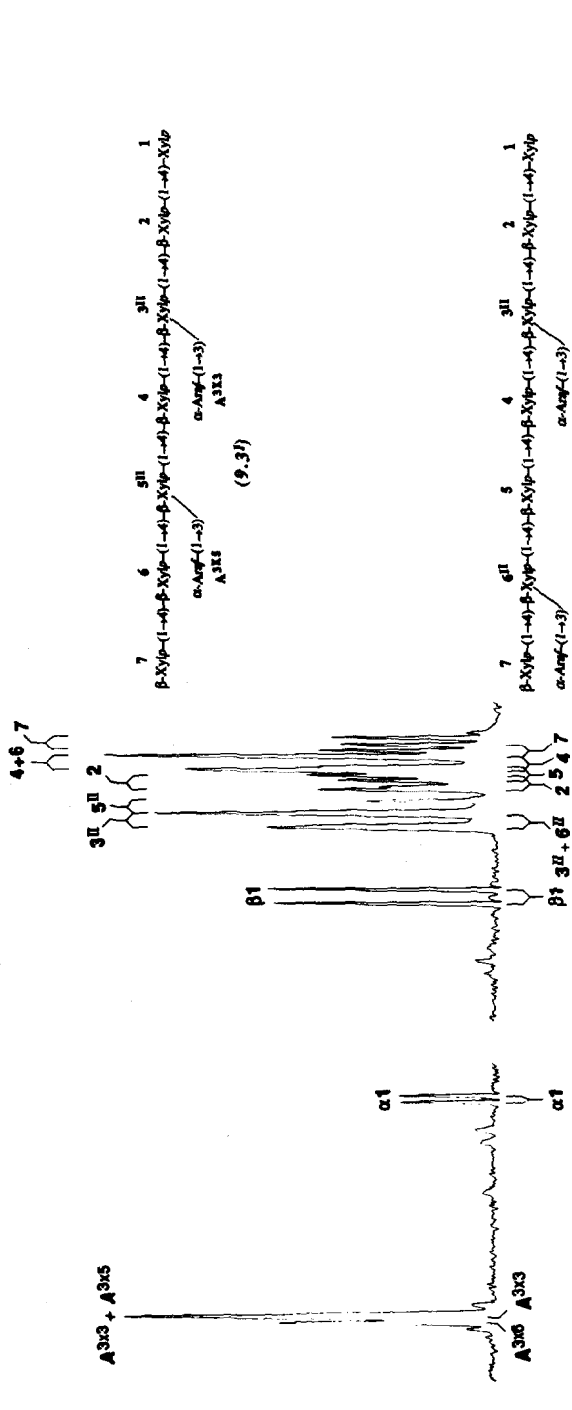


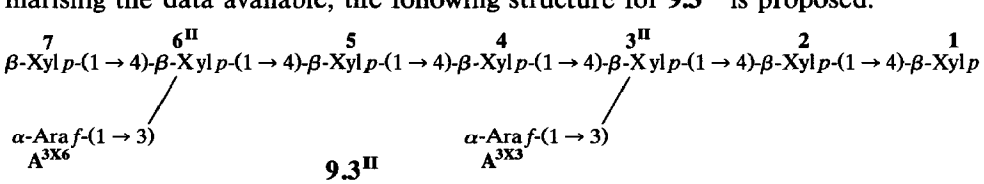
Fig. 4. Resolution-enhanced 600-MHz ^1H NMR spectrum of fraction 9.3. The numbers and letters in the spectrum refer to the corresponding residues in the structures.

TABLE V

Cross-peaks observed at the H-1 track in the ROESY spectra of the diarabinosylxylohexaoses **8.3^{I}** and **8.3^{II}**, measured with a mixing time of 200 ms

Compound	Residue	NOE effect
8.3^{I}	Xyl-**2** H-1	Xyl-**2** H-3,5*ax*; Xyl-**1**β H-4,5*eq*; Xyl-**1**α H-4,5
	Xyl-**3^{II}** H-1	Xyl-**3^{II}** H-3,5*ax*; Xyl-**2** H-4,5*eq*
	Xyl-**4^{II}** H-1	Xyl-**4^{II}** H-3,5*ax*; Xyl-**3^{II}** H-4,5*eq*
	Xyl-**5** H-1	Xyl-**5** H-3,5*ax*; Xyl-**4^{II}** H-4,5*eq*
	Xyl-**6** H-1	Xyl-**6** H-3,5*ax*; Xyl-**5** H-4,5*eq*
	Ara-**A^{3X3}** H-1	Ara-**A^{3X3}** H-2; Xyl-**3^{II}** H-3
	Ara-**A^{3X4}** H-1	Ara-**A^{3X4}** H-2; Xyl-**4^{II}** H-3
8.3^{II}	Xyl-**2** H-1	Xyl-**2** H-3,5*ax*; Xyl-**1**β H-4,5*eq*; Xyl-**1**α H-4,5
	Xyl-**3^{II}** H-1	Xyl-**3^{II}** H-3,5*ax*; Xyl-**2** H-4,5*eq*
	Xyl-**4** H-1	Xyl-**4** H-3,5*ax*; Xyl-**3^{II}** H-4,5*eq*
	Xyl-**5^{II}** H-1	Xyl-**5^{II}** H-3,5*ax*; Xyl-**4** H-4,5*eq*
	Xyl-**6** H-1	Xyl-**6** H-3,5*ax*; Xyl-**5^{II}** H-4,5*eq*
	Ara-**A^{3X3}** H-1	Ara-**A^{3X3}** H-2; Xyl-**3^{II}** H-3
	Ara-**A^{3X5}** H-1	Ara-**A^{3X5}** H-2; Xyl-**5^{II}** H-3

indicated the presence of a second β-Xyl*p*-**4**-(α-Ara*f*-**A^{3X3}**)β-Xyl*p*-**3^{II}**-β-Xyl*p*-**2**-Xyl*p*-**1** reducing unit, belonging to the second diarabinosylxyloheptaose, **9.3^{II}**. The presence of a nonreducing terminal arabinosylxylobiose unit β-Xyl*p*-(α-Ara*f*-**A^{3X6}**)β-Xyl*p* was highlighted by the observed β-Xyl*p* H-1 signals at δ 4.440 and 4.514 in combination with the α-Ara*f* H-1 signal at δ 5.396, resonating at chemical shifts comparable to those for the corresponding residues in **5.4**. The typical H-1 signal at δ 4.468 reflects the presence of an internal, unbranched β-Xyl*p* residue, being neither part of an unsubstituted nonreducing terminal xylobiosyl unit (see **6.4**), nor of an unsubstituted reducing xylotriosyl unit[6]. Summarising the data available, the following structure for **9.3^{II}** is proposed.



9.3^{II}

CONCLUSIONS

The majority of the oligosaccharides purified and characterised in this study were previously described by Hoffmann et al.[6,7]. However, some new structures were found which can be added to the existing ones.

The similarity in oligosaccharides derived from wheat alkali-extractable arabinoxylan by endo-(1 → 4)-β-D-xylanase III and wheat water-soluble arabinoxylan[6,7] suggests not only that the endo-xylanases used are the same type of enzyme but also a structural similarity in the building blocks of these polysaccharides.

Most of the oligosaccharides obtained with endo-(1 → 4)-β-D-xylanase III from *A. awamori* CMI 142717 are different from the oligosaccharides obtained with endo-(1 → 4)-β-D-xylanase I[8] from the same xylanolytic system. The pattern of action of enzymes from the same xylanolytic system will be the subject of future communications.

ACKNOWLEDGMENTS

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