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STRUCTURES OF THE OLIGOSACCHARIDES FROM THE ENZYMIC HYDROLYSIS OF HEMICELLULOSE BY A HEMICELLULASE OF *Ceratocystis paradoxa**

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ABSTRACT

An endo-hemicellulase (HC-II) of *Ceratocystis paradoxa* degraded spear-grass hemicellulose B to a series of mixed oligosaccharides. Four neutral oligosaccharides ($AraXyl_2$, $AraXyl_3$, Xyl_2, and Xyl_3), isolated by preparative paper chromatography, were shown by enzymic and methylation techniques to constitute a series of β-(1→4)-linked D-xylopyranosyl oligomers. The oligosaccharides $AraXyl_2$ and $AraXyl_3$ were identified as *O*-α-L-arabinofuranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose and *O*-α-L-arabinofuranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose, respectively, the latter being a new compound.

INTRODUCTION

Several arabinose–xylose oligosaccharides have been isolated from hydrolysates of hemicellulose formed by the action of microbial hemicellulases. Bishop and Whitaker[1] degraded wheat-straw xylan with a purified cellulase preparation from the fungus *Myrothecium verrucaria* to give a series of mixed arabinose–xylose oligosaccharides of d.p. 3–7, one of which was characterised as 3^2-α-L-arabinofuranosylxylobiose[2]. The first direct evidence was thus provided that arabinose residues are covalently linked to β-(1→4)-linked D-xylose residues in hemicellulose. 3^2-α-L-Arabinofuranosylxylobiose was found[3] in hydrolysates of rye-flour and cocksfoot-grass arabinoxylan obtained using a commercial "hemicellulase" preparation. Goldschmid and Perlin[4] used an enzyme preparation from Streptomyces QMB 814, which contained a xylanase, to degrade wheat arabinoxylan and subsequently isolated a tetrasaccharide (arabinoxylotriose, $AraXyl_3$), which was shown to be 3^2-α-L-arabinofuranosylxylotriose. Takenishi and Tsujisaka[5] used a highly purified xylanase preparation (xylanase I) to hydrolyse rice-straw arabinoxylan, and obtained two oligosaccharides ($AraXyl_2$ and $AraXyl_3$), both containing arabinose and xylose,

*Studies on Hemicellulases: Part IV. For Part III, see Ref. 13.

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which migrated ahead of the corresponding xylose oligosaccharides, xylobiose (Xyl_2) and xylotriose (Xyl_3), on p.c. $AraXyl_2$ and $AraXyl_3$ were found to be 3^1-α-L-arabinofuranosylxylobiose and 3^1-α-L-arabinofuranosylxylotriose, respectively. Beveridge and Richards[6] used cell-free enzyme preparations from rumen to hydrolyse spear-grass hemicellulose B and obtained a series of oligosaccharides in which the arabinose–xylose oligosaccharides migrated (p.c.) ahead of the corresponding xylose oligosaccharides (*cf.* refs. 5 and 7). The earlier workers[1–4] reported that the arabinose–xylose oligosaccharides migrated *behind* the corresponding xylose oligosaccharides. An arabinose–xylose oligosaccharide that migrated ahead of Xyl_2 was found in enzymic hydrolysates of rye-flour and cocksfoot arabinoxylans[3], and wheat-straw xylan[8], but its structure was not identified. It now appears, however, that this component may have been 3-*O*-α-L-arabinofuranosylxylose. Higher, mixed arabinose–xylose oligosaccharides (d.p. >4) have also been reported[1,3–9] to be present in enzymic hydrolysates of hemicelluloses, but their structures have not been determined.

Several xylose oligosaccharides of d.p. 2–7 found in hydrolysates of plant arabinoxylans, arabino-4-*O*-methylglucuronoxylans, and glucuronoxylans formed by the action of endo-xylanases[3–11], constitute a series of β-(1→4)-linked D-xylose oligomers.

We now report on the structures of the oligosaccharides $AraXyl_2$, Xyl_2, and Xyl_3, and a new tetrasaccharide ($AraXyl_3$), which were isolated from the hydrolysate of spear-grass hemicellulose B formed by the action of a hemicellulase (HC-II) from *Ceratocystis paradoxa*.

EXPERIMENTAL

Materials. — Spear-grass hemicellulose B and authentic samples of the arabinose–xylose and xylose oligosaccharides of d.p. 4–6 and 2–5, respectively, were kindly provided by Dr. R. J. Beveridge[6] of this laboratory. The hemicellulase preparations HC-I and HC-II were purified as previously described[12,13]. D-Glucose oxidase (Type V, *Aspergillus niger*) and peroxidase (horse radish) were obtained from Sigma Chemical Co., U. S. A. *o*-Dianisidine dihydrochloride was prepared from purified *o*-dianisidine by treatment with gaseous hydrogen chloride in ethanol.

General methods. — Oligosaccharides were determined by the phenol–sulphuric acid procedure[14], using L-arabinose and D-xylose as standards; and reducing sugars by the Nelson–Somogyi method[15], using D-xylose as the reference standard. D-Glucose was determined enzymically by the D-glucose oxidase–peroxidase–chromogen method[16]. Optical rotations were measured for aqueous solution at 25° with a Perkin–Elmer model 141 polarimeter.

Chromatography. — Samples from hemicellulose and oligosaccharide digests, and acid hydrolysates, were deionized with mixed Amberlite IR-45(HO^-) and IRC-50(H^+) resins prior to chromatography.

Paper chromatography (p.c.) was carried out on Whatman No. 1 paper, using *A*, ethyl acetate–pyridine–water (10:4:3); *B*, ethyl acetate–pyridine–water (2:1:2, upper

layer); *C*, 1-butanol–pyridine–water (6:4:2:5); and *D*,1-butanol–ethanol–water (3:1:1). Sugars were detected with *A*, alkaline silver nitrate[17]; and *B*, *p*-anisidine hydrochloride[18]. R_{XYL} values connote distances moved relative to that of D-xylose.

T.l.c. was carried out on silica gel 60 (0.25-mm thickness, E. Merck, Darmstadt, Germany), using *E*, 1-propanol–nitromethane–water (5:2:3); and *F*, ethyl acetate–65% 2-propanol (65:35). Neutral sugars were detected by charring at 100°/0.5 h after spraying with 9M sulphuric acid. Methylated sugars were separated using *G*, butanone–water (92:8), and detected with spray *B*. R_{TMG} values of methylated sugars refer to migration distances relative to that of 2,3,4,6-tetra-*O*-methyl-D-glucose.

Enzymic degradation of hemicellulose B. — A solution of hemicellulose B (1 g) in acetate buffer (50mM, pH 5.5; 100 ml) was incubated with enzyme solution (purified HC-II, 2 ml) and a few drops of toluene at 35°, until the increase in reducing power corresponded to 32% conversion into xylose (47 h). The digest was next centrifuged (1000 *g*, 15 min), and mercuric acetate (0.1M, 1 ml) was added to the supernatant solution to deactivate the enzyme. P.c. indicated the presence of xylose, a component of R_{XYL} 0.78 previously identified[13] as an arabinose–xylose saccharide of d.p. 3, glucose (R_{XYL} 0.70), xylobiose (Xyl_2, R_{XYL} 0.60), $AraXyl_3$ (R_{XYL} 0.38), and xylotriose (Xyl_3, R_{XYL} 0.31) as the major products. There were also trace amounts of higher oligosaccharides, which were identified by co-chromatography with authentic arabinose–xylose and xylose oligosaccharides of d.p. 3–6 and 2–5, respectively. D-Glucose in the hydrolysate was detected by a positive response in the D-glucose-oxidase test.

Removal of D-*glucose from the hemicellulose digest using* D-*glucose oxidase.* — The supernatant solution from the preceding experiment was adjusted to pH 7.0 (2M NaOH), and concentrated under reduced pressure at 40°, and mercury(II) ions- and acidic sugars were removed by passage through columns of Amberlite IRC 50(H^+) and IRA-400(AcO^-) resins, followed by elution with ~10 bed-volumes of water. The neutral sugar fraction was concentrated to 30 ml, the pH was adjusted to 7, and the D-glucose concentration was determined. D-Glucose oxidase solution (0.1 ml) was added, and the digest was kept at 35° until all of the D-glucose had been converted into D-gluconic acid (17 h). The enzyme reaction was terminated by heating (100°/10 min), and the resulting solution was concentrated, and deionized with mixed exchange resins to remove D-gluconic acid and sodium ions. P.c. of the eluate indicated that glucose had been completely removed. The eluate was concentrated to a syrup and stored at 5°.

Isolation and characterization of the arabinose–xylose and xylose oligosaccharides of d.p. 3–4 and 2–3, respectively. — The neutral sugar components arising from the enzymic degradation of hemicellulose B were separated by preparative p.c. (Whatman No. 17MM paper, solvent *A*, 240 mg loaded onto 4 sheets, running time 8 h). Components having R_{XYL} values of 0.78, 0.60, 0.34, and 0.31, which corresponded to $AraXyl_2$, Xyl_2, $AraXyl_3$, and Xyl_3, respectively, were eluted from the paper with 80% ethanol, and characterised as follows.

$AraXyl_2$ (66.4 mg), $[\alpha]_D^{25}$ −17° (*c* 0.34) (lit.[1–3] $[\alpha]_D$ −19.3°, −14.9°, and 15.3°),

was homogeneous by p.c. (R_{XYL} 0.78, 1.02, 0.83; solvents *A–C*) and t.l.c. (R_{XYL} 1.02; solvent *E*). Partial hydrolysis (5mM sulphuric acid, 100°, 30 min) gave (p.c. solvent *A*) arabinose and Xyl_2, together with traces of xylose. Total hydrolysis (0.5M sulphuric acid, 100°, 4 h) gave arabinose and xylose (t.l.c., solvent *E*) in the ratio of 1.0:1.99, as determined by densitometry. The reaction of $AraXyl_2$ with saturated lime-water for 3 h at 25° did not yield arabinose (p.c., solvent *A*).

$AraXyl_3$ (7.5 mg), $[\alpha]_D^{25}$ −74° (*c* 0.075), contained a glucose disaccharide (~12%) which co-chromatographed with cellobiose (p.c., solvent *D*; t.l.c., solvent *E*). The R_{XYL} values of $AraXyl_3$ and cellobiose were 0.29 and 0.43 in p.c. (solvent *D*), and 0.90 and 0.76 in t.l.c. (solvent *E*). The mixture ran as a single spot on p.c. in solvent *A* (R_{XYL} 0.34) and on t.l.c. in solvent *F* (R_{XYL} 0.50). Partial, acid hydrolysis (5mM sulphuric acid, 100°, 30 min) gave arabinose, Xyl_2, and xylotriose (Xyl_3), together with traces of xylose and glucose (p.c., solvent *A*). Total hydrolysis (0.5M sulphuric acid, 100°, 4 h) gave arabinose, xylose, and glucose (t.l.c., solvent *E*) in the ratios of 1.0:3.2:0.5, as determined by densitometry.

Xyl_2 (100 mg), $[\alpha]_D^{25}$ −25° (*c* 1) (lit.[6] $[\alpha]_D$ −25°), was homogeneous by p.c. (R_{XYL} 0.60, 0.86, 0.69, 0.53; solvents *A–D*) and t.l.c. (R_{XYL} 0.89, 0.56, solvents *E* and *F*). Hydrolysis with 0.5M sulphuric acid at 100° for 4 h gave xylose as the only product (p.c., solvent *A*). Alkaline degradation of Xyl_2 with $Ca(OH)_2$ yielded xylose (p.c., solvent *A*).

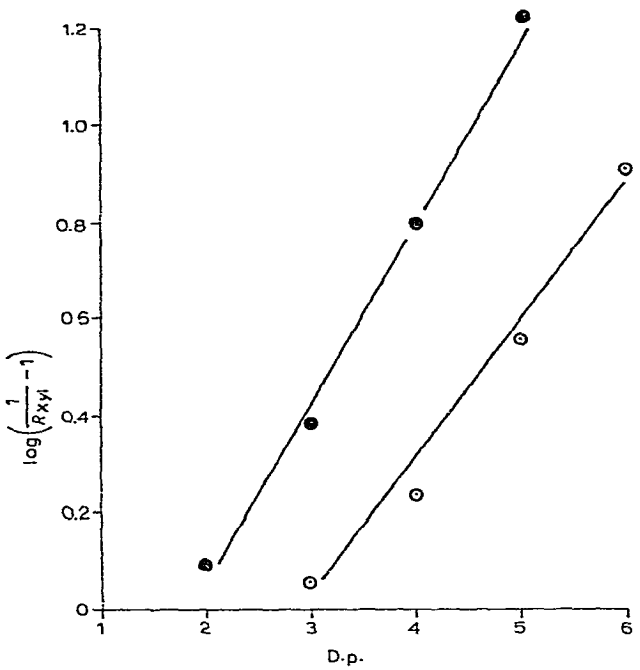


Fig. 1. The relationship between d.p. and R_{XYL} values of arabinose–xylose (⊙——⊙) and xylose (●——●) oligosaccharides on paper chromatography (solvent *A*).

Xyl_3 (8 mg), $[\alpha]_D^{25}$ −60° (*c* 0.1) (lit.[6] $[\alpha]_D$ −61°), was homogeneous by p.c. (R_{XYL} 0.31, 0.25; solvents *A* and *D*) and t.l.c. (R_{XYL} 0.82, solvent *E*). Hydrolysis with 0.5M sulphuric acid at 100° for 4 h gave xylose only (p.c., solvent *A*).

Enzymic hydrolysis of the oligosaccharides by purified hemicellulases from C. paradoxa. — Solutions of arabinose–xylose saccharides [0.5 ml, $AraXyl_2$ (containing 1.68 mg) and $AraXyl_3$ (containing 0.38 mg)] and xylose saccharides (0.5 ml, 2mM), acetate buffer (0.1M, pH 5.5, 0.5 ml), and enzyme solution [HC-I (0.02 ml) and HC-II (0.01 ml)] were incubated at 35°, and monitored for reducing power and by p.c. The results are shown in Table I.

TABLE I

PRODUCTS OF ENZYMIC HYDROLYSIS OF ARABINOSE–XYLOSE AND XYLOSE OLIGOSACCHARIDES

Enzyme	*Substrate*	*Increase in reducing power* (%)	*Degradation products*[a]				
			Xyl	*Ara*	*$AraXyl_2$*	*Xyl_2*	*Xyl_3*
HC-I	$AraXyl_2$	20.0	1	1		0	0
	$AraXyl_3$	32.6	0	2	0	0	3
	Xyl_2	0	0	0	0		0
	Xyl_3	0	0	0	0	0	
HC-II	$AraXyl_2$	0	0	0		0	0
	$AraXyl_3$	37.0	1	0	1	0	0
	Xyl_2	0	0	0	0		0
	Xyl_3	18.0	1	0	0	2	

[a]The figures represent the relative amounts of sugar present, as judged visually on paper chromatograms.

Methylation. — Methylation was carried out on the dried sugars by the procedure of Hakomori[19]. Aqueous solutions of $AraXyl_2$, Xyl_3, and $AraXyl_3$ (1 ml containing 5–10 mg) were reduced to their respective alditols with sodium borohydride (20–30 mg) for 3 h at 25°. Excess of borohydride was decomposed with glacial acetic acid, sodium ions were removed with Amberlite IR-120(H^+) resin, and the resulting solution was concentrated to dryness. Boric acid was removed as the volatile methyl borate by codistillation with methanol (5 × 20 ml).

Solutions of methylsulphinyl anion were prepared[20] by the addition of methyl sulphoxide to sodium hydride. Solutions of the base were prepared in advance, and stored frozen under nitrogen.

Xyl_2 and $AraXyl_2$, hemicellulose B, and the glycitols derived from $AraXyl_2$, Xyl_3, and $AraXyl_3$ were dissolved in methyl sulphoxide, and methylated as described by Conrad[20] and Garegg *et al.*[21]. The methylated products were hydrolysed[21], neutralised ($BaCO_3$), and deionised with mixed resins, and the products were fractionated by t.l.c. (solvent *G*).

Identification of the neutral components in hydrolysates of the methylated oligosaccharides. — The neutral components from the hydrolysis of methylated

hemicellulose B were used as reference standards (Table II) for identification (t.l.c.) of the components formed by hydrolysis of the methylated oligosaccharides. This was possible because the structure of hemicellulose B from spear grass has already been elucidated[22]. Methylated Xyl_2 yielded two components in approximately equal amounts, which co-chromatographed (t.l.c., solvent *G*) with 2,3,4-tri-*O*-methyl-D-xylose (R_{TMG} 1.02) and 2,3-di-*O*-methyl-D-xylose (R_{TMG} 0.77). Each component stained a reddish brown colour with spray *B*.

Methylated xylotriitol yielded two reducing components (spray *B*) in approximately equal amounts, which co-chromatographed with 2,3,4-tri-*O*-methyl-D-xylose and 2,3-di-*O*-methyl-D-xylose.

Methylated $AraXyl_2$ yielded three components in approximately equal amounts, which co-chromatographed with 2,3,5-tri-*O*-methyl-L-arabinose (R_{TMG} 1.04), 2,3-di-*O*-methyl-D-xylose, and 2,4-di-*O*-methyl-D-xylose (R_{TMG} 0.67). Each component stained a reddish brown colour with spray *B*.

The methylated glycitol of $AraXyl_2$ yielded two reducing components in approximately equal amounts, which co-chromatographed with 2,3,5-tri-*O*-methyl-L-arabinose and 2,4-di-*O*-methyl-D-xylose.

The methylated glycitol of $AraXyl_3$ yielded four components in approximately equal amounts, which co-chromatographed with 2,3,5-tri-*O*-methyl-L-arabinose, 2,3-di-*O*-methyl-D-xylose, 2,4-di-*O*-methyl-D-xylose, and 2,3,4,6-tetra-*O*-methyl-D-glucose. The last product arose from the methylated glucose disaccharide which was a contaminant of $AraXyl_3$. With spray *B*, the methylated aldopentoses gave a characteristic reddish brown colour, whereas the glucose derivative stained a yellow-brown colour.

RESULTS AND DISCUSSION

Enzymic hydrolysis of hemicellulose B (spear grass), using purified HC-II, yielded a series of mixed oligosaccharides which were identified, by co-chromatography with authentic sugars, as xylose, a mixed trisaccharide ($AraXyl_2$), glucose, xylobiose (Xyl_2), a mixed tetrasaccharide ($AraXyl_3$), a glucose disaccharide, and xylotriose (Xyl_3) as major products, in order of decreasing mobility in p.c. The glucose and the glucose disaccharide (which co-chromatographed with cellobiose) originated from a glucan constituent in the hemicellulose B (*cf.* ref. 13). This phenomenon has also been observed[25] in hemicellulose digests obtained by using cell-free enzyme extracts of *Epidinium ecaudatum* (rumen protozoan). D-Glucose, which migrated in the vicinity of $AraXyl_2$, was converted by D-glucose oxidase into D-gluconic acid, which was subsequently removed by ion-exchange chromatography. Four oligosaccharides were isolated from the hydrolysate, and tentatively identified by their optical rotations, by their behaviour in p.c. and t.l.c., and on the results of complete and partial acid hydrolysis and enzymic hydrolysis using HC-I and HC-II (see Table I). HC-I degraded $AraXyl_2$ and $AraXyl_3$, liberating arabinose, but did not attack the unbranched Xyl_2 and Xyl_3. HC-II degraded $AraXyl_3$ and Xyl_3, yielding $AraXyl_2$ and

xylose, and Xyl_2 and xylose, respectively, but did not further attack $AraXyl_2$ or Xyl_2. The results are in keeping with the specificity of these enzymes, as demonstrated[12,13] by their action on authentic samples of arabinose–xylose and xylose oligosaccharides.

Spear-grass hemicellulose B is an arabino-4-*O*-methylglucuronoxylan[22]. The methylated polysaccharide was therefore used as a source of reference methylated sugars (see Table II) in the methylation analysis of $AraXyl_2$, Xyl_2, $AraXyl_3$, and Xyl_3. Hydrolysis of methylated $AraXyl_2$ and its derived glycitol yielded 2,3,5-tri-*O*-

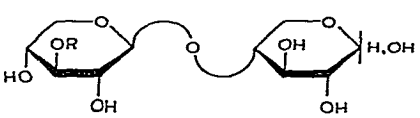
TABLE II

T.L.C. ANALYSIS OF THE NEUTRAL PRODUCTS IN THE HYDROLYSATE OF METHYLATED HEMICELLULOSE B

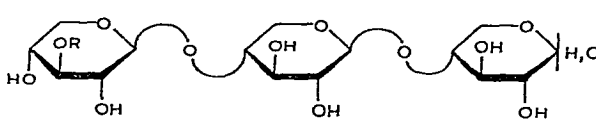
Hydrolysis product	R_F		R_{TMG}	
	Found	*Reported*[23a]	*Found*	*Reported*[23a]
2,3,5-Tri-*O*-methyl-L-arabinose	0.58	0.82	1.04	1.04
2,3,4-Tri-*O*-methyl-D-xylose	0.56	0.80	1.02	1.01
2,3-Di-*O*-methyl-D-xylose	0.43	0.58	0.77	0.63
3-*O*-Methyl-D-xylose	0.27	0.23	0.47	
2-*O*-Methyl-D-xylose	0.22	0.21	0.38	0.22
D-Xylose	0.05	0.06	0.08	0.08

[a]For methylated glycoses separated by p.c.

methyl-L-arabinose, 2,3- and 2,4-di-*O*-methyl-D-xylose, and 2,3,5-tri-*O*-methyl-L-arabinose and 2,4-di-*O*-methyl-D-xylose, respectively, as the reducing products. The formation of 2,3,5-tri-*O*-methyl-L-arabinose shows that the arabinose residue in $AraXyl_2$ is in the furanoid form. Since $AraXyl_2$ is hydrolysed by HC-I (see Table I), the arabinose is probably present as the L isomer and is linked α-(1→3) to xylose, since this enzyme hydrolyses[12] arabinose residues from wheat-endosperm arabinoxylan, which contains[26] α-L-arabinofuranosyl residues (1→3)-linked to the xylan backbone. The formation of 2,3- and 2,4-di-*O*-methyl-D-xylose from $AraXyl_2$ indicates that the xylose residues are (1→4)-linked and are branched at C-3, respectively. The negative rotation of $AraXyl_2$ confirms a preponderantly β-configuration for the D-xylose. The structure therefore assigned to $AraXyl_2$ is 3^2-α-L-arabinofuranosylxylobiose (**1b**). This trisaccharide has been characterised by Bishop[2], and Aspinall and co-workers[3].



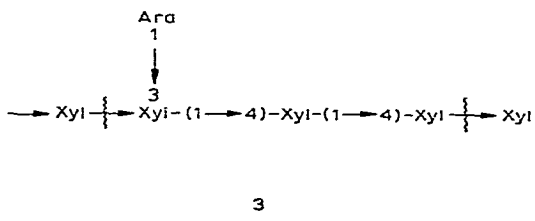
1a R = H
1b R = α-L-Araf



2a R = H
2b R = α-L-Araf

The formation of 2,3,4-tri-*O*-methyl-D-xylose and 2,3-di-*O*-methyl-D-xylose from Xyl_2 and xylotriitol, together with the optical roation data, indicates the presence of pyranoid units, β-(1→4)-linkages, and the absence of branching. The structures **1a** and **2a** are assigned to Xyl_2 and Xyl_3, respectively.

Methylation and hydrolysis of the glycitol from $AraXyl_3$ afforded 2,3,5-tri-*O*-methyl-L-arabinose, 2,4-di-*O*-methyl-D-xylose, and 2,3-di-*O*-methyl-D-xylose, in approximately equal proportions, as the only reducing sugars. Three possible structures can therefore be considered for $AraXyl_3$, which could arise from the cleavages by HC-II of the hemicellulose shown in **3**–**5** (*cf.* ref. 27).



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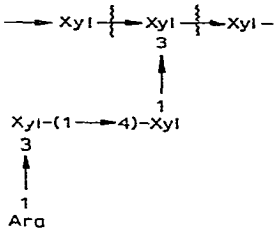

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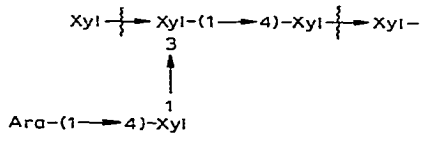

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$AraXyl_3$ was degraded with HC-I and HC-II from *C. paradoxa*. HC-I is an endo-enzyme, which hydrolyses both the β-(1→4)-D-xylopyranosyl and the α-(1→3)-L-arabinofuranosyl linkages of wheat-endosperm arabinoxylan[12], but does not attack linear β-(1→4)-linked D-xylose saccharides smaller than Xyl_4. HC-I should therefore attack $AraXyl_3$ from structures **3** and **4** (but not **5**), liberating arabinose and a xylose trisaccharide. The Xyl_3 liberated co-chromatographed with xylotriose (**2a**) (Table I), whereas the expected, branched Xyl_3 from **4** has [9] an R_{XYL} value different from that of **2a**. The endo-enzyme HC-II hydrolyses[13] β-(1→4)-D-xylopyranosyl linkages in hemicellulose B but not xylobiose (**1a**) or $AraXyl_2$ (**1b**), and should attack only $AraXyl_3$ from structure **3**, liberating xylose and the trisaccharide $AraXyl_2$. The products of enzymic hydrolysis of $AraXyl_3$ by HC-II co-chromatographed (Table I) with $AraXyl_2$ and xylose. It should be noted that both HC-I and HC-II are specific for β-(1→4)-D-xylopyranosyl linkages.

The foregoing data unequivocally show that $AraXyl_3$ is derived from **3**, and has structure **2b**. The tetrasaccharide $AraXyl_3$ (**2b**) is a new compound, and differs from the tetrasaccharides found by Goldschmid and Perlin[4] and by Takenishi and Tsujisaka[5]. The origin and structure of several arabinose–xylose oligosaccharides

isolated from the enzymic hydrolysates of various hemicelluloses are summarised in Table III.

TABLE III

STRUCTURE AND ORIGIN OF OLIGOSACCHARIDES OF D.P. 3–4 ISOLATED FROM THE ENZYMIC HYDROLYSATES OF VARIOUS HEMICELLULOSES

Enzyme and references	*Organism*	*Hemicellulose*	*Oligosaccharide structure*[a]	$[\alpha]_D$ (*degrees*)
Xylanase 1[5]	*Aspergillus niger*	Rice-straw arabinoxylan	3^1-α-L-Ara*f*-Xyl_2	−80
			3^1-α-L-Ara*f*-Xyl_3	−84
Cellulase[2]	*Myrothecium verrucaria*	Wheat-straw arabinoxylan	3^2-α-L-Ara*f*-Xyl_2	−19.3
"Hemicellulase"[3]	Commercial preparation	Cocksfoot-grass arabinoxylan	3^2-α-L-Ara*f*-Xyl_2	−14.9
		Rye-flour arabinoxylan	3^2-α-L-Ara*f*-Xyl_2	−15.3
Xylanase[4]	*Streptomyces* QMB-814	Wheat arabinoxylan	3^2-α-L-Ara*f*-Xyl_3	−75
Rumen cell-free enzymes[6]	Mixed bovine-rumen bacteria and protozoa	Spear-grass hemicellulose B	Unknown, but now suspected to be 3^3-α-L-Ara*f*-Xyl_3 (See Refs. 12 and 13)	−70
Hemicellulase II[b]	*Ceratocystis paradoxa*	Spear-grass hemicellulose B	3^2-α-L-Ara*f*-Xyl_2	−17.3
			3^3-α-L-Ara*f*-Xyl_3	−73.6

[a]For nomenclature, see Ref. 10. [b]This work.

The mobilities (p.c., t.l.c.) of $AraXyl_2$ and $AraXyl_3$ were different from those reported by the earlier workers[1,3,4]. Our arabinose–xylose oligosaccharides migrated ahead of the corresponding xylose oligosaccharides (see Table IV), in agreement with previous results[5–7]. The reverse behaviour was reported by the earlier workers[1,3,4] (see Tables IV and V).

TABLE IV

CHROMATOGRAPHIC DATA FOR $AraXyl_2$ AND Xyl_2

Oligosaccharide	R_{XYL}					
	Solvent A		*Solvent* B		*Solvent* C	
	Found	*Reported*[3]	*Found*	*Reported*[2]	*Found*	*Reported*[5,7]
$AraXyl_2$	0.78	0.40	1.024	0.46	0.83	0.75
Xyl_2	0.60	0.60	0.86	0.61	0.69	0.59

TABLE V

CHROMATOGRAPHIC DATA FOR OLIGOSACCHARIDES ARISING FROM THE ENZYMIC DEGRADATION OF HEMICELLULOSES

Oligosaccharides	R_{XYL}						
	Solvent A	A	A	A	B	C	H[b]
	Ref. 3	*4*	*6*	*This work*	*5,7*	*1*	*8*
XylAra	0.66	—	—	—	—	—	0.49
Xyl_2Ara	0.40	—	—	0.78	0.75	0.46	0.19
Xyl_2	0.60	1[a]	0.58	0.60	0.59	0.61	0.41
Xyl_3Ara	—	3	0.39	0.34	0.41	0.34	—
Xyl_3	0.30	2	0.30	0.31	0.28	—	0.15
Xyl_4Ara	—	4	0.22	0.22	0.20	—	—
Xyl_4	0.14	—	0.15	0.14	—	—	—
Xyl_5Ara	—	5	0.12	0.11	0.08	0.16	—

[a]Order of decreasing chromatographic mobility; no R_{XYL} values reported. [b]1-Butanol–pyridine–water (10:3:3).

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