Note

Isolation and Identification of a New Feruloylated Tetrasaccharide from Bagasse Lignin-Carbohydrate Complex Containing Phenolic Acid

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Problems concerning the mode of linkages between phenolic acids and carbohydrates in gramineous plant cell walls have attracted considerable interest in recent years.^{1,2)} We have demonstrated that ferulic and *p*-coumaric acids released by alkaline treatment of sugar-cane bagasse are esterified to the different molecular species, carbohydrate and lignin moieties, respectively,³⁾ and isolated a feruloylated trisaccharide, *O*-[5-*O*-(*trans*-feruloyl)- α -L-arabinofuranosyl]-(1 $\rightarrow$ 3)-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 4)-D-xylopyranose, from the enzymatic hydrolysate of the bagasse lignin-carbohydrate complex (LCC).⁴⁾ Recently, the same feruloylated trisaccharide has been isolated from *Zea mays* shoots,⁵⁾ barley aleurone layers⁶⁾ and straw,⁷⁾ and wheat bran.⁸⁾ In this paper, we extend the previous studies on the phenolic acid-carbohydrate linkages in bagasse LCC and isolated a new feruloylated tetrasaccharide **1** and its tetrasaccharide fragment **2**.

Water-soluble LCC (LCC-W)³⁾ isolated from depecti-

nated milled sugar-cane bagasse was hydrolyzed with purified Cellulase Onozuka RS (Yakult Pharmaceutical Industry, Co., Ltd.).⁴⁾ The water-soluble material was passed through Dowex 50X8 (H⁺) and extracted twice with chloroform. The aqueous layer was concentrated to a small volume and poured into 5 volumes of ethanol. The ethanol-soluble material was then put on a column of Sephadex LH-20 (57.0 $\times$ 6.0 cm) which was eluted with distilled water. The two fractions having *K_{av}* values of 1.94 and 3.37 contained predominantly feruloylated oligosaccharides. The former fraction contained the feruloylated trisaccharide the structure of which has been previously elucidated.⁴⁾ The latter fraction contained one major feruloylated oligosaccharide having an *R_f* value of 0.38 on TLC using 1-butanol-acetic acid-water (62:15:23, v/v) with slight contamination by compounds having *R_f* values of 0.41 and 0.54. The major compound was purified by preparative TLC and gel filtration on Sephadex G-10 (60.0 $\times$ 1.0 cm) using 50% aqueous dioxane as an eluent to give **1**; yield, 0.28% of the original LCC-W.

Chemical compositional and secondary ion mass spectroscopic data (Table I) indicate that **1** is a pentamer which is composed of one mole of *trans*-ferulic acid, one mole of L-arabinose, and three moles of D-xylose. For analyzing the primary structure of its carbohydrate moiety, **1** was saponified by dilute alkali (0.1 M sodium hydroxide, 20°C, 12 hr) into **2**. The chemical composition and methylation analysis (Table I) indicate that **2** is a tetrasaccharide having L-arabinofuranose and one D-xylopyranose at the non-reducing end units. The remaining D-xylose units were recovered as 2,3-di-*O*- and 2-*O*-methyl D-xylopyranoses. Based on these results, the relative position of **2** could be deduced to be 3²-L-arabinofuranosyl-D-xylotriose or 4²-L-arabinofuranosyl-3¹-D-xylopyranosyl-D-xylobiose. To get further information about the sequence and the configuration of the glycosidic linkages of **2**, ¹H and ¹³C-NMR spectroscopic analyses were done. The ¹³C-NMR signals assigned according to the published data⁹⁾ (Table II) clearly indicated that three xylose residues are linked to each other through (1 $\rightarrow$ 4)-linkages and that an L-arabinofuranosyl res-

TABLE I. CHARACTERISTICS OF THE ISOLATED OLIGOMERS

Components	Oligomers		Methylated sugars ^d	Oligomers	
	1	2		1	2
Compositions ^a			2,3,5-Ara ^c		0.9
L-Arabinose	1.00	1.00	2,3-Ara	1.0	
D-Xylose	2.80	2.84	2,3,4-Xyl	0.8	0.9
<i>trans</i> -Ferulic acid	0.95		2,3-Xyl	1.0	1.0
			2-Xyl	0.9	1.1
M_w^b	722	546			
$\lambda_{\max}$ (water, nm)	325				

^a Values are expressed as a relative molar ratio.^b Measured by secondary ion mass spectra.^c 2,3,5-Ara = 2,3,5-tri-*O*-methylarabinose, etc.^d Values are expressed as a relative molar ratio.TABLE II. ¹³C AND ¹H-NMR DATA FOR THE ISOLATED OLIGOMERS

Oligomers	Residues	Carbon-13 chemical shifts (ppm) ^a and anomeric ¹ J _{C,H} coupling constants (Hz)										
		C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	OMe	
1	X ^α	92.9 (171.2)	71.8	72.2	77.5	59.7						
	X ^β	97.4 (161.1)	74.8	74.8	77.3	63.7						
	X'	102.5 (160.8)	73.9	81.6	74.7	63.8						
	X''	102.5 (160.8)	74.1	76.5	70.1	66.0						
	A	108.7 (174.7)	82.2	78.5	82.9	65.1						
	F	127.6	112.1	148.4	148.7	114.5	124.3	147.5	116.3	169.9	56.6	
2	X ^α	92.9 (170.1)	71.8	72.2	77.5	59.8						
	X ^β	97.4 (161.9)	74.8	74.9	77.4	63.7						
	X'	102.5 (163.2)	73.9	81.5	74.7	63.9						
	X''	102.5 (163.2)	74.2	76.5	70.1	66.0						
	A	108.5 (174.8)	81.5	78.1	85.7	62.2						
Oligomers		Proton chemical shifts (ppm) ^b and coupling constants (Hz)										
		X		X'	X''	A	F					
		H-1	H-1	H-1	H-1	H-1	H-2	H-5	H-6	H-7	H-8	OMe
1		5.19	4.59	4.50	4.46	5.41	7.25	6.90	7.14	7.66	6.42	3.88
		(3.6)	(7.8)	(7.9)	(7.6)	(1.0)	(1.8)	(8.1)	(1.8,8.0)	(16.1)	(16.1)	
	Integral	0.4	0.6	1	1	1	1	1	1	1	1	3
2		5.20	4.60	4.52	4.46	5.40						
		(3.6)	(7.8)	(7.6)	(7.8)	(1.0)						
	Integral	0.4	0.6	1	1	1						

^a In D₂O at 50.3 MHz and 23°C using 1,4-dioxane (67.40 ppm) as an internal standard.^b In D₂O at 200 MHz and 90°C using sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate as an internal standard.

idue is located at O-3 of the intermediate xylopyranosyl residue. The existence of the signal at 108.9 ppm showed that the anomeric configuration of the arabinofuranose residue was α . From the values of one-bond

¹³C-¹H coupling constants, the anomeric configuration of the glycosidic linkage of the D-xylopyranose residues was β . ¹H-NMR spectroscopic data completely confirmed the results of ¹³C-NMR and methylation analy-

ses (Tables I and II). Based on these results, the primary structure of **2** was *O*- β -D-xylopyranosyl-(1 $\rightarrow$ 4)-*O*-[α -L-arabinofuranosyl-(1 $\rightarrow$ 3)]-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 4)-D-xylopyranose. This structure is the same as that isolated from various plants in the family of Gramineae.¹⁰⁻¹²⁾

Next, the mode and point of attachment of the ferulic acid in **1** was analyzed. A comparison of the ¹³C-NMR spectrum of **1** with that of **2** showed appreciable differences in chemical shifts at the C-5 (+2.9 ppm) and C-4 (-2.8 ppm) of the L-arabinofuranosyl residue (Table II). Methylation analysis indicates the presence of 2,3-di-*O*-methyl-L-arabinose instead of 2,3,5-tri-*O*-methyl-L-arabinose (Table I). These results indicate that ferulic acid is linked at O-5 of the L-arabinofuranose. The mode of linkage between ferulic acid and L-arabinofuranose was deduced to be an ester linkage from the IR absorption band at 1710 cm⁻¹ (C=O). **1** also had IR absorption bands at 1620 cm⁻¹ (conjugated aliphatic C=C), 1600 cm⁻¹ and 1510 cm⁻¹ (aromatic C=C), and 890 cm⁻¹ (β -glycosidic linkage of D-xylopyranose residue). Assignments of the signals in ¹H-NMR spectrum of **1** (Table II) showed that ferulic acid has a *trans* configuration from the wide coupling constants of H-7 and H-8 (about 16 Hz). The ratio of signal intensities of anomeric protons of L-arabinofuranose and D-xylopyranose was 1:2, in accordance with the chemical compositional analysis (Table I). Based on these results, the primary structure of **1** was concluded to be *O*- β -D-xylopyranosyl-(1 $\rightarrow$ 4)-*O*-[5-*O*-(*trans*-feruloyl)- α -L-arabinofuranosyl-(1 $\rightarrow$ 3)]-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 4)-D-xylopyranose.

The structures of the bagasse feruloylated oligosaccharides presented in this and previous⁴⁾ papers indicate that the site of feruloylation in bagasse hemicellulose is exclusively the O-5 of L-arabinofuranosyl residue which is

attached to the (1 $\rightarrow$ 4)- β -linked D-xylan backbone at O-3. Further studies are underway to characterize the structure of the remaining feruloylated oligosaccharides produced by enzymolysis of bagasse LCC.

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