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A NEW FERULOYLATED TRISACCHARIDE FROM BAGASSE

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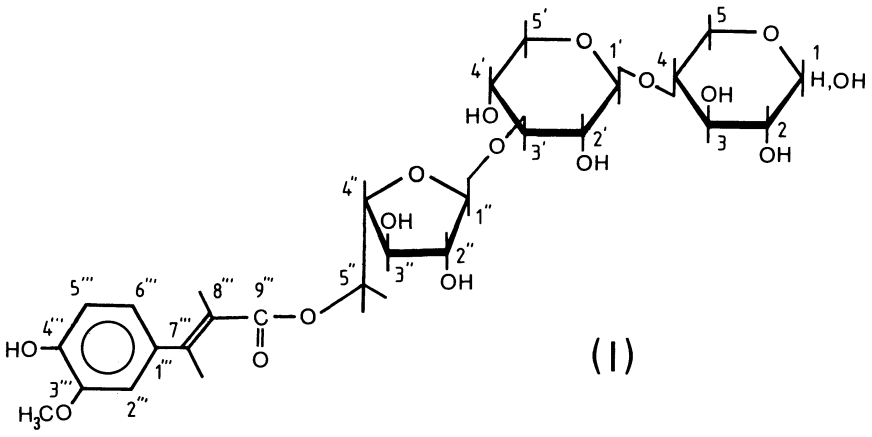
O-(5-O-Feruloyl-α-L-arabinofuranosyl)-(1→3)-O-β-D-xylopyranosyl-(1→4)-D-xylopyranose (I) was isolated from the enzymatic hydrolysate of bagasse lignin-carbohydrate complex containing ferulic acid (LCC-F) and identified from I.R., U.V., G.L.C.-M.S., and N.M.R. spectra.

Presence of ferulic acid esterified to polysaccharide was reported for the first time by Neukom and coworkers.[1] They have shown that ferulic acid is esterified to about one pentose residue in 50, in the arabinoxylans of wheat flour.[2] Since then, similar ferulic acid-carbohydrate complexes have been detected in a wide variety of plants cell-walls including graminae,[3-5] other monocotyledons,[6] dicotyledons[7,8] and grain seeds.[9,10] However, the exact nature of the chemical linkages between ferulic acid and carbohydrate is not fully elucidated. Fry has recently isolated two feruloylated disaccharides of D-galactose and L-arabinose from the primary cell wall of the dicotyledons spinach and deduced their structures as 4-O-(6-O-feruloyl-β-D-galactopyranosyl)-D-galactose and 3-O-(3-O-feruloyl-α-L-arabinopyranosyl)-L-arabinose.[8]

In the course of our studies on the lignin-carbohydrate linkages, a new feruloylated trisaccharide was observed as an enzymatic decomposition product of bagasse lignin-carbohydrate complex containing ferulic acid (LCC-F). The isolation and identification of the compound confirmed the structure to be O-(5-O-feruloyl-α-L-arabinofuranosyl)-(1→3)-O-β-D-xylopyranosyl-(1→4)-D-xylopyranose (I).

LCC-F was isolated from sugar cane bagasse obtained from Daiichi sugar (Mill) Co., Okinawa, as previously described for pine lignin-carbohydrate complex.[11] In this case the product was further purified by reextraction with chloroform to remove lignin rich fraction. The reextraction procedure gave a final yield of 5.8% of the original bagasse. The composition of LCC-F was 0.6% of ferulic acid, 9.0% of acetyl, 24% of Klason lignin, 2.1% of uronic acid and 62.9% of neutral sugar in which L-arabinose and D-xylose are contained in the molar ratio of 1 : 9.

LCC-F was treated with cellulase from *Trichoderma viride* (Onozuka R-10, Yakult Pharmaceutical Industry Co., Ltd., Nishinomiya) in 0.1M sodium acetate buffer (pH 4.6) at substrate and enzyme concentrations of 1.0% and 0.1%, respectively, under toluene atmosphere. The enzyme preparation was purified by gel filtration on Sephadex G-50 to remove lactose added as an extender and lower molecular weight phenolic contaminants. Absence of esterase activity in the enzyme preparation was verified by using chlorogenic acid as a substrate.[12] The precipitate formed



(I)

Table 1. Chemical properties of compound (I)

Chemical composition[a] (molar proportion)		Methylated sugars[b] (molar proportion)	
L-Arabinose	20.7 (1)[e]	2,3-Di-O-methyl-arabinitol	(1)[e]
D-Xylose	44.4 (2)	2,4-Di-O-methyl-xylitol	(1)
trans-Ferulic acid	34.9 (1)	2,3-Di-O-methyl-xylitol	(1)

Molecular weight[c]	λ^{d}_{max} (nm)	R^{1}_{f}	R^{2}_{f}
600 [590.5]	323 ((325))	0.54	0.33

[a]G.L.C. analysis.[11,19] [b]G.L.C.-M.S. analysis.[11,20] [c]Vapour pressure measurement and theoretical value in square brancket. [d]In 80% (v/v) aqueous 1,4-dioxane and trans-ferulic acid in double parenthesis. [e]Molar ratio in parentheses. R^{1}_{f}: Mobility in the solvent; 1-butanol : acetic acid : water = 62 : 15 : 23 (v/v). R^{2}_{f}: Mobility in the solvent; acetone : ethyl acetate : water = 10 : 10 : 1 (v/v).

during enzymatic treatment was removed by centrifugation. The supernatant was passed through a Dowex 50W-X8 (H^{+}) column, lyophilized (yield, 73.3% of the original LCC-F), and subjected to gel filtration on Toyopearl HW-40SF (Toyo soda Co.) using 50% (v/v) aqueous 1,4-dioxane as an eluent. There was overlapping of the two major peaks (K_{av} = 0.48 and 0.57) due to ferulic acid and carbohydrate. The fraction having K_{av} value of 0.57 was further separated into two peaks (K_{av} = 1.99 and 2.66) by column chromatography on Sephadex LH-60 using water as an eluent.

Table 2. ^{1}H-N.M.R. Data for compound (I)[a]

δ[b]	$J_{1,2}$ (Hz)	Integral proton	Assignment[c]
7.14 (d)[d]	1.8	1	H-2"'
6.86 (d)	8.0	1	H-5"'
7.07 (dd)	1.8, 8.0	1	H-6"'
7.56 (d)	16.0	1	H-7"'
6.34 (d)	16.0	1	H-8"'
3.90 (s)		3	O-CH_3
5.26 (d)	1.2	1	H-1"
5.18 (d)	3.6	0.4	H-1α
4.59 (d)	7.4	0.6	H-1β
4.47 (d)	7.0	1	H-1'

[a]In D_2O at 200 MHz and 90°C. [b]In p.p.m. downfield from T.S.P. (sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate) as internal standard. [c]For the source of protons, see (I). [d]Values in parentheses represent the multiplicities of signals.

Table 3. ^{13}C-N.M.R. Data for compound (I)[a]

δ[b]	Assignment[c]	δ[b]	Assignment[c]	δ[b]	Assignment[c]
127.68 (-0.13)[d]	C-1"'	109.01 (+0.13)[d]	C-1"	92.84 (-0.32)[d]	C-1α
112.17 (-0.91)	C-2"'	[172.4][e]		[168.9][e]	
148.38 (-0.25)	C-3"'	81.96 (+0.23)	C-2"	97.33 (-0.10)	C-1β
148.74 (-0.03)	C-4"'	77.67 (-0.17)	C-3"	[161.2]	
114.40 (-1.32)	C-5"'	82.27 (-2.42)	C-4"	71.79 (-0.13)	C-2α
124.18 (+0.23)	C-6"'	68.58 (+6.55)	C-5"	74.80 (-0.06)	C-2β
147.29 (-0.23)	C-7"'	102.55 (-0.08)	C-1'	72.22 (-0.11)	C-3α
116.41 (-0.30)	C-8"'	[160.5]		74.80 (-0.15)	C-3β
178.50 (+7.02)	C-9"'	73.61 (-0.02)	C-2'	77.33 (-0.06)	C-4α
56.72 (-0.45)	O-CH_3	82.65 (+6.70)	C-3'	77.33 (-0.02)	C-4β
		70.10 (-0.03)	C-4'	59.69 (-0.32)	C-5α
		65.84 (-0.07)	C-5'	63.82 (-0.10)	C-5β

[a]In D_2O at 50.3 MHz and 80°C. [b]In p.p.m. relative to internal 1,4-dioxane (67.40 p.p.m. from T.M.S.). [c]For the source of carbons, see (I). [d]The values in parentheses represent the differences of the shifts from those of trans-ferulic acid, methyl α-L-arabinofuranoside, and 4-O-β-D-xylopyranosyl-D-xylose. [e]$J_{C\text{-}1,H\text{-}1}$ values (in Hz) in square branckets.

The fraction having K_{av} value of 2.66 was purified by rechromatography on the same Sephadex LH-60 column to obtain compound (I) in yield of 0.39% of the original LCC-F. On the basis of its chemical properties (Table 1), ^{1}H- and ^{13}C-N.M.R. spectral data (Tables 2 and 3), the structure of compound (I) is O-(5-O-feruloyl-α-L-arabinofuranosyl)-(1→3)-O-β-D-xylopyranosyl-(1→4)-D-xylopyranose. Although ^{1}H-N.M.R. data did not give decisive information about the nature of arabinose residue in (I),[13] with ^{13}C-N.M.R. spectra, it is possible to deduce the ring size, anomeric configuration and ester linked, hydroxyl group of the arabinose residue in (I), confirming the results of methylation analysis (Table 1). Existence of ester carbonyl and conjugated aliphatic olefinic bond are also confirmed by I.R. absorptions at 1715 cm^{-1}, and 1620 cm^{-1}, respectively. The trisaccharide portion of (I) (O-α-L-arabinofuranosyl-(1→3)-O-β-D-xylopyranosyl-(1→4)-D-xylopyranose) was the same as those previously isolated.[14-18] The whole structure of (I) is however completely different from the feruloylated oligosaccharides isolated by Fry.[8] He suggested that these ferulates are attached to pectin. Our present results demonstrate that ferulic acid is directly attached to hemicellulose in bagasse, suggesting the presence of similar ferulic acid-carbohydrate linkage in the feruloylated arabinoxylan previously isolated from wheat flour.[2] The frequency of the esterified ferulic acid is estimated to be one in 15 arabinofuranose residues, in the original LCC-F.

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