Structures of Ferulic Acid Glycoside Esters in Corn Hulls

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1D-NMR (^{1}H and ^{13}C) and 2D-NMR spectroscopy as well as chemical means have been used to determine the structures of three new feruloylated disaccharides derived from acid hydrolysis of corn hulls. The structures of these oligosaccharides were determined to be *O*-(2′-*O*-*trans*-feruloyl-α-L-arabinofuranosyl)-(1→3)-*β*−D-xylopyranose, *O*-[2′-*O*-methoxyl-5′-*O*-(*E*)-feruloyl]-α-L-arabinofuranosyl-(1→3)-*β*-D-xylopyranose, and *O*-[2′-*O*-methoxyl-5′-*O*-(*Z*)-feruloyl]-α-L-arabinofuranosyl-(1→3)-D-*β*-xylopyranose.

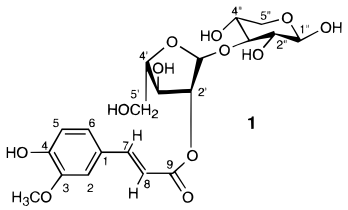
The common phenolic compounds *trans*-ferulic ((*E*)-4-hydroxy-3-methoxycinnamic acid) and *trans*-*p*-coumaric acid ((*E*)-4-hydroxycinnamic acid) are found commonly linked to plant cell-wall components, including lignins, suberins, and oligosaccharides.[1−3] The structures and specific linkages of phenolic acids to plant cell components have received considerable attention because of their role in plant tissue growth and biodegradation.[3,4] Linkages between cinnamic acids and cell-wall polysaccharides are well known in monocotyledons, but are less well known among dicotyledonous plants.[5−12] In monocotyledons, feruloyl and *p*-coumaroyl esters of disaccharides, trisaccharides, tetrasaccharides, and oligosaccharides are known.[13−20] They have been identified, for example, in wheat bran,[13,21] sugar cane bagasse,[14,22,23] maize bran,[15,24] barley,[16] barley straw,[25,26] bamboo shoots,[19,27,28] coastal Bermuda grass shoots,[29] sugar beet pulp,[30] and *Cynodon dactylon*.[5]

In the cell walls of graminaceous plants, phenolic acids are usually esterified at position C-5 to a single α-L-arabinofuranosyl residue. The α-L-arabinofuranosyl residue is, in turn, glycosidically linked to a *β*-D-xylopyranosyl residue that is part of a larger linear xylan chain.[13,14,17,22,26,27,29]

Corn hulls obtained from the wet milling of maize, *Zea mays* (*Gramineae*), are an abundant source of ferulic acid,[31,32] representing as much as 2−4% by dry weight of the hulls.[33] This source alone could provide more than 1 billion pounds per year of ferulic acid from corn hulls, part of the product stream obtained during the corn wet milling process. We are developing biocatalytic approaches to convert abundant and renewable chemical resources, such as ferulic acid, into value-added chemical products.[31] Ferulic acid is readily converted into several value-added chemical products including 4-hydroxy-3-methoxystyrene by enzymatic decarboxylation, vanillic acid by *β*-oxidative removal of the cinnamoyl side chain, and guaiacol by decarboxylation of vanillic acid.[31] This study was undertaken in order to further understand the nature of ferulic acid as it exists within corn hulls.

Results and Discussion

An acidic−EtOH extract of corn hulls (50 g) was fractionated by solvent partitioning and a combination of Si gel column chromatography, followed by further purification through RP-18 flash column chromatography, to yield three new compounds (**1**−**3**).



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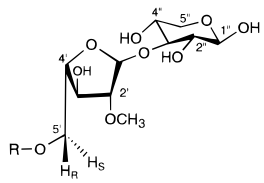

2 R = *trans*-feruloyl
3 R = *cis*-feruloyl

Compound **1** gave UV (bathochromic shifts with NaOH) and IR spectra suggesting the presence of an oxygenated cinnamic acid moiety as a part of the structure. The positive ion HRFABMS molecular ion at *m*/*z* 459.3710 and fragment ions at *m*/*z* 309 and 177 indicated a molecular formula of $C_{20}O_{12}H_{26}$ containing a ferulic acid moiety linked to two pentoses. ^{1}H- and ^{13}C-NMR spectra confirmed the presence of *trans*-ferulic acid with doublets at *δ* 6.32 and 7.57 ($J_{7,8}$ = 16 Hz) with signals for one methoxyl group and three aromatic protons in a typical ABX system. NMR spectra also displayed aromatic sugar proton (*δ* 109.69 and 98.90) signals. Exhaustive 5 M HCl hydrolysis of **1** afforded ferulic acid, arabinose, and xylose, which were identified by TLC and/or PC.

A comparison of the anomeric shifts and coupling constants for the arabinose residue in **1** and methyl α and *β*-arabinofuranoside[34−36] indicated that the ring is in the furanose form and that it is α-linked to xylose. The chemical shift of H-2′ of the arabinofuranosyl residue is about 1 ppm higher than that normally expected for an unsubstituted arabinofuranosyl unit (*δ* 4.04),[34] indicating that the feruloyl group is located at O-2′ of the arabinose moiety. In the ^{13}C-NMR spectrum, the arabinofuranosyl C-2′ was deshielded by *δ* 1.77, compared to the usual chemical shift of *δ* 1.80 for an unsubstituted arabinofuranosyl unit,[37] indicating that the linkage of ferulic acid is at this position. The

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resonance of the arabinofuranosyl C-1 at δ 109.69 indicated that the nonreducing arabinofuranosyl residue is α-linked to the reducing xylopyranoside. For the α and β anomers of this residue, the signals for C-3″ were shifted downfield by 0.35 ppm as compared with the corresponding proton signal of free xylose (δ 3.42).[38] This indicated that the nonreducing xylopyranoside is 3-substituted. The position of this linkage was unambiguously assigned by acetylation analysis as described below.

Heteronuclear $^1H-^{13}C$ correlation spectroscopy (HMBC) allowed for complete assignment of all protons and carbons, including those of the disaccharide residue. In this way the feruloyl carbonyl−carbon signal C-9 at δ 169.83 was correlated with the proton signal at the site of esterification, H-2′ (δ 5.14) of the arabinofuranosyl unit. HMBC also established that the reducing terminal xylopyranosyl unit is linked to the arabinofuranosyl unit at the 3-position because this carbon resonated at δ 82.88 in the ^{13}C-NMR spectrum. Permethylation by Hakomori's method[39] and subsequent acid hydrolysis yielded only 2,4-di-*O*-methyl-β-D-xylose as a totally methylated sugar, derived from a 3-linked xylopyranosyl residue, indicating that it was a terminal sugar linked to arabinose. Acetylation of **1** in the usual way gave hexaacetate **1a**. The ^1H-NMR spectrum of **1a** revealed the presence of 6 Ac signals belonging to one aromatic (δ 2.29) and five aliphatic (δ 2.08, 2.01, 1.99, 1.92, and 1.76) Ac groups. No downfield shift was observed for H-3 of xylose (d, 3.34, J = 9.6 Hz), indicating this to be the site of the interglycosidic linkage. The EIMS spectrum of **1a** exhibited an $[M]^+$ ion peak at *m/z* 694 ($C_{32}H_{38}O_{17}$), with a major fragment at *m/z* 219 (4-*O*-acetyl-feruloyl)$^+$ and the characteristic fragment ion peak at *m/z* 259 (terminal tri-*O*-acetylxylose)$^+$. These ions indicated that compound **1** is a diglycoside of ferulic acid and that terminal sugar is xylose. Compound **1** was thus established as *O*-(2′-*O*-*trans*-feruloyl-α-L-arabinofuranosyl)-(1→3)-β-D-xylopyranose.

As with **1**, FABMS, IR, UV, and NMR spectra all confirmed the presence of a *trans*-ferulic acid moiety in the structure of compound **2.** The FABMS gave a molecular ion of *m/z* 488 compatible with $C_{21}H_{28}O_{13}$ composed of one ferulic acid, one methoxyl, and two pentose residues. By HMBC, the ferulic acid carboxyl carbon (C-9) was correlated with one of the H-5′ sugar protons indicating esterification between ferulate and C-5′ of the L-arabinofuranosyl moiety.[40]

The xylopyranosyl anomeric position possesses a β-hydroxyl group, as indicated by the coupling constant of the anomeric proton (δ 4.59, d, J = 7.8 Hz, H = 1″). The $^1H-^{13}C$ long-range correlation (HMBC) from each anomeric proton confirmed the sugar sequence. In this HMBC spectrum, the methine proton signals at δ 3.61 established that the reducing terminal xylopyranosyl unit was linked to the arabinofuranosyl unit through the 3-position. The assignment of xylose as a terminal sugar was indicated from partial acid hydrolysis, which yielded xylose and gave an EIMS fragment at *m/z* 354 $[M - \text{xylose} - H]^-$. The signals of C-2′ and C-5′ of the arabinofuranosyl residues at δ 90.65 (C-2′) and δ 66.50 (C-5′), and the absence of a signal at δ 62.40 corresponding to C-5 of the unsubstituted arabinofuranosyl residue,[37] indicated that ferulic acid was linked to O-5′ of the arabinofuranosyl residue. This was also confirmed from the one-bond C−H correlation experiment.

The presence of signals at δ 108.73 and δ 97.55 suggested that the anomeric hydroxyl groups of the arabinofuranosyl and xylopyranose residues are α and β, respectively, as indicated by the coupling constants of the corresponding anomeric protons. ^1H- and ^{13}C-NMR spectra showed the presence of an additional methoxyl group per molecule. The 2D-NMR $^1H-^{13}C$ (HMBC) spectrum showed that the methoxyl group was located at C-2′ (δ 90.65) of the arabinofuranosyl residue. Correlation of the ^1H-NMR spectrum with the ^{13}C-NMR spectrum by selective ^1H decoupling showed unambiguously that the methoxyl group was at C-2′.

These assignments were further confirmed by ^1H-NMR spectroscopy, which served to show that the arabinofuranosyl C-5′ and C-2′ were strongly deshielded by δ 1.25 and 2.77, indicating the linkage of ferulic and methoxyl groups at these positions, respectively. A comparison of the anomeric shifts and coupling constants for the arabinose residue in **2** and methyl α and β arabinofuranosyl[34−36] indicate that the ring is in the furanose form and α-linked to xylose.

The reducing xylose residue was substituted at O-3; H-3 was deshielded relative to the corresponding proton in free xylose on comparison with the literature (0.19 ppm).[38] The hydroxyl groups at 1′, 2′, and 5′ of arabinose are substituted based upon the insignificant ^1H-NMR signal shifts that occurred upon peracetylation of **2** to **2a**. The xylose moiety was substituted at position-3 as indicated by the lower-field shift of xylose protons except for that at C-3 upon peracetylation. The chemical shift of the arabinose H-2′ at δ 5.48 indicated the attachment of the methoxyl group at C-2′. Both protons at C-2′ and C-5′ are only slightly shifted (δ 5.48 to 5.56 and δ 4.89 to 4.96, respectively, upon peracetylation. The primary structure of **2** is thus established as *O*-[2′-*O*-methoxyl-5′-*O*-(*E*)-feruloyl]-α-L-arabinofuranosyl-(1→3)-β-D-xylopyranose.

The $[M]^+$ ion peak at *m/z* 488 for $C_{21}H_{28}O_{13}$ suggested that the structure of **3** was similar to **2**. It showed UV absorptions at 215 and 272 nm and IR bands for hydroxyl groups, ester carbonyl, and an aromatic ring. The ^{13}C-NMR spectrum of **3** exhibited chemical shifts from which straightforward assignments could be made. The ^{13}C-NMR spectrum of **3** was very similar to **2**, except for the C-2, C-5, C-6, and C-7 signals of their acyl units**.** Moreover, the ^{13}C chemical shifts for the carbons due to the arabinoxyloyl moiety of **3** are quite similar to those of **2**. The ^1H-NMR spectral data of **3** were also closely correlated with that of **2** with respect to the presence of the oligomer moiety. For **3**, coupled olefinic signals and an aromatic ABX signal pattern for H-2, H-5, and H-6 are readily discerned together with a methoxyl signal at δ 3.89. A careful study of the ^1H-NMR spectrum of **3** indicated the configuration of the double bond of the acyl unit; the corresponding olefinic protons of **3** were located upfield (observed δ 6.85 and 5.81) from the positions of the *trans*-protons of **2** (observed δ 7.38 and 6.46). The smaller coupling constants of these protons (J = 13.1 Hz)[41] in **3** led us to conclude that **3** has a *cis*-configuration at the olefinic double bond of the feruloyl moiety and indicated that the *trans*-feruloyl group in **2** is replaced by a *cis*-feruloyl group in **3**. On the basis of these observations, the

structure of **3** is established as *O*-[2′-*O*-methoxyl-5′-*O*-(*Z*)-feruloyl]-α-L-arabinofuranosyl-(1→3)-β-D-xylopyranose.

Experimental Section

General Experimental Procedures. Flash column liquid chromatography was performed using J. T. Baker glassware with 40 μm Si gel (Baker) and Sepralyte C18, 40 μm as the stationary adsorbent phase. TLC used Si gel 60 F_{245} (Merck) plates; column chromatography, Si gel 60. Ferulated arabinoxylans were detected by 1% vanillin−H_2SO_4, followed by heating at 100 °C for 5 min. UV spectra (λ_{max}) were determined on a Hitachi 340 spectrophotometer. IR spectra (cm^{-1}) were recorded on a Jasco A-202 spectrophtometer. ^{1}H- and ^{13}C-NMR spectra were obtained with a Bruker WM 360 spectrometer operating at 360.13 and 90.56 MHz, respectively. All spectra were obtained in CD_3OD and $CDCl_3$ using TMS as internal standard, with the chemical shifts expressed in δ (ppm) and the coupling constants (*J*) in Hz. Heteronuclear multiple-bond correlation (HMBC) NMR experiments were obtained on a Bruker AMX-600 high-field spectrometer equipped with an IBM Aspect-2000 processor and with software VNMR version 4.1, using TMS as internal standard in appropriate solvents. Fast atom bombardment (FAB) experiments were performed on a VG-ZAB-HF reversed-geometry (BE configuration, where B is a magnetic sector and E is an electrostatic analyzer) mass spectrometer (MS) (VG Analytical, Inc.).

Plant Material. Corn seed hulls of *Zea mays* were provided by Archer Daniels Midland, Inc., Clinton, IA.

Extraction and Isolation. Corn seed hulls (50 g) were dispersed in 2 N H_2SO_4 in EtOH (95%) (3 × 1 L) and the mixture was kept in a boiling H_2O bath at 75 °C for 24 h. The acidified EtOH extracts were concentrated *in vacuo* at 40 °C. The concentrated oily extract was dissolved in H_2O (100 mL) and filtered through Celite. The filtrate and washings were combined and washed with petroleum ether. The concentrated defatted crude extract (6.2 g) was chromatographed on a Si gel column (2.5 × 90 cm), eluting with a CH_2Cl_2−MeOH gradient (8:2−7:3 v/v). Three fractions were collected (A−C). The second fraction (940 mg) was further purified by flash column chromatography (1.5 × 50 cm, Sepralyte-C18, 40 μm) using a mobile phase gradient of MeOH in H_2O (5→35%) to afford compounds **1** (187 mg) and **2** (76 mg). Fraction C (785 mg) was rechromatographed over a Si gel column with $CHCl_3$−MeOH−H_2O (90:10:1−70:30:3) (v/v/v) to yield a mixture of compound **2** (66 mg) and crude **3** (98 mg), which was further purified by flash column chromatography (1.5 × 50 cm, Sepralyte-C18, 40 μm), using H_2O−MeOH mixtures, and gel filtration (Sephadex LH-20, 1.5 × 50 cm) eluted with MeOH, to give pure compound **3** (18 mg) in addition to compound **2** (23 mg).

Compound 1: amorphous white powder; IR (KBr) 3460 cm^{-1} (OH), 1695 cm^{-1} (C=O) 1630 cm^{-1} (C=C), 1590 cm^{-1} (aromatic ring); UV (λ_{max} MeOH) 230 and 279 nm; ^{1}H NMR (CD_3OD, 360 MHz) δ, feruloyl moiety: 7.57 (d, *J* = 16.0 Hz, H-7), 7.18 (d, *J* = 1.8 Hz, H-2), 7.10 (dd, *J* = 8.1/1.8 Hz, H-6), 6.88 (d, *J* = 8.1 Hz, H-5), 6.32 (d, *J* = 16.0 Hz, H-8), 3.78 (s, OCH_3); α-L-arabinofuranose moiety 5.42 (d, *J* = 1.8 Hz, H-1′), 5.14 (d, *J* = 3.4 Hz, H-2′), 4.43 (d, *J* = 3.6 Hz, H-4′), 4.19 (d, *J* = 5.6 Hz, H-3′), 3.98 (dd, *J* = 5.5/11.5 Hz, H-5′$_B$), 3.77 (d, *J* = 5.7 Hz, H-5′$_A$); β-D-xylopyranose moiety 4.49 (d, *J* = 7.8 Hz, H-1″), 4.02 (d, *J* =11.5 Hz, H-5″$_B$), 3.77 (d, *J* = 9.5 Hz, H-3″), 3.65 (d, *J* = 5.5 Hz, H-4″), 3.52 (d, *J* = 9.5 Hz, H-2″), 3.48 (d, *J* = 9.6 Hz, H-5″$_A$); ^{13}C NMR (CD_3OD, 100 MHz) δ, feruloyl moiety 169.83 (s, C-9), 148.78 (s, C-4), 148.18 (s, C-3), 148.14 (d, C-7), 127.57 (s, C-1), 124.48 (d, C-6), 116.06 (d, C-5), 115.00 (d, C-8), 111.90 (d, C-2), 56.90 (s, OCH_3); α-L-arabinofuranose moiety 109.69 (d, C-1′), 83.87 (d, C-4′), 83.57 (d, C-2′), 77.25 (d, C-3′), 63.18 (t, C-5′); β-D-xylopyranose moiety 98.90 (d, C-1″), 73.80 (d, C-2″), 82.88 (d, C-3″), 67.93 (d, C-4″), 63.87 (t, C-5″); positive ion HRFABMS, *m/z* 481.3701 $[M + Na]^+$, 459.3710 $[M + H]^+$, 177 [M − feruloyl]$^+$ $C_{20}H_{26}O_{12}$.

Compound 2: amorphous white powder; IR (KBr) 3460 cm^{-1} (OH), 1695 cm^{-1} (C=O) 1630 cm^{-1} (C=C), 1590 cm^{-1} (aromatic ring); UV (λ_{max} MeOH) 228 and 284; ^{1}H NMR (CD_3OD, 360 MHz) δ, feruloyl moiety 7.38 (d, *J* = 16.5 Hz, H-7), 7.21 (d, *J* = 2.0 Hz, H-2), 6.92 (dd, *J* = 2.0/8.0 Hz, H-6), 6.79 (d, *J* = 8.0 Hz, H-5), 6.46 (d, *J*=16.5 Hz, H-8), 3.73 (s, OCH_3); α-L-arabinofuranose moiety 5.67 (d, *J* = 1.5 Hz, H-1′), 5.48 (d, *J* = 3.4 Hz, H-2′), 4.89 (d, *J* = 12.0 Hz, H-5′$_B$), 4.58 (d, *J* = 3.5 Hz, H-4′), 4.48 (d, *J* = 6.0 Hz, H-5′$_A$), 4.25 (d, *J* = 6.1 Hz, H-3′), 3.90 (s, OCH_3); β-D-xylopyranose moiety 4.59 (d, *J* = 7.8 Hz, H-1″), 4.00 (d, *J* = 11.2 Hz, H-5″$_B$), 3.69 (d, *J* = 5.5 Hz, H-4″), 3.61 (d, *J* = 9.5 Hz, H-3″), 3.41 (d, *J* = 9.5 Hz, H-2″), 3.35 (d, *J* = 9.4 Hz, H-5″$_A$); ^{13}C NMR (CD_3OD, 100 MHz) δ, feruloyl moiety 169.00 (s, C-9), 149.35 (s, C-4), 148.95 (s, C-3), 147.50 (d, C-7), 127.20 (s, C-1), 123.85 (d, C-6), 115.90 (d, C-5), 114.08 (d, C-8), 112.02 (d, C-2), 56.12 (s, OCH_3); α-L-arabinofuranose moiety 108.73 (d, C-1′), 90.65 (d, C-2′), 83.29 (d, C-4′), 78.30 (d, C-3′), 66.50 (t, C-5′), 57.15 (s, OCH_3); β-D-xylopyranose moiety 97.55 (d, C-1″), 83.05 (d, C-3″), 72.48 (d, C-2″), 68.20 (d, C-4″), 65.21 (t, C-5″); positive ion FABMS, M^+ 488, *m/z* 511 $[M + Na]^+$, 177 [M − feruloyl]$^+$ $C_{21}H_{28}O_{13}$.

Compound 3: amorphous white powder; IR (KBr) 3460 cm^{-1} (OH), 1695 cm^{-1} (C=O), 1630 cm^{-1} (C=C), 1590 cm^{-1} (aromatic ring); UV (λ_{max} MeOH) 215 and 272 nm; ^{1}H NMR (CD_3OD, 360 MHz) δ, feruloyl moiety 7.76 (d, *J* = 2.0 Hz, H-2), 7.13 (dd, *J* = 8.3/2.0 Hz, H-6), 6.85 (d, *J* = 13.1 Hz, H-7), 6.78 (d, *J* = 8.3 Hz, H-5), 5.81 (d, *J* = 13.1 Hz, H-8), 3.77 (s, OCH_3); α-L-arabinofuranose moiety 5.58 (d, *J*= 1.5 Hz, H-1′), 5.52 (d, *J* = 3.3 Hz, H-2′), 4.94 (d, *J* = 12.3 Hz, H-5′$_B$), 4.55 (d, *J* = 3.3 Hz, H-4′), 4.52 (d, *J* = 6.0 Hz, H-5′$_A$), 4.37 (d, *J* = 6.0 Hz, H-3′), 3.89 (s, OCH_3); β-D-xylopyranose moiety 4.54 (d, *J* = 7.8 Hz, H-1″), 4.10 (d, *J* = 11.5 Hz, H-5″$_B$), 3.59 (d, *J* = 9.5 Hz, H-3″), 3.58 (d, *J* = 5.5 Hz, H-4″), 3.56 (d, *J* =9.5 Hz, H-5″$_A$), 3.47 (d, *J* = 9.5 Hz, H-2″); ^{13}C NMR (CD_3OD, 100 MHz) δ, feruloyl moiety 168.50 (s, C-9), 149.43 (s, C-4), 148.00 (s, C-3), 145.60 (d, C-7), 128.24 (s, C-1), 126.55 (d, C-6), 117.62 (d, C-5), 115.11 (d, C-2), 116.70 (d, C-8), 56.53 (s, OCH_3); α-L-arabinofuranose moiety 108.36 (d, C-1′), 91.17 (d, C-2′), 82.27 (d, C-4′), 78.48 (d, C-3′), 66.32 (t, C-5′), 57.70 (s, OCH_3); β-D-xylopyranose moiety 97.23 (d, C-1″), 82.56 (d, C-3″), 73.07 (d, C-2″), 67.59 (d, C-4″), 65.10 (t, C-5″); positive ion FABMS, M^+ 488 *m/z* 511 $[M + Na]^+$, 177 [M − feruloyl]$^+$ $C_{21}H_{28}O_{13}$.

Acetylation of Compounds 1 and 2. Treatment of **1** (15 mg) and **2** (20 mg) separately with Ac_2O (1 mL) and pyridine (1 mL) at room temperature overnight, followed by column chromatography over Si gel using

C_6H_6−Me_2CO (9:1−8:2) gave hexaacetate **1a** and pentaacetate **2a**, respectively.

Compound 1 hexaacetate (1a): IR (KBr) cm^{-1}, 1730 (C═O), 1620 (C═C) and 1490 (arom ring); ^1H NMR (600 MHz, $CDCl_3$) feruloyl moiety 7.08 (1H, d, J = 1.8 Hz, H-2), 7.03 (1H, d, J = 8.5 Hz, H-5), 7.14 (1H, dd, J = 8.5, 1.8 Hz, H-6), 7.72 and 6.30 (each 1H, d, J = 15.9 Hz, H-7 and H-8, respectively), **3.89 (3H, s, OCH_3)**; arabinose moiety 5.35 (1H, d, J = 2.0 Hz, H-1′), 5.18 (1H, d, J = 3.5 Hz, H-2′), 5.04 (1H, d, J = 5.5 Hz, H-3′), 5.26 (1H, d, J = 3.5 Hz, H-4′), 4.47 (1H, dd, J = 12.3, 3.5 Hz, H-5′$_A$), 4.15 (dd, J = 12.3, 5.7 Hz, H-5′$_B$); xylose moiety 4.97 (1H, d, J = 7.6 Hz, H-1″), 4.91 (1H, dd, J = 7.6, 9.3 Hz, H-2″), 3.34 (1H, d, J = 9.3 Hz, H-3″), 4.88 (1H, dd, J = 9.3, 5.1 Hz, H-4″), 3.96 (1H, dd, J = 11.9, 9.0 Hz, H-5″$_A$), 4.23 (1H, dd, J = 11.9, 5.1 Hz, H-5″$_B$) 2.29 (1, CH_3COO, arom) and 2.08, 2.01, 1.99, 1.92, 1.76 (5 × CH_3COO, aliph); EIMS, m/z 694 $(M)^+$, calcd for $C_{32}O_{17}H_{38}$, 259 [triacetyl − xylose]$^+$ and 219 [4-*O*-acetylferuloyl]$^+$.

Compound 2 pentaacetate (2a): IR (KBr) cm^{-1}, 1740 (C═O), 1630 (C═C) and 1490 (arom ring); ^1H NMR (600 MHz, $CDCl_3$), feruloyl moiety 7.03 (1H, d, J = 1.8 Hz, H-2), 7.05 (1H, d, J = 8.3 Hz, H-5), 7.11 (1H, dd, J = 8.3, 1.8 Hz, H-6), 7.78 and 6.42 (each 1H, d, J = 16.0 Hz, H-7 and H-8, respectively), 3.79 and 3.88 (each, 3H, s, 2 OCH_3); arabinose moiety 5.72 (1H, d, J = 1.8 Hz, H-1′) 5.56 (1H, d, J = 3.5 Hz, H-2′), 5.12 (1H, d, J = 5.7 Hz, H-3′), 5.29 (1H, d, J = 3.5 Hz, H-4′), 4.59 (1H, d, J = 6.0 Hz, H-5′A), 4.96 (1H, d, J = 12.0 Hz, H-5′$_B$); xylose moiety 4.97 (1H, d, J = 7.6 Hz, H-1″), 4.91 (1H, dd, J = 7.6, 9.3 Hz, H-2″), 3.88 (1H, d, J = 9.3 Hz, H-3″), 4.88 (1H, dd, J = 9.3, 5.1 Hz, H-4″), 3.96 (1H, dd, J = 11.9, 9.0 Hz, H-5″$_A$), 4.23 (1H, dd, J = 11.9, 5.1 Hz, H-5″$_B$), 2.22 (1, CH_3COO, arom) and 2.06, 2.01, 1.95, 1.79 (4 × CH_3COO, aliph); EIMS, m/z 694 $(M)^+$, calcd for $C_{32}O_{17}H_{38}$, 259 [triacetyl − xylose]$^+$ and 219 [4-*O*-acetylferuloyl]$^+$.

Acid Hydrolysis of 1. Compound **1** (20 mg) was dissolved in 5% HCl and heated at 100 °C for 2 h, cooled, and filtered. The filtrate was neutralized by passing it through Dowex #1 (Cl^- form) and evaporating to dryness. The residue was examined for sugars by TLC using EtOAc−AcOH−MeOH−H_2O (60:15:15:10 v/v/v/v) and PC (descending method) using *n*-BuOH−pyridine−H_2O (9:5:4 v/v/v).

Methylation of 1. Compound **1** (10 mg) was methylated by the Hakomori method[39] using NaH (1 g) in DMSO (8 mL), and after stirring the solution at room temperature for 30 min under N_2, MeI (2 mL) was added, and stirring was continued for a further 1 h in the dark. The reaction mixture was poured into iced H_2O and extracted with Et_2O (3 × 50 mL). The combined Et_2O extract was washed with H_2O and then was concentrated under reduced pressure. The methylated products were dissolved in 5 mL 5% HCl in MeOH and heated at 100 °C. After 2 h, 5 mL H_2O was added and the mixture reheated for an additional 1 h. After removing MeOH, the H_2O phase was extracted with $CHCl_3$ (3 × 5 mL). The combined $CHCl_3$ extracts were dried (Na_2SO_4), concentrated, and subjected to TLC using C_6H_6−MeCO (2:1) and C_6H_6−EtOH (4:1). Identification of methylated sugars was made by comparison with authentic samples. 2,3,4-Tri-*O*-methylarabinofuranosyl and 2,4-di-*O*-methylxylopyranoside were identified for **1**.

Partial Acid Hydrolysis of Compound 2. Hydrolysis of compound **2** (5 mg) in refluxing 2 M TFA and filtration of the neutral aqueous phase through an alumina cartridge afforded xylose, which was identified by TLC using Me_2CO−H_2O (9:1); EIMS m/z 354 [M − xylose − H]$^+$.

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