

Synthesis and spectroscopic characterization of hydroxycinnamoylated methyl α -L-arabinofuranosyl-(1 \rightarrow 2)- and (1 \rightarrow 3)- β -D-xylopyranosides

Richard F. Helm ^a and John Ralph ^b

^a USDA-Agricultural Research Service, US Dairy Forage Research Center 1925 Linden Drive, Madison, WI 53706 (USA)

^b Affiliated with the Department of Forestry, University of Wisconsin-Madison, Madison, WI 53706 (USA)

ABSTRACT

A reaction sequence for the preparation of methyl 5-*O*-feruloyl- α -L-arabinofuranosyl-(1 \rightarrow 3)- β -D-xylopyranoside, the companion 5-*O*-*p*-coumaroyl disaccharide, and their (1 \rightarrow 2) analogs has been developed. The (1 \rightarrow 3) hydroxycinnamoylated disaccharides are available in 11 steps from L-arabinose and methyl β -D-xylopyranoside in 17% overall yield (based on methyl β -D-xylopyranoside). The corresponding (1 \rightarrow 2) materials were prepared in 9 steps in > 37% overall yield. Complete spectral characterization provides unambiguous assignments for comparison with analogous materials isolated from plant cell-walls. Conformational aspects of the prepared materials are discussed in relation to coupling-constant information.

INTRODUCTION

The role of ferulic (4-hydroxy-3-methoxy-*trans*-cinnamic) and *p*-coumaric (4-hydroxy-*trans*-cinnamic) acids in the cell-walls of forage grasses is not well understood. The primary site of attachment to polysaccharides is via esterification through the 5-position of α -L-arabinofuranosyl units of arabinoxylans¹⁻⁵, and strong evidence has been presented⁶⁻¹¹ for both the esterification and etherification to lignin. The coupling of lignin to polysaccharide through hydroxycinnamic acids has been proposed as a mechanism by which cell-wall degradation in ruminants is inhibited^{12,13}. The controlled attachment and detachment of polysaccharide chains via dehydrodiferulic acid has been suggested as a mechanism by which cell-wall expansions is moderated¹⁴, while hydroxycinnamoylated polysaccharides have been implicated as the initial site of lignification¹⁵. Thus the

Correspondence to (present address): Dr. R.F. Helm, Department of Wood Science and Forest Products, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA.

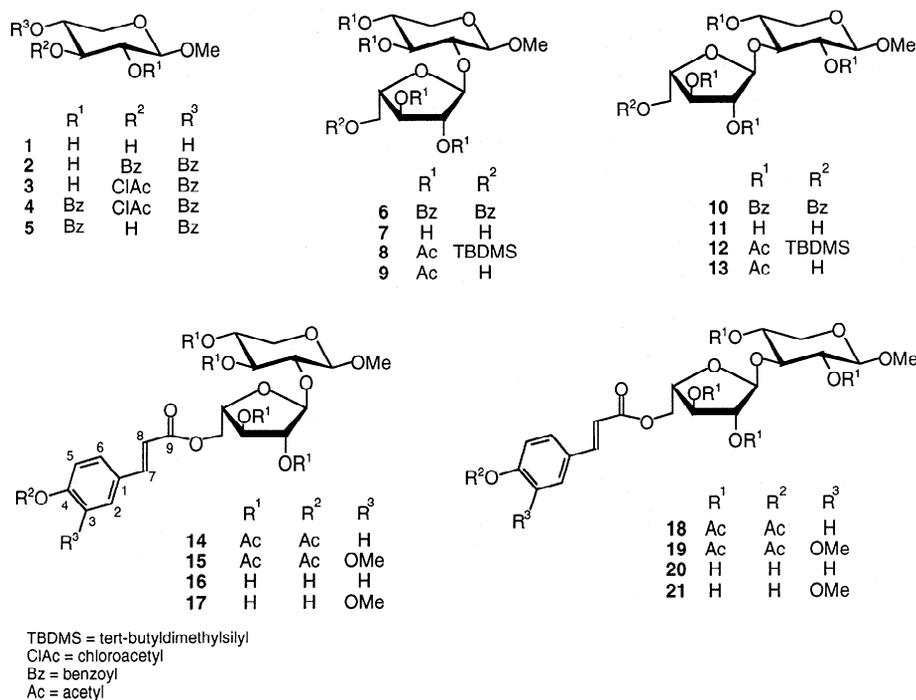
presence of these cross-links, either by dehydrodiferulic acid or a lignin–hydroxycinnamic acid–polysaccharide bridge, would appear to have a definite impact on the three-dimensional nature of the forage grass cell-wall. Covalent bonds that cannot be cleaved by esterases or hydrolases, either due to spatial concerns or the type of linkage (biphenyl or aryl ether, for example) would provide regions of restricted access where utilizable substrates cannot be accessed by degradative enzymes. The recent isolation of a diferul-9,9'-dioyl-linked hexasaccharide¹⁶ as well as the incorporation of methyl 5-*O*-feruloyl- α -L-arabinofuranoside into synthetic lignin preparations by classical free-radical mechanisms¹⁷ do indeed suggest that hydroxycinnamic acids are important structural components of the forage grass walls.

The use of NMR spectroscopy for the determination of the regiochemistry of hydroxycinnamic acid attachment to lignin and polysaccharide in native cell-wall isolates necessitates the synthesis of suitable models for spectral characterization and optimization of correlation experiments. Our previous synthetic endeavors were directed at the preparation of hydroxycinnamoylated methyl glycosides^{18,19} with the acylation site being the primary hydroxyl position. These materials served as substrates for the assessment of esterase specificity²⁰, and methyl 5-*O*-([9'-¹³C]feruloyl)- α -L-arabinofuranoside with coniferyl alcohol was used as a substrate for the incorporation into synthetic DHP lignins¹⁷. The synthetic transformations developed in these studies, in concert with the recently described strategy for the regioselective protection of *D*-xylopyranosides²¹, allowed for the preparation and subsequent spectral characterization of several hydroxycinnamoylated disaccharides. These most recent efforts are the subject of this report.

RESULTS AND DISCUSSION

Synthetic aspects.—The key to the preparation of target compounds **16**, **17**, **20**, and **21** was to prepare the unprotected disaccharides **7** and **11**. Since these disaccharides contain only one primary hydroxyl, selective manipulation of this hydroxyl according to the strategy developed¹⁸ for the selective acylation of methyl α -L-arabinofuranoside was envisaged. Methyl 2,3,5-tri-*O*-benzoyl- α -L-arabinofuranoside was reacted with SnCl₄-dichloromethyl methyl ether in dichloromethane to afford the crystalline α -chloride (82%). Methyl β -*D*-xylopyranoside (**1**) was converted to the 3,4-dibenzoate **2** in one step via dibutyltin oxide-mediated acylation²¹, and glycosylation of **2** and the chloride with silver trifluoromethanesulfonate (silver triflate) and 2,4,6-trimethylpyridine (collidine) gave **6** in 95% yield with a minimum of excess halide (1.1 equiv). Debenzylation (NaOMe, 92%) gave **7** as an amorphous material in 87.5% yield from **1**.

Preparation of the *D*-xylopyranosyl unit of (1 \rightarrow 3)-linked disaccharide **11** was achieved by benzylation and subsequent dechloroacetylation of methyl 4-*O*-benzoyl-3-*O*-chloroacetyl- β -*D*-xylopyranoside (**3**)²¹, which is available from **1** in one step (54% crystalline yield) via dibutyltin oxide-mediated acylation. Benzylation



Scheme 1.

of **3** with *N,N*-(diisopropyl)ethylamine–4-(dimethylamino)pyridine–benzoyl chloride in dichloromethane afforded **4** as a crystalline material in 86% yield without silica gel chromatography. Acylation with benzoyl chloride–pyridine provided a much lower yield of **4**, presumably due to the well-known ability of pyridine to form quaternized compounds with chloroacetate groups²². Dechloroacetylation was accomplished with thiourea in 10:1 absolute ethanol–pyridine at 50°C in almost quantitative yield (95–98%). Glycosylation (silver triflate–collidine) gave **10** in 92% yield, which upon debenzoylation gave crystalline **11**.

The protocol for the preparation of **7** and **11** is an improvement on previous schemes^{23–25} in that nucleophiles **2** and **5** were prepared in one and three steps, respectively, from **1**. The improved yield and stability of 2,3,5-tri-*O*-benzoyl- α -L-arabinofuranosyl chloride relative to the analogous bromide is also an advantage in that the crystalline chloride can be stored for long periods without the degradation. Only minor amounts of the presumed β -L-linked disaccharides (< 2%) were detected in the ¹H spectra of the crude reaction products when glycosylation reactions were performed on a large scale (~ 5 g).

Silylation of the 5-positions of the L-arabinofuranosyl moieties of **7** and **11** with *tert*-butylchlorodimethylsilane occurred cleanly with pyridine as the solvent/base¹⁸. The subsequent addition of acetic anhydride provided **8** and **12** which were isolable by silica gel chromatography. *O*-Desilylation with aq 80% acetic acid gave

the crystalline acetates **9** and **13**. The entire sequence can be performed without purification of the intermediate compounds (**8** and **12**) in the same overall yield (76% from **7** and 74% from **11**). Coupling of **9** and **13** with the appropriate 4-acetoxycinnamoyl chloride in pyridine gave the peracetates **14**, **15**, **18**, and **19** in > 88% yield. The final deprotection was accomplished by treatment with ethanolic pyrrolidine which, after workup, gave **16**, **17**, **20**, and **21** in yields of up to 87%. Neutralization of the ethanolic pyrrolidine solution via passage through a column of strongly acidic ion-exchange resin [Amberlite IRA-120 (H^+)] had to be conducted at 0–4°C to avoid cleavage of the glycosidic linkage, a reaction which occurred extensively at room temperature. This facile acid-catalyzed cleavage of the α -L-arabinofuranosyl linkage is well established²⁶ and emphasizes the need for mild conditions when isolating native arabinoxylans from plant tissue of manipulating synthetic preparations.

Interestingly, while the hydroxycinnamoylated (1 → 2)-linked peracetates (**14** and **15**) were deacetylated in less than 48 h, complete deacetylation of the (1 → 3)-disaccharides (**18** and **19**) required up to 6 days. UV monitoring of the reaction by TLC indicated that there was the rapid elimination of several of the acetate groups, and after 48 h two spots remained. The slower material was that of the desired product, while the slightly faster-migrating material was presumably (for **19**) methyl 5-*O*-feruloyl- α -L-arabinofuranosyl-(1 → 3)-2-*O*-acetyl- β -D-xylopyranoside. Unfortunately the longer reaction time needed for complete deacetylation afforded hydroxycinnamoyl cleavage and thus a lower yield (62–65%) relative to the (1 → 2)-linked materials (78–87%).

Spectroscopic aspects.—Acylated D-xylopyranoside derivative can undergo significant conformational changes in nonpolar aprotic solvents^{27,28}. The phenomenon typically depends on acylate location^{29,30}, and this can often lead to difficulty in assigning anomeric configuration³¹. Our routine analysis of intermediate products led us to discover that **5** has a tremendous solvent dependence with respect to the favored conformation. For example, the $J_{1,2}$ values for **5** in several solvents are as follows: acetone- d_6 , 6.70 Hz; $CDCl_3$; 3.2 Hz; benzene- d_6 , 2.90 Hz. This compares to a $J_{1,2}$ of 3.55 Hz for methyl 2,4-di-*O*-benzoyl- α -D-xylopyranoside²¹ in benzene- d_6 . Calculations based on the work of Petráková and Schraml ($J_{1,2} = 7.1 P + 1$; where $P = {}^4C_1$ population)²⁹ indicate that the 4C_1 conformation is favored for acetone- d_6 (80%) although in benzene- d_6 this conformer only contributes 27% to the averaged structure. Mixtures of benzene- d_6 (or $CDCl_3$) and acetone- d_6 have intermediate $J_{1,2}$ values depending on solvent polarity.

This behavior with pentopyranosyl halides has been attributed to the strong anomeric effect associated with the halide^{27,32}. The methyl aglycon of **5** is also under the influence of the anomeric effect, although to a lesser degree, and this could be the driving force for the molecule to favor the 1C_4 conformation even in the presence of unfavorable 1,3-diaxial interactions. It has also been suggested that preference for the 1C_4 conformation is due to the strength of a “hydrogen bond” which develops between the HO-3 and the methyl aglycon³⁰. Qualitative one-di-

dimensional NOE experiments with **5** in acetone- d_6 reveal the typical H-1–H-3 and H-1–H-5 $_{ax}$ enhancements indicative of the 4C_1 conformer, whereas no enhancement effects were observed in $CDCl_3$. That the C-5 protons do not change their relative positions upon changes in solvent polarity suggests that in nonpolar aprotic solvents (benzene- d_6 and $CDCl_3$) the upfield proton is in the axial position. This is in contrast to the typical situation for D-xylopyranosides where H-5 $_{eq}$ is downfield with respect to H-5 $_{ax}$. The combined results of standard and long-range COSY experiments performed on **5** in benzene- d_6 are shown in Fig. 1. There are several 4J “W-couplings,” namely H-1–H-3, H-2–H-4, H-3–H-5, and H-3–H-5'. That the H-1 and H-3 protons do not have 4J couplings to the same H-5 proton as well as the H-1–H-4 interaction indicate that the averaged conformation is slightly more complicated than a straightforward ${}^1C_4 \leftrightarrow {}^4C_1$ transformation.

The ${}^{13}C$ NMR data for the disaccharides prepared in this study are shown in Table I. The appropriate inverse-detected one-bond (HMQC, phase-sensitive) and long-range (HMBC) correlation experiments confirmed all assignments. The re-

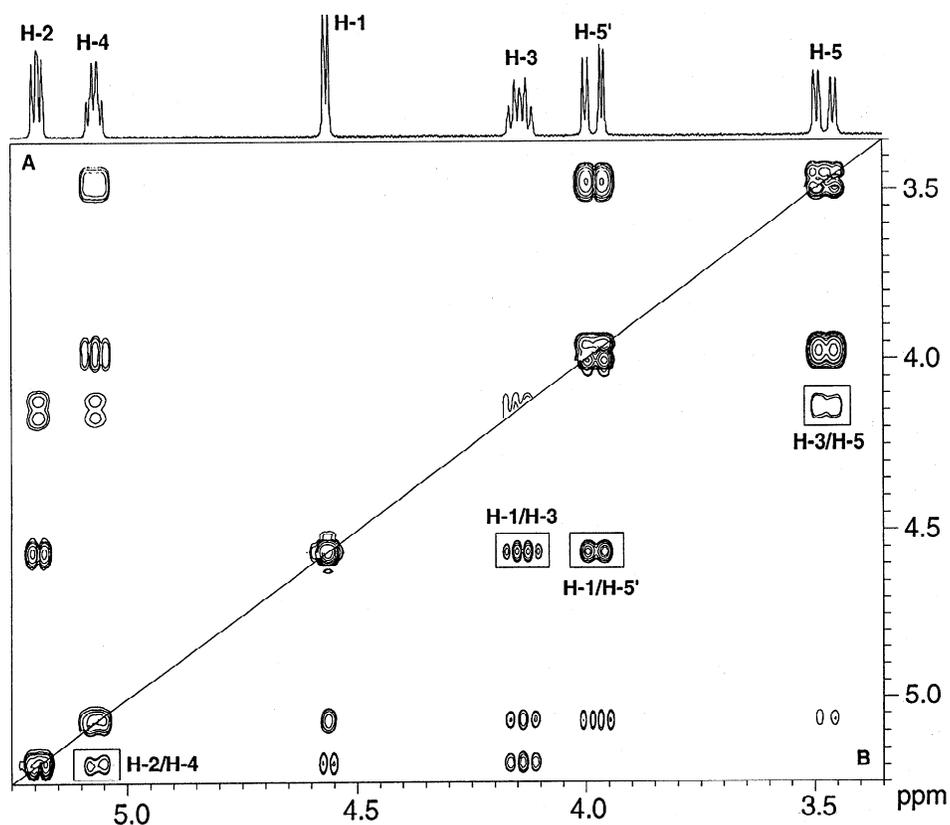


Fig. 1. 1H - 1H COSY spectra of **5** in benzene- d_6 (the aromatic and methoxyl regions are not shown). A, COSY90; B, long-range COSY (delay, 225 ms). The important long-range correlations are indicated. The spectra were not symmetrized.

TABLE I

¹³C NMR chemical shift data ^a for selected disaccharides

Compound ^b	Chemical shift (ppm)									
	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	OCH ₃
6 A	106.9	83.2	78.5	81.9	64.4					
	X	103.7	75.7	74.5	71.2	62.7				56.9
7 A	106.2	78.9	74.7 ^c	82.4	59.2					
	X	100.8	75.9 ^c	73.5	67.0	62.8				55.1
8 A	106.7	82.7	77.5	84.0	63.1					
	X	103.9	75.6	73.9	70.4	62.8				56.8
9 A	106.7	82.6	77.7	84.5	62.2					
	X	104.0	75.6	73.8	70.4	62.8				56.8
10 A	107.4	82.9	78.3	82.3	63.9					
	X	102.7	74.0	77.2	70.9	62.9				56.6
11 A	106.2	79.1	74.4	82.0	59.2					
	X	101.8	70.9	79.8	65.8	62.9				55.2
12 A	107.4	82.4	77.4	84.2	63.3					
	X	102.8	73.1	78.1	70.6	63.1				56.4
13 A	107.4	82.3	77.6	84.5	62.2					
	X	102.8	73.2	78.1	70.6	63.1				56.5
14 A	106.7	82.1	78.0	81.8	64.0					
	X	103.9	75.8	74.0	70.4	62.9				56.9
	C	132.8	130.2	123.3	153.5	123.3	130.2	144.8	118.7	166.6
15 A	106.6	82.0	77.9	81.8	64.0					
	X	103.9	75.7	73.9	70.4	62.9				56.9
	F	134.1	112.4	152.7	142.7	124.1	122.2	145.2	118.8	166.7
16 A	106.4	79.0	75.2	79.8	62.8					
	X	100.9	76.3	73.5	67.0	62.1				55.0
	C	124.2	128.5	113.9	156.3	113.9	128.5	144.3	111.7	167.0
17 ^d A	106.4	79.0	75.2	79.8	62.1					
	(2.5)	(2.7)	(2.4)	(3.0)	(2.8)					
	X	100.9	76.3	73.5	67.0	62.8				55.0
17 F	(3.1)	(1.1)	(3.4)	(3.2)	(3.1)					(1.7)
	124.6	109.1	145.5	145.9	113.4	121.3	144.4	111.7	167.0	53.7
	(2.2)	(2.2)	(3.2)	(4.0)	(2.6)	(2.4)	(2.0)	(3.1)	(1.0)	(2.4)
18 A	107.5	81.8	78.5	82.0	64.0					
	X	102.8	73.1	77.8	70.6	63.1				56.6
	C	132.8	130.2	123.3	153.6	123.3	130.2	144.9	118.6	166.6
19 A	107.5	81.8	77.7	82.0	64.0					
	X	102.8	73.1	78.4	70.6	63.1				56.5
	F	134.1	112.4	152.7	142.8	124.2	122.2	145.3	118.7	166.7
20 A	106.3	79.1	74.8	79.4	61.8					
	X	101.8	70.8	80.2	65.8	62.9				55.2
	C	124.3	128.5	113.9	156.2	113.9	128.5	144.3	111.7	167.1

TABLE I (continued)

Compound ^b	Chemical shift (ppm)									
	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	OCH ₃
21 A	106.3	79.1	74.8	79.3	61.9					
X	101.8	70.7	80.4	65.8	62.9					55.2
F	124.6	109.1	145.5	146.0	113.4	121.4	144.5	111.6	166.9	53.7

^a Recorded at 27°C. Compounds **6**, **8**, **9**, **10**, **12**, **13**, **14**, **15**, **18**, and **19** were in acetone-*d*₆ and referenced to the central solvent peak (29.8 ppm). Compounds **7**, **11**, **16**, **17**, **20**, **21** were in D₂O and referenced to internal acetone (2 μL/mL, 28.225 ppm). ^b A, α-L-arabinofuranosyl; X, β-D-xylopyranosyl; C, *p*-coumaroyl; F, feruloyl. ^c Reversal of values reported in original preparation (ref. 25). ^d Values in parentheses represent the chemical shift differences between 4:1 acetone-*d*₆-D₂O (referenced to the acetone-*d*₆ central peak at 29.8 ppm) and D₂O (4:1 values-D₂O values).

sults compare favorably with those obtained with the hydroxycinnamoylated methyl α-L-arabinofuranosides¹⁸. The C-2 and C-3 carbons of the D-xylopyranosyl moieties of the (1 → 2) and (1 → 3) disaccharides display standard glycosylation shifts, which allows for their facile differentiation. Comparison of the carbon assignments for the hydroxycinnamoylated (1 → 3) disaccharides **20** and **21** with the analogous carbons from hydroxycinnamoylated arabinoxylans¹⁻⁵ and arabinoxylan oligosaccharides³³ isolated from native tissue is somewhat difficult owing to the use of different references and temperatures. Most of the differences can be attributed to the choice of reference, as the chemical-shift differences are generally constant. However, it should be noted that there is a fairly strong solvent effect as can be seen in Table I, compound **17**, where the differences in chemical shifts between 4:1 acetone-*d*₆-D₂O and D₂O are shown. Although there is a standard difference of ~2.2 ppm due to the choice of internal standard, some of the differences cannot be explained solely by the choice of reference. For example, the F4 and X3 carbons differ by 4.0 and 3.4 ppm, respectively; whereas the X2 carbons differ by only 1.1 ppm. Thus the assignment of the carbon chemical shifts of hydroxycinnamoylated arabinoxylan oligosaccharides is tenuous if unambiguous C-H correlation data are not available for the particular solvent used.

Conformational aspects.—Considerable effort has been made in determining the extent to which ¹J_{C-H} data can be used for obtaining conformational information on pyranosidic oligosaccharides³⁴⁻³⁶. These coupling constants are much easier to obtain than the three- and four-bond constants, and empirical relationships have been developed for carbohydrate systems³⁴⁻³⁶. The ¹J_{C-H} values for several disaccharides prepared in this investigation are shown in Table II. The X1 J_{C-H} values were from 162 Hz (**11**) to 167 Hz (**20**) and the X2 and X3 J_{C-H} values were within the range of 145.6–148.3 Hz. These values suggest that the conformation of the D-xylopyranosyl moiety is somewhat insensitive to the position of L-arabinofuranosyl substitution. This is corroborated by the ¹H NMR data shown in Table III as the D-xylopyranosyl ³J_{H-H} coupling constants are also quite similar for the (1 → 2)- and (1 → 3)-linked disaccharides. The ³J_{1,2} proton values in combina-

TABLE II

 $^1J_{C-H}$ values ^a for selected disaccharides in D₂O at 27°C

Carbon ^b	Compound					
	7	11	16	17	20	21
A1	175.9	175.7	176.0	175.8	176.0	175.8
A2	152.2	~151	151.3	151.4	151.5	151.6
A3	148.0	147.1	148.2	147.4	149.0	148.3
X1	163.6	162.1	166.9	163.1	164.2	166.4
X2	146.8	148.3	146.7	~148	146.9	147.0
X3	147.2	145.6	146.6	147.1	147.1	147.8

^a Values are within ± 0.3 Hz. ^b A, α -L-arabinofuranosyl; X, β -D-xylopyranosyl. The number refers to the carbon position.

tion with the J_{gem} and $J_{4,5}$ values indicate a D-xylopyranosyl unit in a 4C_1 conformation, with the unprotected (1 \rightarrow 3) disaccharides in D₂O (**11**, **17**, and **21**) showing a slight preference for the 4C_1 conformer relative to their companion (1 \rightarrow 2) disaccharides (**7**, **16**, and **20**).

The use of the $^1J_{C-H}$ coupling constants for furanosides has not been established due to the rapid interconversion of the furanosyl ring between various envelope and twist forms³⁷. The A1 J_{C-H} values were in the range of 175.7–176.0 Hz (Table II), which compares to an A1 J_{C-H} value of 173.0 Hz for methyl α -L-arabinofuranoside. This difference is probably a manifestation of the anomeric effect associated with the quasial axial directing affect of the methoxyl and/or the stereochemical effect of the D-xylopyranosyl moiety. That there is very little difference between the other L-arabinofuranosyl $^1J_{C-H}$ couplings indicates that furanosidic $^1J_{C-H}$ values are not useful in determining favored conformations^{38,39}.

There is, however, information concerning the general conformational aspects of the L-arabinofuranosyl ring within the 1H NMR data of Table III. The H-5 *proR* and *proS* assignments are based on the deuterium exchange studies of Serianni et al.⁴⁰. It can be readily seen that the L-arabinofuranosyl ring $^3J_{H-H}$ coupling constants change depending on substitution and linkage position, suggesting slightly different pseudorotational itineraries. The conformational aspects of arabinoxylan oligosaccharides have recently been described⁴¹. A fast equilibrium between two favored conformations (N- and S-types) was described for both (1 \rightarrow 3)- and (1 \rightarrow 2)-linked α -L-arabinofuranosyl residues. The (1 \rightarrow 3)-linked furanosyl rings favored equal amounts of the $^4E-^4T_0$ (N) and $^1T_2-^2E$ (S) conformers (Fig. 2). The (1 \rightarrow 2)-linked L-arabinofuranosyl residue (which had a vicinal α -L-arabinofuranosyl moiety attached at the D-xylopyranosyl O-3 position) favors $^4T_3-^4E$ (N) and $^1E-^1T_2$ (S) conformers.

Comparison of the ring proton coupling constants reported by Hoffmann et al.⁴¹ with the disaccharides (**7** and **11**) and hydroxycinnamoylated disaccharides (**16**, **17**, **20**, and **21**) reveal that the (1 \rightarrow 2)-linked materials prepared in this work (**7**, **16**, and **20**) have couplings constants quite similar to the (1 \rightarrow 3)-linked furanosyl

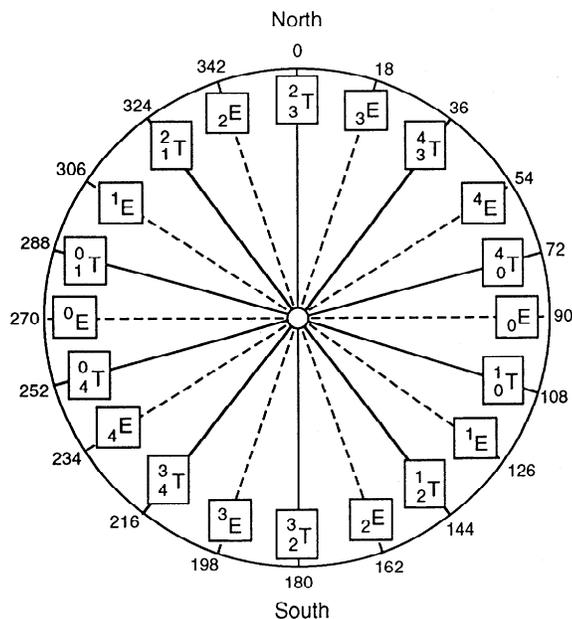


Fig. 2. Pseudorotational pathway of the L-furanose ring (adapted from ref. 41).

moieties of the oligosaccharides⁴¹. The (1 → 3) disaccharides (**11**, **17**, and **21**) have coupling constants very comparable to the oligosaccharide (1 → 2)-linked furanosyl rings. Thus the (1 → 2)-linked disaccharides favor the ${}^4E-{}^4T_0$ (N) and ${}^1T_2-{}^2E$ (S) conformers, whereas the (1 → 3)-linked materials prefer the ${}^4T_3-{}^4E$ (N) and ${}^1E-{}^1T_2$ (S) conformers (Fig. 2). These results suggest that the α -L-arabinofuranosyl ring conformation of arabinoxylans may be influenced by both the xylan chain and vicinal furanosyl xylan substituents.

Methyl α -D-arabinofuranoside in D₂O is considered to favor the ${}^0T_{4-1}E$ conformations^{42,43}. The pseudorotational itinerary for the L-furanoside (mirror image) would then be ${}^4T_0-{}^1E$, which places its averaged conformation intermediate to that of the arabinoxylan oligosaccharides. Comparison of the ${}^3J_{H-H}$ values of the furanoside with the data reported in Table III for disaccharides in D₂O (**7**, **11**, **16**, **17**, **20**, and **21**) indicate that the (1 → 3) disaccharides (**11**, **20**, and **21**), behave quite similarly to the methyl furanoside. The (1 → 2)-linked disaccharides (**7**, **16**, and **20**) have ring-proton coupling constants similar to those reported for methyl α -D-threofuranoside⁴². The tetroside, with respect to L-furanosides, favors the more southerly ${}^0E-{}^2E$ conformers, which experimentally results in lower ${}^3J_{1,2}$ and ${}^3J_{2,3}$ values and a slightly more effective ${}^4J_{1,3}$ coupling.

The comparable results obtained between the synthetic disaccharides described here and monosaccharides^{42,43} and oligosaccharides⁴¹ suggest that a rigid C-4-O-4-C-1-O-1 segment of the furanosyl ring is present as was described in the previous studies. The quasiaxial directing affect of the aglycon and the preference

TABLE III
¹H NMR chemical shift data ^a for selected disaccharides

Compound ^b	Chemical shift in ppm (coupling constant, Hz)							
	H-1	H-2	H-3 ^c	H-4	H-5 _{eq} / <i>proR</i>	H-5 _{ax} / <i>proS</i>		
6	A	5.498 (<1)	5.391 (1.37)	5.601 (5.19, 0.71)	4.841	4.820 (3.32)	4.687 (5.47, nfo)	
	X	4.710 (6.84)	4.104 (8.92)	5.776 (8.83)	5.263	4.301 (5.06)	3.734 (8.90, -11.7)	
7	A	5.265 (<1)	4.157 (1.29)	3.950 (5.50, <1)	4.138	3.809 (3.58)	3.712 (5.56, -12.3)	
	X	4.396 (7.46)	3.397 (8.70)	3.571 (8.90)	3.647	3.970 (5.03)	3.317 (9.72, -11.6)	
8	A	5.104 (0.37)	4.881 (1.40)	5.077 (5.15, 0.74)	4.204	3.836 (nfo)	3.836 (nfo)	
	X	4.404 (7.06)	3.603 (9.30)	5.144 (9.22)	4.805	3.985 (5.29)	3.428 (9.15, -11.7)	
9	A	5.110 (0.39)	4.900 (1.54)	5.016 (5.21, 0.70)	4.203	3.734 (3.93)	3.666 (4.68, -12.0)	
	X	4.404 (7.00)	3.601 (9.23)	5.136 (9.10)	4.803	3.986 (5.29)	3.429 (9.04, -11.7)	
10	A	5.538 (<1)	5.357 (1.33)	5.469 (4.73, 0.69)	4.404	4.471 (3.75)	4.309 (4.26, -11.6)	
	X	4.762 (7.07)	5.535 (~8.7)	4.543 (~8.7)	5.339	4.282 (5.24)	3.720 (9.05, -11.8)	
11	A	5.315 (1.60)	4.185 (3.34)	3.951 (6.00, 0.56)	4.184	3.816 (3.41)	3.702 (5.80, -12.3)	
	X	4.353 (7.87)	3.392 (9.00)	3.593 (8.97)	3.671	3.997 (5.29)	3.352 (10.2, -11.6)	
12	A	5.098 (<1)	4.862 (1.53)	5.039 (4.48, 0.69)	4.097	3.829 (~4.4)	3.829 (~4.4, nfo)	
	X	4.408 (7.51)	4.862 (9.35)	3.902 (8.84)	4.862	3.980 (5.46)	3.367 (9.53, -11.7)	
13	A	5.094 (0.61)	4.874 (1.68)	4.969 (4.96, 0.66)	4.100	3.721 (4.31)	3.662 (5.02, -11.8)	
	X	4.404 (7.52)	4.856 (9.34)	3.898 (8.84)	4.859	3.973 (5.48)	3.366 (9.52, -11.7)	

14	A	5.141 (<1)	4.948 (1.34)	5.035 (5.08, 0.70)	4.448	4.540 (3.28)	4.339 (5.55, -11.7)
	X	4.172 (7.16)	3.360 (9.39)	5.141 (9.09)	4.822	3.995 (5.33)	3.433 (9.24, -11.7)
15	A	5.168 (<1)	4.950 (1.37)	5.029 (5.07, 0.71)	4.449	4.546 (3.23)	4.334 (5.59, -11.8)
	X	4.419 (7.17)	3.634 (9.38)	5.168 (9.13)	4.824	3.921 (5.33)	3.435 (9.25, -11.7)
16	A	5.278 (~1)	4.206 (2.62)	4.009 (5.69, <1)	4.332	4.475 (2.51)	4.271 (6.62, -11.5)
	X	4.352 (7.48)	3.391 (8.98)	3.567 (9.17)	3.644	3.951 (5.20)	3.295 (9.69, -11.6)
17	A	5.281 (1.17)	4.208 (2.61)	4.014 (5.61, <1)	4.345	4.483 (2.63)	4.275 (6.69, -11.6)
	X	4.358 (7.53)	3.391 (8.75)	3.568 (9.03)	3.642	3.950 (5.07)	3.297 (9.75, -11.6)
18	A	5.164 (0.52)	4.926 (1.44)	5.012 (4.23, 0.66)	4.330	4.522 ^d (nfo)	4.330 ^d (nfo)
	X	4.419 (7.54)	4.877 (9.38)	3.927 (8.85)	4.881	3.988 (5.49)	3.379 (9.55, -11.7)
19	A	5.165 (0.50)	4.926 (1.43)	5.011 (4.22, 0.68)	4.326	4.531 ^d (nfo)	4.326 ^d (nfo)
	X	4.418 (7.53)	4.878 (9.34)	3.927 (8.84)	4.883	3.990 (5.48)	3.379 (9.54, -11.7)
20	A	5.323 (1.58)	4.226 (3.34)	4.046 (6.11, 0.48)	4.386	4.453 (2.97)	4.276 (6.15, -11.8)
	X	4.320 (7.86)	3.394 (9.07)	3.561 (8.90)	3.664	3.981 (5.41)	3.322 (10.3, -11.6)
21	A	5.310 (1.62)	4.226 (3.43)	4.043 (6.13, 0.49)	4.380	4.452 (2.91)	4.257 (6.40, -11.7)
	X	4.305 (7.85)	3.390 (9.10)	3.538 (8.86)	3.655	3.975 (5.44)	3.311 (10.4, -11.6)

^a Recorded at 27°C in the solvents indicated in Table I. Samples in acetone-*d*₆ were referenced to the central solvent peak (2.04 ppm), and samples in D₂O were referenced to internal acetone (2 μL/mL, 2.225 ppm). Coupling constants are based on a first-order analysis and are to within ±0.03 Hz; (nfo) indicates non-first order multiplicity prevented an accurate coupling constant determination. Chemical shifts are to within ±0.002 ppm. ^b A, α-L-arabinofuranosyl; X,

of the hydroxymethyl group to be in a quasiequatorial position affords this conformation. The hydroxycinnamate group does not affect the observed ring-proton coupling constants and is probably not a major factor in favored ring conformation. The $J_{4,5}$ and $J_{5R,5S}$ values for **16**, **17**, **20**, and **21** are about 1 Hz different than for the disaccharides (**7** and **11**) and arabinoxylan oligosaccharides. Thus the preferred exocyclic rotomer distribution may be somewhat different than reported by Hoffmann et al.⁴¹ but the electronegativity of the acyl group must be included in the appropriate Karplus equation.

The protected (1 → 2) and (1 → 3) precursors (analyzed in acetone- d_6) have $^3J_{H-H}$ couplings associated with the more southerly N and S conformers. In some cases $^4J_{1,3} > ^3J_{1,2}$ (Table III). However, assignment of favored conformations to these samples is hindered as much less information is available for arabinofuranosides in this solvent. It should be stated, however, that the $^3J_{H-H}$ values observed for methyl α -L-arabinofuranoside in D₂O and acetone- d_6 are quite similar.

Implications.—The preparation of **11**, **20**, **21**, and the feruloylated and *p*-coumaroylated methyl α -L-arabinofuranosides¹⁸ provides the necessary models to begin the task of developing monoclonal antibodies. These antibodies can then be used to search for these types of structures in cell walls. Although at present there is no direct evidence for (1 → 2)-linked feruloylated and *p*-coumaroylated arabinoxylans, there is evidence from methylation analysis for (1 → 4)-D-xylans with substituents at the 2-position⁴⁴⁻⁴⁶. The preponderance of 2,3-disubstituted arabinoxylans isolated from wheat endosperm^{33,41} indicates that 2-*O*-L-arabinofuranosylation can occur on xylan chains. It remains to be determined if the 3-substitution is necessary for 2-substitution to occur, as well as if any or all of these L-arabinofuranosyl moieties can possess 5-*O*-hydroxycinnamoyl substituents.

EXPERIMENTAL

General.—Instrumentation and general procedures were as described previously^{18,21}, unless noted otherwise. All NMR spectra were recorded at 27°C with a Bruker AMX-360 spectrometer fitted with a 5-mm 4-nucleus (QNP) probe with normal geometry. The two-dimensional C–H correlation experiments (both one-bond and long range) were performed with the probe in the inverse mode (HMQC and HMBC, respectively). Chemical shifts are relative to the central solvent peaks of acetone- d_6 (δ_C 29.8 ppm, δ_H 2.04 ppm). Spectra recorded in D₂O were internally referenced to acetone (2 μ L/ml; δ_C 28.225 ppm, δ_H 2.225 ppm)⁴⁷.

Methyl 2,4-di-O-benzoyl-3-O-chloroacetyl- β -D-xylopyranoside (4).—Methyl 4-*O*-benzoyl-3-*O*-chloroacetyl- β -D-xylopyranoside (**3**, 5.61 g, 16.3 mmol)²¹ was dissolved in CH₂Cl₂ (150 mL) and cooled in an ice–water bath. Benzoyl chloride (1.93 mL, 16.6 mmol), *N,N*-(diisopropyl)ethylamine (4.2 mL, 24 mmol), and 4-(dimethylamino)pyridine (1.58 g, 12.9 mmol) were added in respective order. The flask was removed from the bath and the solution stirred for 70 min, at which time TLC (19:1 CHCl₃–EtOAc) indicated complete conversion to a faster moving material.

The solution was washed with cold aq 3% HCl (2 ×) and subsequently with aq NaCl (2 ×). Standard processing and crystallization from MeOH gave **4** as large rhombic crystals in two crops (6.29 g, 86.2%): mp 111.0–112.5°C; $[\alpha]_D -42^\circ$ (c 1.18, acetone); NMR (acetone- d_6): δ_{H} 3.46 (OCH₃), 3.77 (dd, 1 H, $J_{5a,4}$ 8.8, J_{gem} –11.8 Hz, H-5a), 4.13 and 4.19 (2 d, 2 × 1 H, J_{gem} 14.9 Hz, CH₂Cl), 4.35 (dd, 1 H, $J_{5e,4}$ 5.2 Hz, H-5e), 4.84 (d, 1 H, $J_{1,2}$ 6.8 Hz, H-1), 5.24 (dt, 1 H, $J_{3,4} \sim J_{4,5a} \sim 8.8$ Hz, H-4), 5.26 (dd, 1 H, $J_{2,3}$ 8.7 Hz, H-2), and 5.68 (t, 1 H, H-3); δ_{C} 41.3 (CH₂Cl), 56.7 (OCH₃), 62.5 (C-5), 70.6 (C-4), 71.9 (C-2), 73.8 (C-3), and 102.4 (C-1). Anal. Calcd for C₂₂H₂₁ClO₈: C, 58.87; H, 4.72. Found: C, 58.49; H, 4.85.

Methyl 2,4-di-O-benzoyl-β-D-xylopyranoside (5).—Crystalline **4** (4.06 g, 9.05 mmol) was added to a solution of abs EtOH (160 mL) and pyridine (1.6 mL) maintained at 50°C. Thiourea (2.70 g, 35.4 mmol) was added, the mixture was stirred for 5 h, and evaporated to a solid. The crude product was dissolved in an immiscible mixture of 1:1 CH₂Cl₂–3% aq HCl and the separated organic layer was washed with aq NaCl (2 ×). Processing afforded **5** as a foam in > 97% purity and nearly quantitative yield. This material was of adequate purity to be submitted to the next step, although an analytical sample was prepared by silica gel chromatography (19:1 CHCl₃–EtOAc): $[\alpha]_D -40^\circ$ (c 0.98, acetone), lit.²⁹ $[\alpha]_D -40^\circ$ (c 1.0, CHCl₃).

Glycosylation.—Nucleophile **2** (ref. 21) or **5** (1.30 g, 3.5 mmol) was dissolved in CH₂Cl₂ (100 mL) and freshly dried 4A molecular sieve (3.1 g) was added. Silver triflate (1.37 g, 5.35 mmol) and collidine (505 mg, 4.16 mmol) were added, and the solution was cooled to 0°C in an ice–water bath. Crystalline 2,3,5-tri-*O*-benzoyl- α -L-arabinofuranosyl chloride²¹ (1.85 g, 3.85 mmol) was added, and a white precipitate (AgCl) formed immediately. TLC indicated the complete disappearance of nucleophile within 15 min. The solution was stirred for an additional 30 min and subsequently filtered through a pad of Celite and washed successively with aq Na₂SO₃, cold 1.5 M H₂SO₄, and H₂O. The organic layer was dried and processed to afford crude **6** or **10**. Purification by silica gel chromatography (20:1 CCl₄–acetone) gave the desired disaccharide perbenzoates in yields ranging from 92–96%.

Methyl (2,3,5-tri-O-benzoyl- α -L-arabinofuranosyl)-(1 → 2)-3,4-di-O-benzoyl-β-D-xylopyranoside (6).—Compound **6** was obtained as a white foam $[\alpha]_D -34^\circ$ (c 0.66, acetone). Anal. Calcd for C₄₆H₄₀O₁₄: C, 67.64; H, 4.94. Found: C, 67.66; H, 4.91.

Methyl (2,3,5-tri-O-benzoyl- α -L-arabinofuranosyl)-(1 → 3)-2,4-di-O-benzoyl-β-D-xylopyranoside (10).—Compound **10** was obtained as a white foam: $[\alpha]_D -6.1^\circ$ (c 1.46, acetone). Anal. Calcd for C₄₆H₄₀O₁₄: C, 67.64; H, 4.94. Found: C, 67.64; H, 4.83.

Debenzylation.—Perbenzoates **6** and **10** (2.9 g, 3.6 mmol) were suspended in MeOH (100 mL) and NaOMe (100 mg) was added. The reaction was monitored by TLC (6:1 CHCl₃–MeOH) and quenched (after at least 12 h) by the addition of strongly acidic ion-exchange resin. Processing and subsequent silica gel chromatography (6:1 CHCl₃–MeOH) afforded the disaccharides **7** and **11** in 92% yield.

Methyl α -L-arabinofuranosyl-(1 \rightarrow 2)- β -D-xylopyranoside (7).—Compound **7** was obtained as an amorphous powder: $[\alpha]_D -111^\circ$ (*c* 0.72, acetone); lit.²³ $[\alpha]_D -112^\circ$ (*c* 1, H₂O).

Methyl α -L-arabinofuranosyl-(1 \rightarrow 3)- β -D-xylopyranoside (11).—Compound **11** crystallized from acetone as long, thin needles: mp 139.5–140.5°C; $[\alpha]_D -126^\circ$ (*c* 1.1, 1:1 acetone–H₂O); lit.²⁵ mp 135–137°C; $[\alpha]_D -127.5^\circ$ (*c* 1, H₂O).

Methyl 2,3-di-O-acetyl- α -L-arabinofuranosyl-(1 \rightarrow 2)-3,4-di-O-acetyl- β -D-xylopyranoside (9).—Amorphous **7** (813 mg, 2.75 mmol) was dissolved in pyridine (8 mL) and *tert*-butylchlorodimethylsilane (448 mg, 2.97 mmol) was added. When TLC (6:1 CHCl₃–MeOH) indicated complete conversion to a faster-moving material (typically 2–4 h), Ac₂O (1.5 mL) was added and the mixture was left overnight at room temperature. The reaction was quenched with abs EtOH, diluted with CH₂Cl₂ and washed successively with H₂O, cold aq 3% HCl, and H₂O. Processing and silica gel chromatography (19:1 CHCl₃–EtOAc) afforded **8** in 90% yield: $[\alpha]_D -37^\circ$ (*c* 0.97, acetone).

The crude product was typically of adequate purity (> 95%) to continue with the next step without intermediate purification. Crude **8** was treated with aq 80% HOAc (5 mL) for 24 h. The resulting solution was diluted with CH₂Cl₂ and washed successively with H₂O, aq NaHCO₃, and H₂O. Processing and silica gel chromatography (4:1 CHCl₃–EtOAc) gave **9** as a syrupy solid which crystallized from abs EtOH–petroleum ether (974.2 mg, 76% from **7**): mp 98.5–99.5°C; $[\alpha]_D -59^\circ$ (*c* 0.62, acetone). Anal. Calcd. for C₁₉H₂₈O₁₃: C, 49.14; H, 6.08. Found: C, 49.39; H, 6.26.

Methyl (2,3-di-O-acetyl- α -L-arabinofuranosyl)-(1 \rightarrow 3)-2,4-di-O-acetyl- β -D-xylopyranoside (13).—Compound **13** was prepared according to the procedure outlined for **9**. Processing of the silylation–acetylation product gave **12** in 88% yield as a clear syrup: $[\alpha]_D -88^\circ$ (*c* 0.69, acetone). Subsequent O-desilylation and work-up as described for **9** gave **13** which crystallized from abs EtOH–petroleum ether (74% yield from **11**): mp 118–118.5°C; $[\alpha]_D -63^\circ$ (*c* 0.69, acetone). Anal. Calcd. for C₁₉H₂₈O₁₃: C, 49.14; H, 6.08. Found: C, 49.31; H, 6.12.

4-Acetoxycinnamoylation.—Crystalline **9** or **13** (847 mg, 1.82 mmol) was dissolved in pyridine (10 mL) and the mixture was cooled to 0°C. The appropriate 4-acetoxycinnamoyl chloride^{18,19} (2.19 mmol) was added, and the solution was stirred for 30 min at 0°C and subsequently at room temperature 1 h. The crude mixture was diluted with CH₂Cl₂ and washed successively with H₂O, 3% HCl, and H₂O. Processing and silica gel chromatography (4:1 CHCl₃–EtOAc) gave the peracetylated derivatives **14**, **15**, **18**, and **19** in yields ranging from 88–98%.

Methyl 2,3-di-O-acetyl-5-O-(4-acetoxy-p-coumaroyl)- α -L-arabinofuranosyl-(1 \rightarrow 2)-3,4-di-O-acetyl- β -D-xylopyranoside (14).—Compound **14** was isolated as a white foam in 88% yield: $[\alpha]_D -43^\circ$ (*c* 0.89, acetone). Anal. Calcd for C₃₀H₃₆O₁₆: C, 55.21; H, 5.56. Found: C, 55.56; H, 5.72.

Methyl 2,3-di-O-acetyl-5-O-(4-acetoxyferuloyl)- α -L-arabinofuranosyl-(1 \rightarrow 2)-3,4-di-O-acetyl- β -D-xylopyranoside (15).—Compound **15** was isolated as a white foam

in 98% yield: $[\alpha]_D -37^\circ$ (*c* 1.48, acetone). Anal. Calcd for $C_{31}H_{38}O_{17}$: C, 54.55; H, 5.61. Found: C, 54.49; H, 5.63.

Methyl 2,3-di-O-acetyl-5-O-(4-acetoxy-p-coumaroyl)- α -L-arabinofuranosyl-(1 \rightarrow 3)-2,4-di-O-acetyl- β -D-xylopyranoside (18).—Compound **18** was isolated as a white foam in 98% yield: $[\alpha]_D -63^\circ$ (*c* 0.70, acetone). Anal. Calcd for $C_{30}H_{36}O_{16}$: C, 55.21; H, 5.56. Found: C, 55.22; H, 5.66.

Methyl 2,3-di-O-acetyl-5-O-(4-acetoxyferuloyl)- α -L-arabinofuranosyl-(1 \rightarrow 3)-2,4-di-O-acetyl- β -D-xylopyranoside (19).—Compound **19** was isolated as a white foam in quantitative yield: $[\alpha]_D -62^\circ$ (*c* 1.51 acetone). Anal. Calcd for $C_{31}H_{38}O_{17}$: C, 54.55; H, 5.61. Found: C, 54.82; H, 5.58.

Deacetylation.—The procedure was as described previously¹⁸ with the exception that the neutralization was accomplished by passage through an ion-exchange resin maintained at 4°C. The eluate obtained from the ion-exchange column was evaporated to a syrup and purified by silica gel chromatography (6:1 $CHCl_3$ –MeOH) to provide **16**, **17**, **20**, and **21**. The (1 \rightarrow 3) disaccharides required prolonged exposure to the basic solution for complete deacetylation (up to 6 days). This resulted in lower yields due to hydroxycinnamoyl cleavage. The resulting disaccharide (**11**) could be recovered during the chromatographic purification on silica gel.

Methyl 5-O-p-coumaroyl- α -L-arabinofuranosyl-(1 \rightarrow 2)- β -D-xylopyranoside (16).—Compound **16** was isolated as a white foam in 78% yield: $[\alpha]_D -89^\circ$ (*c* 0.71, acetone). Anal. Calcd for $C_{20}H_{26}O_{11}$: C, 54.30; H, 5.92. Found: C, 54.11; H, 5.91.

Methyl 5-O-feruloyl- α -L-arabinofuranosyl-(1 \rightarrow 2)- β -D-xylopyranoside (17).—Compound **17** was isolated as a white foam in 87% yield: $[\alpha]_D -84^\circ$ (*c* 0.78, acetone). Anal. Calcd for $C_{21}H_{28}O_{12}$: C, 53.39; H, 5.97. Found: C, 53.60; H, 6.09.

Methyl 5-O-p-coumaroyl- α -L-arabinofuranosyl-(1 \rightarrow 3)- β -D-xylopyranoside (20).—Compound **20** was isolated as a white foam in 65% yield: $[\alpha]_D -92^\circ$ (*c* 0.94, acetone). Anal. Calcd for $C_{20}H_{26}O_{11}$: C, 54.30; H, 5.92. Found: C, 54.68; H, 6.02.

Methyl 5-O-feruloyl- α -L-arabinofuranosyl-(1 \rightarrow 3)- β -D-xylopyranoside (21).—Compound **21** was isolated as a white foam in 62% yield: $[\alpha]_D -86^\circ$ (*c* 1.14, acetone). Anal. Calcd for $C_{21}H_{28}O_{12}$: C, 53.39; H, 5.97. Found: C, 53.55; H, 6.04.

ACKNOWLEDGMENTS

Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable. This work was supported in part by a grant from the USDA Competitive Grants Program (Plant Growth and Development No. 90-37261-5617). The authors would like to thank the USDA-Agricultural Research Service and the U.S. Dairy Forage Research Center for funding the purchase of the Bruker AMX-360 spectrometer, which was used throughout the course of this investigation.

REFERENCES

- 1 A. Kato, J. Azuma, and T. Koshijima, *Chem. Lett.*, (1983) 137–140.
- 2 Y. Kato and D.J. Nevins, *Carbohydr. Res.*, 137 (1985) 139–150.
- 3 I. Mueller-Harvey, R.D. Hartley, P.J. Harris, and E.H. Curzon, *Carbohydr. Res.*, 148 (1986) 71–85.
- 4 T. Ishii and T. Hiroi, *Carbohydr. Res.*, 196 (1990) 175–183.
- 5 T. Ishii and T. Hiroi, *Carbohydr. Res.*, 206 (1990) 297–310.
- 6 A. Scalbert, B. Monties, J.-Y. Lallemand, E. Guittet, and C. Rolando, *Phytochemistry*, 24 (1985) 1359–1362.
- 7 A. Scalbert, B. Monties, C. Rolando, and A. Sierra-Escudero, *Holzforschung*, 40 (1986) 191–195.
- 8 T. Kondo, K. Mizuno, and T. Kato, *Can. J. Plant Sci.*, 71 (1990) 495–499.
- 9 T.B.T. Lam, K. Iiyama, and B.A. Stone, *Phytochemistry*, 29 (1990) 429–433.
- 10 K. Iiyama, T.B.T. Lam, and B.A. Stone, *Phytochemistry*, 29 (1990) 733–737.
- 11 T.B.T. Lam, K. Iiyama, and B.A. Stone, *Phytochemistry*, 31 (1992) 1179–1183.
- 12 R.D. Hartley and D.E. Akin, *J. Sci. Food Agric.*, 49 (1989) 405–411.
- 13 H. Jung, *Agron. J.*, 81 (1989) 33–38.
- 14 S.C. Fry and J.G. Miller, *ACS Symp. Ser.*, 399 (1989) 33–46.
- 15 E. Yamamoto, G.H. Bokelman, and N.G. Lewis, *ACS Symp. Ser.*, 399 (1989) 68–88.
- 16 T. Ishii, *Carbohydr. Res.*, 219 (1991) 15–22.
- 17 J. Ralph, R.F. Helm, S. Quideau, and R.D. Hatfield, *J. Chem. Soc., Perkin Trans. 1*, in press.
- 18 R.F. Helm, J. Ralph, and R.D. Hatfield, *Carbohydr. Res.*, 229 (1992) 183–194.
- 19 R.D. Hatfield, R.F. Helm, and J. Ralph, *Anal. Biochem.*, 194 (1991) 25–33.
- 20 R.D. Hatfield, B. Uicker, R.F. Helm, and J. Ralph, *Plant Cell Physiol.*, in preparation.
- 21 R.F. Helm, J. Ralph, and L. Anderson, *J. Org. Chem.*, 56 (1991) 7015–7021.
- 22 C.P.J. Glaudemans and M.J. Bertolini, *Methods Carbohydr. Chem.*, 8 (1970) 271–275.
- 23 J. Hirsch, E. Petráková, and J. Schraml, *Carbohydr. Res.*, 131 (1984) 219–226.
- 24 S. Koto, N. Morishima, K. Takenaka, C. Uchida, and S. Zen, *Bull. Chem. Soc. Jpn.*, 58 (1985) 1464–1468.
- 25 J. Hirsch, E. Petráková, and M. Hricovíni, *Chem. Papers*, 43 (1989) 395–402.
- 26 K.C.B. Wilke, *Adv. Carbohydr. Chem. Biochem.*, 39 (1979) 215–264.
- 27 P.L. Durette and D. Horton, *Carbohydr. Res.*, 18 (1971) 57–80.
- 28 P.L. Durette and D. Horton, *Carbohydr. Res.*, 18 (1971) 403–418.
- 29 E. Petráková and J. Schraml, *Coll. Czech. Chem. Commun.*, 48 (1983) 877–888.
- 30 A. De Bruyn, M. Anteunis, R. van Rijsbergen, M. Claeysens, and P. Kovác, *J. Carbohydr. Chem.*, 1 (1982-3) 301–309.
- 31 O. Kanie, T. Takeda, N. Hada, and Y. Ogihara, *J. Carbohydr. Chem.*, 10 (1991) 561–568.
- 32 I. Tvaroška and T. Bleha, *Adv. Carbohydr. Chem. Biochem.*, 47 (1989) 45–123.
- 33 R.A. Hoffmann, J.P. Kamerling, and J.F.G. Vliegthart, *Carbohydr. Res.*, 226 (1992) 303–311.
- 34 M. Hricovíni and I. Tvaroška, *Magn. Res. Chem.*, 28 (1990) 862–866.
- 35 I. Tvaroška, *Carbohydr. Res.*, 206 (1990) 55–64.
- 36 I. Tvaroška and F.R. Taravel, *Carbohydr. Res.*, 221 (1991) 83–94.
- 37 N. Cyr and A.S. Perlin, *Can. J. Chem.*, 57 (1979) 2504–2511.
- 38 K. Mizutai, R. Hasai, M. Nakamura, O. Tanaka, and H. Matsuura, *Carbohydr. Res.*, 185 (1989) 27–38.
- 39 P.C. Kline and A.S. Serianni, *J. Am. Chem. Soc.*, 112 (1990) 7373–7381.
- 40 G.D. Wu, A.S. Serianni, and R. Barker, *J. Org. Chem.*, 48 (1983) 1750–1757.
- 41 R.A. Hoffmann, J. van Wijk, B.R. Leeftang, J.P. Kamerling, C. Altona, and J.F.G. Vliegthart, *J. Am. Chem. Soc.*, 114 (1992) 3710–3714.
- 42 A.S. Serianni and R. Barker, *J. Org. Chem.*, 49 (1984) 3292–3300.
- 43 S.J. Angyal, *Carbohydr. Res.*, 77 (1979) 37–50.
- 44 J.A. Lomax, A.H. Gordon, and A. Chesson, *Carbohydr. Res.*, 122 (1983) 11–22.
- 45 A. Chesson, A.H. Gordon, and J.A. Lomax, *Carbohydr. Res.*, 141 (1985) 137–147.
- 46 J.A. Lomax, A.H. Gordon, and A. Chesson, *Carbohydr. Res.*, 138 (1985) 177–188.
- 47 J.F.G. Vliegthart, L. Dorland, and H. van Halbeek, *Adv. Carbohydr. Chem. Biochem.*, 41 (1985) 209–374.