Lignin–carbohydrate model compounds. Reactivity of methyl 3-*O*-(α-L-arabinofuranosyl)-β-D-xylopyranoside and methyl β-D-xylopyranoside towards a β-*O*-4-quinone methide †

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The reactivity of a disaccharide, namely methyl 3-*O*-(α-L-arabinofuranosyl)-β-D-xylopyranoside, and that of a monosaccharide, methyl β-D-xylopyranoside towards a lignin β-*O*-4 quinone methide were compared as a model reaction for the formation of lignin–carbohydrate complexes (LCCs). Benzyl ethers were formed *via* a β-*O*-4 quinone methide giving 4 diastereomers of each product. All three secondary hydroxy groups of methyl β-D-xylopyranoside but only the primary hydroxy group of the arabinofuranosyl moiety of methyl 3-*O*-(α-L-arabinofuranosyl)-β-D-xylopyranoside became etherified to the benzyl position of the lignin β-aryl ether model compound. The LCC model compounds were isolated as individual diastereomers or mixtures of diastereomers using silica gel and HPLC chromatography and they were all characterized by NMR spectroscopy and MS.

Introduction

Numerous investigations have been made to elucidate the resistance of wood lignin and carbohydrates to chemical and biochemical treatments. The results support the existence of lignin–carbohydrate complexes (LCCs), and covalent bonding of lignin to cell-wall hemicelluloses has been suggested.[1] Isolated LCC preparations have been investigated using cleavage reactions such as acid or base treatment, methylation analysis or treatment with oxidants, and benzyl ether, ester and glycosidic lignin–carbohydrate bonds have been suggested.[2–5] It has been suggested that softwood lignin is connected to arabinose sidechains and xylan backbone of arabino-4-*O*-methylglucuronoxylan.[2] Hardwood lignin has been indicated to bond to the xylopyranosyl units of glucuronoxylan.[4] However, different bond types still need to be carefully studied and a series of lignin–carbohydrate model compounds with different linkages will make it possible to interpret the results of research on lignin–carbohydrate bonds more correctly.

Lignin–carbohydrate benzyl ether bonds are thought to be formed *via* quinone methides, which play an important role during biosynthesis of lignin. Carbohydrates can attack quinone methides of lignin, and benzyl ether-type lignin–carbohydrate bonds are formed.[6] We have studied the conditions of formation of lignin–carbohydrate benzyl ether bonds using model compounds. Model compounds for LCCs can give valuable information about possible lignin–carbohydrate bonds in Nature and also about LCC structures which may be formed during pulping processes.

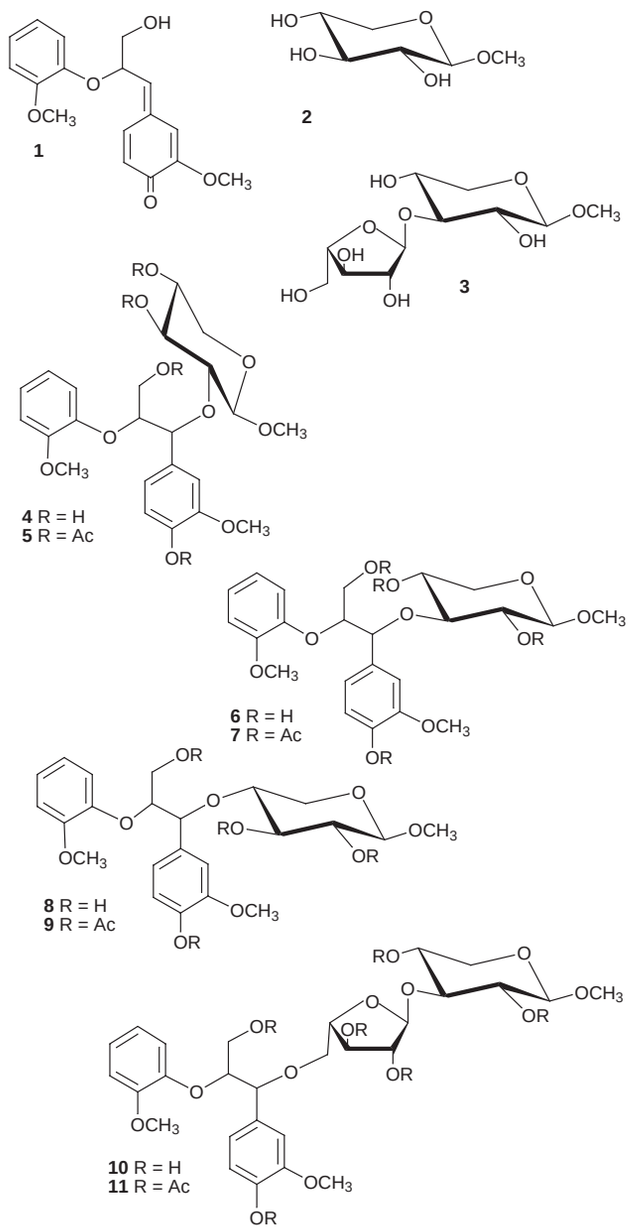
We have previously studied lignin–carbohydrate bond formation with the quinone methide from guaiacyl glycerol β-guaiacyl ether and two monosaccharides, methyl α-L-arabinofuranoside and methyl α-D-galactopyranoside in organic solution catalysed by PTSA.[7] These monosaccharides, which are in Nature attached to the backbone of hemicelluloses, reacted predominantly through primary hydroxy groups with the quinone methide of the lignin model but some secondary C-3 hydroxy groups of methyl α-D-galactopyranoside were reactive also. In this study we have used methyl β-D-xylopyranoside and methyl 3-*O*-(α-L-arabinofuranosyl)-β-D-xylopyranoside, structural units of arabinoglucuronoxylan under similar conditions. We report here, for the first time, that the disaccharide is able to form lignin–carbohydrate model compounds with the β-*O*-4-quinone methide. LCC model compounds were separated to give diastereomers or mixtures of diastereomers, and these were characterized by NMR spectroscopy.

† The IUPAC term for quinone methides is quinomethanes.

Results and discussion

We compared the reactivity of a β-*O*-4-type quinone methide towards methyl β-D-xylopyranoside and methyl 3-*O*-(α-L-arabinofuranosyl)-β-D-xylopyranoside in organic solvent in the presence of catalytic amount of PTSA according to the protocol of Sipilä and Brunow.[8] In these non-aqueous conditions all of the secondary hydroxy groups of methyl β-D-xylopyranoside were reactive and lignin–carbohydrate benzyl ether model compounds **4**, **6** and **8** were formed in 48–56% combined yield. However, when we connected the arabinofuranosyl group to the C-3 hydroxy group of methyl β-D-xylopyranoside only the primary hydroxy group of the arabinofuranosyl moiety of methyl 3-*O*-(α-L-arabinofuranosyl)-β-D-xylopyranoside etherified to the benzyl position of the lignin model. Compound **10** (four diastereomers) was formed in up to 36% yield. Reaction of the secondary hydroxy groups of arabinofuranosyl and xylopyranosyl moieties of the disaccharide did not produce detectable LCC model compounds. This is consistent with the previous result that methyl α-L-arabinofuranoside (as a monosaccharide) reacts only through the primary C-5 hydroxy group with the lignin model.[7]

LCC model-compound formation *via* β-*O*-4-type quinone methide having a chiral centre in the propyl sidechain always produces a mixture of *erythro*- and *threo*-diastereomers when carbohydrates attach to the C_α-position of the lignin model (Scheme 1). Combination of flash and HPLC reversed-phase column chromatography enabled us to separate the 12 methyl xyloside LCC model diastereomers to individual diastereomers or a mixture of diastereomers. The methyl arabinoxyloside LCC model diastereomers were separated to give pairs of *erythro* and *threo* diastereomers by flash and HPLC normal-phase column chromatography. Assignments of the *erythro*- and *threo*-LCC model compounds were made from ^{1}H NMR signals of the propyl sidechain protons in analogy with previous work on β-*O*-4 lignin model compounds.[9]



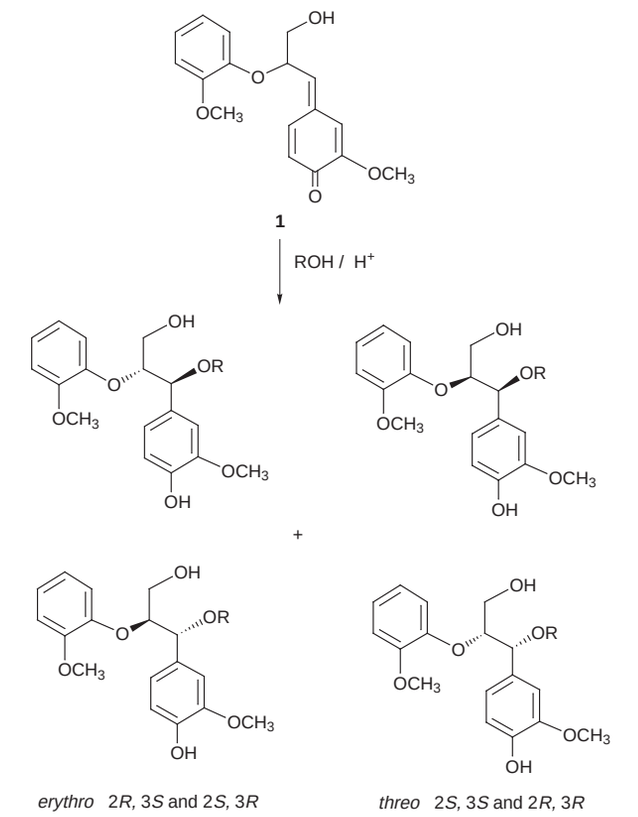
Experimental

General

The lignin β-*O*-4 dimer 1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol was prepared by benzylation of acetovanillone, bromination, coupling with guaiacol, hydroxymethylation, reduction of ketone and debenzylation (62% overall yield).[10,11] The quinone methide **1** was prepared from 1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-propane-1,3-diol using the TMSBr method.[12,13] Methyl β-D-xylopyranoside **2** was purchased from Sigma and L-(+)-arabinose from Fluka. Methyl β-D-xylopyranoside was partially protected by benzylation to methyl 2,4-di-*O*-benzyl-β-D-xylopyranoside.[14] 2,3,5-Tri-*O*-benzoyl-α-L-arabinofuranosyl bromide was prepared by methylation of L-(+)-arabinose,[7,15] benzoylation and bromination[16] (2 h, acetic acid as solvent). The glycosyl bromide was purified by filtering with dry methylene dichloride through a patch of silica gel. The glycosylation step of methyl 2,4-di-*O*-benzyl-β-D-xylopyranoside and 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl bromide was promoted by silver triflate[17] and the disaccharide, methyl 2,4-di-*O*-benzyl-3-*O*-(2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl)-β-D-xylopyranoside, was formed in 70–80% yield. Removal of the benzoyl and then benzyl protecting groups gave the methyl 3-*O*-(α-L-arabinofuranosyl)-β-D-xylopyranoside[18–20] (Scheme 1). The opposite deprotection order resulted in incomplete debenzylation. No glycoside bond breaking of the disaccharide was observed in the deprotection step. LCC samples were acetylated with pyridine and acetic anhydride (1:1) at RT overnight.

Table 1 Optical rotations and purities of acetylated LCC model compounds **5**, **7**, **9** and **11**

Compound	Purity (%) by ^{1}H NMR (contaminated with)	[*α*] in acetone	*c*
5 *e*-1	30 (**9** *t*-2)	$[\alpha]_D^{20}$ −5.7	1.0
5 *e*-2	55 (**9** *e*-2)	$[\alpha]_D^{20}$ 5.3	1.0
5 *t*-1	69 (**9** *t*-2)	$[\alpha]_D^{21}$ −2.2	0.5
5 *t*-2	45 (**9** *t*-1)	$[\alpha]_D^{21}$ −30.0	0.9
7 *e*-1	97	$[\alpha]_D^{20}$ −2.6	0.8
7 *e*-2	38 (**7** *t*-1, *t*-2)	$[\alpha]_D^{20}$ −13.2	0.2
7 *t*-1, *t*-2	35 and 35 (**7** *e*-1)	$[\alpha]_D^{20}$ −11.1	1.0
9 *e*-1	93	$[\alpha]_D^{22}$ −10.6	0.7
9 *e*-2	60 (**5** *e*-2)	$[\alpha]_D^{22}$ −19.7	0.7
9 *t*-1	91	$[\alpha]_D^{20}$ 4.6	0.2
9 *t*-2	89	$[\alpha]_D^{22}$ −22.9	0.2
11 *e*-1, *e*-2	32 and 46 (**11** *t*-1, *t*-2)	$[\alpha]_D^{21}$ −52.1	0.9
11 *t*-1, *t*-2	41 and 57	$[\alpha]_D^{21}$ −42.3	0.5



Scheme 1 Formation of lignin–carbohydrate benzyl ether model compounds.

Analytical TLC was performed on silica gel 60 F_{254} plates (Merck) and compounds were visualized by charring (9:1 H_2SO_4–formalin) and heating. Silica gel 60, 230–400 mesh (Merck) was used for flash column chromatography. Semi-preparative HPLC experiments were carried out on a Waters liquid chromatograph consisting of a Waters 600E multisolvent delivery system and a Waters 996 photodiode array detector. Components were monitored by measuring the absorption at 260 nm. A Merck LiChrospher Si60 (5 μm) column and a Merck LiChrospher RP-18e (5 μm) column were used for HPLC separation. Mass spectra were run on a JEOL JMS-SX102 instrument. Optical rotations were recorded on a JASCO DIP-1000 polarimeter, with $[\alpha]_D$-values given in units of 10^{-1} deg cm^2 g^{-1}, and are given in Table 1.

NMR spectra (Tables 2–5) were recorded in [$^2H_6$]acetone

Table 2 ^{1}H NMR data for lignin–methyl xyloside and lignin–methyl arabinoxyloside benzyl ethers

	H-1 (1H)	H-2 (1H)	H-3 (1H)	H-4 (1H)	H-5 (2H)	H^{α} (1H)	H^{β} (1H)	H^{γ} (2H)	OCH_3 Ar	OCH_3 Xyl	Ar (7H)
4, *e*-1	4.14 (d, *J* 7.1)	3.23 (dd, *J* 6.8, 7.9)	3.28–3.33 [a]	3.46–3.52 [a]	3.13–3.19 (1H, [a]) 3.74–3.82 (1H, [a])	5.06 (d, *J* 6.4)	4.30 (dt, *J* 3.7, 6.5)	3.72–3.80 (1H, [a]) 3.96 (1H, dd, *J* 4.1, 12.1)	3.78 (s) 3.83 (s)	3.11 (s)	6.74–7.11
4, *e*-2	4.30 (d, *J* 7.0)	3.05 (dd, *J* 7.0, 8.7)	3.37–3.41 [a]	3.37–3.41 [a]	3.16 (1H, m) 3.72–3.85 (1H, [a])	5.13 (d, *J* 6.4)	4.33 (m)	3.72–3.85 (2H, [a])	3.77 (s) 3.82 (s)	3.39 (s)	6.73–7.32
4, *t*-1	4.13 (d, *J* 7.0)	3.14–3.20 [a]	3.46–3.56 (m)	3.46–3.56 [a]	3.14–3.20 (1H, [a]) 3.79 (1H, dd, *J* 4.8, 11.4)	5.12 (d, *J* 6.4)	4.39 (dt, *J* 4.5, 6.4)	3.38 (1H, m) 3.70 (1H, d, *J* 4.2, 11.9)	3.84 (s) 3.85 (s)	3.16 (s)	6.74–7.14
4, *t*-2	4.30 (d, *J* 7.1)	3.04 (dd, *J* 7.1, 8.6)	3.40–3.49 (m)	3.34–3.40 [a]	3.15 (1H, dd, *J* 9.1, 10.8) 3.72–3.82 (1H, [a])	5.21 (d, *J* 5.4)	4.35 (m)	3.38–3.46 (1H, [a]) 3.56 (1H, dd, *J* 4.1, 11.9)	3.81 (s) 3.84 (s)	3.41 (s)	6.75–7.28
6, *e*-1	4.10 (d, *J* 6.4)	3.26 (m)	3.34 (m)	3.73–3.81 [a]	3.23 (1H, dd, *J* 8.8, 11.3) 3.83–3.90 (1H, [a])	5.13 (d, *J* 6.9)	4.32 (m)	3.84–3.90 (1H, [a]) 3.96 (1H, dd, *J* 3.7, 12.0)	3.76 (s) 3.82 (s)	3.36 (s)	6.73–7.24
6, *e*-2	4.19 (d, *J* 6.2)	3.44–3.50 (m)	3.29 (m)	3.46–3.53 [a]	3.15 (1H, dd, *J* 8.8, 11.3) 3.76–3.80 (1H, [a])	5.09 (d, *J* 7.2)	4.35 (m)	3.76–3.80 (1H, [a]) 3.91 (1H, dd, *J* 4.2, 12.2)	3.74 (s) 3.81 (s)	3.40 (s)	6.71–7.26
6, *t*	4.06 (d, *J* 6.9) 4.13 (d, *J* 6.9)	3.16–3.22 [a] 3.35–3.43 [a]	3.29 (m)	3.46–3.53 [a] 3.66–3.72 [a]	3.12 (1H, dd, *J* 9.4, 11.4) 3.18 (1H, dd, *J* 9.5, 11.3) 3.74–3.84 (2H, [a])	5.21 (d, *J* 6.7) 5.25 (d, *J* 6.0)	4.41 (m)	3.40–3.50 (2H, [a]) 3.74–3.84 (2H, [a])	3.82 (s) 3.83 (s) 3.84 (s)	3.35 (s) 3.39 (s)	6.74–7.26
8, *e*-1	4.05 (d, *J* 7.4)	3.14 (dd, *J* 7.5, 8.9)	3.53 (m)	3.38 (m)	3.09 (1H, dd, *J* 9.7, 11.0) 3.47 (1H, dd, *J* 5.3, 11.1)	4.86 (d, *J* 7.7)	4.32 (dt, *J* 3.2, 7.7)	3.80–3.88 (1H, [a]) 3.96 (1H, dd, *J* 3.4, 12.3)	3.72 (s) 3.83 (s)	3.34 (s)	6.72–7.07
8, *e*-2	4.15 (d, *J* 6.8)	3.11 (dd, *J* 6.8, 8.2)	3.49 (m)	3.27–3.41 [a]	3.27–3.41 (1H, [a]) 4.08 (1H, m)	4.82 (d, *J* 5.8)	4.26–4.36 [a]	3.72 (1H, dd, *J* 3.7, 11.8) 3.75–3.81 (1H, [a])	3.79 (s) 3.83 (s)	3.38 (s)	6.73–7.30
8, *t*-1	4.05 (d, *J* 7.4)	3.08–3.17 [a]	3.44–3.55 [a]	3.30 (dd, *J* 5.1, 8.5)	3.08–3.17 (1H, [a]) 3.44–3.55 (1H, [a])	4.98 (d, *J* 6.3)	4.36 (m)	3.44–3.55 (1H, [a]) 3.74 (1H, dd, *J* 4.8, 11.8)	3.84 (s)	3.34 (s)	6.77–7.10
8, *t*-2	4.13 (d, *J* 6.9)	3.11 (m)	3.35–3.50 [a]	3.35–3.50 [a]	3.27 (1H, dd, *J* 8.7, 11.4) 4.05 (1H, dd, *J* 4.1, 11.3)	4.83 (d, *J* 6.5)	4.29 (dt, *J* 6.0, 4.0)	3.35–3.63 (2H, [a])	3.83 (s) 3.84 (s)	3.37 (s)	6.77–7.26
10, *e*						4.64 (d, *J* 6.2) 4.64 (d, *J* 6.4)	4.33 (m)	3.70–3.85 (2H, [a])	3.76 (s) 3.82 (s)		6.75–7.12
ara	5.29 (s), 5.31 (s)	4.04 (m)	3.93 (m)	4.23 (m)	3.46–3.65 (2H, [a])						
xyl	4.13 (d, *J* 7.4)	3.27 (m)	3.46–3.65 [a]	3.46–3.65 [a]	3.20 (1H, dd, *J* 9.9, 11.3) 3.80–3.88 (1H, [a])					3.41 (s)	
10, *t*						4.66 (d, *J* 5.9) 4.72 (d, *J* 5.4)	4.31 (m)	3.37–3.44 (1H, [a]) 3.65 (1H, dd, *J* 4.9, 11.5)	3.82 (s) 3.83 (s)		6.77–7.14
ara	5.28 (s), 5.35 (s)	4.00 (m)	3.93 (m)	4.23 (m)	3.54–3.61 (2H, [a])						
xyl	4.12 (d, *J* 7.4)	3.27 (m)	3.40–3.53 [a]	3.40–3.53 [a]	3.19 (1H, dd, *J* 9.7, 11.4) 3.81–3.88 (1H, [a])					3.41 (s) 3.41 (s)	

[a] Signals overlapped.

Table 3 ^{1}H NMR data for peracetates of lignin–methyl xyloside and lignin–methyl arabinoxyloside benzyl ethers

	H-1 (1H)	H-2 (1H)	H-3 (1H)	H-4 (1H)	H-5 (2H)	H^{α} (1H)	H^{β} (1H)	H^{γ} (2H)	OCH_3 Ar	OCH_3 Xyl	Ar	$(COCH_3)$
5, *e*-1	4.31 (d, *J* 6.7)	3.57 (dd, *J* 6.7, 8.5)	5.27 (m)	4.85 (dd, *J* 5.4, 8.6)	3.44 (1H, dd, *J* 7.1, 9.1) 3.92–3.99 (1H, [a])	5.07 (d, *J* 4.5)	4.52 (dt, *J* 4.0, 7.2)	4.25 (1H, dd, *J* 3.7, 11.8) 4.34 (1H, dd, *J* 6.2, 11.8)	3.81 (s) 3.82 (s)	3.41 (s)	6.69–7.29	1.88–2.21
5, *e*-2	4.44 (d, *J* 7.1)	3.32–3.42 [a]	5.06 (m)	4.63–4.77 [a]	3.32–3.42 (1H, [a]) 3.94 (1H, dd, *J* 5.5, 11.5)	4.94 (d, *J* 6.2)	4.63–4.77 [a]	4.24–4.35 (2H, m)	3.71 (s) 3.78 (s)	3.49 (s)	6.77–7.23	1.91–2.21
5, *t*-1	4.34 (d, *J* 7.0)	3.64 (dd, *J* 6.9, 8.9)	5.19 (m)	4.83 (m)	3.41 (1H, dd, *J* 9.0, 11.7) 3.94 (1H, dd, *J* 6.4, 11.7)	5.11 (d, *J* 5.1)	4.62 (m)	3.95 (1H, dd, *J* 5.3, 11.7) 4.12 (1H, dd, *J* 3.9, 11.7)	3.82 (s) 3.85 (s)	3.14 (s)	6.82–7.24	1.84–2.22
5, *t*-2	4.49 (d, *J* 7.2)	3.35–3.41 [a]	5.15 (m)	4.70 (m)	3.35–3.41 (1H, [a]) 3.92–4.00 (1H, [a])	5.07 (d, *J* 4.8)	4.73 (m)	3.92–4.00 (1H, [a]) 4.21–4.28 (1H, [a])	3.80 (s) 3.82 (s)	3.47 (s)	6.82–7.18	1.90–2.21
7, *e*-1	4.34 (d, *J* 6.4)	4.81 (dd, *J* 6.3, 7.9)	3.80–3.86 [a]	4.92 (m)	3.38 (1H, dd, *J* 8.0, 11.9) 4.09 (1H, dd, *J* 4.8, 11.8)	5.04 (d, *J* 4.6)	4.59 (dt, *J* 4.4, 6.3)	4.22 (1H, dd, *J* 4.2, 11.7) 4.32 (1H, dd, *J* 6.2, 11.7)	3.78 (s) 3.82 (s)	3.33 (s)	6.79–7.17	1.89–2.22
7, *e*-2	4.31 (d, *J* 7.0)	4.93 (dd, *J* 6.5, 8.1)	3.78–3.88 [a]	4.81 (dd, *J* 7.2, 8.5)	3.24–3.36 (1H, [a]) 3.94–4.06 (1H, [a])	5.07 (d, *J* 5.8)	4.51 (dt, *J* 3.9, 6.7)	4.19 (1H, d, *J* 3.8) 4.30 (1H, dd, *J* 6.7, 11.8)	3.81 (s) 3.84 (s)	3.32 (s)	6.82–7.23	1.89–2.23
7, *t*-1, *t*-2	4.43 (d, *J* 6.5) 4.48 (d, *J* 6.3)	5.00 (dd, *J* 6.4, 7.8)	3.78–3.88 [a]	4.81 (dd, *J* 7.2, 8.5)	3.24–3.36 (2H, [a]) 3.94–4.06 (2H, [a])	5.04 (d, *J* 6.3) 5.05 (d, *J* 5.3)	4.56–4.67 [a]	3.86–3.96 (2H, [a]) 4.06–4.16 (2H, [a])	3.82 (s) 3.83 (s) 3.84 (s) 3.85 (s)	3.37 (s) 3.39 (s)	6.82–7.23	1.86–2.22
9, *e*-1	4.40 (d, *J* 7.5)	4.74 (dd, *J* 7.5, 9.1)	5.17 (m)	3.76–3.84 [a]	3.25 (1H, dd, *J* 9.6, 11.7) 3.66 (1H, dd, *J* 5.3, 11.7)	4.91 (d, *J* 5.5)	4.57 (dt, *J* 5.7, 4.0)	4.29 (2H, m)	3.77 (s) 3.82 (s)	3.34 (s)	6.76–7.21	1.91–2.22
9, *e*-2	4.48 (d, *J* 7.6)	4.65 (dd, *J* 7.8, 9.5)	5.11 (m)	3.70–3.78 [a]	3.51 (1H, dd, *J* 10.1, 11.6) 4.42 (1H, dd, *J* 5.3, 11.5)	4.96 (d, *J* 5.2)	4.60 (m)	4.30 (2H, m)	3.80 (s) 3.81 (s)	3.41 (s)	6.76–7.12	1.88–2.21
9, *t*-1	4.41 (d, *J* 7.6)	4.70 (dd, *J* 7.5, 9.3)	5.15 (m)	3.70–3.80 [a]	3.31 (1H, m) 3.70–3.80 (1H, [a])	4.93 (d, *J* 5.7)	4.58 (dt, *J* 5.7, 4.1)	3.96 (1H, dd, *J* 5.9, 11.7) 4.13 (1H, dd, *J* 4.2, 11.7)	3.81 (s) 3.82 (s)	3.35 (s)	6.79–7.29	1.86–2.22
9, *t*-2	4.41 (d, *J* 7.7)	4.65 (dd, *J* 7.7, 9.6)	5.03 (m)	3.69 (dd, *J* 5.3, 9.1)	3.33–3.40 (1H, [a]) 4.27 (1H, dd, *J* 6.6, 7.7)	4.85 (d, *J* 6.8)	4.61 (m)	3.89 (1H, dd, *J* 5.4, 11.8) 4.10 (1H, dd, *J* 4.2, 11.9)	3.82 (s) 3.83 (s)	3.38 (s)	6.82–7.79	1.89–2.22
11, *e*						4.76 (d, *J* 5.5)	4.62 (m)	4.31 (1H, dd, *J* 3.5, 6.6)	3.76 (s)		6.77–7.28	1.92–2.22
ara	5.13 (s)	4.83–4.90 [a]	5.11 (m)	4.22 (m)	3.69 (4H, m)	4.77 (d, *J* 5.3)		4.35 (1H, dd, *J* 3.5, 6.4)	3.77 (s)			
xyl	4.41 (d, *J* 7.5)	4.83–4.90 [a]	3.86–4.01 [a]	4.83–4.90 [a]	3.35 (2H, dd, *J* 3.9, 9.5) 3.86–4.01 (2H, [a])			4.41–4.45 (1H, [a]) 4.45 (1H, dd, *J* 3.5, 5.8)	3.82 (s) 3.83 (s)	3.38 (s)		
11, *t*						4.82 (m)	4.64 (m)	4.05 (2H, dd, *J* 6.3, 11.6)	3.81 (s)		6.80–7.28	1.91–2.22
ara	5.11 (s)	4.83–4.89 [a]	5.05 (m)	4.20 (m)	3.69 (4H, m)			4.28 (2H, dd, *J* 4.2, 11.7)	3.81 (s)			
xyl	4.40 (d, *J*, 7.5)	4.83–4.89 [a]	3.90 (m)	4.83–4.89 [a]	3.30 (2H, m) 3.97 (2H, dd, *J* 5.4, 11.6)				3.82 (s) 3.83 (s)	3.38 (s) 3.38 (s)		

[a] Signals overlapped.

Table 4 ^{13}C NMR data for lignin–methyl xyloside and lignin–methyl arabinoxyloside benzyl ethers

Compound	C-1	C-2	C-3	C-4	C-5	C^{α}	C^{β}	C^{γ}	OCH_3		Ar (12C)
									Ar	Xyl	
4, *e*-1	104.9	81.6	78.7	70.8	66.0	82.6	85.5	61.3	56.2	56.3	112.2–151.0
4, *e*-2	105.9	78.1	76.1	70.7	66.1	80.8	86.0	62.2	56.2	56.4	113.0–151.7
4, *t*-1	104.7	81.5	77.7	70.5	66.1	82.7	85.3	61.0	56.1, 56.2	56.3	112.4–151.4
4, *t*-2	106.0	78.5	75.2	70.7	66.1	80.6	86.0	62.1	56.2, 56.3	56.4	112.7–151.4
6, *e*-1	105.1	72.7	82.5	70.8	65.4	81.9	85.4	61.4	56.2	56.3	112.3–151.7
6, *e*-2	104.8	73.6	81.6	69.6	65.1	81.8	85.3	61.8	56.1	56.4	112.4–151.5
6, *t*-1, *t*-2	105.1, 105.4	73.0, 74.8	83.4, 83.7	69.6, 71.4	65.7, 65.9	82.2, 82.6	85.4	61.4, 61.6	56.1, 56.4	56.6	112.4–151.1
8, *e*-1	105.4	74.3	77.3	78.7	65.0	82.9	84.7	61.6	56.3	56.5	112.3–151.7
8, *e*-2	105.3	74.0	75.3	76.0	63.0	79.5	85.8	61.4	56.2	56.4	112.2–151.7
8, *t*-1	105.4	74.1	77.5	78.7	64.9	83.4	85.1	61.4	56.1, 56.2	56.5	112.1–151.0
8, *t*-2	105.4	74.0	75.4	76.2	63.0	80.2	86.3	61.8	56.2, 56.3	56.4	112.3–151.5
10, *e*						82.1	85.2, 85.3	61.5	56.3		112.2–151.8
ara	109.5	82.1	79.0	84.7, 85.0	70.0, 70.2						
xyl	105.7	74.2	82.3	69.6	66.3					56.7	
10, *t*						82.2, 82.5	85.7, 86.1	61.5	56.2, 56.3		111.9–151.7
ara	109.4, 109.6	81.6	79.1	85.1, 85.5	70.0, 70.1						
xyl	105.8	74.4, 74.5	81.9, 82.0	69.7	66.3					56.7	

with the central solvent peak serving as the internal reference (δ_H 2.04, δ_C 29.8) with Varian Inova 300 MHz instrument; *J*-values are given in Hz. The spectra were measured at 27 °C, non-spinning. Samples with multiplicity problems with OH–OD exchange were exchanged with D_2O prior to measurements. Inverse-detected ^1H–^{13}C correlation HMQC spectra were measured according to the method of Summers *et al.*[21] The delay for polarization transfer between ^{13}C and ^1H was set for an assumed $^1J_{C-H} = 140$ Hz and a relaxation delay of 0.9 s was used between scans. The spectral width in F2 was set to 3 kHz and to 18 kHz in F1. GARP-1 decoupling was used in the ^{13}C channel during acquisition. 128 or 160 Time increments and 32 scans per increment were collected by the hypercomplex method. The spectra were processed using 90°-shifted squared sinebell functions in both domains prior to Fourier transformation. Homonuclear Hartman–Hahn spectra, HOHAHA, were recorded using the method of Griesinger *et al.*[22] The spectral width was 3 kHz (F1 = F2). A relaxation delay of 2.0 s was used between scans. Spin-lock periods were 30–120 ms (MLEV-17). 160 Time increments and 16 or 32 scans per increment were collected by the hypercomplex method. The spectra were processed using 90°-shifted squared sinebell functions in both domains prior to Fourier transformation. Homonuclear nuclear Overhauser enhancement (NOESY) spectra were recorded using the standard NOESY pulse sequence on the Varian V-NMR software. The spectral width was 2 kHz (F1 = F2). A relaxation delay of 2.0 s was used between scans. Mixing time was 1.0 s. 128 Time increments and 32 scans per increment were collected by the hypercomplex method. The spectra were processed using 90°-shifted squared sinebell functions in both domains prior to Fourier transformation.

Methyl 2,4-di-*O*-benzyl-3-*O*-(2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl)-β-D-xylopyranoside

Methyl 2,4-di-*O*-benzyl-β-D-xylopyranoside (4.62 mmol) was dissolved in methylene dichloride (10 ml). Powdered 4 Å molecular sieves (2 g) were added and the solution was cooled to 0 °C. 2,3,5-Tri-*O*-benzoyl-α-L-arabinofuranosyl bromide (6.23 mmol) was added in methylene dichloride (5 ml), followed by silver triflate (10.85 mmol) and collidine (10.39 mmol).[17] Stirring in argon was continued at 0–5 °C in the dark for 2.5 h. The mixture was filtered, and then washed successively with aq. $Na_2S_2O_3$ and aq. NH_4Cl. Drying (Na_2SO_4) and evaporation gave a foamy solid. Purification by silica gel chromatography using 7 : 1 toluene–ethyl acetate as eluent gave, in 70–80% yield, methyl 2,4-di-*O*-benzyl-3-*O*-(2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl)-β-D-xylopyranoside.

Methyl 3-*O*-(α-L-arabinofuranosyl)-β-D-xylopyranoside 3

Methyl 2,4-di-*O*-benzyl-3-*O*-(2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl)-β-D-xylopyranoside was debenzoylated with sodium methoxide in methanol. After 2 h the reaction mixture was quenched with ion-exchange resin (Amberlite IR-120, H^+ form), filtered and evaporated. Purification on silica gel with 7 : 1 toluene–ethyl acetate and 6 : 1 methylene dichloride–methanol gave methyl 3-*O*-(α-L-arabinofuranosyl)-2,4-di-*O*-benzyl-β-D-xylopyranoside. Hydrogenolysis over palladium (10%) on carbon in acetic acid–methanol–water (10 : 9 : 1) for 6 h gave methyl 3-*O*-(α-L-arabinofuranosyl)-β-D-xylopyranoside in 90–95% yield, δ_C (75 MHz) 56.7 (OCH_3, Xyl), 62.7 (Ara-5), 66.3 (Xyl-5), 69.6 (Xyl-4), 74.4 (Xyl-2), 78.6 (Ara-3), 81.4 (Ara-2 and Xyl-3), 87.3 (Ara-4), 105.7 (Xyl-1) and 109.3 (Ara-1).

Formation of LCC model compounds[8]

The quinone methide of 1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol (1 mol equiv.), a monosaccharide or disaccharide (5 mol equiv.) and PTSA (0.1 mol equiv.) were dissolved in dry DMF (5 ml) and the mixture was stirred in argon for 4 h. The reaction mixture was poured into distilled water. LCC and unchanged lignin model were extracted with chloroform, and the extract was dried over Na_2SO_4 and evaporated to dryness. TLC indicated that water phases contained monosaccharide or disaccharide only. The yield of methyl xyloside LCC model compounds was 48–56% and that of methyl arabinoxyloside LCC model compounds 30–36%.

Purification of LCC models

Methyl xyloside LCC model compounds were fractionated on silica gel using methylene dichloride–methanol gradient starting from 3% methanol and ending at 10%. The *erythro*-diastereomers of the C-3 adduct eluted first on silica gel, followed by the *threo*-diastereomers of the same product. The *erythro*- and *threo*-diastereomers of the C-2 and C-4 adducts eluted as a mixture. Flash fractions typically were composed of 2–4 isomers, which were purified further by HPLC RP-18 column using methanol–water (pH 6) gradient as mobile phase. Combination of flash and HPLC chromatography enabled the separation of LCC models as individual diastereomers or a mixture of diastereomers.

Table 5 ^{13}C NMR and MS data for peracetates of lignin–methyl xyloside and lignin–methyl arabinoxyloside benzyl ethers

									OCH_3					
	C-1	C-2	C-3	C-4	C-5	C^{α}	C^{β}	C^{γ}	Ar	Xyl	Ar	($CO\underline{C}H_3$)	($\underline{C}OCH_3$)	HRMS: M^+
5, *e*-1	104.7	80.5	73.1	70.2	62.3	82.4	82.9	63.1	56.3, 56.4	56.5	112.9–152.1	20.5–20.6	168.9–170.7	634.2275
5, *e*-2	105.4	78.9	73.3	70.5	62.8	82.5	81.1	64.2	56.2, 56.4	56.8	113.6–152.4	20.5–20.8	168.9–170.8	634.2267
5, *t*-1	105.0	79.6	73.5	70.3	62.6	81.5	81.0	63.8	56.2, 56.2	56.7	112.6–151.8	20.5–20.6	168.9–170.7	634.2266
5, *t*-2	105.2	80.2	73.5	70.5	62.8	82.3	80.6	64.2	56.2	56.9	113.2–151.8	20.4–20.6	169.0–170.7	634.2271
7, *e*-1	102.2	72.2	78.3	71.6	61.8	82.8	82.5	63.4	56.2	56.1	112.8–152.0	20.5–21.0	169.0–170.8	634.2265
7, *e*-2	102.6	71.8	79.4	72.1	61.7	83.2	83.4	63.1	56.1, 56.2	56.3	112.4–152.2	20.5–20.9	169.0–170.8	634.2263
7, *t*-1, *t*-2	102.2	71.5	79.6	72.4	62.0	82.3	81.5	63.9	56.1, 56.2	56.3	111.9–152.2	20.5–20.9	169.0–170.8	634.2280
	102.3		80.0	72.5	62.3									
9, *e*-1	102.4	72.2	74.4	76.9	64.5	82.4	82.2	63.6	56.0, 56.2	56.6	112.7–152.2	20.4–20.9	169.0–171.0	634.2246
9, *e*-2	102.7	72.5	74.6	77.8	64.2	82.7	82.0	62.9	56.2	56.6	112.5–152.0	20.4–20.6	169.0–170.8	634.2267
9, *t*-1	102.4	72.4	74.6	76.6	64.6	82.6	81.3	63.7	56.2, 56.3	56.6	112.9–152.1	20.5–20.7	168.9–170.8	634.2255
9, *t*-2	102.6	72.4	74.3	77.4	64.2	83.4	81.0	63.3	56.2	56.6	112.9–152.5	20.4–20.6	169.0–170.9	634.2278
11, *e*						82.2	82.0	63.7	56.2		112.6–152.2	20.5–21.1	168.9–170.9	LRMS: M^+
ara	107.3	82.4	77.8	82.9	69.7, 69.8									850
xyl	102.8	73.1	77.9	70.5	63.1, 63.5					56.5				
11, *t*						82.4	81.5	64.1	56.2		112.7–152.2	20.5–21.1	168.9–170.7	850
ara	107.3	82.0	77.7	82.9	69.8									
xyl	102.8	73.2	78.0	70.5	63.1					56.5				

LRMS: **11** *e*-1 and *e*-2; *m*/*z* (%) 42 (100), 641 (97), 209 (22), 139 (22), 231 (20), 447 (10), 850 (M^+ 8), 387 (4). LRMS: **11** *t*-1 and *t*-2; *m*/*z* (%) 42 (100), 641 (60), 209 (17), 139 (17), 231 (15), 447 (6), 850 (M^+ 5), 387 (3).

Methyl arabinoxyloside LCC model compounds containing 4 diastereomers were prepurified on silica gel with 12 : 1 methylene dichloride–methanol. Flash fractions were enriched to afford a pair of *erythro*- and *threo*-isomers by HPLC on a silica phase column using 4 : 1 methylene dichloride–propan-2-ol as eluent.

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