Synthesis and Rearrangement Reactions of Ester-Linked Lignin–Carbohydrate Model Compounds

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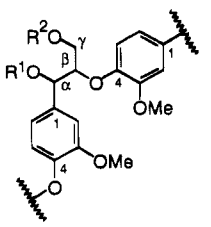
A series of ester-linked lignin–carbohydrate model compounds has been synthesized, which represent possible ester linkages of lignin to hemicellulose in wood through the carboxylic acid group of 4-*O*-methyl-α-D-glucopyranosiduronic acid moieties. The *threo* and *erythro* isomers of these compounds were prepared through an appropriate combination of protective and stereoselective transformations. Spectroscopic characterization of these esters revealed that the uronosyl group migrated between the primary (γ) and benzylic (α) positions of the lignin side chain in both acidic and neutral conditions. It has been determined that the migration equilibrium favors γ- over α-esters, with the reaction possibly proceeding through a six-membered ring intermediate. That uronosyl migrations occur favoring the γ-position suggests that the classical view of lignin–xylan ester linkages in wood needs to be reevaluated.

Keywords: *Acyl migration; lignin–carbohydrate complexes; NMR*

INTRODUCTION

Lignin and hemicellulose are theorized to be intimately associated with one another through covalent and noncovalent interactions (Fengel and Wegener, 1989). One type of covalent linkage thought to be present is an ester linkage between a xylan uronic acid moiety and the lignin polymer. Since the principal hemicellulose of temperate zone hardwoods is *O*-acetyl-(4-*O*-methylglucurono)xylan (Timell, 1967), for such a linkage to occur the uronosyl moiety of this polymer would be esterified to a lignin hydroxyl. Indeed, evidence has been reported for the existence of the ester linkages between lignin and 4-*O*-methyl-α-D-glucopyranosiduronic acid in lignin–carbohydrate complexes (LCC) isolated from hardwoods (Takahashi and Koshijima, 1988; Watanabe and Koshijima, 1988; Imamura *et al.*, 1994).

Results from these investigations support ester linkages between lignin and hemicellulose which form in accordance with the classical hypothesis of lignin biosynthesis (Freudenburg, 1968). In this scenario, lignin quinone methide intermediates undergo a nucleophilic attack by the carboxylic acid group of a uronic acid, affording a benzylic ester, or the so called α-ester (structure A). In contrast to this result, it has been



Structure	R^1	R^2
A	acyl	H
B	H	acyl
C	H	H

reported that maize rind lignin contains substantial amounts of *p*-coumaric acid esterified exclusively at the primary position of the lignin propyl side chain (γ-ester; structure B) (Ralph *et al.*, 1994). This linkage cannot be explained by a quinone methide mechanism, implying a distinct biologically controlled pathway may be responsible. If such a process exists in maize, it is also possible that lignin–hemicellulose ester linkages are under some level of enzymatic control, affording linkages such as structure B.

In addition to this regiochemical issue, stereochemical questions pertaining to lignin–polysaccharide structure have yet to even be considered. The most abundant lignin structural unit, the β-*O*-4 (structure C), has two chiral centers in the propyl side chain and hence can exist as a pair of diastereomers: *erythro* and *threo*. It has been reported that almost equal amounts of *erythro* and *threo* isomers exist in softwood spruce lignin, whereas *erythro* isomers are predominant in hardwood lignin (Lundquist, 1980; Nimz *et al.*, 1984; Hauteville *et al.*, 1986). It has been reported that various stereoselectivities were observed in the reaction between several carboxylic acids and a β-*O*-4 quinone methide (Sipilä and Brunow, 1991; Ralph and Young, 1983; Nakatsubo *et al.*, 1976). Whether or not an α-ester linkage between the naturally abundant 4-*O*-methyl-α-D-glucopyranosiduronic acid moiety of hardwood xylans and the lignin β-*O*-4 α-position has a stereochemical preference is not known.

In efforts to elucidate these regiochemical and stereochemical questions, we have prepared several α- and γ-ester model compounds between methyl 4-*O*-methyl-α-D-glucopyranosiduronic acid and the *threo* and *erythro* isomers of the lignin dimer, 1-[4-(benzyloxy)-3-methoxyphenyl]-3-hydroxy-2-(2-methoxyphenoxy)propan-1-ol. During the course of this work, we observed migration of the 4-*O*-methyl α-D-glucopyranosiduronate group between α- and γ-hydroxyl groups of the lignin dimer. The chemical aspects of the preparation of these compounds, their spectroscopic characterization, and the kinetics of the migration are presented here, as well as the implications these results have on our understanding of lignin–xylan ester linkages in hardwoods.

EXPERIMENTAL PROCEDURES

Moisture sensitive reactions were performed under an atmosphere of dry nitrogen, and evaporations were performed

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under reduced pressure at temperatures not exceeding 42 °C. Standard processing implies drying of the organic solvent with either Na_2SO_4 or $MgSO_4$, filtration, and evaporation of the solvent under diminished pressure. Trace solvent removal was accomplished under higher vacuum (25–75 mTorr). The NMR spectra were recorded at 400 MHz (Varian Unity 400) at 27 °C unless indicated otherwise, and chemical shifts (ppm) are relative to the central solvent peak of acetone-d_6 (^{13}C, 29.8 ppm; ^1H, 2.04 ppm). Thin-layer chromatography (TLC) was performed with AlugramSil-G/UV_{254} plates (Macherey-Nagel) with UV light or by charring (5% H_2SO_4 in 95% EtOH). Column chromatography was with silica gel 60 (230–400 mesh, Whatman) using standard flash chromatography (Ace Glass).

Methyl (Methyl 4-*O*-methyl-α-D-glucopyranosid)uronate (2). Diazomethane in Et_2O (Furniss *et al.*, 1989) was added to the solution of **1** (0.560 g, 2.52 mmol; Li and Helm, 1995) in methanol until the yellow color of diazomethane persisted, at which time the excess diazomethane was destroyed with acetic acid. The solution was evaporated to a syrup and purified by silica gel chromatography ($CHCl_3$/EtOAc 1:1) to afford **2** (0.554 g, 2.34 mmol) in 93% yield.

Methyl [Methyl 2,3-di-(*O*-triethylsilyl)-4-*O*-methyl-α-D-glucopyranosid]uronate (3). Chlorotriethylsilane (0.80 mL, 4.78 mmol) was added dropwise to a solution of **2** (0.418 g, 1.77 mmol) and imidazole (0.602 g, 8.85 mmol) in DMF (4 mL). The solution was stirred at room temperature (1 h) and then poured into a separatory funnel that contained Et_2O/aqueous $NaHCO_3$ (30:30 mL). The aqueous phase was extracted with Et_2O (4 × 20 mL), and the combined ether phases were processed in a standard fashion to afford a thin syrup which was purified by silica gel chromatography (hexane/EtOAc 15:1) to yield **3** (0.699 g, 1.50 mmol, 85%): NMR (acetone-d_6, ambient temperature) δ_H 0.67 [12H, q, J = 8.2 Hz, $Si(CH_2CH_3)_3$], 0.98 [9H, t, J = 7.9 Hz, $Si(CH_2CH_3)_3$], 0.99 [9H, t, J = 7.9 Hz, $Si(CH_2CH_3)_3$], 3.19 (1H, dd, $J_{3,4}$ = 8.7 Hz, $J_{4,5}$ = 9.9 Hz, H-4), 3.35 (3H, s, 1-OCH_3), 3.36 (3H, s, 4-OCH_3), 3.54 (1H, dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 9.2 Hz, H-2), 3.76 (3H, s, Ac), 3.80 (1H, t, $J_{3,4}$, $J_{2,3}$ = 8.9 Hz, H-3), 3.92 (1H, d, $J_{4,5}$ = 9.9 Hz, H-5), 4.64 (1H, d, $J_{1,2}$ = 3.5 Hz, H-1); δ_C 5.55, 5.81 [$Si(CH_2CH_3)_3$], 7.04, 7.28 [$Si(CH_2CH_3)_3$], 52.58 (6-OCH_3), 55.53 (1-OCH_3), 60.72 (4-OCH_3), 71.14 (C-5), 74.56 (C-2), 74.91 (C-3), 83.30 (C-4), 101.40 (C-1), 170.71 (C-6).

Methyl 2,3-Di-(*O*-triethylsilyl)-4-*O*-methyl-α-D-glucopyranosiduronic Acid (4). To a solution of **3** (0.097 g, 0.209 mmol) in methanol (2 mL) was added aqueous NaOH (1 N, 0.42 mL, 0.42 mmol). The mixture was stirred until the starting material had disappeared (monitored by TLC), at which time the solution was poured into a separatory funnel that contained a mixture of Et_2O (20 mL) and cold 0.05 N HCl (70 mL). The aqueous phase was extracted with Et_2O (3 × 20 mL), and the combined ether phase was washed with water (1×) and processed in a standard fashion to afford **4** (0.088 g, 0.195 mmol, 94%). Crude **4** was submitted to the subsequent coupling reactions without further purification.

***erythro*-1-[4-(Benzyloxy)-3-methoxyphenyl]-3-(benzoyloxy)-2-(2-methoxyphenoxy)propan-1-ol (7e).** Compound **5** (0.190 g, 0.37 mmol) was dissolved in EtOAc (4 mL) and cooled in an ice–water bath. Ethereal $Zn(BH_4)_2$ (5 mL; Helm and Ralph, 1993) was added, and the reaction was monitored by TLC ($CHCl_3$/EtOAc 19:1). When the reaction was complete, aqueous NH_4Cl (1 mL) was added to quench the reductant. The solution was diluted with EtOAc and washed with aqueous NH_4Cl (3×). Standard processing and silica gel chromatography ($CHCl_3$/EtOAc 19:1) afforded **7e** (0.173 g, 0.337 mmol, 91%) with >90% *erythro* selectivity.

***erythro*-1-[4-(Benzyloxy)-3-methoxyphenyl]-3-(benzoyloxy)-1-(1-ethoxy)ethoxy-2-(2-methoxyphenoxy)propane (8e).** Compound **7e** was dissolved in CH_2Cl_2 (5 mL) and cooled in an ice–water bath. Ethyl vinyl ether (0.16 mL, 1.67 mmol) was added and followed by adding a trace amount of *p*-toluenesulfonic acid monohydrate (Helm and Ralph, 1993). The reaction was stirred for 1 h (TLC indicated that the reaction was complete in 10 min), and aqueous $NaHCO_3$ was added to quench the reaction. The solution was diluted with EtOAc and washed with water (2×). Standard processing provided **8e** (0.192 g, 0.33 mmol, 99%), which was submitted to subsequent reactions without further purification.

***erythro*-1-[4-(Benzyloxy)-3-methoxyphenyl]-1-(1-ethoxy)ethoxy-2-(2-methoxyphenoxy)propan-3-ol (9e).** A solution of **8e** (0.192 g, 0.327 mmol) and sodium methoxide (10 mg) in methanol (10 mL) was stirred overnight at room temperature. The solution was poured into a separatory funnel which contained aqueous NH_4Cl (30 mL) and $CHCl_3$ (30 mL). The aqueous phase was extracted with $CHCl_3$ (3 × 30 mL), and the combined $CHCl_3$ layer was washed with water (1×) and processed in standard fashion. Trace methyl benzoate was removed by three additions and evaporations of water to the syrup. The syrup was subsequently dried under high vacuum to give **9e** (0.155 g, 0.321 mmol, 98%).

***threo*-3-Acetoxy-1-[4-(benzyloxy)-3-methoxyphenyl]-1-(1-ethoxy)ethoxy-2-(2-methoxyphenoxy)propane (11t).** The protection of the α-hydroxyl in **10t** (0.207 g, 0.458 mmol) was accomplished as described for **8e**, to afford **11t** as a syrup (0.238 g, 0.454 mmol, 99%).

***threo*-1-[4-(Benzyloxy)-3-methoxyphenyl]-1-(1-ethoxy)ethoxy-2-(2-methoxyphenoxy)propan-3-ol (12t).** Deacetylation of **11t** (0.210 g, 0.400 mmol), as described for **9e**, gave **12t** quantitatively.

***erythro*-3-[4-(Benzyloxy)-3-methoxyphenyl]-3-hydroxy-2-(2-methoxyphenoxy)propyl (Methyl 4-*O*-methyl-α-D-glucopyranosid)uronate (16e).** Dicyclohexylcarbodiimide (DCC, 0.047 g, 0.228 mmol) was added to a solution of **4** (0.051 g, 0.113 mmol) in dry CH_2Cl_2 (distilled from CaH_2, 1 mL), followed successively by a solution of **9e** (0.0656 g, 0.136 mmol) in dry CH_2Cl_2 (1 mL) and 4-(dimethylamino)pyridine (DMAP) (0.028 g, 0.229 mmol). The reaction was stirred overnight, subsequently filtered through Celite, and washed with $CHCl_3$. The filtrate was processed to give crude **15e**, which was dissolved in ethanolic 1% HCl (2 mL) and stirred for 0.5 h at room temperature. The reaction was terminated by adding saturated aqueous $NaHCO_3$, and the neutralized reaction mixture was transferred to a separatory funnel, diluted with brine (20 mL), and extracted with $CHCl_3$ (4 × 30 mL). The combined $CHCl_3$ layer was processed in a standard fashion to give a syrup. The syrup was purified with preparative TLC (hexane/*i*-PrOH 2:1) to afford **16e** (0.050 g, 0.081 mmol, 72% based on **4**).

***erythro*-3-Acetoxy-3-[4-(benzyloxy)-3-methoxyphenyl]-2-(2-methoxyphenoxy)propyl (Methyl 2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosid)uronate (17e).** Acetic anhydride (0.0498 g, 0.046 mL, 0.488 mmol) and DMAP (0.0143 g, 0.117 mmol) were added to a solution of **16e** (0.020 g, 0.0325 mmol) in dry CH_2Cl_2 (1 mL). The reaction was stirred overnight, diluted with $CHCl_3$, washed with cold 3% HCl (2×) and water (1×), and subsequently processed in standard fashion to afford **17e** (0.0229 g, 0.0309 mmol, 95%).

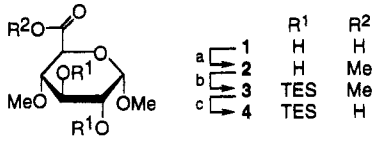
***threo*-3-[4-(Benzyloxy)-3-methoxyphenyl]-3-hydroxy-2-(2-methoxyphenoxy)propyl (Methyl 4-*O*-methyl-α-D-glucopyranosid)uronate (16t).** The reaction of **4** (0.0926 g, 0.2054 mmol) with **12t** (0.1190 g, 0.2465 mmol), as described for **15e**, gave crude **15t**, which was deprotected, as described for **16e**, to afford **16t** as a syrup (0.0858 g, 0.1397 mmol, 68% based on **4**).

***threo*-3-Acetoxy-3-[4-(benzyloxy)-3-methoxyphenyl]-2-(2-methoxyphenoxy)propyl (Methyl 2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosid)uronate (17t).** Compound **16t** (0.0289 g, 0.047 mmol) was peracetylated with acetic anhydride (0.072 g, 0.067 mL, 0.71 mmol) and DMAP (0.021 g, 0.17 mmol), as described for **17e**, to afford **17t** (0.0327 g, 94%).

***erythro*-1-[4-(Benzyloxy)-3-methoxyphenyl]-3-hydroxy-2-(2-methoxyphenoxy)propyl (Methyl 4-*O*-methyl-α-D-glucopyranosid)uronate (21e).** The reaction of **4** (0.0496 g, 0.1100 mmol) with **13e** (0.0637 g, 0.1320 mmol; Helm and Ralph, 1993), as described for **15e**, gave crude **18e**, which was deprotected for 20 min, as described for **16e**, to afford **21e** (0.0282 g, 0.0459 mmol, 42%) and **16e** (0.0188 g, 0.0306 mmol, 28%). The overall yield of **21e** and **16e** was 70%.

***erythro*-3-Acetoxy-1-[4-(benzyloxy)-3-methoxyphenyl]-2-(2-methoxyphenoxy)propyl (Methyl 2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosid)uronate (22e).** Compound

Scheme 1. Synthesis of the Uronic Acid Portion of the LCC Models[a]



[a] a, CH_2N_2/MeOH; b, TES-Cl/imidazole; c, 1N, NaOH(aq) in MeOH.

(**21e**) (0.0282 g, 0.0459 mmol) was peracetylated, as described for **17e**, to afford **22e** (0.0313, 0.0423 mmol, 92%).

***threo*-1-[4-(Benzyloxy)-3-methoxyphenyl]-3-chloroacetoxy-2-(2-methoxyphenoxy)propyl (Methyl 4-*O*-methyl-α-D-glucopyranosid)uronate (20t).** The reaction of **4** (0.0851 g, 0.1888 mmol) with **14t** (0.1104 g, 0.2266 mmol; Helm and Li, 1995), as described for **15e**, gave crude **19t**, which was purified with silica gel chromatography (hexane/EtOAc 2:1) to afford **19t** (0.1077 g, 0.117 mmol, 62%). The desilylation of **19t** (0.0910 g, 0.0989 mmol) was accomplished as described for **16e**, to afford crude **20t**, which was subsequently purified by preparative TLC (EtOAc) to give **20t** (0.0581 g, 0.0841 mmol, 85%).

***threo*-1-[4-(Benzyloxy)-3-methoxyphenyl]-3-hydroxy-2-(2-methoxyphenoxy)propyl (Methyl 4-*O*-methyl-α-D-glucopyranosid)uronate (21t).** The mixture of **20t** (0.0550 g, 0.0796 mmol) and thiourea (0.024 g, 0.318 mmol) in EtOH (95%, 2 mL) was stirred at 70 °C for 2 h. The reaction mixture was diluted with water and then extracted with $CHCl_3$ (4×). The combined chloroform solution was processed in a standard fashion to give a syrup which was purified with preparative TLC (EtOAc) to give **21t** (0.0323 g, 0.0525 mmol, 66%).

***threo*-3-Acetoxy-1-[4-(benzyloxy)-3-methoxyphenyl]-2-(2-methoxyphenoxy)propyl (Methyl 2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosid)uronate (22t).** Compound **21t** (0.0252 g, 0.0410 mmol) was peracetylated, as described for **17e**, to afford **22e** (0.0279, 0.0423 mmol, 92%).

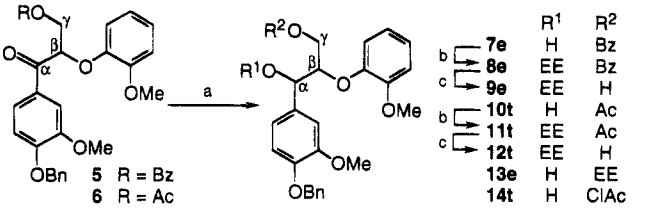
Quinone Methide Reactions. 1-(4-Hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol (0.51 g, 0.158 mmol) was converted to the quinone methide in $CHCl_3$ (8 mL) as described by Ralph and Young (1983). The solution was dried and filtered (total volume ca. 15 mL of $CHCl_3$), and a solution of **1** (0.090 g, 0.403 mmol) in DMF (1.5 mL) was added. The mixture was kept in the dark at room temperature for 4 days and subsequently evaporated under reduced pressure to remove the $CHCl_3$ and under high vacuum to remove the DMF. The syrup was purified by preparative TLC ($CHCl_3$/MeOH 6:1) to afford a *threo:erythro* mixture of 1-(4-hydroxy-3-methoxyphenyl)-3-hydroxy-2-(2-methoxyphenoxy)propyl (methyl 4-*O*-methyl-α-D-glucopyranosid)uronate (0.022 g, 0.042 mmol, 26%). In a separate experiment the above procedure was followed except that 1,8-bis(dimethylamino)naphthalene (Proton-Sponge, 2 mg, 0.0098 mmol) was added to the uronic acid/quinone methide solution and the reaction was stopped after 2 days. The yield of the α-ester was increased slightly (33%) and the purified product was a 1:1 mixture of *threo:erythro* isomers.

RESULTS AND DISCUSSION

Synthetic Aspects and Spectroscopic Characterization. The carbohydrate portion of the lignin–carbohydrate esters was prepared as shown in Scheme 1. Compound **1** (Li and Helm, 1995) was reacted with diazomethane in methanol to afford **2**. Subsequently, silylation with chlorotriethylsilane using imidazole as the base produced **3** (Berkowitz *et al.*, 1993). The target uronic acid **4** was prepared by saponification of **3** with 1 N NaOH followed by neutralization with 0.05 N HCl. Crude **4** was submitted to the subsequent coupling reactions without further purification.

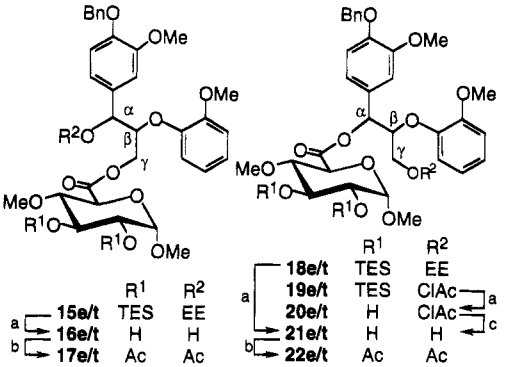
The lignin portion was prepared in the following way (Scheme 2). Compound **5** was reduced with zinc borohydride (Helm and Ralph, 1993) to afford the *erythro*

Scheme 2. Synthesis of the Lignin Portion of the LCC Models[a]



[a] a, $Zn(BH_4)_2$ or DIP-Cl; b, ethyl vinyl ether/*p*-TsOH; c, NaOMe/MeOH.

Scheme 3. Preparation of the LCC Models[a]



[a] a, ethanolic 1% HCl; b, Ac_2O/DMAP; c, thiourea/95% EtOH, 70 °C.

isomers **7e** in 91% yield. A benzoate protecting group was used instead of acetate to avoid acyl migration from the γ-hydroxyl to the α-hydroxyl during zinc borohydride reduction. Protection of the α-hydroxyl in **7e** with ethyl vinyl ether/*p*-toluenesulfonic acid cleanly produced **8e**. Debenzoylation of **8e** with sodium methoxide in methanol afforded **9e**, which was submitted to the coupling reaction without further purification. The *threo* isomers **10t** were prepared through the reduction of **6** with diisopinocampheylchloroborane (DIP-Cl) in THF (Helm and Li, 1995). As with the preparation of **8e** and **9e**, the ethoxyethylidenation of **10t** and subsequent deacetylation afforded **11t** and **12t**, respectively. Crude **12t** was submitted to the coupling step without further purification.

The lignin–carbohydrate esters were prepared by the coupling of lignin and carbohydrate moieties as depicted in Scheme 3. The DCC-mediated esterification (Hassner and Alexanian, 1978) of **4** with **9e**, **12t**, **13e** (Helm and Ralph, 1993), and **14t** (Helm and Li, 1995) afforded **15e**, **15t**, **18e**, and **19t**, respectively. These compounds were deprotected (ethanolic 1% HCl) without characterization to afford **16e**, **16t**, **21e**, and **20t**, respectively. The yield range of this two-step process was 53–72%. Dechloroacetylation of **20t** with thiourea in 95% ethanol produced **21t** in 66% yield (Cook and Maichuk, 1970). Peracetylation of **16e**, **16t**, **21e**, and **21t** with acetic anhydride and DMAP in CH_2Cl_2 afforded **17e**, **17t**, **22e**, and **26t**, respectively, in 92–95% yield.

Selected ^1H NMR data for the final products are shown in Table 1. Placement of an optically active unit (the uronic acid) on a pair of enantiomers (either the *threo* or *erythro* isomers) affords a mixture of diastereomers. Thus, the NMR spectra of the products show "peak doubling" due to the two diastereomers present. When extraction of spectroscopic data for each diastereomer was possible, the data are listed separately in Table 1. Assignment of the signals to individual diastereomers was not possible. For α-esters, the coupling

Table 1. Selected ^{1}H NMR Data for α- and γ-Esters and Their Corresponding Peracetates[a]

	γ-esters		α-esters		γ-esters OAc		α-esters OAc	
	16e	**16t**	**21e**	**21t**	**17e**	**17t**	**22e**	**22t**
α	4.96	4.96	6.06	6.11	6.05	6.11	6.14	6.14
($J_{\alpha,\beta}$)	(5.2)	(5.0)	(5.0)	(6.0)	(5.2)	(6.1)	(4.6)	(6.9)
α	4.96	4.97	6.06	6.13	6.06	6.13	6.14	6.16
($J_{\alpha,\beta}$)	(5.0)	(5.0)	(4.9)	(5.8)	(5.2)	(6.0)		(7.0)
β	4.64[b]	4.60	4.64[b]	4.60	4.90[b]	4.84[b]	4.90[b]	4.88
γ_1	4.43[c]	4.04	3.66[d]	3.47	4.43[c]	4.06	4.25	4.00
($J_{\gamma 1,\beta}$)		(6.0)		(5.0)		(5.4)	(4.0)	(5.3)
γ_1	4.43[c]	4.09	3.66[d]	3.50	4.43[c]	4.11	4.18	4.03
($J_{\gamma^1,\beta}$)		(6.3)		(5.1)		(5.5)	(4.3)	(5.4)
γ^2	4.43[c]	4.37	3.76[e]	3.67[f]	4.43[c]	4.39	4.25	4.23
($J_{\gamma^2,\beta}$)		(3.3)				(3.3)	(6.1)	(3.9)
γ^2	4.43[c]	4.41	3.76[e]	3.67[f]	4.43[c]	4.43	4.29	4.25
($J_{\gamma^2,\beta}$)		(3.4)				(3.4)	(6.2)	(4.0)
H-1	4.62[b]	4.65	4.64[b]	4.62	4.90[b]	4.92[b]	4.90[b]	4.87
($J_{1,2}$)	(3.7)	(3.7)		(3.7)		(3.4)		(3.7)
H-1	4.63	4.66	4.64[b]	4.64	4.90[b]	4.93	4.90[b]	4.90
($J_{1,2}$)	(3.7)	(3.7)		(3.5)		(3.5)		(3.5)
H-2	3.39[g]	3.43	3.39	3.40	4.74	4.74	4.65	4.73
($J_{2,3}$)		(10.2)	(9.7)		(10.3)	(10.3)		(10.4)
H-2	3.39[g]	3.44	3.43	3.40	4.76	4.78	4.70	4.76
($J_{2,3}$)		(11.0)	(9.7)		(10.2)	(10.3)		(10.4)
H-3	3.66	3.68	3.66[d]	3.67[f]	5.37	5.37	5.38	5.36
($J_{3,4}$)	(9.2)	(9.3)			(9.3)	(9.3)	(9.3)	(9.3)
H-3	3.66	3.68	3.66[d]	3.67[f]	5.37	5.38	5.39	5.37
($J_{3,4}$)	(9.0)					(9.3)	(9.2)	(9.2)
H-4	3.23	3.26	3.22	3.18	3.54	3.56	3.57	3.55
($J_{4,5}$)	(10.8)	(10.0)	(10.1)	(9.9)	(9.8)	(9.9)	(10.7)	(9.9)
H-4	3.24	3.31	3.22	3.27	3.55	3.57	3.62	3.57
($J_{4,5}$)	(9.8)	(10.0)		(9.9)	(9.9)	(9.9)	(9.9)	(9.8)
H-5	3.88	3.90	3.95	3.94	4.01	4.04	4.12	4.05
H-5	3.88	3.92	3.96	3.95	4.03	4.08	4.14	4.09

[a] All spectra were recorded at 27 °C in acetone-d_6 (peracetates) or acetone-d_6/D_2O (9:1; unacetylated). Due to the complex nature of these diastereomeric mixtures, complete resolution of all chemical shifts and coupling constants was not possible, nor was the assignment of the signals to the individual diastereomers. The discernible values are listed and coupling constants are to ±0.2 Hz. [b] Signals of H-1 and β-H overlap. [c] Signals of γ^1-H and γ^2-H overlap. [d] Signals of H-3 and γ^1-H overlap. [e] Signals of γ^2-H and ArOMe overlap. [f] Signals of H-3 and γ^2-H overlap. [g] Signals of H-2 and 4-OMe overlap.

constants ($J_{\alpha,\beta}$) of *threo* isomers are larger than those of *erythro* isomers, and this trend was also observed in the peracetylated products. The chemical shifts of γ^1-H and γ^2-H (with γ^2-H referring to the downfield γ-proton) in *erythro* isomers are much closer than those in *threo* isomers. The resonance signals of γ^1-H and γ^2-H in **16e**, **21e**, and **17e** were completely overlapped, prohibiting first-order analyses of coupling constants. The resonance signals of H-1 and β-protons also overlapped in the *erythro* isomers, whereas they were separated in the *threo* isomers. The $J_{\gamma 1,\beta}$ values are typically larger than $J_{\gamma^2,\beta}$ values in *threo* isomers, while the opposite is true of the *erythro* isomers, *i.e.* $J_{\gamma^1,\beta} < J_{\gamma^2,\beta}$. The chemical shifts of β-protons in the *threo* isomers are upfield relative to those of their *erythro* counterparts. The α-proton chemical shifts of *threo* isomers are slightly downfield relative to those of the *erythro* isomers in the α-esters.

Table 2. Selected ^{13}C NMR Data for the α- and γ-Esters and Their Corresponding Peracetates[a]

	γ-esters		α-esters		γ-esters OAc		α-esters OAc	
carbon	**16e**	**16t**	**21e**	**21t**	**17e**	**17t**	**22e**	**22t**
α	72.83	72.92	75.80	76.41	74.76	75.59	76.20	77.32
			75.90	76.64	74.91	75.72	76.32	77.41
β	82.86	82.71	82.82	83.17	80.32	80.84	80.32	80.29
	82.75	82.78	83.04	83.36	80.50	81.03	80.66	80.75
γ	64.88	64.89	60.80	60.87	64.75	65.21	63.25	63.53
	65.01	65.09				65.26		63.65
C-1	101.14	101.01	101.04	100.96	98.18	98.24	98.22	98.12
		101.03		101.01			98.28	98.20
C-2	72.71	72.40	72.43	72.41	71.45	71.45	71.40	71.41
		72.42				71.48	71.45	71.46
C-3	74.14	73.82	73.87	73.86	71.87	71.90	71.90	71.82
			73.98	74.06	71.92	71.95	72.03	72.14
C-4	81.73	81.57	81.41	81.34	79.56	79.60	79.49	79.41
			81.82	82.07	79.67	79.69	79.89	80.08
C-5	70.87	70.77	70.95	70.92	70.75	70.68	70.90	70.76
	70.99	70.88		71.01		70.88		70.99
C-6	170.13	170.11	168.99	169.26	168.95	168.77	167.97	168.10
	170.16	170.06	169.21	169.69		168.89		168.41
$PhCH_2$	71.30	71.17	71.04	71.08	71.34	71.35	71.15	71.32
			71.08				71.33	
1-OCH_3	55.68	55.69	55.66	55.58	55.83	55.85	55.83	55.68
				55.66		55.88		55.85
4-OCH_3	60.42	60.39	60.30	60.24	60.33	60.40	60.32	60.17
				60.44			60.38	60.40
ArOCH_3	56.07	55.99	56.12	56.04	56.19	56.21	56.18	56.23
	55.16	56.14		56.12	56.24	56.24	56.24	
				56.20				

[a] The chemical shifts are in ppm and were measured at 27 °C in acetone-d_6 (peracetates) or acetone-d_6/D_2O (9:1; unacetylated). Carbon designations are based on standard lignin and carbohydrate nomenclature and are not assigned to individual diastereomers.

The above information can be used to differentiate *threo* and *erythro* isomers. The unacetylated α-esters have characteristic peaks (α-protons) at *ca.* 6.0 ppm, and no peaks between 4.1 and 4.5 ppm, whereas the γ-esters have characteristic peaks (γ-protons) at 4.1–4.5 ppm and no peaks at *ca.* 6.0 ppm. Such information can aid in the identification of α- and γ-esters in native LCC complexes, but it is imperative that NMR data of unacetylated material be obtained.

The selected ^{13}C NMR data are shown in Table 2. As a general rule, the chemical shifts of the α-carbon in *threo* isomers are downfield from their *erythro* counterparts. Differentiation of α-esters and γ-esters can easily be achieved by the downfield shifts exhibited by the binding carbon of glucopyranosiduronate. For instance, the chemical shifts of α-carbons change from 72.83–72.92 ppm in γ-esters to 75.80–76.64 ppm in the α-esters; the chemical shifts of γ-carbons change from 64.88–65.09 ppm in γ-esters to 60.80–60.87 ppm in α-esters. This effect is lost upon peracetylation, which also increases the chemical shifts of the C-1 carbon (*ca.* 3 ppm) as well as all other ring carbons (*ca.* 1–2 ppm) except C-5 in the uronate moiety. It is also interesting to note that the C-4 signals in each pair of the isomers are more significantly different from all other carbon signals in the α-esters (**21e**, **22e**, **21t**, **22t**) but not in the γ-esters.

Uronosyl Migration. During the deprotection of **15e** and **15t**, it was observed that the uronate group

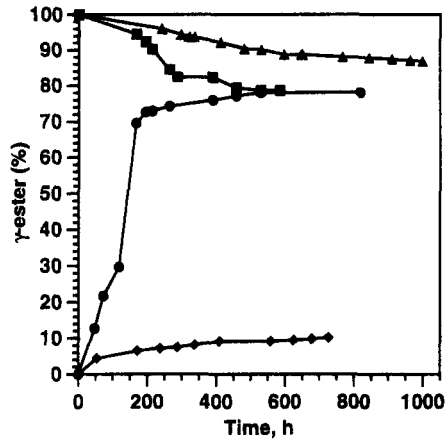


Figure 1. Percent γ-ester present in an NMR tube experiment over time. Starting material: ■, *erythro* γ-ester (**16e**); ▲, *threo* γ-ester (**16t**); ●, *erythro* α-ester (**21e**); ◆, *threo* α-ester (**21t**).

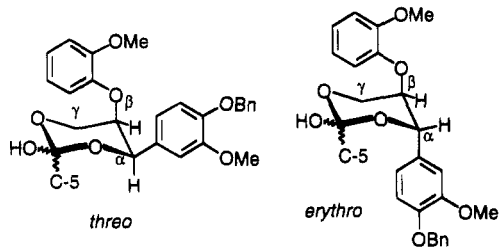


Figure 2. Intermediates for the uronosyl migration. C-5 indicates the 5-position of the carbohydrate ring. Note the orientation of the aryl rings in the *threo* and *erythro* conformations.

would migrate from the γ-position to the α-position if the reaction time was sufficiently long (>30 min). The same deprotection procedure applied to **18e** afforded a mixture of α-ester **21e** and γ-ester **16e**. If **18e** was stirred in ethanolic 1% HCl for an hour, the major compound was γ-ester **16e**, not α-ester **21e**. It was subsequently found that migration also would occur under neutral conditions. Compounds **16e, 16t, 21e,** and **21t** were dissolved in NMR tubes with acetone-d_6/D_2O (9:1), and their ^1H NMR spectra were recorded over the course of several weeks. The ratio of γ-esters to α-esters was acquired by integration of α-protons of the α- and γ-esters, and the results are shown in Figure 1.

The *erythro* isomers, whether starting from the α- or γ-esters, formed an equilibrium mixture in which the γ-ester predominated (80% γ-ester). However, in the time frame and solvent conditions investigated, it appears that the *threo* isomers are much less susceptible to the migration, as convergence to an equilibrium value starting from **16t** or **21t** has yet to occur. The difference in the rates of migration may be explained by examining the intermediates of the migration shown in Figure 2. Classical 1,3-diaxial interactions will occur only between the α-aryl group and the carbohydrate ring. If it is assumed that this interaction does not occur due to high energetics, the strongest interactions will be between the guaiacyl and phenyl rings. These moieties are gauche (*cis*) to each other in the *threo* intermediate and would thus be of higher energy when compared to the *erythro* structure, where the two groups can assume an anti (*trans*) relationship. The diaxial orientation is shown for the *erythro* isomers as this is the favored conformation for lignin model α,γ-phenylboronates and acetals (Ralph and Young, 1983).

This migration phenomenon has significant implications with respect to temperate zone hardwood LCC structures. If one were to assume that the LCC ester linkages are formed through the quinone methide intermediate, initial nucleophilic attack of the 4-*O*-methyl-α-D-glucopyranosiduronic acid moiety of glucuronoxylan would form an α-ester. The uronosyl group would then migrate from the α-position to the γ-position to form the equilibrium mixture. Although the time scale of the migration reaction may be long on the laboratory scale (Figure 1), it is very short with respect to the average lifespan of a temperature zone hardwood. The migration would be further aided by the acidic conditions present in the heartwood of hardwoods (brought about by cleavage of acetate groups). Therefore, LCC isolation protocols as well as procedures utilizing DDQ methodology for quantitating LCC esters may need to be modified (Imamura *et al.*, 1994). The chemical and enzymatic susceptibility of the materials prepared in this study will be the subject of a separate investigation.

To examine the chemistry of uronate/quinone methide coupling and the potential for migration, the reaction of methyl 4-*O*-methyl-α-D-glucopyranosiduronic acid (**1**) and the quinone methide of 1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol was performed. The quinone methide was prepared according to the procedure of Ralph and Young (1983) and was left exposed to methyl 4-*O*-methyl-α-D-glucopyranosiduronic acid in chloroform/DMF for 4 days. The α-ester fraction was purified, and ^1H NMR spectra were obtained for this sample in acetone-d_6/D_2O (9:1) over the course of several days. The initial spectrum revealed almost equal amounts of the *threo* and *erythro* isomers with a barely detectable amount of the γ-esters. Subsequent spectra showed the appearance of an increased amount of the γ-esters, approximately 8% after 100 h. The majority of the γ-esters were the *erythro* diastereomers, as would be expected from the results displayed in Figure 1.

Summary. Several α- and γ-ester LCC models and their peracetates which accurately depict the theorized ester linkages between lignin and xylans in temperate zone hardwoods have been prepared. The characterization of these compounds by NMR spectroscopy facilitated the differentiation between α-ester and γ-esters, as well as the *threo* and *erythro* isomers. During the course of these studies it was discovered that the 4-*O*-methyl-α-D-glucopyranosiduronate would migrate between the α- and γ-positions of the lignin side chain. The migration of *erythro* isomers relative to that of the *threo* isomers was attributed to differences in the cyclic intermediate of transesterification. It was also found that equal amounts of *threo* and *erythro* α-esters were formed from the reaction of the β-*O*-4 quinone methide and methyl 4-*O*-methyl-α-D-glucopyranosiduronic acid, and the transformation of these α-esters to γ-esters was also observed. Therefore, on the basis of this study, it is proposed that if α-esters exist in hardwood cell walls (Imamura *et al.*, 1994), γ-esters will be present in higher concentrations. This potentially rules out enzyme involvement in the formation of uronosyl–lignin esters in wood.

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LITERATURE CITED

Berkowitz, D. B.; Eggen, M.; Shen, Q.; Sloss, D. G. Synthesis of (α,α-difluoroalkyl)phosphonates by displacement of primary triflates. *J. Org. Chem.* **1993**, *58*, 6174–6176.

Cook, A. F.; Maichuk, D. T. Use of chloroacetic anhydride for the protection of nucleoside hydroxyl groups. *J. Org. Chem.* **1970**, *35*, 1940–1943.

Fengel, D.; Wegener, G. *Wood: Chemistry, Ultrastructure, Reactions*; de Gruyter: Berlin, 1989; pp 234–238.

Freudenberg, K. In *Constitution and Biosynthesis of Lignin*; Freudenberg, K., Neish, A. C., Eds.; Springer-Verlag: Berlin, 1968; pp 47–116.

Furniss, B. S.; Hannaford, A. J.; Smith, P. W. G.; Tatchell, A. R. In *Vogel's Textbook of Practical Organic Chemistry*, 5th ed.; Wiley: New York, 1989; pp 430–433.

Hassner, A.; Alexanian, V. Direct room temperature esterification of carboxylic acids. *Tetrahedron Lett.* **1978**, *46*, 4475–4478.

Hauteville, M.; Lundquist, K.; von Unge, S. NMR studies of lignins. ^{1}H NMR spectroscopic investigation of the distribution of *erythro* and *threo* forms of *β-O*-4 structures in lignins. *Acta Chem. Scand.* **1986**, *B40*, 331–335.

Helm, R. F.; Li, K. Complete *threo*-stereospecificity for the preparation of *β-O*-4 lignin model dimers. *Holzforschung* **1995**, in press.

Helm, R. F.; Ralph, J. Stereospecificity for the zinc borohydride reduction of α-aryloxy-β-hydroxy ketones. *J. Wood Chem. Technol.* **1993**, *13*, 593–601.

Imamura, T.; Watanabe, T.; Kuwahara, M.; Koshijima, T. Ester linkages between lignin and glucuronic acid in lignin-carbohydrate complexes from *Fagus crenata*. *Phytochemistry* **1994**, *37*, 1165–1173.

Li, K.; Helm, R. F. A practical synthesis of methyl 4-*O*-methyl-α-D-glucopyranosiduronic acid. *Carbohydr. Res.* **1995**, in press.

Lundquist, K. NMR studies of lignins. 4. Investigation of spruce lignin by ^{1}H NMR Spectroscopy. *Acta Chem. Scand.* **1980**, *B34*, 21–26.

Nakatsubo, F.; Sato, K.; Higuchi, T. Enzymic dehydrogenation of p-coumaryl alcohol. IV. Reactivity of the quinonemethide. *Mokuzai Gakkaishi* **1976**, *22*, 29–33.

Nimz, H. H.; Tschirner, U.; Stable, M.; Lehmann, R.; Schlosser, M. Carbon-13 NMR spectra of lignins, 10. Comparison of structural units in spruce and beech lignin. *J. Wood Chem. Technol.* **1984**, *4*, 265–284.

Ralph, J.; Young, R. A. Stereochemical aspects of addition reactions involving lignin model quinone methides. *J. Wood Chem. Technol.* **1983**, *31*, 161–181.

Ralph, J.; Hatfield, R. D.; Quideau, S.; Helm, R. F.; Grabber, J. H.; Jung, H.-J. G. Pathway of *p*-coumaric acid incorporation into maize lignin as revealed by NMR. *J. Am. Chem. Soc.* **1994**, *116*, 9448–9456.

Sipilä, J.; Brunow, G. On the mechanism of formation of noncyclic benzyl ethers during lignin biosynthesis. Part 4. The reactions of *β-O*-4 type quinone methide with carboxylic acids in the presence of phenols. The formation and stability of benzyl esters between lignin and carbohydrates. *Holzforschung* **1991**, *45*, 9–14 (Suppl.).

Takahashi; Koshijima, T. Ester linkages between lignin and glucuronoxylan in a lignin-carbohydrate complex from beech (*Fagus crenata*) wood. *Wood Sci. Technol.* **1988**, *22*, 231–241.

Timell, T. E. Recent progress in the chemistry of wood hemicelluloses. *Wood Sci. Technol.* **1967**, *1*, 45–70.

Watanabe, T.; Koshijima, T. Evidence for an ester linkage between lignin and glucuronic acid in lignin carbohydrate complexes by DDQ oxidation. *Agric. Biol. Chem.* **1988**, *52*, 2953–2955.

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