Holzforschung, Vol. 65, pp. 131–143, 2011 • Copyright © by Walter de Gruyter • Berlin • New York. DOI 10.1515/HF.2011.028

Delineating pMDI model reactions with loblolly pine via solution-state NMR spectroscopy. Part 1. Catalyzed reactions with wood models and wood polymers

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Abstract

To better understand adhesive interactions with wood, reactions between model compounds of wood and a model compound of polymeric methylene diphenyl diisocyanate (pMDI) were characterized by solution-state NMR spectroscopy. For comparison, finely ground loblolly pine sapwood, milled-wood lignin and holocellulose from the same wood were isolated and derivatized with the pMDI model compound. One-bond $^{13}C–^{1}H$ correlation (HSQC) experiments on derivatized and dissolved ball-milled wood revealed which hydroxyl group positions of the cell wall polymers reacted with the pMDI model compound to form carbamates. The chemical shifts of the derivatized model compounds correspond precisely to the chemical shifts of derivatized wood polymers. These model experiments will be taken as a basis in the next phase of our research (Part 2), in which the reactions of pMDI model compounds will be studied with intact wood cell walls under conditions similar to those used in oriented strand-board production.

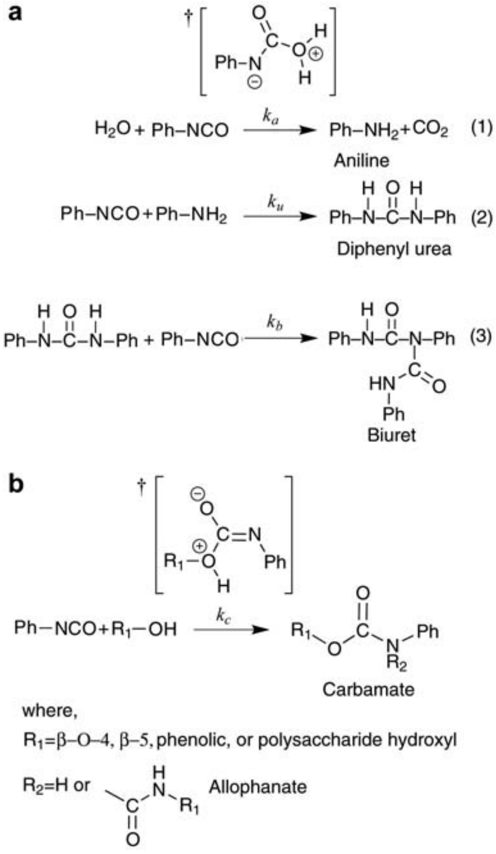
Keywords: β-aryl-ether; ball-milled wood (MW); carbamate; cellobiose; holocellulose; heteronuclear single quantum coherence (HSQC); methyl 4-*O*-methyl-β-D-glucopyranoside; milled-wood lignin (MWL); phenylcoumaran; phenyl isocyanate; pinoresinol; *Pinus taeda*; polymeric methylene diphenyl diisocyanate (pMDI); solution-state NMR.

Introduction

Derivatization of lignocellulosics is common for modifying their chemical, physical and biological properties. Modification through carbamylation (i.e., the derivatization of wood with isocyanates) decreases water absorption and produces modified wood with improved mechanical strength (Clermont and Bender 1957; Baird 1969; Rowell and Ellis 1979). Isocyanates, particularly polymeric methylene diphenyl diisocyanate (pMDI), are known to impart considerable moisture durability, decreasing thickness swelling and providing high flexural strength to oriented strand-board, laminated strand-lumber and exterior particleboard. It has been thought that MDI reacts almost exclusively with wood polymers *in situ* (Deppe and Ernst 1971), purporting that the principal bonding mechanism is one of wood carbamate crosslinks. However, MDI has also been repeatedly shown to react competitively with water (Wittmann 1976; Weaver and Owen 1995; Wendler and Frazier 1996; Rosthauser et al. 1997; He and Yan 2005) and, thus, form urea-type products, which are capable of intermolecular hydrogen bonding (Born and Hespe 1985).

pMDI reactivity

Aryl isocyanates are well known to readily react with alcohols to form carbamates (Saunders and Slocombe 1948). For wood, the major reactions occur between the isocyanate and free or bound water in the cells, and/or the wood polymer hydroxyls. Figure 1 shows the two main types of competing mechanisms expected during pMDI cure; phenyl isocyanate is presented for simplicity. Phenyl isocyanates react with water to form carbamic acid, which quickly expels CO_2 to form an aromatic amine at a rate k_a. The reaction rate between the aromatic amine and other isocyanate molecules exceeds that of water or wood, and a urea is formed at a rate k_u. Further reaction of the urea with phenyl isocyanate can occur, forming a biuret at a rate k_b. It has been demonstrated that $k_u > k_a > k_b$ and that k_u is highly dependent on H_2O concentration and temperature (Tiger et al. 1971). When the ratio of water to isocyanate is low, the phenyl isocyanate has the ability to react with hydroxyl groups of wood polymers leading to a carbamate group at a rate k_c. The rate of an isocyanate reacting with methanol is approximately two times slower than that with water (Tiger et al. 1969). The carbamate can react with another isocyanate to form an allophanate linkage. Because of their structural similarities, as noted in Figure 1, the difficulty has been in distinguishing between urea, biuret, carbamate and allophanate structures in wood-



$H_2O + Ph{-}NCO \xrightarrow{k_a} Ph{-}NH_2 + CO_2$ (1)

Figure 1 The major reactions between phenyl isocyanate and (a) water, (b) wood. The rate constants are: k_a, aniline formation; k_u, urea formation; k_b, biuret formation; k_c, carbamate formation. The † symbol refers to the intermediate structures.

pMDI bondlines (Wendler and Frazier 1996; Zhou and Frazier 2001; Bao et al. 2003).

Homogeneous carbamylation of wood cell wall polymers

Cellulose carbamylation, first described and characterized by means of static light scattering and viscometry (Burchard and Husemann 1961), has since become a routine derivatization method for determining the molecular weight distribution of cellulose (Valtasaari and Saarela 1975). Cellulose carbamates have been characterized, to some extent, by solution-state NMR spectroscopy, but these studies have been limited to one-dimensional ^{1}H and ^{13}C experiments. Moreover, carbamylated whole plant cell walls have not been characterized; this is necessary to provide a dataset for the application of powerful NMR spectroscopy techniques for advanced wood analysis and to enhance our understanding of the reactivity between wood and isocyanates.

Many homogeneous carbamylation methods for cellulose exist, but several give urea-type byproducts or catalyze depolymerization side reactions, which can mask proper characterization of the desired carbamate (Diamantoglou and Kuhne 1988; Evans et al. 1991; Berthold et al. 2004). The byproducts can be separated from cellulose carbamates through precipitation (Mormann and Michel 2002).

Lignin carbamylation has been the focus of much less attention compared to polysaccharides. Studies on lignin-based polymers have been reviewed by Reichelt and Poller (1981). For example, lignin isolated by mineral acids was reacted in dioxane with 1,6-hexamethylene diisocyanate and 2,4-toluene diisocyanate catalyzed by triethylamine at 100°C and the derivative was characterized by IR spectroscopy (Jaunzems et al. 1968a,b). Reaction of lignin model compounds with isocyanates have rarely been investigated, and most studies have not used particularly representative models of structural units in lignins. 2,4-Toluene diisocyanate was reacted with isoeugenol, ethylveratrylcarbinol and ethylguaiacylcarbinol at NCO:OH ratios of 1:1 and 2:1 in dioxane in the presence of ferric acetylacetonate (FeAA) as catalyst (Mozheiko et al. 1973). It was found by IR spectroscopy that the benzylic hydroxyl group reacted both at 40°C and 100°C, whereas the phenolic hydroxyl reacted only at 100°C. Another study examined the reactions of 1,6-hexamethylene diisocyanate with simple lignin model compounds (isoeugenol, phenol, *p*-hydroxybenzaldehyde, vanillin and guaiacol) without catalysts at room temperature and 100°C and reported quantitative yields for all derivatizations (Detoisien et al. 1985). The reaction products were characterized by IR and ^{1}H NMR spectroscopy. The chemical shift of the N–H group of the urethane was between 5.00 and 5.75 ppm; however, urea products would also have N–H signals in this range.

The structure of carbamylated wood cell wall polymers has not been confirmed unambiguously. An essential step toward better characterizing isocyanate reactivity with wood would be to prove that a carbamate involving a hydroxyl group of a cell wall polymer is in fact formed. Carbamylation of isolated wood polymers is necessary so that the individual reactivity of polymers within the complex cell wall matrix can be delineated. The adhesive bonding mechanism between pMDI and wood cannot be discerned without structural confirmation of carbamate and/or urea formation. Therefore, the mechanisms involved in pMDI-wood bonding have to be clarified. First of all, it is crucial to know whether or not covalent bonding is a probable contributing mechanism.

Objective

The objective of this study was to obtain the necessary data to allow delineation of pMDI reactions with wood. Carbamylated cell wall polymers of wood should be characterized by high-resolution solution-state NMR spectroscopy. The approach can be subdivided into five parts. (1) Synthesizing wood model compounds (e.g., dimeric lignin model β-aryl ether, phenylcoumaran and pinoresinol compounds, and a simple model for the internal units of cellulose, methyl 4-*O*-methyl-β-D-glucopyranoside); (2) reacting the wood model compounds with an excess of a pMDI model (phenyl isocyanate) in the presence of a catalyst to form model carbamates; (3) reacting limited equivalents of phenyl isocyanate with model compounds to determine regioselectivity of hydroxyl groups; (4) homogeneously reacting isolated wood polymers (e.g., milled-wood lignin, holocellulose and ball-milled wood) with phenyl isocyanate in the presence of a catalyst to form wood polymer carbamates; and (5) characterizing the carbamylated products by solution-state NMR experiments to provide a chemical shift dataset for carbamates formed in the cell wall (Yelle et al. 2011).

Materials and methods

Materials

Phenyl isocyanate and *N*,*N*-dimethylacetamide (DMAc) (both distilled over 3 Å molecular sieves), the catalysts – ferric acetoacetonate (FeAA) and dibutyltin dilaurate (DBTDL) –, dioxane and toluene were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Ball-milled loblolly pine (referred to as MW) was prepared by further milling Wiley-milled loblolly pine (80-mesh, 400 mg) in a Retsch PM100 planetary ball-mill with ZrO_2 balls and vessel at 600 rpm milling for 4 h. Milled wood lignin (MWL) was prepared from MW via a dioxane:water (v/v=96:4) extraction (Björkman 1956). Holocellulose from loblolly pine was prepared using sodium chlorite according to Jayme (1942). The lignin model dimers – β–*O*–4-dehydroconiferyl alcohol (''β-aryl ether''), β–5-dehydroconiferyl alcohol (''phenylcoumaran'') and β–β-dehydroconiferyl alcohol (''pinoresinol'') – were prepared by standard methods (Miksche et al. 1966; Quideau and Ralph 1994). Dimethylsulfoxide-d_6 (DMSO-d_6) (99.9%), guaiacol and D(+)-cellobiose (98%) were supplied by Acros Organics (Geel, Belgium). *N*-Methylimidazole-d_6 (NMI-d_6) was synthesized and characterized as previously described (Yelle et al. 2008). The cellulose model – methyl 4-*O*-methyl-β-D-glucopyranoside – was synthesized in analogy to the methods Kuhn et al. (1955) and Bouveng et al. (1957).

Reactions with phenyl isocyanate

Reactions were carried out under nitrogen in solution with 0.001 mM DBTDL or 0.01 mM FeAA as catalyst. The model compounds and wood polymers were dried in a vacuum oven overnight at 45°C and stored over P_2O_5. Reaction times varied from 2 h for model compounds to 12 h for wood polymers. Reactions of model compounds were monitored for completeness by thin-layer chromatography (TLC) with ethyl acetate:chloroform (v/v=1:1) as the liquid phase. Work-up of the reacted model compounds was as follows: (1) dry methanol was added to quench excess isocyanate; (2) saturated NH_4Cl (5 ml) was added to render the solution slightly acidic and allow improved partitioning of the products into organic solvent; (3) the products were extracted with ethyl acetate (3×3 ml); (4) the organic layer was dried over $MgSO_4$; and (5) solvents were removed by rotary evaporation at reduced pressure. Wood polymer reactions were considered complete when the reacted polymers went fully into solution. At that point, excess phenyl isocyanate was removed (half of the original volume) with a miniature distillation apparatus under vacuum (5 mbar) with the receiving flask immersed in dry-ice slurry. DMAc was added to bring the solution back to its original volume. Carbamylated wood polymers were then precipitated by adding the solutions drop-wise to a stirred MeOH/H_2O mixture (v/v=5:1), the precipitate filtered onto a 0.2 μm nylon membrane and washed further with the MeOH/H_2O mixture. The solid was vacuum-dried at 45°C and analyzed via NMR spectroscopy. The filtrate, containing methyl carbamate and diphenyl urea, was isolated via vacuum distillation; both crude products were purified by means of preparative TLC with ethyl acetate:chloroform (v/v=1:1) as the liquid phase. The structures of the purified compounds were confirmed via ^{1}H and ^{13}C NMR spectroscopy.

Theoretical quantification of wood polymer hydroxyls

The NCO:OH ratios used in wood polymer reactions were based on data of percent acetyl content of MW where 40.6%-gain (by weight) was achieved. Because the hydroxyl groups are converted to *O*-acetyl groups via acetylation on a 1:1 molar basis, the reactive hydroxyl content of MW is equal to the acetyl content of the acetylated MW. This is calculated based on the molecular weight of an acetyl (43.05 mg mmol^{-1}) (e.g., 100 mg ball-milled wood has 0.94 mmol of -OH groups, or 9.4 mmol g^{-1}). For MWL, a 27.0%-gain (by wt.) was achieved after acetylation. For 100 mg of MWL, there are 0.63 mmol of -OH groups, or 6.3 mmol g^{-1}. For holocellulose, a 43.2%-gain was achieved after acetylation. For 100 mg of holocellulose, there are 1.00 mmol of -OH groups, or 10.0 mmol g^{-1}.

Solution-state NMR spectroscopy

The lignin model compounds were dissolved in acetone-d_6 (500 μl) and cellulose model compounds were dissolved in DMSO-d_6 (500 μl) and the usual suite of one- and two-dimensional experiments were acquired at 25°C on a Bruker (Rheinstetten, Germany) DPX-250 (62.9 MHz for the ^{13}C) instrument using standard Bruker pulse programs. Chemical shift assignments of model compounds were confirmed based on the Lignin NMR Database (Ralph et al. 2004) and heteronuclear multiple-bond correlation (HMBC) experiments (Bruker pulse program, inv4gslplrnd), with an 80-ms long-range coupling delay. For the wood polymers, heteronuclear single quantum coherence (HSQC) experiments (Bruker pulse program, invietgpsi) were acquired at 55°C on a Bruker DMX-500 instrument equipped with a cryogenically cooled 5-mm TXI ^{1}H/^{13}C/^{15}N gradient probe with inverse geometry (proton coils closest to the sample). Samples (60 mg each) were added to 5 mm diameter NMR tubes and dissolved in 500 μl of DMSO-d_6 (400 μl DMSO-d_6+100 μl NMI-d_6 was added only when analyzing holocellulose and ball-milled wood in the non-derivatized state). The central DMSO solvent peak served as an internal reference ($\delta_C=39.5$ ppm, $\delta_H=2.49$ ppm). Parameters for HSQC experiments for wood polymers: sweep width, 6.4–2.8 ppm in F_2 (^{1}H) with 718 data points (acquisition time, 200 ms) and 110–40 ppm in F_1 (^{13}C) using 256 increments (F_1 ''acquisition time'' 14.5 ms). The number of scans was 200 with a 1-s interscan delay, the d_{24} delay was set to 1.72 ms ($\sim 0.25/J$, where J is an average 145 Hz ^{13}C–^{1}H coupling constant), and the total acquisition time was 18 h. Processing involved typical matched Gaussian apodization in F_2 and a squared cosine-bell apodization in F_1. Chemical shift assignments of wood polymers were confirmed with model compound data and literature sources.

Results and discussion

Model compound reactions

Reaction conditions were adjusted to provide good yields for the model compounds, as presented in Table 1; the reaction products were purified for analysis.

The cellobiose octa-carbamate structure was characterized and confirmed with ^{13}C, HSQC and HMBC NMR spectra, and data in the literature (Gagnaire et al. 1976). The ^{13}C spectrum showed more than 8 peaks in the carbamate car-

Table 1 Reaction conditions, yields and derivatized positions for wood model compounds reacted with phenyl isocyanate.

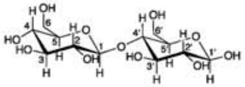
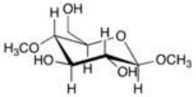
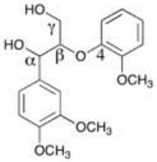
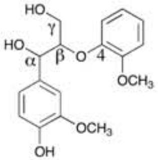
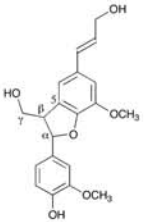
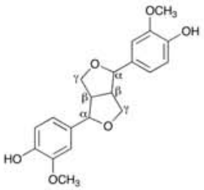
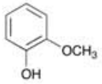
Model compound		Equivalents (NCO:OH)	Solvent	Catalyst	Temp (°C)	Molar yield	
Name	Structure					%	Position
D(+)-Cellobiose		1:1	DMAc	DBTDL	60	86	C1′, C2, C2′, C3, C3′, C4, C6, C6′
Methyl 4-*O*-methyl-β-D-glucopyranoside		1:3	DMAc	DBTDL	60	27	C2
		2:3	DMAc	DBTDL	60	36	C2
						12	C2+C6
β-Aryl ether, **A1** (etherified)		1:1	Dioxane	FeAA	40	10	γ
		1:1	DMAc	DBTDL	40	80	γ
						9	α
β-Aryl ether, **A2** (free-phenolic)		1:1	DMAc	DBTDL	40	63	γ+α
		1:1	Toluene	DBTDL	40	92	γ+α+phenolic
Phenylcoumaran, **B**		1:1	DMAc	DBTDL	40	13	γ+γ′
		1:1	Toluene	DBTDL	40	44	γ+γ′+phenolic
Pinoresinol, **C**		1:1	Toluene	DBTDL	40	56	Phenolic
Guaiacol		1:1	Toluene	DBTDL	40	90	Phenolic

Table 2 ^{1}H and ^{13}C chemical shifts for wood model compounds after derivatization with phenyl isocyanate, isolation and purification[a].

Structure	^{1}H (δ, ppm)	^{13}C (δ, ppm)
D(+)-cellobiose octa-carbamate (DMSO-d_6)		
H1, C1	5.08	100.90
(β) H1′, C1′	5.96	91.87
(α) H1′, C1′	6.30	89.13
H2, C2	4.83	72.01
(β) H2′, C2′	5.29	72.68
(α) H2′, C2′	4.99	70.04
H3, C3	5.35	73.84
(β) H3′, C3′	4.99	71.25
(α) H3′, C3′	5.67	69.89
H4, C4	5.00	68.31
(β) H4′, C4′	3.98	77.63
(α) H4′, C4′	4.18	77.50
H5, C5	3.97	72.12
(β) H5′, C5′	4.12	72.94
(α) H5′, C5′	4.22	71.07
$H6_a$, $H6_b$, C6	4.08, 3.93	61.40
(β) $H6_a'$, $H6_b'$, C6′	4.62, 4.19	62.50
(α) $H6_a'$, $H6_b'$, C6′	4.37, 4.20	62.50
-NH-CO-	9.21, 9.40, 9.45, 9.50, 9.58, 9.60, 9.75, 9.84	151.20, 151.99, 152.29, 152.35, 152.39, 152.68 152.95, 153.19
Methyl 4-*O*-methyl-β-D-glucopyranoside, 2-OH derivative (DMSO-d_6)		
H1, C1	4.32	100.81
H2, C2	4.49	73.77
H3, C3	3.50	73.77
H4, C4	3.07	79.16
H5, C5	3.23	75.32
H6, C6	3.64, 3.54	59.97
-NH-CO-	9.64	152.75
Methyl 4-*O*-methyl-β-D-glucopyranoside, 2,6-OH derivative (DMSO-d_6)		
H1, C1	4.40	100.93
H2, C2	4.55	73.78
H3, C3	3.58	72.55
H4, C4	3.15	79.50
H5, C5	3.59	73.86
H6, C6	4.30	62.98
-NH-CO-	9.66, 9.78	152.73, 154.10
A1 α-derivative (acetone-d_6)		
Aα	6.12	75.73
Aβ	4.50	84.87
Aγ	3.71, 3.55	61.13
-NH-CO-	8.84	153.34
A1 γ-derivative (acetone-d_6)		
Aα	4.97	73.27
Aβ	4.52	84.36
Aγ	4.40, 4.01	64.23
-NH-CO-	8.78	154.05
A2 γ+α+phenolic-derivative (acetone-d_6)		
Aα	6.18	75.08
Aβ	4.92	80.26
Aγ	4.49, 4.11	63.10
Phenolic G4	–	140.70
-NH-CO-	8.91, 8.88, 9.16	153.33, 154.10, 152.30
B γ+γ′+phenolic-derivative (acetone-d_6)		
Bα	5.69	87.84
Bβ	3.84	51.78
Bγ	4.52	65.97
Bγ′	4.75	65.76

Table 2 (Continued)

Structure	^{1}H (δ, ppm)	^{13}C (δ, ppm)
Phenolic G4	–	140.91
-NH-CO-	8.88, 8.68, 9.17	154.23, 154.29, 152.14
C phenolic-derivative (acetone-d_6)		
Cα	4.80	86.11
Cβ	3.16	55.32
Cγ	4.29, 3.93	72.43
Phenolic G4	–	140.86
-NH-CO-	9.16	152.27

[a]Underlined structures denote derivatized position(s).

Table 3 Reaction conditions, weight percent gains (WPGs) and products recovered for loblolly pine wood polymers reacted with phenyl isocyanate.

Polymer	NCO:OH (mmol)	Solvent	Catalyst	Temp. (°C)	WPG (%)		Products recovered (mmol)		
					Theo.	Actual	Polymer carbamate	Methyl carbamate	Diphenyl urea
Ball-milled wood	2:1	DMAc	DBTDL	60	127	280	0.97	0.95	0.10
Milled wood lignin	2:1	DMAc	DBTDL	40	85	184	0.65	0.62	0.08
Holocellulose	2:1	DMAc	DBTDL	60	135	261	0.91	0.39	0.06

bonyl region, meaning that there were possibly other types of carbonyl groups present (for example, of the urea-type). The long-range HMBC experiments, which correlate ^{13}C and ^{1}H atoms 2 or 3 bonds away, confirmed the signals between 151.2 ppm and 153.2 ppm (carbamate carbonyl carbon) to be correlations to ring protons.

With the cellulose model compound methyl 4-*O*-methyl-β-D-glucopyranoside, it was instructive to determine the regioselectivity of derivatization at each hydroxyl group. At a ratio NCO:OH = 1:3, the ^{13}C and HSQC NMR of the product showed that the 2-OH position reacts first. When the NCO:OH ratio was increased to 2:3, the HSQC revealed that 2-OH reacted before 6-OH. Accordingly, the order of reactivity found in this tin-catalyzed model system was 2-OH > 6-OH > 3-OH. A primary hydroxyl group is generally thought to be kinetically favored over a secondary hydroxyl group. However, regioselective preference for secondary hydroxyl acetylation has been observed previously with unprotected saccharides (Sugihara 1953; Kurahashi et al. 1999). Delocalization of positive charge through intramolecular hydrogen bonding (e.g., between the 2-OH and 3-OH groups) could play a role in regioselectivity (Kurahashi et al. 1999). Intramolecular coordination via dibutyltin at the 2-*O* and 3-*O* positions should also promote subsequent electrophilic attack (Ogawa and Matsui 1981). Migration of a carbamyl group from the 6-OH to 2-OH is another possibility (Bouveng 1961).

Initial reactions between phenyl isocyanate and etherified β-ether model compound **A1** were performed in dioxane as solvent and FeAA as catalyst at 40°C (conditions in Table 1). The γ-carbamate was obtained in an unexpectedly low 10% yield. With an increased amount of isocyanate and at a temperature of 100°C, free-phenolic β-aryl ether model compound **A2** was expected to fully derivatize at the γ-, α- and phenolic positions as described previously (Mozheiko et al. 1973). This reaction resulted in five products that were shown by NMR to be degraded fragments of the model compound along with some starting material. These results prompted us to explore other known carbamylation systems, such as DMAc with DBTDL, a system that had been tested for derivatizing cellulose (Meyer-Stork et al. 1992). Another

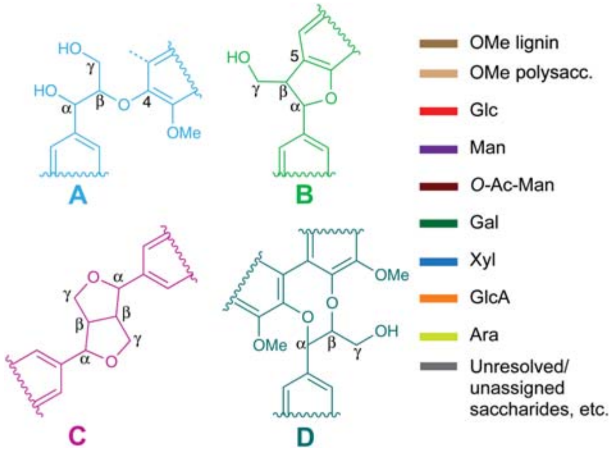


Figure 2 The key to the wood polymer structures represented in Figures 3, 4 and 5. All contour colors can be matched to their respective structures: (A) β-aryl ether, cyan; (B) phenylcoumaran, light green; (C) pinoresinol, raspberry; (D) dibenzodioxocin, sea green; (OMe lignin) lignin methoxyls, brown; (OMe polysacc.) polysaccharide methoxyls, tan; (Glc) β-D-glucopyranosyl units, red; (Man) β-D-mannopyranosyl units, purple; (*O*-Ac-Man) acetylated man, maroon; (Gal) β-D-galactopyranosyl units, forest green; (Xyl) β-D-xylopyranosyl units, blue; (GlcA) 4-*O*-methyl-α-D-glucuronic acid, orange; (Ara) α-L-arabinofuranosyl units, chartreuse; and unresolved or unassigned saccharides, gray.

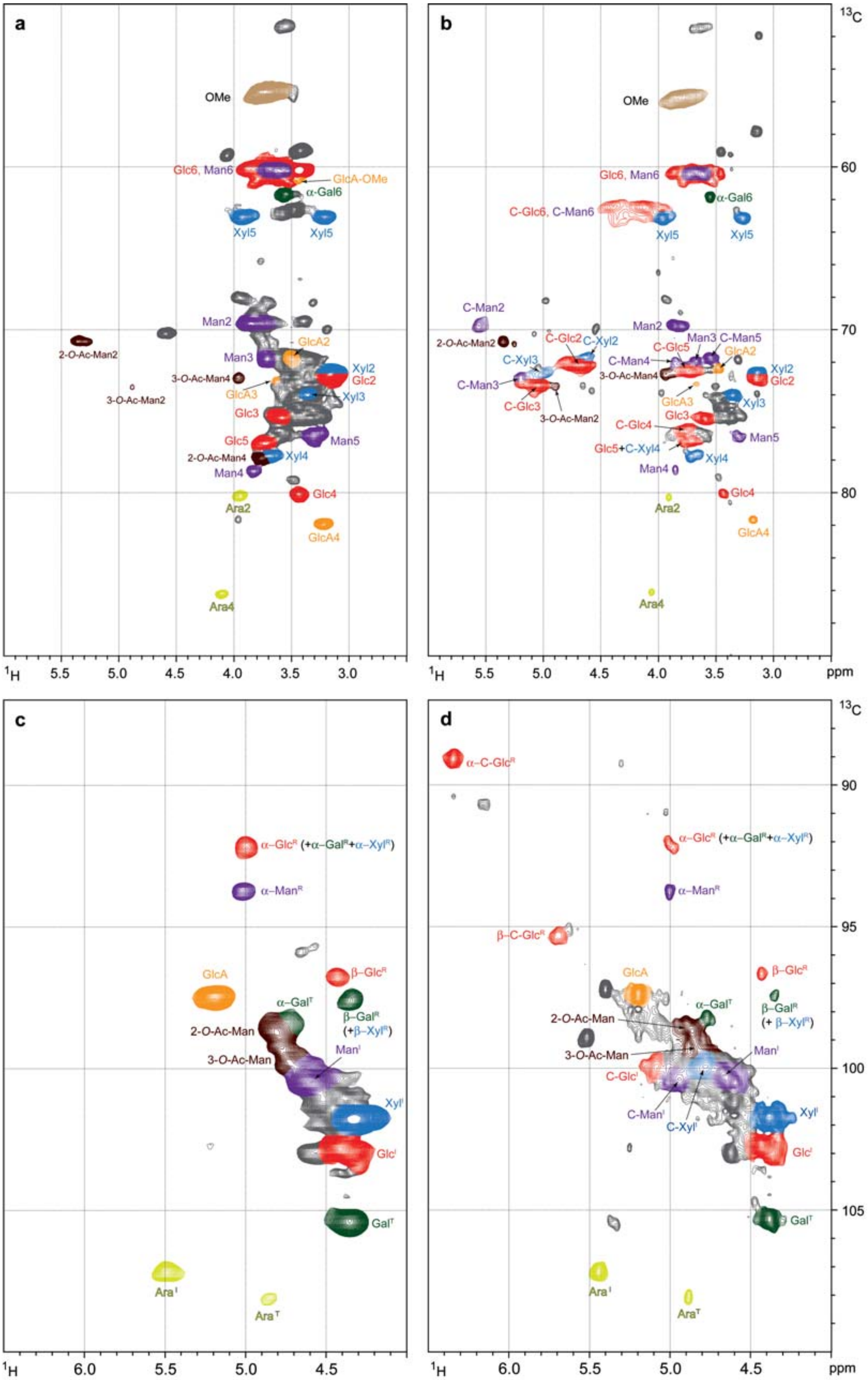


Figure 3 HSQC spectra of loblolly pine holocellulose: (a) non-derivatized aliphatics, (b) partially derivatized aliphatics, (c) non-derivatized anomerics, (d) partially derivatized anomerics. Colored contours correspond to specific polysaccharides shown in Figure 2. Contours labeled with a ''C'' (e.g., C-Glc) represent carbamylated structures.

Table 4 ^{1}H and ^{13}C chemical shifts of non-derivatized cell wall structures of loblolly pine in DMSO-d_6.

Structure	^{1}H (δ, ppm)	^{13}C (δ, ppm)	Ref.
Polysaccharide aliphatics in holocellulose and ball-milled wood			
Glc2	3.14	72.88	(1)
Glc3	3.59	75.33	(1)
Glc4	3.44	80.16	(1)
Glc5	3.73	76.89	(1)
Glc6	3.79, 3.40	60.07	(1)
Man2	3.86	69.65	(2)
Man3	3.71	71.76	(2)
Man4	3.83	78.67	(2)
Man5	3.31	76.41	(2)
Man6	3.67, 3.56	60.13	(2)
2-*O*-AcMan2	5.36	70.63	(3)
3-*O*-AcMan3	4.89	73.54	(3)
2-*O*-AcMan4	3.77	77.81	(3)
3-*O*-AcMan4	3.97	72.96	(3)
Xyl2	3.12	72.64	(4)
Xyl3	3.36	73.97	(4)
Xyl4	3.69	77.73	(4)
Xyl5	3.93, 3.21	63.11	(4)
α-Gal6	3.55	61.72	(3)
GlcA2	3.51	71.71	(5)
GlcA3	3.60	73.08	(5)
GlcA4	3.22	81.89	(5)
GlcA5	4.33	72.60	(5)
GlcA-OMe	3.48	60.95	(5)
Ara2	3.95	80.17	(6)
Ara4	4.11	86.20	(6)
Ac-CH_3	2.02	20.61	(7)
Polysaccharide anomerics in holocellulose and ball-milled wood			
Glc^{I}	4.39	102.85	(1)
α-Glc^{R}	4.94	92.31	(1)
β-Glc^{R}	4.41	96.66	(1)
Man^{I}	4.57	100.56	(2)
α-Man^{R}	4.98	93.83	(2)
Xyl^{I}	4.31	101.83	(4)
β-Xyl^{R}	4.32	97.52	(4)
α-Xyl^{R}	4.99	92.05	(4)
α-Gal^{T}	4.84	98.54	(2)
β-Gal^{I}	4.33	105.40	(3)
α-Gal^{R}	4.99	92.69	(3)
β-Gal^{R}	4.31	97.34	(3)
Ara^{I}	5.50	107.19	(6)
Ara^{T}	4.86	108.13	(6)
GlcA	5.20	97.50	(3)
Lignin structures in milled-wood lignin and ball-milled wood			
Aα	4.79	71.24	(8)
Aβ	4.36	83.89	(8)
Aγ	3.49, 3.67	59.96	(8)
Bα	5.48	87.22	(8)
Bβ	3.47	53.23	(8)
Bγ	3.91	63.24	(8)
Cα	4.66	84.96	(8)
Cβ	3.06	53.60	(8)
Cγ	3.78, 4.11	71.00	(8)
Phenolic G4	–	148.34	(8)
-OMe	3.70	55.37	(8)

Key to references: (1) van Hazendonk et al. (1996), Capek et al. 2000, Hannuksela and du Penhoat (2004); (2) Capek et al. (2000), Hannuksela and du Penhoat (2004); (3) Hannuksela and du Penhoat (2004); (4) Teleman et al. (2000); (5) Teleman et al. (2000); Hannuksela and du Penhoat (2004); (6) van Hazendonk et al. (1996), Hannuksela and du Penhoat (2004); (7) Capek et al. (2000), Teleman et al. (2000), Hannuksela and du Penhoat (2004); (8) Ralph et al. (2004).

advantage of this system is that reactions between phenyl isocyanate and alcohols lead exclusively to carbamates (Wong and Frisch 1987). In the new system, model compound **A1** yielded 80% of γ-carbamate (66% *threo*, 14% *erythro*) and 9% of α-carbamate. Additional experiments were performed with model compound **A2** to attempt side-chain and phenolic derivatization. With DBTDL as catalyst and with a NCO:OH ratio of 1:1, γ- and α-hydroxyls were derivatized, leaving the phenolic position non-derivatized; the ^{13}C NMR signal of the aromatic C4 did not indicate any carbamylation of phenolic OH (4-OH). It is obvious that the phenolic position of lignin model compounds are the least reactive under the conditions employed.

The low reactivity of the phenolic position could be partially attributable to hydrogen bonding. Isocyanate-alcohol reactions are highly dependent on hydrogen bonding complexes between the alcohol, phenyl isocyanate and aprotic solvents. The electron donor number (DN) of the aprotic solvent is indicative in this regard (Gutmann 1978), which can predict catalysis and inhibition effects (Ephraim et al. 1958; Tiger and Éntelis 1965; Oberth and Bruenner 1968; Suarez et al. 1982; Chang and Chen 1987). Solvents having higher polarity and a higher capacity for forming hydrogen bonds with the alcohol appeared to result in a lower reaction rate through the increased formation of solvent-alcohol complexes. The proposed mechanisms for successful carbamate formation suggest that: (1) the alcohol forms a hydrogen-bonding complex with the isocyanate and (2) the non-associated species of the alcohol and aprotic solvent solvates the acidic hydrogen in the complex to form an ion-pair (Chang and Chen 1987). According to this ion-pair mechanism, dioxane exhibits strong hydrogen bonding complexes with alcohols and has a low DN value, thus giving an inhibitory effect. DMAc, having strong hydrogen bonding power and a high DN value, solvates the active hydrogen and gives a catalytic effect; however, given the difficulty of derivatizing the phenolic position of model compound **A2** in DMAc, these hypotheses seem inadequate. Although the mechanism of non-catalyzed reactions with isocyanate-alcohol systems is well understood, tin-catalyzed reactions remain unclear. It seems that other factors as well as the hydrogen bonding and DN value of DMAc, in conjunction with the DBTDL catalyst, give an inhibitory effect in phenolic derivatization with these lignin model compounds. It is possible therefore that DMAc interferes with DBTDL dissociation and/or that DMAc in combination with DBTDL decreases the concentration of the isocyanate-phenolic complex.

An aprotic solvent with low hydrogen bonding capability was then applied to derivatize the phenolic position. Toluene, having a hydrogen bonding index of 1.5 (compared to 9.7 for dioxane and 12.3 for DMAc), has been used in industrial processes with DBTDL/alcohol systems to produce carbamates and polyurethanes. Kinetic studies have demonstrated that isocyanate-alcohol complexes are stable within these systems (Majumdar et al. 2000). Phenolic derivatization of guaiacol with 1 equivalent of phenyl isocyanate at 40°C with DBTDL yielded the guaiacol carbamate almost quantitatively. The ^{13}C NMR spectra of guaiacol and guaiacol carbamate revealed the phenolic OH group derivatization: the signal of C4 quaternary carbon at 148.3 ppm was shifted versus the derivatized C4 quaternary carbon at 140.8 ppm.

Further reactions with the lignin model compound **A2** were performed with a NCO:OH ratio of 1:1 to obtain the fully derivatized β-aryl ether carbamate in high yield. The structure was confirmed with ^{13}C, HSQC and HMBC NMR. Toluene as the solvent, instead of DMAc, conveniently allowed formation of the desired **A2** tri-carbamate, which crystallized as the reaction progressed. The carbamylation reaction of phenylcoumaran **B** in toluene gave the γ- and phenolic carbamate in reasonable yield (44%); this structure was confirmed by ^{13}C, HSQC and HMBC NMR. The pinoresinol **C** carbamylation reaction yielded the phenolic bis-carbamate in reasonable yield (56%) and its structure was confirmed by the presence of its carbamate carbonyl signal at 152.3 ppm in the ^{13}C NMR spectrum. Table 2 summarizes the ^{13}C and ^{1}H chemical shift data for the derivatized model compounds.

Reaction of wood polymers

For reacting wood polymers, the wood swelling solvent DMAc along with the DBTDL catalyst was necessary to carbamylate homogeneously. Two key steps for successfully isolating the carbamylated wood polymers include: (1) removal of as much moisture from the initial wood polymers as possible before reaction and (2) removal of excess isocyanate from solution at low temperature after the reaction is complete. The reaction conditions and products recovered are compiled in Table 3. It can be deduced from the weight percent gain (WPG) of the MW, MWL and holocellulose poly-

Table 5 ^{1}H and ^{13}C chemical shifts for loblolly pine wood polymers after derivatization with phenyl isocyanate in DMSO-d_6.

Structure	^{1}H (δ, ppm)	^{13}C (δ, ppm)	Ref.
Polysaccharide aliphatics in holocellulose and ball-milled wood			
Glc2	4.66	72.28	(1)
Glc3	5.04	73.51	(1)
Glc4	3.74	76.21	(1)
Glc5	3.75	72.50	(1)
Glc6	4.38, 4.10	62.55	(1)
Man2	5.55	69.80	(2)
Man3	5.03	72.33	(2)
Man4	3.84	72.11	(2)
Man5	3.53	71.77	(2)
Man6[a]	4.4, 4.1	62.6	(2)
Xyl2	4.62	71.71	(3)
Xyl3	4.98	72.77	(3)
Xyl4[b]	3.8	76.6	(3)
Xyl5	3.95, 3.25	63.21	(3)
-NH-CO-(Glc)	8.77, 8.88, 9.10	152.00, 152.40, 153.5	(4)
Polysaccharide anomerics in holocellulose and ball-milled wood			
Glc$^{\text{I}}$	5.09	100.02	(1)
β-Glc$^{\text{R}}$	5.65	95.33	(1)
α-Glc$^{\text{R}}$	6.40	89.06	(1)
Man$^{\text{I}}$	4.92	100.06	(2)
Xyl$^{\text{I}}$	4.76	100.02	(3)
Lignin structures in milled wood lignin and ball-milled wood			
Aα	6.14	74.46	(5)
Aβ	4.86	79.22	(5)
Aγ	4.42, 4.06	63.20	(5)
Bα	5.54	87.26	(6)
Bβ	3.77	50.11	(6)
Bγ	4.43	64.66	(6)
Cα	4.65	84.90	(7)
Cβ	3.05	53.65	(7)
Cγ	4.13, 3.76	71.01	(7)
-NH-CO-	8.63, 8.67, 8.70, 8.76, 8.94, 9.18	152.43, 152.55, 153.44, 153.65, 153.92, 154.26	(8)

[a] Overlapping with Glc6 contours.

[b] Overlapping with non-derivatized Glc5 contour.

Key to references: (1) D(+)-cellobiose octa-carbamate; (2) Hedenström et al. (2009); (3) Vincendon (1993); (4) Mormann and Michel 2002; (5) **A2** γ+α+phenolic-derivative; (6) **B** γ+γ′+phenolic-derivative; (7) **C** phenolic-derivative; (8) **A1**, **A2**, **B** and **C** derivatives.

mers that other products in addition to polymer carbamates had formed. Nevertheless, the polymer carbamates were isolated quantitatively based on theoretical molar calculations described in the methods section. The methyl carbamate recovered from the wood polymer reactions came from quenching excess isocyanate with methanol. Diphenyl urea was also recovered. Accordingly, some moisture must have been present during the reactions, even though the wood polymers and reagents were dried rigorously. The amount of diphenyl urea in the product gave an estimate of the water level in the starting wood polymer that was most likely tightly bound in the wood cell wall. That is, when there is an excess of phenyl isocyanate, for every mole of water in the reactant solution 1 mol of diphenyl urea is produced. For example, the initial moisture content of the MW, MWL and holocellulose were estimated to be 1.8%, 1.4% and 2.1%, respectively.

NMR spectroscopy of wood polymers

Dissolution of the carbamylated wood polymer samples was a key factor in obtaining NMR spectra with well-dispersed peaks. For the cellulosic wood polymers (i.e., carbamylated MW and carbamylated holocellulose), complete dissolution only occurred once heated to 55°C. The carbamylated MWL, by contrast, dissolved readily at 25°C.

Figure 2 is the key to the polymer structures for all HSQC spectra. Figure 3 shows aliphatic and anomeric regions of HSQC spectra from non-derivatized and carbamylated loblolly pine holocellulose. The non-derivatized holocellulose spectra (Figure 3a and c) display several correlations that were previously assigned (Yelle et al. 2008; Kim and Ralph 2010). Further tentative assignments were made from these spectra in the aliphatic and anomeric regions for (1 → 4)-linked β-D-glucopyranosyl (Glc, cellulose) units (van Hazendonk et al. 1996; Capek et al. 2000; Hannuksela and du Penhoat 2004), (1 → 4)-linked β-D-mannopyranosyl (Man) units (Capek et al. 2000; Hannuksela and du Penhoat 2004) and its 2-*O*- and 3-*O*-acetylated moieties (Hannuksela and du Penhoat 2004), (1 → 4)-linked β-D-xylopyranosyl (Xyl) units (Teleman et al. 2000), (1 → 6)-linked α-D-galactopyranosyl (Gal) units (Hannuksela and du Penhoat 2004), 4-*O*-methyl-α-D-glucuronic acid (GlcA) units (Teleman et al. 2000; Hannuksela and du Penhoat 2004) and (1 → 3)-linked α-L-arabinofuranosyl (Ara) units (van Hazendonk et al. 1996; Hannuksela and du Penhoat 2004). The ^{1}H and ^{13}C chemical shifts for the non-derivatized wood polymers are listed in Table 4, whereas Table 5 summarizes the ^{13}C and

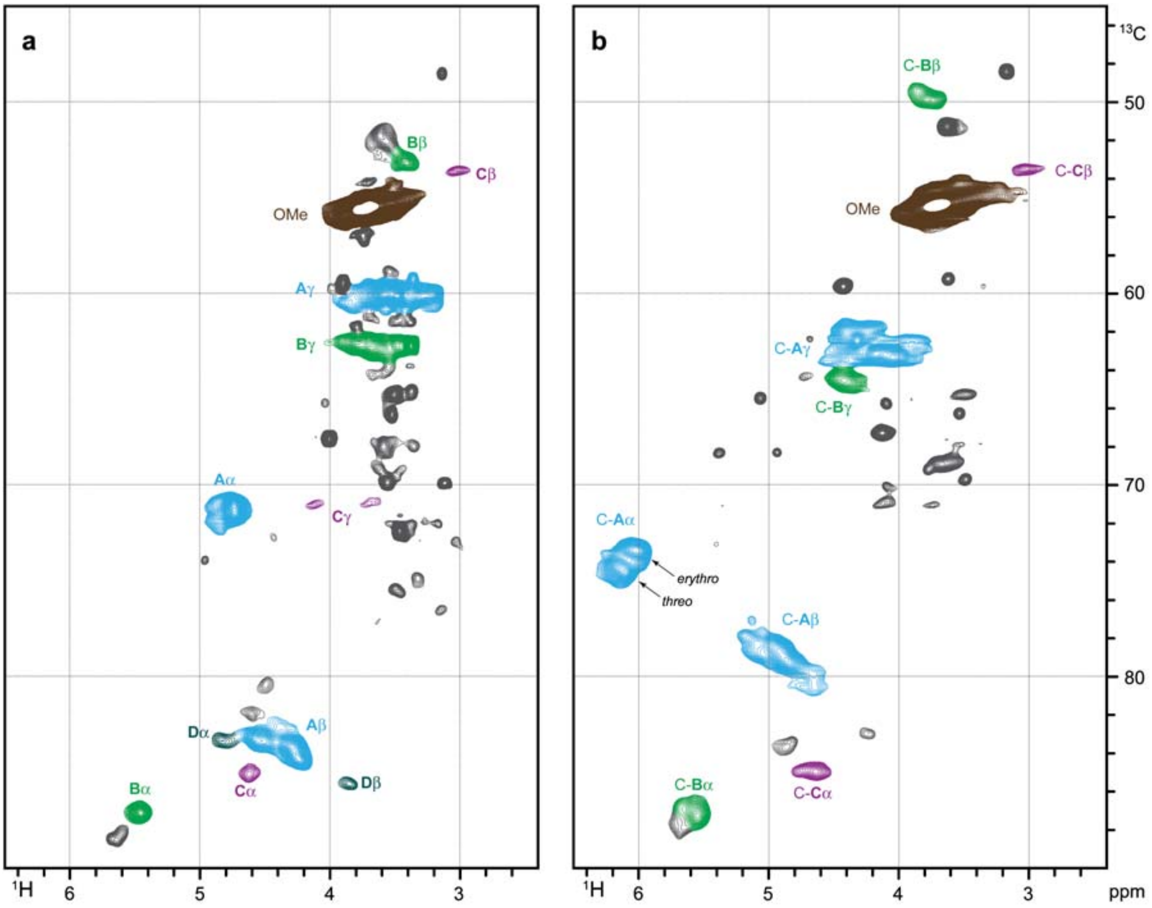


Figure 4 HSQC spectra of loblolly pine milled wood lignin: (a) non-derivatized, (b) derivatized with phenyl isocyanate. Colored contours correspond to specific lignin structures shown in Figure 2. Contours labeled with a ''C'' (e.g., C-**Aα**) represent carbamylated structures.

^{1}H signal assignments for the derivatized wood polymers. It is evident that the chemical shifts are dramatically different and distinct for each hydroxylated H/C position in wood polymers. The chemical shifts of carbamylated wood polymers are directly comparable to those of the corresponding model compounds (Table 2), indicating that the chemical shifts of model compounds can be used to identify derivatized structures in wood polymers.

From the carbamylated holocellulose spectra (Figure 3b and d), it is evident that only partial carbamylation has occurred. The Glc and Man H6/C6 correlations, as well as the H2/C2, H3/C3, H4/C4 and H5/C5 correlations, are consistent with derivatized Glc (Mormann and Michel 2002), non-derivatized Glc units, derivatized Man (Hedenström et al. 2009) and non-derivatized Man. Similarly for Xyl, the H2/C2, H3/C3 and H4/C4 correlations are consistent with a derivatized Xyl, xylan-2,3-bis(phenyl carbamate) (Vincendon 1993) and non-derivatized Xyl. Other polysaccharides such as Ara, Gal and GlcA showed no evidence here of derivatization. Anomeric (i.e., H1/C1) correlations visible in Figure 3d are also consistent with derivatized and non-derivatized Glc as evidenced by the presence of derivatized and non-derivatized α- and β-reducing end-groups of cellulose, indicating that obtaining complete derivatization along the main chain of polysaccharides under catalyzed conditions is not a straightforward process, even at the (presumably more accessible) ends of the cellulose chains. According to volume integration of the Glc reducing end-group contours (β-C-Glc^{R} + α-C-Glc^{R} vs. β-Glc^{R} + α-Glc^{R}), 72 ± 0.4% of the Glc end-groups were derivatized in the carbamylated holocellulose.

HSQC spectra for non-derivatized and derivatized MWL and MW, respectively, are depicted in Figures 4 and 5. Figures 4b and 5b give clear evidence of complete lignin side-chain carbamylation; β-aryl ether (C-A), phenylcoumaran (C-B) and pinoresinol (C-C) correlations are consistent with those in carbamylated lignin model compounds (Table 2). To investigate phenolic derivatization, one-dimensional ^{13}C NMR spectra were acquired on the derivatized and non-derivatized MWL. Signals at 152.55 ppm and 152.42 ppm of the carbamylated MWL spectra confirmed that phenolic derivatization occurred; lignin side-chain carbamates were detected between 153.4 ppm and 154.3 ppm. The major polysaccharide correlations in the carbamylated ball-milled wood spectra (C-Glc, C-Man and C-Xyl) were found to be consistent with correlations from the carbamylated D(+)-cellobiose and carbamylated holocellulose.

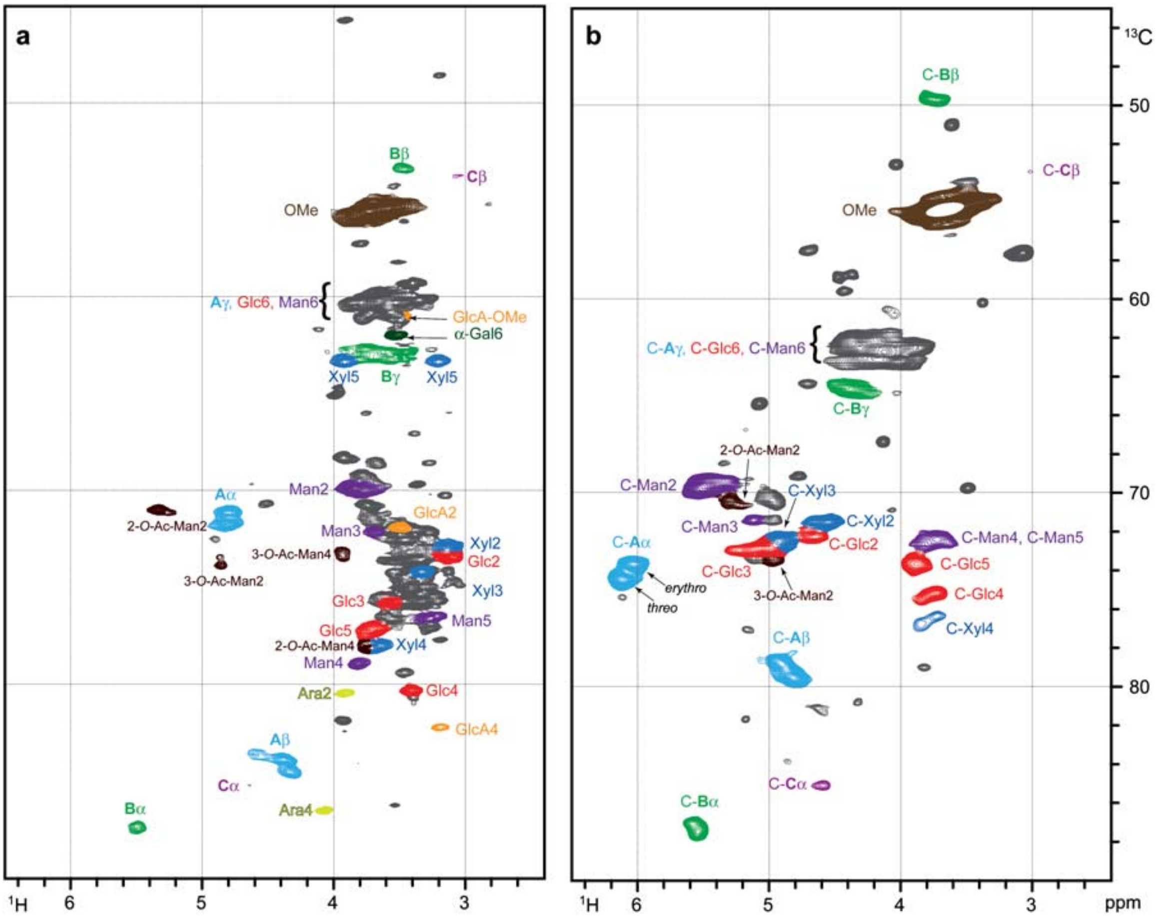


Figure 5 HSQC spectra of loblolly pine ball-milled wood: (a) non-derivatized, (b) derivatized with phenyl isocyanate. Colored contours correspond to specific lignin structures shown in Figure 2. Contours labeled with a ''C'' (e.g., C-**Aα**) represent carbamylated structures.

Conclusions

Reactions between the pMDI model compound, phenyl isocyanate, and wood polymers were analyzed to elucidate where and to what degree carbamates formed between isocyanates and cellulose, hemicelluloses and lignin in loblolly pine. It was found that lignin model compound reactions in DMAc with DBTDL as the catalyst were able to derivatize side-chain hydroxyls, but not the phenolic hydroxyl; however, in toluene/DBTDL systems, under identical molar ratios of NCO:OH, the phenolic OH groups were derivatized in good yields. Reactions with lignin and polysaccharide model compounds produced carbamate derivatives that displayed characteristic chemical shifts in solution-state NMR. These chemical shifts, and the shift differences between derivatized and non-derivatized components, permitted us to identify the degree and regiochemistry of derivatization of wood polymer hydroxyls for each cell wall component. Dissolution of carbamylated cellulosic polymers at 55°C allowed for good peak dispersion in HSQC spectra. Two-dimensional ^{13}C–^{1}H correlation NMR spectroscopy therefore is effective to characterize carbamylated and non-derivatized polymer structures in plant cell walls.

Acknowledgements

We thank Dr. Hoon Kim, Dr. Fachuang Lu (Department of Biochemistry, University of Wisconsin-Madison) and Dr. Takuya Akiyama (Wood Chemistry Laboratory, Department of Biomaterial Sciences, The University of Tokyo, Japan) for their enlightening discussions on lignin chemistry and plant cell wall dissolution. We thank Sally Ralph and Kolby Hirth (U.S. Forest Products Laboratory) for their insightful discussions on the NMR experiments. This research made use of 500 MHz instruments at the U.S. Dairy Forage Research Center and National Magnetic Resonance Facility at the University of Wisconsin-Madison. Support for this research came from the Office of Science (BER), U.S. Department of Energy, Interagency agreement No. DE-AI02-06ER64299 and in part by the DOE Great Lakes Bioenergy Research Center (DOE Office of Science BER DE-FC02-07ER64494).

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Received May 21, 2010. Accepted October 11, 2010.