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ORIGINAL

Origin of the acetylated structures present in white birch (*Betula pendula* Roth) milled wood lignin

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Abstract The occurrence and nature of acetate groups in the milled wood lignin (MWL) isolated from birch (*Betula pendula* Roth) has been addressed by spectroscopic (2D-NMR) and chemical degradative (derivatization followed by reductive cleavage, DFRC) methods. Considerable amounts of acetate groups were present in the MWL preparation. However, 2D-NMR analysis indicated that the lignin polymer is not extensively acetylated and that the major part of the acetate groups is attached to the xylan moieties present in the MWL preparation. Nevertheless, evidence of the presence of minor acetylation of the γ-carbon of the lignin side chain (<3% of both syringyl and guaiacyl lignin units) was provided by DFRC analysis.

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Introduction

It has recently been demonstrated that a fraction of the terminal hydroxymethyl groups in the lignin units in certain types of lignins, including wood lignins, are acetylated, and in some cases to a high extent (Ralph 1996; Lu and Ralph 2002, 2008; del Río et al. 2007, 2008; Martínez et al. 2008). In a previous study on milled wood lignin (MWL) isolated from white birch (*Betula pendula* Roth), considerable amounts of acetate groups were found to be present (Lundquist et al. 1980; Lundquist 1987). This fact raised the question as to whether the lignin moiety from birch is eventually acetylated at the γ-carbon, as occurs in other woods (del Río et al. 2007), and this structural characteristic was biased in previous studies. Therefore, in the present paper, the origin of the acetate groups in the MWL from birch was studied. For this purpose, the authors used bidimensional nuclear magnetic resonance (2D-NMR), which is a useful tool for lignin structural studies, and DFRC (derivatization followed by reductive cleavage) degradation, which can give information on the extent of acetylation of the γ-carbon of the lignin side chain. In particular, DFRC is a simple and powerful method, which selectively and efficiently cleaves α-ether and β-ether linkages but leaves γ-esters intact allowing the analysis of native γ-acylated lignin (Lu and Ralph 1997a, b, 1998). However, the DFRC method uses acetylating reagents that interfere in the analysis of native acetates in lignin, but with appropriate modification (Ralph and Lu 1998; del Río et al. 2007), it is possible to obtain significant information and clues about the occurrence and extent of lignin acetylation.

Experimental

MWL isolation

The preparation of MWL from birch has been described by Björkman and Person (1957). Neutral carbohydrate composition in MWL was determined by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD), using a Dionex ICS-3000 system equipped with a CarboPac PA1 (4 × 250 mm) analytical column, after acid hydrolysis according to Theander and Westerlund (1986). Xylan was the prevalent carbohydrate constituent accounting for 5% of the MWL preparation.

Preparation of model compounds

Compounds 1 and 2 (Fig. 1) were obtained by acetylation of 1-(3,4 dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol, prepared according to Ibrahim and Lundquist (1994), and 2-(2,6-dimethoxyphenoxy)-1-(3,4,5-trimethoxyphenyl)-1,3-propanediol, prepared according to von Unge et al. (1988). Brief treatment of these compounds with acetic anhydride according to the procedure described by Paulsson et al. (1996) gave 1 and 2 as predominating products.

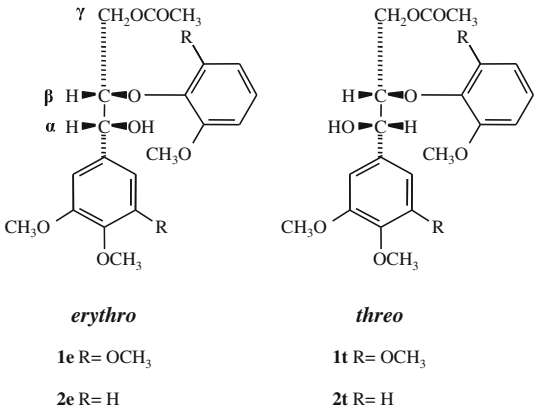

Fig. 1 Model compounds representative of γ-acetylated lignin units

^{1}H NMR spectra

^{1}H NMR spectra were recorded at 400 MHz on a Varian Unity 400 instrument, at 20°C. DMSO-d_6 was used as solvent [internal reference, $(CH_3)_4Si$)]. For lignin spectra, the delay time was set to 5 s to secure quantitative results.

^{1}H NMR spectrum of compound 1e: δ 1.77 (3H, s, CH_3CO), 3.63 (3H, s, OCH_3), 3.73 (6H, s, OCH_3), 3.76 (6H, s, OCH_3), 4.05 (1H, dd, $J = 2.5$ and 11.6 Hz, H_γ), 4.30 (1H, dd, $J = 6.4$ and 11.6 Hz, H_γ), 4.36 (1H, ddd, $J = 2.5$, 4.6 and 6.4 Hz, H_β), 4.93 (1H, dd, $J = 4.6$ and 4.8 Hz, H_α), 5.57 (1H, d, $J = 4.8$ Hz, OH), 6.64 (2 H, d, $J = 8.4$ Hz, H–Ar), 6.65 (2 H, s, H–Ar), 6.97 (1 H, t, $J = 8.4$ Hz, H–Ar).

^{1}H NMR spectrum of compound 1t: δ 1.85 (3H, s, CH_3CO), 3.65 (3H, s, OCH_3), 3.73 (6H, s, OCH_3), 3.75 (6H, s, OCH_3), 3.86 (1H, dd, $J = 6.3$ and 11.8 Hz, H_γ), 4.07 (1H, dd, $J = 3.5$ and 11.8 Hz, H_γ), 4.34 (1H, ddd, $J = 3.5$, 4.5 and 6.3 Hz, H_β), 4.84 (1H, dd, $J = 4.5$ and 4.5 Hz, H_α), 5.42 (1H, d, $J = 4.5$ Hz, OH), 6.66 (2 H, d, $J = 8.4$ Hz, H–Ar), 6.80 (2 H, s, H–Ar), 6.99 (1 H, t, $J = 8.4$ Hz, H–Ar).

^{1}H NMR spectrum of compound 2e: δ 1.89 (3H, s, CH_3CO), 3.712 (3H, s, OCH_3), 3.714 (3H, s, OCH_3), 3.718 (3H, s, OCH_3), 4.22 (1H, dd, $J = 3.3$ and 11.9 Hz, H_γ), 4.27 (1H, dd, $J = 5.9$ and 11.9 Hz, H_γ), 4.53 (1H, ddd, $J = 3.3$, 5.9 and 5.9 Hz, H_β), 4.78 (1H, dd, $J = 4.6$ and 5.9 Hz, H_α), 5.64 (1H, d, $J = 4.6$ Hz, OH), 6.77-7.03 (7H, m, H–Ar).

^{1}H NMR spectrum of compound 2t: δ 1.91 (3H, s, CH_3CO), 3.72 (3H, s, OCH_3), 3.73 (3H, s, OCH_3), 3.76 (3H, s, OCH_3), 3.82 (1H, dd, $J = 7.1$ and 11.9 Hz, H_γ), 4.11 (1H, dd, $J = 3.2$ and 11.9 Hz, H_γ), 4.56 (1H, ddd, $J = 3.2$, 4.9 and 7.1 Hz, H_β), 4.80 (1H, dd, $J = 4.6$ and 4.9 Hz, H_α), 5.62 (1H, d, $J = 4.6$ Hz, OH), 6.82–7.06 (7H, m, H–Ar).

2D-NMR spectroscopy

2D-NMR spectra of underivatized MWL were recorded at 25°C in a Bruker AVANCE 500 MHz, equipped with a z-gradient triple resonance probe. Around 40 mg of MWL was dissolved in 0.75 ml of deuterated dimethylsulfoxide (DMSO-d_6),

and HSQC (heteronuclear single quantum correlation) spectrum was recorded. The spectral widths were 5,000 and 13,200 Hz for the ^{1}H and ^{13}C dimensions, respectively. The number of collected complex points was 2,048 for ^{1}H dimension, with a recycle delay of 5 s. The number of transients was 64, and 256 time increments were always recorded in ^{13}C dimension. The $^{1}J_{CH}$ used was 140 Hz. The *J*-coupling evolution delay was set to 3.2 ms. Squared cosine-bell apodization function was applied in both dimensions. Prior to Fourier transformation, the data matrixes were zero filled up to 1,024 points in the ^{13}C dimension. The central solvent (DMSO) peak was used as an internal reference (δ_C 39.5; δ_H 2.49). HSQC cross-signals were assigned by comparison with the literature (Ralph et al. 1999, 2004; Liitiä et al. 2003; Capanema et al. 2004, 2005; Ibarra et al. 2007a, b; del Río et al. 2008, 2009; Martínez et al. 2008; Rencoret et al. 2008, 2009).

A semiquantitative analysis of the intensities of the HSQC cross-signal intensities was performed (Liitiä et al. 2003). Since the cross-signal intensity depends on the particular $^{1}J_{CH}$ value, as well as on the T_2 relaxation time, a direct analysis of the intensities is elusive. Thus, integration was performed separately for the different regions of the spectra, which contain signals corresponding to chemically analogous C–H pairs, with similar $^{1}J_{CH}$ coupling values. In the aliphatic-oxygenated region, inter-unit linkages were estimated from C_α-H_α correlations, except for structures J described below where the C_γ-H_γ correlations were used, and the relative abundance of side chains involved in the different substructures and terminal structures were calculated. In the aromatic region, C–H correlations from syringyl (S) and guaiacyl (G) units were used to estimate the S/G ratio, taking into account that, on average, there are 2.5 protons in G units and two protons in S units (Larsson and Miksche 1971). The number of acetate groups in xylan moieties was roughly estimated based on integration of the acetate signal and comparison with the peak due to the anomeric protons.

DFRC analysis

A modification of the standard DFRC method by using propionyl instead of acetyl reagents (DFRC′) was used (Ralph and Lu 1998; del Río et al. 2007). Lignins (10 mg) were stirred for 2 h at 50°C with propionyl bromide in propionic acid (8:92, v/v). The solvents and excess of bromide were removed by rotary evaporation. The products were then dissolved in dioxane/propionic acid/water (5:4:1, v/v/v), and 50 mg powdered Zn was added. After 40-min stirring at room temperature, the mixture was transferred into a separatory funnel with dichloromethane and saturated ammonium chloride. The aqueous phase was adjusted to pH < 3 by adding 3% HCl, the mixture was vigorously mixed, and the organic layer was separated. The water phase was extracted two more times with dichloromethane. The combined dichloromethane fractions were dried over anhydrous $NaSO_4$, and the filtrate was evaporated in a rotary evaporator. The residue was subsequently propionylated for 1 h in 1.1 ml of dichloromethane containing 0.2 ml of propionic anhydride and 0.2 ml pyridine. The propionylated lignin degradation compounds were collected after rotary evaporation of the solvents and subsequently analyzed by GC/MS.

The GC/MS analyses were performed with a Star 3400 GC (Varian) equipped with a Saturn 2000 ion trap detector (Varian) using a 12 m × 0.25 mm i.d., 0.1 μm, DB-5HT length capillary column (J&W Scientific, Folsom, CA, USA). The oven was heated from 50 (held 0.2 min) to 100°C at 30°C/min, then raised to 300°C at 5°C/min, and held for 5 min at the final temperature. The injector and transfer line were kept at 300°C. Helium was used as the carrier gas at a rate of 2 ml/min.

Results and discussion

The HSQC spectrum of the birch MWL preparation is shown in Fig. 2, and the main lignin substructures found are also depicted in Fig. 2. Cross-signals from both lignin and carbohydrates were observed in the HSQC spectrum, and their assignments are listed in Table 1. Interestingly, the spectrum exhibits a strong cross-signal at δ_C/δ_H 20.6/2.00, assigned to methyl units in acetate groups, in agreement with the relatively high amounts of acetates in this MWL (Lundquist et al. 1979, 1980; Lundquist 1987). Acetylated units are known to occur in lignins of different origins (Ralph 1996; Lu and Ralph 2002, 2008; del Río et al. 2007, 2008; Martínez et al. 2008), and this could be an explanation for the occurrence of acetate groups in the birch MWL. In order to assess the possible presence of acetate groups in the lignin polymer, model compounds representative of lignin units with acetyl groups attached to the γ-carbon of the side chain (Fig. 1) were prepared and examined. Model compound 1 is representative of the major part of the acetylated units found in lignins (Ralph 1996; Lu and Ralph 2002, 2008; del Río et al. 2007, 2008; Martínez et al. 2008). The location of the acetate signals in the model compounds 1e (δ_H 1.77), 1t (δ_H 1.85), 2e (δ_H 1.89), and 2t (δ_H 1.91) differs significantly from the signal at δ_H 2.00 observed in the spectrum of the birch MWL. However, the location of the acetate signals in the spectrum agrees well with those of the acetate groups in acetylated xylan fractions isolated from birch wood (Teleman et al. 2002).

The side-chain region of the HSQC spectrum, which gives information about the inter-unit linkages present in the structure of these lignins, can also provide additional information concerning the possible acetylation of birch MWL. The spectrum showed prominent signals corresponding to β-O-4′ aryl ether linkages. Interestingly, the most characteristic C_γ-H_γ correlation signal in γ-acylated β-*O*-4′ substructures to be found around δ_C/δ_H 63.8/3.83-4.30 (del Río et al. 2008; Martínez et al. 2008) was not detected in the HSQC spectrum, indicating the absence of γ-acetylated lignin units. Instead, the C_γ-H_γ correlations in β-O-4′ substructures were observed at δ_C/δ_H 59.54/3.38-3.71 and corresponded to normal hydroxylated γ-carbon units. The C_α-H_α correlations in β-O-4′ substructures were observed at δ_C/δ_H 72.3/4.86 (structure A), while the C_β-H_β correlations were observed at δ_C/δ_H 86.0/4.11 (for structures linked to an S unit) and 83.5/4.28 (for structures linked to a G unit), indicating also the absence of γ-acetylated lignin units. Other signals observed in the spectrum corresponded to resinol (β-β′) substructures (B), phenylcoumaran (β-5′) substructures (C), spirodienone (β-1′, α-O-α′) substructures (D), β-O-4′ substructures bearing a C_α carbonyl group (E), minor amounts of conventional open β-1′ structures (F), and *p*-hydroxycinnamyl alcohol end-groups

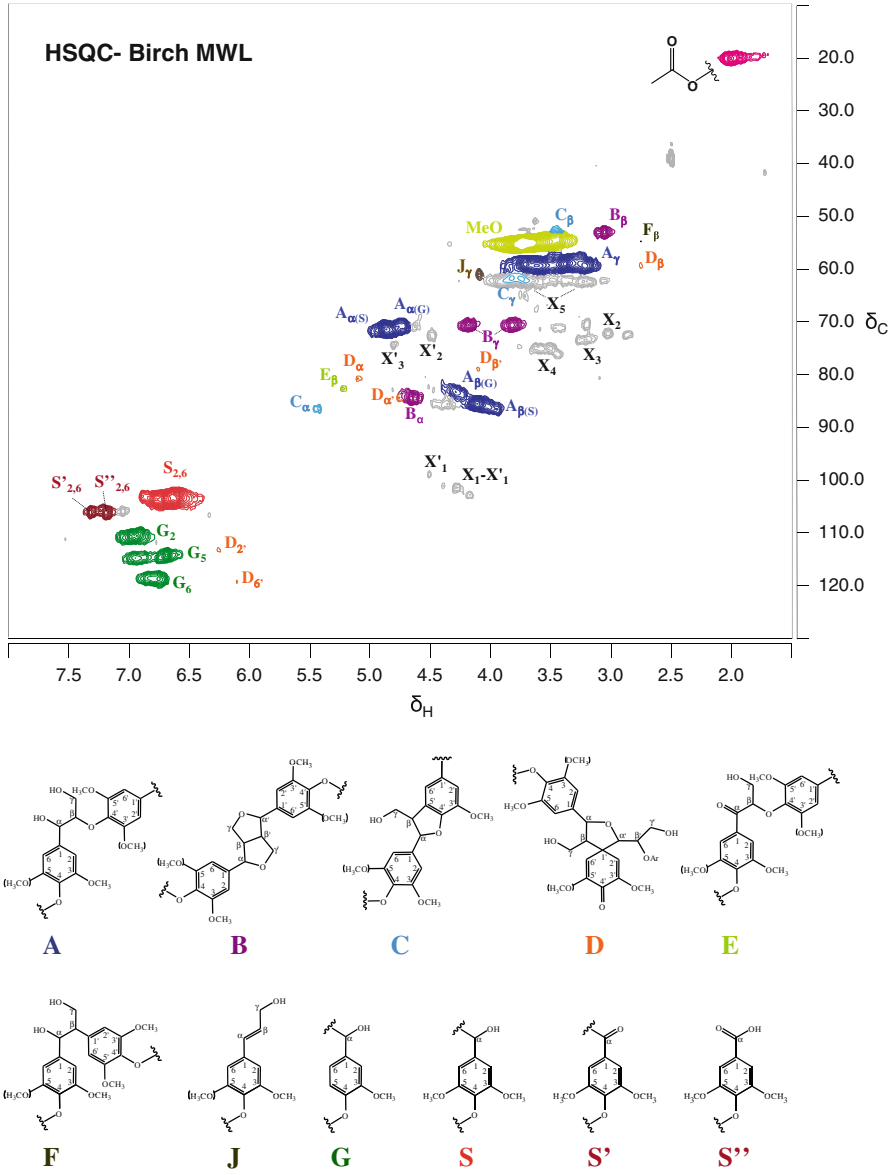

Fig. 2 HSQC NMR (δ_C/δ_H 10–130/1.5–8.0 ppm) spectrum of the MWL from birch. The lignin substructures identified are also shown: *A* *β*-*O*-4′ linkages; *B* resinol structures formed by *β*-*β*′, *α*-*O*-*γ*′, and *γ*-*O*-*α*′ linkages; *C* phenylcoumaran structures formed by *β*-5′ and *α*-*O*-4′ linkages; *D* spirodienone structures formed by *β*-1′ and *α*-*O*-*α*′ linkages; *E* *β*-*O*-4′ linkages oxidized at the *α*-carbon; *F* conventional open *β*-1′ structures; *J* *p*-hydroxycinnamyl alcohol end-groups; *G* guaiacyl units; *S* syringyl units; *S*′ oxidized syringyl units bearing a carbonyl group at C_α; *S*″ oxidized syringyl units bearing a carboxyl group at C_α. Labels for the main polysaccharide units: *X* xylopyranose or glucopyranose units; *X*′ acetylated xylopyranose or glucopyranose units. See Table 1 for the assignments of the different lignin and carbohydrate cross-signals

Table 1 Assignment of main lignin ^{13}C-^{1}H correlation signals in the HSQC spectrum of the MWL from birch shown in Fig. 1

Labels	δ_C/δ_H (ppm)	Assignment
Lignin cross-signals		
B_β	53.4/3.06	C_β-H_β in β-β' (resinol) substructures (B)
C_β	53.4/3.45	C_β-H_β in β-5′ (phenylcoumaran) substructures (C)
F_β	54.8/2.75	C_β-H_β in β-1′ substructures (erythro forms) (F)
A_γ	59.5/3.38-3.71	C_γ-H_γ in β-*O*-4′ substructures (A) and others
J_γ	61.4/4.10	C_γ-H_γ in cinnamyl alcohol end-groups (J)
C_γ	62.0/3.75	C_γ-H_γ in β-5′ (phenylcoumaran) substructures (C)
D_β	59.8/2.75	C_β-H_β in β-1′ (spirodienone) substructures (D)
B_γ	71.1/3.82 and 4.18	C_γ-H_γ in β-β' (resinol) substructures (B)
$A_{\alpha(S)}$	71.8/4.87	C_α-H_α in β-*O*-4′ substructures linked to a S unit (A)
$A_{\alpha(G)}$	71.3/4.77	C_α-H_α in β-*O*-4′ substructures linked to a G unit (A)
$D_{\beta'}$	79.3/4.11	$C_{\beta'}$-$H_{\beta'}$ in β-1′ (spirodienone) substructures (D)
D_α	81.1/5.10	C_α-H_α in β-1′ (spirodienone) substructures (D)
E_β	82.9/5.22	C_β-H_β in C_α-oxidized β-*O*-4′ substructures (E)
$A_{\beta(G)}$	83.5/4.29	C_β-H_β in β-*O*-4′ substructures linked to a G unit (A)
B_α	84.6/4.66	C_α-H_α in β-β' (resinol) substructures (B)
$A_{\beta(S)}$	86.0/4.11	C_β-H_β in β-*O*-4′ substructures linked to a S unit (A)
$D_{\alpha'}$	84.7/4.76	$C_{\alpha'}$-$H_{\alpha'}$ in β-1′ (spirodienone) substructures (D)
C_α	86.8/5.42	C_α-H_α in β-5′ (phenylcoumaran) substructures (C)
$S_{2,6}$	103.9/6.68	C_2-H_2 and C_6-H_6 in syringyl units (S)
$S'_{2,6}$ $S''_{2,6}$	106.3/7.32 and 7.20	C_2-H_2 and C_6-H_6 in C_α-oxidized syringyl units (S′ and S″)
G_2	110.8/6.96	C_2-H_2 in guaiacyl units (G)
$D_{2'}$	113.5/6.26	$C_{2'}$-$H_{2'}$ in β-1′ (spirodienone) substructures (D)
G_5	114.9/6.70 and 6.94	C_5-H_5 in guaiacyl units (G)
G_6	118.9/6.76	C_6-H_6 in guaiacyl units (G)
$D_{6'}$	118.9/6.08	$C_{6'}$-$H_{6'}$ in β-1′ (spirodienone) substructures (D)
Carbohydrate cross-signals		
X_5	62.8/3.19 and 3.87	C_5-H_5 in β-D-xylopyranoside
X_2	72.6/3.03	C_2-H_2 in β-D-xylopyranoside
X'_2	73.1/4.49	C_2-H_2 in 2-*O*-acetyl-β-D-xylopyranoside
X_3	73.8/3.22	C_3-H_3 in β-D-xylopyranoside
X'_3	74.7/4.79	C_3-H_3 in 3-*O*-acetyl-β-D-xylopyranoside
X_4	75.3/3.52	C_4-H_4 in β-D-xylopyranoside
X'_1	99.4/4.49	C_1-H_1 in 2-*O*-acetyl-β-D-xylopyranoside
X'_1/X_1	101.6/4.27	C_1-H_1 in 3-*O*-acetyl-β-D-xylopyranoside/β-D-xylopyranoside

(J; Fig. 2, Table 1). The main cross-signals in the aromatic region of the HSQC spectrum correspond to the aromatic rings of the S and G lignin units. No signals for *p*-hydroxyphenyl (H) lignin units could be detected. The relative abundances of the main lignin inter-unit linkages (referred to as the total side chains) present in the

Table 2 Structural characteristics (relative abundance of the main inter-unit linkages, as percentage of side chains involved, and H/G/S composition) observed from the HSQC spectrum of the MWL from birch

	Birch MWL
Linkage relative abundance (%)	
β-O-4′ alkyl-aryl ethers (A)	69
Resinols (B)	17
Phenylcoumarans (C)	3
Spirodienones (D)	4
β-O-4′ oxidized at Cα (E)	3
β-1′ structures (F)	2
Cinnamyl alcohol end-groups (J)	2
H:G:S ratio	0:34:66

MWL from birch, as well as the relative molar abundance of the H, G, and S units, were calculated from the HSQC spectrum and are shown in Table 2. The HSQC data shown above indicate a predominance of S over G lignin units (S/G 1.94) and a predominance of β-O-4′ aryl ether linkages followed by resinol structures and minor proportions of other structures (i.e., spirodienones, phenylcoumarans, etc.,). In addition, the HSQC data clearly indicate that the lignin moiety is not extensively acetylated at the γ-position of the side chain, as shown above, and that it cannot be at the origin of the acetate groups present in the birch MWL preparation.

On the other hand, important signals from carbohydrates were also clearly present in the HSQC spectrum (Fig. 2), in agreement with the presence of ca. 5% xylans in the birch MWL preparation. Cross-signals from carbohydrates are observed in two differentiated regions of the spectrum, in the aliphatic-oxygenated region and in the region corresponding to the correlations of the anomeric carbon. The aliphatic-oxygenated region of the spectrum shows strong signals from carbohydrates, including acetylated hemicelluloses. Among them, it is particularly evident the presence of signals from *O*-acetylated xylans (3-*O*-acetyl-β-D-xylopyranoside, X'_3 and 2-*O*-acetyl-β-D-xylopyranoside, X'_2) at δ_C/δ_H 74.7/4.79 and 73.1/4.49, respectively. Other signals in this region correspond to C_2-H_2, C_3-H_3, C_4-H_4, and C_5-H_5 correlations of xylans (β-D-xylopyranoside, X_2, X_3, X_4, X_5). The anomeric carbon correlation (C_1-H_1) signals corresponded to both (C-2 and/or C-3) acetylated (X'_1) and unacetylated (X_1) xylan moieties. The number of acetate groups in xylan moieties was roughly estimated based on integration of the acetate signal and comparison with the peak due to the anomeric protons and indicated about 7 acetate groups per 10 xylose units, including both monoacetylated (at C-2 or C-3) and diacetylated (at C-2 and C-3) units. This is in fair agreement with data for birch xylan reported in the literature (Sjöström 1993). The HSQC spectrum therefore confirmed the significant acetylation of the xylan moiety present in the birch MWL preparation. The above cross-signals are also visible in other xylan-containing MWL spectra and are especially evident in the spectra of whole lignocellulosic samples, analyzed at the gel state (Rencoret et al. 2009; Kim et al. 2008; Kim and Ralph 2010). Therefore, it is clear from the HSQC data shown above

that the lignin moiety is not extensively acetylated (at the γ-position of the side chain) and that the major part of the acetate groups present in this MWL preparation are attached to xylan moieties.

However, the presence of minor acetylation of the γ-carbon of the lignin side chain in the birch MWL could be observed by the modified-DFRC analysis. The DFRC degradation method cleaves α- and β-ether linkages in the lignin polymer leaving γ-esters intact (Lu and Ralph 1997a, b, 1998) and is an appropriate and sensitive method for the analysis of native γ-acylated lignin. However, the original DFRC degradation method does not allow the analysis of native acetylated lignin because the degradation products are acetylated during the degradation procedure, but by substituting acetylating reagents with propionylating reagents (DFRC′), it is possible to obtain information about the occurrence and extent of native lignin acetylation (Ralph and Lu 1998; del Río et al. 2007). Figure 3 shows the chromatogram of the DFRC′ products released from the MWL from birch. The DFRC′ released the *cis*- and *trans*- isomers of G- (*c*-G and *t*-G) and S-type (*c*-S and *t*-S) lignin monomers (as their propionylated derivatives) arising from normal γ-OH units in lignin. In addition, the presence of γ-acetylated guaiacyl (*c*-G_{ac} and *t*-G_{ac}) and syringyl (*c*-S_{ac} and *t*-S_{ac}) lignin units, arising from originally acetylated lignin units, could also be clearly observed in the chromatogram of the DFRC′ degradation

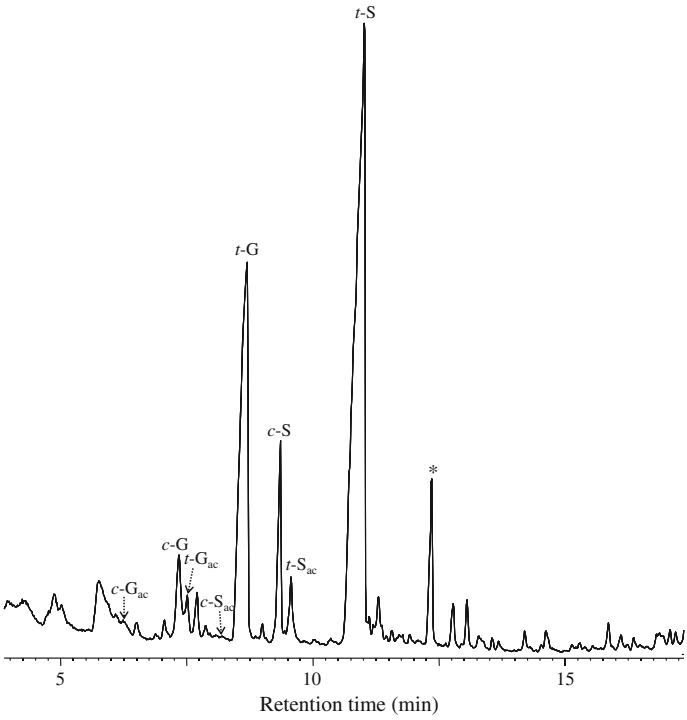

Fig. 3 Chromatogram of the DFRC′ degradation products of the MWL from birch. *c*-G, *t*-G, *c*-S and *t*-S are the *cis*- and *trans*-guaiacyl and syringyl monomers, respectively. *c*-G_{ac}, *t*-G_{ac}, *c*-S_{ac} and *t*-S_{ac} are the originally acetylated *cis*- and *trans*-guaiacyl and syringyl monomers, respectively

products of the birch MWL, indicating that some acetylation occurred at the γ-carbon of the lignin side chain. The DFRC′ analyses indicated that the extent of acetylation was low and that only 2.7% of S-monomers and only 2.9% of G-monomers were γ-acetylated. This very low acetylation degree is similar to that reported in a previous paper (del Río et al. 2007) for other wood MWL (e.g., from aspen) and differs from that reported, e.g., for *Carpinus betulinus* MWL that, as found also for several nonwoody lignins, has up to 45% of its S units γ-acetylated (but only 3% of the G units). The concerns about the possibility that the minor acetylated lignin units present in the MWL from birch might be artifacts formed during the MWL isolation process must be dismissed and is possible to demonstrate that the acetate groups attached to the γ-carbon of the lignin side chain arise from originally pre-acetylated lignin monomers. The evidence comes from the β–β' coupling reactions. If the γ-carbon of a monolignol is pre-acetylated, the formation of the normal β–β' resinol structures cannot occur because the absence of free γ-hydroxyls needed to re-aromatize the quinone methide moiety. Instead, new tetrahydrofuran structures are formed from the β–β' homo- and cross-coupling of

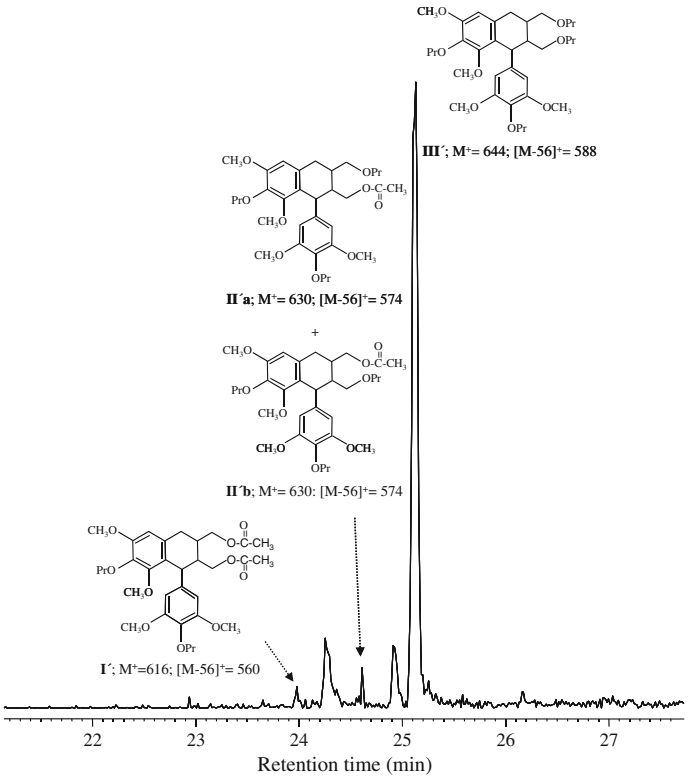

Fig. 4 Detail of the reconstructed chromatogram (sum of the characteristic ions at *m/z* 560, 574 and 580) of the DFRC′ degradation products of the MWL from birch, showing the presence of aryltetralin β-β' products containing two (I′), one (II′a and II′b), and no (III′) native acetates, with indication of their molecular weight and base peak in their mass spectra

two sinapyl (acetylated and non-acetylated) monolignols (Lu and Ralph 2002, 2008; del Río et al. 2007, 2008). Figure 4 shows the reconstructed chromatogram (sum of the single-ion chromatograms of the respective base peaks) of the DFRC′ degradation products of the tetrahydrofuran dimers arising from the β-β' coupling of the acetylated and non-acetylated sinapyl monolignols. Interestingly, compounds derived from the DFRC′ of homo-coupling (I′) and cross-coupling (II′a and II′b) of sinapyl acetate were clearly observed, although in low amounts, in the MWL from birch, indicating that in this lignin a small amount of sinapyl alcohol is pre-acetylated and behaves like a real monolignol participating in post-coupling reactions, as also occurs in other lignins.

The low level of lignin acetylation observed by DFRC′, which is undetectable in the HSQC spectrum, cannot be responsible for the considerable amounts of acetate groups reported in this MWL preparation (Lundquist et al. 1980; Lundquist 1987) and the strong acetate cross-signal observed in the HSQC spectrum. It is then possible to conclude that the acetate groups attached to the xylan moieties are at the origin of the relatively high amounts of acetate groups present in the birch MWL.

Conclusion

Considerable amounts of acetate groups have been found to occur in birch MWL. Spectroscopic (HSQC) and chemical degradative (DFRC) analyses concluded that the acetylation degree of the lignin moiety is only minor (*ca.* 3% of G and S lignin units), while the xylan moiety present in the birch MWL preparation (*ca.* 5% of the MWL) is significantly acetylated (*ca.* 7 acetate groups per 10 xylose units) and responsible for the major part of the acetate groups in this MWL.

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