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A Biomimetic Route to Lignin Model Compounds *via* Silver (I) Oxide Oxidation

2. NMR Characterization of Non-Cyclic Benzyl Aryl Ether Trimers and Tetramers

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Keywords

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Summary

The non-cyclic α-O-4 benzyl aryl ethers, *erythro*-guaiacylglycerol-α,β-bis-coniferyl ether, *erythro*-guaiacylglycerol-α-dehydrodiconiferyl-β-coniferyl-bis-ether and *erythro*-guaiacylglycerol-α-pinoresinol-β-coniferyl-bis-ether, isolated from the silver(I) oxide-mediated oxidative coupling of coniferyl alcohol in aqueous solutions, were fully characterized by the use of 1D and 2D NMR techniques. The application of 2D HMQC-TOCSY and delayed COSY experiments were found to be a useful complement and/or alternative to the application of the usual routine 2D homo- and heteronuclear correlation experiments for the structural elucidation and chemical shift assignments of these oligomeric lignols.

Introduction

Non-cyclic α-O-4 linked benzyl aryl ethers were first proposed as lignin intermediates by Freudenberg and Friedmann (1960) on the basis of their results from the dehydrogenation of coniferyl alcohol with manganese dioxide. The trilignol **5** (guaiacylglycerol-α,β-bis-coniferyl ether) and the tetralignol **6** (guaiacylglycerol-α-dehydrodiconiferyl-β-coniferyl-bis-ether) were isolated and their structures confirmed *via* derivatization of their phenolic hydroxyl groups with 2,4-dinitrofluorobenzene. These *p*-hydroxybenzyl aryl ethers are susceptible to hydrolysis of their α-O-4 bond, but would be stabilized by etherification during lignin biosynthesis (Freudenberg 1968). Dehydrogenation of their phenolic hydroxyl groups and subsequent radical coupling lead to stable *p*-alkoxybenzyl aryl ethers and thus the incorporation of α-O-4 units into lignins. Spruce milled wood lignin isolates (MWLs) have been estimated by chemical analyses to contain about 2–4 non-cyclic *p*-hydroxybenzyl aryl ethers and 5–9 non-cyclic *p*-alkoxybenzyl aryl ethers per 100 phenylpropanoid lignin units (Freudenberg *et al.* 1964; Adler *et al.* 1966, 1968). More recent analyses of lignins by NMR spectroscopy have indicated that the above estimates should be revised downwards (Lundquist 1980; Adler *et al.* 1987; Ede *et al.* 1990; Ede and Brunow 1992). Nimz and Lüdemann (1974) had reported the ^{13}C-NMR chemical shifts of the 2,4-dinitrophenyl ethers of the hydrogenated and acetylated derivatives of **5** and **6**, and assigned signals in ^{13}C-NMR spectra of MWLs from both soft- and hardwoods, as well as DHP lignins, to the side-chain carbons in non-cyclic benzyl aryl ether structures (Nimz and Lüdemann 1976; Nimz *et al.* 1981, 1982). On the basis of studies on the reactivity of simple quinone methides towards hydroxy compounds, Leary (1980) claimed that non-cyclic α-O-γ benzyl alkyl ethers would be more frequent in lignins than non-cyclic α-O-4 benzyl aryl ethers, and thus doubted Nimz's interpretations of ^{13}C-NMR lignin spectra with respect to the nature of the non-cyclic benzyl ether bonds occurring in lignins (Nimz 1981; Leary 1982; Leary *et al.* 1983; Leary and Sawtell 1984; Nimz *et al.* 1984). The exclusive formation of non-cyclic α-O-4 benzyl aryl ethers during phenol/alcohol competing additions to quinone methide lignin model compounds has however recently been reported (Sipilä and Brunow 1991; Quideau and Ralph 1994).

This paper describes the NMR characterization of the non-cyclic benzyl aryl ethers **5, 6** and **7** (guaiacylglycerol-α-pinoresinol-β-coniferyl-bis-ether), readily accessible as secondary products of the Ag_2O-mediated oxidative coupling of coniferyl alcohol in aqueous solutions (Quideau and Ralph 1994). This oxidizing system was chosen to mimick the initial conditions of the *p*-hydroxycinnamyl alcohol polymerization.

Experimental

Compounds **5a–7a** were obtained as described in the preceding paper (Quideau and Ralph 1994). Acetylations were performed using the acetic anhydride/4-(dimethylamino)pyridine procedure (Ralph *et al.* 1992) and methylation of **6a** was performed with

diazomethane generated from N-nitrosomethylurea (Fieser and Fieser 1967). NMR experiments were performed at 300 K, using 10–20 mg of sample in ca. 0.45 cm^3 of acetone-d_6 (unless otherwise noted), on a Bruker AMX-360 360 MHz narrow-bore instrument fitted with a 5 mm 4-nucleus (QNP) probe with normal geometry. The central solvent signals were used as internal reference (^{1}H, 2.04 ppm; ^{13}C, 29.80 ppm). Two-dimensional NMR spectra were obtained using standard pulse sequence programs. The phase sensitive HMQC-TOCSY spectrum in Figure 2 was run by using Bruker's inv4mltp pulse program (Lerner and Bax 1986) with an isotropic mixing period of 60 ms. A 2 K × 256 data matrix was acquired using 64 transients per t_1 increment (*ca.* 14 h run time). Squared cosine-bell apodization was applied in both dimensions, the matrix was zero-filled and Fourier transformed to a final size of 1 K × 1 K real points. The delayed COSY spectrum in Figure 3 was run by using Bruker's cosylr pulse program (Bax and Freeman 1981) with 90° pulses of 10.7 μs and a delay of 400 ms. 1 K data points were recorded in t_2 and 256 increments of 16 transients in t_1. The data matrix was zero-filled to a final size of 1 K × 512 real points and sine-bell apodization was applied in both dimensions prior to Fourier transformation. Full data for compounds **5–7**, recorded in acetone-d_6 (here), dimethylsulfoxide-d_6 and $CDCl_3$, are given in the pending NMR database of plant cell wall model compounds (Ralph *et al.* 1993).

Results and Discussion

The proton and carbon chemical shift assignments of compounds **5–7** given in Tables 1 and 2 were determined from analysis of the usual combination of routine 1 D experiments (^{1}H, ^{13}C and DEPT 135), 2 D ^{1}H-^{1}H (COSY's and TOCSY) and inverse-detected one-bond (HMQC, Bax and Subramanian 1986) and long-range (HMBC, Bax and Summers 1986) 2 D ^{1}H-^{13}C chemical shift correlation experiments. The assignment of the *erythro (anti)* configuration for these non-cyclic benzyl aryl ethers is based upon the chemical shifts of the non-equivalent methylene γ-protons of the phenylpropanoid unit A (Johansson and Miksche 1972; Helm and Ralph 1992). Chemical shifts and coupling constants of γ-proton resonances overlapping with methoxyl proton resonances in compounds **5a, 6c** and **7a** were obtained from analysis of reprocessed rows of TOCSY spectra (Helm and Ralph 1992). Whereas **5a** and **7a** were quite stable, the underivatized tetramer **6a** was partially degraded after a few hours in acetone-d_6 solution, preventing its detailed NMR characterization. NMR criteria of the side-chain carbons and protons of units A and C of **6a** were given in the preceding paper (Quideau and Ralph 1994). Compounds **6** have four centers of asymmetry. The *trans* configuration of the 2,3-dihydrobenzofuran moiety (i.e. 2*SR*, 3*RS*) and the *erythro (anti)* configuration of the 1,2-diaryloxypropane moiety (1*RS*, 2*SR*) limit the number of

Table 1. Selected ^{1}H NMR data for the *erythro (anti)* non-cyclic benzyl aryl ethers[a]

Compound	$\alpha_A(J_{\alpha\beta})$	β_A	$\gamma_A(J_{\gamma\beta})$ (J_{gem})	$\alpha_C(J_{\alpha\beta})$	β_C	$\gamma_C(J_{\gamma\beta})$ (J_{gem})
5a	5.46 (5.5)	4.56	3.81 (7.1) 3.93 (4.1) (11.7)	–	–	–
5b	5.62 (5.3)	4.87	4.45 (3.8) 4.53 (5.9) (11.8)	–	–	–
6b	5.62 (5.4)	4.88	4.46 (3.8) 4.53 (5.8) (11.9)	[c]5.490 (6.9) 5.494 (6.9)	3.72	[c]4.285 (7.5) 4.291 (7.5) 4.40 (5.4) (11.1)
6c[b]	5.48 (5.8)	4.62	3.80 (6.5) 3.91 (4.9) (11.9)	5.51 (5.8)	3.43	3.69 3.80
6d	5.54 (5.2)	4.87	4.44 (3.7) 4.52 (6.2) (11.8)	[c]5.483 (6.9) 5.487 (6.9)	3.70	[c]4.283 (7.5) 4.289 (7.5) 4.39 (5.5) (11.1)
7a	5.45 (5.6)	4.55	3.82 (6.5) 3.92 (5.2) (11.5)	4.63	3.04	3.77 4.17
7b	5.60 (5.6)	4.86	4.45 (3.8) 4.53 (5.9) (11.9)	4.66 [d]4.74 (4.5)	3.06	3.83 4.17 [d]4.23

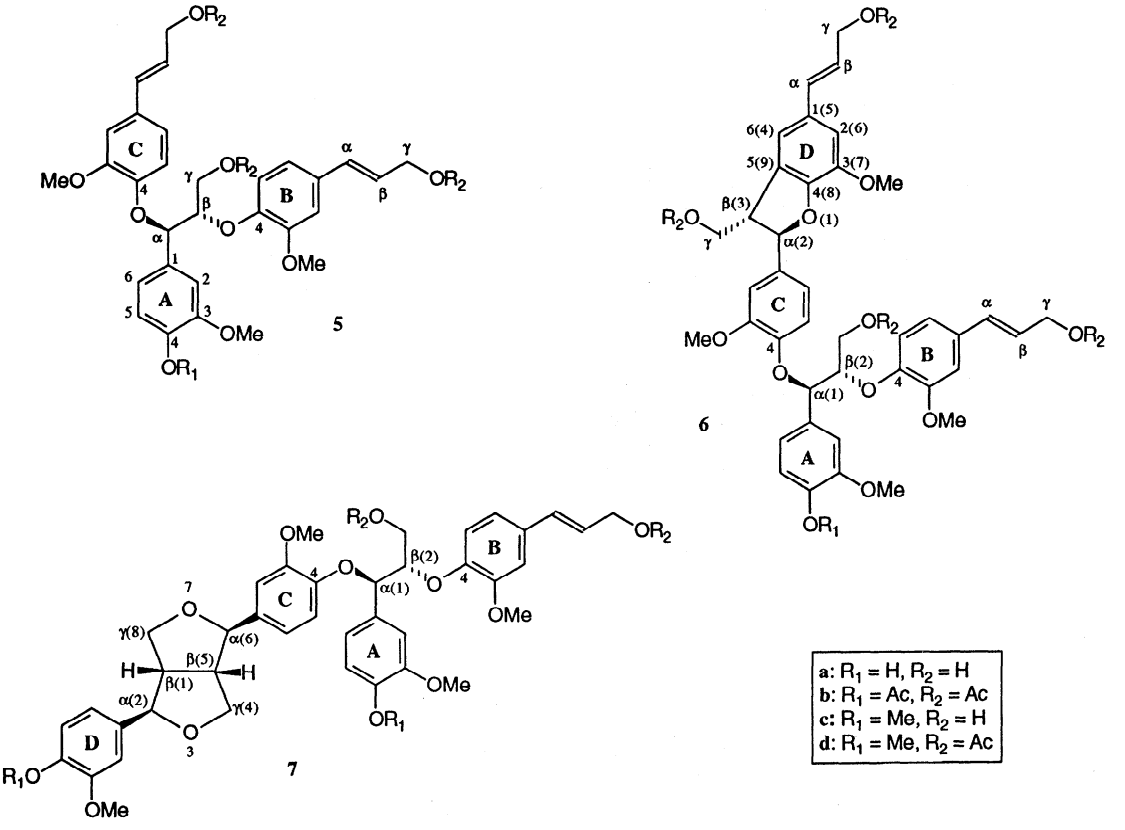
[a] Chemical shifts are given in ppm (acetone-d_6) and data in parentheses are coupling constants in Hz; see Fig. 1 for proton designations. Chemical shifts are quoted to three decimal places, when two resolvable shifts are close, to indicate the dispersion between them. [b] In acetone-d_6 : D_2O (9 : 1). [c] Compounds **6** and **7** are diastereomeric mixtures (see text and Fig. 1 for details); some proton resonances consist of two sets of lines, one for each diastereomer, e.g. for the α-proton and one of the γ-protons of unit C (α_C and γ_C) in **6b.** [d] unit D.

Table 2. ^{13}C NMR chemical shifts for the *erythro (anti)* non-cyclic benzyl aryl ethers[a,b]

	Carbon	5a	5b	6b	6c[c]	6d	7a	7b
Unit **A**	α	81.15	80.85	80.73	80.05	80.89	81.21	80.88
					80.10		81.20	80.86
	β	85.31	81.74	81.63	83.86	81.73	85.38	81.73
	γ	61.75	63.56	63.52	61.20	63.86	61.82	63.62
	1	130.39	137.52	137.49	130.94	130.90	130.53	137.72
	2	112.11	112.74	112.66	112.04	112.28	112.21	112.77
				112.69		112.31	112.23	112.79
	3	148.10	152.12	152.05	149.45	150.20	148.15	152.10
	4	147.18	140.65	140.56	149.48	150.23	147.24	140.62
	5	115.23	123.26	123.24	111.67	112.22	115.28	123.25
	6	121.49	120.51	120.45	120.96	120.95	121.51	120.51
				120.43		120.98	121.53	120.53
Unit **B**	α	129.85	134.22	134.20	130.02	134.26	129.88	134.23
	β	129.45	123.18	123.10	128.64	123.04	129.50	123.14
	γ	63.25	65.37	65.36	62.77	65.38	63.27	65.38
	1	132.74	132.34	132.23	132.11	132.08	132.78	132.27
	2	110.93	111.32	111.17	110.71	111.28	111.05	111.31
	3	151.71	151.91	151.82	150.83	151.81	151.75	151.86
	4	148.61	148.66	148.56	147.98	148.83	148.66	148.67
	5	119.04	119.23	119.11	117.55	118.91	119.06	119.11
	6	120.15	120.50	120.46	119.99	120.48	120.17	120.51
Unit **C**	α	129.85	134.22	88.57	87.80	88.64	86.37	86.27
				88.61		88.65		86.28
	β	129.22	122.99	51.04	54.21	51.06	55.15	55.11
					54.28	51.08		
	γ	63.25	65.37	65.84	64.18	65.90	72.23	72.48
	1	132.05	131.73	135.92	136.05	135.73	136.27	136.85
				135.94	136.08	135.74	136.29	136.86
	2	110.74	111.16	111.28	110.89	111.45	111.35	111.47
				111.36	110.98	111.47	111.43	111.57
	3	151.17	151.43	151.30	150.58	151.41	151.08	151.27
				151.33		151.42	151.12	151.31
	4	147.77	148.20	147.96	147.30	148.11	147.61	147.42
					147.31			
	5	117.02	117.41	117.29	116.32	117.41	116.79	117.29
					116.41	117.46	116.83	117.32
	6	119.90	120.45	119.02	118.40	119.05	118.88	118.90
				119.11	118.42	119.11	118.96	119.01
Unit **D**	α			134.69	130.57	134.71	86.58	86.20
							86.60	86.22
	β			122.13	127.73	122.19	55.15	55.36
	γ			65.48	62.85	65.49	72.23	72.37
	1			131.41	131.83	131.46	134.10	141.84
	2			112.02	111.35	112.16	110.59	111.07
	3			145.32	144.75	145.37	148.30	152.24
	4			149.22	148.27	149.29	146.85	140.08
	5			128.99	129.64	129.06	115.50	123.43
	6			116.24	115.96	116.31	119.61	118.62

[a] Chemical shifts are given in ppm (acetone-d_6); see Fig. 1 for carbon designations. [b] Split signals in compounds **6** and **7** are due to chemical shift differences between the carbon resonances of the two diastereomers (see text and Fig. 1 for details). [c] In acetone-d_6 : D_2O (9 : 1).

isomers of **6** to two diastereomeric pairs of enantiomers. Similarly, the 2-*cis*,6-*cis* configuration of the *cis*-fused 2,6-diaryl-3,7-dioxabicyclo[3.3.0]octane system (1*SR*, 2*RS*, 5*SR*, 6*RS*) and the 1*RS*, 2*SR* configuration of the 1,2-diaryloxypropane moiety for compounds **7** give rise to two racemic diastereomers (Fig. 1). As can be seen in Table 1, two sets of lines were observed for the resonances of some protons in compounds **6b** and **6d**. For instance, an 8-peak pattern was observed for the highfield γ-proton of the unit C of compounds **6b** and **6d**. A 600 MHz ^1H-NMR spectrum of **6b** indicated that this pattern was not the consequence of some resolved long-range coupling, but was due to two diastereomeric γ-protons, the dd's resonating at 4.285 and 4.291 ppm (Table 1). In the ^{13}C-NMR spectra of compounds



Compound			1,2-Diaryloxypropane	2,3-dihydrobenzofuran	2,6-Diaryl-3,7-dioxabicyclo[3.3.0]octane
6	d	e	1R, 2S	2R, 3S	
			1S, 2R	2S, 3R	
		e	1R, 2S	2S, 3R	
			1S, 2R	2R, 3S	
7	d	e	1R, 2S		1S, 2R, 5S, 6R
			1S, 2R		1R, 2S, 5R, 6S
		e	1R, 2S		1R, 2S, 5R, 6S
			1S, 2R		1S, 2R, 5S, 6R

Fig. 1. Non-cyclic α-O-4 benzyl aryl ethers isolated from the oxidative coupling of coniferyl alcohol in aqueous solutions. The atom designations are based on standard lignin nomenclature. Compounds **6** and **7** are diastereomeric pairs of racemates. The systematic numbering given in parentheses for individual fragments in structures **6** and **7** is employed with the assignment of the R/S stereo-descriptors, which follows CIP nomenclature. "e" and "d" stand for "enantiomer" and "diastereomer", respectively. Compound numbering maintains consistency with the previous paper (Quideau and Ralph 1994).

6b–d, 7a and **7b** (Table 2), the signals of some carbons, and especially those of the C-units, were split into pairs of lines with reduced intensity because of the chemical shift differences between the carbon resonances of the two diastereomers.

The aromatic carbon chemical shifts of the tetramers **6–7** were the most difficult to assign because of the congested aromatic proton regions. Analysis of COSY spectra allowed identification of aromatic protons 5 and 6 belonging to the same ring. Ring assignments were then determined from analysis of long-range HMBC spectra, in which the quaternary aromatic carbons 1 and 4 strongly correlated to aromatic and side-chain protons *via* two and three bonds (e.g. in **6b**, the A-1 carbon correlated to the A-2, A-5, α_A and β_A protons, the C-1 carbon with the C-2, C-5, α_C and β_C protons, the A-4 carbon with the A-2, A-6 and A-5 protons, the D-4 carbon with the D-2, D-6 and α_C protons). The three-bond correlations between the C-4 carbon and the α_A proton, as well as between the B-4 carbon and the β_A proton, are noteworthy since our confirmation of the α,β-bis-O-4-aryl ether structure of compounds **5–7** was based on these observations. The aromatic carbons 2, 5 and 6 were then easily assigned from analysis of the one-bond HMQC spectra.

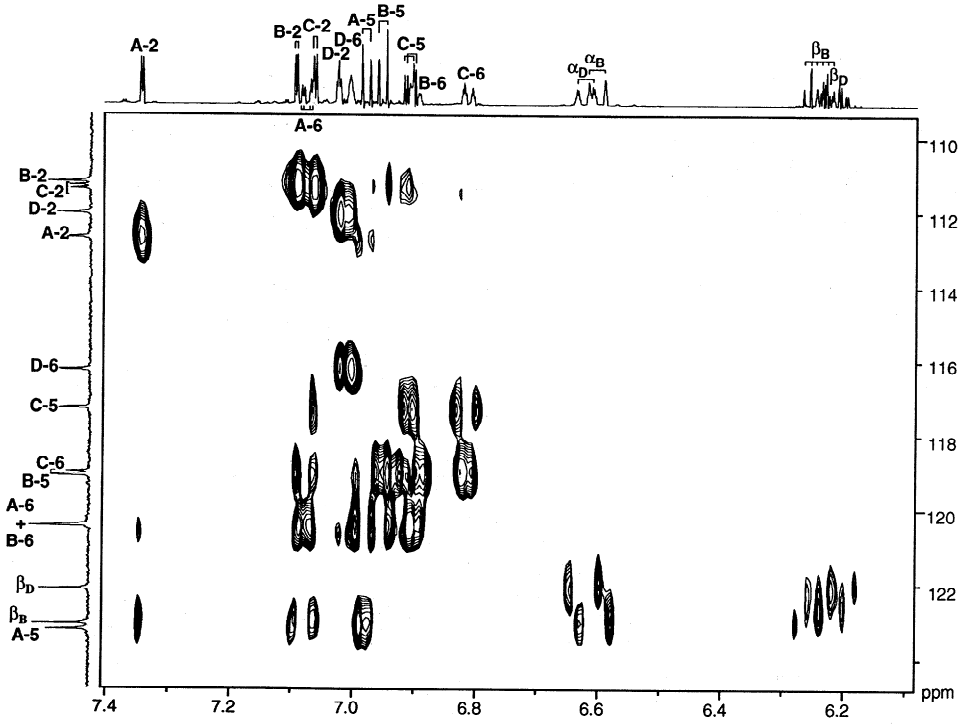


Fig. 2. Aromatic region of the phase-sensitive HMQC-TOCSY spectrum of **6b** (diastereomeric mixture) showing correlations between the aromatic carbons and the protons of their respective rings.

A combined HMQC-TOCSY experiment (Lerner and Bax 1986) was shown to be very useful for the characterization of the investigated compounds since it provided, in a single experiment (see Experimental), the information gathered from COSY's, TOCSY and HMQC experiments, while confirming the HMBC correlation assignments. The aromatic region of the phase-sensitive HMQC-TOCSY spectrum of compounds **6b** is displayed in Figure 2. All aromatic carbons 5 and 6 exhibit intense responses with their directly attached proton *via* the one-bond ^{1}H-^{13}C coupling, and with the other protons of their respective rings *via* the TOCSY magnetisation transfer between protons in the same coupling network. For instance, the C-5 carbon correlates to the C-5 proton, and to the C-6 and C-2 protons. Ring assignments can be determined from the HMBC spectrum, as outlined above, and proton assignments can be deduced from the multiplicity of the proton resonances [e.g. C-5 (d, *ca.* 8 Hz), C-6 (dd, *ca.* 8 and 2 Hz), C-2 (d, *ca.* 2 Hz)]. Two signals can be observed in the 1 D spectra for the C-5 and C-6 protons, as well as for the C-2 and C-6 carbons, because of the chemical shift differences between the resonances of the two diastereomers of **6b** (Fig. 1). Similarly, the vinylic protons α_D and β_D each exhibit two signals.

Another experiment that we have found particularly useful for the characterization of such oligomeric lignin model compounds is the delayed COSY experiment, in which a fixed delay is incorporated to the standard COSY sequence at the end of the evolution period and before the beginning of acquisition, allowing for the enhancement of the intensity of the ^{1}H-^{1}H long-range coupling cross-peaks (Bax and Freeman 1981). This long-range version of the COSY experiment has recently been applied to the characterization of ester-linked lignin-hydroxycinnamyl model compounds (Helm and Ralph 1993). A portion of the delayed COSY spectrum of compounds **7a**, recorded by using an optimized delay of 400 ms (see Experimental) is shown in Figure 3. Intense cross-peaks are observed between the side-chain and aromatic proton resonances of the respective phenylpropanoid units. The α_A proton correlates to the A-2 and A-6 protons *via* four bonds, as well as to the A-5 proton *via* five bonds. Similar connectivities are observed for the α_C, α_D and the vinylic α_B proton resonances, allowing the complete assignment of the aromatic proton region. More important are the cross-peaks correlating the β_A proton to the B-5 proton, and the α_A proton to the C-5 proton. These ^{5}J connectivities similarly linked the three phenylpropanoid units A, B and C in all compounds, providing us with the unambiguous establishment of their α,β-bis-O-4-aryl ether structure.

Low intensity ^{13}C-resonances assignable to the α- and β-carbons of non-cyclic benzyl aryl ethers are observed in the 80–85 ppm range of 1 D ^{13}C-NMR spectra of various underivatized and acetylated lignins (Nimz *et al.* 1974; Lüdemann and Nimz 1974b;

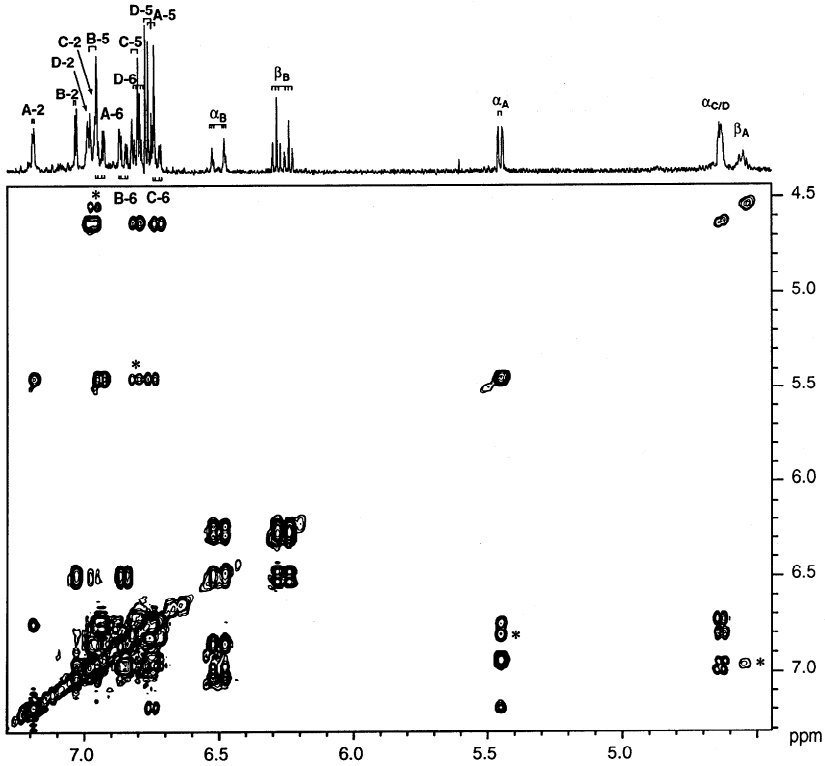


Fig. 3. Portion of the delayed COSY spectrum of **7a** (diastereomeric mixture). The data matrix was not symmetrized. The stars "*" indicate the 5-bond proton connectivities linking the three phenylpropanoid units A, B and C (i.e. B-5/β_A and C-5/α_A cross-peaks).

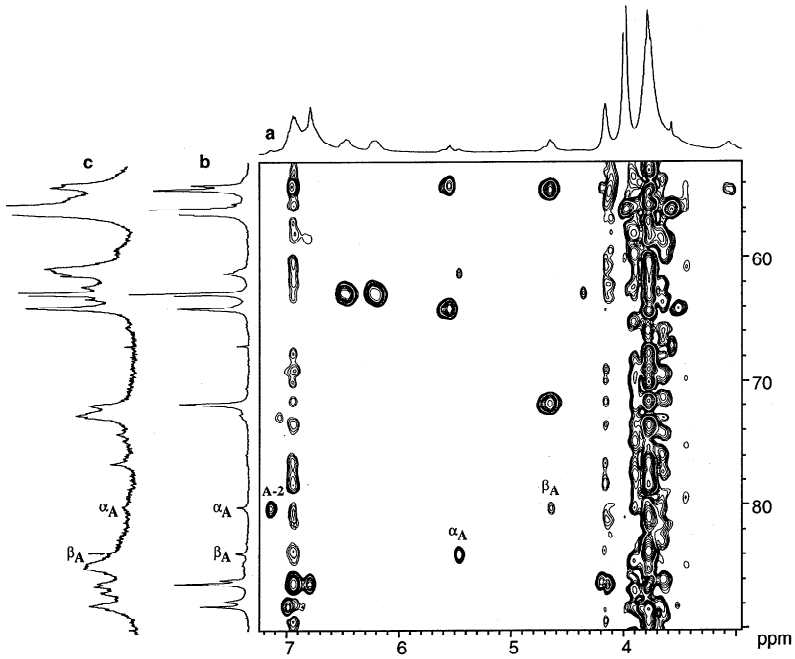


Fig. 4. Aliphatic region of the HMBC spectrum of an unacetylated coniferyl alcohol "Zulauf-like" DHP, showing two-bond correlations between the α and β protons and carbons, as well as a characteristic three-bond correlation between the A-2 proton and the α carbon of the phenylpropanoid unit A in non-cyclic benzyl aryl ethers of types **6** and **7**. **a**: 1D ^{1}H spectrum of the "Zulauf-like" DHP. **b**: 1D ^{13}C spectrum of the "Zulauf-like" DHP. **c**: 1D ^{13}C spectrum of a "Zutropf" DHP showing the α and β carbon signals indicating the presence of non-cyclic benzyl aryl ethers of types **6** and **7** (the expected two- and three-bond ^{1}H-^{13}C correlations were not observed in the corresponding HMBC spectrum).

Nimz and Lüdemann 1976; Gagnaire and Robert 1977; Nimz *et al.* 1981, 1982, 1984). The α_A and β_A carbons chemical shifts given here for all non-cyclic benzyl aryl ether lignin model compounds **(5–7)** are within this range (Table 2). Unlike the α_A and β_A carbons of the acetylated 2,4-dinitrophenyl derivatives, reported by Nimz and Lüdemann (1974) to both resonate at 80.7 ppm in acetone-d_6, the corresponding carbons in compounds **5b, 6b, 6d** and **7b** gave separated signals at about 80.8 and 81.7 ppm, respectively (Table 2). Whereas the β-carbon signals of diastereomeric guaiacylglycerol-β-guaiacyl ethers, which are respectively observed at about 80.6 and 80.3 ppm for the *threo* and *erythro* isomer (Nimz and Lüdemann 1976; Quideau and Ralph 1994), may overlap with the α-carbon signal of non-cyclic α-O-4 benzyl aryl ethers, a distinct signal at about 81.7 (i.e. the β_A carbon resonance) could be diagnostic of the presence of α-O-4 units in acetylated spruce lignin. The α_A and β_A carbons respectively resonate at about 81.2 and 85.3 ppm for the unacetylated guaiacyl derivatives **5a** and **7a**, whereas the corresponding carbons of the veratryl derivative **6c** gave signals at 80.1 and 83.9 ppm, respectively. Although poorly resolved, and initially assigned to the β-carbons in β-aryl ethers with carbonyl group at the α-position, 85.5–85.2 and 82.9–83.3 ppm carbon resonances were observed (Nimz *et al.* 1974) in 25 MHz ^{13}C-NMR spectra of unacetylated coniferyl alcohol DHPs and spruce MWLs. The utilization of higher-field NMR spectrometers is necessary to obtain sufficient resolution of the above signals, which otherwise may overlap with the strong β-aryl ether carbons signals. The β-carbon signals of the *threo* and *erythro* guaiacylglycerol-β-guaiacyl ether model compounds resonate at 86.2 and 85.4 ppm, respectively, in acetone-d_6 : D_2O (9 : 1) (Lüdemann and Nimz 1974a; Quideau and Ralph 1994). ^{13}C-NMR spectra of coniferyl alcohol DHPs, recorded in our laboratories at 90 MHz, have shown relatively weak but distinct signals assignable to the side-chain carbons in non-cyclic benzyl aryl ethers (Fig. 4). On the basis of the NMR data reported here and the results described in the preceding paper (Quideau and Ralph 1994), the relatively weak signals observed in the 80–85 ppm range of 1D ^{13}C-NMR spectra of lignins are presumed to be due to non-cyclic benzyl aryl ethers.

Based on the results of lignin degradation by mild hydrolysis and thioacetic acid treatment, Nimz incorporated up to 28% of non-cyclic α-O-4 benzyl aryl ethers in his structural scheme of beech lignin, and pointed out that the frequency of these structural units would be lower in MWLs than in native lignins because of the cleavage of the α-O-4 bonds during MWL preparation (Nimz 1974). Accordingly, DHP lignins have been reported to contain more α-O-4 units than MWLs (Brunow and Wallin 1981). Surprisingly, recent 2D NMR studies of lignins (Ede *et al.* 1990; Ede and Brunow 1992) revealed weak correlations assignable to α-O-4 units in MWLs by COSY experiments, whereas no such correlations were observed in COSY, TOCSY and HMQC spectra of DHP and suspension culture (SC) lignins. However, low sensitivity and relatively poor resolution due to rapid relaxation of the protons and the polydisperse nature of the lignin polymer must be taken into account when using 2D NMR techniques for lignin structural studies, especially when aimed at detecting diastereomeric structures which are present in low amounts. In other words, the absence of corresponding correlations does not unambiguously prove the non-existence of a specific structure. The fact that the non-cyclic α-O-4 benzyl aryl ethers are present in low concentration in lignins does not diminish the importance of their structural role. They would act as cross-linking units allowing important increase of the molecular mass of the lignin polymer by an alternative mechanism which does not depend on enzyme-catalyzed oxidative coupling (e.g. 5-5 and 4-O-5 couplings) of preformed lignin units. Also, we have observed that, in addition to the predominant formation of β-5- and β-β-coupled dilignols, peroxidase-catalyzed dehydrogenative polymerization of the "bulk" type ("Zulaufverfahren" DHPs) (Freudenberg 1956; Lai and Sarkanen 1975) led to the formation of significant amounts of α-O-4 units of types **6** and **7**, as indicated by 1D and 2D NMR analyses. In particular, the two-bond correlations (e.g. C-β_A/H-α_A and Cα_A/H-β_A) and the diagnostic three-bond correlation (i.e. C-α_A/$H_{A\text{-}2}$) were readily detected in the corresponding HMBC spectra (Fig. 4). Although ^{13}C-NMR analysis of "Zutropfverfahren" DHPs, which are obtained by a more "end-wise" type dehydrogenative polymerization, indicated the presence of low amounts of structures of types **6** and **7** (Fig. 4c), the signal to noise ratio was sufficiently low that analogous 2D NMR experiments did not reveal the expected correlations.

Conclusions

Two-dimensional HMQC-TOCSY and delayed COSY experiments were found to be useful complements or alternatives to the routine 2D hetero- and homonuclear correlation experiments for the structural elucidation and chemical shift assignments of oligomeric lignols **5–7.** The contribution to the structure of native lignins of these non-cyclic benzyl aryl ethers, obtained from the oxidative coupling of coniferyl alcohol, still remains a controversial subject.

From the conditions of formation of the benzyl aryl ethers **5–7**, we have proposed in the preceding paper

(Quideau and Ralph 1994) that they would preferably accumulate during initial lignification, e.g. in the compound middle lamella of the cell wall in which the lignin polymer is thought to be more of the "bulk" type. NMR studies of MWLs have failed to provide unambiguous evidence of the occurrence of these α-O-4 linked units in lignin. However, it has recently been reported that MWLs originate from secondary wall lignin, which would be formed via a more "end-wise" type polymerization, rather than from middle lamella lignin (Terashima *et al.* 1992; Maurer and Fengel 1992a, b). It is plausible that their formation is completely suppressed due to the preferential formation of benzyl alcohols, but this may imply that the pH value of the cell wall is *ca.* 4, as noted by Sipilä (1990).

Application of 2D NMR techniques to the selective study of a middle lamella lignin, isolated by non-degradative procedures and ideally ^{13}C-labelled at strategic positions, in conjunction with the development of chemical schemes aimed at selectively cleaving non-cyclic α-ether bonds, is necessary to confirm the occurrence of the α-O-4 benzyl aryl ether units in lignins.

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