

# NMR Studies of Lignins. 7.

## <sup>1</sup>H NMR Spectroscopic Investigation of the Distribution of *Erythro* and *Threo* Forms of $\beta$ -*O*-4 Structures in Lignins

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The distribution of *erythro* and *threo* forms of  $\beta$ -*O*-4 structures in spruce and birch lignin was elucidated by <sup>1</sup>H NMR examination of lignin samples (methylated and/or acetylated) and stereoisomers of 11 lignin model compounds of the  $\beta$ -*O*-4 type. It was found that similar amounts of *erythro* and *threo* forms of such structures were present in spruce lignin while *erythro* forms dominated in birch lignin.

Formulas A-D and A'-D' exemplify structural elements in lignin (acetylated) with phenylpropane units attached to each other by  $\beta$ -*O*-4 linkages. Such linkages constitute very roughly half of the intermonomer connections in lignins. Structural elements A-D and A'-D' can have the *erythro* or the *threo* configuration. <sup>13</sup>C NMR studies suggest that about equal amounts of the two diastereomeric forms are present in spruce lignin.<sup>1,2</sup> Similar results could be derived from <sup>1</sup>H NMR studies.<sup>3,4</sup> Applied to birch lignin, the same approach indicated a rather large proportion of *erythro* forms of  $\beta$ -*O*-4 structures.<sup>3</sup> Interestingly, recent <sup>13</sup>C NMR studies<sup>2</sup> suggested a prevalence of *erythro* forms of  $\beta$ -*O*-4 structures in beech lignin, the composition of which resembles that of birch lignin.

The conclusions drawn in earlier <sup>1</sup>H NMR studies about the distribution of *erythro* and *threo* forms of  $\beta$ -*O*-4 structures in lignins were primarily based on studies of the signal from H<sub>a</sub> in acetate derivatives.<sup>3,4</sup> We have now been able to corroborate the earlier results by extended model

compound studies and examinations of lignin derivatives prepared by methylation of phenolic groups and subsequent acetylation. The <sup>1</sup>H NMR data for lignin model compounds of the  $\beta$ -*O*-4 type given in this paper (Table 1) also provide a good basis for the stereochemical assignment of such compounds.

Spruce lignin consists almost entirely of guaiacyl units. This implies that the main types of  $\beta$ -*O*-4 structures which have to be considered are the *erythro* and *threo* forms of structures A and A'. Since birch lignin is composed of nearly equal amounts of guaiacyl units and syringyl units, one has to take into account a substantially larger number of different  $\beta$ -*O*-4 structures, namely the *erythro* and *threo* forms of structures A-D and A'-D'. The model compound data given in Table 1 cover most of these structural possibilities. It can be concluded from data given in Table 1 that the signal from H<sub>a</sub> is shifted 0.03–0.06  $\delta$  units upfield when a *p*-acetoxy group is replaced by a *p*-alkoxy group. A similar shift is observed when going from *threo* to *erythro* (Table 1 and Refs. 5 and 6). Application of these generalizations together with the data given in Table 1 makes it possible to derive  $\delta$  values for the positions of the

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Table 1. NMR data for lignin model compounds of the  $\beta$ -O-4 type (solvent, CDCl<sub>3</sub>).

Compound	$\delta$ /p.p.m. vs. TMS (J/Hz)						
	H <sub>a</sub>	H <sub>b</sub> <sup>a</sup>	H( $\gamma_1$ )	H( $\gamma_2$ )	H <sub>c</sub>	OCH <sub>3</sub>	CH <sub>3</sub> CO
1a (erythro)	6.02 (5.1)	4.70	4.23 (4.0, 11.8)	4.42 (5.9, 11.8)		3.79, 3.86, 3.87	2.02, 2.07
1a (threo)	6.08 (7.0)	4.65	4.01 (5.7, 11.8)	4.28 (4.0, 11.8)		3.83, 3.86, 3.87	2.00, 2.01
1b (erythro)	6.08 (5.4)	4.67	4.25 (4.0, 11.8)	4.46 (5.9, 11.8)		3.77, 3.81	2.02, 2.09, 2.30
1b (threo)	6.12 (6.4)	4.63	4.06 (5.8, 11.9)	4.31 (4.5, 11.9)		3.81, 3.82	1.99, 2.05, 2.30
1c (erythro) <sup>b</sup>	6.01 (5.3)	4.69	4.21 (3.9, 11.9)	4.41 (5.9, 11.9)	5.02		
1c (threo) <sup>b</sup>	6.07 (6.8)	4.63	4.01 (5.8, 12.0)	4.27 (4.1, 12.0)	5.04		
1d (threo)	6.11 (6.3)	4.62	4.06 (5.7, 11.8)	4.31 (4.6, 11.8)	5.03	3.81, 3.82	1.99, 2.05, 2.09, 2.29
2a (threo)	6.09 (6.6)	4.50	3.93 (4.6, 11.9)	4.32 (4.5, 11.9)	2.29	3.76 <sup>c</sup> , 3.82, 3.84 <sup>c</sup>	1.99 <sup>c</sup>
2b (threo)	6.12 (6.2)	4.47	3.97 (4.4, 11.8)	4.36 (5.2, 11.8)	2.30	3.74, 3.80	2.00, 2.02, 2.32
2c (threo)	6.12 (6.2)	4.53	3.98 (4.6, 11.8)	4.36 (4.8, 11.8)	5.02	3.77, 3.80	2.00, 2.01, 2.10, 2.32
3a (erythro)	6.00 (5.7)	4.69	4.27 (4.3, 11.7)	4.44 (5.8, 11.7)		3.83 <sup>c</sup> , 3.86 <sup>c</sup>	2.04, 2.09
3a (threo)	6.05 (6.5)	4.63	4.05 (5.9, 11.8)	4.31 (4.3, 11.8)		3.83 <sup>c</sup> , 3.85 <sup>c</sup>	2.01, 2.05
3b (erythro) <sup>b</sup>	6.05 (5.7)	4.67	4.29 (4.1, 12.0)	4.46 (5.4, 12.0)			2.04, 2.09, 2.32
3b (threo)	6.09 (6.2)	4.62	4.09 (5.7, 11.8)	4.33 (4.6, 11.8)		3.80 <sup>c</sup> , 3.81	2.00, 2.06, 2.32
3c (erythro) <sup>b</sup>	6.04 (5.7)	4.66	4.28 (4.0, 12.0)	4.45 (5.5, 12.0)	5.02		
3c (threo)	6.08 (6.2)	4.61	4.08 (5.7, 11.8)	4.32 (4.5, 11.8)	5.03	3.81 <sup>d</sup>	2.00, 2.07, 2.10, 2.32
4 (threo)	6.15 (6.2)	4.52	3.95 (4.5, 11.9)	4.35 (4.8, 11.9)	5.02	3.77 <sup>c</sup> , 3.81	1.99, 2.00, 2.10, 2.30

<sup>a</sup>Multiplet. <sup>b</sup>From analysis of a diastereomeric mixture; data for methoxyl and/or acetyl groups are lacking. <sup>c</sup>H. <sup>d</sup>H.

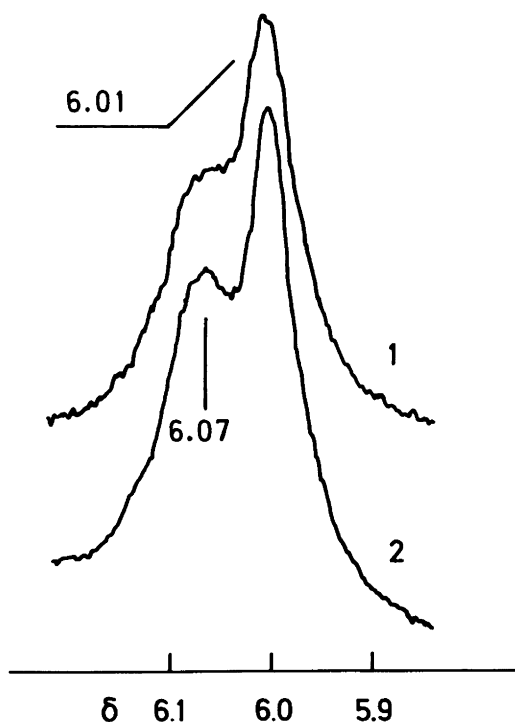


Fig. 1. Range  $\delta$  5.9–6.1 of the  $^1\text{H}$  NMR spectrum of acetylated birch lignin. Spectrum 2 appeared on irradiation at  $\delta$  4.6.

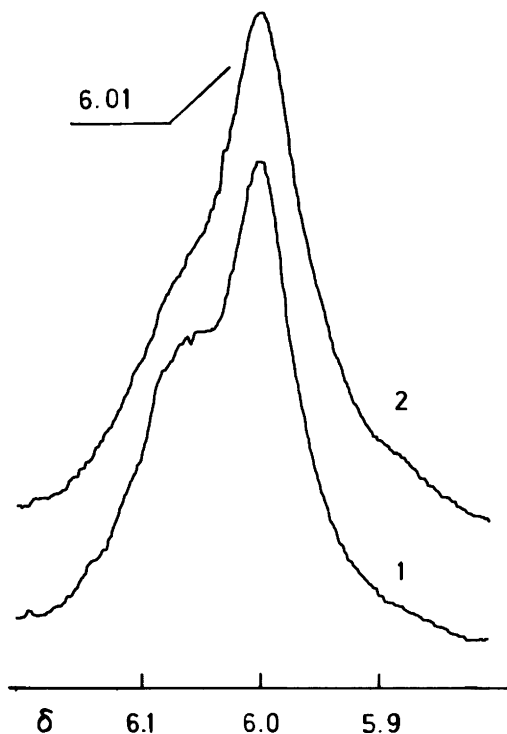


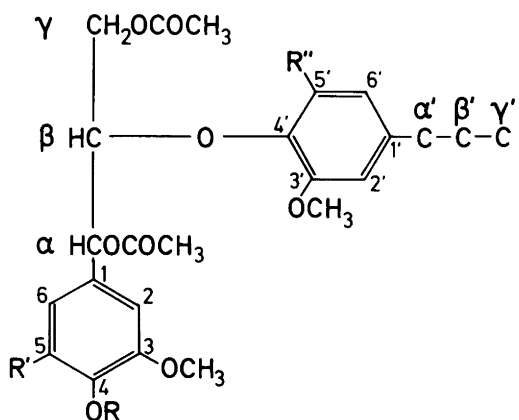
Fig. 2. Range  $\delta$  5.9–6.1 of the  $^1\text{H}$  NMR spectrum of methylated and acetylated birch lignin (Spectrum 2). For comparison, the corresponding range of the spectrum of acetylated birch lignin is included in the figure (Spectrum 1).

signals from  $\text{H}_\alpha$  in all the structural elements A-D and A'-D'.

Fig. 1 shows the region of the  $^1\text{H}$  NMR spectrum of acetylated birch lignin in which signals from  $\text{H}_\alpha$  in  $\beta$ -O-4 structures are located (the highest point of the peak appears at  $\delta$  6.01; the complete spectrum is given in Ref. 7). Decoupling by irradiation at  $\delta$  4.6 (the position for  $\text{H}_\beta$  in  $\beta$ -O-4 structures, cf. Table 1) results in changes of the peak at  $\delta$  6.01; it is resolved into two peaks ( $\delta$  6.01 and  $\delta$  6.07) separated by a shallow minimum at  $\delta$  6.04 (Fig. 1). Peak positions (cf. Table 1) together with the decoupling experiments show that the lignin peak at  $\delta \approx 6$  is due to signals from  $\text{H}_\alpha$  in  $\beta$ -O-4 structures. The signal from  $\text{H}_\alpha$  in all the *threo* forms (and some *erythro* forms) of structures A-D and A'-D' should be located above  $\delta$  6.04; this could be concluded from the data in Table 1 together with applications of the

generalizations discussed above. Accordingly, only signals from *erythro* forms will appear below  $\delta$  6.04. The highest point ( $\delta$  6.01) of the lignin peak is found below this  $\delta$  value. It follows from what has been said above that this suggests a predominance of *erythro* forms of  $\beta$ -O-4 structures in birch lignin. Additional support for this conclusion comes from the fact that the spectrum of acetylated birch lignin exhibits a peak at  $\delta$  4.437 which could be attributed to  $\text{H}_\gamma$  in *erythro* forms of  $\beta$ -O-4 structures (Table 1).

*Erythro* forms of  $\beta$ -O-4 structures with *p*-acetoxy groups (formulas A'-D') do not contribute to the fraction of the lignin peak located below  $\delta$  6.04, since such groups cause a downfield shift of the signal from  $\text{H}_\alpha$  to values above  $\delta$  6.04. This is apparently a drawback in estimates of the *threo/erythro* ratio, although the amount of units with phenolic groups (or *p*-acetoxy groups) is rather

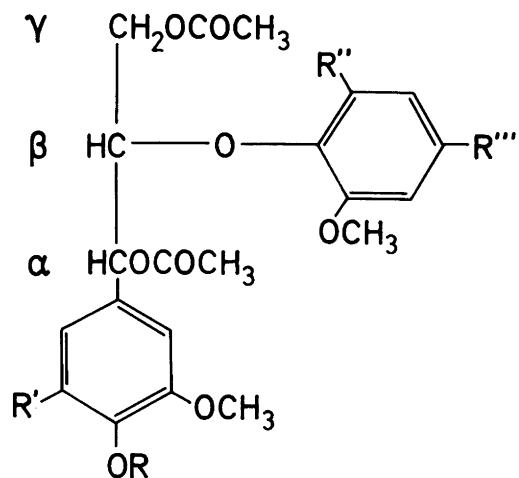


- A R = C in adjacent unit, R' = R'' = H  
 A' R = COCH<sub>3</sub>, R' = R'' = H  
 B R = C in adjacent unit, R' = R'' = OCH<sub>3</sub>  
 B' R = COCH<sub>3</sub>, R' = R'' = OCH<sub>3</sub>  
 C R = C in adjacent unit, R' = OCH<sub>3</sub>, R'' = H  
 C' R = COCH<sub>3</sub>, R' = OCH<sub>3</sub>, R'' = H  
 D R = C in adjacent unit, R' = H, R'' = OCH<sub>3</sub>  
 D' R = COCH<sub>3</sub>, R' = H, R'' = OCH<sub>3</sub>

small in birch lignin (about 18 %<sup>7</sup>). To eliminate this source of error we methylated the phenolic groups prior to acetylation. The types of  $\beta$ -O-4 structures in this derivative are limited to those represented by formulas A-D. As could be expected, the fraction of the lignin peak which is located below  $\delta$  6.04 is larger in this derivative (Fig. 2). This strengthens the arguments for a predominance of *erythro* forms of  $\beta$ -O-4 structures in birch lignin. It should, however, be noted that the peak still exhibits an inflexion at about  $\delta$  6.07 which indicates that the amount of *threo* forms present is not negligible.

The distribution of *threo* and *erythro* forms in spruce lignin has earlier been discussed on the basis of the contributing signals from H <sub>$\alpha$</sub>  in structures A and A'.<sup>4</sup> We examined methylated and acetylated spruce lignin. In this derivative only structures of type A have to be considered. The lignin peak from H <sub>$\alpha$</sub>  in  $\beta$ -O-4 structures ( $\delta \approx 6.05$ ) was found to consist of approximately equal contributions from *erythro* ( $\delta(H_{\alpha})$  6.01) and *threo* ( $\delta(H_{\alpha})$  6.07) forms which is in agreement with earlier results.<sup>4</sup>

The stereochemical assignment of the *erythro* and *threo* forms of compounds 1a and 1b has been firmly established in earlier work (cf. Ref.



- 1a. R = CH<sub>3</sub>, R' = R'' = R''' = H  
 1b. R = COCH<sub>3</sub>, R' = R'' = R''' = H  
 1c. R = CH<sub>3</sub>, R' = R'' = H, R''' = CH<sub>2</sub>OCOCH<sub>3</sub>  
 1d. R = COCH<sub>3</sub>, R' = R'' = H, R''' = CH<sub>2</sub>OCOCH<sub>3</sub>  
 2a. R = CH<sub>3</sub>, R' = R'' = OCH<sub>3</sub>, R''' = CH<sub>3</sub>  
 2b. R = COCH<sub>3</sub>, R' = R'' = OCH<sub>3</sub>, R''' = CH<sub>3</sub>  
 2c. R = COCH<sub>3</sub>, R' = R'' = OCH<sub>3</sub>, R''' = CH<sub>2</sub>OCOCH<sub>3</sub>  
 3a. R = CH<sub>3</sub>, R' = OCH<sub>3</sub>, R'' = R''' = H  
 3b. R = COCH<sub>3</sub>, R' = OCH<sub>3</sub>, R'' = R''' = H  
 3c. R = COCH<sub>3</sub>, R' = OCH<sub>3</sub>, R'' = H, R''' = CH<sub>3</sub>OCOCH<sub>2</sub>  
 4. R = COCH<sub>3</sub>, R' = H, R'' = OCH<sub>3</sub>, R''' = CH<sub>2</sub>OCOCH<sub>3</sub>

8). <sup>1</sup>H NMR spectral comparisons with these compounds could therefore be used for the assignment of the *threo* and *erythro* forms of the other compounds in Table 1. The stereochemical relationships have actually already previously been elucidated for several of the compounds by the same method.<sup>5,6</sup> A modern high frequency spectrometer was used in the present work. This considerably facilitates comparative studies of the current type, since the spectra are well resolved (the improved resolution of the signals from H <sub>$\gamma$</sub>  is particularly notable) and very accurate spectral data can be obtained.

Additional conclusions regarding <sup>1</sup>H NMR spectral properties of  $\beta$ -O-4 model compounds could be drawn from the data in Table 1. An exchange of the hydrogen at C-1' for a substituent more resembling a lignin side-chain (CH<sub>2</sub>O-COCH<sub>3</sub>) leaves the signals from H <sub>$\alpha$</sub> , H <sub>$\beta$</sub> , and H <sub>$\gamma$</sub>  practically unaffected. A replacement of the

same hydrogen atom for a methyl group causes a slight upfield shift (0.06  $\delta$  units) of the signal from  $H_\beta$ .

### Experimental

*Lignin model compounds* were prepared according to procedures described in Refs. 6, 9 and 10 or modifications of them.<sup>11</sup>

*Methylation of lignin from birch (Betula verrucosa)*. The procedure described by Fenn and Kirk<sup>12</sup> was adapted. Milled wood lignin from birch (0.63 g),  $K_2CO_3$  (0.48 g), and methyl iodide (0.21 ml) in DMF (5 ml) were stirred magnetically overnight (argon atmosphere). Methyl iodide (0.21 ml) was then added and stirring continued for 3 h. The DMF solution was dripped into 300 ml of an aqueous solution of 1%  $Na_2SO_4$  acidified with a few drops of acetic acid (magnetic stirring). The precipitate formed was centrifuged off and dried. The product was methylated a second time according to the same procedure.  $^1H$  NMR examination of the acetate derivative of the product from two methylations showed that the methylation of phenolic groups was almost complete [the peak due to phenolic acetate ( $\delta \approx 2.3$ ) was practically absent].

$^1H$  NMR spectra were recorded on a 270 MHz

instrument working in the pulse Fourier mode (Bruker WH 270). Chloroform-*d* was used as solvent (internal reference, TMS). Temperature, 300 K. Number of scans (lignin spectra), 1000.

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