NMR Studies of Lignins. 4. Investigation of Spruce Lignin by ¹H NMR Spectroscopy

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The structure of milled wood lignin from spruce (*Picea abies*) has been investigated by ¹H NMR spectroscopy, using a 270 MHz instrument. New evidence for the presence of β-O-4 structures (*erythro* and *threo* forms) as major constituents of the lignin is presented. Spectral characteristics which could be attributed to β-5 and divanillyltetrahydrofuran structures have been investigated. It was found that, at most, a few percent of the lignin units are involved in pinoresinol structures. Signals from different types of hydroxyl, formyl, and aromatic groups have been analyzed.

Previous papers in this series describe the study of lignins and lignin model compounds by ¹H NMR spectroscopy, using new techniques.[1-3] The present investigation is a continuation of these studies and reports results from structural analyses of lignin from spruce (*Picea abies*) by ¹H NMR spectroscopy. The spectrum of milled wood lignin from spruce (acetate derivative) is given in Fig. 1. The location and assignments of the peaks in the spectrum are summarized in Table 1. From here on data from Table 1 are used without reference. The results obtained in the present work are largely in accord with earlier ¹H NMR spectral studies of spruce lignin.[4,5] However, the use of a 270 MHz instrument and new techniques have greatly increased the amount of available structural informa-
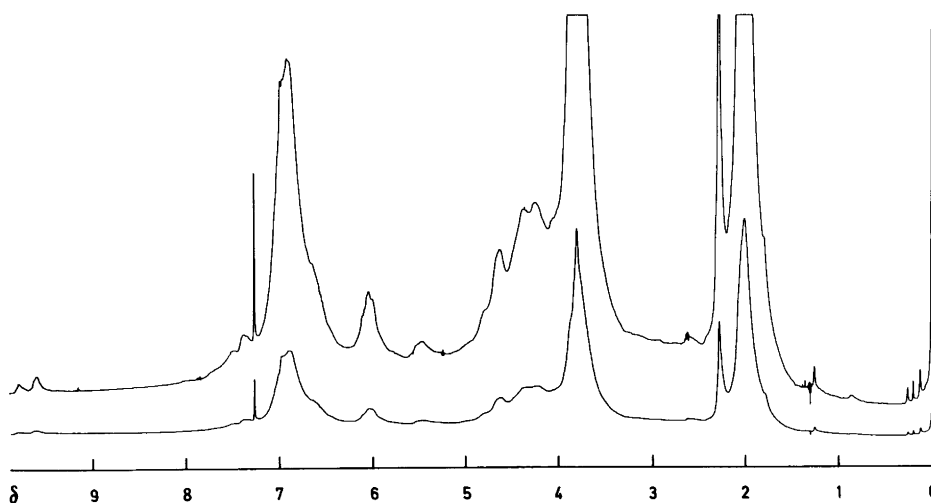

Fig. 1. ¹H NMR spectrum of acetylated milled wood lignin from spruce. For peak positions and assignments of peaks, see Table 1.

Table 1. Assignments of signals in the ^{1}H NMR spectrum of acetylated spruce lignin (Fig. 1). Several peaks are broad and have irregular shapes; δ values given always refer to the highest point of the peak.

δ Value/ppm	Assignment
1.26	Hydrocarbon contaminant
2.01	Aliphatic acetate
2.28	Aromatic acetate
2.62	H_α in β-β structures of type A
3.81	Protons in methoxyl groups
4.27	H_γ in several types of structures [3]
4.39	H_γ in, primarily, β-O-4 structures (*erythro* forms) and β-5 structures [3]
4.65	H_β in β-O-4 structures (methylene protons in cinnamyl alcohol units)
5.49	H_α in β-5 structures (H_α in structures of type *4*, H_β in aryloxypropiophenones)
6.06	H_α in β-O-4 and β-1 structures (certain vinyl protons)
6.93	Aromatic protons (certain vinyl protons)
7.29	Chloroform (solvent)
7.41	Aromatic protons in benzaldehyde units and vinyl protons on the carbon atoms adjacent to aromatic rings in cinnamaldehyde units
7.53	Aromatic protons in benzaldehyde units
9.64	Formyl protons in cinnamaldehyde units
9.84	Formyl protons in benzaldehyde units

tion. The lignin preparation examined was obtained according to an extraction procedure which provides lignins with a low carbohydrate content[6] and signals from protons in carbohydrates could not be detected in the lignin spectra.

Acetyl groups. Peaks at δ 2.01 and 2.28 are due to acetyl groups. Integrations suggest a total of 1.45 acetyl groups/phenylpropane unit; this figure should correspond to the number of hydroxyl groups in the original lignin preparation. It is worth noting that this result agrees fairly well with ^{1}H NMR spectrometric measurements of hydroxylic groups in non-derivatized lignin from *Sequoia sempervirens*.[7] The peak at δ 2.28 (aromatic acetate) was found to correspond to 0.26 phenol groups/phenylpropane unit (certain phenol groups in biphenyl structures are not included in this estimate[4]).

$\beta - \beta$ *structures of type A (7 or 8).* The peak at δ 2.62 may be attributed to H_α in $\beta - \beta$ structures

of type A (either 7 [3] or 8 [8]). Integrations suggest that $1 - 2\%$ of the phenylpropane units are involved in such structures. Spectral and degradative evidence for the occurrence of structures of types *7* and *8* in lignins has accumulated during the past few years, but biosynthetic support for their existence has not been obtained.[9,8,10]

β-5 *structures (5).* Model compound data[3] suggest that the peak at δ 5.49 is due to H_α in β-5 structures. Support for this was obtained by decoupling experiments. Thus irradiation at δ 3.77 (but not at δ 3.67 or 3.87) which corresponds to the position of the signal from H_β in β-5 structures[3] resulted in a sharpening of the 5.49 peak. Furthermore, an additional peak appeared at δ 5.54 which was somewhat lower in intensity than the 5.49 peak and was separated from this by a shallow minimum (δ 5.52). Model compound data[3] may be interpreted to indicate that the 5.49 peak corresponds to 4-alkoxy-substituted structures of type 5 while the 5.54 peak corresponds to 4-acetoxy-substituted structures of the same type. H_α in structures of type *4* would also contribute to the 5.49 peak since the signals from such protons can be expected to be located at about δ 5.40. (In appropriate model compounds for 4-acetoxysubstituted structures of type *4* the signals from H_α are located at δ 5.39 (*erythro* form) and δ 5.46 (*threo* form);[11] 4-alkoxy substituents should lower the δ values by ≈ 0.05 δ units.[3]) H_β in aryloxypropiophenones ($\delta \approx 5.6$) contributes to a small extent to the 5.49 peak.[1] The decoupling experiments together with the position and shape of the 5.49 peak suggest that this peak is mainly due to signals from H_α in β-5 structures. Calculated as H_α in β-5 structures, the integral of the 5.49 peak corresponds to $11 \pm 5\%$ of the side chains.

β-O-4 *structures (3).* The peaks at δ 6.06 (H_α) and 4.65 (H_β) can essentially be attributed to β-O-4 structures.[3] This could be confirmed in decoupling experiments. Irradiation at δ 4.65 resulted in changes of the 6.06 peak. Peaks appeared at δ 6.01, 6.06, and 6.11 (Fig. 2). Model compound data[3] may be interpreted to indicate that the 6.01 peak is due to H_α in *erythro* forms of β-O-4 structures with a 4-alkoxy substituent and the 6.06 peak is due to the corresponding structures of the *threo* form and, in addition, 4-acetoxy-substituted structures of the *erythro* form. Finally, the 6.11 peak can be attributed to *threo* forms of β-O-4 structures with a 4-acetoxy substituent. Quantitative estimates suggest about equal amounts of *erythro* and *threo*
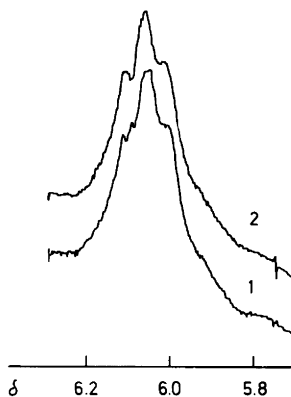
Fig. 2. Range δ 5.8 — 6.2 of the ^{1}H NMR spectrum of acetylated spruce lignin. Spectrum 2 appeared on irradiation at δ 4.61 (or 4.66). Irradiation at δ 4.70 (or 4.57 or δ values more distant from the range δ 4.61 — 4.66) gave spectrum *1*.

forms of β-O-4 structures or, possibly, some predominance of the *threo* form of such structures. It should be noted that changes of the 6.06 peak were not observed on irradiation at δ 4.70 (Fig. 2). This δ value corresponds to the position of signals from methylene protons in cinnamyl alcohol groups[1] and decoupling can be expected to affect the signal from vinyl protons at adjacent carbon atoms which are located at about δ 6.16.[1] The fact that no changes were observed near this δ value, excludes the presence of an appreciable number of cinnamyl alcohol groups in spruce lignin. This agrees with previous investigations.[5] H_α in β-1 structures (6) contribute to the 6.06 peak;3 there seem to be few such structures (see below). Thus, as far as we know, structural elements other than β-O-4 structures affect the 6.06 peak very little. This fact justifies the above interpretation of the decoupling results in terms of the occurrence of different types of β-O-4 structures. Integrations of the 6.06 peak suggest that 30 — 50 % of the side chains are involved in β-O-4 structures (3). It appears from what has been said previously that side chains in β-1 structures and cinnamyl alcohol groups are included in this estimate.

Aromatic groups. The peak at δ 6.93 is due to aromatic protons (signals from certain vinyl protons give a small contribution to this peak). There is an inflexion between δ 6.6 and 6.7 which in part (see below) can be explained by signals from aromatic protons in biphenyls.[4] It was observed in
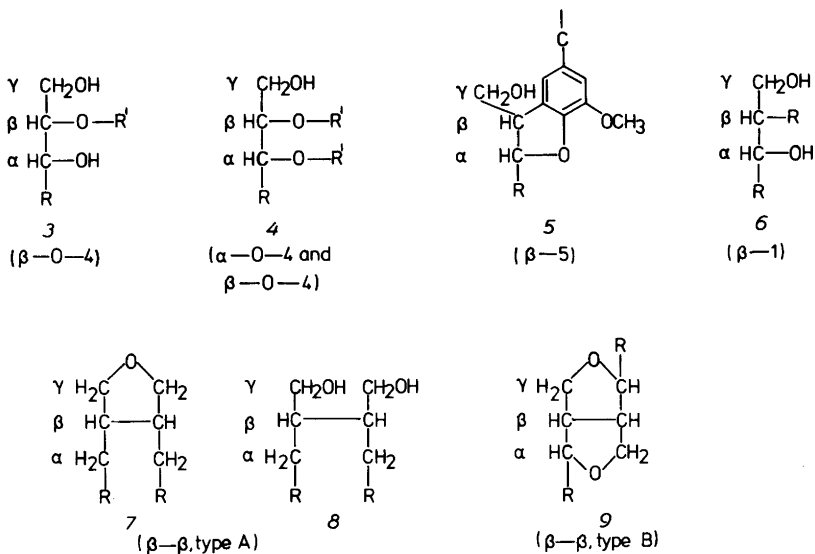
model compound studies[12] that the signal from at least one of the aromatic protons was located at around δ 6.6 in compounds where aromatic rings were separated by one (diphenylmethanes) or two (β-5 or β-1 structures) carbon atoms. Signals from aromatic protons in syringyl units and diaryl ethers are also located at approximately this δ value. Thus it seems as though structural elements in lignins containing units of type 2 or other types of units with a substituent in the 5 position contain aromatic protons which give signals at relatively high field ($\delta \approx 6.6$). It is difficult to evaluate the quantitative importance of the inflexion at δ 6.60 in the lignin spectra but it is apparent that a rather small portion of the signals from aromatic protons are located at approximately this δ value. This fact, together with the above-mentioned model compound data, suggests that the proportion of units of type *1* is larger than that of units with a substituent in the 5 position (either C (2) or O).

1 R = H
2 R = C
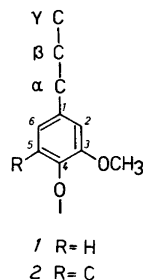
This could also be concluded from measurements of the total signal from aromatic protons. The peaks at δ 7.41 and 7.53 can be explained by signals from protons in units containing carbonyl groups.[1]

Formyl groups. The signals at δ 9.64 and 9.84 are attributed to formyl groups. Detailed arguments for such assignment have been published elsewhere.[1]

β-β structures of type B (9). Separate peaks from β-β structures of type B would most likely appear at δ 3.10 (H_β) and 4.76 (H_α).[3] The lignin spectrum does not exhibit any peak at δ 3.10, but there is an inflexion at about δ 4.80. Attempts failed at obtaining evidence for the occurrence of structures of type *9* by decoupling experiments. The inflexion at δ 4.80 may alternatively be caused by H_β in structures of type *4*.[11] Primarily on the basis of the absence of a 3.10 peak in the lignin spectrum it can be concluded that the number of β-β structures of type B is small in spruce lignin. This is in agreement with studies of spruce lignin by degradative

Y CH₂OH Y CH₂OH   Y CH₂OH   Y CH₂OH
β HC—O—R' β HC—O—R'   β HC   OCH₃ β HC—R
α HC—OH   α HC—O—R'   α HC—O   α HC—OH
R   R   R   R

3   4   5   6
(β—O—4)   (α—O—4 and   (β—5)   (β—1)
      β—O—4)

Y H₂C   CH₂   Y CH₂OH CH₂OH   Y H₂C   CH
β HC——CH   β HC——CH   β HC——CH
α H₂C   CH₂   α H₂C   CH₂   α HC   CH₂
R   R   R   R   R

7   8   9
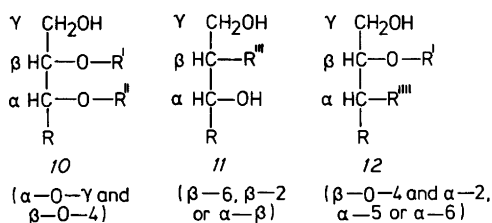(β—β, type A)   (β—β, type B)

R = C–1 in 1 or 2
R'= C–4 in 1 or 2

methods[9] as well as by ^{13}C NMR spectroscopy.[10] ^{1}H NMR spectroscopic studies of birch lignin provided clear evidence for the occurrence of β-β structures of type B in birch lignin.[2] Thus the frequency of such structures seems to be much larger in birch lignin than in spruce lignin. This was further supported by examinations of non-derivatized lignins in dioxane-d_8 – D₂O (5:1). Under these conditions a distinct peak appeared at δ 4.71 in the birch lignin spectrum (H$_\alpha$ in β-β structures of type B[3]) while the spectrum of spruce lignin did not exhibit any peaks near this δ value. Peaks at δ 4.87 (H$_\alpha$) and 4.30 (H$_\beta$) which appeared in the spruce lignin spectrum can be attributed to β-O-4 structures.[3]

β-1 structures (6). The spectrum of acetylated spruce lignin shows no characteristics that can be attributed to structures of type *6*. Examinations of non-derivatized lignin in dioxane-d_8 – D₂O (5:1) provided some qualitative support[3,12] for the occurrence of such structures. Tentative estimates suggest that the proportion of side chains in β-1 structures was less than 5 %.

Comments. The ^{1}H NMR spectral studies presented in this paper provide independent evidence for the occurrence of β-O-4, β-5, and β-β structures of type A in spruce lignin. Quantitative estimates

indicate that 37—68 % of the side chains are involved in these types of structures, the contribution from β-O-4 structures being strongly predominant. The proportion of side chains with formyl groups has previously been determined as 9 %.[1] It appears from what has been said in earlier sections that the sum of the β-O-4 structures (*3*) and β-5 structures (*5*) as determined by integrations of the 5.49 and 6.06 peaks include side chains in β-1 structures (*6*), structures of type *4*, cinnamyl alcohol units, and aryloxypropiophenones. It seems plausible to conclude from the ^{1}H NMR spectral studies that the number of side chains in these latter types of structures is rather small and probably constitutes less than 15 % of the side chains. The failure to detect signals from β-β structures of type B makes it probable that only a very small percentage of the side chains is involved in such structures.

The estimates made account at most for 80 % of the side chains. At least 20 % of the side chains can therefore probably be found in other types of structures than those discussed above. Any peaks or other characteristics which can be attributed to such residual side chains have not been detected in the spruce lignin spectrum. However, in this connection it is of interest to discuss how additional (in

Y CH₂OH
β HC—O—R'
α HC—O—R"
  R
10
(α—O—Y and
β—O—4)

Y CH₂OH
β HC—R'''
α HC—OH
  R
11
(β—6, β—2
or α—β)

Y CH₂OH
β HC—O—R'
α HC—R''''
  R
12
(β—O—4 and α—2,
α—5 or α—6)

R   = C-1 in *1* and *2*
R' = C-4 in *1* and *2*
R" = C$_y$ in *1* and *2*
R''' = C-2, C-6 or C$_\alpha$ in *1* or *2*
R''''= C-2 or C-6 in *1* or *2*, C-5 in *1*.

some cases hypothetical) structural elements can be expected to influence the lignin spectrum. Small amounts of units with glycerol side chains may be present in lignins;[13,14] side chain protons in such units can be expected to contribute to the 5.49 as well as to the 6.06 peak. The absence of peaks at about δ 3 seems to exclude the presence of any large number of structures of the 2-benzyl-2-aryloxy-ethanol type.[15,3] The occurrence of α-O-γ structures of type *10* in lignins has been discussed.[16] The signal from H$_\alpha$ in model compounds for such structures is located at about δ 5.2 [a mixture of the diastereomers of compound *13* showed signals due to H$_\alpha$ at δ 5.17 ($J = 7$ Hz) and 5.22 ($J = 6$ Hz)]. The lignin spectrum does not exhibit any peak near this δ value and it seems therefore unlikely that a substantial number of such structures is present in lignin. As shown by lignin degradation studies using oxidative[17,18] and reductive methods,[19,20] lignins contain structural elements with side chains linked to aromatic rings in modes other than those mentioned above as well as structural elements involving α-β linkages. Little is known of these additional types of lignin structures. Formulae *11* and *12* exemplify conceivable structures for such lignin elements. In our opinion the biosynthesis of these structural elements may involve reduction of

quinone methides (*cf.* Ref. 2) or radical—quinone methide reactions (structures *11* and *12* might have been formed in such reactions). It has previously been suggested that similar or related lignin structures are formed in "radical exchange reactions" or acid catalyzed reactions, possibly during the "aging" of the lignin.[21,17,18,16] On the basis of model compound data and extrapolations thereof (Ref. 3, data for compound *14* are given in Experimental) signals from H$_\alpha$ in units of type *11* can be expected to contribute to the 5.49 or the 6.06 peak in the lignin spectrum, while signals from side chain protons in units of type *12* are located at lower δ values.

Finally, "residual side chains" would include side chains involving carboxylic or ester groupings[5,22] and side chains in quinonoid structures[23] or their conversion products. However, very little is known about these complex groups of side chains and it is therefore difficult to make any statements about their influence on the lignin spectrum.

EXPERIMENTAL

¹H NMR spectra were recorded with a 270 MHz instrument working in the pulse Fourier mode (Bruker WH 270). Solvents were chloroform-*d* (internal reference TMS) and dioxane-d_8—D$_2$O (5:1) (internal reference was the sodium salt of 3-(trimethylsilyl)propanesulfonic acid). Temperatures were at about 300 K. The concentrations of the lignin samples were 50—100 mg in 0.5 ml solvent. Integrations were performed with docosane as the internal standard; the integer of the signal from the methylene protons in docosane (δ 1.26) was used as reference for quantitative determinations.

¹H NMR spectrum of the diacetate of diisoeugenol (*14*). (δ units, solvent CDCl₃): 1.00 (3H, t, $J = 7.3$ Hz; H'$_\gamma$), 1.06 (3 H, d, $J = 7$ Hz; H$_\gamma$), 1.74 (2 H, m; H'$_\beta$), 2.25 (3 H, s; CH₃CO), 2.30 (3 H, s; CH₃CO), 2.51 (1 H, m; H$_\beta$), 2.95 (1 H, m; H'$_\alpha$), 3.74 (3 H, s, OCH₃), 3.82 (1 H, d, $J = $? Hz; H$_\alpha$), 3.84 (3 H, s; OCH₃), 6.5—7.0 (5 H, m; aromatic protons).

Acetylations were performed as described in Ref. 1.
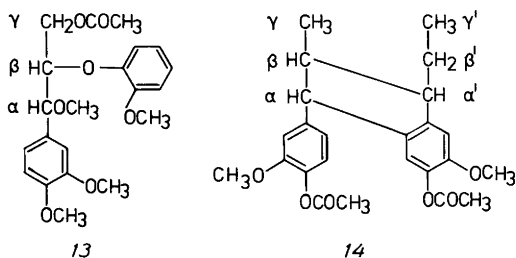
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Y CH₂OCOCH₃
β HC—O—⟨aryl⟩
α HCOCH₃ OCH₃
  ⟨ring⟩ OCH₃
  OCH₃
13

Y CH₃   CH₃ Y'
β HC   CH₂ β'
α HC   CH α'
CH₃O   OCH₃
  OCOCH₃ OCOCH₃
14

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