NMR Studies of Lignins. 8. Examination of Pyridine-d_5 Solutions of Acetylated Lignins from Birch and Spruce by ^{1}H NMR Spectroscopy

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The ^{1}H NMR spectra of acetylated milled wood lignins from spruce and birch in pyridine-d_5 solution have been analysed in structural terms on the basis of data from examinations of lignin model compounds. The results confirm and complement the observations made in previous ^{1}H NMR studies with chloroform and acetone as solvents. In pyridine solution, the methoxyl signal is fairly well separated from other signals and could be used for rough estimations of the methoxyl contents in lignins. Signals which could be specifically attributed to *erythro* forms of arylglycerol-β-syringyl ethers, syringaresinol structures and phenolic guaiacyl groups could be identified in the birch lignin spectrum. The structural significance of these signals is discussed.

The ^{1}H NMR spectra of lignins (for examples, see Ref. 1) have a less resolved character than the corresponding ^{13}C NMR spectra (for recent ^{13}C NMR studies of lignins, see, e.g., Refs. 2–6). This is partly compensated for by the very favorable signal/noise ratio which can be achieved in ^{1}H NMR spectroscopy (see, e.g., Ref. 7). Solvent effects offer a possibility to circumvent the limitations to interpretation caused by superimposition of signals in ^{1}H NMR spectra of lignins. Hitherto, lignin acetates have been examined in this series with chloroform and acetone as solvents.[1a,b,d] This study describes an examination of lignin acetates in pyridine-d_5 solution. An exchange of chloroform or acetone for pyridine as solvent causes fairly large changes in signal positions in spectra of lignin acetates.

The ^{1}H NMR spectra of acetylated milled wood lignins from spruce and birch are shown in Fig. 1. The interpretations of the spectra given in Tables 1 and 2 are made on the basis of the lignin model compound data in Table 3.

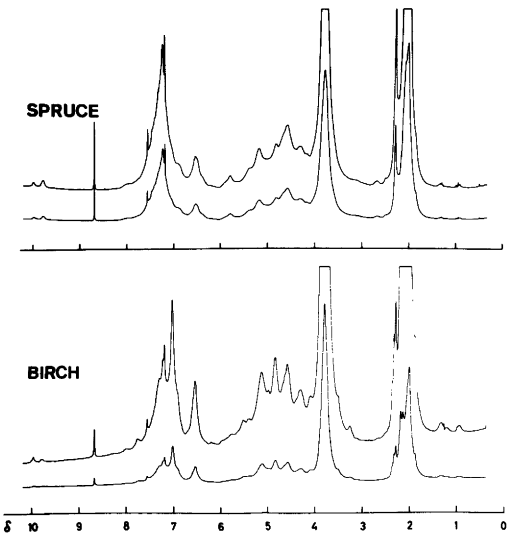


Fig. 1. ^{1}H NMR spectra of acetylated milled wood lignin from spruce and birch in pyridine-d_5 solution.

Table 1. Assignment of signals in the ^{1}H NMR spectrum of acetylated birch lignin (Fig. 1). Solvent, pyridine-d_5. Several peaks are broad and have irregular shapes; δ values given always refer to the highest point of the peak. The position of some inflections and less well defined peaks are given in parentheses.

δ Value/ ppm	Assignment
(1.83)	Unknown
1.96	Aliphatic acetate
2.08	Acetate in xylan, benzylic acetate in primarily *erythro* forms of arylglycerol-β-guaiacyl ethers (A,A′,C,C′)
2.14	Benzylic acetate in *erythro* forms of arylglycerol-β-syringyl ethers (B,B′, D,D′)
2.24	Aromatic acetate (guaiacyl type)
2.28	Aromatic acetate (syringyl type)
3.23	Hβ in β-β structures of the syringaresinol type (cf. *6b*)
3.77	CH_3O-
4.08	Hγ in β-β structures of the syringaresinol type (cf. *6b*)
4.30	Hγ in *threo* forms of β-*O*-4 structures, Hγ in β-β structures
4.56	Hγ in *threo* and *erythro* forms of β-*O*-4 structures (Hγ in β-5 and β-1 structures)
4.83	Hγ in *erythro* forms of arylglycerol-β-syringyl ethers (B,B′, D,D′) (Hγ in noncyclic benzyl aryl ethers, α-aryloxypropiophenones, cinnamyl alcohol end groups, and *threo* forms of β-1 structures)
4.98	Hα in β-β structures; Hβ in certain *threo* forms of β-*O*-4 structures, cf. Table 3
5.12	Hβ in β-*O*-4 structures
5.40	Unknown
5.52	Xylan
(5.78)	Hα in β-5 structures
(5.97, 6.20)	Hα in α-aryloxypropiophenones and noncyclic benzyl aryl ethers
6.55	Hα in β-*O*-4 structures (Hα, Hβ in cinnamyl alcohol end groups, Hα in β-1 structures)
7.03	Aromatic protons
7.25, 7.33	Aromatic protons
7.21, 7.58, 8.72	Pyridine (solvent)
9.84, 10.01	Formyl protons

Table 2. Assignment of signals in the ^{1}H NMR spectrum of acetylated spruce lignin (Fig. 1). Solvent, pyridine-d_5. Several peaks are broad and have irregular shapes; δ values given always refer to the highest point of the peak. The positions of some inflections and less well defined peaks are given in parentheses. Spruce lignin consists almost entirely of guaiacyl units and, therefore, only data from guaiacyl models (*1a, 1b, 5a, 6a, 7, 8a, 8b, 9a, 10*) have been considered in the interpretation.

δ Value/ ppm	Assignment
1.96	Aliphatic acetate
2.24	Aromatic acetate
2.63	Ar-CH_2- (cf. Ref. 1c)
(3.08)	Hβ in β-β structures of the pinoresinol type (cf. *6a*)
3.76	CH_3O-
4.29	Hγ in *threo* forms of β-*O*-4 structures (Hγ in β-β structures)
4.55	Hγ in *erythro* and *threo* forms of β-*O*-4 and in β-5 structures (Hγ in β-1 structures and *threo* forms of noncyclic benzyl aryl ethers)
4.80	Hγ in noncyclic benzyl aryl ethers, coniferyl alcohol units, *threo* forms of β-1 structures, and α-aryloxypropiophenones
5.16	Hβ in β-*O*-4 structures and noncyclic benzyl aryl ethers
(5.35)	Unknown
5.78	Hα in β-5 structures
6.53	Hα in β-*O*-4 structures (Hα, Hβ in coniferyl alcohol units, Hα in β-1 structures)
(6.92)	Aromatic protons
7.24	Aromatic protons
7.20, 7.56, 8.72	Pyridine (solvent)
9.81, 10.01	Formyl protons

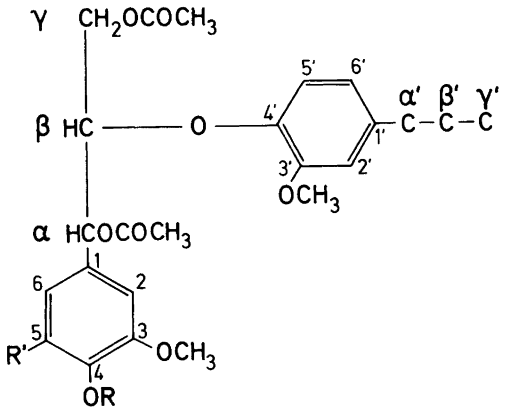
The occurrence of *erythro* forms of β-O-4 structures of types B, B′, D, and D′ (arylglycerol-β-syringyl ethers) in birch lignin

Birch lignin consists of nearly equal amounts of syringyl and guaiacyl units. Therefore, one has to take into account several types of β-*O*-4 structures (A-D and A′-D′) in appraisal of the structure of acetylated birch lignin. The signals in the birch lignin spectrum at δ 2.14 (benzylic acetate) and δ 4.83 (Hγ) (Fig. 1) could be attributed to

Table 3. NMR data for lignin model compounds in pyridine solution.

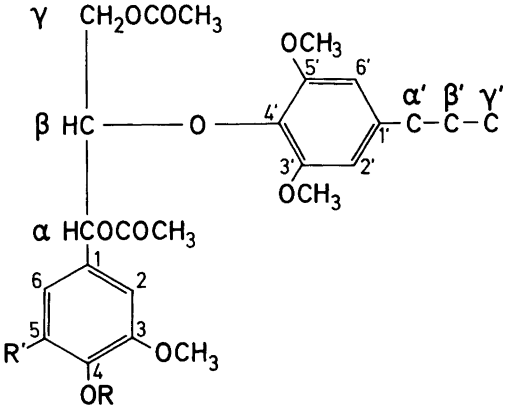
Compound	δ/ppm vs. TMS (*J*/Hz)						
	H_α	H_β[a]	$H(\gamma_1)$	$H(\gamma_2)$	$H_{\alpha'}$	OCH_3	CH_3CO
1a (*erythro*, type A)	6.50 (5.2)	5.19	4.54 (4.0, 11.8)	4.73 (6.0, 11.8)		3.71[b], 3.76	1.97, 2.06
1a (*threo*, type A)	6.56 (6.9)	5.15	4.32 (5.7, 11.9)	4.60 (3.7, 11.9)		3.72, 3.73, 3.74	1.95, 2.01
1b (*erythro*, type A′)	6.52 (5.1)	5.12	4.51 (4.1, 11.9)	4.71 (5.7, 11.9)		3.70, 3.71	1.96, 2.06, 2.23
2a (*erythro*, type B)	6.59 (4.6)	5.07	4.63 (3.6, 11.9)	4.89 (6.1, 11.9)	5.17	3.74[b], 3.76[b], 3.87	1.96, 2.05, 2.16
2b (*erythro*, type B′)	6.61 (4.6)	5.08	4.64 (4.1, 11.8)	4.86 (5.7, 11.8)	2.23	3.70[b], 3.75[b]	1.97, 2.16, 2.28
2c (*erythro*, type B′)	6.63 (4.7)	5.13	4.63 (4.0, 11.9)	4.88 (5.7, 11.9)	5.17	3.71[b], 3.76[b]	1.97, 2.05, 2.16, 2.29
2c (*threo*, type B′)	6.66 (6.7)	5.04	4.30 (4.6, 11.9)	4.72 (4.5, 11.9)	5.19	3.72[b], 3.75[b]	1.98, 2.03, 2.05, 2.28
3a (*erythro*, type C)	6.50 (5.3)	5.21	4.58 (3.9, 12.0)	4.75 (5.8, 12.0)		3.71, 3.73[b], 3.89	1.97, 2.09
3a (*threo*, type C)	6.57 (6.6)	5.17	4.36 (5.6, 11.9)	4.63 (4.0, 11.9)		3.72, 3.75[b], 3.87	1.95, 2.05
4a (*erythro*, type D)	6.54 (4.3)	5.12	4.57 (3.7, 11.8)	4.85 (6.4, 11.8)		3.72, 3.73[c]	1.93, 2.13
4a (*threo*, type D)	6.64 (7.5)	5.05	4.23 (4,6, 11.9)	4.69 (3.4, 11.9)		3.71, 3.72, 3.73[b]	1.97[b]
4b (*erythro*, type D′)	6.61 (4.5)	5.06	4.56 (4.0, 11.8)	4.85 (5.9, 11.8)		3.69, 3.72[b]	1.94, 2.12, 2.24
4b (*threo*, type D′)	6.65 (6.6)	4.99	4.23 (4.4, 11.9)	4.70 (4.3, 11.9)		3.70, 3.71[b]	1.96, 1.98, 2.25
4c (*threo*, type D′)	6.64 (6.6)	5.00	4.25 (4.6, 11.9)	4.69 (4.4, 11.9)	5.19	3.71, 3.74[b]	1.96, 2.00, 2.05, 2.24
5a (*erythro*)	6.57 (6.7)	3.76	4.48 (6.8, 11.2)	4.65 (7.0, 11.2)		3.66, 3.70	1.91, 1.97, 2.23, 2.24
5a (*threo*)	6.39 (8.0)	3.75	4.64 (5.4, 11.2)	4.82 (7.2, 11.2)		3.63, 3.65	1.96, 2.11, 2.20, 2.21
5b (*erythro*)	6.62 (6.9)	3.81	4.55 (6.8, 11.2)	4.69 (6.9, 11.2)		3.66, 3.73[b]	1.93, 1.99, 2.23, 2.29
6a	4.95 (4.0)	3.10	4.01 (3.6, 9.1)	4.29 (6.9, 9.1)		3.76[b]	2.28[b]
6b	5.00 (4.0)	3.20	4.08 (3.7, 9.1)	4.37 (6.9, 9.1)		3.76[d]	2.32[b]
7	5.79 (6.9)	3.87	4.50 (7.6, 11.1)	4.63 (5.3, 11.1)	2.68	3.67, 3.86	2.00, 2.03, 2.26
8a (*erythro*)	5.99 (5.7)	5.20	4.84 (3.5, 11.8)	4.97 (5.5, 11.8)	5.11	3.68, 3.69, 3.79	1.90, 2.00, 2.21
8b (*threo*)	5.92 (4.9)	5.24	4.44 (6.4, 11.7)	4.81 (4.2, 11.7)	2.47	3.68, 3.71, 3.75	1.92, 2.22
9a		6.20	4.86 (6.7, 11.8)	4.99 (3.8, 11.8)		3.69, 3.76, 3.77	1.96
9b		5.95 (5.6)	4.93[e] (5.6)			3.67[b], 3.78, 3.79	1.92
10	6.73 (1.2, 15.9)	6.36 (6.5, 15.9)	4.82[e] (1.2, 6.5)			3.74, 3.79	2.04

[a]Multiplet, when no *J* values are given. [b]6 H. [c]9 H. [d]12 H. [e]2 H.



A R=C in adjacent unit, R′=H
A′ R=$COCH_3$, R′=H
C R=C in adjacent unit, R′=OCH_3
C′ R=$COCH_3$, R′=OCH_3

(Arylglycerol-β-guaiacyl ethers)

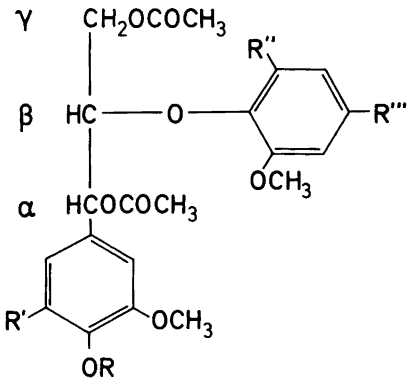


B R=C in adjacent unit, R′=OCH_3
B′ R=$COCH_3$, R′=OCH_3
D R=C in adjacent unit, R′=H
D′ R=$COCH_3$, R′=H

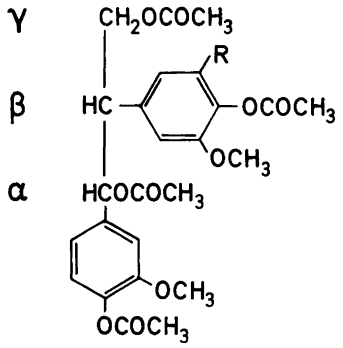
(Arylglycerol-β-syringyl ethers)

erythro forms of β-*O*-4 structures of types B, B′, D and D′ (arylglycerol-β-syringyl ethers) (cf. Table 3). Signals around δ 4.83 are also exhibited by noncyclic benzyl aryl ethers, α-aryloxypropiophenones, cinnamyl alcohol end groups and *threo* forms of β-1 structures (Table 3). The contributions from these structural elements to the 4.83 peak are, however, probably small. Noncyclic benzyl aryl ethers and α-aryloxypropiophenones should in addition give rise to signals around δ 6 (Table 3). There are only small signals around this δ value in both the birch and spruce lignin spectra (Fig. 1). Previous studies[1c] have shown that the number of cinnamyl alcohol end groups and β-1 structures is relatively small in birch lignin as well as spruce lignin. (Recent ^1H NMR studies of underivatized lignins have provided some evidence for the occurrence of small amounts of β-1 structures in lignins; the number of β-1 side chains may be 1–2 % in spruce lignin and perhaps as much as 5 % in birch lignin[8]). *It is, in this context, of interest to note that the spectrum of spruce lignin, which is practically lacking in syringyl units, exhibits only a comparatively small peak (δ 4.80) in the spectral region in which the 4.83 peak in the birch lignin spectrum is located and no peak at δ 2.14 (Fig. 1, Tables 1 and 2).*

The spectrum of acetylated birch lignin in chloroform solution[1a] shows a signal at δ 2.13 which could be specifically attributed to benzylic

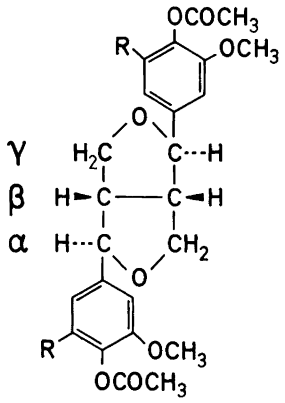


1a. R=CH_3, R′=R″=R‴=H
1b. R=$COCH_3$, R′=R″=R‴=H
2a. R=CH_3, R′=R″=OCH_3, R‴=CH_2OCOCH_3
2b. R=$COCH_3$, R′=R″=OCH_3, R‴=CH_3
2c. R=$COCH_3$, R′=R″OCH_3, R‴=CH_2OCOCH_3
3a. R=CH_3, R′=OCH_3, R″=R‴=H
4a. R=CH_3, R′=H, R″= OCH_3, R‴=H
4b. R=$COCH_3$, R′=H, R″=OCH_3, R‴=H
4c. R=$COCH_3$, R′=H, R″=OCH_3, R‴=CH_2OCOCH_3 (β-*O*-4)



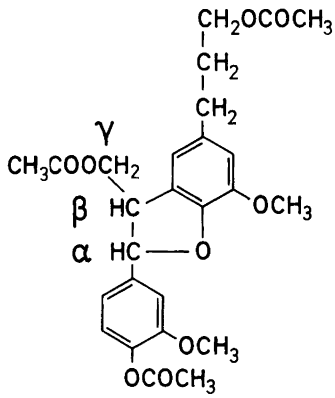
5a R=H
5b R=OCH_3

(β-1)



6a R=H
6b R=OCH_3

(β-β)



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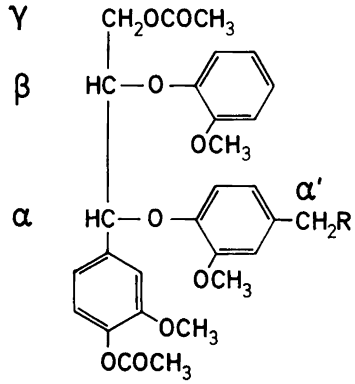
(β-5)

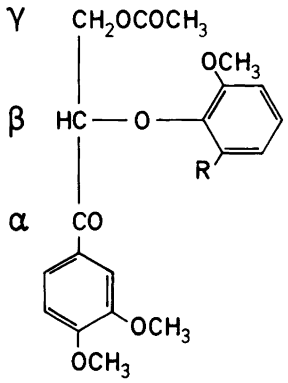
acetate in *erythro* forms of β-*O*-4 structures of the arylglycerol-β-syringyl ether type according to recent model compound studies.[8] (The contribution from aromatic acetate in certain biphenyl structures to the current peak[1a] can be neglected since the number of such structures is in all likelihood small in birch lignin.)

To summarize, our studies provide evidence for the presence of *erythro* forms of arylglycerol-β-syringyl ethers (B, B′, D, and D′) as a relatively prominent type of structure in birch lignin. This may be the explanation for the overall (i.e. taking into account *erythro* forms of β-*O*-4 structures A-D and A′-D′) predominance of *erythro* forms of β-*O*-4 structures in birch lignin reported earlier.[7] It is, in this connection, of interest to



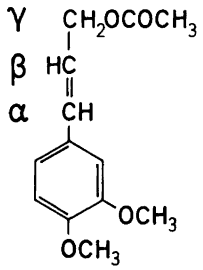
8a R=$OCOCH_3$
8b R=CH_3

(Non-cyclic benzyl aryl ethers)



9a R=H
9b R=OCH_3

(α-Aryloxypropiophenones)



10

(Cinnamyl alcohol end groups)

note that, according to recent ^{13}C NMR studies,[4] *erythro* forms of arylglycerol-β-syringyl ethers are prevalent in beech lignin.

The *erythro/threo* ratio of β-*O*-4 structures in lignins is of interest in connection with questions concerning the biosynthesis of lignins (Refs. 9–12) and the reactivity of lignins (see, e.g., Ref. 2).

β-β Structures of the syringaresinol and pinoresinol types

The signal from the Hβ in the acetates of syringaresinol [(*6b*) (δ(Hβ) 3.20)] and pinoresinol [(*6a*) (δ(Hβ) 3.08)] are located at different δ values. The spectrum of birch lignin exhibited a peak at δ 3.23, suggesting the presence of β-β structures of the syringaresinol type; no signal indicating the presence of β-β structure of the pinoresinol type is discernible. Keeping the biosynthesis of lignins in mind, this is not a very unexpected result. Sinapyl alcohol is prone to form syringaresinol on enzymic oxidation, while such oxidation of coniferyl alcohol to a large extent results in formation of products other than pinoresinol.[11,13,14]

The spruce lignin spectrum exhibits a very weak signal at δ 3.08 which suggests the presence of only a few structural elements of the pinoresinol type in spruce lignin. This is in accordance with other ^1H NMR studies.[1d]

Estimation of the methoxyl content in lignins

When pyridine is used as a solvent, the methoxyl signals are fairly well separated from other signals in the spectra of lignin acetates (Fig. 1) (only the comparatively small signals from Hβ in β-5 and β-1 structures would interfere; xylan signals contributed to some extent in the current region of the birch lignin spectrum); consequently, integrations of the methoxyl peak could be used for estimations of the methoxyl content in lignins. Provided that the methoxyl content of the lignin samples have been determined by other means (e.g. microanalysis), the results obtained by NMR analysis could be used for quantitative evaluation of the other signals in the lignin spectra (see below).

Phenolic groups of the guaiacyl and syringyl types in birch lignin

It appears from Table 3 that the signal from aromatic acetate is located at different δ values in guaiacyl acetates ($\delta \approx 2.24$) and syringyl acetates ($\delta \approx 2.29$). The appearance of the aromatic acetate peaks (corresponding to phenolic groups in underivatized lignin) in the birch lignin spectrum (Fig. 1) indicates that there are more guaiacyl acetate groups than syringyl acetate groups in acetylated birch lignin. This result is in accordance with conclusions drawn from oxidative degradations of birch lignin.[15]

Comparisons with results from ^1H NMR studies of lignin acetates in chloroform and acetone solution

New results which could be derived from ^1H NMR studies of lignin acetates in pyridine solution have been discussed above. It is noteworthy that the pyridine spectra, in addition, confirm and complement the conclusions drawn in preceding papers in this series. For example, the pyridine spectra support the opinion that the quantitative role of α-aryloxypropiophenones and noncyclic benzyl aryl ethers is small in spruce as well as birch lignin (absence of significant signals in the spectral region around δ6; Fig. 1, Table 3). It should be kept in mind, however, that lignin model compounds with syringyl groups representing noncyclic benzyl aryl ethers have not been examined and this makes the conclusion regarding the occurrence of such structures in birch lignin uncertain. Integrations of the peak due to Hα in β-*O*-4 structures and calculations using the methoxyl signal as reference gave a figure for the number of β-*O*-4 linkages in birch lignin (40–50 %) which is in good agreement with earlier results.[1a] A corresponding estimation of the number of β-*O*-4 linkages in spruce lignin gave a somewhat lower figure (30–40 %) than the one obtained in previous ^1H NMR studies (30–50 %).[1b] A proportion of β-*O*-4 structures amounting to 30–40 % in spruce lignin is compatible with results derived from ^{13}C NMR investigations.[3]

Experimental

Lignin model compounds. The arylglycerol-β-

syringyl ethers corresponding to acetate derivatives *4a* and *4b* were prepared using α-lithiated carboxylic acid intermediates according to a procedure previously described for the synthesis of veratrylglycerol-β-guaiacyl ether.[16,17] α-Aryloxypropiophenone *9b* was prepared by DDQ oxidation of veratrylglycerol-β-syringyl ether (*4a*, OH instead of $OCOCH_3$) followed by acetylation.[17]

The ^1H NMR spectra were recorded on a 270 MHz instrument working in the pulse Fourier mode (Bruker WH270). Pyrine-d_5 was used as solvent (internal reference, TMS). The signal from water appears at about δ5 in pyridine solution: it has been shown that the peaks around δ5 in the lignin spectra (Fig. 1) are not caused by the presence of water. Temperature, 300–305 K. Number of scans (lignin spectra), 1000.

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References

1. (a) Lundquist, K. *Acta Chem. Scand. B 33* (1979) 27; (b) Lundquist, K. *Acta Chem. Scand. B 34* (1980) 21; (c) Lundquist, K. *Acta Chem. Scand. B 35* (1981) 497; (d) Lundquist, K., Paterson, A. and Ramsey, L. *Acta Chem. Scand. B 37* (1983) 734.
2. Kringstad, K. P. and Mörck, R. *Holzforschung 37* (1983) 237.
3. Robert, D. R. and Brunow, G. *Holzforschung 38* (1984) 85.
4. Nimz, H. H., Tschirner, U., Stähle, M., Lehmann, R. and Schlosser, M. *J. Wood Chem. Technol. 4* (1984) 265.
5. Lapierre, C., Monties, B., Guittet, E. and Lallemand, J. Y. *Holzforschung 38* (1984) 333; Scalbert, A., Monties, B., Lallemand, J. Y., Guittet, E. and Rolando, C. *Phytochemistry 24* (1985) 1359.
6. Bardet, M., Foray, M.-F. and Robert, D. *Makromol. Chem. 186* (1985) 1495.
7. Hauteville, M., Lundquist, K. and von Unge, S. *Acta Chem. Scand. B 40* (1986) 31.
8. Lundquist, K. and von Unge, S. *Unpublished data.*
9. Erickson, M. and Miksche, G. E. *Acta Chem. Scand. 26* (1972) 3085.
10. Nakatsubo, F., Sato, K. and Higuchi, T. *Mokuzai Gakkaishi 22* (1976) 29.
11. Yamasaki, T., Hata, K. and Higuchi, T. *Mokuzai Gakkaishi 22* (1976) 582.
12. Ralph, J. and Young, R. A. *J. Wood Chem. Technol. 3* (1983) 161.
13. Tanahashi, M., Takeuchi, H. and Higuchi, T. *Wood Res. 61* (1976) 44.
14. Freudenberg, K. In: Freudenberg, K. and Neish, A. C. *Constitution and Biosynthesis of Lignin,* Springer, Berlin 1968, p. 73.
15. Larsson, S. and Miksche, G. E. *Acta Chem. Scand. 25* (1971) 647.
16. Lundquist, K. and Remmerth, S. *Acta Chem. Scand. B 29* (1975) 276.
17. Brunow, G., Lundquist, K., Sipilä, J. and von Unge, S. *To be published.*

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