Lignin Stereochemistry and its Biosynthetic Implications

Knut Lundquist,[1] Vratislav Langer,[2] Shiming Li,[1] and Rolf Stomberg.[3]

[1]Department of Forest Products and Chemical Engineering, Chalmers University of Technology, SE-41296 Göteborg, Sweden

[2]Department of Environmental Inorganic Chemistry, Chalmers University of Technology, SE-41296 Göteborg, Sweden

[3]Department of Chemistry, Division of Inorganic Chemistry, Göteborg University, SE-41296 Göteborg, Sweden

ABSTRACT

Study of the stereochemistry of lignins constitutes one approach to the elucidation of the biosynthesis of lignins. Results from stereochemical studies directed to an understanding of lignin biosynthesis are summarized. The distribution of the diastereomeric forms of different types of structural elements in lignins was determined. It can be expected that the distribution of diastereomers is unaffected during the isolation of lignin or that an equilibration occurs during this procedure. The compositions of equilibrium mixtures of representative lignin model compounds, obtained by acid-catalysed equilibration, were therefore determined. The results showed that the distribution of diastereomers in lignins differs from that of the equilibrium mixtures but agrees fairly well with that found in reaction mixtures obtained on oxidation of *p*-hydroxycinnamyl alcohols *in vitro*. These results reinforce the opinion that "random" polymerization of *p*-hydroxycinnamyl alcohols via radicals plays a role in the biosynthesis of lignins. Aiming at an elucidation of the "secondary structure" of lignins the conformation of a variety of crystalline lignin models was determined by X-ray crystallography. It was found that the bulky aromatic groups in many cases tend to be far apart from each other in the conformations adopted. This suggests that repulsion between aromatic groups plays a role. However, it is evident that other factors also influence the conformations of the model compounds examined.

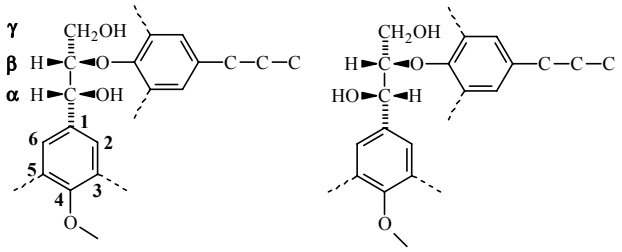
INTRODUCTION

Freudenberg and coworkers (1) studied the enzyme-catalysed oxidative polymerization of *p*-hydroxycinnamyl alcohols. They prepared dehydrogenation polymers that exhibit striking similarities to lignins. However, the dehydrogenation polymers obtained are not identical with isolated lignin samples. There are several possible explanations for this (2). A recent publication (3) describes the preparation of dehydrogenation polymers that are similar to isolated lignin samples but there are still significant differences. Studies of the stereochemistry of the structural elements in lignins offer one possibility to elucidate the biosynthesis of lignins. In this presentation results from such studies are compared with those expected from experiments with model compounds and from examinations of products obtained on enzymic oxidation of *p*-hydroxycinnamyl alcohols. Acid-catalysed equilibration of diastereomeric forms of structural elements in lignins may occur in the plant or during the isolation of lignin. The composition of the equilibrium mixtures of the diastereomeric forms of a variety of lignin model compounds have therefore been determined. Aiming at an elucidation of the "secondary structure" of the lignin molecules we have studied the conformation of a number of lignin model compounds representative of different types of lignin structures. We have in particular focused on model compounds representative of different types of arylglycerol β-aryl ethers (**1**).

RESULTS AND DISCUSSION

Lignin stereochemistry

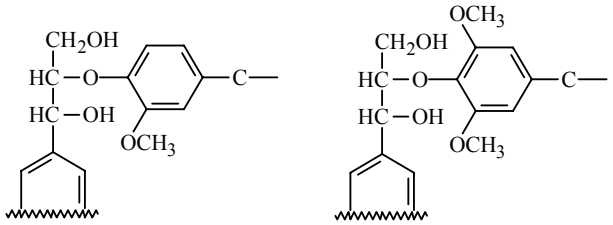
It is in general assumed that lignins are optically inactive and that the structural elements in lignins are "racemic." The correctness of this assumption has been confirmed in recent studies (4,5). It is important to recognize that the occurrence of "racemic" structural elements in lignins by no means excludes an uneven distribution of different diastereomeric forms.



The distribution of *erythro* (**1a**) and *threo* (**1b**) forms of arylglyceryl β-aryl ethers has been studied by a variety of methods (6-10). The results concur in the respect that they suggest about equal amounts of the diastereomeric forms in softwood lignins and that *erythro* forms (**1a**) are predominant in hardwood lignins.

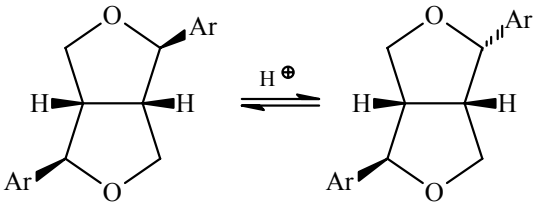
Guaiacylpropane units are prevalent in softwood lignins. Acid-catalysed equilibration of lignin models of the arylglycerol β-guaiacyl ether type (**2**) gave reaction mixtures consisting of about equal amounts of the *erythro* and *threo* forms (11,12), *i.e.* the distribution of diastereomers in the equilibrium mixtures is similar to that found in softwood lignins. It cannot be concluded from this that an equilibration has occurred, since equal amounts of the diastereomeric forms are obtained on the

addition of water to model quinone methides under suitable conditions (11).



2 **3**

Most hardwood lignins are composed of about equal amounts guaiacylpropane and syringylpropane units and some 60-70 % of the arylglycerol β-aryl ethers in such lignins have the *erythro* configuration (**1a**). The *erythro*/*threo* ratio in equilibrium mixtures of arylglycerol β-syringyl ethers (**3**) is 55:45 (11). It is evident from the equilibration experiments with models of β-guaiacyl ether type and β-syringyl ether type that the distribution of β-ether diastereomers in hardwood lignins does *not* correspond to the equilibrium mixture. Recent 2D NMR studies of a hardwood lignin (13) illustrate in a very clear way that the predominance of *erythro* forms (**1a**) is due to the presence of large amounts of *erythro* forms (**1a**) of arylglycerol β-syringyl ethers (**3**). The *erythro* β-syringyl ether/*threo* β-syringyl ether ratio was estimated to be 3-4. This ratio is in accordance with results from *in vitro* studies of the stereochemistry of the addition of water to quinone methides (11) and with results from studies of dehydrogenation polymers (9,14); the assignment of the peak at δ 75.4 in the ^{13}C NMR spectra of DHPs in ref. 14 should be changed to Cα in *erythro* forms (S.A. Ralph, personal communication, 2003). As expected from the high *erythro* β-syringyl ether/*threo* β-syringyl ether ratio the *erythro* β-aryl ether/*threo* β-aryl ether ratio is high in lignins in which syringylpropane units predominate (15,16).

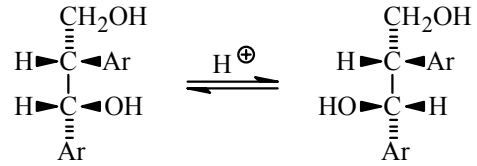


4 **5**

Ar= 4-hydroxy-3-methoxyphenyl or
4-hydroxy-3,5-dimethoxyphenyl

Fig. 1. Acid-catalysed isomerization of pinoresinol (or syringaresinol).

Acidolytic treatment of pinoresinol (or syringaresinol) (**4**) results in an equilibrium mixture consisting of about equal amounts of the starting material and its *epi* form (**5**) (Fig. 1). Pinoresinol (or syringaresinol) structures are the predominating type of β-β structures in lignins and enzymic oxidation (*in vitro*) of coniferyl alcohol (or sinapyl alcohol) almost solely produces pinoresinol (or syringaresinol) as far as β-β linked products are concerned. Studies on the occurrence of different types of β-β structures in lignins (and the distribution of their diastereomeric forms) have been reviewed (17).



threo ***erythro***

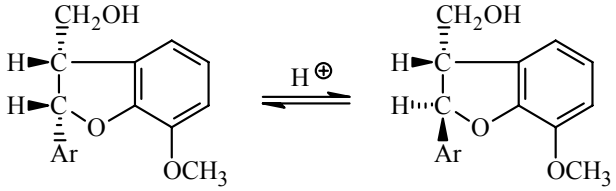
6 **7**

Ar= 3,4-dimethoxyphenyl

Fig. 2. Acid-catalysed equilibration of stereoisomeric forms of a model compound representative of β-1 structures in lignins.

The diastereomeric forms (**6** and **7**) of a model representative of β-1 structures, 1,2-bis(3,4-dimethoxyphenyl-1,3-propanediol, gave on prolonged treatment (≈7 days) with 0.2 M HCl in dioxane-water (1:1) at room temperature an equilibrium mixture (Fig. 2) in which there is a slight excess of the *threo* form (*erythro* form/*threo* form ratio 44:56) (18). Studies of the distribution of diastereomeric forms of β-1 structures in softwood lignins indicate that the number of *erythro* forms is larger than the number of *threo* forms (18). Results from ozonation studies suggest the presence of both *erythro* and *threo* forms of β-1 structures in lignins (19).

Acidolysis of β-5 models **8**/**9** showed that only a few percent of the *cis* form (**8**) (20) are present in the equilibrium mixture (Fig. 3) (21). The half-life period of **8** on refluxing with 0.1 M HBr in dioxane-water (9:1) was



cis ***trans***

8 **9**

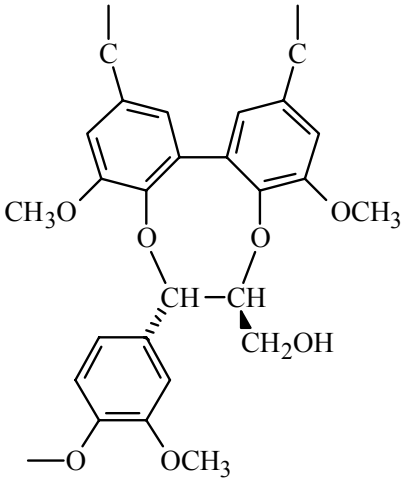
Ar= 3,4-dimethoxyphenyl

Fig. 3. Acid-catalysed equilibration of stereoisomeric forms of a model compound representative of β-5 structures in lignins.

about 30 min. Phenylcoumarans formed by oxidative phenol coupling have the *trans* configuration at the furan

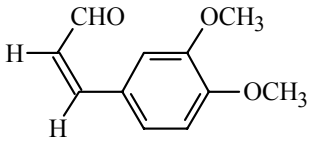
ring [no *cis* isomer is present in oxidation products of *iso*eugenol (22), attempts to detect *cis*-dehydrodiconiferyl alcohol in the reaction mixture obtained on oxidation of coniferyl alcohol failed]. The ^{1}H NMR spectrum of the acetate of **8** exhibits a signal at δ 5.87 (20). No signal can be discerned at this position in spectra of lignin acetates. Such spectra clearly reveal the presence of *trans* phenylcoumaran structures (23). It can be concluded that the number of *cis* phenylcoumaran structures in lignins is negligible compared to the number of *trans* phenylcoumaran structures. Ozonation studies show that *trans* forms of β-5 structures predominate in lignins but the presence of small amounts of *cis* forms could not be excluded for sure (19).

The occurrence of dihydrodibenzodioxocin structures in lignins has fairly recently been demonstrated (24). The *trans* form (**10**) dominates in lignins as well as in mixtures of model compounds representative of such structures prepared by methods involving enzymic oxidation (25).



10

Signals at ≈δ 9.6 in ^{1}H NMR spectra of lignin acetates can be attributed to the formyl group in *trans* forms of cinnamaldehyde end groups. The formyl proton signal of a model compound (**11**) representative of *cis* forms of such end groups is located at δ 10.02 (26). There is no signal at this position in the lignin spectra and, consequently, *cis* forms of cinnamaldehyde end groups are not present in detectable amounts in lignins.

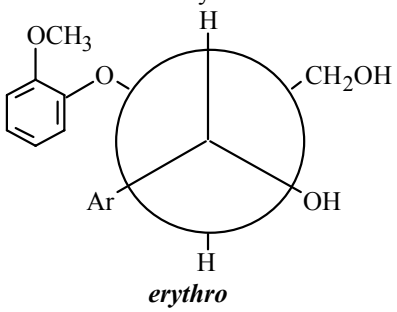


11

To summarize, the distribution of stereoisomers in lignins is in accordance with the hypothesis that lignins are produced by oxidative polymerization (*via* radicals) of *p*-hydroxycinnamyl alcohols. The distribution of diastereomers agrees fairly well with that expected from oxidation experiments with *p*-hydroxycinnamyl alcohols *in vitro* and deviates in most cases from that corresponding to equilibrium mixtures. As far as stereochemistry is concerned it seems that the polymerization process leading to lignin in plants is similar to that leading to dehydrogenation polymers when *p*-hydroxycinnamyl alcohols are oxidized *in vitro.*

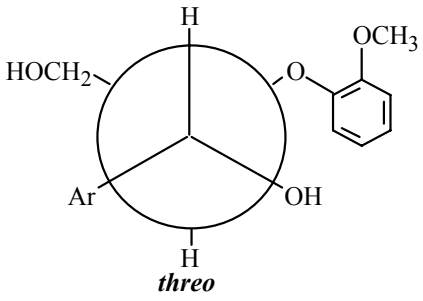
"Secondary structure" of lignins

Aiming at an elucidation of the "secondary structure" of lignins we have studied the conformation of a variety of crystalline lignin model compounds using X-ray crystallography. We have in particular focused on models representative of lignin structures of the arylglycerol β-aryl ether type (**1**). The conformation of such model compounds is largely determined by the torsion angles C_{aryl}-Cα-Cβ-O and C_{aryl}-O-Cβ-Cα.



erythro

12a Ar= 3,4-dimethoxyphenyl
12b Ar= 4-hydroxy-3-methoxyphenyl
12c The triacetate of **12b**



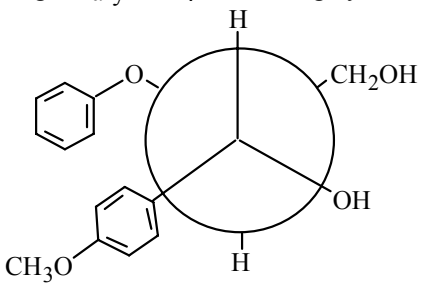
threo

13a Ar= 4-hydroxy-3-methoxyphenyl
13b Ar= The triacetate of **13a**
13c Ar= 4-hydroxy-3,5-dimethoxyphenyl
13d Ar= 3,4-dimethoxyphenyl

Fig. 4. Newman projections showing the approximate orientation of the different groups in the crystal structures of arylglycerol β-guaiacyl ethers.

In the crystal structures of the *erythro* forms of a series of β-guaiacyl ethers (**12**) (27) and the *erythro* β-phenyl ether

14 (28) the C_{aryl}-Cα-Cβ-O angle is about 60° (Fig. 4) and the angle C_{aryl}-O-Cβ-Cα is roughly -140°.



erythro

14

A more complex model of *erythro* β-guaiacyl ether type adopts a similar conformation (29). In the crystal structures of the *threo* forms of β-guaiacyl ethers **13** (30,31) the angle C_{arvl}-Cα-Cβ-O is about 180° (Fig. 4)

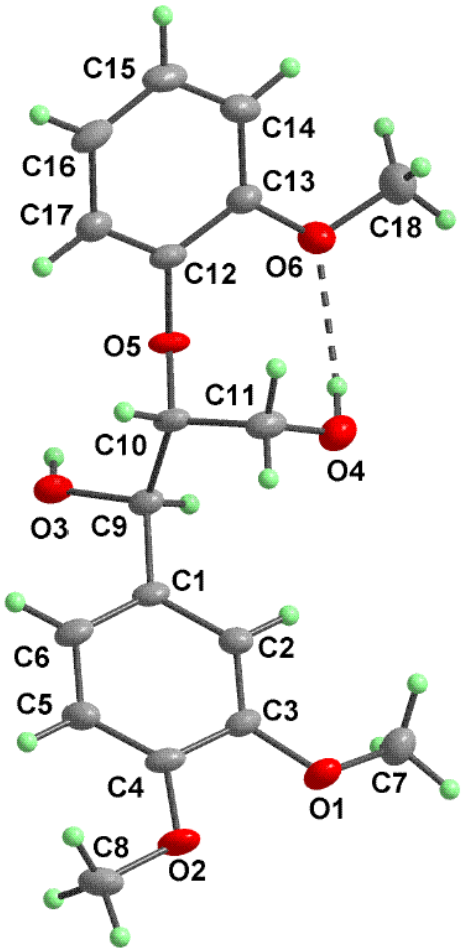


Fig. 5. Perspective drawing of *threo*-1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propandiol (**13d**). An intramolecular hydrogen bond is indicated.

and the C_{aryl}-O-Cβ-Cα angles are ranging from 105° to 165° implying that the aromatic rings are almost as far apart as possible. A perspective drawing of **13d** is shown in Fig. 5.

It is notable that the aromatic rings are interrelated in a similar way in the crystal structures of the *erythro* β-syringyl ether model **15** (27) and the *threo* β-syringyl ether model **16** (32) (Fig. 6); the C_{aryl}-O-Cβ-Cα angles are about -150 °. A perspective drawing of **16** is shown in Fig. 7.

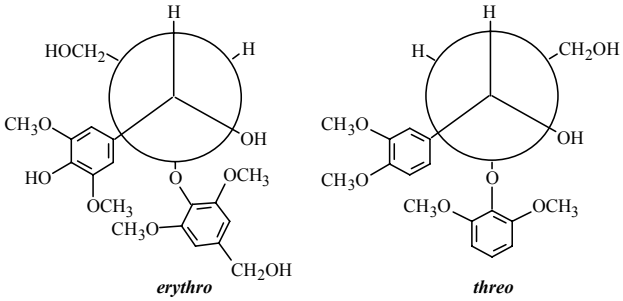


Fig. 6. Newman projections of the *erythro* syringyl ether model **15** and the *threo* syringyl ether model **16** showing the approximate orientations of the different groups.

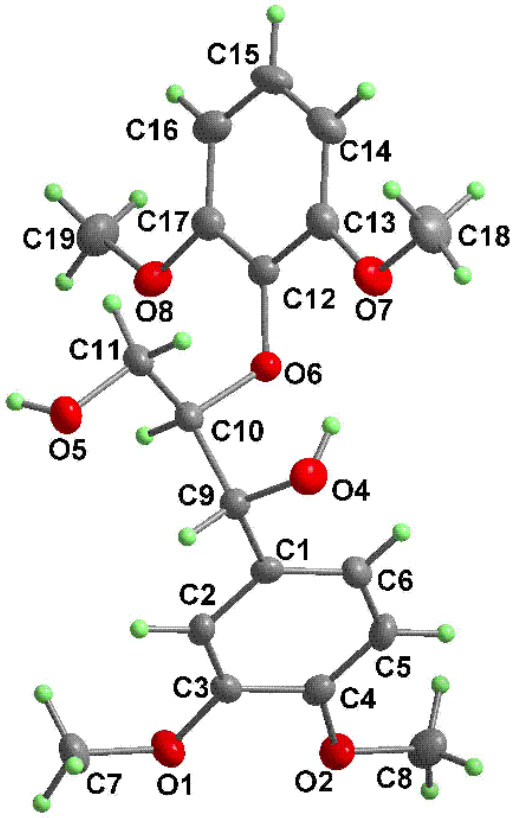
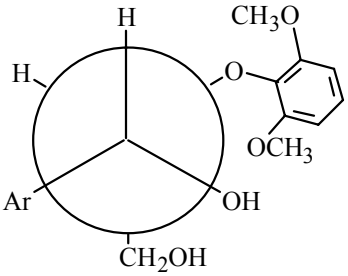


Fig. 7. Perspective drawing of *threo*-2-(2,6-dimethoxyphenoxy)-1-(3,4-dimethoxyphenyl) -1,3-propandiol (**16**).

The conformations in the crystal structures of the *erythro* β-syringyl ether models **17** (33) and **18** (34) are similar (and differ from that of **15**): the C_{aryl}-Cα-Cβ-O angles are about 180° (Fig. 8) and the C_{aryl}-O-Cβ-Cα angles are about -80 °.



erythro

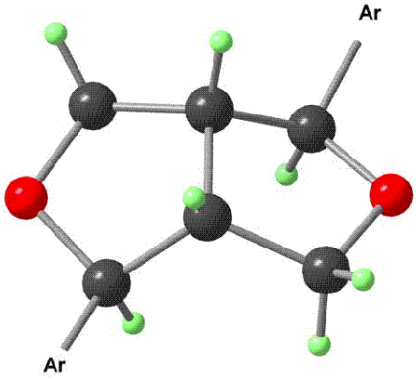
17 Ar= 3,4,5-trimethoxyphenyl
18 Ar= 4-hydroxy-3,5-dimethoxyphenyl

Fig. 8. Newman projections showing the approximate orientation of the different groups in the *erythro* syringyl ether models **17** and **18**.

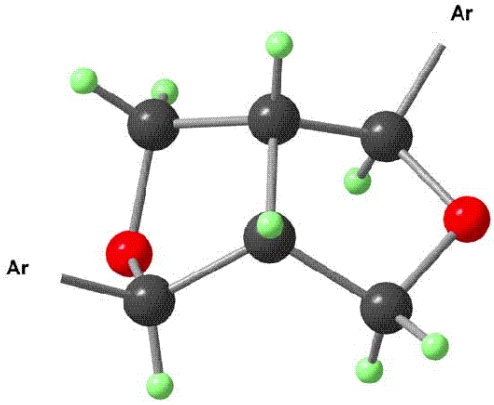
The conformations of lignin models of the arylglycerol β-aryl ether type have been subjected to computational studies. Recent results from such studies agree very well with those obtained from crystal structure determinations as concerns *threo* forms of β-guaiacyl ethers (**13**) while the conformation adopted by *erythro* forms of such ethers (**12**) (and the *erythro* β-phenyl ether **14**) is not very favored according to the calculations (35,36). Using other approaches Elder (37 and T. Elder, personal communication, 2002) obtained calculated results for the *erythro* forms that are in accordance with the crystal structures. The conformation of the *erythro* syringyl ether **15** is the one expected from computational studies (36). The conformation adopted by the *threo* β-syringyl ether **16** in the crystals is also fairly probable according to calculations (36). The conformation adopted by the *erythro* syringyl ethers **17** and **18** in the crystals deviates from those expected based on computational studies (36).

We have recently studied the conformation of pinoresinol, a model representative of lignin structures of the β-β type. X-ray crystallography showed that there are two favored conformations of the central dioxabicyclooctane ring system: in (+)-pinoresinol the five-membered rings adopt envelope conformations with the oxygen atoms as "flaps" (17) while in racemic pinoresinol the five-membered rings adopt envelope conformations with the benzylic carbons as "flaps" (38) (Fig. 9).

We think that crystal structure determinations together with computational studies of lignin models provide a basis for the elucidation of the shape ("secondary structure") of the lignin polymers.



pinoresinol in the racemate



(+)-pinoresinol

Fig. 9. Conformations of pinoresinol in the crystals of (+)-pinoresinol and the racemic form pinoresinol. Ar= 4-hydroxy-3-methoxyphenyl.

REFERENCES

1. K. Freudenberg, The constitution and biosynthesis of lignin, in Constitution and biosynthesis of lignin K. Freudenberg, A.C. Neish, Eds. (Springer, Berlin, 1968) pp. 47-122.
2. G. Brunow, K. Lundquist, Comparison of a synthetic dehydrogenation polymer of coniferyl alcohol with milled wood lignin from spruce, using ^{1}H NMR spectroscopy, *Paperi ja Puu* **62** 669-672 (1980).
3. N. Terashima, R.H. Atalla, S.A. Ralph, L.L. Landucci, C. Lapierre, B. Monties, New preparation of lignin polymer models under conditions that approximate cell wall lignification. II. Structural characterization of the models by thioacidolysis, *Holzforschung* **50**, 9-14 (1996).
4. J. Ralph, J. Peng, F. Lu, R.D. Hatfield, R.F. Helm, Are lignins optically active?, *J. Agric. Food Chem.* **47**, 2991-2996 (1999).
5. T. Akiyama, K. Magara, Y. Matsumoto, G. Meshitsuka, A. Ishizu, K. Lundquist, Proof of the presence of racemic forms of arylglycerol-β-aryl ether structure in lignin: studies on the stereo structure of lignin by ozonation, *J. Wood Sci.* **46** 414-415 (2000).

6. K. Lundquist, NMR studies of lignins. 3. ^{1}H NMR spectroscopic data for lignin model compounds, *Acta Chem Scand., Ser. B* **33**, 418-420 (1979); M. Hauteville, K. Lundquist, S. von Unge, NMR studies of lignins. 7. ^{1}H NMR spectroscopic investigation of the distribution of *erythro* and *threo* forms of β-O-4 structures in lignins, *Acta Chem. Scand., Ser. B* **40**, 31-35 (1986).
7. H.H. Nimz, U. Tschirner, M. Stähle, R. Lehmann, M. Schlosser. Carbon-13 NMR spectra of lignins, 10. Comparison of structural units in spruce and beech lignin, *J. Wood Chem. Technol.* **4**, 265-284 (1984).
8. Y. Matsumoto, A. Ishizu, J. Nakano, Studies on chemical structure of lignin by ozonation, *Holzforschung* **40** (Suppl.), 81-85 (1986).
9. B. Saake, D.S. Argyropoulos, O. Beinhoff and O. Faix, A comparison of lignin polymer models (DHPs) and lignins by ^{31}P NMR spectroscopy, *Phytochemistry* **43**, 499-507 (1996).
10. R.M. Ede, J. Ralph, Assignment of 2D TOCSY spectra of lignins: the role of lignin model compounds, *Magn. Reson. Chem.* **34**, 261-268 (1996).
11. G. Brunow, O. Karlsson, K. Lundquist, J. Sipilä, On the distribution of the diastereomers of the structural elements in lignins: the steric course of reactions mimicking lignin biosynthesis,*Wood Sci. Technol.* **27**, 281-286 (1993).
12. K. Lundquist, S. Li. 1997, Lignin reactions under acid and neutral conditions in Recent Res. Devel., in Agricultural & Food Chem. Vol. 1 S.G. Pandalai, Ed. (Trivandrum, India, 1997) pp. 15-27.
13. M. Bardet, D. Robert, K. Lundquist, S. von Unge, Distribution of *erythro* and *threo* forms of different types of β-O-4 structures in aspen lignin by ^{13}C NMR using the 2D INADEQUATE experiment, *Magn. Reson. Chem.* **36**, 597-600, 1998.
14. L.L. Landucci, S.A. Ralph, K.E. Hammel, ^{13}C NMR Characterization of guaiacyl/syringyl and syringyl dehydrogenation polymers, *Holzforschung* **52**, 160-170 (1998).
15. J. Ralph, An unusual lignin from kenaf, J. Nat. Prod. **59** , 341-342 (1996).
16. T. Akiyama, D.S. Nawawi, Y. Matsumoto, G. Meshitsuka, The distribution of diastereomeric forms of β-O-4 structures among species, To be presented at 12th ISWPC.
17. K. Lundquist, R. Stomberg, On the occurrence of structural elements of the lignan type (β-β-structures) in lignins. The crystal structures of (+)-pinoresinol and (±)-*trans*-divanillyltetrahydrofuran, *Holzforschung* **42**, 375-384 (1988).
18. S. Li, K. Lundquist, Analysis of hydroxyl groups in lignins by ^{1}H NMR spectroscopy, *Nord. Pulp Pap. Res. J.* **16**, 63-67 (2001).
19. N. Habu, Y. Matsumoto, A. Ishizu, J. Nakano, The role of the diarylpropane structure as a minor constituent in spruce lignin, *Holzforschung* **44**, 67-71 (1990).
20. S. Li, T. Iliefski, K. Lundquist, A.F.A. Wallis, Reassignment of relative stereochemistry at C-7 and C-8 in arylcoumaran neolignans, *Phytochemistry* **46**, 929-934 (1997).
21. S. Li, K. Lundquist, Acid reactions of lignin models of β-5 type, *Holzforschung* **53**, 39-42 (1999).
22. K.V. Sarkanen, A.F.A. Wallis, Stereospecific formation of *trans*-phenylcoumarans by dehydrogenative dimerisation of *trans*- and *cis*-isoeugenol, *Chem. Commun.*, 298-299 (1969).
23. K. Lundquist, ^{1}H NMR spectral studies of lignins. Results regarding the occurrence of β-5 structures, β-β structures, non-cyclic benzyl aryl ethers, carbonyl groups and phenolic groups, *Nord. Pulp Pap. Res. J.* **7**, 4-8, 16, (1992).
24. Ä. Ämmälahti, G.Brunow, M. Bardet, D. Robert, I. Kilpeläinen, Identification of side-chain structures in a poplar lignin using three-dimentional HMCQ-HOHAHA NMR spectroscopy, *J. Agric. Food Chem.* **46**, 5113-5117 (1998).
25. P. Karhunen, P. Rumakko, A. Pajunen, G. Brunow, Synthesis and crystal structure determination of model compounds for the dibenzodioxocine structure occurring in wood lignin, *J. Chem. Soc. Perkin Trans. I ,* 2303-2308 (1996).
26. S. Li, K. Lundquist. Reactions of lignin-related cinnamaldehydes and cinnamyl alcohols with borane and sodium tetrahydridoborate, *Acta Chem. Scand*. **49**, 64-67 (1995).
27. R. Stomberg, K. Lundquist, Stereochemistry of lignin structures of the β-O-4 type. Crystal structures of model compounds, *Nord. Pulp Pap. Res. J.* **9**, 37-43 (1994).
28. V. Langer, K. Lundquist, Unpublished data.
29. J.-P. Roblin, H. Duran, E. Duran, L. Gorrichon, B. Donnadieu, X-ray structure of a trimeric 5,5'-biaryl/*erythro*-β-O-4-ether lignin model: evidence for through-space weak interactions, *Chem. Eur. J.* **6**, 1229- 1235 (2000).
30. K. Lundquist, S. Li, R. Stomberg, Stereochemistry of lignin structures of the arylglycerol β-aryl ether type. Crystal structures of the *threo* form of guaiacylglycerol β-guaiacyl ether and its triacetate, *Nord. Pulp Pap. Res. J.* **11**, 43-47 (1996).
31. V. Langer, K. Lundquist, *threo*-1-(3,4-Dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol, *Acta Cryst.* **E58**, o431-o435 (2002).
32. V. Langer, K. Lundquist, R. Stomberg, S. von Unge, *threo*-2-(2,6-Dimethoxyphenoxy)-1-(3,4-dimethoxyphenyl)-1,3-propanediol, *Acta Cryst.* **E58**, o90-o92 (2002).
33. V. Langer, K. Lundquist, *erythro*-2-(2,6-Dimethoxyphenoxy)-1-(3,4,5-trimethoxyphenyl)-1,3-propanediol, *ActaCryst.* **E57**, o1219-o1221 (2001).
34. V. Langer, S. Li, K. Lundquist, *erythro*-2-(2,6-Dimethoxyphenoxy)-1-(4-hydroxy-3,5-dimethoxyphenyl)-1,3-propanediol, *Acta Cryst.* **E58**, o42-o44 (2002).
35. J.P. Simon, K.-E. L. Eriksson, Computational studies of the three-dimentional structure of guaiacyl β-O-4 lignin models, *Holzforschung* **52**, 287-296 (1998).
36. S. Besombes, D. Robert, J.-P. Utille, F.R. Taravel, K. Mazeau, Molecular modeling of syringyl and *p*-hydroxyphenyl β-O-4 dimers. Comparative study of the computed and experimental conformational properties of lignin β-O-4 model compounds, *J. Agric. Food Chem.* **51**, 34-42 (2003).
37. T.J. Elder, M.L. McKee, S.D. Worley, The application of molecular orbital calculations to wood chemistry. V. The formation and reactivity of quinone methide intermediates, *Holzforschung* **42**, 233-240 (1988).
38. R. Stomberg, V. Langer, S. Li, K. Lundquist, The racemate of pinoresinol, *Acta Cryst.* **E57**, o692-o694 (2001).