Thermally Induced Homolytic Scissions of Interunitary Bonds in a Softwood Lignin Solution: A Spin-Trapping Study

T. Seino,[1] A. Yoshioka,[1] M. Takai,[1] M. Tabata[2]

[1]Department of Biotechnology, Graduate School of Engineering, Hokkaido University, Sapporo, 060-8628 Japan
[2]Department of Molecular Chemistry, Graduate School of Engineering, Hokkaido University, Sapporo, 060-8628 Japan

ABSTRACT: Unstable chemical species, that is, radicals generated by the thermal treatment of a dimethyl sulfoxide (DMSO) solution of the lignin of a softwood, Yezo spruce (*Picea jezoensis* Carr.), were studied in detail with an electron spin resonance (ESR) method combined with a spin-trapping technique. An unstable secondary carbon radical ($\sim$CH $\cdot$) in the solution was trapped as a stable nitroxide spin adduct [R—(N—O $\cdot$)—CH$\sim$ ($R = tert$-butyl benzene)] when the DMSO solution was heat-treated in the presence of a spin-trapping reagent [2,4,6-tri-*tert*-butylnitrosobenzene (BNB)] at about 40°C. This meant that alkyl phenyl ether bonds ($\sim$CH—O-phenyl), known as interunitary lignin bonds, were homolytically scissioned by the thermal treatment in the lignin solution. A detailed analysis of the ESR spectrum revealed that three kinds of radicals—primary ($\sim$CH$_2$ $\cdot$), secondary ($\sim$CH $\cdot$), and tertiary ($\sim$C $\cdot$) carbon radicals—were trapped as stable spin adducts at about 60°C, although the phenoxy radical (Ph—O $\cdot$) was not trapped by the BNB spin trap as the counter radical of the secondary carbon radical. This suggested that a fairly large steric hindrance existed between the so-called guaiacoxy radical with a methoxy group in the ortho position and the BNB molecule bearing two butyl groups as bulky moieties in the ortho positions. However, the phenoxy radicals in the lignin solution were stable up to about 60°C. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 93: 2136–2141, 2004

Key words: ESR/EPR, Spin-Trapping, BNB

INTRODUCTION

The scission mechanism of alkyl phenyl ether bonds (β-O-4 and α-O-4 bonds) as interunitary linkages in wood lignin has been studied by many chemists from the point of view of pulping processes. Because the efficient fabrication of high-quality pulps requires delignification in not only woods but also in unbleached pulps, many basic studies on delignification have been carried out, including chemical oxidation with oxygen[1] and KMnO$_4$,[2] hydrolysis with dioxane and water,[3] and photodegradation.[4] The efficient rupturing of the ether bond (e.g., β-O-4 and α-O-4 bonds) in wood lignin is needed not only to produce high-quality pulp but also to reduce the amount of chlorine dioxide (ClO$_2$) used in the bleaching process. Because lignin-rich paper may yellow on the surface upon light exposure, the efficient fabrication of high-quality pulps is necessary. Therefore, it is very important to determine what kinds of unstable chemical species are produced by the rupturing of β-O-4 and α-O-4 bonds as the major and minor linkages, respectively, connecting the three-dimensional interunitary linkages of wood lignin, which are partly shown in Scheme 1.

We recently reported that the alkyl phenyl ether bonds of the lignin of a hardwood, *Fagus crenata* Blume, and a softwood, *Picea jezoensis* Carr., could be homolytically scissioned by ultrasonic irradiation, and the resulting secondary carbon radicals ($\sim$CH $\cdot$) as the counter radicals of the phenoxy radicals (Ph—O $\cdot$) were successfully identified with an electron spin resonance (ESR) method combined with a spin-trapping reagent [2,4,6-tri-*tert*-butylnitrosobenzene (BNB)].[5,6] In this study, ESR combined with a spin-trapping technique was also applied to trap and identify unstable lignin radicals that were thermally generated from a dimethyl sulfoxide (DMSO) solution of *P. jezoensis* Carr. lignin involving a BNB spin trap in the temperature range of 24–60°C. In this report, we show that three kinds of radicals—primary ($\sim$CH$_2$ $\cdot$), secondary ($\sim$CH $\cdot$), and tertiary ($\sim$C $\cdot$) carbon radicals—can be observed as stable nitroxide [R—(N—O $\cdot$)—CH$_n\sim$ ($R = tert$-butyl benzene and $n = 1$ or 2)] and aniline [R—N—O—C$\equiv$] spin adducts when the lignin solution is thermally treated in the presence of BNB at 60°C. To the best of our knowledge, such unstable species in lignin (e.g., alkyl carbon radicals and phenoxy radicals) have not been identified in detail with ESR.

Correspondence to: M. Takai (takai@dove-mc.eng.hokudai.ac.jp) or M. Tabata (tabata@eng.hokudai.ac.jp)
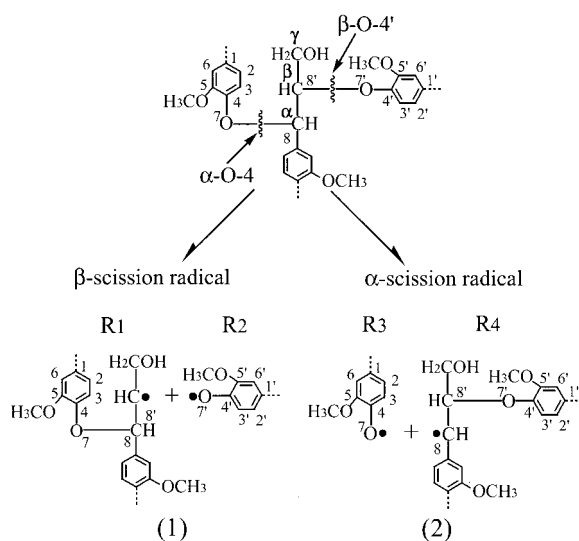
Scheme 1 Possible structure of the chemical species produced by ruptures of β-O-4 and α-O-4 bonds in lignin (R_1–R_4).

EXPERIMENTAL

Materials

A Wiley mill (Fritsch P-15, Idar-Oberstein, Germany) was used to prepare wood powders (50-mesh) of Yezo spruce (*P. jezoensis* Carr.) at Hokkaido, Japan. The obtained wood powders were extracted with a mixed solvent of ethanol and benzene (1/2 v/v) for 6 h, and this was followed by drying at room temperature for 48 h *in vacuo* at about 10^{-3} Torr. The crude lignin, known as milled wood lignin (MWL), of the softwood Yezo spruce was prepared according to the literature,[7] and the obtained lignin was purified with the so-called Lundquist method[8] and was dried at room temperature for 48 h *in vacuo* at about 10^{-3} Torr. The acetylation of MWL was performed to characterize the structure according to Chen and Robert's method.[9] The spin-trapping reagent BNB was synthesized according to the literature[10] and was used after purification.

Preparation of the lignin solution

Lignin (250 mg) was dissolved in 0.5 mL of DMSO (Junsei Chemical, Tokyo, Japan) at room temperature to prepare a homogeneous solution including 0.6 mg of BNB, and a DMSO solution was poured into a flat quart ESR cell.

^{13}C-NMR

A ^{13}C-NMR spectrum of the acetylated lignin (350 mg) dissolved in 3 mL of a mixed solvent of D_2O and CD_3COCD_3 (Cambridge Isotype Laboratories, Inc., Andover, MA) (1/9 v/v) was recorded on an NMR

spectrometer (Bruker MSL-400, Ettlingen, Germany) at room temperature.

Gel permeation chromatography (GPC)

The molecular weights of the acetylated lignin were measured with a gel permeation chromatograph (JASCO GPC-900, Tokyo, Japan) with Shodex KF-804L columns and with tetrahydrofuran (THF, Wako Pure Chemical Industries, Osaka, Japan) as an eluent and polystyrene as a standard.

ESR

ESR spectra were recorded with an x-band ESR spectrometer (JEOL FE1XG, Tokyo, Japan) with a temperature-control unit at 9.4 GHz with 100-kHz field modulation and were observed in the temperature range of 24–60°C. ESR spectral simulations were performed with a Macintosh computer to estimate the parameters of the ESR spectra observed at 60°C.

RESULTS AND DISCUSSION

^{13}C-NMR

The ^{13}C-NMR spectrum of the acetylated Yezo spruce MWL is shown in Figure 1; the chemical shifts on the aromatic ring are shown from 160 to 100 ppm. The peaks at 110–121 and 145–152 ppm are ascribed to the guaiacyl unit, the typical feature of softwood lignin.[11] Peaks at 154 ppm due to the syringyl unit were not observed. Therefore, the obtained lignin was considered softwood lignin.

GPC

The number-average molecular weight and polydispersity (weight-average molecular weight/number-average molecular weight) of acetylated MWL were estimated to be 2600 and 1.58, respectively, with GPC with THF as an eluent and polystyrene as a standard.

ESR analysis

Figure 2(a) shows the ESR spectra of a solution of Yezo spruce lignin (250 mg) and DMSO (0.5 mL) containing BNB (0.6 mg); a singlet with a *g* value of 2.0041 and a Gaussian line width of $\Delta H_{msl} = 5.1$ G can be observed (g value is defined as the parameter which characterizes the magnetic environment of unpaired electrons). The observed spectral parameters indicate that the spectrum could be assigned to the phenoxy radicals (Ph—O ·); this was previously observed for solid pristine lignin and wood powders.[12] The small peaks were generated with the peak of Ph—O · when the BNB solution of the Yezo spruce lignin was heated to
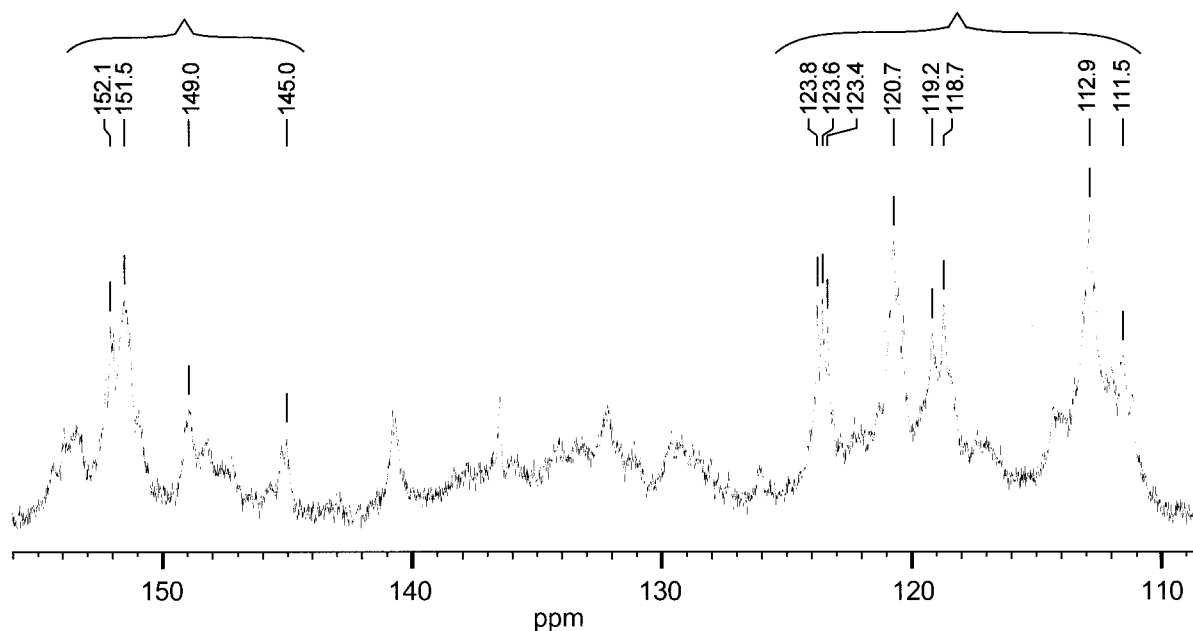
Figure 1 ^{13}C-NMR spectrum of acetylated Yezo spruce (*P. jezoensis* Carr.) lignin.

40°C [see Fig. 2(b)]. However, the observed ESR spectrum did not provide enough information for us to determine what kind of an unstable radical was trapped by the BNB trap reagent because of the broader line width spectrum. Figure 3(a) shows the ESR spectrum of the lignin solution observed at 60°C. Computer simulations of the observed spectral components SA I, SA II, and SA III [SA = spin adduct; Fig. 3(b–d)] and the singlet component IV [Fig. 3(e)] were carried out to determine the exact spectral parameters. The ESR parameters used for the best fit spectrum are summarized in Table I. The best fit spectrum of SA I was obtained with the following parameters assumed: a *g* value of 2.0068, isotropic hyperfine coupling constants (a) of 12.0 G for one nitrogen (1N) with a nucleus spin $I = 1$ and 20.0 G for each β proton (H_β, which corresponds to a proton bonded to the β-carbon separated by one chemical bond from a nitrogen with
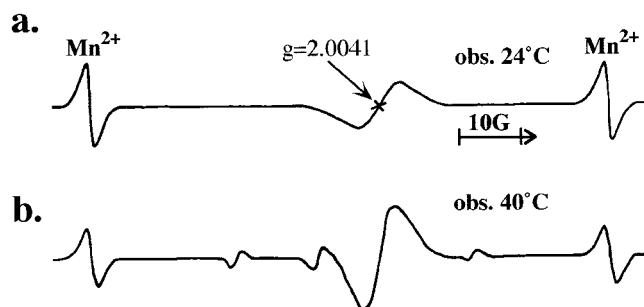

a.

Figure 2 ESR spectra taken before and after the heat treatment of a DMSO solution of the lignin with BNB: (a) at 24°C (room temperature) before heating and (b) at 40°C.
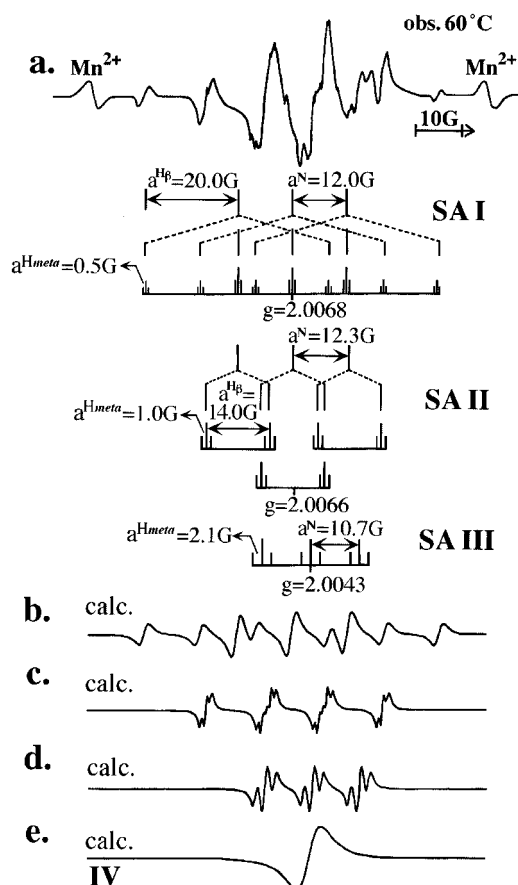

Figure 3 (a) ESR spectra of the spin adducts, observed at 60°C, of a DMSO solution of the lignin with BNB, (b) a simulated spectrum of SA I, (c) a simulated spectrum of SA II, (d) a simulated spectrum of SA III, and (e) a simulated spectrum of phenoxy radical IV.

TABLE I
ESR Parameters Observed and Calculated for the Spin Adducts and Phenoxy Radicals Generated by the Heat Treatment of DMSO Solution of P. jezoensis Carr. Lignin at 60°C in the Presence of BNB

	a^N (G)	$a^{H\beta 1}$ (G)	a^{Hmeta} (G)	g	ΔH_{msl} (G)	$\theta_1(°)$	$\theta_2(°)$
SA I	12.0	20.0	0.5	2.0068	1.5	30.2	150.2
SA II	12.3	14.0	1.0	2.0066	0.7	43.7	—
SA III	10.7	—	2.1	2.0043	1.0	—	—
IV	—	—	—	2.0045	5.0	—	—

an unpaired electron), and a ΔH_{msl} value of 1.5 G. The component SA I was considered the spin adduct formed by the trapping of a primary carbon radical ($\sim CH_2 \cdot$) because it was observed as a peak of the triple–triplet. Each peak of the triple–triplet of SA I was split into a small triplet with a small separation of 0.5 G and a relative intensity of 1:2:1, as shown in the stick diagrams and simulated spectra. The 1:2:1 intensity was ascribed to an equal coupling with two protons due to the meta protons in the tri-*tert*-butyl benzene ring because of their known coupling constant (ca. 0.8 G).[13] $\sim CH_2 \cdot$ was created by the hydrogen abstraction of the active secondary carbon radical, that is, α- and/or β-hydrogen abstractions from a methoxy methyl group ($\sim OCH_3$) in the guaiacyl moieties of the lignin mother molecules during the heating of the lignin solution at 60°C (see Scheme 2). The agreement between the experimental and simulated spectra was satisfactory, except for the central part of the spectrum (discussed later). The simulated spectrum for SA II was also calculated with the following parameters assumed: a g value of 2.0066, isotropic hyperfine coupling constants of 12.3 (1N), 14.0 (1H$_\beta$), and 1.0 G for two meta protons (2H$_{meta}$), and a ΔH_{msl} value of 0.7 G. SA II was assigned to a spin adduct formed by the trapping of a secondary carbon radical ($\sim CH \cdot$) because the spin adduct was observed as a peak of the

double–triplet. The same two kinds of radical species, that is, the secondary carbon radicals $R_1SA(1\beta)$ = $\sim C\beta H \cdot$, known as the β-scission radical, and $R_4SA(1\alpha)$ = $\sim C\alpha H \cdot$, known as the α-scission radical, could be produced by the rupture of the β-O-4 and α-O-4 bonds of the mother lignin molecules when they were heat-treated in the DMSO solution at 60°C. Each radical could be trapped, forming the corresponding spin adducts, that is, $R_1SA(I_\beta)$ and $R_4SA(I_\alpha)$, as shown in Scheme 3, although the observed ESR spectra do not provide enough information to determine which ether bond was selectively ruptured by the heat treatment because of the fairly broad line widths. The center-line shape of the observed spectrum, that is, the third, fourth, and fifth lines shown in Figure 3(a), did not coincide with that of the simulated spectra of SA I and SA II. The simulated spectrum for SA III was also calculated with a g value of 2.0043, isotropic hyperfine coupling constants of 10.7 (1N), and 2.1 G (2H$_{meta}$), and a ΔH_{msl} value of 1.0 G. Therefore, SA III was attributed to the spin adduct of tertiary carbon radicals ($\sim C \cdot$) because this spin adduct did not show any β-proton couplings. The simulated spectrum of SA III shown in Figure 3(a) satisfies the component of the central part, that is, the third and fifth lines in the observed spectrum, although the fourth-line spectrum intensity did not coincide with the simulated spectra of SA I, SA II, and SA III. $\sim C \cdot$ was created by the hydrogen abstraction of the active secondary carbon
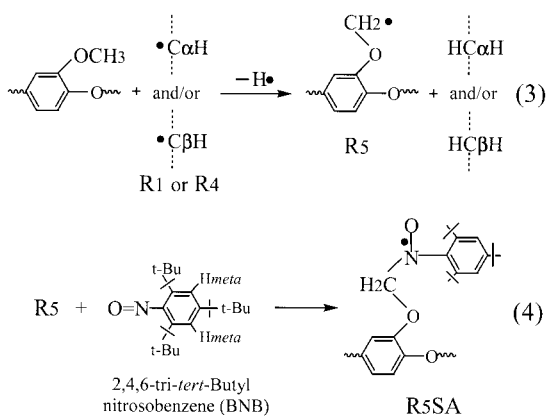
Scheme 2 Possible hydrogen abstractions from a methoxy methyl group in the lignin guaiacyl moieties induced by the secondary carbon radicals (R$_5$) and the resulting spin adducts (R$_5$SA).
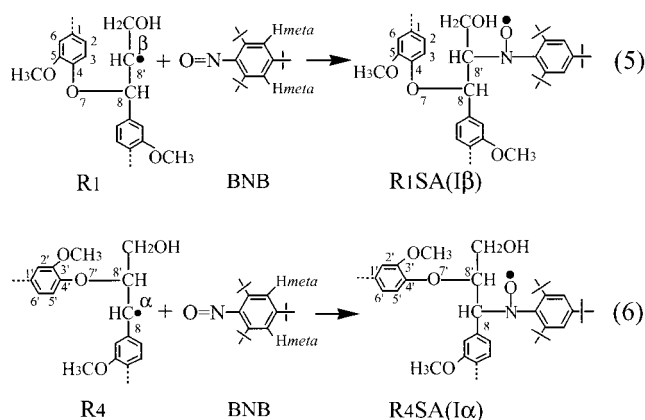
Scheme 3 Possible structures of the secondary carbon radicals trapped by a BNB spin trap (R$_1$SA and/or R$_4$SA).

$$(7)$$

R1 or R4   R6α   R6β

$$(8)$$
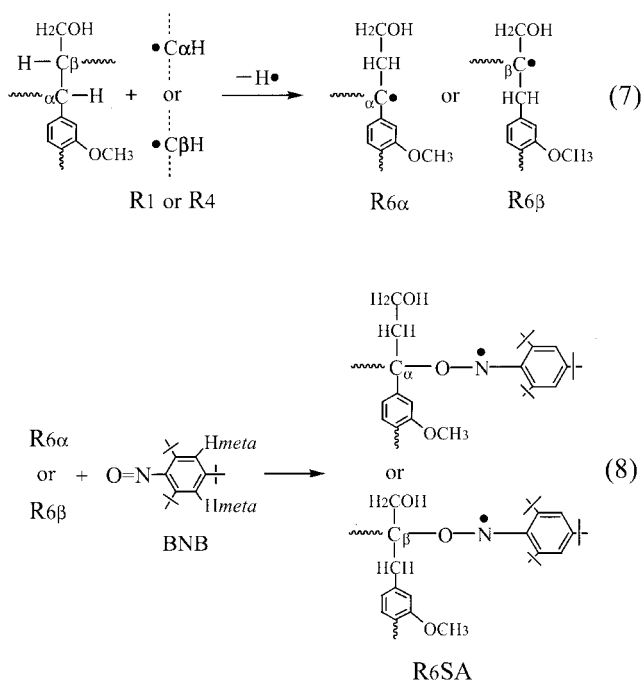
Scheme 4 Possible hydrogen abstractions from the propane chain in the lignin guaiacyl moieties induced by the secondary carbon radicals (R₆α and R₆β) and the resulting spin adducts (R₆SA).

radical, that is, α- and/or β-hydrogen abstraction, from the so-called propane chain in the guaiacyl moieties of the lignin molecules during the heating of the lignin solution up to 60°C (see Scheme 4). On the other hand, component IV was observed as a broad singlet (g = 2.0045 and ΔH_{msl} = 5.0 G), as shown in Figure 3(a). This singlet spectrum was assigned to the guaiacoxy radical (Ph—O ·) as reported for the solid wood and lignin.[14] This suggests that Ph—O · was not trapped to form either the nitroxide spin adduct or the aniline spin adduct, but the radical was stable up to 60°C [see eqs. (9) and (10) in Scheme 5]. In other words, the nitroxide and/or the aniline spin adducts of the guaiacoxy radical may have been unstable and easily decayed, even if it was formed because of the peroxide. Thus, the simulated spectra of SA I, SA II, SA III, and IV shown in Figures 3(b–e) clearly coincide with those of the observed spectra shown in Figure 3(a).

In previous articles,[5,6] we reported that the alkyl phenyl ether bonds of the lignin of *P. jezoensis* Carr. were homolytically scissioned by ultrasonic irradiation, and then the resulting secondary carbon radicals (~CH ·) as the counter radical of the phenoxy radical (Ph—O ·) and tertiary carbon radical (~C ·) were successfully identified by the method called ESST. However, in this thermally induced experiment, the primary carbon radical (~CH₂ ·) was also found to be trapped by the BNB spin trap, producing the corresponding stable spin adducts (see Table I).

There were fundamental differences in the cleavage mechanism of the alkyl phenyl ether bonds in the lignin degradation reactions induced by ultrasonic irradiation and thermal treatment at 60°C. In the case of ultrasonic degradation, the alkyl phenyl ether bonds in the lignin solution were ruptured at random through so-called ultrasonic cavitation. For thermal-treatment degradation, the alkyl phenyl ether bonds may have been regularly ruptured from most weakest part of the lignin network in the lignin solution because the thermal energy was uniformly added to the lignin molecule solution.[3] This is clearly supported by the fact that the observed ESR parameters of the spin adducts of the radicals generated by each treatment were not the same, thereby indicating different scission mechanisms even at 60°C.

Local structure of the lignin

We examined whether the local structure of lignin molecules in solutions of Yezo spruce and Japanese beech could be deduced by the ESR method combined with the spin-trapping method when the so-called McConnell equation was applied (see Fig. 4).[15] The deduced local structure of the lignin molecules is shown in Figure 4. The local molecular structure is important because the structure may reflect features of each lignin, like finger prints, that are necessary to classify the various types of wood lignin. The spatial conformations of the spin adducts, R₅SA and R₁SA(Iβ), produced by the primary carbon radical and the secondary carbon radical were calculated with the McConnell equation [eq. (11)] as shown in Figure 4(a,b), respectively. The dihedral angles θ_1 and θ_2 between the p_z orbital of an unpaired electron and H_{β^1} and H_{β^2} bonds were estimated to be 30.2 and 150.2° (i.e., 30.2° + 120°), respectively, as shown in Figure 4(a) [B_0 (isotropic interaction) was 0, and B (anisotropic interaction) was assumed to be 26.8 G]).[16] The dihedral angle θ between the p_z orbital of unpaired electron and H_{β^1} bond was also estimated to be 43.7°, as shown in Figure 4(b):

$$(9)$$

R(2or3)SA

$$(10)$$

R(2or3)SA
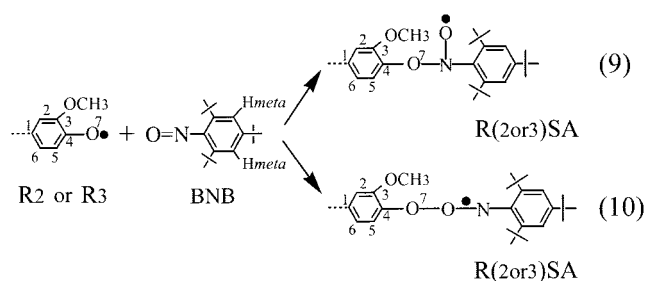
Scheme 5 Possible structure of the phenoxy radicals trapped by a BNB spin trap (R₂SA or R₃SA).
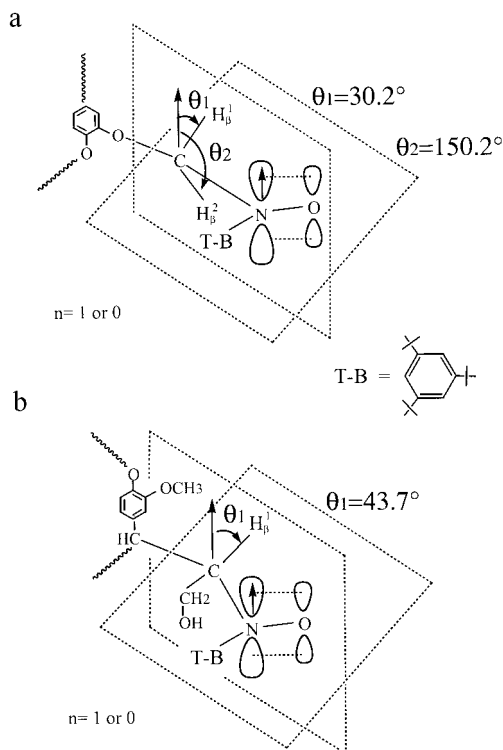
Figure 4 Perspective view of the spin adducts of (a) the primary carbon radical created by the hydrogen abstraction of the active secondary radical and (b) the secondary carbon radical produced by the rupture of the β-O-4 bond in the lignin.

$$a^{H_{\beta 1} \text{ and } H_{\beta 2}} = B_0 + B \cos^2 \theta \qquad (11)$$

where $H\beta 1$ and $H\beta 2$ is defined as hyperfine coupling a constant of β proton bonded to the β-carbon, B_0 is 0 and B is 26.8 G.

CONCLUSIONS

An ESR method combined with a spin-trapping reagent (BNB) was successfully applied to detect the unstable chemical species generated by the heat treatment of a DMSO solution of the lignin of the softwood Yezo spruce (*P. jezoensis* Carr.). The three kinds of radicals—the primary carbon radical ($\sim CH_2 \cdot$), secondary carbon radical ($\sim CH \cdot$), and tertiary carbon radical ($\sim C \cdot$)—were thermally generated from the DMSO solution of the lignin at 60°C and trapped by a

BNB spin trap to produce the corresponding stable spin adducts. $\sim CH \cdot$ was considered the counter radical of the phenoxy radical (Ph—O ·), which was previously observed in solid lignin and wood powders when subjected to photoirradiation,[12] heat treatment,[17] and milling.[18] However, on the basis of these data, we also concluded that the observed ESR spectra did not provide enough information to determine which alkyl phenyl ether bond (i.e., the α-O-4 or β-O-4 bond) was selectively ruptured by the heat treatment. The observed ESR spectra possessed a wider line width in the spin adduct (SA II), and this precluded a detailed analysis. These findings suggested that hydrogen abstraction from the secondary carbons together with recombination between the resulting secondary carbon radicals was efficiently reduced, and this allowed efficient delignification in an economical pulping process.

References

1. Kratzl, K.; Gratzl, J.; Claus, P. Lignin Structure and Reactions; Advances in Chemistry Series 59; American Chemical Society: Washington, DC, 1966; p 157.
2. Freudenberg, K.; Janson, A.; Knopf, E.; Haag, A. Ber Dtsch Chem Ges 1936, 69, 1415.
3. Sano, Y. Mokuzai Gakkaishi 1975, 21, 508.
4. Gierer, J.; Stephen, Y. L. Svensk Papperstidn 1972, 75, 233.
5. Yoshioka, A.; Seino, T.; Takai, M.; Tabata, M. Holzforschung 2000, 54, 357.
6. Seino, T.; Yoshioka, A.; Fujiwara, M.; Chen, K.-L.; Erata, T.; Tabata, M.; Takai, M. Wood Sci Technol 2001, 35, 97.
7. Björkman, A. Svensk Papperstidn 1956, 59, 477.
8. Lundquist, K.; Ohlsson, B.; Simonson, R. Svensk Papperstidn 1977, 80, 143.
9. Chen, C.-L.; Robert, D. In Methods in Enzymology: Biomass; Wood, W. A.; Kellogg, S. T., Eds.; Academic: San Diego, 1988; Vol. 161, Part B, p 137.
10. Okazaki, R.; Hosogai, T.; Hashimoto, M.; Inamoto, N. Bull Chem Soc Jpn 1969, 42, 3559.
11. Lüdemann, H. D.; Nimz, H. Makromol Chem 1974, 175, 2409.
12. Hon, D. N.-S.; Ifju, G.; Feist, W. C. Wood Fiber 1980, 12, 121.
13. Tabata, M.; Sohma, J.; Shiotani, M.; Heusinger, H. Radiat Phys Chem 1980, 16, 369.
14. Seino, T.; Yoshioka, A.; Fujiwara, M.; Chen, K.-L.; Erata, T.; Tabata, M.; Takai, M. Mokuzai Gakkaishi 2000, 46, 342.
15. (a) Heller, C.; McConnell, H. M. J Chem Phys 1960, 32, 1535; (b) Windle, J. J.; Kuhnle, J. A.; Beck, B. H. J Chem Phys 1969, 50, 2630.
16. Watanabe, O.; Tabata, M.; Sohma, J. Macromolecules 1984, 17, 1139.
17. Hatakeyama, H.; Nakano, J. J Cellul Chem Technol 1970, 4, 281.
18. Kleinert, T. N.; Morton, J. R. Nature 1962, 196, 334.