ELUCIDATION OF THE BONDS BETWEEN CELLULOSE AND DEHYDROGENATION POLYMER WITH CARBON-13 ISOTOPIC TRACER METHOD

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In this study, the DHP-cellulose complexes (DHPCC) were prepared from coniferin-[α-^{13}C] and cellulose. The CP/MAS indicated that the linkage between lignin and cellulose was a benzyl ether bond. The DHPCC was hydrolyzed with cellulase. The enzymatic hydrolyzates were fractionated into water-soluble and water-insoluble fractions. The water-soluble fractions were fractionated by adsorption chromatography. Comparing the ^{13}C NMR spectra of the labeled water-insoluble fractions and LC-rich water-soluble fractions with those from the unlabeled DHPCC, it was possible to observe significant enhancement in the signal intensities of the α-carbons. The main substructures of the DHPCC were *β*-O-4, *β*-*β*, *β*-5, *β*-1 and coniferyl alcohol. Furthermore, *β*-O-4 units were linked with cellulose at the α-position through benzyl ether bonds and acetal bonds.

Keywords: dehydrogenation polymer, cellulose, carbon-13 isotopic tracer, coniferin, ^{13}C NMR

INTRODUCTION

Cellulose, hemicellulose and lignin are the three main components of plant cell walls. Lignin, which is covalently linked to polysaccharides in the plant cell walls, could make the process of pulping and bleaching more difficult.[1-3] Many studies[4-7] on the bonds between lignin and hemicellulose reported that benzyl ether bonds and benzyl ester bonds were the main LC bonds. Nakano also thought that lignin could be bound to cellulose in unbleached kraft pine pulp and these bonds could be the main obstacle during bleaching.[8] In 1996, Karlsson and Westermark[9] demonstrated that lignin was chemically bound to cellulose in kraft pulp using size exclusion chromatography (SEC). Then, Lawoko *et al*.[10] reported that at least 90% of the residual lignin in softwood kraft pulp was chemically bound to carbohydrates. A major proportion (92%) of the lignin-carbohydrate complexes (LCC) in softwood kraft pulp was bound to xylan and glucomannan, whereas a minor proportion (8%) was linked to cellulose. Although these studies suggested that lignin may be chemically bound to cellulose, details on what types of bonds occur between these molecules and the mechanisms of how these bonds form are not clear.

In this study, DHPs were prepared in the presence of cellulose by simulating natural conditions[11-13] in the laboratory to better understand these bonds. Firstly, coniferin or coniferin-[α-^{13}C] was added to the cellulose solution in the presence of peroxidase, glucose oxidase and β-glucosidase. Then, the DHPCCs were obtained and analyzed by gel-adsorption chromatography and ^{13}C NMR spectroscopy.

EXPERIMENTAL

Preparation of ^{13}C-labeled DHP-cellulose complexes (DHPCC)

Coniferin-[α-^{13}C] was synthesized according to the method of N. Terashima *et al*.[14]

Coniferin-[α-^{13}C] (1.6 g) was dissolved in 0.2 M acetate buffer (160 mL, pH=4.9). Cellulose (1.6 g) was pretreated with 85% phosphoric acid.[15-16] Under sterile conditions, β-glucosidase (46.4 mg, from almonds, 6.3 μg/mg, Fluka), glucose oxidase (62.4 mg, Type II from *Aspergillus niger*, 15,500 units/g, Sigma), and peroxidase (4.4 mg, Type II from horseradish, 158 purpurogallin units/mg, Sigma) were dissolved in

sterile water and added to the pretreated cellulose. Over a period of 24 h, a coniferin [α-^{13}C] solution was added to the mixture of enzymes and cellulose with a tube pump with air-bubbling at room temperature. Then the reaction was continued for 5 days at 30 °C. The crude product was obtained by centrifugation and thorough washing with water. After freeze-drying, the crude products were washed with dichloroethane/ethanol (2/1, v/v) and then 8 M urea in boric acid/borax buffer. The purified products (DHPCCs, 1.464 g) were washed completely with water and freeze-dried. The yield of DHPCC was 59.9%.

Preparation of enzyme-degraded DHPCCs

DHPCCs (1.2 g) were suspended in an enzyme solution containing 0.4% cellulase (Onozuka RS, 1.036 g, Yakult Pharmaceutical Ind. Co., Nishinomiya, Japan), which was dissolved in a buffer (pH=4.5) composed of 0.5 M sodium acetate and 0.5 M acetic acid. A few drops of toluene were added as preservative. The mixture was agitated on a shaker at 50 °C for 170 h. After centrifugation, the water-soluble fractions and water-insoluble fractions were collected. Water-insoluble fractions were called DHPCC-IS (0.246 mg) and the yield of DHPCC-IS was 20.5%.

Isolation of water-soluble DHPCC from the water-soluble fraction of enzyme-degraded DHPCC

The solution of the enzyme-degraded DHPCCs was separated by gel-adsorption chromatography on a Toyopearl HW-40F column. First, distilled water was used as the mobile phase. The eluant was collected. The sugar content of each fraction was measured by UV at 490 nm with the phenol-sulfuric acid method.[17] The amount of lignin was measured by UV at 280 nm.[18] The mobile phase was changed to 50% dioxane solution when the water-soluble portions were completely eluted. Then the water-soluble fragments (called DHPCC-WS, 0.152 mg) containing abundant low molecular lignin-carbohydrate complexes were eluted and analyzed with the same method as mentioned above. The yield was 12.7%.

FT-IR determination

Infrared spectra were determined using an FT-IR 710 infrared spectrophotometer (Nicolet, Madison, WI, USA). Dried samples (1~2 mg) were milled into powder with a diameter less than 1 mm. A certain amount of the powder was dispersed in spectroscopic grade KBr and subsequently pressed into disks using 10 tons of pressure for 1 min. A total of 100 scans with a 2 cm^{-1} resolution were signal averaged and stored; the wave number range scanned was 4000-500 cm^{-1}.

CP/MAS ^{13}C-NMR determination

The CP-MAS ^{13}C NMR spectra were recorded on a VARIAN Infinityplus-400 (Bruker Instrument, Inc., Billerica, MA, USA) equipped with CP-MAS accessories. Dipolar decoupling was systematically used during the acquisition sequence. The samples were spun at a rate of 7 kHz at room temperature, the accumulation of 4331 scans was used to obtain a satisfactory signal to noise ratio. The optimal contact time was 50.0 μs, spectral width – 25.0 kHz and acquisition time – 3.0 ms.

^{13}C NMR spectroscopy

Both water-soluble and water-insoluble samples (100 mg) were dissolved in DMSO-d_6 and put in a ϕ 5 mm probe tube. The ^{13}C NMR spectra were recorded on a BRUKER Advance-600 NMR spectrometer at 150 MHz at 50 °C. The pulse angle was 90° with a 1.75 s pulse delay time. A total of 20,000 scans was accumulated.

RESULTS AND DISCUSSION

FT-IR determination of DHPCC

FT-IR is one of the most common methods to determine the structure features of all chemicals, due to its high sensitivity, selectivity and accuracy.[19] The FT-IR spectra of DHPCC before and after purification are presented in Fig. 1, which clearly shows that the signals at 1600 cm^{-1} and 1510 cm^{-1}, corresponding to the aromatic ring[20-21] in the FT-IR spectra of DHPCC before purification are stronger than those of DHPCC after purification. This is due to the removal of DHP during DHPCC treatment with dichloroethane/ethanol (2/1, v/v) and 8 M urea in boric acid/borax buffer. Also, the signal at 1730 cm^{-1}, which corresponds to non-conjugated C=O stretching vibration[22] in the FT-IR spectra of DHPCC before purification, disappeared in the FT-IR spectra of DHPCC after purification. But there was an adsorption peak at 1500 cm^{-1} in DHPCC after purification. These data suggest that the lignin content in DHPCC after purification decreased, but there still was an aromatic ring structure in DHPCC. These aromatic compounds were bonded to cellulose in strong physical or chemical ways.

CP/MAS ^{13}C-NMR determination of DHPCC

CP/MAS NMR have been reported in the study of cell wall structure for cellulose, hemicellulose, lignin and even extractives.[23-26]

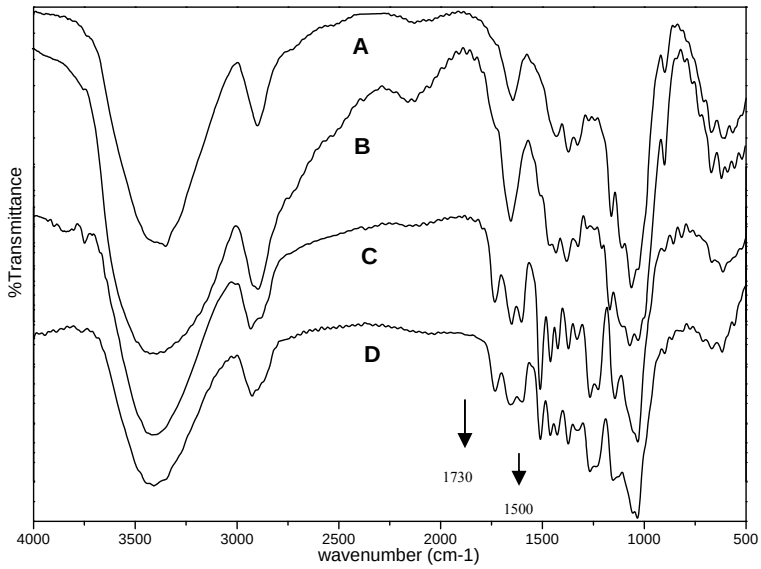


Figure 1: FT-IR spectra of DHPCC (DHP-Cellulose Complexes); A: DHPCC-Cont. (after purification); B: DHPCC-[α-^{13}C] (after purification); C: DHPCC-Cont. (before purification); D: DHPCC-[α-^{13}C] (before purification)

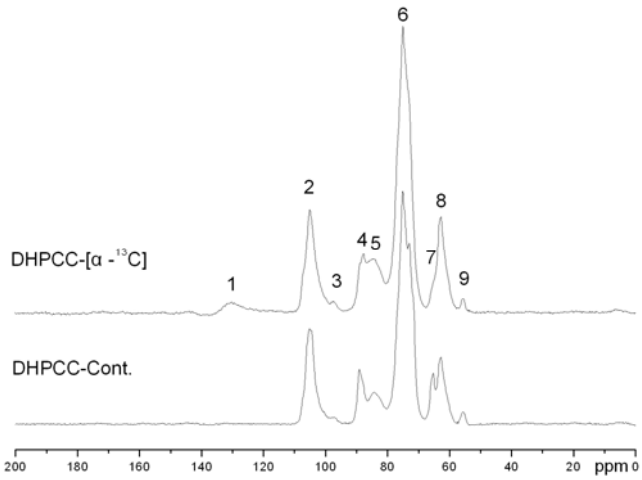


Figure 2: CP/MAS ^{13}C-NMR spectra of DHPCC (DHP-cellulose complexes)

CP/MAS ^{13}C-NMR spectra of DHPCC-[α-^{13}C] and DHPCC-Cont. are shown in Fig. 2, and the assignment of signals in CP/MAS ^{13}C-NMR spectra is presented in Table 1.

In Fig. 2, it can be seen that the weak signal at 131.4 ppm (No. 1), which was due to the structure of α-C of coniferyl alcohol, C1 of guaiacyl connected with Cα of HC=C, was strengthened. The strengthened signal was from α-C of coniferyl alcohol, which proved that there were a few coniferyl alcohol structures in DHPCC. Signal No. 2 (105 ppm) was a strong single peak. It came from C1 of cellulose.[24] The signal at 87.9 ppm (No. 4) came from Cα (β-5) and C4 of cellulose. The signal at 84.6 ppm (No. 5) was strengthened after ^{13}C labeling, and was assigned to the structure of Cα (β-β) and Cα connected with polysaccharides in the benzyl ether bond. The data proved that, in DHPCC, part of lignin was connected with polysaccharides by the benzyl ether bond. In the spectra, the strongest peak was at 75.1 ppm (No. 6) and was also strengthened after ^{13}C labeling. It could be concluded that this signal came not only from C2,

C3 and C5 of cellulose,[28] but also from Cα(β-O-4). It was easy to see that the signal at 63 ppm (No. 8) was strengthened after ^{13}C labeling, which proved the existence of β-1 substructure. The weak signal at 56 ppm (methoxyl) indicated that most of the DHP had been removed after the treatment with dichloroethane/ethanol (2/1, v/v). Only a small amount of DHP connected with cellulose by chemical bond was still retained in DHPCC, which is the reason for using the carbon-13 isotopic tracer method to determine the existence of lignin and the connection between lignin and cellulose.

According to the above data, it could be concluded that the main structure of lignin in the DHP-cellulose complex included β-O-4 units, β-β units, β-5 units and β-1 units, with minor coniferyl alcohol and cinnamic alcohol structure. DHP was connected with cellulose by a benzyl ether bond.

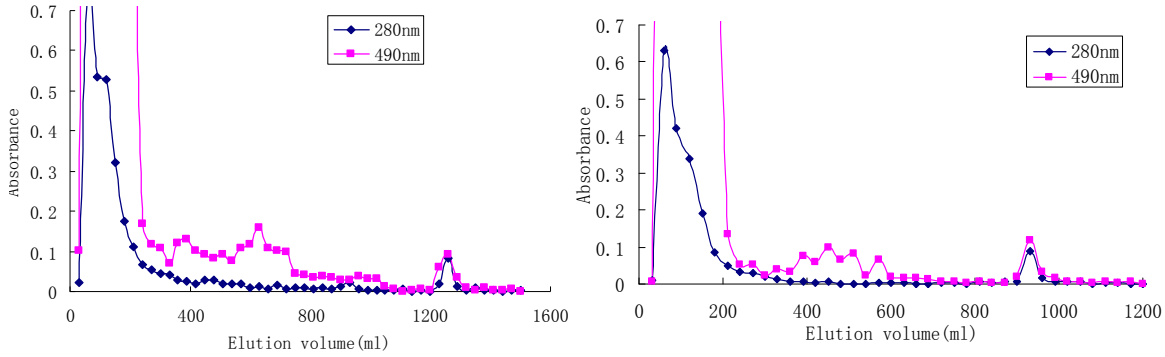
Analysis of cellulase-degraded DHPCC

Isolation of DHPCC-WS fraction by gel-adsorption chromatography

As shown in Fig. 3a and 3b, the water-soluble DHPCC had significant absorption of UV light (280 nm) and visible light (490 nm). In the first stage of separation, there was a maximum peak, which mainly came from soluble lignin fragments, enzyme-degraded polysaccharides and residual enzyme. In the last stage of separation by aqueous dioxane elution, the fraction also absorbed UV light at 280 nm and 490 nm. It is believed that this fraction consisted of lignin and carbohydrates, which were from the DHP-cellulose complexes. By gel-adsorption chromatography separation, the DHPCC-WS fraction could be obtained, as shown in Figs. 3 and 4. The results agree with those of Ohnishi.[29]

Table 1
Analysis of CP/MAS ^{13}C-NMR spectra of DHPCC

No.	δ, ppm		Assignment
	DHPCC-Cont.	DHPCC-[α-^{13}C]	
1	Not clear	131.4	α-C of coniferyl alcohol, C1 of guaiacyl connected with Cα of HC=C
2	105.2	105	C1 of cellulose
3	97.1	97.4	unknown
4	89.1	87.9	Cα(β-5), Cβ(β-O-4), C4 of cellulose
5	84.3	84.6	Cα(β-β), Cα connected with polysaccharides in methyl phenyl ether bond, Cβ(β-O-4 with α-CO)
6	75.2	75.1	Cα(β-O-4), C2, C3 and C5 of cellulose
7	65.3	65.1	Cγ of phenylcoumaran and cinnamic alcohol, C6 of glucose
8	62.8	63	Cα/Cβ(β-1), Cγ(β-5, β-1, β-O-4), γ-C of cinnamic alcohol, C6 of cellulose
9	55.6	55.6	OCH_3



(a) (b)

Figure 3: Adsorption chromatograms of enzymatic hydrolyzates from DHPCC (a) and DHPCC-[α-^{13}C] (b)

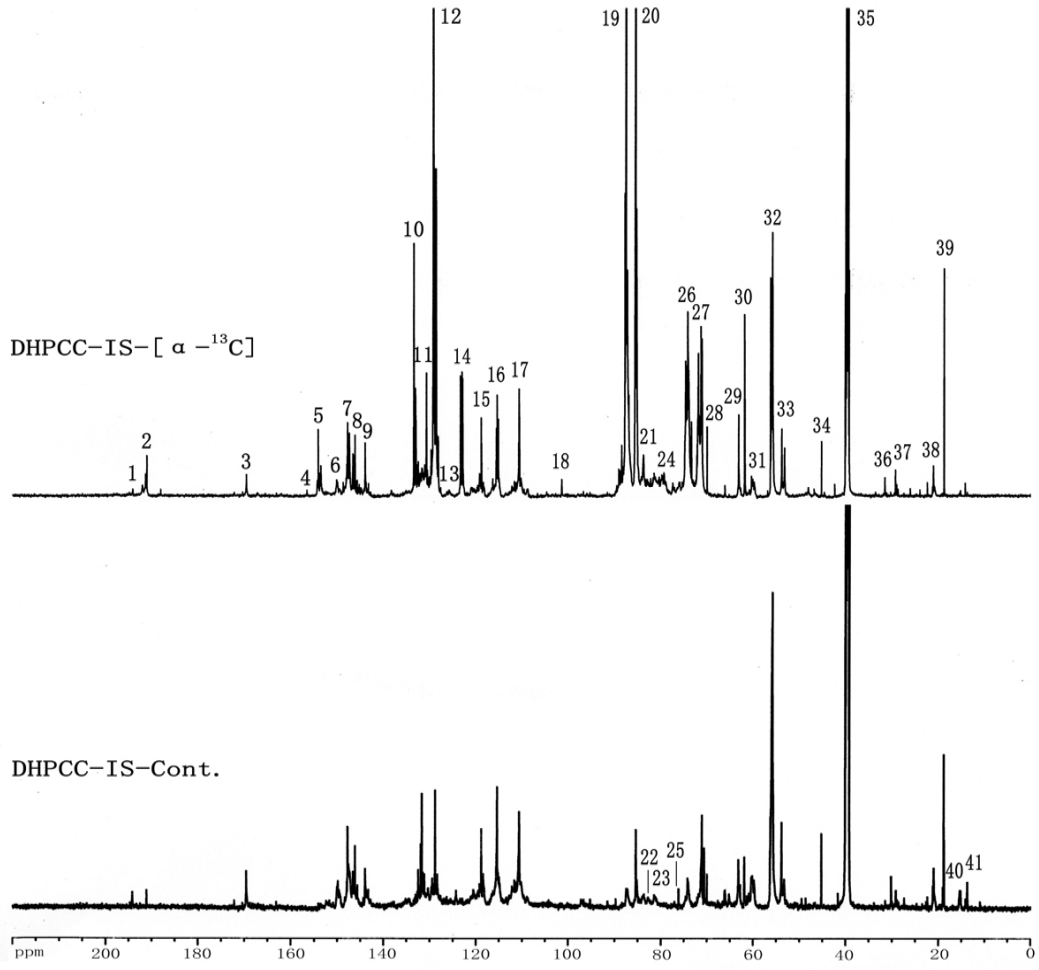


Figure 4: ^{13}C-NMR spectra of DHPCC-IS (water-insoluble fractions of enzyme-degraded DHPCC)

^{13}C NMR spectra of ^{13}C-enriched DHPCC-IS

In order to gain a more complete understanding of the structures in DHPCC-IS, qualitative ^{13}C NMR spectra of DHPCC-IS with or without ^{13}C labeling were investigated. The ^{13}C NMR spectra of DHPCC-IS-Cont. and DHPCC-IS-[α-^{13}C] are shown in Fig. 4. Comparing the two spectra, the signal No. 2 (191.1 ppm) was enhanced by the α-^{13}C-enrichment. This was assigned to the C-α in the structures of the aldehyde group.[30] A significantly enhanced signal No. 12 at 130 ppm by the α-^{13}C-enrichment was related to the C-α in the structures of coniferyl alcohol.[31] From the intensity of this peak, it was concluded that DHPCC contained a large amount of coniferyl alcohol subunits. Signal No. 18 at about 101.5 ppm was assigned to the α-carbon of the lignin side chain with acetal linkage to carbohydrates.[27]

Signal No. 19 (87.4 ppm) and signal No. 20 (85.4 ppm) were greatly enhanced in the spectrum of the ^{13}C-enriched sample. Signal No. 19 (87.4 ppm) was assigned to the C-α of the β-5 structures[26] and signal No. 20 (85.4 ppm) was assigned to the C-α of the β-β structures.[30] The slightly increased signals No. 21-23 at about 81.5-83.9 ppm were assigned to the C-α in the benzyl ether linkages to carbohydrates.[27,30] Signal No. 24 at about 80.1 ppm was produced by the C-α of the β-O-4 units with α-CO groups.[32] Two strongly enhanced signals No. 26 and No. 27 were observed at about 74.2 ppm and 71.8 ppm. Signal No. 26 was assigned to the C-α of the *threo* type β-O-4 structures. Signal No. 27 was assigned to the *erythro* type β-O-4 structures.[33] Signal No. 29 (δ 63.4 ppm) was assigned to the C-γ of the β-5 structures and signal No. 30 (δ 61.9 ppm) was produced by the C-γ of the β-O-4 structures.[33] The assignment of the ^{13}C-NMR signals of DHPCC-WS is presented in Table 2.

Table 2
Assignment of ^{13}C-NMR signals of DHPCC-IS

No.	Assignment	δ, ppm	
		DHPCC-IS-[α-^{13}C]	DHPCC-IS-Cont.
1	γ-CHO of coniferyl aldehyde	194.7	194.6
2	α-CHO	191.4	191.3
3	-COO-(ferulic acid ester)	169.6	169.6
4	C3 of guaiacyl with α-CO, C3/C4 of guaiacyl with α-ether bond	156.4	—
5	Cα of coniferyl aldehyde	154.1	154.1
6	C3/C4 of guaiacyl	150.2	150.1
7	C3/C5 of etherified guaiacyl	147.9	147.7
8	C4 of non-etherified guaiacyl	146.1	146.1
9	etherified 5-5' C4/C4'	143.9	143.9
10	C1 of non-etherified guaiacyl in β-O-4	133.4	132.4
11	Cα of coniferol alcohol	130.7	130.3
12	Cα of coniferol alcohol	129.2	128.8
13	etherified guaiacyl with α-CO	126	126
14	C1 of p-hydroxyphenyl	123.4	124
15	C6 of guaiacyl	118.8	118.8
16	C5 of guaiacyl	115.4	115.5
17	C2 of guaiacyl	110.7	110.6
18	Cα connected with polysaccharides in acetal bond, C1 of cellulose	101.5	—
19	Cα(β-5)	87.4	87.3
20	Cα(β-β)	85.4	85.4
21-23	Cβ(β-O-4), Cα connected with polysaccharides in benyl ether bond	81.5-83.9	81.3-83.8
24	Cα of β-O-4 with α-CO groups	80.1	—
25	C3(non-reducing glucose)	76.1	76.1
26	Cα(β-O-4, threo type), C2, C3, C5 of cellulose	74.2	74.1
27	Cα(β-O-4, erythro type)	71.1-71.9	71.1
28	C4(non-reducing glucose)	70.2	70.5
29	Cγ(β-5), Cγ(β-O-4 with α-carbonyl)	63.4	63.3
30	Cγ(β-O-4), Cγ of coniferyl alcohol	61.9	61.9
31	Cγ(β-O-4)	60.3	60.2
32	-OCH3	56.3	56.3
33	Cβ(β-β)	53.9	53.8
34	unknown	45.1	45.2
35	DMSO-d6	40	40
36	-CH2-(G-CH2-CH2-CH2OH)	31.5	—
37	-CH2-(C5-CH2-C5)	29.1	29
38	-CH3(acetyl group)	20.9	20.9
39	unknown	18.7	18.7
40	unknown	15.3	15.3
41	CγH3	13.9	13.7

^{13}C NMR spectra of ^{13}C-enriched water soluble DHPCC (DHPCC-WS)

Fig. 5 shows the ^{13}C NMR spectra of the water-soluble DHPCCs prepared from [α-^{13}C]-labeled coniferin and unlabeled coniferin. The assignment of all signals is provided in Table 3. The signals at about 163.5-174.2 ppm were due to carbonyl groups[30] and the signals at about 126-136 ppm were assigned to the carbons in the benzene units in the DHP.[27] Signals No. 16 (88.7 ppm) and No. 17 (82.3 ppm) could be clearly observed and were assigned to the Cα of the α-O-4 units (Capanema *et al*.)[30] and the etherified C-α to carbohydrates. This indicated that the DHP from water-soluble DHPCC was linked to cellulose with a benzyl ether bond. The signal at

about 72.6 ppm (No. 18), which was assigned to the Cα of the *erythro* type β-O-4 units,[27,30] was increased by the ^{13}C-enrichment. A strong signal No. 16 (63.1 ppm) in the ^{13}C-labeled sample was assigned to the Cγ of the β-1 units.[33]

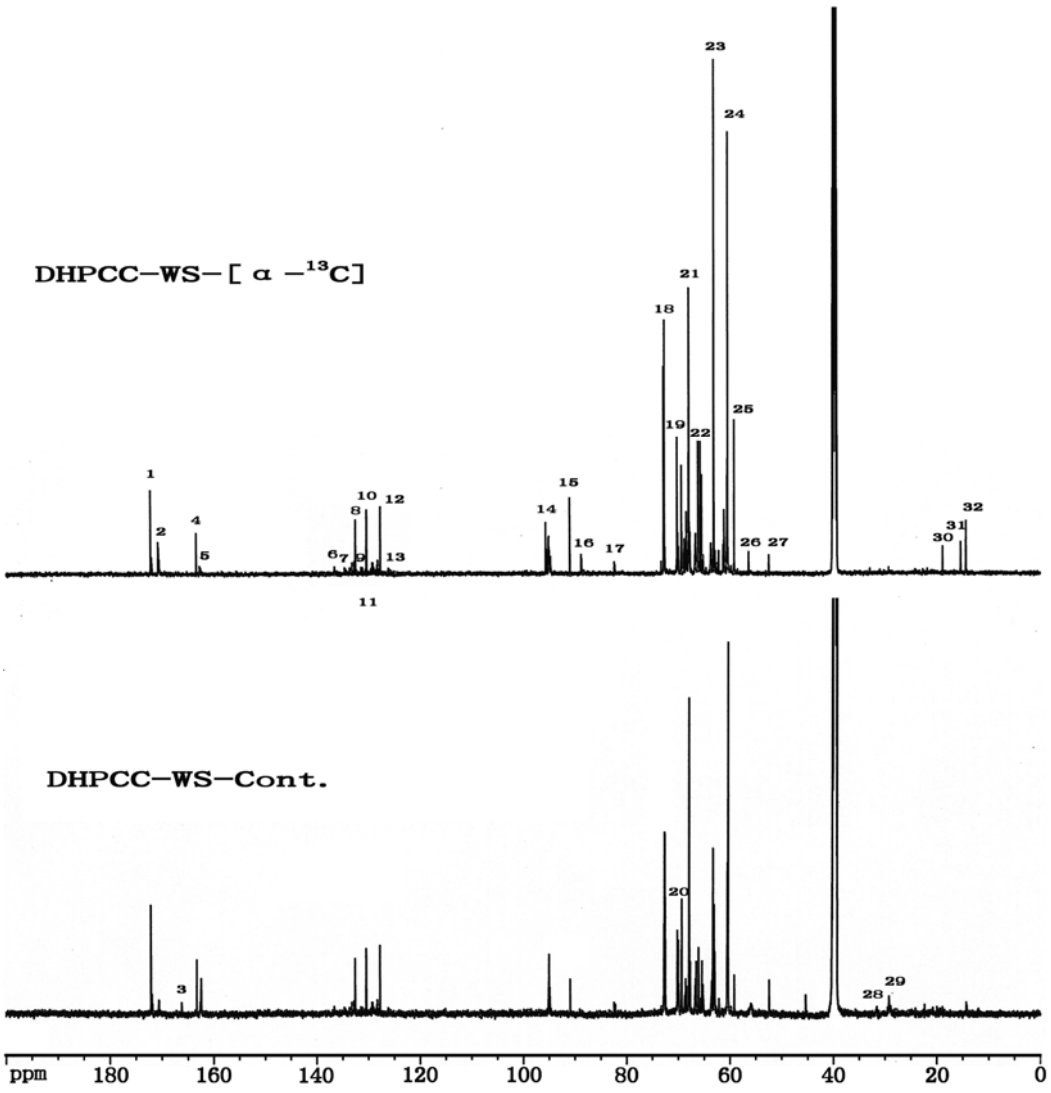


Figure 5: ^{13}C-NMR spectra of DHPCC-WS (water-soluble fractions of enzyme-degraded DHPCC)

Table 3
Assignment of ^{13}C-NMR signals of DHPCC-WS

No.	Assignment	δ, ppm	
		DHPCC-WS-[α-^{13}C]	DHPCC-WS-Cont.
1	-COO-(aliphatic acetyl group)	172.1	172.1
2	-COO-(aliphatic acetyl group)	170.5	170.5
3	C=O of aromatic acid	—	167.2
4	C=O of carboxy group	163.3	163.3
5	unknown	162.3	162.4
6	C1 of guaiacyl ring in etherified β-O-4	136.1	136.1
7	C1 of guaiacyl ring in non-etherified β-O-4	133.4	132.4
8	unknown	132.6	132.6

9	C1 of guaiacyl ring in non-etherified β-O-4	131.7	131.6
10	Cα of coniferyl alcohol	130.4	130.3
11	Cβ of coniferyl aldehyde, C2/C6 of p-hydroxyphenyl	128.3	128.2
12	C1 of ferulic acid	127.8	127.8
13	C6 of etherified guaiacyl with α-CO	126	126
14	unknown	95.1-95.6	95.1
15	C4 of cellulose	90.9	90.9
16	(α-O-4) Cα	88.7	—
17	Cα connected with polysaccharides in benzyl ether bond	82.3	82.3
18	Cα(β-O-4, erythro type)	72.6	72.6
19	C4(non-reducing glucose)	70.2	70.2
20	C5 of acetylated glucosyl	69.3	69.4
21	unknown	67.8	67.8
22	unknown	65.4-66.1	65.3-66.1
23	Cγ(β-1), Cγ of coniferyl alcohol	63.1	63.3
24	Cγ(β-O-4)	60.4	60.5
25	unknown	59.1	59.1
26	-OCH3	56.1	56
27	unknown	52.6	52.5
28	Cα of guaiacyl propane type	31.4	31.5
29	-CH2-(C5-CH2-C5)	29.1	29.2
30	unknown	18.8	18.6
31	unknown	15.4	—
32	$C\gamma H_3$	14.3	—

CONCLUSION

(1) DHP-cellulose complexes can be successfully obtained from dehydrogenation polymerization of coniferin-[side chain-(α-^{13}C)] and cellulose catalyzed by lignin peroxidase, glucose oxidase and β-glucosidase.

(2) Insoluble DHPCC and water-soluble DHPCC from the treatment of DHPCC with cellulase were lignin-rich and suitable for ^{13}C NMR analysis of the LCC linkages.

(3) ^{13}C NMR spectra showed that β-O-4 units were linked with cellulose at the α-position through benzyl ether bonds and acetal bonds. The carbon-13 isotopic tracer method provided detailed and reliable information about the chemical bonds between lignin and cellulose.

(4) The main substructures of the DHPCCs were found to be β-O-4, β-β, β-5, β-1 and coniferyl alcohol substructures.

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REFERENCES

[1] J. Y. Zhu, X. J. Pan and G. S. Wang *et al*., *Bioresource Technol*., **100**, 2411 (2009).
[2] M. E. Himmel, S. Y. Ding and D. K. Johnson *et al*., *Science*, **315**, 804 (2007).
[3] D. Ibarra, J. C. del Río and A. Gutiérrez *et al*., *J. Anal. Appl. Pyrol*., **74**, 116 (2005).
[4] N. N. Das, S. C. Das and A. S. Dutt *et al*., *Carbohyd. Res*., **94**, 73 (1981).
[5] N. Takahashi and T. Koshijima, *Wood Sci. Technol*., **22**, 231 (1988).
[6] Ö. Eriksson and B. O. Lindgren, *Sven Papperstidn*., **80**, 59 (1977).
[7] M. Balakshin, E. Capanema and H. Gracz *et al*., *Planta*, **233**, 1097 (2011).
[8] A. Isogai, A. Ishizu and J. Nakano, *J. Wood Chem. Technol*., **7**, 463 (1987b).
[9] O. Karlsson and U. Westermark, *J. Pulp Pap. Sci*., **22**, J397 (1996).
[10] M. Lawoko, G. Henriksson and G. Gellerstedt, *Holzforschung*, **57**, 69 (2003).
[11] H. T. Yang and Y. M. Xie, *Chem. Ind. Forest Prod*., **28**, 1 (2008).

[12] H. T. Yang, Y. M. Xie and J. Y. Fan *et al*., *Chem*. *Ind*. *Forest Prod*., **27**, 11 (2007).
[13] N. Terashima, R. H. Atalla and S. A. Ralph *et al*., *Holzforschung*, **49**, 521 (1995).
[14] N. Terashima, S. A. Ralph and L. L. Landucci, *Holzforschung*, **50**, 151 (1995).
[15] H. Boerstoel, H. Maatman and J. B. Westerink *et al*., *Polymer*, **42**, 7371 (2001).
[16] M. Pagliaro, *Carbohyd*. *Res*., **315**, 350 (1999).
[17] T. Masuko, A. Minami and N. Iwasaki *et al*., *Anal*. *Biochem*., **339**, 69 (2005).
[18] S. L. Shi, in "Analysis and Measurement of Pulp and Paper", edited by S. L. Shi and F. W. He, China Light Industry Press, 2008, pp. 49-51.
[19] B. Hortling, T. Tamminen and E. Kentä, *Holzforschung*, **51**, 405 (1997).
[20] N. B. Colthup, L. H. Daly and S. E. Wiberley, "Introduction to Infrared and Raman Spectroscopy", Academic Press Limited, San Diego, USA, 1990.
[21] F. Xu, J.-X. Sun and R. C. Sun *et al*., *Ind*. *Crop Prod*., **23**, 180 (2006).
[22] J. Dorado, J. A. Field and G. Almendros *et al*., *Appl*. *Microbiol*. *Biot*., **57**, 205 (2001).
[23] R. H. Atalla, J. C. Gast and D. W. Sindorf *et al*., *J*. *Am*. *Chem*. *Soc*., **102**, 3249 (1980).
[24] M. Bardet, M. F. Foray and Q. K. Tran, *Anal*. *Chem*., **74**, 4386 (2002).
[25] T. J. Bootten, P. J. Harris and L. D. Melton *et al*., *Carbohyd*. *Res*., **343**, 221 (2008).
[26] C.-M. Popescu, P. T. Larsson and C. Vasile, *Carbohyd*. *Polym*., **83**, 808 (2011).
[27] Y. M. Xie, S. Yasuda and H. Wu *et al*., *J*. *Wood Sci*., **46**, 130 (2000).
[28] N. Atichokudomchai, S. Varavinit and P. Chinachoti, *Carbohyd*. *Res*., **58**, 383 (2004).
[29] J. Ohnishi, T. Watanabe and T. Koshijima, *Photochemistry*, **31**, 1185 (1992).
[30] E. A. Capanema, M. Y. Balakshin and J. F. Kadla, *J*. *Agric*. *Food Chem*., **52**, 1850 (2004).
[31] R. Gu and Y. M. Xie, *Paper Science & Technology*, **20**, 1 (2001).
[32] R. Gu, Master Degree Thesis, South China University of Technology, 2002, pp. 44-47.
[33] H. T. Yang, Y. M. Xie and L. Yao *et al*., *Cellulose Chem*. *Technol*., **41**, 361 (2007).