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Original

Lignin-carbohydrate Complexes from *Albizia falcata* (L.) Back.*[1]

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Abstract——Lignin-carbohydrate complexes (LCCs) have been extracted from tropical hard wood of *Albizia falcata* (L.) Back. by a modified Björkman method. After extraction of milled wood lignin (MWL) from the hard wood, LCCs were isolated by successive extraction of the residual wood meal with water (LCC-WE) and 4% NaOH (LCC-AE). NMR and methylation analysis of the LCCs indicated that carbohydrate moiety of LCC-AE was composed of 4-*O*-methyl-glucuronoxylan while polyose component of LCC-WE consisted of glucomannan and xylan. Analysis of neutral fraction from LCC-WE revealed that this LCC contained β-1,4-linked xylan chain bearing no glucuronic acid residue. In addition, it has been clarified by DDQ-oxidation that the glucuronoxylan was directly bound to the lignin through C-2 and C-3 positions of xylose unit by ether linkage.

Keywords: Lignin-carbohydrate complex, *Albizia falcata*, DDQ.

Introduction

A tropical Leguminosae, *Albizia falcata* (L.) Back. is widely planted in Southeast Asia region, and utilized for raw materials of pulp, mouldings, furniture and woodenware. To expand usage of this fast growing wood to structural building materials, Subiyanto et al. processed *A. falcata* wood into particleboad, and reported its mechanical and dimentional properties[1]. Regarding chemical analysis of *A. falcata*, Yatagai *et al.* isolated α-spinasterol, auercetin, taxifolin and fustin from methanol extracts of the hard wood, in order to elucidate the origin for irritating action on human mucocus membranes by *A. falcata* saw dust[2].

Because *A. falcata* is a low-density and fast growing species, conversion of this wood to useful chemicals is attractive from a view point of effective utilization of tropical biomass. Standing on this view point, we analyzed chemical structure of polyose component in lignin-carbohydrate complexes (LCCs) obtained from the hard wood. Furthermore, chemical linkages between lignin and the polyoses are analyzed using oxidative cleavage of benzyl

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ether linkages with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[3,4].

2. Experimental

2.1 General methods

Carbon-13 NMR spectra of LCCs were recorded on a Varian XL-200 Spectrometer (50.3 MHz) in D_2O at room temperature. Chemical shifts were measured relative to internal 1,4-dioxane (67.4 ppm downfield from TMS). Carbon-13 NMR spectra of lignin were recorded in DMSO-d_6 at 50°C. Signals were assigned on the basis of references by BARDET *et al.*[5,6] Methylatlon analysis was done by the method of HAKOMORI[7]. Each methylated sugar was converted to alditol acetates and anlyzed by GC and GC-MS on capillary columns of (a) Ulbon HR-SS-10 and (b) Silicon OV-225. Lignin content was determined by acetyl bromide method[8]. Uronic acid content was determined by *m*-hydroxybiphenyl method[9]. UV spectra were measured in 50% dioxane (LCC-AE, LCC-SW and LCC-WE) or in water (C-1-M and C-1-A). Specific rotation was determined in 10% NaOH (LCC-AE, C-1-A and LCC-WE), water (C-1-M) or 50% aqueous dioxane (LCC-SW), respectively.

2.2 Isolation of Lignin-carbohydrate complexes

Albizia wood (*Albizia falcata* (L.) Back.) obtained in Bandung, Indonesia, was extracted stepwisely with an ethyl alcohol-benzene mixture (1 : 2, v/v), and 0.25% aqueous potassium acetate according to the method described before[10]. The wood meal was dried and then vibromilled for 48 hr under nitrogen atmosphare with external cooling by tap water. Crude milled wood lignin (MWL) fraction was extracted twice from the wood meal with 80% aqueous dioxane for 48 hr at room temperature. Ligni-rich LCC fraction (LCC-SW) was separated from the extract according to the method reported by AZUMA[11]. Purification of MWL was done by the method of Björkman[12]. LCCs were extracted from the wood residue with cold water (20°C) for 12 hr and then with hot water (80°C) for 5 hr. Both extracts were combined, concentrated to a small volume and precipitated from 5 times its volume of ethyl alcohol to give a water-soluble LCC (LCC-WE). LCC-WE was then fractionated into neutral (C-1-M) and acidic (C-1-A) subfractions by anion-exchange chromatography on DEAE-Sephadex A-50 (CO_3^{2-} form). An alkali-soluble LCC fraction was extracted from the wood residue with 4% NaOH for 5 hr. The extract was neutralized with acetic acid, dialyzed, concentrated and precipitated with 5 volumes of ethyl alcohol to give LCC-AE.

2.3 Enzymatic degradation of LCCs

LCC-AE was hydrolyzed at 45°C for 72 hr at a substrate concentration of 1% and the enzyme concentration of 0.1% with a mixture of two commercial cellulases, Cellulosin AC-40 (*Aspergillus niger*, Ueda Kagaku Kogyo Co. Ltd.) and Meicellase (*Trichoderma viride*, Meiji Seika Co. Ltd.) which had been purified by gel filtration on Bio-Gel P-2. The enzymatic

hydrolyzates were heated in a boiling water bath to inactivate the enzymes, and then centrifuged to separate water-soluble and insoluble materials. The precipitate was washed 5 times with distilled water. Enzyme-treated LCC fragments (AE-F) were obtained from the precipitate by extraction with 50% aqueous dioxane for 48 hr.

2.4 Binding site analysis of LCCs

The enzyme-treated LCC fragments (AE-F) were acetylated with pyridine and acetic anhydride at 40°C for 18 hr. The acetylated LCC fragments were then oxidized with DDQ in a refluxing dichloromethane-water mixture (18 : 1, v/v) for 2 hr[3,4]. After reaction, the reaction mixture was partitioned between chloroform and water ten times and the organic layer was separated and passed through a sodium sulfate column, evaporated below 30°C and dried *in vacuo*. The DDQ-oxidized LCC fragments were methylated for 3 hr with methyl trifluoromethanesulfonate according to the Prehm method[13]. The methylated sample was hydrolyzed with 2 M trifluoroacetic acid at 100°C for 3 hr in a sealed tube. The hydrolyzates were evaporated and then converted to alditol acetates and analyzed by GC-MS and GC on capillary columns coated with (a) Ulbon HR-SS-10 and (b) Silicon OV-225 at 210°C.

3. Results and Discussion

We reported previouly that a large amount of water-soluble lignin-carbohydrate complexes could be extracted from finely divided wood meal of *Pinus densiflora,* and that chemical properties of the water-soluble LCCs[10] were similar to those of Björkman LCC[14,15]. In this paper, we applied this method to a tropical hard wood of *Albizia falcata.*

Albizia falcata was treated with a refluxing ethyl alcohol-benzen mixture and then with 0.25% potassium acetate to remove wood extractives and pectic substances, respectively. The wood meal was dried and then vibromilled for 48 hr under nitrogen atomosphare. Crude milled wood lignin (MWL) fraction was extracted from the finely divided wood meal with 80% aqueous dioxane at room temperature. The extract was separated to lignin-rich LCC fraction and MWL by dialysis and centrifugation. The MWL fraction obtained was further purified by Björkman method[12].

Water-soluble LCC (LCC-WE) was obtained from the residual wood meal by extraction with water at 20°C and 80°C. Both LCC extracts were combined and fractionated into neutral and acidic subfractions by anion-exchange chromatography on DEAE-Sephadex A-50 (Fig. 1). As shown in Table 1, yield of LCC-WE was 3.3% based on the weight of wood meal previously extracted with 80% aqueous dioxane. The yield of LCC-WE from *Albizia* wood was about one third, compared with the result of *Pinus densiflora* reported previously[10]. This is because main component of hemicelluloses from *Albizia falcata* is glucuronoxylan, while that of *Pinus densiflora* consists of water-soluble acetylglucomannan. Because yield of LCC-WE was lower, compared with the case of *Pinus*

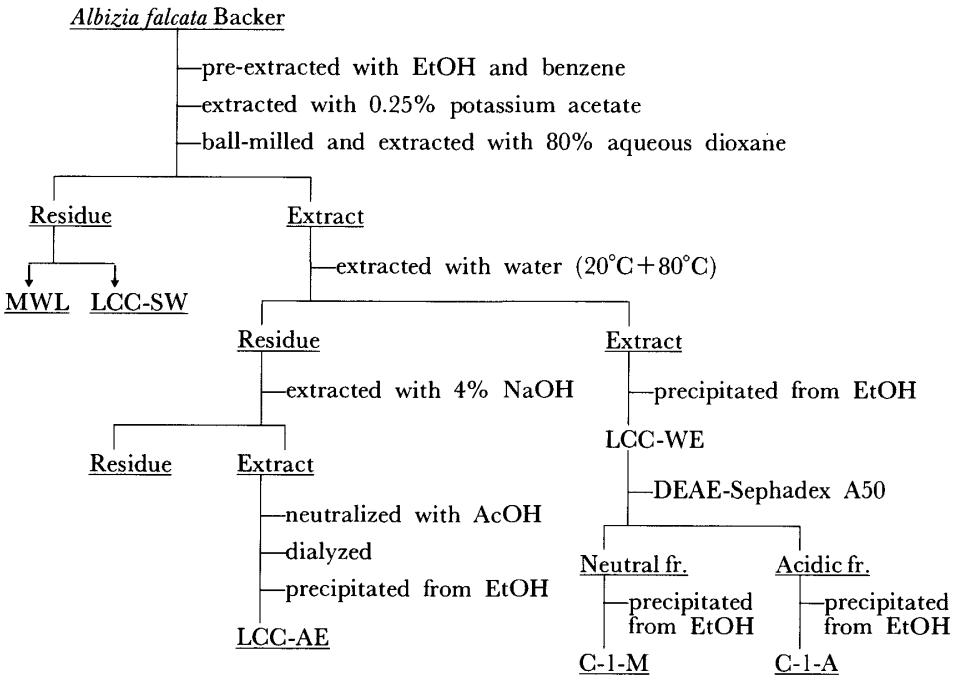

Fig. 1. Preparation of *Albizia* LCCs.

Table 1. Chemical composition and properties of *Albizia* LCCs.

Components	Lignin-carbohydrate complexes				
	LCC-SW	LCC-AE	LCC-WE	C-1-M	C-1-A
Recovery (%)	2.6[1]	8.8[1]	3.3[1]	12.5[2]	24.8[2]
Carbohydrate content (%)					
Neutral surgar	62.3	75.0	78.3	94.8	73.8
Uronic acid	14.7	14.7	11.9	0.0	19.2
Lignin content (%)	22.5	8.5	5.8	2.2	6.4
$[\alpha]_D^{20}$	−19.0°	−53.5°	−15.0°	−5.1°	−26.0°
a_{280} ($l \cdot g^{-1} \cdot cm^{-1}$)	3.90	1.39	0.71	0.08	0.76
a_{280}/a_{260}	1.26	1.02	1.13	1.14	1.09

1 : Values are expressed as weight percetages of the wood meal extracted with 80% aqueous dioxane. 2 : Values are expressed as weight percentages of LCC-WE.

densiflora, we extracted alkali-soluble LCC fraction from the residual wood with 4% NaOH. As a result, alkali-soluble LCC fraction could be extracted in a yield of 8.8%. Chemical properties of these LCC fractions are summarized in Table 1. Neutral sugar compositions are shown separately in Table 2. The neutral sugar composition of LCC-AE suggests that carbohydrate moiety of this LCC fraction consists of glucuronoxylan. Lignin content of this fraction was 8.5% (Table 1, Fig. 2). To analyze carbohydrate moiety of *Albizia* LCCs, methylation analysis was carried out (Table 3). In the methylation analysis of LCC-AE, LCC-WE and C-1-M, 2,3-di-*O*-methyl-xylose was detected as internal and reducing-end units of 1,4-linked xylan chain. Non-

Table 2. Neutral sugar composition of *Albizia* LCCs.

Monosaccharides	Lignin-carbohydrate complexes				
	LCC-SW	LCC-AE	LCC-WE	C-1-M	C-1-A
L-Arabinose	0.7	0.6	1.9	0.7	2.3
D-Xylose	94.9	94.4	48.0	18.6	79.6
D-Mannose	1.0	1.3	23.2	38.3	4.5
D-Galactose	1.2	0.8	5.1	1.U	7.8
D-Glucose	2.2	2.9	21.8	40.7	5.8

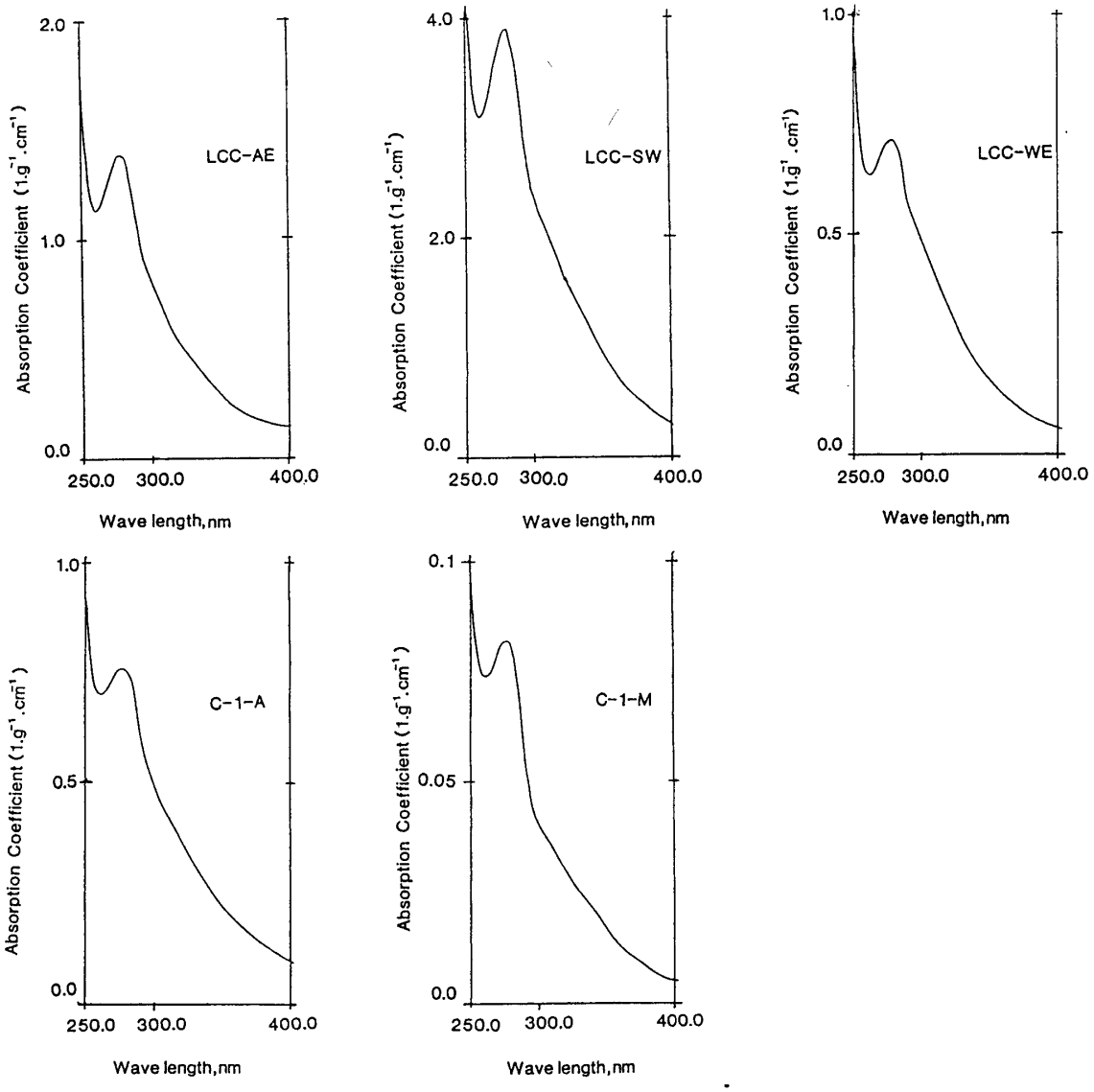

Fig. 2. UV spectra of *Albizia* LCCs.

Table 3. Methylation analysis of *Albizia* LCCs.

Methylated sugars (as alditol acetate)	T[2]	Lignin-carbohydrate complexes		
		LCC-AE	LCC-WE	C-1-M
2,3,5-Ara[1]	0.543	trace	1.0	0.1
2,3,4-Xyl	0.699	1.3	0.8	0.4
2,3,4,6-Man+2,3,4,6-Glc	1.000	0.1	3.4	11.9
2,3,4,6-Gal	1.198	0.1	0.9	trace
2,3-Xyl	1.421	93.7	47.2	19.9
2,3,6-Man	1.921	0.1	23.3	25.7
2,3,6-Gal	2.139	0.1	4.9	5.3
2,3,6-Glc	2.291	2.1	18.5	36.6
2-Xyl+3-Xyl	2.405	2.5	trace	0.1

1 : 2,3,6-Man=1,4,5-*O*-acetyl-2,3,6-tri-*O*-methyl-D-mannitol, etc. Values are expressed as relative molar percentages of the total partially methylated sugars identified. 2 : Retention time relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, on column ULBON HR-SS-10, isothermal at 210°C.

reducing end unit of the xylan chain was also identified as 2,3,4-tri-*O*-methyl xylose. The percentage of both methyl ethers was more than 96% in the fraction of LCC-AE. Degree of polymerization (DP) of the xylan main chain is calculated to be 72. On the other hand, the existence of glucomannan in LCC-WE and C-1-M was confirmed by 2,3,6-tri-*O*-methyl glucose, 2,3,6-tri-*O*-methyl mannose, 2,3,4,6-tetra-*O*-methyl glucose and 2,3,4,6-tetra-*O*-methyl mannose. Carbon-13 NMR spectra of the LCCs supported this structure. As shown in the methylation analysis, all of the main signals in the spectrum of LCC-AE were assigned as carbon atoms from 4-*O*-methylglucuronoxylan[16,17]. No other polysaccharides were detected below background noise. On the other hand, signals ascribed to acetylglucomannan[18] was detected as major component in the spectrum of LCC-WE. Furthermore, as indicated in the methylation analysis, β-1,4-linked xylan unit was detected in the neutral LCC fraction (Fig. 3). Because uronic acid could not be detected by *m*-hydroxybiphenyl method, it is evident that *Albizia falcata* contains neutral xylan chain as one of its hemicellulose comopnent.

Because a part of lignin is soluble in alkaline solution, we examined whether the glucuronoxylan is directly bound to the lignin in the fraction of LCC-AE. To achieve this, we degraded carbohydrate component of LCC-AE with a mixture of cellulases from *Trichoderma viride* and *Aspergillus niger,* and then the cellulase-degraded LCC fragments (AE-F) were isolated by extraction with 50%aqueous dioxane. Yield of the LCC fragments was 9.4% based on the weight of LCC-AE. Carbon-13 NMR spectrum of AE-F revealed that the alkali-soluble LCC fraction contains typical hard wood lignin which is composed of both guaiacyl and syringyl nuclei (Fig. 4). To examine chemical bonds between the lignin and glucuronoxylan, AE-F was then subjected to the binding site analysis using DDQ-oxidation. Namely, AE-F was (1) acetylated, (2) DDQ-oxidized, (3) methylated by Prehm method, (4) hydrolyzed (5) converted to alditol

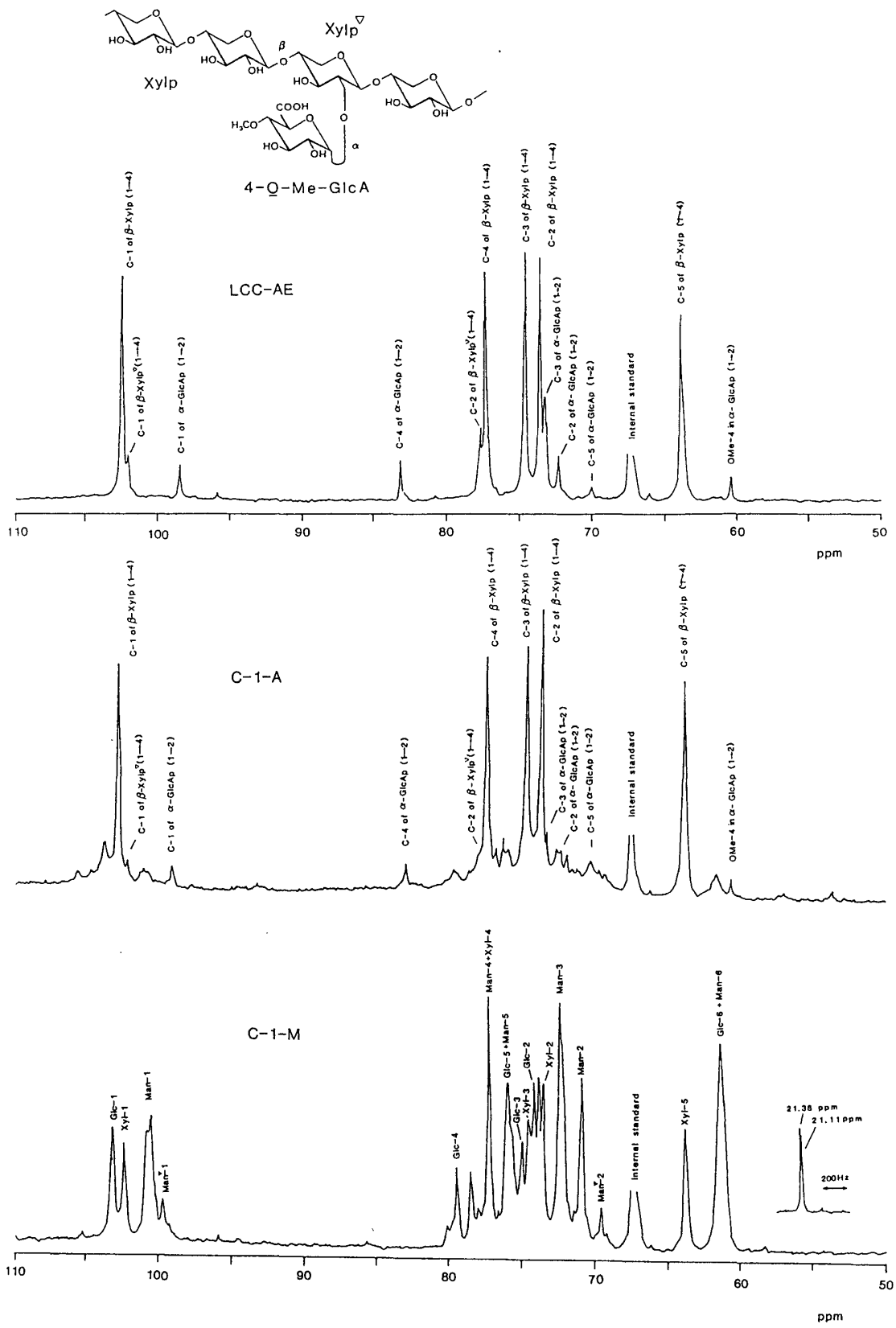

Fig. 3. Carbon-13 NMR spectra of *Albizia* LCCs.

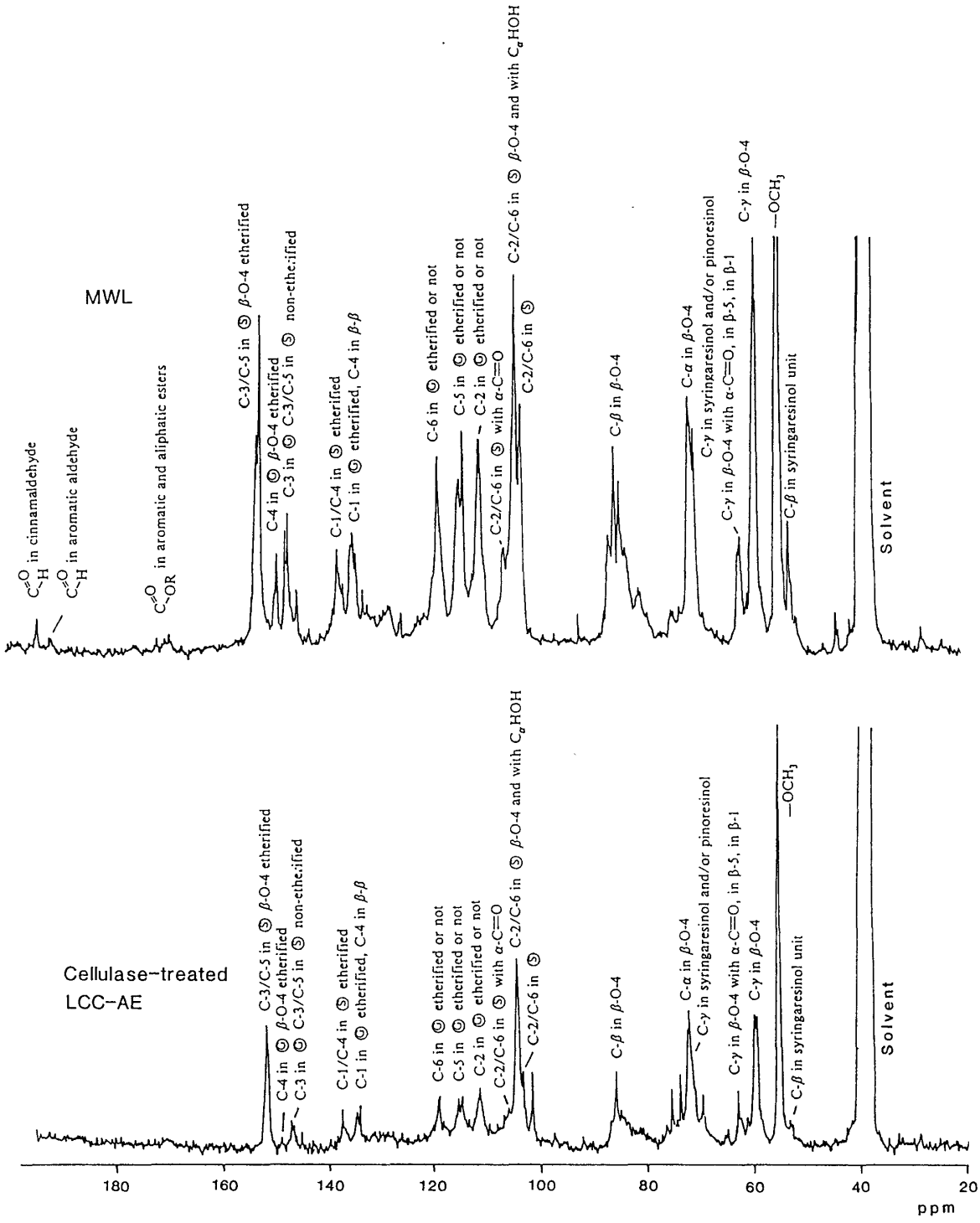

Fig. 4. Carbon-13 NMR spectra of MWL and cellulase-treated LCC fragments from *Albizia falcata*.

acetates and then analyzed. As a result, 2-*O*-methyl and 3-*O*-methyl xylose were detected (Fig. 5). Because it has been confirmed that DDQ does not decompose glycosidic bonds between sugar residues but cleave benzyl ether bonds between sugar and α-position of

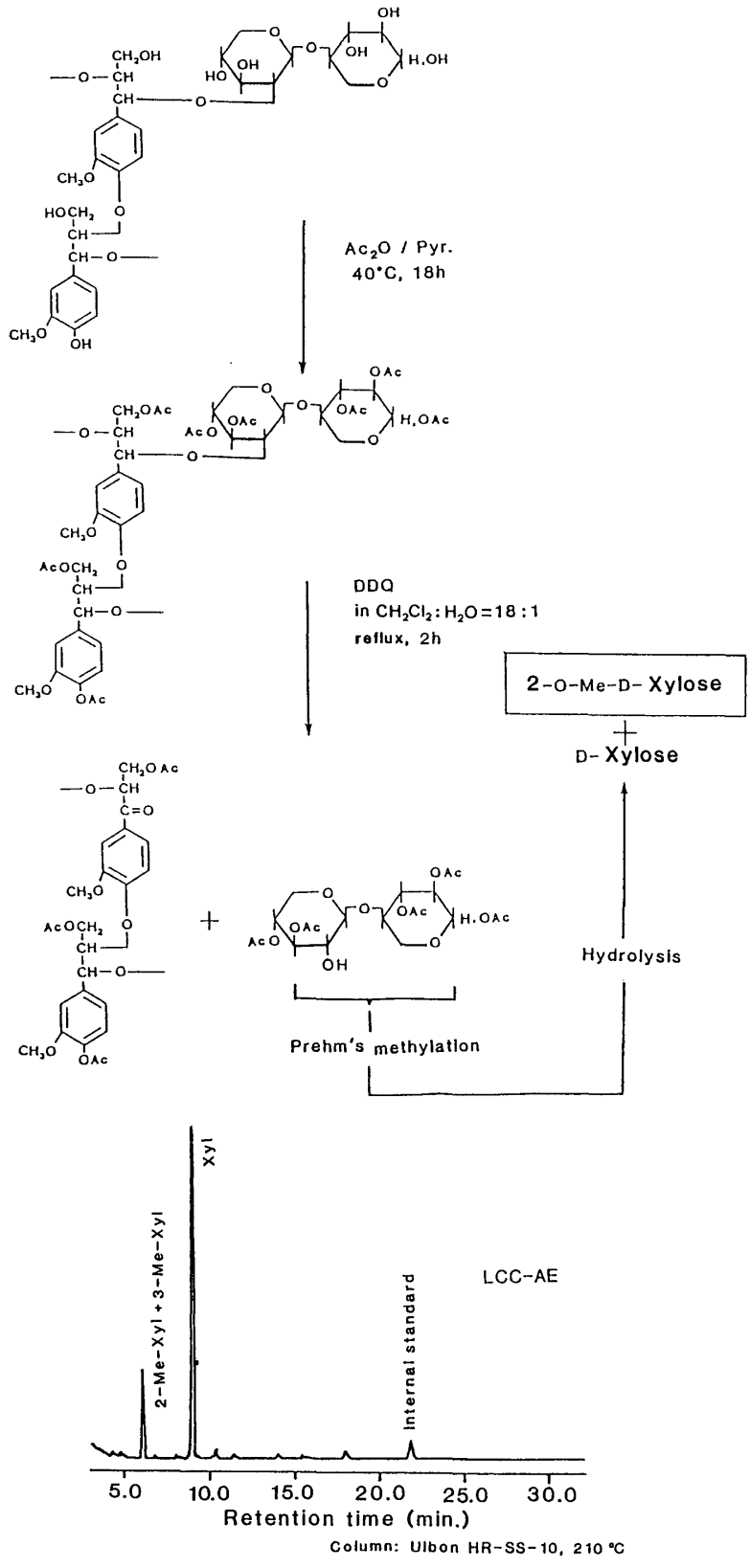

Fig. 5. Binding site analysis of LCC-AE from *Albizia falcata*.

lignin[3,4], it is evident that the glucuronoxylan was directly bound to the lignin by the ether bonds in *Albizia falcata* wood. This result is consistent with our previous report that ether bonds were formed between xylan and DHP during dehydrogenative polymerization of coniferyl alcohol in the presence of the xylan *in vitro*[19].

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