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Binding Sites of Carbohydrate Moieties toward Lignin in "Lignin-Carbohydrate Complex" from Pinus densiflora Wood

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Partially enzyme-degraded lignin-carbohydrate complex (LCC) was subjected to acetylation, followed by oxidation with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ), which is comfirmed to decompose α- and conjugated γ-ether to release the corresponding alcohol. Methylation analysis of the liberated sugar acetates showed that glucomannan preferably binds to lignin at C-6 position of mannose and glucose residues and that arabinoglucuronoxylan at C-2 and C-3 position of xylose.

Methylation analysis of carbohydrates has been widely utilized to elucidate the fine structure of lignin-carbohydrate complex (LCC).[1-3] But until now, little attention has been given to the cleavage of alkali-labile lignin-carbohydrate linkages. The most probable mode of linkages, benzyl ethers don't withstand in a strong nucleophilic media where current methylation analysis are conducted. For example, the powerful Hakomori-methylation technique destroys all of alkali-labile linkages that must be important in the over all architecture of LCC. We now present a new analytical method effective for both alkali-labile and acid-sensitive lignin-carbohydrate linkages by applying the methylation with methyl trifluoromethanesulfonate , in conjunction with oxidative cleavage of lignin-carbohydrate linkages by the action of DDQ.

The finely divided wood meal (Pinus densiflora Sieb. et Zucc.), previously extracted with ethanol and benzene mixture (1:2, v/v) and depectinated with potassium acetate, was treated with 80% aqueous dioxane for 48 h at room temperature. The residual wood meal was further extracted with cold water (20 ℃) and then hot water (80 ℃) for 17 hr in total to give LCC-WE. LCC-WE was then fractionated into neutral (C-1-M) and acidic fractions (C-1-A) through DEAE-sephadex A-50 (carbonate form) by using distilled water and 1 M ammonium carbonate as eluents, respectively. C-1-A was subjected to enzymatic hydrolysis by using two types of cellulase preparations which were purified from Cellulosin AC (Aspergillus niger, Ueda Kagaku Kogyo Co., Ltd.) and Meicelase (Trichoderma viride, Meijiseika Co., Ltd.) by means of salting-out from aqueous ammonium sulfate and gel filtration on Bio-Gel P-2. The enzymatic hydrolysis of C-1-A was carried out sequentially at

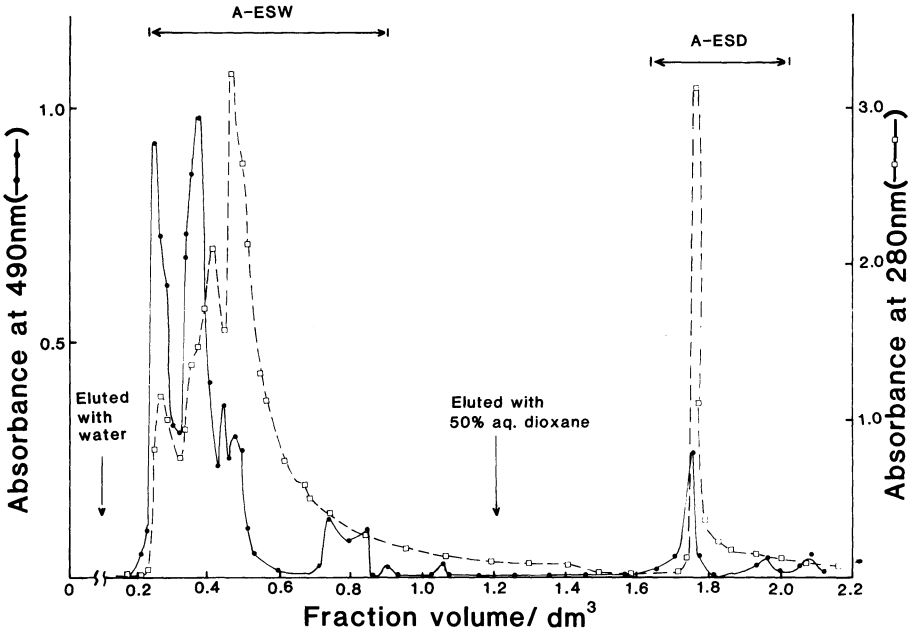
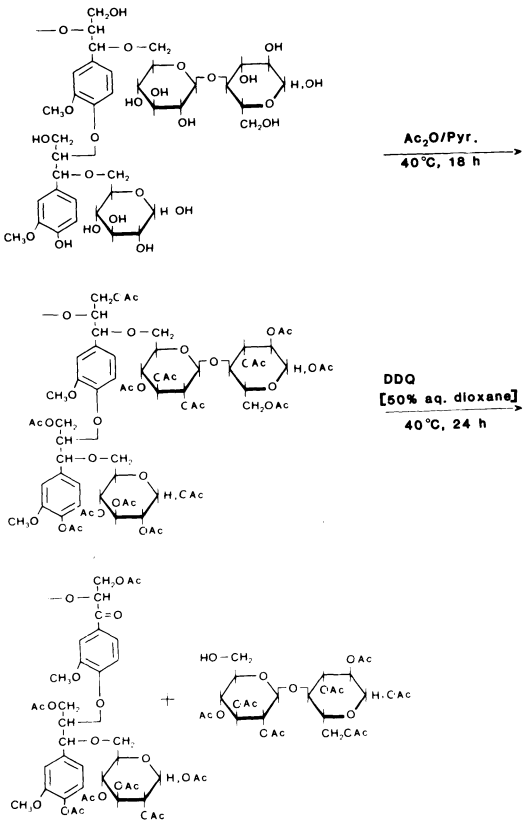


Fig. 1. Adsorption chromatogram of enzymatic hydrolyzate of C-1-A on Toyopearl HW-40 S column.



Scheme 1. Removal of carbohydrate moiety by the action of DDQ.

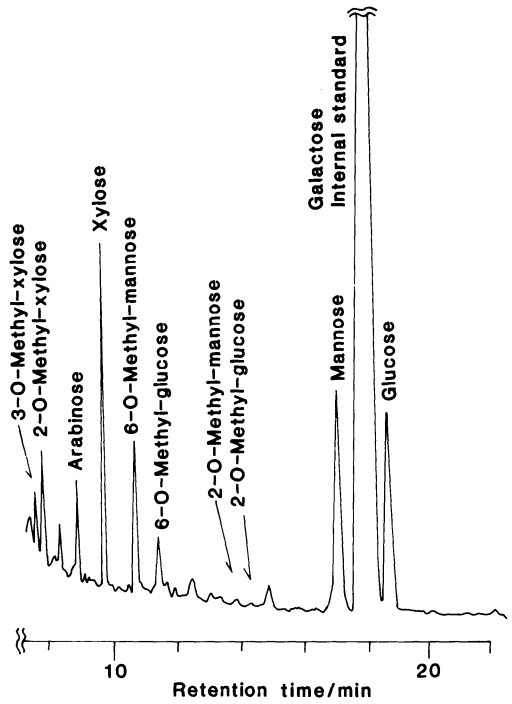


Fig. 2. Gas-liquid-chromatogram of methylated sugars released from A-ESD acetylated, DDQ-decomposed, and methylated successively.

40 ℃ for 72 h in each in 0.1 M sodium acetate buffer (pH 4.8). The hydrolyzate was boiled for 5 min, dialyzed against distilled water, and then centrifuged to remove water-insoluble materials. LCC fraction (A-ESD) was isolated from the supernatant by adsorption chromatography on Toyopearl HW-40 S (4 cm x 45 cm) according to the method described before.[4] The elution profile was shown in Fig. 1. Chemical analysis of the fragments showed that A-ESD contained 8.2% of neutral carbohydrates, consisting 7.5% L-arabinose, 17.6% D-xylose, 48.7% D-mannose, 7.7% D-galactose, and 18.6% D-glucose in rerative mole percent.

Location and frequency of binding sites of carbohydrates to lignin was analyzed by using the following new approach. The LCC fraction A-ESD was acetylated with acetic anhydride and pyridine at 40 ℃ for 18 h in order to protect hydroxyl group. The fully acetylated A-ESD was then treated at 40 ℃ for 24 h in 50% aqueous dioxane with an equal amount of DDQ, which is inert to acetyl group[5] and glycosidic linkages between saccharides,[6] while oxidatively splits off both benzyl ether[7] and conjugated γ-ether attached to an electron-donative benzene skeleton. As shown in Scheme 1, carbohydrates linked to α-position of etherified guiacyl nuclei of Pine lignin must be released by the action of DDQ, while those linked to p-acetoxyl lignin units cannot be released due to the electron-withdrawing inductive effects of the acetyl group. Provided that we can mark the hydroxyl group of carbohydrates derived from the cleaved lignin-carbohydrate linkages, binding sites of carbohydrates to lignin could be analyzed. Based on above views, we tried the methylation analysis of carbohydrates released after the DDQ-decomposition.

After the DDQ-oxidation was terminated by addition of ascorbic acid, carbohydrates released were methylated with methyl trifluolomethanesulfonate at 50 ℃ for 3 h according to the Prehm's methylation technique.[8] The methylated carbohydrates were hydrolyzed, and then converted to alditol acetate form. The resulting partially methylated alditol acetates were separated by GLC using a capillary column (25 m x 0.28 mm) coated with SP-1000 at 210 ℃. Component sugars corresponding to each peak were identified by their retention time and by GLC-MS using the fragmentometry technique at m/z 45, 59, 89, 117, 131, 161, 175, 189, 203, 205, 217, 233, 261, 305, and 333. The gas-liquid chromatogram of the methyl ethers are shown in Fig. 2. Methylation analysis of A-ESD predominantly gave 1,2,3,4,5-penta-O-acetyl-6-O-methyl mannitol and 1,2,3,4,5-penta-O-acetyl-6-O-methyl glucitol in the ratio of 2:1 (Table 1). 1,3,4,5-Tetra-O-acetyl-2-O-methyl xylitol and 1,2,4,5-tetra-O-acetyl-3-O-methyl xylitol were also identified as main components of methylated pentoses probably originating from arabinoglucuronoxylan-lignin complex. Linkages at C-2 in mannose and glucose residues with lignin moiety were substantiated based on retention time and mass-fragmentometry at m/z 117. These results demonstrate that glucomannan binds to lignin predominantly at C-6 and slightly at C-2 positions of glucomannan main chain and that arabinoglucuronoxylan at C-2 and C-3 positions of xylose main chain through α- or γ-carbon atoms of etherified guiacylpropane units of the lignin moiety.

Table 1. Methyl ether of monosaccharides from the hydrolyzate of methylated A-ESD

Methylated sugars	Mol% a)
2-Xylose	9.2
3-Xylose	24.2
Total xylose	33.4
2-Mannose	2.8
6-Mannose	41.6
Total mannose	44.4
2-Glucose	2.0
6-Glucose	20.3
Total glucose	22.3

a) Based on total methylated sugar components identified.

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