CHEMISTRY LETTERS, pp. 1737 - 1740, 1984.

EXISTENCE OF BENZYLATED CARBOHYDRATE MOIETY IN LIGNIN-CARBOHYDRATE COMPLEX FROM PINE WOOD

Tetsuo KOSHIJIMA, Takashi WATANABE, and Jun-ichi AZUMA*
Section of Wood Chemistry, Wood Research Institute,
Kyoto University, Gokasho, Uji, Kyoto 611

Using the reaction of 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) which acts on O-methoxybenzyl ether to liberate alcohol quantitatively, a part of sugar moieties contained in conifer lignin-carbohydrate complex (LCC) was confirmed to link to lignin moiety at α-carbon of phenylpropane units through benzyl ether bond.

Recently, the formation of lignin-carbohydrate linkages through quinone methide intermediates has been confirmed by Leary[1] who demonstrated that the etherification of vanillyl alcohol with sugars proceeds at any of their free hydroxyl groups even in neutral aqueous solution. The similar synthetic reactions to provide O-hydroxybenzyl ethers and esters have been reported in this decade.[2-4] However, none of them have determined the linkage types of the lignin-carbohydrate bonds on the actually isolated lignin-carbohydrate complex (LCC). Oikawa et al.[5] reported in 1982 that 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) reacts with 4-methoxybenzyl and 3,4-dimethoxybenzyl ethers selectively at room temperature to give alcohols. Provided that the cleavage is specific to the O-methoxybenzyl ether linkages, it would be capable to give direct evidence of the existence of benzylic lignin-carbohydrate linkages in LCC. Seven LCC model compounds (1-7) were synthesized to determine the optimum conditions for liberating carbohydrate component by treatment with DDQ in 50% aqueous dioxane which was thought to be the most suitable solvent for LCC. Using a model compound (1), quantitative recovery of methyl β-D-glucopyranoside was obtained under the condition (b) in Table 1. The quantities of monosaccharides released from the LCC models by reacting with DDQ under the condition (b) were listed in Table 2. It is obvious from the tables that model compounds having benzyl ether-linked sugar reacts with DDQ to give any of monosaccharides but not at all from others. Since disaccharides (8 and 9) could be quantitatively recovered after DDQ treatment, glycosidic linkages between monosaccharides were concluded to be inert to this treatment and oxidation of carbohydrates suggested to be caused by treatment with DDQ in anhydrous dioxane under reflux for 72 h[6] did not operate under the present conditions.

Although a large amount of LCC is needed for characterization of linkages between lignin and carbohydrate because of the limitations of the frequency of these linkages, the previous LCC preparation methods[7-9] suffered from the time consuming and rather tedious operations. To overcome these defects, we now

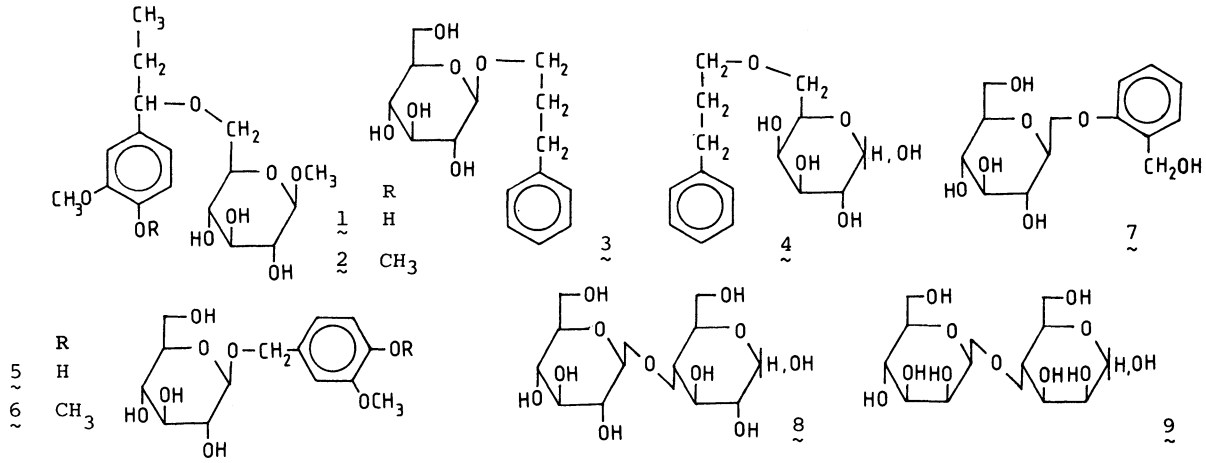

Table 1. Reaction of DDQ with methyl 6-O-(1'-guaiacyl-propan-1'-yl)-β-D-glucopyranoside (1)

Condition	Solvent	Reaction time / h	Temperature °C	Molar ratio of DDQ to 1	Methyl β-D-glucoside released (%)[a]
(a)	50% aq. dioxane	1	50	2	99.9
(b)	"	24	40	2	98.7
(c)	"	1	40	2	40.8
(d)	"	1	40	1	31.2
(e)	"	1	25	2	25.7
(f)	methanol	1	25	2	45.0

a) Weight percent of methyl β-D-glucopyranoside originally present in 1.

Table 2. Amount of monosaccharides released from LCC model compounds and disaccharides by the action of DDQ under the condition (b) in Table 1.

Model compound	1	2	3	4	5	6	7	8	9
Monosaccharide released (%)[a]	98.7	0.2	0	0	68.3	38.4	0	0	0

a) Weight percent of methyl β-D-glucopyranoside, galactose, mannose and glucose originally present in (1, 2), 4, 9, and (3, 5-8).

present a new convenient method for the isolation of a large amount of LCC. The finely divided AKAMATSU (Pinus densiflora S. and Z.) wood, previously extracted with ethanol-benzene (1 : 2, v/v) and depectinated with 0.25% aqueous potassium acetate, was treated twice with 80% aqueous dioxane for 48 h at room temperature. The extracts were combined and dialyzed against distilled water to remove lignin. The aqueous part was freed from residual lignin by extraction with chloroform to give LCC-SW. The residual wood meal was further extracted with distilled water and then with hot water (80 °C) for 17 h in total. The extractives were combined and precipitated with 5 vol. of ethanol to give LCC-WE. The yields of LCC-SW and LCC-WE were 2.9% and 9.3% of the depectinated wood meal, respectively. The neutral fraction (C-I-M) of LCC-WE was isolated by ion-exchange chromatography on DEAE-Sephadex A-50 (carbonate form). Both LCC-SW and C-I-M were subjected to enzymatic hydrolysis at 40 °C for 48 h by using a cellulase preparation (Meicellase CEPB-5042 from Trichoderma viride, Meijiseika Co., Ltd.) at substrate and enzyme concentrations of 2.5% and 0.25%, respectively. This enzyme preparation contained various activities of hemicellulases together with cellulolytic activities,[10] and splitted completely the benzyl glycosidic linkages of 5 and 6. The water soluble portion was extracted with methanol. The methanol-soluble fraction was applied to a column of Toyopearl HW-40SF. After the unadsorbed materials were throughly washed out with distilled water, the adsorbed LCC-oligomers were eluted with 50% aqueous dioxane to give two different oligomers; SW-ED from LCC-SW and M-SD from C-I-M. The chemical compositions of these LCC oligomers are: 60.2% lignin, 40.3% neutral sugars consisting of 75.6% D-mannose, 4.2% D-galactose, and 20.2% D-glucose in relative weight percent, 1.43% phenolic hydroxyl and no uronic acid in M-SD; 67.8% lignin, 27.1% neutral sugars consisting of 6.3% L-arabinose, 16.9% D-xylose, 54.2% D-mannose, 2.0% D-galactose and 20.7% D-glucose in relative weight percent, 1.8% phenolic hydroxyl and 1.7% uronic acid in SW-ED.

LCC-oligomers (50.2 mg of M-SD and 43.6 mg of SW-ED) solubilized in 10.0 ml of 50% aqueous dioxane were allowed to react with equal amount of DDQ under the condition (b) in Table 1. Gel-filtration chromatography on Bio-gel P-2 column was conducted with DDQ-treated products of M-SD and SW-ED using distilled water as an eluant. Table 3 indicates release of monosaccharides from both LCC-oligomers: D-mannose and D-glucose are predominant in M-SD, while D-glucose and L-arabinose are preferential in SW-ED. In addition, (1→4)-β-D-manno-oligosaccharides with degree of polymerization of 2-6 were also detected in both cases of M-SD and SW-ED.

These results show an unambiguous evidence of existence of benzylated sugar components in soft wood LCC, which link to α-carbon of guiacylpropane unit probably with primary hydroxyl at C-6 in hexopyranose or C-5 in pentofuranose in the mode of ether linkages. Ether linkage of this type is, however, not considered in the case of D-xylopyranose, which remains undissolved question. From the reaction with model compounds used here, splitting-off of the sugar components attached to guiacylpropane with free phenolic hydroxyl seem to be favorable, so most of sugars liberated from the LCC would be possibly link to such type of lignin structure in LCC.

Table 3. Monosaccharides liberated from the LCC-oligomers by the action of DDQ

Component	LCC-oligomers	
	M-SD	SW-ED
Total neutral monosaccharides (%)[a]	0.55	1.44
Neutral sugar composition (%)[b]		
L-Arabinose	0	26.4
D-Xylose	0	19.1
D-Mannose	50.9	14.8
D-Galactose	4.1	3.9
D-Glucose	45.0	35.7

a) Weight percent of original LCC-oligomers.
b) Relative weight percent.

References

1) G. J. Leary, D. A. Sawtell, and H. Wong, Holzforschung, 37, 11 (1983).
2) Y. Katayama, N. Morohoshi, and T. Haraguchi, Mokuzai Gakkaishi, 26, 414 (1980).
3) M. Tanahashi and T. Higuchi, Proc. 26th Symp. Lignin Chem. (Japan), 1981, 29.
4) K. Tanaka, F. Nakatsubo, and T. Higuchi, Mokuzai Gakkaishi, 22, 589 (1976).
5) Y. Oikawa, T. Yoshida, and O. Yonemitsu, Tetrahedron Lett., 23, 885 (1982).
6) J. Gierer and I. Norén, Holzforschung, 36, 123 (1982).
7) A. Björkman, Svensk Papperstidn., 60, 329 (1957).
8) F. Yaku, Y. Yamada, and T. Koshijima, Holzforschung, 30, 148 (1976).
9) J. Azuma, N. Takahashi, and T. Koshijima, Carbohydr. Res., 93, 91 (1981).
10) J. Azuma, F. Tanaka, and T. Koshijima, Wood Res., 69, 22 (1983).

(Received July 24, 1984)