Ester linkages between lignin and glucuronoxylan in a lignin-carbohydrate complex from beech (Fagus crenata) wood

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Summary. A water-soluble lignin-carbohydrate complex (LCC) isolated from beech (Fagus crenata) MWL was investigated. Results from gel filtration chromatography and the infrared spectrum of the LCC treated with alkali under mild conditions indicated that the LCC contained alkali-labile bonds. Decrease of uronic acid content and the detection of 4-O-methylglucose in the sodium borohydride-reduced LCC suggested the presence of an ester linkage between lignin and glucuronic acid in the glucuronoxylan. Conductometric titration also indicated the existence of glucuronic acid ester linked to lignin. From these results, it is concluded that the LCC contained an ester linkage between lignin and glucuronoxylan and that about one-third of the glucuronic acid present in the LCC was involved in this ester linkage.

Introduction

Water-soluble, lignin-carbohydrate complexes (LCC) originating from various plants have been investigated by several researchers (Merewether, Samsuzzaman 1972; Hartley 1973; Brice, Morrison 1982). We have also studied the LCC isolated from pine and beech woods and established a standard isolation method of LCCs from wood (Azuma et al. 1981, 1985). Chemical and physical analysis of the LCCs isolated according to above method indicated that they consisted of the typical lignin and hemicellulose originally presented in the parent softwood and hardwood, respectively (Azuma et al. 1981, 1985). In recent reports on LCC, much attention has been directed to the type of bonds linking the lignin to the carbohydrate. Various kinds have been proposed, such as ether (Joseleau, Gancet 1981; Iversen 1985), glycosidic (Neilson, Richards 1982; Joseleau, Kesraoui 1986) and ester linkages (Obst 1982; Das et al. 1984). We have recently demonstrated that there is a benzyl ether bond in beech LCC based on results obtained by alkaline and 2,3-dichloro 5,6-dicyanobenzoquinone (DDQ) treatment (Takahashi, Koshijima 1988).

It has been believed that a detection of 4-O-Me-glucose from reduced LCC by using aqueous sodium borohydride was an evidence for an ester linkage. Comtat et al. (1974) have reported isolation of 4-O-Me-glucose from a glucuronoxylan obtained from a reduced aspen holocellulose. However, 4-O-methyl-glucose can also be derived from a uronic acid lactone, and it is therefore not known whether the original 4-O-Me-d-glucuronic acid residue in a glucuronoxylan is linked to
lignin or not. There have been few quantitative analyses of these ester linkages reported and it is not clear whether all of the 4-O-Me-glucose detected takes part in ester linkages.

In this paper, we report evidence indicating a strong possibility that glucuronic acid in the LCC of hardwood actually participates in forming an ester linkage to lignin. The amount of this type of uronic acid residue is approximately one-third of total uronic acid present in the LCC.

Experimental

Materials and general methods

LCC was prepared from beech (*Fagus crenata*) MWL by the method described previously (Azuma et al. 1985). Xylan (4-O-Me-glucuronic acid/xylose molar ratio: 1/11.6, 8.5% acetyl) was also obtained from the same beech wood according to the method of Timell (1960). The water soluble LCC thus prepared was passed through a Dowex50W-X8(H⁺) column to remove cations.

Distilled water used for all experiments was deionized and decarbonated by boiling. Uronic acid was determined by both the modified carbazole method (Galambos 1967) and the harmine-cysteine method (Wardi et al. 1974). Acetyl content was estimated by detecting the acetic acid released after alkaline treatment, as described below by using g.l.c with 20% of tetramethyl-cyclobutaneiodiopase-4% phosphoric acid on chromosorb-W column at 90°C. Infrared spectra were measured using KBr discs with a JASCO IR-810 spectrophotometer. NMR spectra were measured by using a Varian XL-200 NMR spectrometer in d-chloroform and d-water. Component sugars and 4-O-Me-glucose were detected by g.l.c, on SP-1000 S.C.O.T, glass (25 m×0.28 ram) and 3% ECNSS-M column (2 m×0.3 cm) after hydrolysis with 2 M TFA for 1 hour at 121°C.

Alkaline treatment

The LCC was treated with 1.0 N sodium hydroxide for 2 hours at an ambient temperature with stirring. The clear solution was neutralized with hydrogen chloride. In order to prepare the sample (LCC-A) for i.r. spectroscopy, the resulting turbid solution was dialyzed and lyophilized. For gel-filtration, dioxane was added to the neutralized solution and the resultant clear solution transferred to a gel-filtration column.

Protection of phenolic hydroxyl groups

LCC was dispersed in 90% aqueous dioxane, to which diazomethane in ether was added. The turbid solution was kept for 7 days with vigorous stirring during the reaction, new diazomethane being added every 24 hours. After 7 days the solvent was removed by evaporation. The reaction product did not show the chemical shift of aromatic acetyl proton by ¹H-NMR measurement (Ludwig et al. 1964).