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RAPID COMMUNICATION

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Proof of the presence of racemic forms of arylglycerol-β-aryl ether structure in lignin: studies on the stereo structure of lignin by ozonation

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Recent rapid progress of the biosynthetic study of lignan, especially finding a new type of protein that regulates the stereo structure of lignan,[1] seems to "re-create" an interest in the investigation of optical activity of lignin, although lignin has long been assumed to be optically inactive.

The arylglycerol-β-aryl ether substructure in lignin is known to consist of two diastereomers: *erythro* and *threo* forms. Each diastereomer is assumed to be a mixture of two enantiomers: the D form and the L form. If this structure is produced as a dimer by simple radical coupling of two coniferyl alcohol radicals and no other factor affects this process, the formation of asymmetric carbon at the β-position must be a racemic process. However, when a monomer radical combines with the already grown oligomeric structure, the formation of asymmetric carbon at the β-position of this structure must be affected by other asymmetric carbons already present in oligomer parts. Lignin synthesis is believed to proceed in the polysaccharide matrix. In such a situation, formation of an asymmetric carbon in lignin could be affected by optically active carbohydrates, which may result in enrichment of one enantiomer of a certain lignin substructure. For these reasons the possibility of the presence of an optically active lignin substructure still cannot be excluded.

In this report, we tried to obtain information about the optical activity of arylglycerol-β-aryl ether substructures in lignin by measuring the optical activity of erythronic and threonic acids, which are obtained as ozonation products[2,3] of two diastereomers, *erythro* and *threo* forms of this substructure, respectively. These two acids retain the stereo structure of the original diastereomers. Fig. 1 outlines this work.

Beech milled wood lignin (MWL) (30 mg) extracted from 24-h milled wood was dissolved in 30 ml of ozonation solvent (acetic acid/water/methanol, 16:3:1 v/v/v), and oxygen containing 3% ozone was bubbled into the solution for 120 min at 0°C. The reaction mixture was reduced with sodium thiosulfate, and the solvent was completely removed. The reduced sample was saponified with 20 ml 0.1 M NaOH solution, and erythronic and threonic acids were obtained. The alkali-treated sample was passed through an ammonium form cation-exchange resin to convert organic acids in ozonation products to ammonium salts. The ozonation products were then concentrated and subjected to high-performance liquid chromatography (HPLC) analysis. The concentrations of erythronic and threonic acids in this sample were 45 and 27 mM, respectively; they were determined by gas chromatography (GC) analysis as trimethylsilylated derivatives (column: GL science NB1). The erythronic and threonic acids in these ozonation products were separated by HPLC (column: BIO-RAD HPX87H and Yokogawa CHA-E11 connected in series; eluent: 0.01 M sulfuric acid; flow rate 0.3 ml/min). Their optical activities were measured directly by an on-line optical rotation detector (Jasco OR-990). For a comparison, standard solutions containing optically active authentic samples (D-erythronic acid and L-threonic acid) at the same concentrations as the ozonation products were subjected to the same HPLC analysis. If tetronic acids in the ozonation products showed optical activity, it was concluded that arylglycerol-β-aryl ether substructure was not present in the

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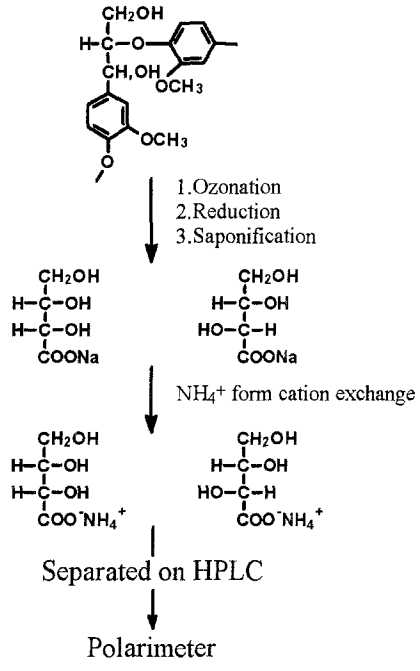

Fig. 1. Outline of the work described herein

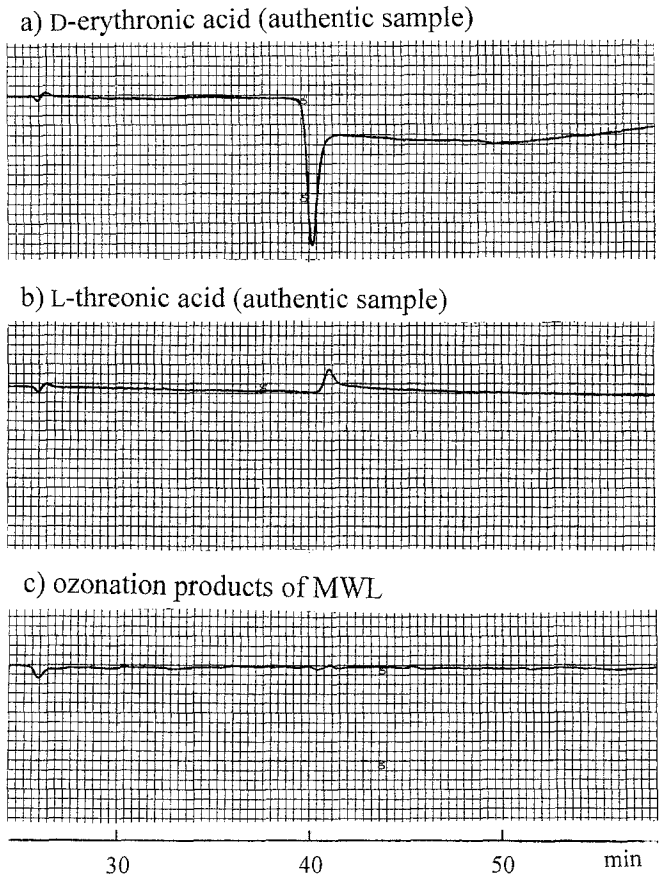

Fig. 2. High performance liquid chromatography (HPLC) profile of ozonation products and authentic sample detected by an optical rotatory detector. **a** D-Erythronic acid 45 mM. **b** L-Threonic acid 27 mM. **c** Ozonation products of milled wood lignin (MWL) containing 45 mM erythronic acid and 27 mM threonic acid

racemic form; if the tetronic acids were optically inactive, the arylglycerol-β-aryl ether substructure was deemed present as the racemic form.

The chromatogram in Fig. 2 shows the HPLC profile obtained by the optical rotation detector. Fig. 2a, and 2b show the optical activity of authentic samples of 45 mM D-erythronic acid and 27 mM L-threonic acid, respectively. Figure 2c shows the optical rotation profile of the ozonation products. Because the concentration of erythronic acid in these ozonation products is equal to that of the authentic sample in Fig. 2a, if erythronic acid in the ozonation products were optically active or rich in one enantiomer, a peak should be observed at a detectable height at the elution volume corresponding to this acid. As is clearly shown, the optical rotation profile of the ozonation products is almost flat. Even when a small fluctuation in the baseline, which cannot be avoided with this kind of chromatography was taken into consideration, excess of one enantiomer was not found in a detectable range, strongly suggestsing that the erythronic acid obtained as the ozonation product of the *erythro* form of arylglycerol-β-aryl ether substructure is optically inactive, and the *erythro* form of arylglycerol-β-aryl ether substructure is present as the racemic form in lignin. As to the *threo* form of the arylglycerol-β-aryl ether substructure, because L-threonic acid shows only a small peak compared with that of D-erythronic acid, a clear conclusion could not be reached.

The results of the *erythro* form alone, however, seem good enough to support the widely accepted hypothesis that the formation of lignin proceeds in a racemic process. Recently, Ralph et al.[4] reported that β-5 and β-β type dimers of pine lignin obtained by the derivatization followed by reductive cleavage method (DFRC) showed no optical activity. Our results and their results well complement each other. The optical activity of other ozonation products including threonic acid is now under investigation.

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References

1. Davin LB, Wang HB, Crowell AL, Bedgar DL, Martin DM, Sarkanen S, Lewis NG (1997) Stereoselective bimolecular phenoxy radical coupling by an auxiliary (dirigent) protein without an active center. Science 275:362–366
2. Matsumoto Y, Ishizu A, Nakano J (1986) Studies on chemical structure of lignin by ozonation. Holzforschung 40(Suppl):81–85
3. Matsumoto Y, Minami K, Ishizu A (1993) Structural study on lignin by ozonation: the *erythro* and *threo* ratio of the β-O-4 structure indicates how lignin polymerizes. Mokuzai Gakkaishi 39:734–736
4. Ralph J, Peng J, Lu F, Hatfield RD, Helm RF (1999) Are lignins optically active? J Agric Food Chem 47:2991–2996