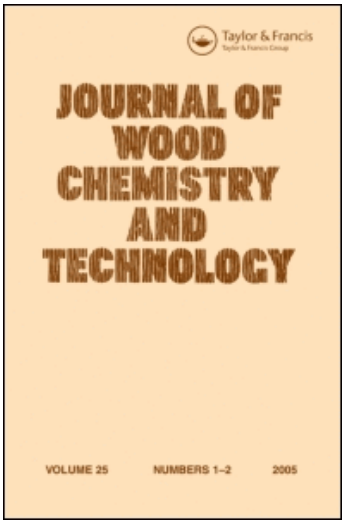

Journal of Wood Chemistry and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

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To cite this Article Terashima, Noritsugu , Fukushima, Kazuhiko , Tsuchiya, Satoshi and Takabe, Keiji(1986) 'Heterogeneity in Formation of Lignin. VII. An Autoradiographic Study on the Formation of Guaiacyl and Syringyl Lignin in Poplar', Journal of Wood Chemistry and Technology, 6: 4, 495 — 504
To link to this Article: DOI: 10.1080/02773818608085241
URL: http://dx.doi.org/10.1080/02773818608085241

JOURNAL OF WOOD CHEMISTRY AND TECHNOLOGY, 6(4), 495-504 (1986)

HETEROGENEITY IN FORMATION OF LIGNIN. VII. AN AUTORADIOGRAPHIC STUDY ON THE FORMATION OF GUAIACYL AND SYRINGYL LIGNIN IN POPLAR

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ABSTRACT

Precursors of lignin biosynthesis, ferulic acid and sinapic acid labeled by ^{3}H or ^{14}C were administered to the differentiating xylem of poplar shoots. Biochemical interconversions between syringyl and guaiacyl type intermediates involved in the biosynthetic pathway were suppressed by administration of precursors in the dark. The process of heterogeneous deposition of guaiacyl and syringyl lignin on the cell wall was visualized by autoradiography. Guaiacyl lignin was deposited in the early stage of the xylem differentiation on the vessel wall followed by deposition of syringyl lignin on the fiber cell wall.

INTRODUCTION

Hardwood lignins consist mainly of guaiacyl and syringyl residues. It was shown that the ratio of guaiacyl to syringyl is different in the different morphological regions of white birch by UV microscopy[1-3] and by bromination-TEM-EDXA[4]. The fiber secondary wall contains predominantly syringyl residues, while the vessel secondary wall and middle lamella including cell corner regions consist mostly of guaiacyl residues. This heterogeneous nature of harwood lignin was confirmed by the chemical characterization

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of lignins in the various tissue fractions separated from mechanical pulp of birch.[5,6]

When hardwood type DHP was treated under kraft cooking conditions, syringyl moiety was degraded to low molecular products faster than guaiacyl moiety.[7] Similar results were observed in kraft cooking of poplar wood which contained guaiacyl or syringyl residues labeled selectively by ^{14}C[7]. The tracer experiments indicated that the residual lignin in the pulp originates largely from guaiacyl lignin. Behavior of lignin in the cell wall during pulping was greatly affected by heterogeneity not only in macromolecular structure but also in distribution among different morphological regions.

In this work, the process of heterogeneous deposition of guaiacyl and syringyl lignin in differentiating xylem of poplar was visualized by microautoradiography, and heterogeneity was estimated semi-quantitatively by counting the silver grains.

EXPERIMENTAL

Materials

A two-year-old stem of Oxford poplar (Populus Maximowiczii x Populus berolinensis) grown in the campus of Nagoya University was cut and stored in the dark for 3 days. A V-shaped groove, 2 mm wide and 5 mm long, was made on the stem with a razor blade under red light. The bottom of the groove reached to the differentiating xylem.

Radioactive precursors of lignin biosynthesis, ferulic acid-[β-^{14}C], sinapic acid-[β-^{14}C], ferulic acid-[ring-2-^{3}H] and sinapic acid-[ring-2-^{3}H] were prepared by the method described in the previous papers.[8-10]

Administration of precursors

About 130 μCi/3 μmol of ^{14}C-labeled precursor or 3 μCi/10 μmol of ^{3}H-labeled precursor dissolved in 60 μl of phosphate

buffer (0.5 mol, pH 7.0) was added dropwise to the fine glass wool packed into the groove under red light and metabolized in the dark. After 2 hours, a drop of 3% glutaraldehyde in phosphate buffer was added to the groove, and a small block of xylem near the groove was cut and fixed again in 3% glutaraldehyde overnight in the refrigerator. A part of the xylem tissue was dehydrated through a graded ethanol series, and embedded in epoxy resin. Another part of the tissue containing ^{3}H-labeled lignin was used for radioassay.

Radioassay

Xylem tissue was milled (80 mesh) and extracted thoroughly with ethanol-benzene for 13 hours and with acetone for 8 hours followed by extraction with boiling water for 14 hours. The dried wood meal (60 mg) was heated with 2N sodium hydroxide (1 ml) and nitrobenzene (60 µl) in a stainless steel bomb containing two small steel balls with shaking at 170°C for 2 hours. The cooled mixture was centrifuged and the supernatant was extracted with ether (3 x 3 ml). The aqueous layer was acidified and extracted again with ether (3 x 3 ml). Dried ether solution was evaporated, and the oxidation products were separated by silica gel thin layer chromatography (20 x 20 cm) with n-hexane-isoamyl alcohol-acetic acid (400:64:1 v/v) as the developer. Vanillin and syringaldehyde were extracted from the gel with methanol. The specific radioactivities of the aldehydes were determined by liquid scintillation counting and ultraviolet spectrophotometry of the methanol extracts.

Autoradiography

Two µm thick transverse sections were cut on a Sorvall JB-4 microtome equipped with a glass knife from an epon embedded segment of the xylem part. They were mounted on glass-slides and covered with Kodak AR-10 stripping film. The glass-slides were stored in a refrigerator for 2 weeks (^{14}C-labeled sections) or 6 months (^{3}H-labeled sections). They were developed with Kodak D-19

and fixed with Fuji Fix. The sections were stained with toluidine blue O, and microphotographs were taken with Nikon Fluophot microscope equipped with Nikon FX-35A camera.

The analysis of silver grain distributions in the microphotographs were made with the aid of ZEISS IBAS 1 image analyzer.

RESULTS AND DISCUSSION

In the previous work[7], it was shown that interconversions between syringyl and guaiacyl units were unavoidable when ferulic acid or sinapic acid were administered to poplar shoots in the light, while the interconversions were suppressed in the dark or under irradiation of red light for a short time.

Table 1 shows the results of radioassay of syringaldehyde-[ring-2-^{3}H] (S) and vanillin-[ring-2-^{3}H] (V) obtained by nitrobenzene oxidation of the ^{3}H-labeled poplar xylem used for autoradiographic examinations. From the molar ratios of S to V and their specific radioactivities, it is possible to estimate the distribution of label among syringyl and guaiacyl units in the lignin.

These results indicate that when labeled sinapic acid and ferulic acid were administered in the dark, 73% and 80% of the total labeled C_6-C_3 units belonged to syringyl and guaiacyl units respectively.

Microautoradiography can visualize the incorporation of radioactivity into cell walls in the newly formed xylem (Fig.1). The radioactivity from the ferulic acid was selectively incorporated into the newly formed vessel wall in the early stage of xylem differentiation (Fig.1,a), and 80% of the silver grains were assigned to guaiacyl residue. In the xylem to which sinapic acid was administered (Fig.1,b), activity was incorporated mainly into fiber wall, which lignified later than vessel wall.

Fig.2 shows an example of autoradiograms of poplar xylem to which ^{3}H-labeled precursors were administered. The general

TABLE 1

Distribution of Label among Syringyl and Guaiacyl Units in Poplar Lignin

Precursor administered	Mol. S/V	Specific radioactivity μCi/μmolx10^3	S/V	Distribution of label (%)
Sinapic acid-[ring-2-^{3}H]	1.57	S:0.339 V:0.190	1.76	Syringyl:73 Guaiacyl:27
Ferulic acid-[ring-2-^{3}H]	1.83	S:0.235 V:1.691	0.14	Syringyl:20 Guaiacyl:80

pattern of incorporation of radioactivity was similar to those observed in autoradiograms of differentiating xylem of cottonwood administered with ferulic acid-[ring-2,5,6-^{3}H] by Saleh, Leney and Sarkanen[11]. Because low-energy beta emitter ^{3}H gives rise to an autoradiogram of higher resolution than ^{14}C, autoradiograms as shown in Fig.2 are more suitable than those in Fig.1 for semiquantitative measurement of radioactivity in different morphological regions by silver grain counting method.

Incorporation of radioactivity from sinapic acid-[ring-2-^{3}H] into the newly formed vessel and fiber wall is shown in Fig.3. The activity from sinapic acid was partly incorporated into the vessel wall in the early stage of xylem differentiation. In the fiber wall, a marked incorporation of activity was observed in the later stage of the differentiation. Even greater differences between the vessel and fiber were observed in the incorporation pattern of activity from ferulic acid-[ring-2-^{3}H] shown in Fig.4.

The vessel wall lignifies earlier than the fiber wall and incorporates more ^{3}H from ferulic acid than from sinapic acid, while the fiber wall incorporates more from sinapic acid. The period of incorporation from sinapic acid seemed to be longer than that from ferulic acid.

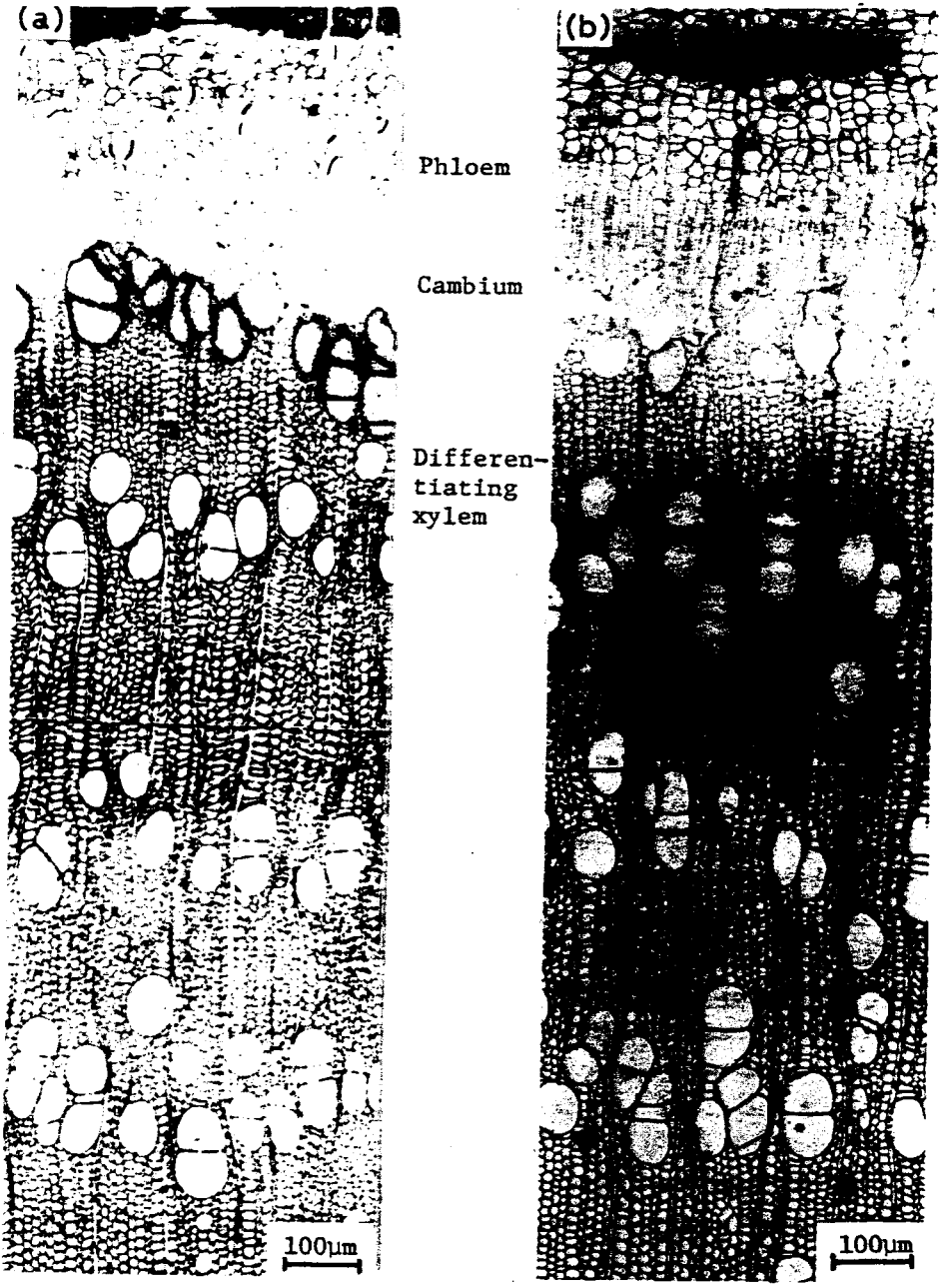

FIGURE 1. Autoradiographs of differentiating xylem of poplar administered with ferulic acid-[β-^{14}C] (a) or sinapic acid-[β-^{14}C] (b).

FIGURE 2. A part of autoradiograph of differentiating xylem of poplar administered with ferulic acid-[ring-2-^{3}H], employed for silver grain counting.

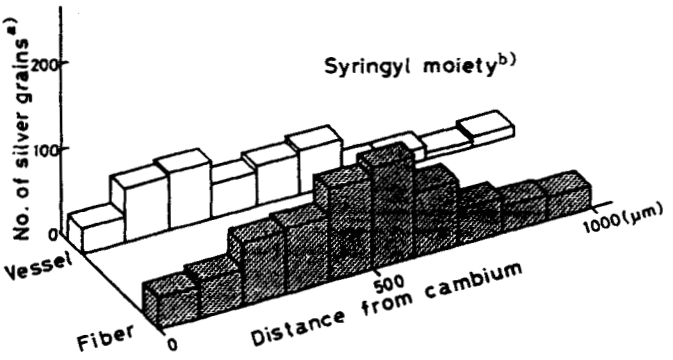

FIGURE 3. Formation of syringyl lignin in differentiating xylem of poplar.
a) Number of silver grains per 100μm length of cell wall on microautoradiogram.
b) Radioactive structures derived from sinapic acid-[ring-2-^{3}H] administered as a precursor.

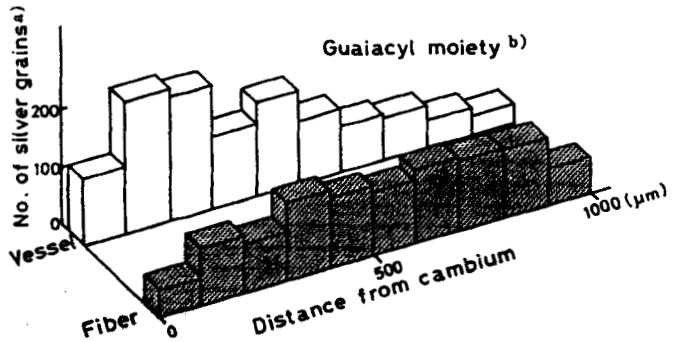

FIGURE 4. Formation of guaiacyl lignin in differentiating xylem of poplar.
a) Number of silver grains per 100μm length of cell wall on microautoradiogram.
b) Radioactive structures derived from ferulic acid-[ring-2-^{3}H] administered as a precursor.

These results indicates that vessel wall lignin is mainly made up of guaiacyl units containing minor amount of syringyl units while fiber wall lignin consists of syringyl units as major building stones.

By bromination-TEM-EDXA method, Saka and Goring estimated the guaiacyl:syringyl ratio of birch lignin at 88:12 and 12:88 for secondary wall lignin of vessel and fiber respectively.[4]

Microautoradiographic examinations visualized the process of heterogeneous formation of lignin in differentiating xylem of poplar. The results suggest that the heterogeneity is caused by (1) the differences in start and speed of lignification between vessel and fiber wall and (2) difference in the deposition type between guaiacyl and syringyl lignin. The formation of guaiacyl lignin is active in the early stage of xylem differentiation, while syringyl lignin deposits in the middle stage.

ACKNOWLEDGEMENTS

The authors thank Prof. H. Harada and Dr. M. Fujita, Kyoto University, for technical support in microautoradiography.

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