FORMATION AND STRUCTURE OF LIGNIN

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Lignin is a natural organic polymer existing as an essential constituent of cell wall in almost all land plants. In the early stage of evolution of plant, formation of lignin might be a kind of antidotal process against harmful phenolic metabolites. However, during the evolution, formation of lignin seems to acquired positive and essential meaning for maintenance of plant life as follows.

Lignin contributes to ----

(1) Low permeability of water across the cell walls in the conducting tissues, which enable internal transport of water, nutrients and metabolites.

(2) Rigidity of cell wall. Lignin, hemicellulose and cellulose constitute a kind of reinforced plastics, which is very strong and stable for thousands of years.

(3) Rigidity of tissue. Lignin acts as a strong binder between cells forming rigid tissues to support plant body.

(4) Resistibility ot the microbial attack.

The lignin contents of woods are usually in the range from 15 to 35%. Thus, among natural polymers, lignin is the second to carbohydrates in natural abundance. Fig. 1 shows the schematic model of lignin structure proposed by Glasser.[1]

Because of its highly complicated structure, elucidation of the true nature of lignin has been one of the most difficult problems for organic chemists long time. However, application of modern analytical methods and modern concepts of biogenesis made rapid and significant progress in the systematic understanding of lignin structures and reactions, though the complete and detailed elucidation of the true nature of lignin is still open to future research.

1. Formation and distribution of lignin in the cell wall

Lignin is formed by dehydrogenative polymerization of three kinds of aromatic alcohols, coniferyl alcohol (I), sinapyl alcohol (II) and p-hydroxycinnamyl alcohol (III) (Fig. 2).

These aromatic alcohols are derived from glucose via the well known biosynthetic pathway called shikimic acid pathway (Fg. 3).[2] Cellulose and hemicellulose are also derived from glucose which was generated from carbon dioxide and water by photosynthesis.

Gymnosperm lignins are derived mainly from coniferyl alcohol (I), while angiosperm lignins are derived from sinapyl alcohol (II) and coniferyl alcohol (I), and grass lignins are mixed polymer of (I), (II) and (III).

If ferulic acid, a good precursor of lignin biosynthesis, is labeled by radioisotope such as ^{14}C or ^{3}H, and administered to plant shoots, the lignin in the newly formed xylem part is also labeled. So, we can trace the formation of lignin in the cell wall by detecting and measuring the radioactivity by autoradiography or other suitable methods.

It was shown by the tracer experiments and ultraviolet microscopic observations that lignification is initiated in the differentiating wood cells from the primary walls adjacent to the cell corners, and extends to the intercellular areas, primary and secon-

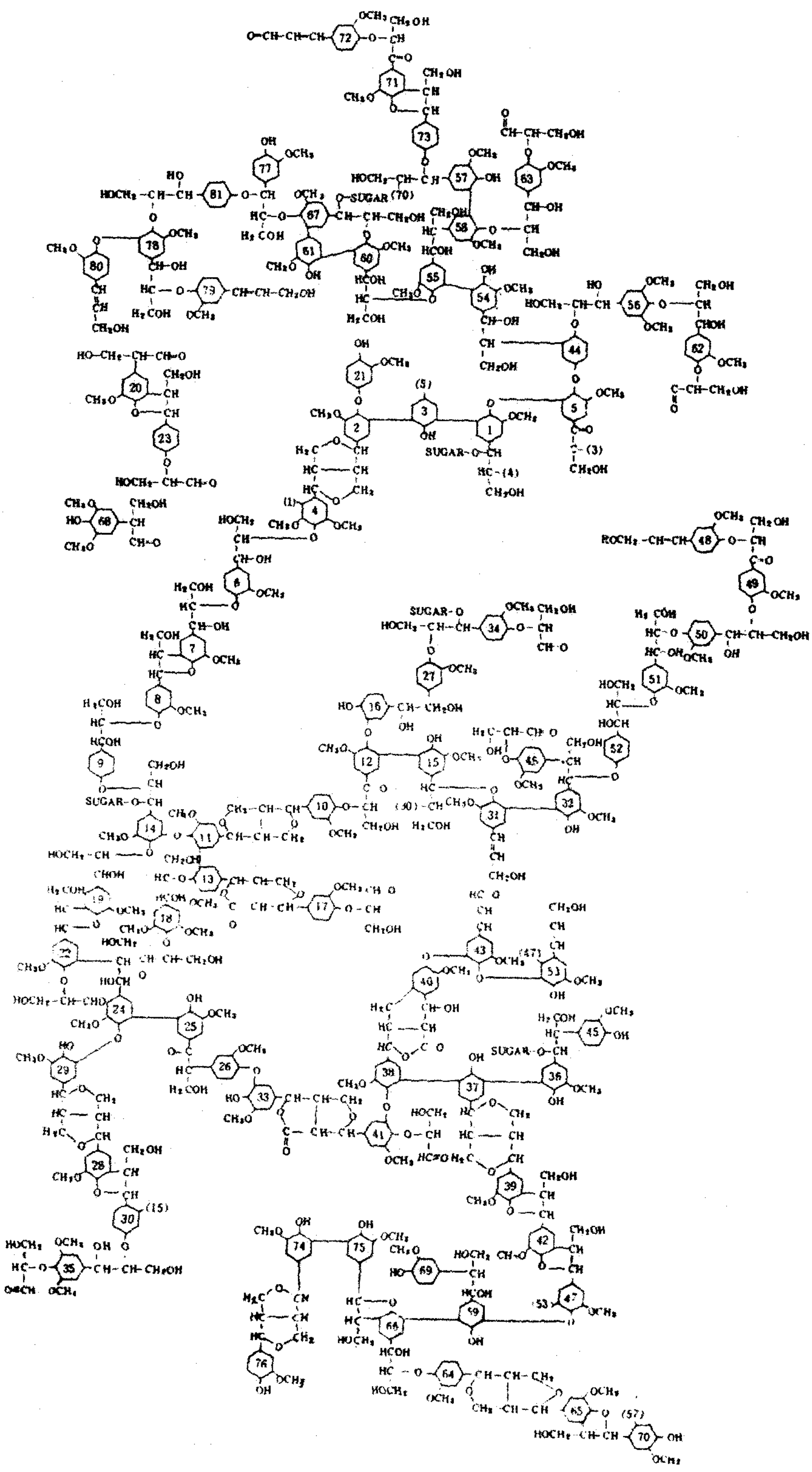
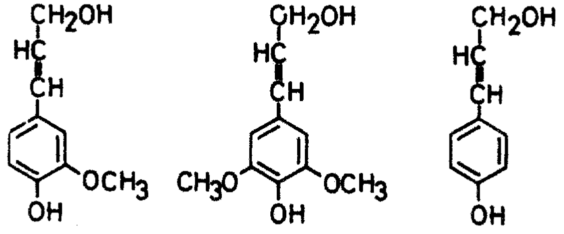


Fig. 1. Schematic model of lignin structure.[1)]



I II III

Fig. 2. ρ-Hydroxycinnamyl alcohols; the primary building stones of lignin.

dary walls.

The intercellular lignin plays an important role as a binder between the cells, and lignin in the secondary walls constitutes lignin-hemicellulose plastic reinforced by cellulosic fibers.

The cellulose microfibrils and lignin are considered to be arranged in a laminar fashion parallel to the middle lamella as shown in this schematic representation, Fig. 4[3])

The interlamellar distances have been estimated at 7.1 nm in spruce and 8.6 nm in silver fir.

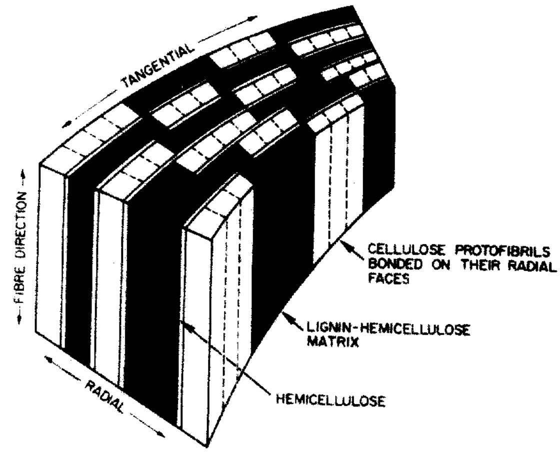


Fig. 4. Schematic of the interrupted lamella model for the ultrastructural arrangement of lignin and carbohydrate in the wood cell wall.[3])

Fig. 5 is the schematic representation of lignin distribution across the double cell wall.[4]) The lignin concentration in middle lamella is very high, and reach about 100% in the cell corner, but the amount of lignin is minor due to the small volume of the

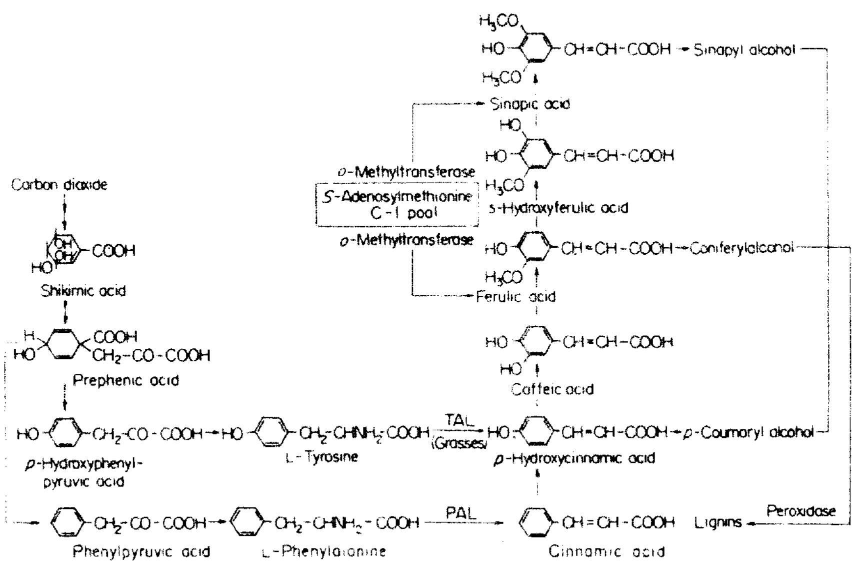


Fig. 3. Biosynthetic pathway of lignin.[2])

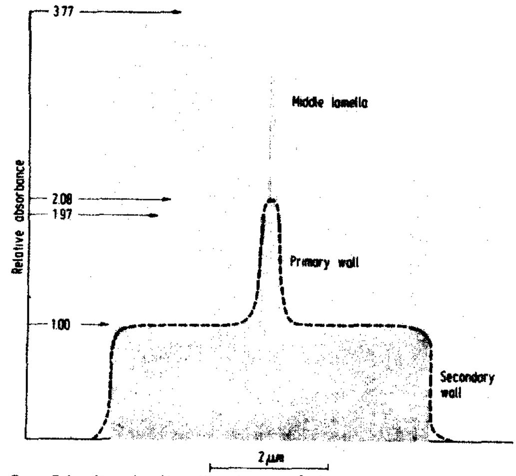


Fig. 5. Idealized distribution of lignin across the double cell wall.[4)]

middle lamellae, which may be only about 0.1 µm thick in the case of spruce. Thus a large part of lignin is distributed almost evenly in the secondary wall.

2. Structure of lignin

The first step of formation of macromolecular lignin is the enzymic dehydrogenation of p-hydroxy-cinnamyl alcohol derivatives to generate phenoxy radical which can be exist as four kinds of resonace structure (Fig. 6).

Coupling of two radicals Rα ① and Rδ ② forms a dimeric quinone methide ③ , to which water is added to form a dimer ⑤ (Fig. 7).

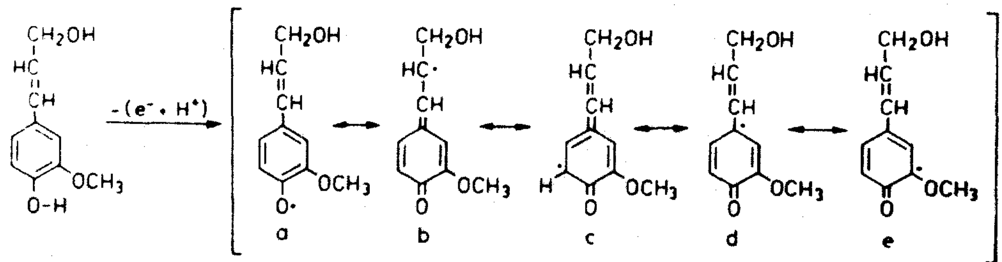


Fig. 6. Formation of monolignol radicals.

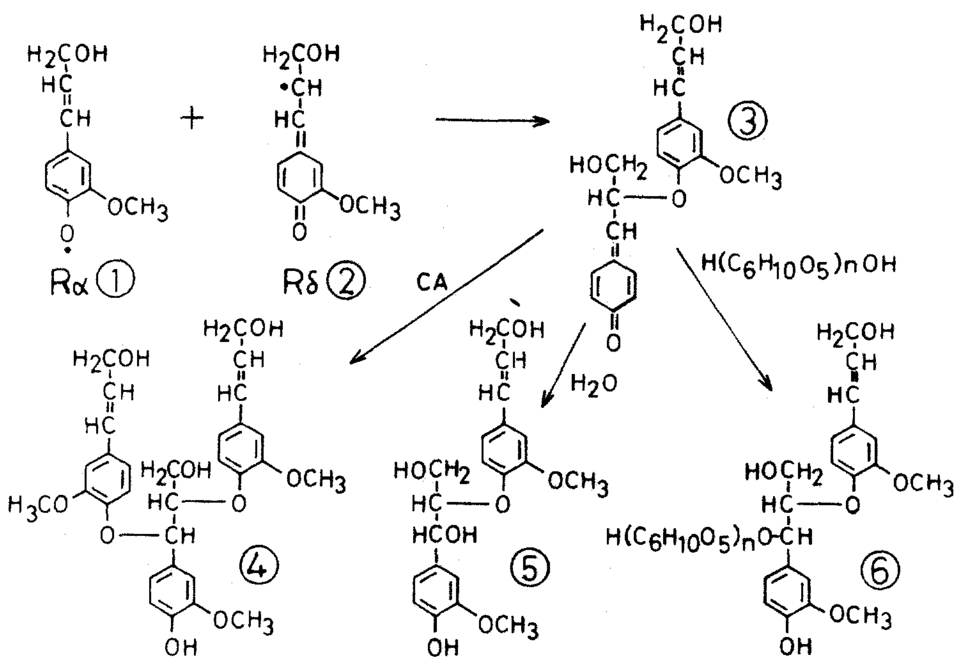


Fig. 7. Polmerization of monolignols.

If coniferyl alcohol is added to the dimeric quinone methide, a trimer (4) is formed.

If addition reaction is occurred between the quinone methide and carbohydrate instead of water or alcohol, lignin-carbohydrate bond will be formed as shown in compound (6) in Fig. 7.

The knowledge on the true nature of this bond between lignin and carbohydrate is essential for better understanding of chemical properties of cell wall as well as reactions during pulping and bleaching. However, the detailed and complete elucidation of true nature of this bond is still open for future research.

Rearrangements affords another types of linkages between building stones.

Repetition of dehydrogenation, coupling and addition reactions forms almost infinite high molecule of lignin with complicated structure of three dimensional network.

Fig. 8 shows the schematic model of structure of beech lignin proposed by Nimz.[5] It should be noted that this is not a exact chemical structure of lignin but a schematic model of lignin structure.

It is impossible to isolate pure lignin which comprises same size of molecule with same chemical structure, because there are no structural regularity and definite limitation of molecular size in formation of lignin. In the case of lignin, a schematic model which represents average contents of various building stones, various types of linkages and functional groups is often referred instead of true chemical structure. This special situation of lignin is completely different from that of other natural organic polymer such as cellulose, starch and protein.

Because the mode of polymerization is greatly affected by the change of reaction conditions during the cell wall development, structure of lignin varies with the age of the cell, temperature, plant hormone

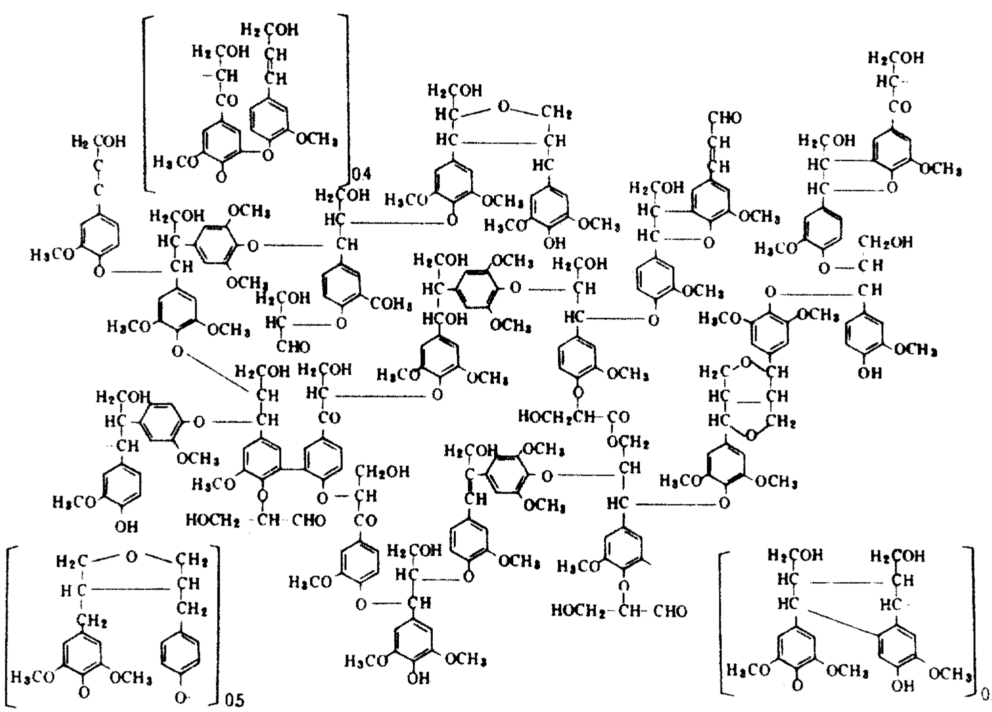


Fig. 8. Schematic model of beech lignin.[5]

balance, gravity and light.

When ferulic acid ①, one of the precursors of lignin biosynthesis, is labeled with ^3H and ^{14}C as shown in Fig. 9, and administered to a plant shoot, radioactive lignin is formed in the cell wall. A part of ^3H is removed from the lignin according to the amount of condensed structure ⑤, ⑥ and ⑦ which is formed by the linkage at position 5 of aromatic nucleus.[6-8]

After administration for 2 weeks, the shoot is barked and cut radially into wedges, and arranged on the freezing sample stage of sliding microtome. The irregular edges are cut off. These are fixed again on the sample stage upside-down. Tangential sections of 100μ thick are prepared from cambium toward pith by sliding microtome (Fig. 10).

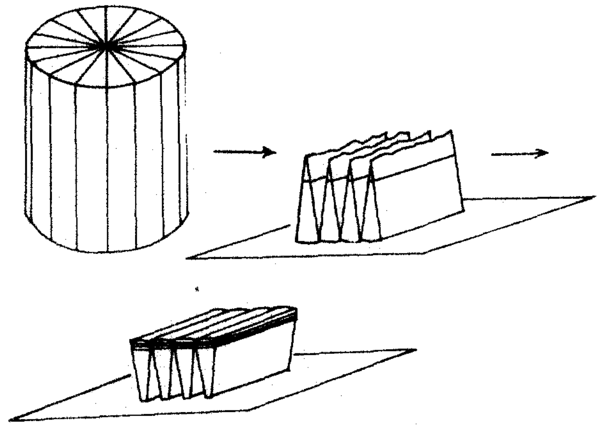


Fig. 10. Preparation of tangential microtome sections of various stages of cell wall development from cambium to pith.

Fig. 11 shows general pattern of lignin contents and incorporation of radioactivity in these sections. Ordinates are lignin contents (absorption) or radioactivity. Abscissa represents distance from cambium, which corresponds to development of cell wall. Those sections of newly formed xylem up to 300μ

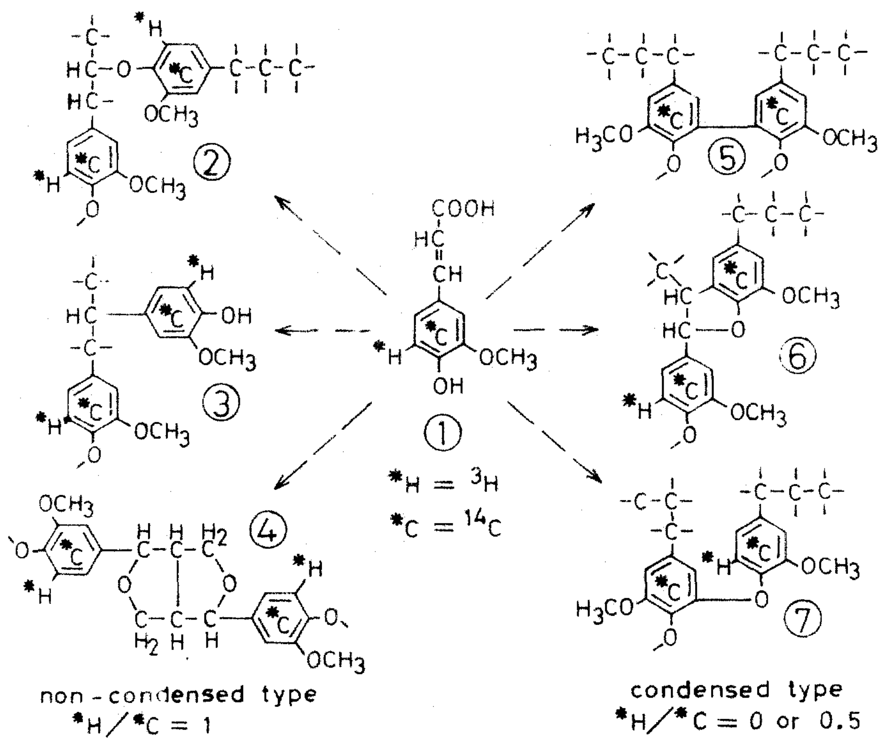


Fig. 9. Incorporation of radioactivity from double labeled lignin precursor ① into non-condensed and condensed type structure in lignin.

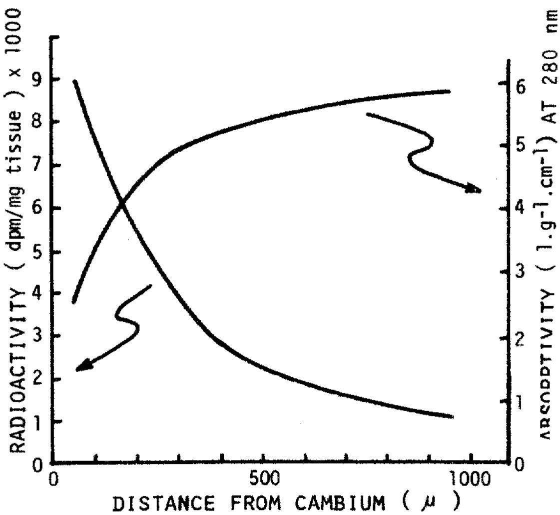


Fig. 11. Incorporation of lignin precursor (radioactivity) and lignin content (absorptivity) in xylem.

from cambium correspond to the tissue of early stage of cell wall development. The sections from 300μ to 600μ and sections from 600μ to 1000μ represent middle stage and late stage of cell wall development respectively.

Fig. 12 shows the change of $^3H/^{14}C$ ratio during the cell wall development in pine shoot. The fact that the $^3H/^{14}C$ ratios of the sections near cambium are lower than that of other parts means that lignin containing larger amount of condensed type structures are formed in the early stage of cell wall development than in the late stage. The lignin formed in the early stage may also contain larger amount of lignin-carbohydrate linkage and will be more resistant in removing lignin from the secondary wall during pulping.

The lignin formed at high temperature contains larger amount of condensed type structure than that formed at low temperature.

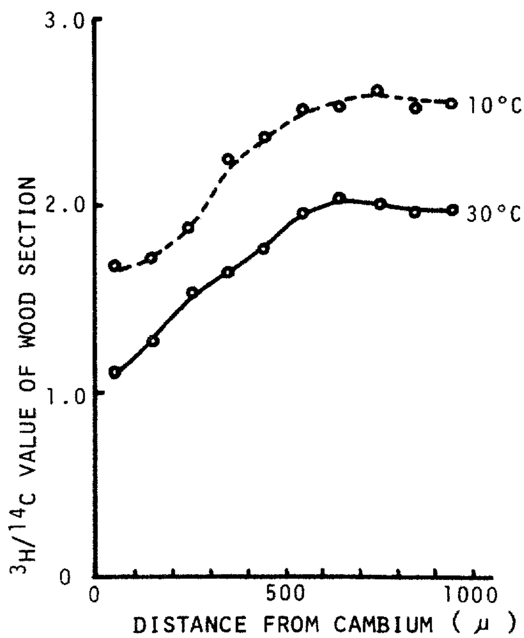


Fig. 12. Formation of condensed type structure in pine.

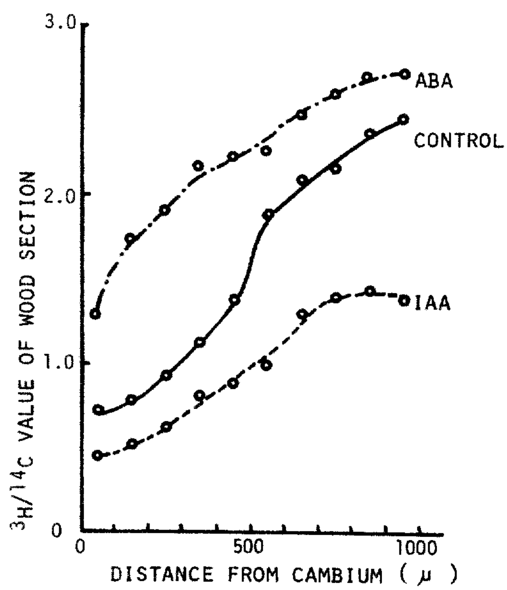


Fig. 13. Effect of plant hormone on formation of condensed type structure in pine.
ABA : Abscisic acid
IAA : Indoleacetic acid

In under part of branch of inclined stem of coniferous wood, namely compression wood, lignin contains more condensed type structure than that in other parts. This heterogeneity was found to be caused by gravity via the change of plant hormone balance.

Increase or decrease of condensed type structure was caused by application of plant hormone on a plant shoot held vertically (Fig. 13).

As the structure of lignin is thus heterogeneous in every respects within the lignin macromolecule, within the cell wall, within the tissue and within the whole tree, lignins never crystallize even after a very long period of time. Heterogeneity of lignin structure contributes to the great stability of mechanical properties of wood. Thus wood, unlike synthetic polymers never become aged and fragile in thousands of years as seen in old wooden buildings or in giant redwood.

Heterogeneity of lignin may also contribute to antibiotic properties of wood. Usually, every linear organic polymers which have regular repeating units are readily decomposed by microorganisms. But, three dimensional and heterogeneous network of lignin structure strongly resist an adaptive attack by microorganisms.

Formation of amorphous and heterogeneous lignin is thus essential for plants not only to support their body but also to maintain their life for a long period of time.

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