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Lignin from Oil Palm Seedlings

(Research note)

By

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Summary : The chemical characteristics of lignin from oil palm seedlings (*Elaeis guineensis* Jacq.) were investigated in comparison with those from matured trunks. Milled wood lignin from oil palm seedlings contained more protein than that from matured trunks. The content of *p*-hydroxybenzoic acid ester was highest in parenchymatous lignin in matured trunks. Lignin from matured vascular bundles was richer in syringyl units than those from seedlings and matured parenchyma which had a syringyl/guaiacyl ratio of about 1.0. Enzyme lignin had a higher syringyl/guaiacyl ratio than milled wood lignin indicating that residual lignin is rich in syringyl units. The polysaccharides contained in milled wood lignin consisted mainly of xylose residues whereas an appreciable part of enzyme lignin, especially that obtained from seedlings, consisted of glucan. The low yield of seedling lignin indicated a possibility of greater linkage between the carbohydrate and the lignin moiety than in the case of matured wood lignin.

1. Introduction

In tropical areas a great number of oil palm trunks will be produced over the next decade but on economically feasible method of utilizing them has not yet been established. Disposal of these trunks causes insect and fungal infestaion problems and their utilization is necessary to reduce the replanting costs. Although many studies have been reported, basic characterization of this plant has not been fully carried out. Therefore, elucidation of its chemical properties may give some clues about possible end uses. It is well known that oil palm trunks contain a lot of carbohydrates such as starch. However, little is known about their lignin properties. The lignin of the oil palm trunk is characteristic in containing *p*-hydroxybenzoic acid ester and the chemical structure differs slightly in vascular bundles and parenchyma (TOMIMURA, 1992). The lignin is heterogeneously distributed in the tissues and also at each growth stage. Since oil palm seedlings are morphologically quite different from matured wood, it is expected that the chemical properties of seedlings are also different. The objective of this work is to investigate the lignin from oil palm seedlings in comparison with that from matured trunks and thus obtain more information about this plant.

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2. Experiments

2.1 Plant material

Oil palm seedlings (*Elaeis guineensis* Jacq.) were harvested after one year growth of germinated seeds in pots in a greenhouse. Wood meal was prepared from whole plants, excluding seeds (Fig. 1).

Wood samples from 25-year-old oil palm trunks were obtained from Socfin Palm Oil State in Batang Berjuntai, Selangor, Malaysia. Samples were debarked and cut into small chips which were then crushed in order to separate vascular bundles from parenchyma.

2.2 Determination of starch content

Wood meal under 200 mesh size was prepared for determination. Starch content was determined as described by HUMPHREYS (1961).

2.3 Determination of protein content

Protein content was determined by alkaline hydrolysis followed by the KCN-ninhydrin method (FUKUDA, 1976).

2.4 Preparation of milled wood lignin (MWL)

The extractives free wood meal was milled for 100 hours using a vibratory ball mill. The MWL was extracted and purified according to the standard method of BJÖRKMAN (1956).

2.5 Preparation of enzyme lignin (EL)

After extraction of MWL, enzyme lignin was prepared by treating the residue with a commercial cellulase preparation derived from *Trichoderma viride* (Meicellase, Meiji seika Co. Ltd.). The extraction and purification of the enzyme lignin were according to the method of SCALBERT (1986).

2.6 Alkaline nitrobenzene oxidation

Nitrobenzene (0.24 m*l*) and 2N potassium hydroxide (4 m*l*) were added to the lignin sample (10 *mg*) in a stainless micro tube and the mixture was heated for 2 hours at 160℃ with continuous vigorous shaking. After cooling, the solution was then extracted with ethyl ether to remove the residual nitrobenzene. The solution was then acidified with hydrochloric acid and extracted again with ethyl ether. The extracts was dried over anhydrous sodium sulfate and evaporated to dryness. The resultant phenols were converted to trimethylsilyl (TMS) derivatives and analysed by gas chromatograph (GC) using acetovanillone as an internal standard. GC was performed using Shimazu GC-14A with a capillary column HiCAP-CBP1.

2.7 Sugar analysis

The sugar composition of the samples was determined by HPLC using the spent liquor from Klason lignin preparation. The sugar composition of MWL was determined by HPLC with the hydrolysate by 2M trifluoroacetic acid at 120℃ for one hour. HPLC was performed using Shimazu LC-3A with column ISA07/S2504.

2.8 ^{13}C NMR spectroscopy

About 100 mg of samples were dissolved in 0.5 m*l* DMSO-d_6 and spectra were obtained with JEOL JNM GXS-400.

2.9 Measurement of IR spectra of lignin

IR spectra of lignin were measured as KBr discs using JASCO FT/IR-3 spectrophotometer.

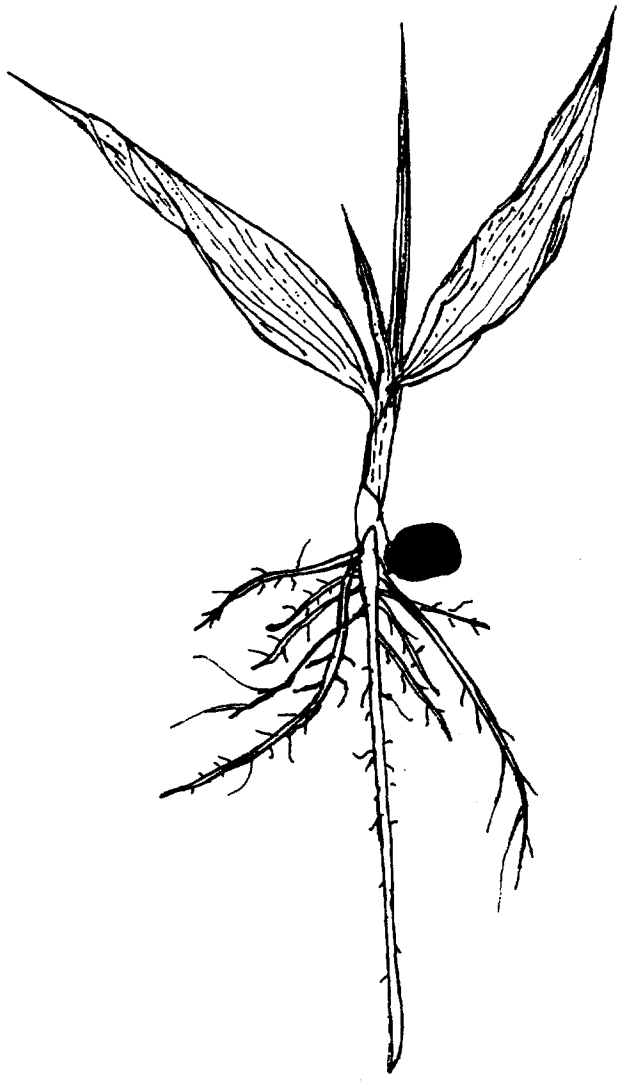

Fig. 1 An oil palm seedling

3. Results and discussion

Table 1 shows the results of analyses of oil palm samples. The ash content was considerably high in seedlings. Klason lignin content was also high in seedlings but this may be because of protein contamination. Protein is usually included to some extent in young plant tissues and in this case seedlings contained more protein than matured wood. Starch content of seedlings was not as high as that of matured parenchyma. Matured wood stored a large amount of starch in its parenchyma, but seedlings did not contain so much starch. In the preparation of sample meal, matured wood was divided into two fractions (parenchyma and vascular bundles) by crushing and screening but it was difficult to divide seedling samples by this method whole seedlings were

used as samples.

Figures 2 and 3 show IR spectra of lignin samples. The absorption band at 770 cm^{-1} has been used as a specific signal attributed to *p*-hydroxybenzoic acid ester structure in poplar lignin (VENVERLOO, 1971 ; LAPIERRE, 1981). Although all lignin preparations had strong absorption in this area, the lignins from matured parenchyma showed the strongest absorption among them. This was in good accordance with the result of the previous report(TOMIMURA, 1992) that milled wood lignin from parenchyma contained a lot of *p*-hydroxybenzoic acid ester structures. The difference between milled wood lignin and enzyme lignin was not so clear from the spectra.

Figures 4 and 5 show the ^{13}C NMR spectra of the samples. Both milled wood lignin and enzyme lignin from seedlings had several signals ranging from 30 ppm to 35ppm which might be derived from protein (IIJIMA, 1991). Since the seedlings contained a considerable amount of protein (Table 1), lignin samples from seedlings may be contaminated with protein. These peaks were not observed in the spectra of milled wood lignin (TOMIMURA, 1992) or enzyme lignin from matured trunk. After alkaline hydrolysis these peaks remained while the peaks at 20 ppm and 172 ppm attributed to the acetyl group in hemicellulose and those at 166ppm and 162 ppm attributed to *p*-hydroxybenzoic acid ester disappeared. The spectra of enzyme lignin showed the same tendency and were not so different from those of milled wood lignin.

The results of nitrobenzene oxidation of milled wood lignin and enzyme lignin are shown in Figures 6 and 7. The main products were vanillin, syringaldehyde, *p*-hydroxybenzoic acid and a small amount of syringic acid and vanillic acid. Part of these phenolic acids could be bonded to lignin as ester groups (TOMIMURA, 1992). Lignins from matured parenchyma contained the largest amount of *p*-hydroxybenzoic acid among the samples. This was in good accordance with the results from IR spectra. Matured parenchyma lignin is rich in *p*-hydroxybenzoic acid ester structures. This tendency was similar to those of sugarcane and bamboo. In the case of sugarcane, the lignin in parenchyma contains more phenolic acids, such as ferulic acid and *p*-coumaric acid as an ester structure, than that in vascular bundles, and matured tissues contain more phenolic acids than young tissues (HE, 1990). Cinnamic acid is extracted in higher yield from bamboo parenchyma than vascular bundles with alkali (HIGUCHI, 1966). Lignins from vascular bundles gave syringaldehyde and syringic acid in relatively higher yields on nitrobenzene oxidation. Matured vascular bundle lignin is of the syringyl rich type. On the other hand, milled wood lignins from seedlings and matured parenchyma yielded as much vanillin as syringaldehyde, indicating that these are syringyl-guaiacyl type lignins. In the enzyme lignin samples the ratio of syringyl unit/guaiacyl units was relatively high in comparison with that

Table 1. Analyses of oil palm samples (%)

	Seedlings	Parenchyma (Matured trunk)	Vascular bundles (Matured trunk)
Ash	6.7	2.9	2.2
Klason lignin (Acid soluble lignin)	26.2 (3.5)	20.0 (4.5)	15.7 (3.9)
Starch	7.6	55.5	2.4
Protein	5.9	0.8	0.7

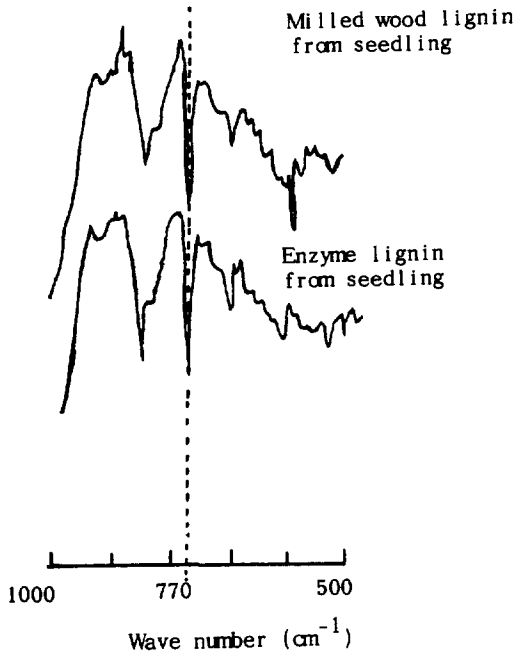

Fig. 2 IR spectra of seedling lignin

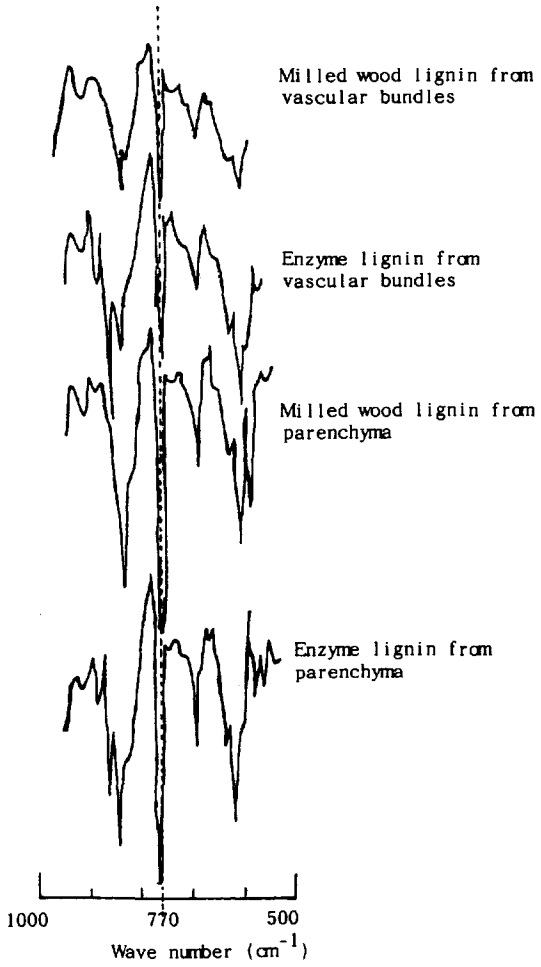

Fig. 3 IR spectra of matured trunk lignin

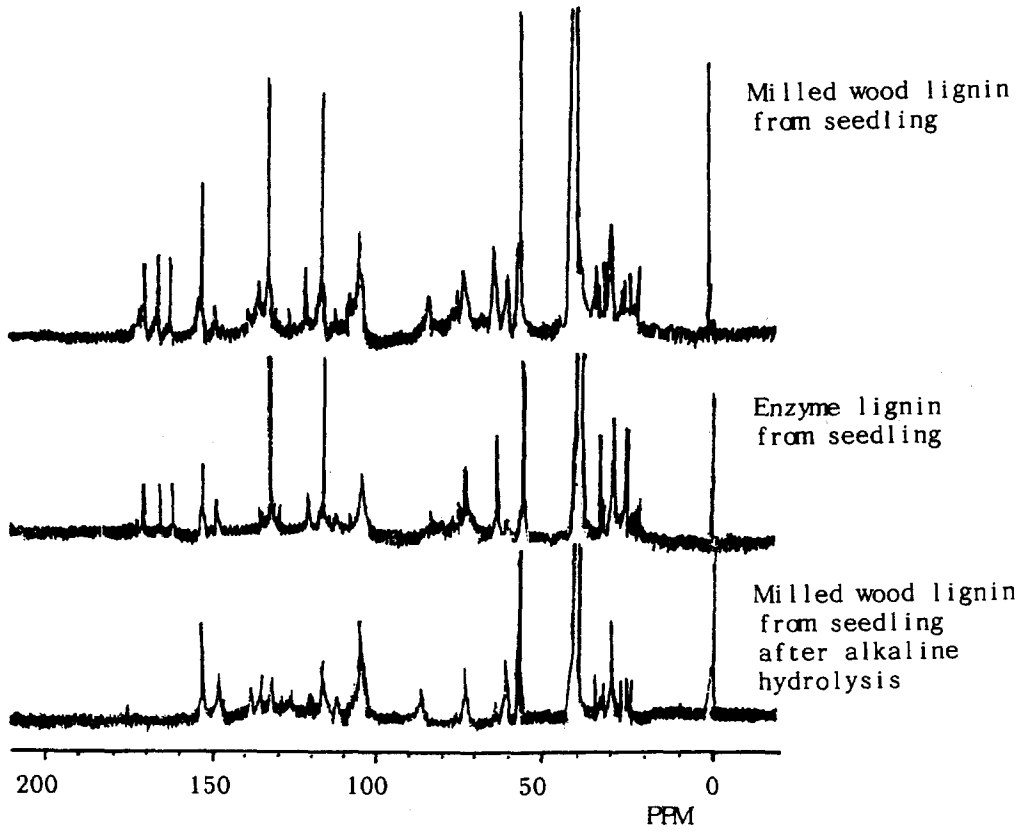

Fig. 4　^{13}C-NMR spectra of seedling lignin

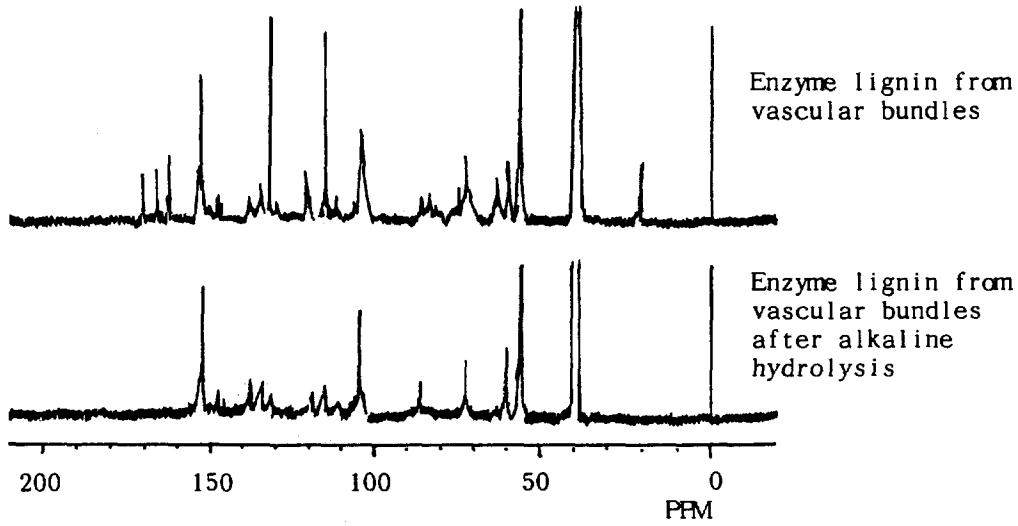

Fig. 5　^{13}C-NMR spectra of enzyme lignin from matured trunk

from milled wood lignin. This means residual lignins contain more syringyl units than milled wood lignins. This was the same tendency as poplar lignin (LAPIERRE, 1981, 1984) but was slightly different from wheat lignin. In the case of wheat lignin, the ratio of guaiacyl unit/syringyl units of milled wood lignin is almost identical to that of enzyme lignin (SCALBERT, 1986).

Figure 8 shows the sugar composition of each sample. In both matured wood and seedlings the main components were glucose and xylose. A small amount of arabinose was also observed in seedlings and matured parenchyma. Figure 9 shows the sugar composition of polysaccharides included in milled wood lignin samples. All lignin samples contained about 5% polysaccharides. In milled wood lignin the main component was xylose, but seedling lignin showed relatively high glucose content. Figure 10 shows the sugar composition of polysaccharides associated with enzyme lignin. In this case the main component was xylose, but the percentage of glucose was also quite high. In the case of enzyme lignin from seedlings in particular, the glucose content was almost equal to that of xylose. Moreover, seedling enzyme lignin contained more arabinose and mannose than matured wood lignin. This indicates that the seedling polysaccharides associated with lignin are different from those of matured wood lignin. These results were not the same as those obtained for wheat, a typical monocotyledon. A study on wheat straw lignin revealed that the main sugar components of enzyme lignin are xylose, arabinose and glucose (SCALBERT, 1985) and that mannose is not included. Although the main component is also xylose, arabinose content is high and glucose content is low in comparison with oil palm enzyme lignin. Oil palms are monocotyledons but their chemical properties seem to be somewhat different from other groups in lignin structure (TOMIMURA, 1992) and polysaccharides.

The yields of milled wood lignin and enzyme lignin were quite different for seedlings and matured wood. In matured wood, the yield of milled wood lignin was about 6% and enzyme lignin 2.5%. On the other hand, in seedlings the yield of milled wood lignin was only 2% and

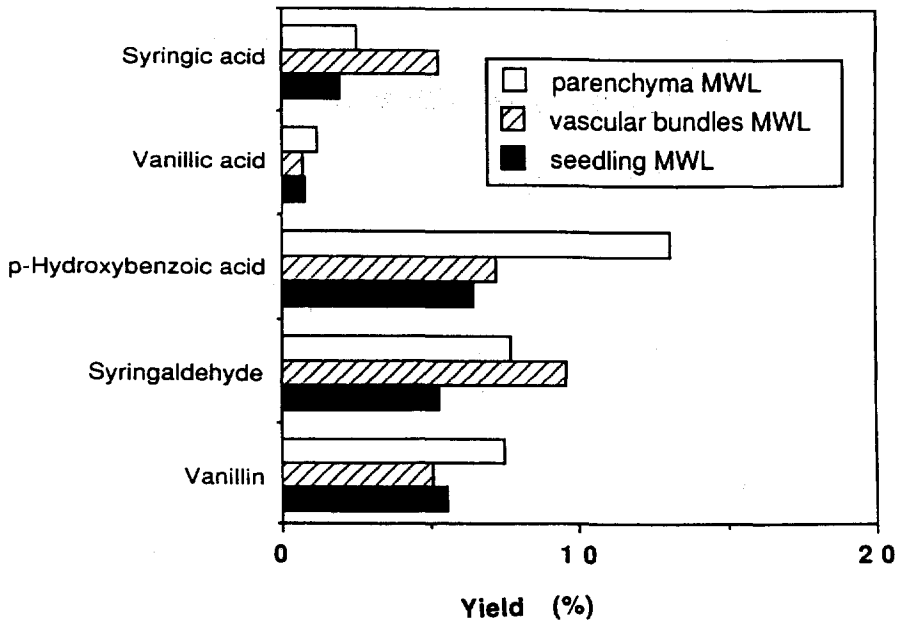

Fig. 6 Results of nitrobenzene oxidation of milled wood lignin

enzyme lignin 7%. Treating residual seedlings with cellulase released a considerable amount of lignin which could not be extracted as milled wood lignin. This indicated a possibility of seedling lignin being highly bonded to the carbohydrare moiety.

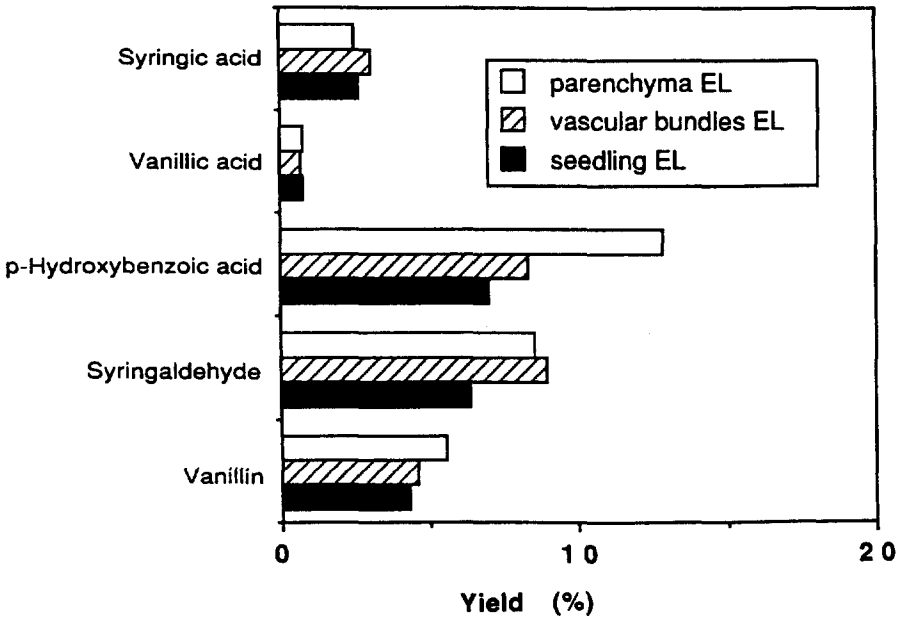

Fig. 7　Results of nitrobenzene oxidation of enzyme lignin

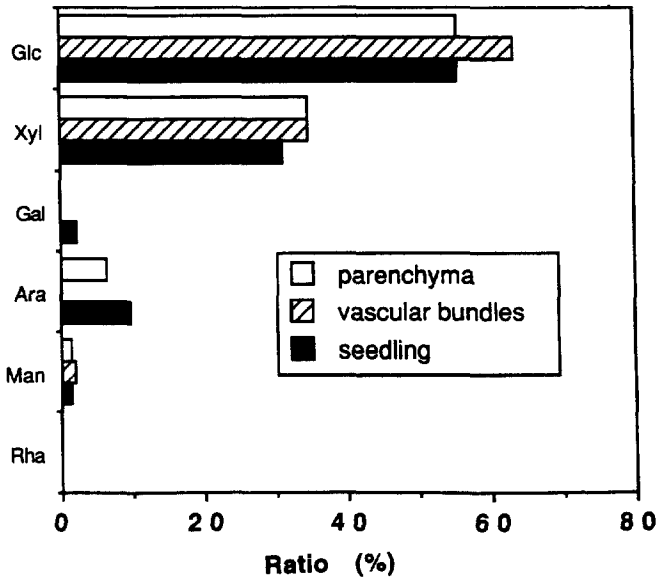

Fig. 8　Sugar composition of seedling and matured trunk

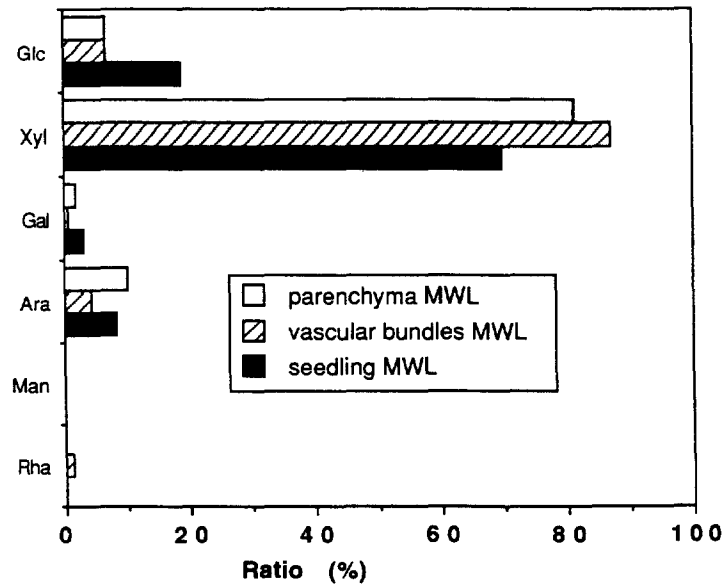

Fig. 9 Sugar composition of milled wood lignin

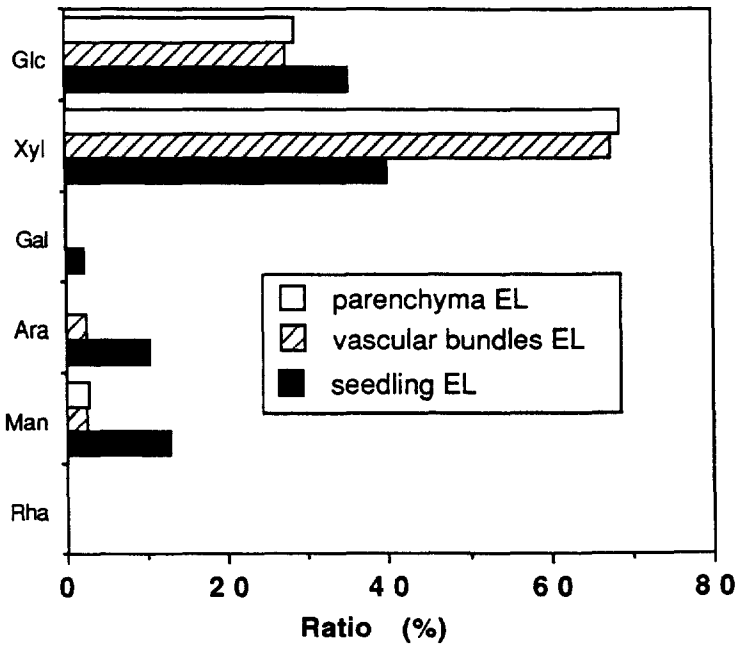

Fig. 10 Sugar composition of enzyme lignin

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References

BJÖRKMAN, A. : Studies on finely divided wood. I. Extraction of lignin with neutral solvents, Svensk Papperstidn., **59**, 477-485 (1956)

FUKUDA, T. *et al.* : Studies on tissue culture of tree-cambium. Ⅲ. Separation of lignin-carbohydrate-protein complex from poplar callus tissue, Mokuzai Gakkaishi, **22**, 112-118 (1976)

HE, L. *et al.* : Formation and structure of lignin in monocotyledons. Ⅲ. heterogeneity of sugarcane (*Saccharum officinarum* L.) lignin with respect to the composition of structural units in different morphological regions, J. Wood Chem. and Technol., **10**, 435-459 (1990)

HIGUCHI, T. *et al.* : Differences of Chemical Properties of Lignins of Vascular Bundles and of Parenchyma Cells of Bamboo, Mokuzai Gakkaishi, **12**, 173-178 (1966)

HUMPHREYS, F.R. *et al.* : A method for determination of starch in wood, Anal. Chim. Acta, **24**, 66-70 (1961)

IIJIMA, T. *et al.* : The Properties and Chemical Components of Mangroves IV. Milled wood lignin from mangrove wood and viviparous seed, Mokuzai Gakkaishi, **37**, 834-840 (1991)

LAPIERRE, C. *et al.* : Photosynthetically ^{13}C-Labelled Poplar Lignins. ^{13}C NMR Experiments, Holzforschung, **38**, 333-342 (1984)

———— : Evidence of lignin heterogeneity by extraction of poplars wood, Proceeding of ISWPC, V, 35-39 (1981)

SCALBERT, A. *et al.* : Ether linkage between phenolic acids and lignin fractions from wheat straw, Phytochemistry, **24**, 1 359-1 362 (1985)

———— : Comparison of Wheat Straw Lignin Preparations. I. Chemical and Spectroscopic Characterizations, Holzforschung, **40**, 119-127 (1986)

TOMIMURA, Y. : Chemical Characteristics of Oil Palm Trunk, Bull. For. & For. Prod. Res. Inst., **362**, 133-142 (1992)

VENVERLOO, C.J. : The Lignin of *Populus nigra* L. cv. 'Italica' and Some Other *Salicaceae*, Holzforschung, **25**, 18-24 (1971)

(研究資料)

オイルパーム実生のリグニンについて

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摘　　要

発芽後1年目のオイルパーム実生から磨砕リグニン(MWL)を調製し，さらにその抽出残渣を酵素分解して，酵素分解リグニン(EL)を得た。また比較のため25年生の成熟材を維管束と柔組織に分割した上で，それぞれからMWLとELを調製し，実生からのものとの違いを検討した。実生からのリグニンは成熟材からのリグニンに比較してタンパク質が多く含まれていた。成熟材柔組織からのリグニンは最も多くのp-オキシ安息香酸エステルを含み，一方成熟材維管束からのリグニンは最も多くのシリンギル核に富んでいた。実生と成熟材柔組織からのMWLはシリンギル核/グアヤシル核の比がほぼ等しく1であった。またすべての試料でELはMWLよりもシリンギル核の割合が多く，残渣リグニンはシリンギル核に富むことが示唆された。材に含まれる糖の構成割合は大きな差が見られなかったが，リグニン中に含まれる糖はMWLの場合キシロースが圧倒的に多いがELではグルコースもかなりの割合を占めるようになり，特に実生の場合キシロースとグルコースの割合は，ほぼ等しかった。また成熟材では磨砕により抽出されるMWLの収率が高く，ELの収率が低いのに反して，実生の場合MWLの収率が低く，酵素分解で得られるELの収率が高いことから，実生のリグニンは成熟材のリグニンよりも糖と結合している部分が多いと推定された。

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