Variation in Chemical Components with wood Age of Keruing mempelas (*Dipterocarpus crinitus*)

By

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Summary : To investigate the effect of wood age on the chemical components of *D. crinitus*, three trees of different age (10, 30 and 50 year-old) were collected from the same place and their chemical characteristics of extractives and lignin were examined.

The successive extraction using n-hexane, ethyl-ether, acetone and methanol showed that the total extractives generally increased with the wood age. From the more than twenty compounds in the n-hexane extractive fraction, the main compounds were found to be hydroxydammarenone-II, ocotillone-I, ocotillone-II, dammarendiol and ocotillol-I by direct comparison with authentic samples using thin layer and gas chromatographical methods. The results indicated that the ratio of ocotillone-I and-II to hydroxydammarenone-II increased with the age of wood.

Klason lignin contents increased from 25.8% in the 10 year-old tree to 28.9% (sapwood) and 30.7% (heartwood) in the 50 year-old tree. The differences in the Klason lignin contents of alkali extractive-free samples, however, were within 1% among these different wood age samples. Alkali soluble lignin increased with the wood age from 2.8 to 4.2% in the sapwood and 6.1% in the heartwood. It shows that the high lignin contents were caused by the presence of alkali soluble lignin. The ratio of syringaldehyde to vanillin (S/V) obtained from the products of the nitrobenzene oxidation of both the wood-meals and the dioxane-lignin reveals that the younger tree had higher S/V values than the older ones, which suggests that the formation of syringyl-nuclei in lignin would decrease with wood age.

The results indicated that wood age had a significant effect on the chemical constituents of *D. crinitus.*

1. Introduction

It has been reported that tropical hardwoods contain more resinous materials and lignin than temperate hardwoods1)~3). These properties sometimes affect the wood color and decayresistance of the timbers as well as contributing to pitch problems and higher chemical consumption in pulping4), 5). OGIYAMA et al.6), 7) reported that the lignin-like substances (for example, polyphenol) in lauan (*Shorea* spp.), which could be extracted with aqueous 1% NaOH, would be included in the Klason lignin.

It is widely believed that tropical hardwoods have properties lying between those of softwoods and temperate hardwoods based on lignin contents, methoxyl group contents and characteristic intensity ratio in IR spectra of lignin8), 9). This observation is also supported by the S/V ratio of milled wood lignin on nitrobenzene oxidation10).

The *Dipterocarpus* species tested belongs to the family of Dipterocarpaceae which is an important genus of trees growing in South-East Asia. Some extractives in *Dipterocarpus* species have been reported by several workers11)~13). Many of the species in this family

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contain resin, which is often seen in large quantities in freshly cut logs.

It has been reported that there are clear differences in chemical composition and fibre morphology between imported tropical hardwoods (normally called tropical wood in Japan) and Malaysia hardwoods[14]. One possible reason for the differences could be the wood age factor between the two materials.

In this report, the effect of wood age on the chemical characteristics of extractives and lignin in a tropical hardwood as represented by Dipterocarpus crinitus was investigated.

2. Experimental

2.1 Wood samples

The samples were collected from the Merlimau Forest Reserve within an area of 1 square km (Jasin, Malacca, Malaysia). The estimated ages of the trees were taken from the records of the forest station. Identification of the species samples was done by the wood anatomy section of FRIM. Disks (20 mm thick) tested were cut from the breast height of the logs. They were converted into wood meal in the Wiley mill.

2.2 Organic solvent extraction

Extractions were done in a Soxhlet extractor with a 200 ml distillation flask and filter paper thimbles. Air dry wood meal (20 g, 40~60 mesh) were extracted successively with n-hexane, ethyl ether, acetone and methanol for 6 hrs. The solvents were evaporated in a rotary vacuum evaporator, and the residue was dried in a vacuum desicator.

2.3 Thin layer chromatography

Thin layer chromatography was carried out on silica gel (0.25 mm, Merk 5626) with n-hexane/ethyl acetate (8/2 and 7/3) or 15% ethyl acetate in benzene as developing solvents. 1%-Vanillin in concentrated sulphuric acid was used as the spray reagent.

2.4 Preparation of model compounds

Dammarendiol and ocotillol-I were prepared by the following procedure: Hydroxydammarenone-II (100 mg), or ocotillone-I (20 mg) was dissolved in 5 ml of methanol (or ethyl ether). Then three drops of 2% Na_2CO_3 solution and 50 mg of sodium-borohydride ($NaBH_4$) were added in the solution. The mixture was left to stand overnight. The solid precipitated was filtered and purified by means of a small scale column chromatograph on silica gel. An attempt to crystallize the compound was unsuccessful.

2.5 Gas chromatography (GC)

GC was performed on a Shimadzu GC-9 A chromatograph with a flame ionization detector. Separation for n-hexane extractives was made on a glass column (100 × 0.2 cm) packed with 2% OV-1 on chromosorb W (AW-DMCS) 80-100 mesh, with nitrogen as carrier gas at a flow rate of 50 ml/min. The column temperature was maintained at 245°C. For nitrobenzene oxidation products, separation was made on a glass colum (200 × 0.2 cm) packed with 2% OV-17 on Chromosorb W (AW-DMCS) 60-80 mesh, with nitrogen as carrier gas at a flow rate of 50 ml/min. The column temperature was programmed at 4°C/min from 160°C to 240°C. Trimethylsilyl (TMS) derivatives of the samples were prepared by Bis (trimethylsilyl)-acetamide and N-(Trimethylsilyl)-imidazol.

2.6 Preparation of dioxane-lignin (DL)

The extractive-free wood meal (4 g) was placed in a 1 liter, two necked flask fitted with a reflux condenser and a nitrogen bubbler. The solvent (dioxane/water, 9/1, 200 ml, containing 1.44 g HCl) was slowly added. The reaction mixture, under nitrogen, was heated over a water-bath and refluxed at 90-98°C for 4 hrs. The extract was concentrated under reduced pressure until a gummy material appeared. The lignin was precipitated by the addition of the extract to vigorously stirred chilled water (500 m*l*). The precipitated lignin was separated by centrifugation and decantation, washed thoroughly with water, separated again, and dried in a vacuum desiccator over phosphoric anhydride. The lignin was then added into stirred ethylether (250 m*l*). The lignin precipitated was separated by centrifugation, washed with ether and petroleum ether, and dried. The yield amounted to 30-50% of the Klason lignin contents of the wood.

2.7 Measurement of functional groups

The unconjugated phenolic hydroxyl group was calculated from the ionization difference spectra according to GOLDSHMID's method15). The alpha-carbonyl group was determined by the reduction difference spectra according to the method of ADLER et al16). The ultra-violet spectra were recorded on a Hitachi, model U100-30 spectrometer.

2.8 Alkaline-nitrobenzene oxidation

The mixture of lignin or wood meal (DL 50 mg, or wood meal 200 mg), nitrobenzene (1 m*l*) and 2 N NaOH (6 m*l*) in a micro-stainless steel tube (10 m*l*) was heated at 160°C for 2 hrs with vigorous shaking. After cooling, the contents in the autoclaves were filtered and wasshed with 5 m*l* of 1 N NaOH.

The combined filtrate and washing was extracted with ethyl ether (3 × 50 m*l*) to remove residual nitrobenzene. The solution was acidified to pH 2.5 with concentrated HCl and then extracted with ethyl ether (3 × 30 m*l*). The extract was dried over anhydrous sodium sulphate and evaporated dry.

3. Results and discussion

Several successive extractions using n-hexane, ethyl ether, acetone, and methanol were performed on samples from the three trees and from different locations across the stem in the medium-aged and old trees. The objective was to study the variation in extractives contents with tree age and between the sapwood and heartwood.

As shown in Table 1 and Fig. 1, the total amount of extractives in the sapwood generally increased with wood age from 2.4% in the 10 year-old tree to 3.0% in the medium-aged tree and 3.7% in the 50 year-old tree. In addition, the heartwood samples contained higher total extractives than the sapwood in the old tree. The amounts of n-hexane soluble fraction, which included neutral compounds such as paraffin, fatty acid, ester, terpenoid etc, varied from 0.28 to 1.16%, and it increased slightly with wood age.

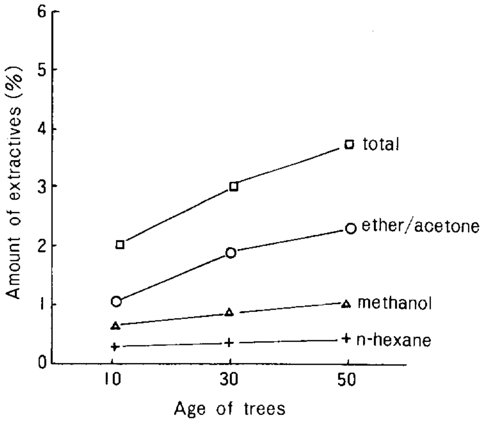


Fig. 1. Relationship between wood age and the amount of extractives in the sapwood of *D. crinitus.*

The results of the distribution of n-hexane and ethyl ether/acetone extracts across the wood disc revealed a different

Table 1. Yields of n-hexane, ether, acetone and methanol extractives from *Dipterocarpus crinitus*.

Sample name	Yield of extractives (%)				
	n-Hexane	Ether	Acetone	Methanol	Total ( )
Y	0.28	0.09	1.25	0.81	2.43
M-a	1.16	0.15	0.78	0.62	2.71 (2.77)
M-b	0.69	0.08	1.07	0.99	2.83
M-c	0.40	0.11	1.56	1.00	3.07 (2.98)
M-d	0.30	0.07	1.58	0.93	2.88
O-a	0.83	0.24	2.25	1.03	4.35 (4.84)
O-b	0.56	0.13	3.17	1.47	5.33
O-c	0.41	0.10	2.47	1.40	4.38 (3.70)
O-d	0.39	0.13	1.66	0.80	2.98

Note) Y; 10, M; 30, and O; 50 year-old trees. a and b are inner and outer heartwood, c and d are inner and outer sapwood, respectively. Figures in parentheses show the average in sap-and heartwood.

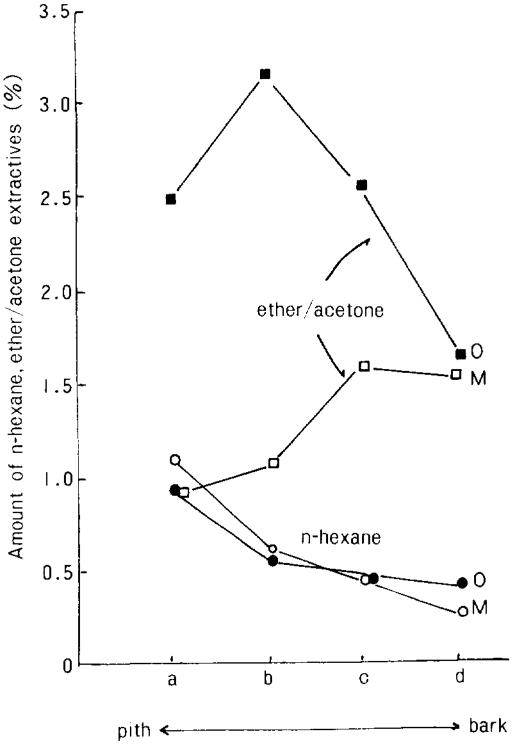


Fig. 2. Distribution of n-hexane and ether/acetone extractives in the cross section of *Dipterocarpus crinitus*.

Note) a,b inner and outer heartwood, c,d inner and outer sapwood, respectively.
(M : 30 year-old tree, O : 50 year-old tree)

tendency. For example, the n-hexane extractives decreased abruptly from the pith to the bark. The amount of ethyl ether/acetone extracts indicated an increasing trend in the 30 year-old tree and a decreasing trend in the 50 year-old tree from the pith to the bark (Fig. 2).

Thin layer chromatogram and gas-chromatograms of the n-hexane extractives are illustrated in Fig. 3, 4 and 5, respectively.

The thin layer chromatogram indicated that more than twenty compounds existed in the n-hexane extractives. The main compounds were found to be hydroxydammarenone-II, ocotillone-I, ocotillone-II, dammarendiol and ocotillol-I in comparison with their authentic specimens both at RF values and color reaction. Dammarendiol and ocotillol-I were synthesized from hydroxydammarenone-II and ocotillone-I on reduction in the usual way. The same components were also found when another developing solvent (15 % ethyl acetate in benzene) was applied for TLC analysis. The presence of these compounds in *Dipterocarpus* species has been reported[11, 12].

The chemical structures of the compounds identified are illusrated in Fig. 6.

Gas-chromatography of n-hexane extractives was carried out on trimethyl silylated derivatives treated with the mixture of Bis (trimethylsilyl) acetamide and N (trimethylsilyl) - imidazol. Trials on the separation of ocotillone-I and -II were unsuccessful. The results indicated that the ratio of ocotillone-I and -II to hydroxydammarenone-II increased from 34.0% in the 10 year-old tree to 79.4% (sapwood) and 45.4% (heartwood) in the 50 year-old tree (Table 2). The results suggest that the formation of ocotillone-I and/or -II in wood extractives increased with wood age. However, it was found that there were clearly different trends within the sapwood and heartwood in the formation of wood components. In addition to the amounts of the extractives, there was wide variation in chemical constituents with wood age. Further investigations on the extracts of ethyl ether, acetone and methanol will be continued in future work.

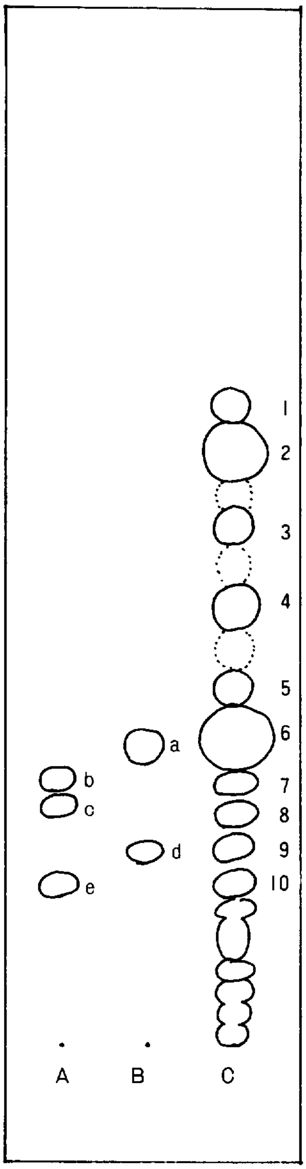


Fig. 3. Thin layer chromatogram of n-hexane extractives from *D. crinitus* (50 year-old, heartwood) and authentic compounds (a : hydroxydammarenone-II, b : ocotillone-I, c : ocotillone-II, d : dammarendiol, and e : ocotillol-I).

Note) Rf values of spot No. 6 to 10 correspond to a to e, respectively.

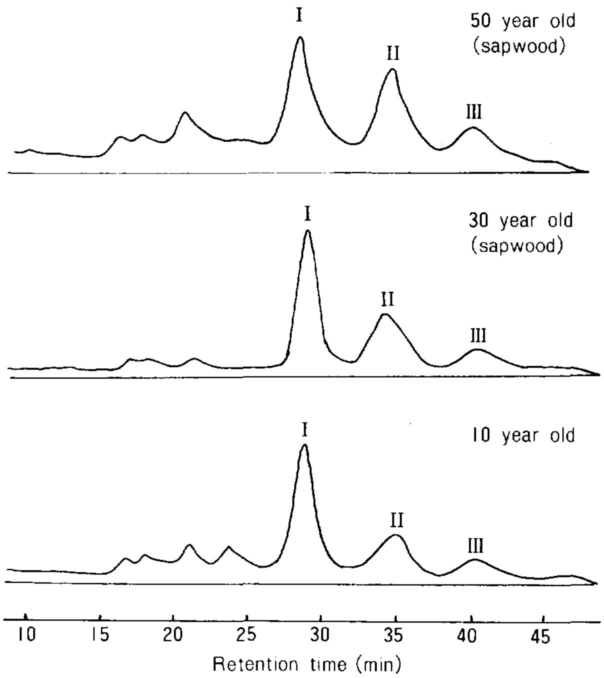


Fig. 4. Gas chromatograms of n-hexane extractives (trimethylsilylated) from *D. crinitus*.

Note) Condition ; glass column 100 × 0.2 cm, Packing ; 2% OV-1 on Chromosorb-W (AW-DMCS) 80-100 mesh. Temp. ; 245°C. Carrier ; N_2 50 ml/min. Peak I ; hydroxydammarenone-I, Peak II ; ocotillone-I and II, Peak III ; unknown.

Table 2. Relationship between wood age and the ratio of hydroxydammarenone-II to ocotilone-I and -II.

Sample	Peak II/Peak I ratio	
	Sapwood	Heartwood
Y	34.0	—
M	61.4	33.1
O	79.7	45.4

Note) Peaks I and II corespond to hydroxydammarenone-II and ocotillone-I and -II, respectively. Y, M and O are 10, 30 and 50 year-old trees (see Figures 4 and 5).

The other chemical components such as Klason lignin, acid soluble lignin, 1% NaOH solubles and alkali soluble lignin were determined on the extractive-free wood meal and shown in Table 3. For *D. crinitus*, the Klason lignin content increased from 25.8% in the 10 year-old tree to 28.9% (sapwood) and 30.7% (heartwood) in the 50 year-old trees. The lignin content in each heartwood sample was higher than in the sapwood of the same tree. Acid soluble lignin varied from 1.5 to 1.9%, but the influence of wood age was very small. 1% NaOH solubles, however, decreased with wood age from 9.7% in the 10 year-old tree to 8.4 % (heartwood) and 6.7% (sapwood) in the 50-year old trees. This indicates that the young tree probably contained more low molecular weight carbohydrates. The differences in Klason lignin content of alkali extractive-free samples, however, were within 1% among these different wood age samples. Alkali soluble lignin increased with wood age from 2.8

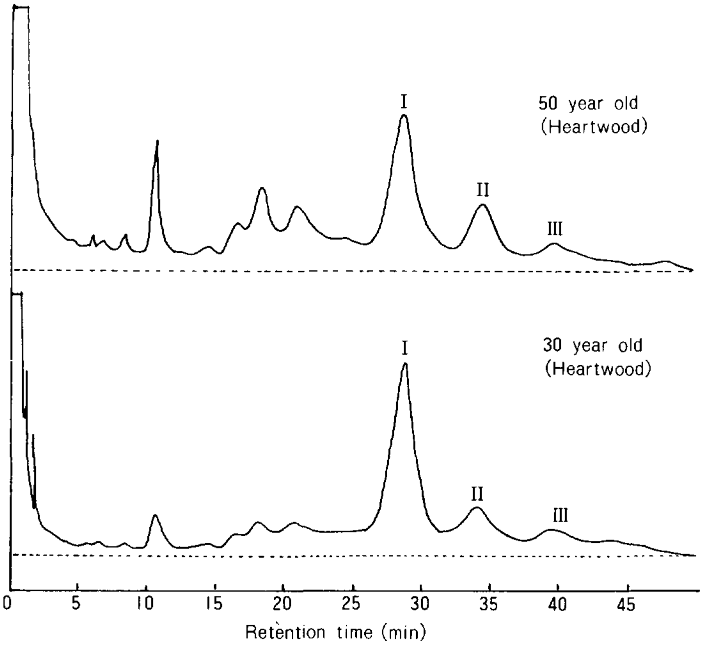
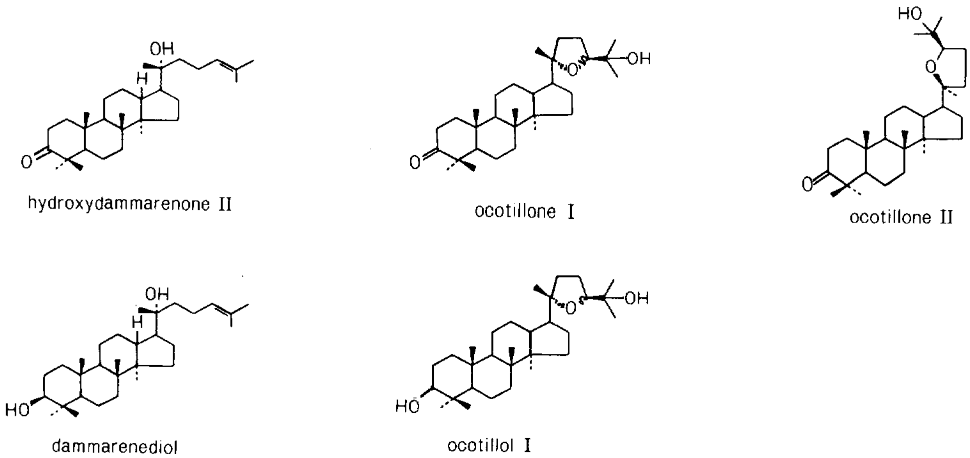


Fig. 5. Gas chromatograms of n-hexane extractives (trimethyl silylated) of *Dipterocarpus crinitus*.

Note) Condition ; glass column 100 × 0.2 cm, Packing ; 2% OV-1 on Chromosorb W, Temp ; 245°C, Carrier gas ; N_2 50 ml/min.
Peak I ; hydroxydammarenone-II, peak II ; ocotillone-I and II, peak III ; unknown.


hydroxydammarenone II

ocotillone I

ocotillone II

dammarenediol

ocotillol I

Fig. 6. Chemical structures of triterpenoid compounds found in the n-hexane extractives from *D. crinitus*.

Note) Structures of ocotillone-I and ocotillol-I not yet determined.

Table 3. Klason lignin contents and yields of alkali soluble lignin.

Sample	Klason-lignin-contents (%)*1		C. 1%-NaOH soluble from wood (%)*1	D. Alkali soluble lignin (%)*3
	A. Before 1%-NaOH extraction*2	B. After 1%-NaOH extraction		
Y	25.76 (1.49)	25.43	9.69	2.79
M-H	28.14 (1.87)	25.20	8.51	5.08
M-S	26.59 (1.84)	24.81	6.89	3.49
O-H	30.72 (1.64)	26.85	8.42	6.13
O-S	28.86 (1.92)	26.40	6.73	4.24

Note) *1 Based on the pre-extracted wood by weight.
*2 Acid soluble lignin.
*3 D = A − (100 − C) × B/100
Y, M, and O are 10, 30, and 50 year-old trees.
H : Heartwood, S : Sapwood.

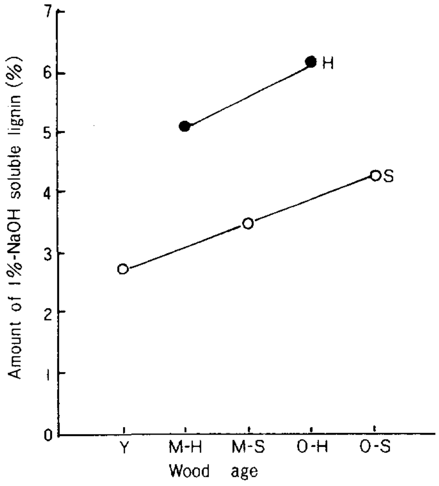


Fig. 7. Relationship between 1% alkali soluble lignin and wood-age of *Dipterocarpus crinitus* (Y ; 10, M ; 30 and O ; 50 year-old, H ; heartwood, S ; Sapwood).

% in the young tree to 4.2% (sapwood) and 6.1% (heartwood) in the old tree (Fig. 7). It shows that the increase in lignin content as the tree aged was caused by the increase in alkali soluble lignin. A similar tendency was observed in the case of *Shorea* spp. (lauan)[6].

To investigate the chemical characteristics of the lignin from the different wood age samples, dioxane-lignins were prepared and analysed as to their functional groups such as unconjugated phenolic hydroxyl and alpha-carbonyl groups. The ionization and the reduction difference spectra of the dioxane-lignin from the 30 year-old *D. crinitus* are shown in Fig. 8. According to the ionization difference spectra, the phenolic hydroxyl groups in the lignin slightly increased with wood age from 1.2 to 1.7% based on the original dioxane-lignin, but the differences between sap- and heartwood were very small. In contrast, the alha-carbonyl group contents were almost similar varying 0.21 to 0.25% in all the samples (Table 4).

Alkaline nitrobenzene oxidation was performed both on the wood meal and the dioxane-lignin to investigate the basic units of the lignin structures. The amounts of each aldehyde contained in the mixture were determined. Fig. 9 shows an example of the gas-chromatograms, which were conducted on the ether soluble fraction (trimethyl silylated) obtained from nitrobenzene oxidation products of *D. crinitus* (50 year-old, heartwood) using an internal standard method.

No p-hydroxybenzaldehyde was found in

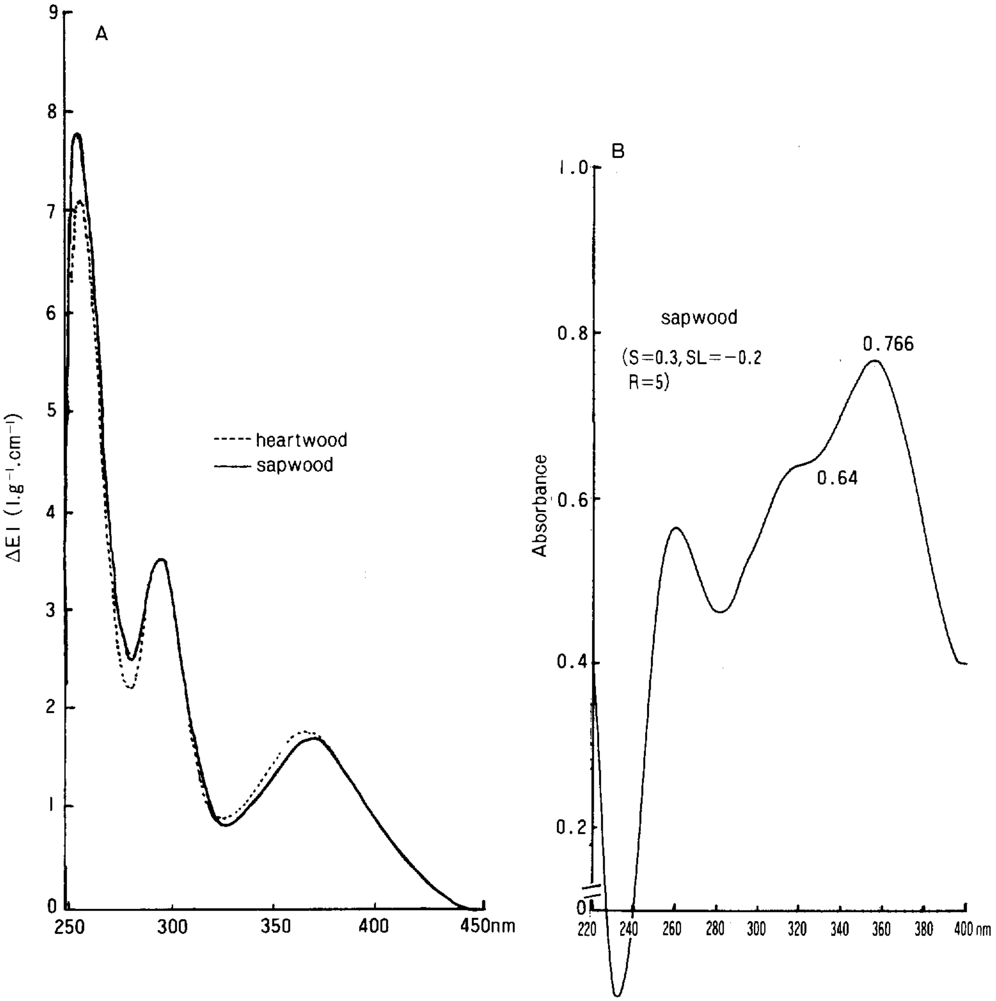


Fig. 8. Ionization difference spectra (A) and reduction difference spectrum (B) of the dioxane-lignin from 30 year-old *D. crinitus*.

Table 4. Amounts of unconjugated phenolic hydroxyl and alpha-carbonyl groups in the dioxanelignin of *D. crinitus*.

Sample	Dioxane-water (9 : 1) solubles (%)	Phenolic hydroxyl group (%)¹⁾	alpha-carbonyl group (%)¹⁾
Y	37.6	1.21	0.22
M-H	33.8	1.47	0.23
M-S	36.1	1.46	0.20
O-H	28.1	1.68	0.21
O-S	36.5	1.73	0.25

Note) Y, M and O are 10, 30 and 50 year-old trees. H and S are heartwood and sapwood.
Dioxane water solubles is based on wood meal (ovendried).
1); based on dioxane lignin.

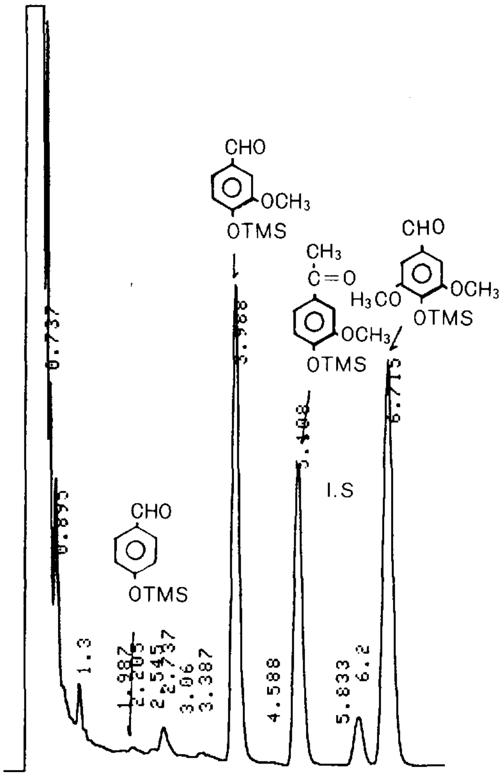


Fig. 9. Gas chromatogram of ether soluble fraction (trimethyl silylated) obtained from nitro-benzene oxidation of *D. crinitus* (50 year-old sapwood wood meal).

Table 5. Yields of vanillin (V), syringaldehyde (S), and S/V ratio of nitrobenzene oxidation products from *D. crinitus*.

Sample	V	S	S/V	V+S	Yield (%)
DL-Y	4.23	6.08	1.43	10.31	20.62
DL-M-H	4.84	4.88	1.01	9.72	19.44
DL-M-S	4.62	5.40	1.17	10.02	20.04
DL-O-H	5.90	6.56	1.34	11.46	22.92
DL-O-S	6.56	6.04	0.92	12.60	25.20
WM-Y	3.42	5.68	1.66	9.10	17.66
WM-M-H	6.54	8.28	1.26	14.82	26.35
WM-M-S	5.80	7.96	1.37	13.79	25.98
WM-O-H	8.26	7.62	0.92	15.88	25.85
WM-O-S	9.06	8.70	0.96	17.76	30.76

DL : Dioxane-lignin, WM : Wood-meals, Y : 10 year-old, M : 30 year-old, and O : 50 year-old. H : Heartwood S : Sapwood, V : Vanillin, S : Syringaldehyde.

all oxidation products of *D. crinitus*. Yields of vanillin (V), syringaldehyde (S) and S/V ratio are shown in Table 5. The yield of V and S in the oxidation products varied from 19.4 to 25.2% in dioxane-lignin, and from 17.7 to 30.8% in wood meals (based on Klason lignin). The wood meal sample from the 10 year-old tree showed a low yield compared to other samples. However, these yields were not unexpected in comparison with data reported previously[17].

S/V ratio decreased with wood age from 1.43 to 0.92% in dioxane-lignin, and from 1.66 to 0.92% in wood meals. The higher ratio of syringaldehyde to vanillin (S/V) in the younger tree compared to the older ones suggests that the formation of syringyl-nuclei in lignin decreased with wood age.

The results indicated that wood age had a significant effect on the chemical constituents of *D. crinitus*.

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クルイン（*Dipterocarpus crinitus*）材成分の樹齢による変化

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摘要

熱帯材の化学的特性を明らかにするため，同一環境下で生育した樹齢の異なるクルイン材（*Dipterocarpus crinitus*, 10, 30および50年生）を供試料とし抽出成分およびリグニンの化学特性を調べた。

n-ヘキサン，エチルエーテル，アセトンおよびメタノールによる逐次抽出物の合計は樹齢とともに増大した。n-ヘキサン抽出物の主成分はHydroxydammarenone-Ⅱ，Ocotillone-Ⅰ，Ocotillone-Ⅱ，DammarendiolおよびOcotillol-Ⅰで，Ocotillone-Ⅰ＋Ⅱ/Hydroxydammarenone-Ⅱの比が樹齢とともに増加していることが特徴的に認められた。

クラソンリグニン含量は10年生の25.8%から，50年生の28.9%（辺材），30.7%（心材）と樹齢とともに増加し，それぞれ心材の方が高リグニン量を示した。アルカリ可溶性リグニン量は樹齢とともに2.8%から4.2%（辺材）および6.1%（心材）に増加し，樹齢により増加するリグニン量はアルカリ可溶性リグニンの増加に由来していることが明らかにされた。各試料の木粉，ジオキサンリグニンのニトロベンゼン酸化分解物より得られるSringaldehyde (S) とVanillin (V) の比 (S/V) を分析した結果，10年生の1.66から50年生の0.96（木粉）に減少し，樹齢の若い材の方が高い値を示し，リグニン中のシリンギル核の形成能が樹齢とともに低下する可能性が示唆された。

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