Lignin Analysis in Some Tropical Hardwoods Using Ultraviolet Microscopy

By

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顕微分光測光による数種熱帯産材のリグニン分析

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ABSTRACT

The lignins of some tropical hardwoods in Xishuangbanna, Yunnan province, Southern China, were investigated using a UV microspectrophotometry and other means. UV spectra of thin sections, VIS spectra after Mäule and wiesner reactions, Klason lignin contents and S/V molar ratios with nitrobenzene oxidation were obtained from the samples which were extracted with ethanol-benzene or with ethanol-benzene and 1% NaOH.

The results were as follows: 1) Lignins in all the elements of the species examined were rich in guaiacyl. 2) The alkali soluble fraction of lignins was abundant in parenchyma cells. 3) In the species with a high Klason lignin content, S/V radio values were extremely low and increased after alkali extraction. 4) The absorbance of fiber in the species with high klason lignin content was higher than that with normal klason lignin content.

A wider survey will be needed to confirm lignin heterogeneity in tropical hardwoods.

Key words: Lignin analysis, Histochemistry, Ultraviolet microspectrophotometry, Tropical hardwoods.

INTRODUCTION

Lignins of tropical hardwoods are different from those of temperate hardwoods, and they have been classified into types Lt and Ls, respectively, by KAWAMURA, et al. (1963, 1964, 1965). We are now investigating their micromorphology by SEM observation of some Yunnan hardwoods, which were collected from South China. In addition we are also

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investigating the UV-absorption spectra of wood elements in these trees because of such lignin heterogeneity. We must recognize that there are technical limitations in identifying lignin characteristics by using ultraviolet microscopy. It will be useful, however, to emphasize the heterogeneity of lignins among species and the elements of tropical hardwoods for the study of tree phylogeny and others.

This is a preliminary study of about 5 species selected from 71 species, the microstructures of which were observed using scanning electron microscopy (Wu et al. 1989). An ultimate object of our investigation is the mapping out of the lignin histochemical characteristics of all species. And thus, we tried in this study to establish and to survey some useful methods using ultraviolet microspectrophotometry and chemical analysis.

MATERIALS AND METHODS

Five tropical species in southern Yunnan Province in China. *Duabanga grandiflora, Toona ciliata, Anthocephalus chinensis, Gmelina arborea* and *Callicarpa poilanei*, were used in this study. They are common hardwoods in the Xishuangbanna District. *Duabanga grandiflora* (Sonneratiaceae) and *Anthocephalus chinensis* (Rubiaceae) are common fast growth species in southern China. *Toona ciliata* (Meliaceae) is one of the useful commercial timber varieties having beautiful color and excellent wood properties, *Gmelina arborea* (Verbenaceae) is also one of the most useful timber varieties and is similar to true teak (*Tectona grandis*) being in the same family. *Callicarpa poilanei* (Verbenaceae) is not so highly regarded for its timber, but it is important for some medical uses (Table 1).

Table 1. List of species examined.

Family	Species
Sonneratiaceae	*Duabanga grandiflora* (ROXB.) ex DC. WALP.
Meliaceae	*Toona ciliata* ROEM.
Rubiaceae	*Anthocephalus chinensis* (LAM.) RICH ex WALP.
Verbenaceae	*Gmelina arborea* ROXB.
	Callicarpa poilanei P. DOP.

Small block samples were taken from the outer xylem of living trees grown at Xishuangbanna, and wood samples collected at the Kunming Institute of Botany, Yunnan Institute of Tropical Botany, the Academy of Sciences of China, Southwest Forestry College and Forest Institute of Yunnan were used for supplementary experiments.

The experimental diagram is shown in Fig. 1. Cross sections of 10μm thickness were used for the Mäule and Wiesner reactions. After the small blocks were subject to extraction in an ethanol-benzene(1 : 2) mixture for 6 hours, 1μm sections of the blocks were taken for UV microspectroscopy. Wood flour was used for the determination of the Klason lignin content and for nitrobenzene oxidation after the extraction of the ethanol-benzene mixture. To extract the alkali soluble fraction in the lignin, some of the small blocks and wood flour were extracted by 1% sodiumhydroxide(NaOH) during 2 hours after the extraction by ethanol-benzene, and were examined for UV spectroscopy and chemical tests.

For the Mäule reaction, the cross sections, which were cut into 10μm thickness, were

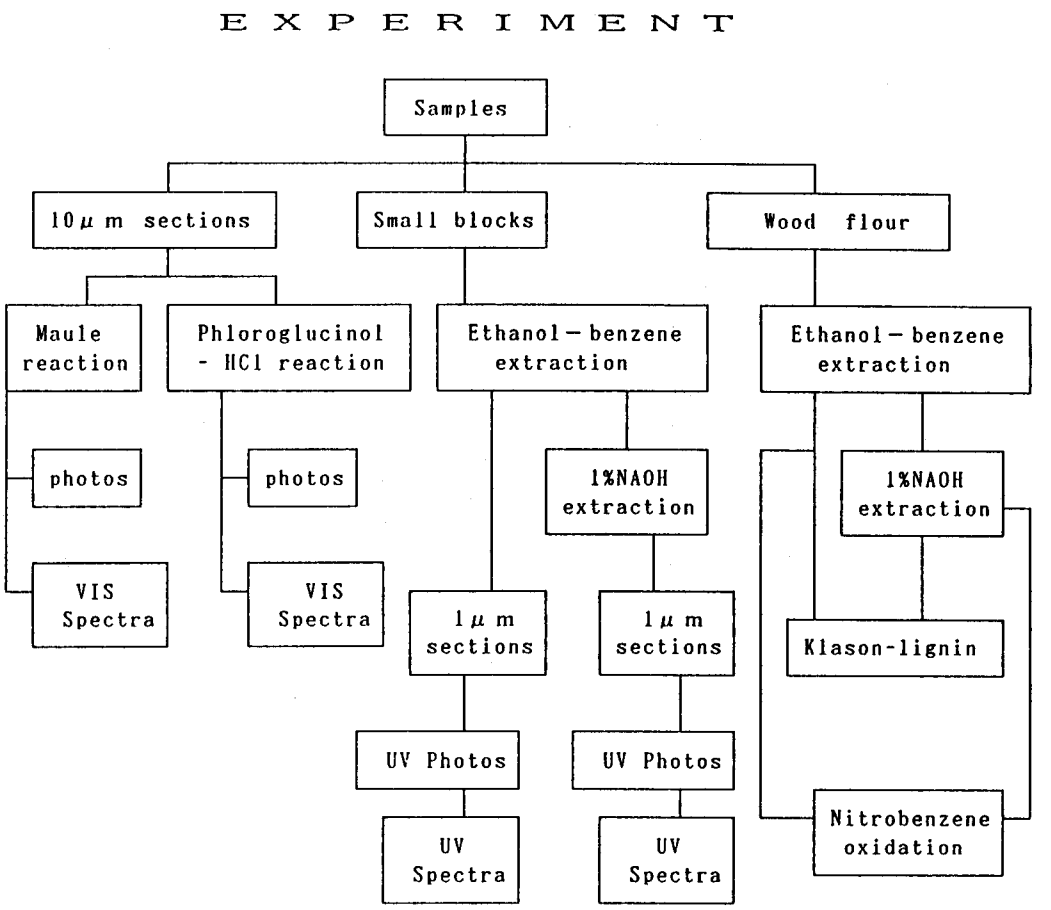


Fig. 1. Diagram of experimental process.

left in 1% aqueous potassium permanganate (5 minutes), rinsed in distilled water (2 or 3 times), immersed in 3% hydrochloric acid, rinsed in water again, and mounted on slides just after immersion in strong ammonium. For the Wiesner reaction, the cross sections mounted on slides were rinsed in a few drops of 0.2% phloroglucinol in ethanol and treated with a few drops of concentrated hydrochloric acid.

Microspectrophotometrical analysis was conducted using an UV microspectrophotometer (Carl Zeiss UMSP 80). Visible light microspectrophotometry was carried out under the following conditions: measurement at 5 points of the secondary walls of fibers and vessels, and of the cell corner regions just after the staining of the lignins because of the rapidity of fading; 1.25μm spot size, 5nm band width, 400-700nm wave range, and 5nm scanning step width, measurements were repeated 3 times (TAKABE and FUKAZAWA 1989). Maximum absorbance was observed at 520nm in the Mäule positive and at 565nm in the Wiesner positive reaction.

UV absorption spectra of fibers, vessel elements, ray parenchyma and cell corners among the fibers were taken at a spot diameter of 1.25μm for fibers and a spot size of

0.5μm for other elements, a band width of 5nm, a wave range of 250-300nm, and a scanning step width of 1nm, measurements were repeated 3 times and were taken at 10 positions for each constituent.

RESULTS AND DISCUSSION

1. Color reaction

It is reported that the Mäule and Wiesner positive reactions shown red or red-purple color; the former is due to the presence of syringyl nucleus while the latter is due to the presence of coniferyl aldehyde groups in the guaiacyl lignin. The absorption maxima were observed about 520nm and 550nm, respectively, according to the model substances (NAKANO 1979).

All 5 species examined in this study did not show a red color in the Mäule reaction (the microphotographs are omitted here). Fig. 2 is the visible ray absorption spectra in a microportion of a *Toona* species. Peculiar peaks at 520nm of Mäule positive were not seen in any components. That there was a negative Mäule reaction in all the species examined is a question to be resolved. SRIVASTAVA (1966) reported that the secondary xylem of Austrobaileya scandens was the only Mäule negative among a large number of samples examined. However, she mentioned that the intensity of lignin reaction in wood cells of different species varied a great deal.

Fig. 3 is an example of the visible ray absorption spectra of Wiesner reaction in *Toona* species. Every component had a peak at 560nm. Thus, every component and every

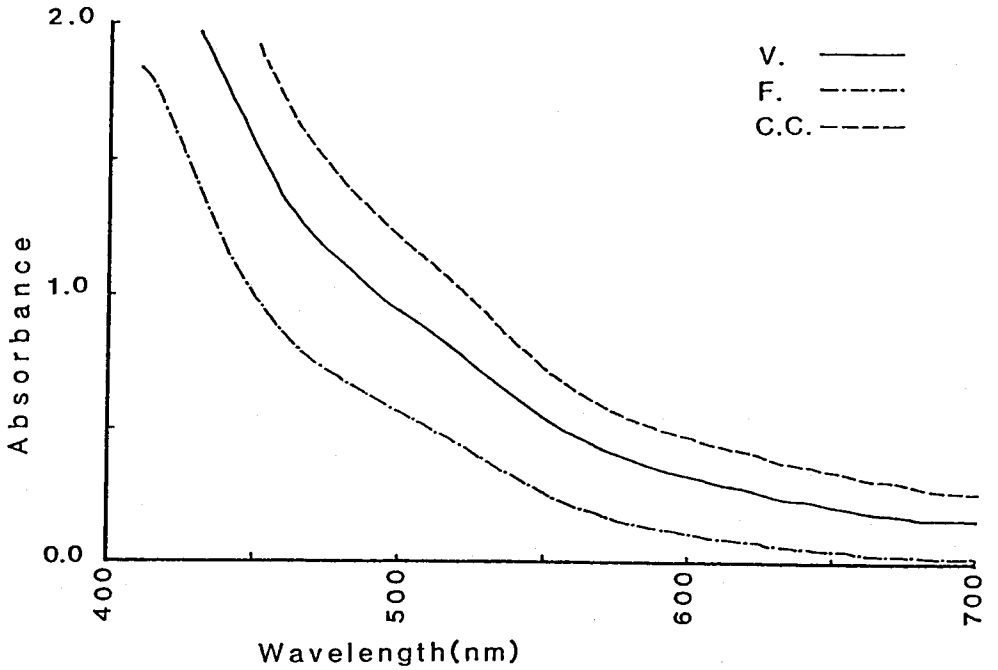


Fig. 2. Abstrption spectra of Mäule reaction in *Toona ciliata*.
V: Vessel F: Fiber C.C.: Cell corner among fibers.

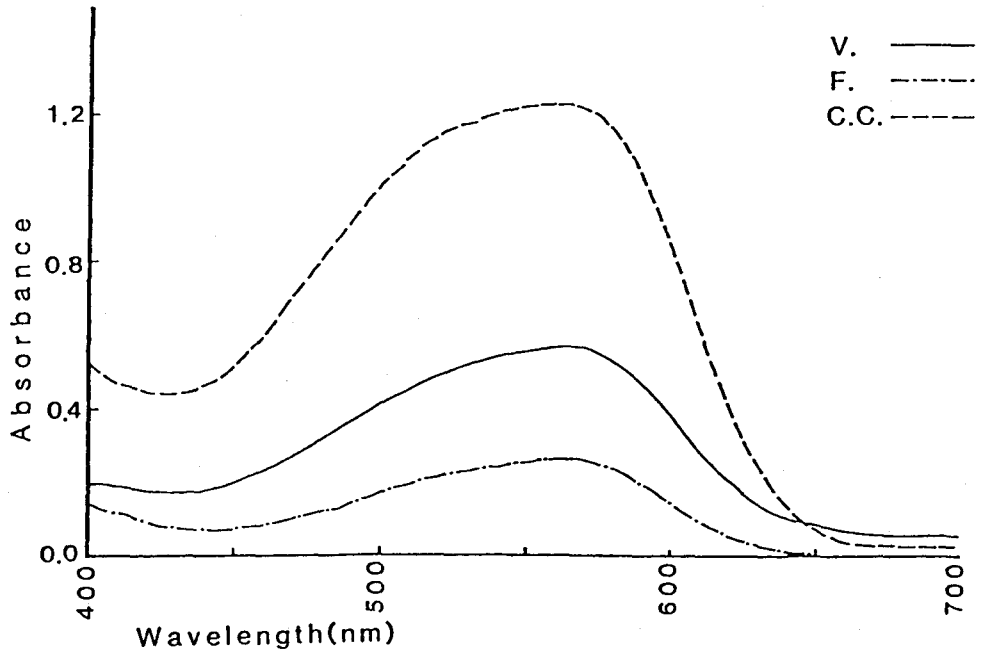


Fig. 3. Absorption spectra of Wiesner reaction in *Toona ciliata*.
V: Vessel F: Fiber C.C.: Cell corner among fibers.

species manifested a typical Wiesner reaction.

2. UV absorption spectra

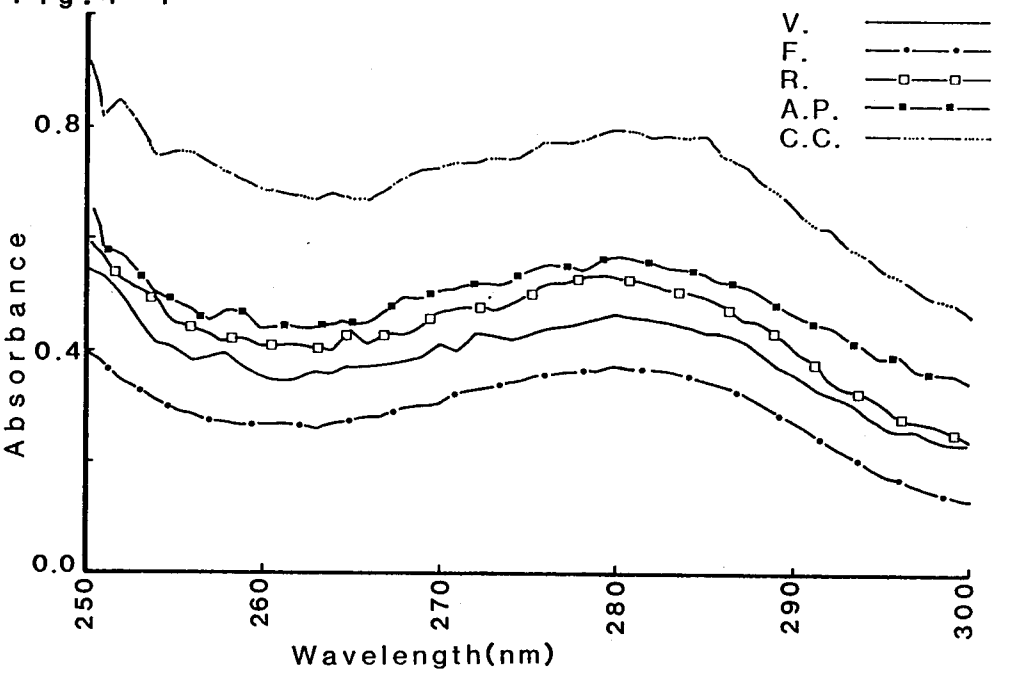
The UV absorption spectra examined in the cell walls of fibers, in vessel elements, in ray and axial parenchyma, and in the cell corners among fibers are shown in Fig. 4 (1-5). The proportion of the UV-absorption rate at 280nm to that at 274nm was calculated and the results are shown in Table 2. These were the results from the samples which only were extracted with ethanol-benzene.

In *Duabanga grandiflora* (Fig. 4-1), the absorption peaks for each element were about 280nm and the ratio (280nm/274nm) for fibers was 1.08; the ratio for the other elements were about 1.10 (Table 2). In *Toona ciliata* (Fig. 4-2), the absorption peaks of the elements were found to be from 278nm to 280nm and the ratio (280nm/274nm) for the fibers was 1.06. In *Anthocephalus chinensis* (Fig. 4-3), all components also had peaks from 278nm to 280nm and the ratio (280nm/274nm) for the fibers was 1.05; the rest were from 1.05 to 1.07. *Gmelina arborea* also had absorption maxima at 280nm and 281nm in all components (Fig. 4-4) and the ratios were from 1.04 to 1.07. *Callicarpa poilanei* had the same tendency (Fig. 4-5).

From these graphs, it is suggested that the lignins in all elements of the samples are belong to the guaiacyl type. In temperate zone hardwoods, it is now generally accepted that the lignins of vessel elements were guaiacyl type, and that the lignins of the secondary walls of fibers and parenchyma cells and the middle lamella among fibers were rich in

UV Spectra of Duabanga grandiflora

Fig. 4 - 1



UV Spectra of Toona ciliata

Fig. 4 - 2

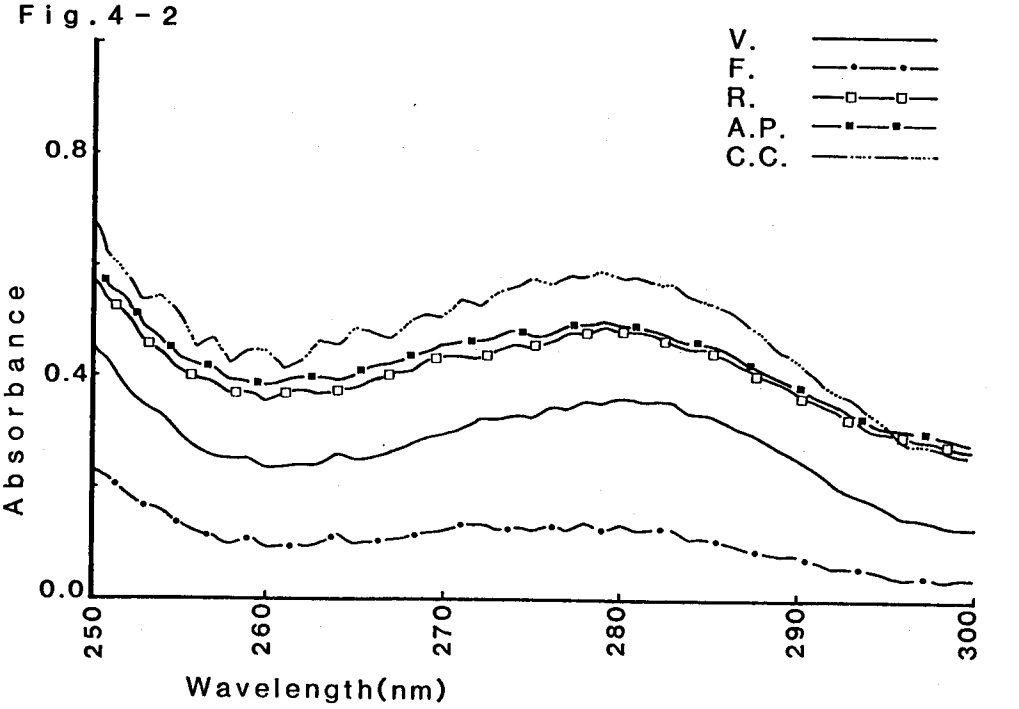


Fig. 4-3 UV Spectra of Anthocephalus chinensis

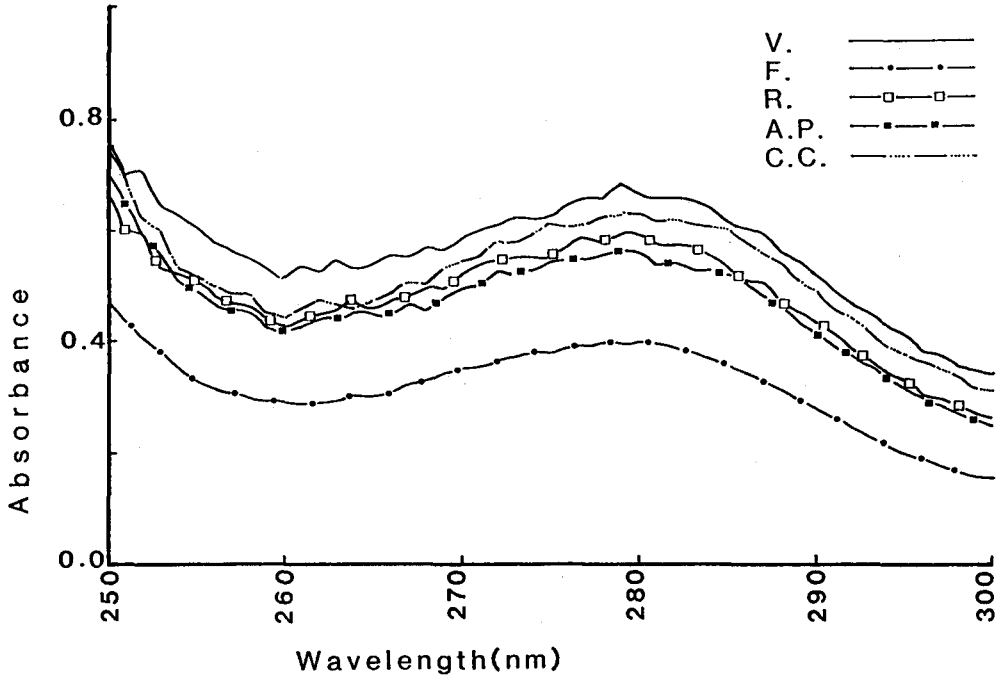
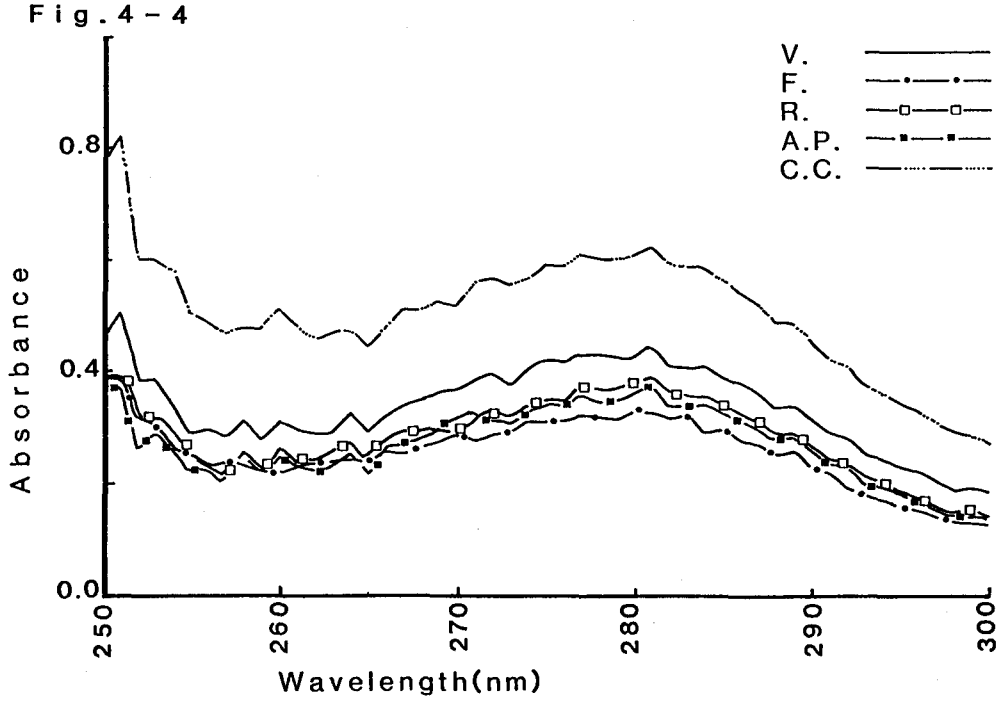


Fig. 4-4 UV Spectra of Gmelina arborea



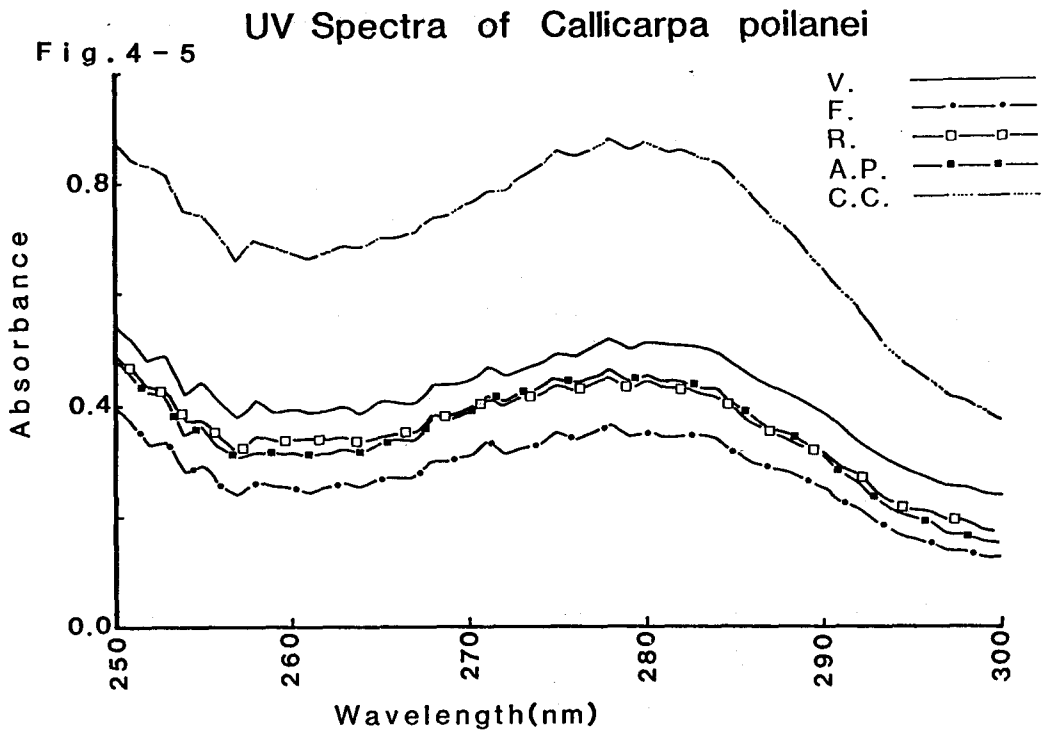


Fig. 4(1-5). UV-absorption spectra in the cell walls of fibers, vessel elements, ray and axial parenchyma and in the cell corners among fibers of all species examined.
V.: Vessel F.: Fiber R.: Ray A.P.: Axial parenchyma C.C.: Cell corner among fibers.

Table 2. The ratio of UV-absorption spectra at 280nm to that at 274nm in the different morphological regious of woods examined.

Sample	Elements				C.C.
	F.	V.	R.	P.	
Duabanga grandiflora	1.08	1.11	1.09	1.10	1.07
Toona ciliata	1.06	1.10	1.06	1.01	1.03
Anthocephalus chinensis	1.05	1.06	1.07	1.05	1.07
Gmelina arborea	1.04	1.05	1.10	1.10	1.07
Callicarpa poilanei	1.06	1.08	1.07	1.06	1.06

syringyl type or syringyl-guaiacyl type. Guaiacyl lignins exhibit a maximum absorbance at 280nm (MUSHA and GORING 1975), while syringyl lignins exhibit the same at 274nm-(FUKAZAWA 1988), the ratio of guaiacyl residues to syringyl residues in the secondary walls of fibers were different from wood to wood as shown by the ratios of UV absorbance at

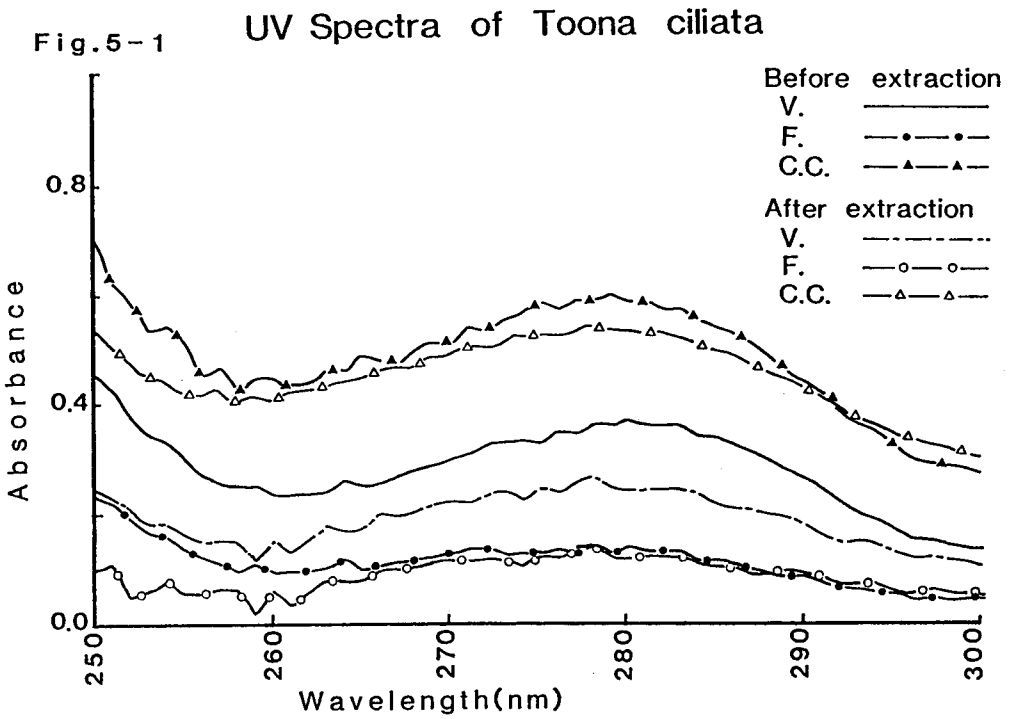
280nm to 274nm, but the ratio for fibers were near to that for vessels shown in Table 2.

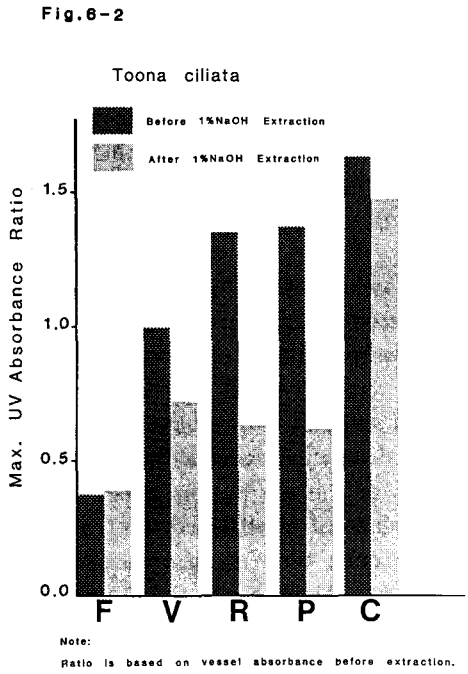
It follows that the lignin composition of the fibers in the species examined is similar to that of the vessels. The results also indicate that guaiacyl lignins were abundant in the cell walls of the different morphological regions of woods examined. On the other hand, the lignins in tropical hardwoods, it can be said, should be classified as a distinct group of low-syringyl lignins (SARKANEN 1971). Further experiments and a comparison of the constuents are described later.

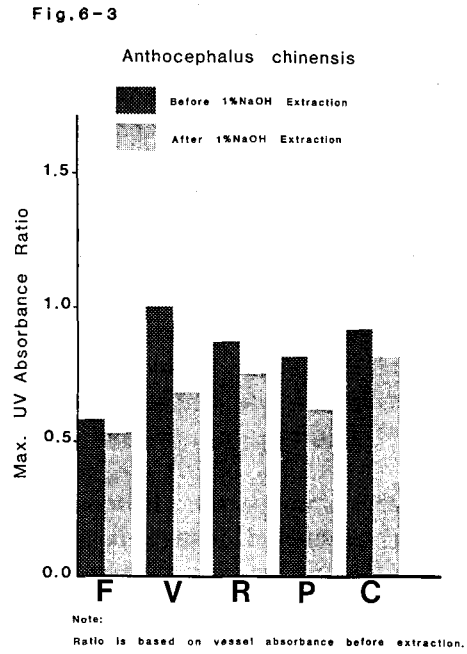
3. Alkali soluble fraction of lignin

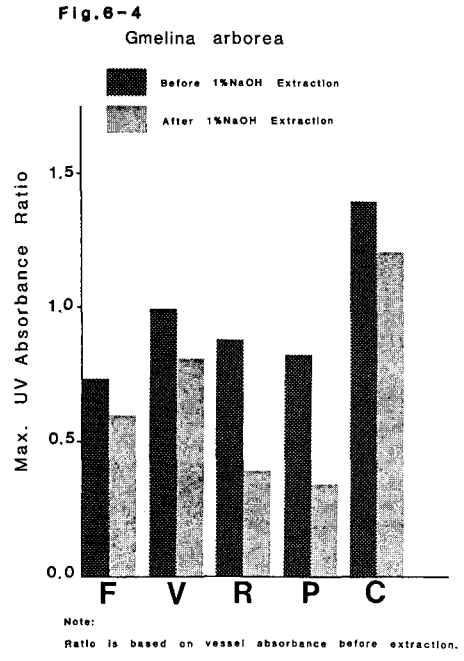
Besides the type of lignin present, the characteristics of tropical hardwood lignins were investigated. OGIYAMA and TANIGUCHI (1971) discovered the presence of an easily soluble alkali fraction in tropical hardwoods lignin and their content amounted to about 10% of the wood and 30% of the Klason lignin content of the extracted wood. extraction with 1% sodiumhydroxide was conducted for the preextracted samples with ethanol-benzene.

Fig. 5(1-2) illustrates examples of the difference in absorption spectra before and after extraction with 1% NaOH in *Toona ciliata*. Shifts of the absorption peaks were not recognized in anyspectra. However, a marked decrease of absorbance after alkali extraction was observed in the axial and ray parenchyma cells, while no difference was found between them in the fiber walls and cell corner regions. The manner and amount of the decrease should differ among the species and cell components although a strict comparison is not possible due to the different sections examined. The absorbance ratios among the elements based on the absorbance of vessel elements before alkali extraction was discussed as shown in Fig. 6(1-5).









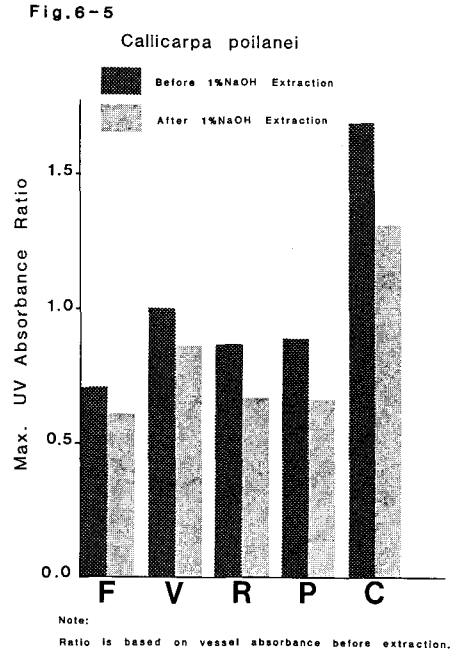


Fig. 6(1-5). The comparison of maximum UV-absorption, which before and after alkali extraction, in the different morphological regions of all species examined.
F: Fiber V: Vessel R: Ray
P: Axial parenchyma C: Cell corner

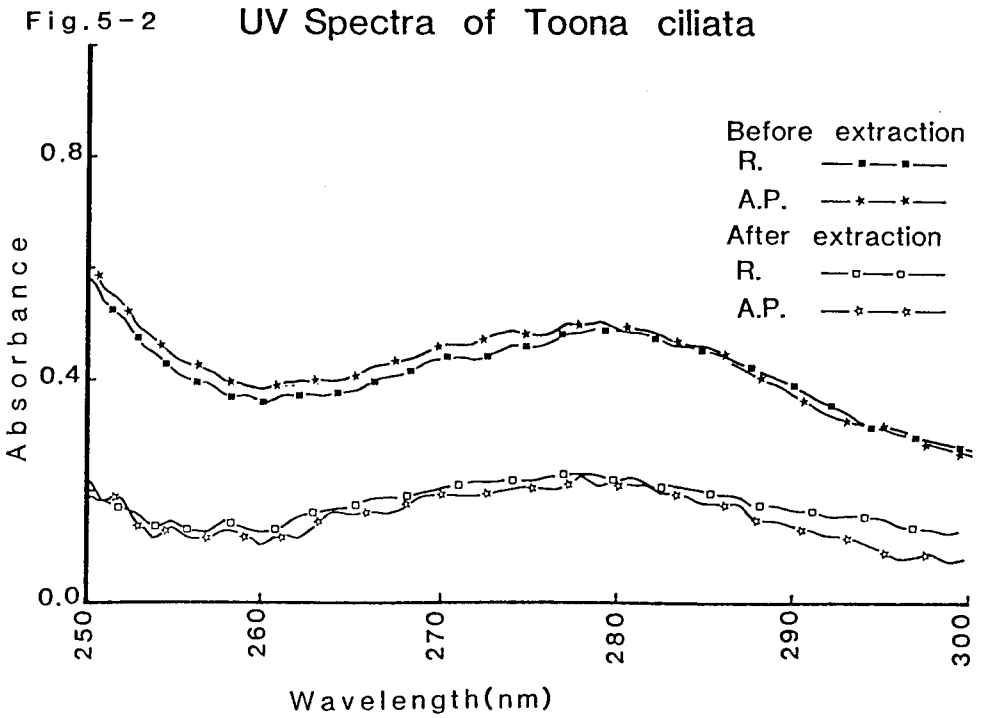
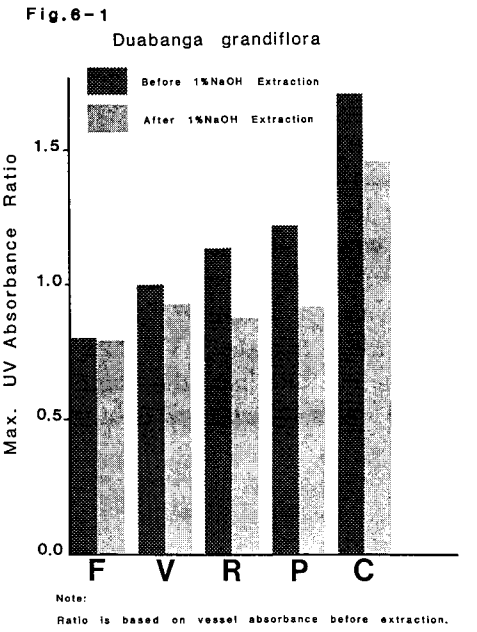


Fig. 5(1-2). The comparison of UV-absorption spectra in *Toona ciliata* which before and after alkali extraction with 1% NaOH.
V.: Vessel F.: Fiber C.C.: Cell corner
R.: Ray A.P.: Axial parenchyma.

The absorbance ratio of vessel elements to the cell corner regions is 1.7, for parenchyma cell the ratio is 1.4 and for fiber the ratio is 0.4 in *Toona*, as shown in Fig. 6-1. This shows a decrease of 50% against the previous extraction in the parenchyma cells, as well as a 30% decrease in the vessel elements and a 10% decrease in the cell corner regions. No decrease was seen in the fibers. *Duabanga grandiflora* (Fig. 6-1) had the same pattern of decrease, but it was of a lesser magnitude than that for *Toona ciliata*, namely, 25% in the parenchyma cells, 15% in the cell cornen regions after alkali extraction. Both *Toona* and *Duabanga* had a higher lignin content in their parenchyma cells than in their vessels before alkali extraction, but this tendency diminished after alkali extraction.



In the other 3 species, *Gmelina, Callicarpa* and *Anthocephalus*, the lignin content in ray and axial parenchyma cells was higher than that of vessel elements. In the case of Gmelina (Fig. 6-3), the lignins in parenchyma cells decreased about 60% more than those of other cells after alkali extraction. *Callicarpa* (Fig. 6-4) showed the same pattern as *Gmelina*, but the rate of decrease was not so high, and lignins in cell corners decreased to the same degree as those in parenchyma cells. In *Anthocephalus*, the decrease of lignins in vessels was rather high compared to that of parenchyma cells.

4. klason lignin content and S/V values

Table 3 shows the syringaldehyde-vanillin ratio and Klason lignin content by using chemical methods. The results also confirm rich guaiacyl lignins in these species.

The determination of S/V values offers a convenient method for comparing lignins, and they have been reported for a large number of trees, ranging from 0.35 to 5.2 (SARKANEN 1971). according to SARKANEN, primitive dicotyledons such as Trochodendraceae have low S/V values range from 0.35 to 1.7. In tropical hardwoods, the Klason lignin content averages about 28.7% as compared with the average lignin content of 22.1% for temperate zone hardwoods, and the S/V values are usually below 1.0 (SARKANEN 1971, KAWAMURA & HIGUCHI 1965).

Table 3. Klason lignin content and syringaldehyde/vanillin (S/V) molar ratio from nitro-benzene oxidation of woods examined.

Sample	Klason Lignin (%)	S/V ratio	
		Before extraction	After extraction
Duabanga grandiflora	27.88	0.32	0.45
Toona ciliata	16.91		
Anthocephalus chinensis	20.76	0.53	0.58
Gmelina arborea	21.98	0.44	0.40
Callicarpa poilanei	25.50	0.26	0.37

The S/V values given in Table 3 are below 1.0, showing the predominance of guaiacyl propane units. This also shows that the more the Klason lignin content increases, the lower the S/V values become. *Duabanga* and *Callicarpa* had a high Klason lignin content, their values being the same as the values reported for tropical hardwood by others. Their S/V values are lower than those for *Anthocephalus* and *Gmelina* which had same Klason lignin content as temperate zone hardwoods, and they increased after alkali extraction. In contrast to this, the S/V values of *Anthocephalus* and *Gmelina* did not change after alkali extraction. Perhaps the vanillin component of lignins was more easily extracted in *Duabanga* and *Callicarpa* than in *Anthocephalus* and *Gmelina*.

要　　約

一般的に，広葉樹リグニンはシリンギル型とグアイアシル型からなり，組織細胞の種類や部位によって構成単位の分布が異なる。道管壁と木繊維の細胞間層のリグニンは大部分がグア

イアシル型からなり，木繊維の二次壁はシリンギル型が豊富であり，柔細胞壁リグニンは両型の混合である。また，川村らは，熱帯産広葉材のリグニンは温帯産広葉材のものとかなり性質が異なっていると報告している。筆者らは中国雲南産の主要広葉樹材の組織構造を調べてきた。今回，その中の数種の熱帯産材の道管壁，木繊維二次壁，放射・軸方向柔細胞壁及び木繊維の細胞間層におけるリグニンの構造について顕微分光学的方法で分析した。

試料は雲南省現地で生立木の辺材部からとった小ブロック及び中国昆明植物研究所，雲南省林業科学院と西南林学院から提供された材鑑を用いた。試料はアルコール・ベンゼン抽出とアルカリ抽出したものの2通りを行った。試料から1 μm 厚さの木口面切片を作成し，Zeiss 社製の紫外線顕微分光光度計 UMSP-80 を用いて，UV スペクトルを測定した。また，フロログルシン-塩酸呈色反応とモイレ呈色反応を行い，クラソンリグニンを定量し，S/V 比はアルカリ・ニトロベンゼン酸化によって求めた。

実験された熱帯産材のリグニン構成単位の分布は温帯産のものとかなり異なっている。(1)道管壁：温帯産材と同じ，相対的な吸光度は木繊維細胞間層より低いが，木繊維と柔細胞の二次壁より高い。最大吸収波長はほぼ 280 nm に位置して，グアイアシル型リグニンに富むことが示唆された。(2)木繊維細胞間層：吸光度は最も高く，最大吸収波長も 280 nm とその前後に出現し，グアイアシル型に富むことが示唆された。(3)放射・軸方向柔細胞壁：同樹種放射柔細胞壁の UV スペクトルは軸方向柔細胞壁の UV スペクトルとよく一致していた。最大吸収波長の位置は，道管壁と似て，280 nm 前後にあり，グアイアシル型に富むことが示唆された。(4)木繊維二次壁：最大吸収波長位置及び 280/274 比は道管壁とよく似ており，温帯広葉材と異なって，グアイアシル型に富むことが示唆された。以上の結果を確認するために Mäule と Wiesner 呈色反応を行って，その結果は，UV スペクトルからの結果を支持するものであった。また，熱帯材試料中に含まれる抽出物の影響を検討してみたが，各組織の最大吸収波長位置の測定に及ぼす影響はほぼ認められなかった。しかし，放射・軸方向柔細胞壁のリグニンの量が多く減少したことがはっきりと示された。これに対して，木繊維の方はほとんど減らなかった。アルカリに溶出するリグニン質は柔細胞壁に多く存在すると考えられる。

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