

Studies on the Lignins of *Podocarpus*, *Gnetum*, *Drimys* and *Pseudowintera**

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Abstract—The lignins of 7 species in Podocarpaceae, 2 *Gnetum*'s, a *Drimys* and 2 *Pseudowintera*'s are characterized in respect of methoxyl content, IR spectrum, acidolysis, and permanganate oxidation of the ethylated wood powder. The relative absorptivities ($A/A_{1505\text{ cm}^{-1}}$) of characteristic maxima, and the ratio of syringyl component to guaiacyl component in acidolysis and permanganate oxidation showed that *Podocarpus* lignin is mostly composed of guaiacyl lignin and that lignins of *Gnetum*, *Pseudowintera* and *Drimys* are composed of guaiacyl-syringyl lignins with increasing amounts of syringyl component in order.

Introduction

CREIGHTON and HIBBERT¹⁾ found that lignins of *Gnetum* and some of *Podocarpus* give both vanillin and syringaldehyde in nitrobenzene oxidation, and are composed of guaiacyl-syringyl lignin. It is known, however, that the lignins of other species of *Podocarpus* e. g. *P. macrophylla*, *P. chinensis* and *P. nagi* yield no detectable syringaldehyde in nitrobenzene oxidation and are composed of guaiacyl lignin.

The lignin of Gnetales which contains both tracheids and vessels as in angiosperm woods gives both vanillin and syringaldehyde and a characteristic IR spectrum of typical angiosperm wood lignin²⁾.

On the other hand, *Drimys* and *Trochodendron aralioides* which are both known to contain no vessels and are considered to be rather primitive angiosperms give both vanillin and syringaldehyde¹⁾.

Since GORING et al.³⁾ reported that secondary walls of birch wood fibers are mainly composed of syringyl lignin, differentiation of cambial cells to fibers but not to vessels may be related to the initiation of the biosynthesis of syringyl lignin, although no information is available.

In the present investigation lignins of some other *Podocarpus*, *Gnetum*, *Drimys* and two *Pseudowintera*'s which contain no vessels are characterized as a part of the study on the relation between lignin and plant phylogeny.

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Experimental

Plant material—*Libocedrus plumosa*, *Agathis australis*, *Dacrycarpus dacrydioides*, *Dacrydium cupressinum*, *Phyllocladus glaucus*, *Podocarpus hallii*, *Podocarpus totara*, *Podocarpus spicatus*, *Podocarpus ferrugineus* (conifers), *Pseudowintera axillaris*, and *Pseudowintera colorata* (vessel-free angiosperm) were all obtained from Forest Products Division, Forest Research Institute, Rotorua, New Zealand by the courtesy of Dr. M. UPRICHARD. Two species in Gnetales, and a *Drimys* sp. (vessel-free angiosperm) collected from Boruneo were given by Dr. M. TAMURA, College of Liberal Arts, Osaka University and Dr. M. HORTA, College of Liberal Arts, Kyoto University. *Cryptomeria japonica* and *Fagus crenata* as references of conifer and angiosperm were used, respectively.

Wood powder (60~80 mesh) was extracted with ethanol-benzene (1 : 2) by a Soxhlet's extractor for 48 hr and with hot water for 40 hr successively before use.

Determination of lignin and its methoxyl group—Lignin was determined by a modified Klason method and methoxyl by a modified Zeisel method, respectively.

Acidolysis⁴⁾—Wood powder (100 mg) was suspended in 10 ml of a mixture of dioxane-water (9 : 1) containing 0.2 N hydrogen chloride and refluxed for 4 hr. The reaction mixture was filtered and washed with a small amount of dioxane, and the filtrate was added dropwise into 150 ml of water with stirring. The precipitated dioxane lignin was filtered and the filtrate was neutralized to pH 3 with NaHCO₃ solution. The filtrate was then extracted with CHCl₃ (35 ml × 3). The extract was dried over anhydrous Na₂SO₄ and evaporated *in vacuo*. The acidolysis monomers were converted to TMS derivatives and analyzed by GC-MS. Column; OV-17, 2% (2 m), 198°C.

IR spectrum of the lignin—IR spectrum of lignin was taken as KBr tablet by a Jasco model IR-S, and the relative absorptivities ($A/A_{1505\text{ cm}^{-1}}$) of characteristic maxima were calculated.

Permanganate oxidation of ethylated wood powder—Wood powder was ethylated with diethyl sulfate and 10 N NaOH at 60~65°C according to the Kirk's method⁵⁾. To the ethylated wood powder (1.4 g) was added *tert*-butanol-H₂O (1 : 1, 120 ml) and 3% Na₂CO₃ (100 ml). Then, reaction mixture was kept at 80~85°C and 1% KMnO₄ (500 ml) was added dropwise. After 4 hr the excess of KMnO₄ was decomposed with ethanol and MnO₂ formed was filtered off. The filtrate was concentrated to one half volume to which 30% H₂O₂ (20 ml) was added, and the solution was kept at 50°C for 5 min. to complete the oxidation. Then the reaction mixture was acidified with sulfuric acid and extracted with CHCl₃-acetone (1 : 1, 100 ml × 2) and subsequently with CHCl₃ (50 ml × 1). The extract

was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The aromatic acids thus obtained were methylated to their methyl esters with diazomethane and analyzed by GC-MS. Column; OV-17, 2% (2 m), 182°C.

Results and Discussion

1. Lignin and its methoxyl content

Lignin and its methoxyl contents are given in Table 1.

Table 1. The contents of lignin and its methoxyl group.

		Klason lignin (%)	Methoxyl (%)	Mäule reaction
Cupressaceae	<i>Libocedrus plumosa</i>	(1) 34.2	15.8	—
Araucariaceae	<i>Agathis australis</i>	(2) 30.0	14.7	—
Podocarpaceae	<i>Dacrycarpus dacrydioides</i>	(3) 29.7	16.6	—
	<i>Dacrydium cupressinum</i>	(4) 31.7	14.8	—
	<i>Phyllocladus glaucus</i>	(5) 29.2	16.9	—
	<i>Podocarpus ferrugineus</i>	(6) 29.1	15.5	—
	<i>Podocarpus hallii</i>	(7) 34.2	15.1	—
	<i>Podocarpus spicatus</i>	(8) 29.7	15.1	—
	<i>Podocarpus totara</i>	(9) 35.9	14.7	—
Gnetaceae	<i>Gnetum</i> sp. (1)	(10) 29.7	13.0	+
	<i>Gnetum</i> sp. (2)	(11) 35.0	13.1	+
	<i>Drimys</i> sp.	(12) 32.9	16.6	+
Winteraceae	<i>Pseudowintera axillaris</i>	(13) 33.3	19.2	+
	<i>Pseudowintera colorata</i>	(14) 35.6	18.3	+
Taxodiaceae	<i>Cryptomeria japonica</i>	(15) 31.0	16.1	—
Fagaceae	<i>Fagus crenata</i>	(16) 23.1	19.1	+

The lignin contents of New Zealand conifers are located in 29.1 to 35.9%. *Podocarpus totara*, *P. hallii*, and *Libocedrus plumosa* gave especially higher values. CREIGHTON et al.⁶⁾ reported 34.9% and 39.9% for the lignin contents of *P. acutifolium* and *P. macrophyllus*, respectively. LEOPOLD and MALMSTRÖM⁷⁾ also found 35.6% for the lignin content of *P. totara*.

These results are in agreement with the present investigation indicating the occurrence of higher amounts of lignin in these *Podocarpus* trees.

The lignin contents of two *Pseudowintera*'s and *Drimys* as vessel-free angiosperm were exceptionally higher than those of ordinary angiosperm woods and rather corresponded to those of conifers.

The methoxyl contents of the lignins of woods in Podocarpaceae were between 14.7 and 16.9% in agreement with those of ordinary conifers. On the other hand the lignins of *Gnetum* and *Drimys* gave considerably lower methoxyl contents than

those of the lignin of ordinary angiosperm woods suggesting the occurrence of non-methoxylated contaminants and/or lower amounts of syringyl component in the lignins, although Mäule test of these woods were positive. *Cryptomeria japonica* and *Fagus crenata* used as reference of conifer and angiosperm wood gave 31.0 and 23.1% of lignin and 16.1 and 19.1% of methoxyl, respectively.

2. IR spectrum of Klason lignin

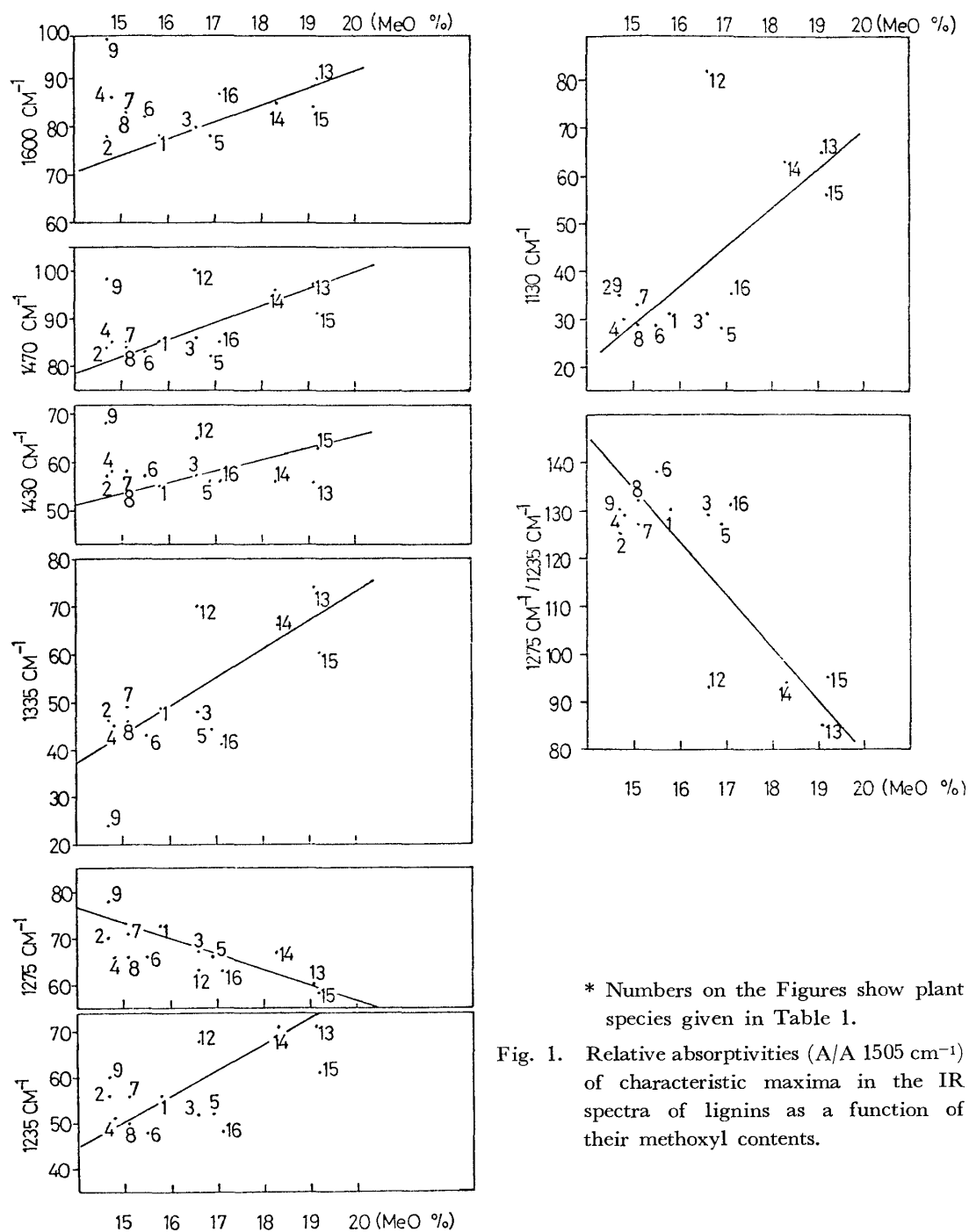
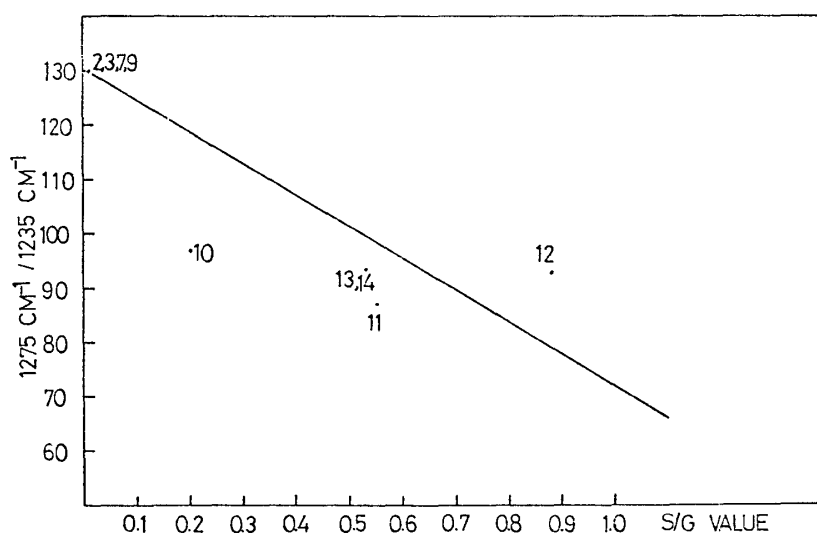


Fig. 1. Relative absorptivities ($A/A_{1505 \text{ cm}^{-1}}$) of characteristic maxima in the IR spectra of lignins as a function of their methoxyl contents.

The relative absorptivities ($A/A_{1505\text{ cm}^{-1}}$) of characteristic maxima in the IR spectra of Klason lignins as a function of their methoxyl contents are shown in Fig. 1. Absorptivities of maxima dominated by syringyl nuclei at 1130, 1235, 1335, 1430, 1470 and 1600 cm^{-1} all showed an ascending relationship with the methoxyl content in the lignins, whereas the maximum at 1275 cm^{-1} , typical of guaiacyl nuclei, showed a descending relationship as found by SARKANEN et al.⁸⁾. However, the lignins of *Gnetum*'s (10, 11) and *Drimys* (12) especially the former gave exceptionally higher absorptivities in relation to its methoxyl content at the all wave numbers, and which suggests that the non-methoxylated contaminants contained lowered the proper methoxyl contents, although previous extraction of these wood samples with 0.5% NaOH at 75°C for 80 min. was not effective to remove the contaminants.

In agreement with this idea, the relative absorptivities ($A_{1275\text{ cm}^{-1}}/A_{1235\text{ cm}^{-1}}$) which was found to reflect the ratio of guaiacyl lignin to syringyl lignin by KAWAMURA et al.⁹⁾ showed a descending relationship with the ratio of syringylpropanones to guaiacylpropanones determined by gas-chromatography of acidolysis monomers of these woods (Fig. 2).



* Numbers on the Figure shows plant species given in Table 1.

Fig. 2. Relative absorptivities ($A_{1275\text{ cm}^{-1}}/A_{1235\text{ cm}^{-1}}$) in the IR spectra of lignins as function of their S/G value in acidolysis.

3. Acidolysis

Gas-chromatograms of the acidolysis products of *Podocarpus ferrugineus*, *Gnetum* sp. (1), *Drimys* sp. and *Pseudowintera axillaris* are shown in Figs. 3~6. 1-Hydroxyl-(3-methoxyl-4-hydroxyphenyl)-2-propanone, 2-hydroxy-1-(3-methoxy-4-hydroxyphenyl)-1-propanone were identified by GC-MS in the acidolysis monomers of conifers, whereas in *Gnetum*, *Pseudowintera* and *Drimys* the corresponding syringylpropanones were found in addition to the above guaiacylpropanones.

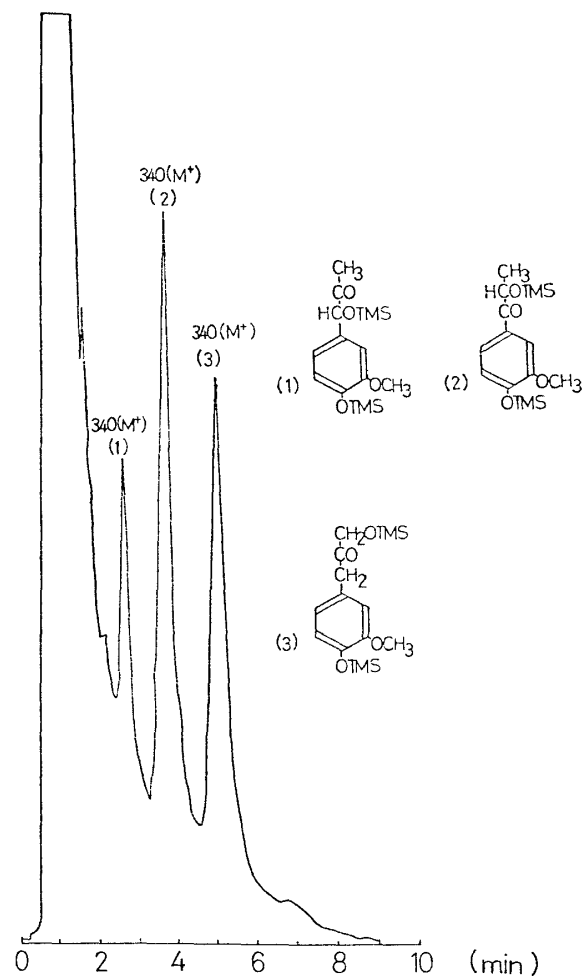


Fig. 3. Gas chromatogram of the acidolysis monomers of wood (*Podocarpus ferrugineus*).
Column: OV-17, 2% (2 m), 198°C.

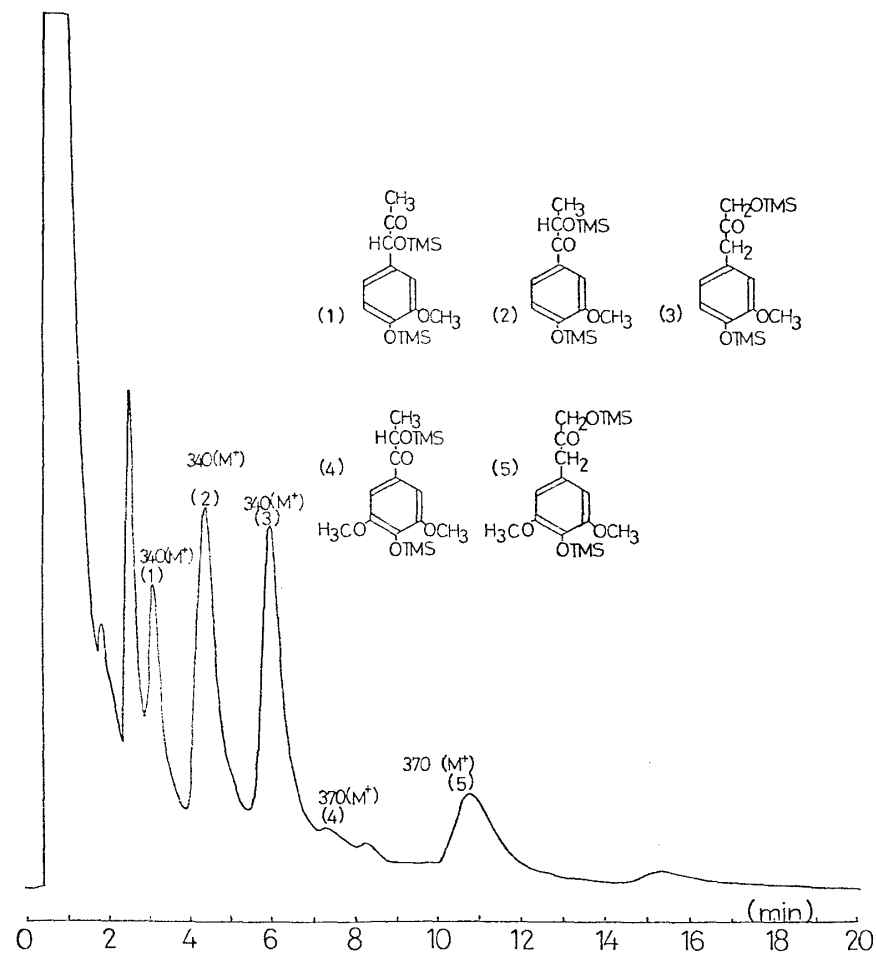


Fig. 4. Gas chromatogram of the acidolysis monomers of wood (*Gnetum* (1)). Column: OV-17, 2% (2 m), 198°C.

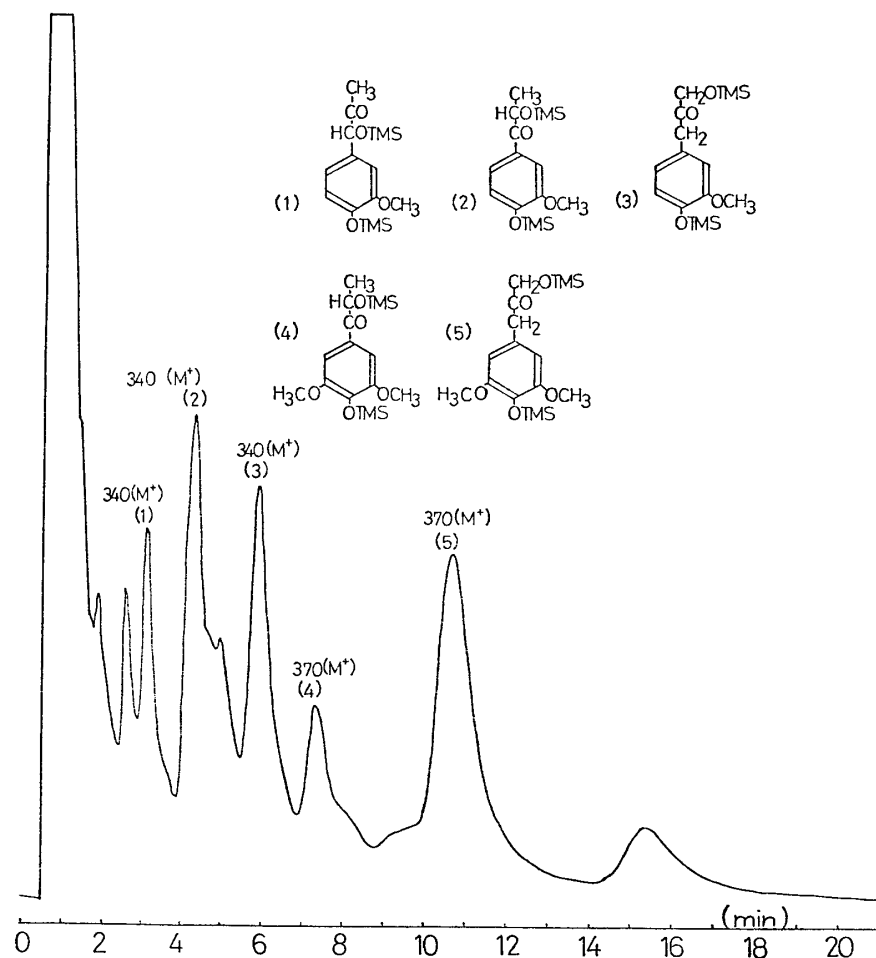


Fig. 5. Gas chromatogram of the acidolysis monomers of wood (*Drimys*).
Column: OV-17, 2% (2 m), 198°C.

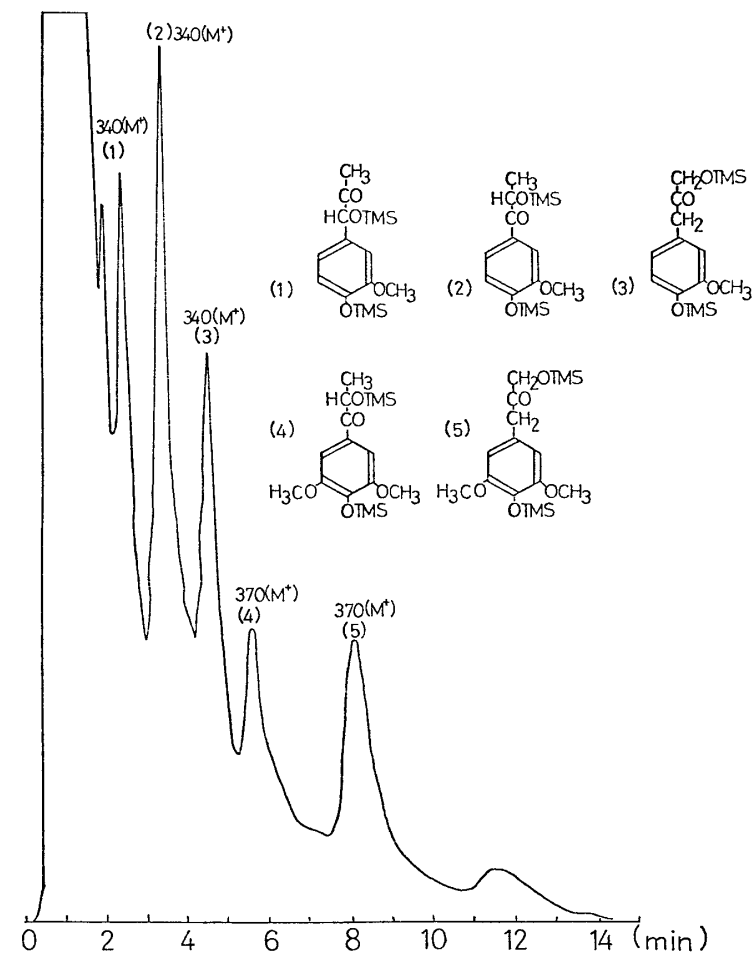


Fig. 6. Gas chromatogram of the acidolysis monomers of wood (*Pseudowintera axillaris*).
Column: OV-17, 2% (2 m), 198°C.

Table 2. The ratio of syringylpropanones to guaiacylpropanones in the acidolysis monomers.

<i>Libocedrus plumosa</i>	0.00
<i>Agathis australis</i>	0.01
<i>Dacrycarpus dacrydioides</i>	0.01
<i>Dacrydium cupressinum</i>	0.00
<i>Phyllocladus glaucus</i>	0.00
<i>Podocarpus ferrugineus</i>	0.01
<i>Podocarpus hallii</i>	0.01
<i>Podocarpus totara</i>	0.01
<i>Gnetum sp. (1)</i>	0.20
<i>Gnetum sp. (2)</i>	0.55
<i>Drimys sp.</i>	0.88
<i>Pseudowintera axilaris</i>	0.53
<i>Pseudowintera colorata</i>	0.53
<i>Cryptomeria japonica</i>	0.00
<i>Fagus crenata</i>	1.15

Table 3. The ratios of 4-ethoxybenzoic, 4-ethoxy-5-methoxyisophthalic and 4-ethoxy-3,5-dimethoxybenzoic acids to 4-ethoxy-3-methoxybenzoic acid in permanganate oxidation of ethylated wood powder.

		P*	G-5**	S***
Cupressaceae	<i>Libocedrus plumosa</i>	0.01	0.35	0.00
Araucariaceae	<i>Agathis australis</i>	0.14	0.40	0.01
Podocarpaceae	<i>Dacrycarpus dacrydioides</i>	0.00	0.39	0.01
	<i>Dacrydium cupressinum</i>	0.00	0.57	0.00
	<i>Phyllocladus glaucus</i>	0.00	0.32	0.00
	<i>Podocarpus ferrugineus</i>	0.00	0.51	0.01
	<i>Podocarpus hallii</i>	0.01	0.44	0.01
	<i>Podocarpus spicatus</i>	0.00	0.26	0.00
	<i>Podocarpus totara</i>	0.01	0.51	0.00
	<i>Gnetum sp. (1)</i>	0.02	0.33	0.16
Gnetaceae	<i>Gnetum sp. (2)</i>	0.01	0.10	0.32
	<i>Drimys sp.</i>	0.00	0.19	0.86
Winteraceae	<i>Pseudowintera axillaris</i>	0.01	0.29	0.73
	<i>Pseudowintera colorata</i>	0.01	0.69	0.95
Cupressaceae	<i>Chamaecyparis pisifera</i>	0.07	0.40	0.00
Pinaceae	<i>Pinus densiflora</i>	0.01	0.42	0.00
Fagaceae	<i>Fagus crenata</i>	0.01	0.17	0.80

* P, 4-ethoxybenzoic acid

** G-5, 4-ethoxy-5-methoxyisophthalic acid

*** S, 4-ethoxy-3,5-dimethoxybenzoic acid

From the peak areas of these guaiacylpropanones and syringylpropanones the S/G ratio was calculated as shown in Table 2. In conifers such as *Podocarpus*, the ratios were very small indicating that the lignins are composed of mainly guaiacylpropane, whereas two *Pseudowintera*'s gave exactly the same value, 0.53, and *Drimys*, 0.88 which was comparable to that of *Fagus crenata* (1.15). The ratios in *Gnetum*'s except other conifers were smallest, although the values varied between two species.

4. Permanganate oxidation of ethylated wood powder

Table 3 shows the ratio of 4-ethoxybenzoic (P), 4-ethoxy-5-methoxyisophthalic (G-5) and 4-ethoxy-3,5-dimethoxybenzoic (S) acids to 3-methoxy-4-ethoxybenzoic acid (V) calculated from the peak areas on the gas-chromatograms of the oxidation products. The amount of P was generally very small except a little higher amount in *Agathis australis*. The relative amounts of G-5 which ascribes to the condensed guaiacyl component of lignins were generally much higher in conifers than angiosperm woods, 0.26~0.57 in Podocarpaceae, 0.29 and 0.69 in *Pseudowintera* but rather small in *Drimys*.

The ratio of syringyl (S) to guaiacyl (V+G-5) is given in Table 4 which shows that the ratio in *Drimys* is comparable to that in *Fagus crenata* and followed by *Pseudowintera* and *Gnetum* in harmony with the S/G ratio in acidolysis of these woods.

Table 4. The ratio of syringyl (S)* to guaiacyl (V**+G-5***) derivatives in the permanganate oxidation.

<i>Gnetum</i> sp. (1)	0.12
<i>Gnetum</i> sp. (2)	0.29
<i>Pseudowintera axilaris</i>	0.56
<i>Pseudowintera colorata</i>	0.56
<i>Drimys</i> sp.	0.72
<i>Fagus crenata</i>	0.68

* S, 4-ethoxy-3,5-dimethoxybenzoic acid

** V, 4-ethoxy-3-methoxybenzoic acid

*** G-5, 4-ethoxy-5-methoxyisophthalic acid

Conclusion

1. The lignins of woods in Podocarpaceae were found to be composed of mainly guaiacyl lignin as in ordinary conifers.
2. The lignins of *Pseudowintera*'s are composed of guaiacyl-syringyl lignins but the amounts of syringyl component are smaller than that of ordinary angiosperm woods.
3. The lignins of *Gnetum*s are composed of guaiacyl-syringyl lignins but the amounts of syringyl component are further smaller than that of *Pseudowintera*'s.
4. The lignin of *Drimys* is comparable to that of *Fagus crenata* in respect to the S/G ratios in acidolysis and permanganate oxidation.

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