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| Title | Studies on the Hydrolysis of Hardwood Lignin |
| Author(s) | AOYAMA, Masakazu |
| Citation | 北海道大學農學部 演習林研究報告, 37(1), 261-285 |
| Issue Date | 1980-01 |
| Doc URL | http://hdl.handle.net/2115/21029 |
| Type | bulletin (article) |
| File Information | 37(1)_P261-285.pdf |

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# Studies on the Hydrolysis of Hardwood Lignin*

By

**Masakazu AOYAMA****

# 広葉樹リグニンの加水分解に関する研究*

青 山 政 和**

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* Recieved July 31, 1979

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## 1. Introduction

Lignin, one of the major constituents of wood, is a natural organic polymer. It is produced as an abundant by-product in the waste liquor at the pulp manufacturing, and estimated that the annual world production of lignin amounts to fifty million tons. Any effective utilization of lignin, however, has not been developed as yet. One of the main factors obstructing the lignin utilization may be found in its chemical structure which has irreversible linkages between building units. Namely, lignin differs from the other natural polymers such as polysaccharides and proteins, in the manners of bonding among the structural elements, which are formed as the results of random coupling of the monomeric precursors. It is of course very significant to develop the effective utilization of lignin as a potential organic resource, as well as to elucidate chemical structure of lignin in the field of phytochemistry.

In the history of lignin research, the biosynthetic studies by K. FREUDENBERG and his coworkers were exceedingly epochal. Earlier, P. KLASON stated the opinion that lignin is a condensation product of cinnamic alochol, without the experimental evidence. In relation to this assumption, FREUDENBERG *et al.* investigated in detail the enzymatic dehydrogenation of coniferyl alcohol, in which many important low molecular intermediates were isolated and their chemical structures were elucidated[1]. From these results, FREUDENBERG proposed the mechanism of lignin formation which is initiated by enzymatic dehydrogenation of *p*-hydroxycinnamic alcohols followed by coupling of their radicals to each other. However, it should be noted that the enzymatic dehydrogenation product of coniferyl alcohol (DHP, Dehydrierungspolymerisat) is so-called "Kunstlignin", and it is now necessary to establish the linkage patterns of lignin by direct proofs from protolignin.

First direct proofs for protolignin were obtained from oxidations by alkaline nitrobenzene[2,3] and permanganate[4], and from hydrogenolysis[5,6] and ethanolysis[7], indicating that lignin is aromatic and composed of phenylpropane derivatives. Later, permanganate oxidation of lignin was studied again in detail by FREUDENBERG and CHEN[8], giving 26 degradation products, in which the acids with diphenyl ether and $C_5$–$C_6$ type biphenyl linkages were newly found. This procedure was further modified by MIKSCHE *et al.*[9] and yields of the products were remarkably increased.

Hydrogenolysis with the objective of gaining information about the linkage pattern in lignin has been carried out by SAKAKIBARA *et al.*[10]. They isolated 28 di- and trilignols from hydrogenolysis products of Ezomatsu (*Picea jezoensis*), Karamatsu (*Larix leptolepis* GOLD.) and Yachidamo (*Fraxinus mandshurica* RUPR.) lignins, in which $C_\alpha$–O–$C_\gamma$[11], $C_\beta$–$C_6$[12] and phenylisochroman[13] type new dilignols are involved. Recently, LEE and PEPPER[14] isolated a trilignol from spruce hydrogenolysis products. On the other hand, NIMZ *et al.* isolated many degradation products from spruce[15] and beech[16,17] wood lignins by treatment with thioacetic acid, followed by reduction with Raney Ni. From the relative yields of dilignols, a constitutional scheme[18] for beech wood lignin was proposed, which was supported by the $^{13}C$–NMR spectrum of beech wood lignin.

Acid degradation of lignin (acidolysis) has been carried out by LUNDQUIST[19]. From the reaction mixture of spruce milled wood lignin, phenylcoumarone[20] and diarylpropane derivatives[21] were isolated, indicating the presence of phenylcoumaran and 1, 2-bis-diarylpropane structures in lignin. These acidolysis products, however, may undergo secondary reactions such as rearrangement and cleavage at side chain and condensation.

On the other hand, mild hydrolysis of lignin was carried out by NIMZ and SAKAKIBARA *et al.*. Earlier, SAKAKIBARA and NAKAYAMA[22] isolated cinnamic alcohols and aldehydes from dioxane-water hydrolysis products of spruce, beech and poplar wood lignins. Further, it was also indicated that protolignin gave cinnamic alcohols under the hydrolysis conditions, while lignin preparations treated with mineral acid or alkali could not give any cinnamic alcohols[23]. FREUDENBERG *et al.*[24] isolated dehydrodiconiferyl alcohol and D, L-pinoresinol which represent major intermediates in the enzymatic dehydrogenation products of coniferyl alcohol, from hydrolysis products with methanolic hydrochloric acid at 20°C. NIMZ[25] isolated guaiacylglycerol-$\beta$-coniferyl ether from spruce hydrolysis products with 2% acetic acid at 100°C. Further, guaiacylglycerol-$\beta$-guaiacylglycerol ether[26], guaiacylglycerol-$\beta$-coniferyl aldehyde ether and guaiacylglcerol-$\beta$-vanillin ether[27], and tri and tetralignols[28] which contain $C_\beta$–O–$C_4$ and $C_\beta$–$C_1$ linkages were isolated by NIMZ from spruce hydrolysis products with water at 100°C. SANO and SAKAKIBARA[29,30] isolated two trilignols, one of which contains $C_\beta$–$C_5$ and $C_\beta$–$C_1$ linkages, from dioxane-water hydrolysis products of spruce lignin. From beech hydrolysis products, NIMZ isolated D, L-syringaresinol and three different 1, 2-bis-diarylpropane-1, 3-diols[31~33]. OMORI and SAKAKIBARA isolated D, L-medioresinol and syringylglycerol-$\beta$-syringylglycerol ether[34], a lignan type dilignol containing $\alpha$-carbonyl and guaiacylglycerol-$\beta$-syringaresinol ether[35] and syringylglycerol-$\beta$-syringaresinol ether[36] from ash wood

lignin hydrolysis products. All these compounds, obtained by mild hydrolysis as well as hydrogenolysis, confirm the biogenesis of lignin proposed by FREUDENBERG.

Therefore, lignin chemical structure has been considerably cleared up by many direct proofs from protolignin. Some intermediates of the enzymatic dehydrogenation products of coniferyl alcohol, however, have not been isolated as yet. Further, the results of permanganate oxidation of protolignin[8,9] suggest the presence of unknown linkages between the structural units in lignin.

The present work deals with the hydrolysis of hardwood (*Quercus mongolica* FISCH. var. *grosseserrata* REHD. et WILS.) lignin with dioxane and water, and is carried out to obtain further information about linkage pattern in lignin molecule. So far, ten dilignols involving four new ones were isolated and their chemical structures were elucidated.

The author would like to acknowledge the continuing guidance and encouragement of Dr. Akira SAKAKIBARA, Professor of Wood Chemistry, Faculty of Agriculture, Hokkaido University. The author also wishes to thank Dr. Tsutomu KAYAMA, Dr. Takashi SASAYA, Dr. Yoshihiro SANO, Messrs. Hirotake TAKEYAMA and Keiji MIKI, Faculty of Agriculture, Hokkaido University, for their helpful advice.

This manuscript comprises a part of the thesis submitted to Hokkaido University in partial fulfilment of the requirement for the degree of Doctor of Agriculture.

## II. Experimental

### II. 1 Preparation of wood meal

Mizunara (*Quercus mongolica* FISCH. var. *grosseserrata* REHD. et WILS.) wood meal extracted with acetone-water (9 : 1), benzene-ethanol (2 : 1) and 95% ethanol for 48 hr, respectively, was used for this experiment. The moisture content and Klason lignin amount to 11.1% and 23.3%, respectively. The extractive free wood meal was subjected to hydrolysis with dioxane-water (7 : 13) mixture under following conditions: hydrolysis temperature 180°C, reaction time 20 min. The total hydrolyzed wood meal was five kg (air dry weight).

### II. 2 Fractionation of hydrolysis mixture

The hydrolysis mixture was fractionated according to Fig. 1. The ether soluble fraction (Fraction ES, yield 113 g) was subjected to gel filtration (Sephadex LH-20, dioxane-water 1 : 1 as an eluant), giving seven fractions (ES•A-ES•G). The acetone soluble fraction (Fraction AS, yield 147 g) was also subjected to gel filtration (Sephadex LH-20, methanol as an eluant), giving six fractions (AS•A-AS•F).

### II. 3 Isolation of dilignols

#### II. 3. 1 Isolation of compound-A (D, L-Syringaresinol)

ES•E fraction (20 g) obtained by gel filtration was separated by a silica gel column (*n*-hexane-acetone 2 : 1), giving 11 fractions (ES•E•1-ES•E•11). These fractions were monitored by TLC on silica gel using a solvent mixture of toluene —formic acid— ethyl formate (5 : 1 : 4, abbr. GT-I). The chromatogram was colored by spraying 50% sulfuric acid followed by heating at 105°C.

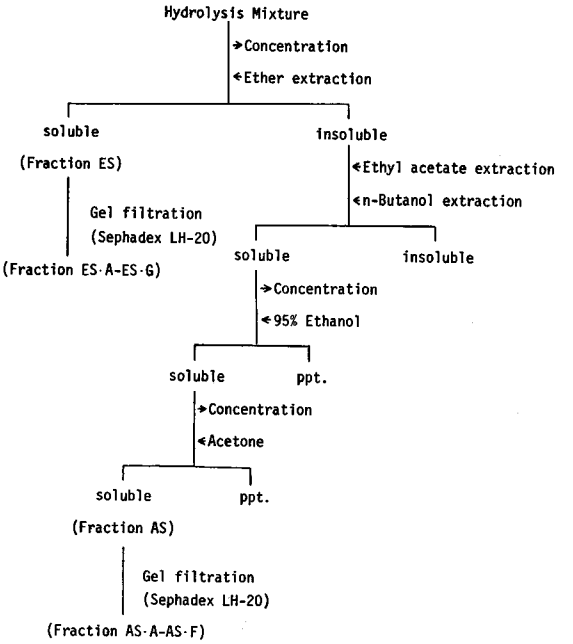



**Fig. 1.** Fractionation of hydrolysis mixture.

ES•E•7 and ES•E•8 fractions were further chromatographed on a silica gel column (dichloroethane-methanol 50 : 1) and a cellulose column (xylene-dimethylformamide 15 : 1) to give needles. The compound was recrystallized from ethanol to give colorless needles (mp. 173.5–174.5°C, yield 2.5 g). UV $\lambda_{max}^{95\%\ EtOH}$ nm (log $\varepsilon$) : 239 (4. 34), 272 (3. 58), 281 (3. 47). UV $\lambda_{max}^{0.1N\ NaOH}$ nm (log $\varepsilon$) : 261 (4. 38), 290 (4.02). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3440, 1615, 1525, 1455, 1430, 1380, 1320, 1250, 1160, 1110, 1065, 1045, 1020. *Anal.* Calcd. for $C_{22}H_{26}O_8$: C, 63.15; H, 6.26. Found: C, 63.24; H, 6.35.

### II. 3. 2 Isolation of compound-B (D, L-Episyringaresinol)

Fractions ES•E•7 and ES•E•8 from which compound-A had been removed was chromatographed on a silica gel column (chloroform-ethyl acetate 8 : 1), giving needles. The compound was recrystallized from ethanol to give colorless needles (mp. 192–193°C, yield 164 mg). UV $\lambda_{max}^{95\%\ EtOH}$ nm (log $\varepsilon$) : 238 (4.31), 271 (3.77), 281 (3.58). UV $\lambda_{max}^{0.1N\ NaOH}$ nm (log $\varepsilon$) : 260 (4.38), 291 (4.02). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3450, 1615, 1525, 1465, 1430, 1390, 1355, 1335, 1255, 1210, 1160, 1120, 1110. *Anal.* Calcd. for $C_{22}H_{26}O_8$: C, 63.15; H, 6.26. Found: C, 63.00; H, 6.23.

### II. 3. 3 Isolation of compound-C (D, L-Medioresinol)

ES•E•6 fraction (530 mg) was separated by a silica gel column (*n*-hexane-acetone 5 : 1), giving a fraction which revealed a spot at Rf value 0.44 on TLC (GT-I). This fraction was further chromatographed on a polyamide column (water as an eluant) to give a fraction which contained impurities in small amount. Then, this fraction was acetylated and chromatographed on a silica gel column (*n*-hexane-

ethyl acetate 2 : 1), giving needles. The compound was recrystallized from ethanol to give colorless needles (mp. 184.5–185.5°C, yield 47 mg). *Anal.* Calcd. for $C_{25}H_{28}O_9$ (as diacetate): C, 63.55; H, 5.97. Found: C, 63.68; H, 6.01.

**II. 3. 4 Isolation of compound-D (3-Hydroxymethyl-2-(4-hydroxy-3, 5-dimethoxyphenyl)-4-(4-hydroxy-3, 5-dimethoxybenzoyl)-tetrahydrofuran)**

ES•E•11 fraction (8.6 g) was separated by three different silica gel columns (benzene-methanol 20 : 1, benzene-ethyl acetate 3 : 2 and 1 : 1), giving a fraction which revealed a spot at Rf value 0.28 on TLC (GT-I). This fraction was further chromatographed on a silica gel column (benzene-ethyl acetate 1 : 1) to give a syrup (yield 33 mg). UV $\lambda^{95\%\ EtOH}_{max}$ nm (log $\varepsilon$): 299 (3.99). UV $\lambda^{0.1N\ NaOH}_{max}$ nm (log $\varepsilon$): 253 (4.23), 366 (4.20). IR $\nu^{KBr}_{max}$ $cm^{-1}$: 3400, 1660, 1610, 1515, 1460, 1420, 1325, 1215, 1160, 1110. The compound was acetylated and crystallized from ethanol to give colorless needles (mp. 74.5–76°C). *Anal.* Calcd. for $C_{28}H_{32}O_{12}$ (as triacetate): C, 59.99; H, 5.75. Found: C, 60.88; H, 6.41.

**II. 3. 5 Isolation of compound-E (6-Oxo-2-(4-hydroxy-3, 5-dimethoxyphenyl)-3, 7-dioxabicyclo-[3, 3, 0]-octane)**

ES•E•6 fraction was separated by a silica gel column (*n*-hexane-acetone 5 : 1), giving a fraction which revealed a spot at Rf value 0.40 on TLC (GT-I). This fraction was further chromatographed on a silica gel column (*n*-hexane-acetone 5 : 1) to give needles. The compound was recrystallized from methanol to give colorless needles (mp. 200–200.5°C, yield 29 mg). UV $\lambda^{95\%\ EtOH}_{max}$ nm (log $\varepsilon$): 238 (3.81), 271 (3.09), 280 (2.98). UV $\lambda^{0.1N\ NaOH}_{max}$ nm (log $\varepsilon$): 261 (3. 94), 288 (3. 67). IR $\nu^{KBr}_{max}$ $cm^{-1}$: 3400, 1765, 1615, 1520, 1460, 1430, 1390, 1370, 1340, 1230, 1165, 1120, 1110, 1060, 1005. *Anal.* Calcd. for $C_{14}H_{16}O_6$: C, 59.99; H, 5.75. Found: C, 59.72; H, 5.79.

**II. 3. 6 Isolation of compound-F (4, 4′-Dihydroxy-3, 3′-dimethoxy-stilbene)**

Fraction ES•G gave yellow needles after gel filtration. The compound was recrystallized for ethanol to give colorless needles (mp. 221–224°C, yield 93 mg). UV $\lambda^{Methyl\ cellosolve}_{max}$ nm (log $\varepsilon$): 297 (4.14), 308 (4.21), 335 (4.42). UV $\lambda^{0.1N\ NaOH}_{max}$ nm (log $\varepsilon$): 240 (4.28), 320 (4.24), 369 (4.51). IR $\nu^{KBr}_{max}$ $cm^{-1}$: 3390, 1605, 1600, 1515, 1465, 1430, 1385, 1330, 1280, 1230, 1160, 1115, 1030. The compound is unstable in atmosphere at room temperature. Therefore, it was acetylated and crystallized from chloroform-petroleum ether mixture to give colorless needles (mp. 228.8°C). *Anal.* Calcd. for $C_{20}H_{20}O_6$ (as diacetate): C, 66.60; H, 5.71. Found: C, 66.87; H, 5.73.

**II. 3. 7 Isolation of compound-G (1, 2-Bis-(4-hydroxy-3, 5-dimethoxyphenyl)-propane-1, 3-diol)**

Fraction AS•D (54 g) was separated by a silica gel column by gradient development, giving 11 fractions (AS•D•1–AS•D•11). Elution began with a solvent mixture of *n*-hexane-acetone (4 : 1), and then the proportion of acetone increased gradually. Fractions from AS•D•5 to AS•D•7 were combined and chromatographed on a silica gel column (*n*-hexane-acetone 4 : 1). The fraction, contained mainly of a

compound which gave a spot at Rf value 0.12 on TLC (GT-I), was further separated by a silica gel column (benzene-methanol 14 : 1) to give amorphous powder. The compound was crystallized from methanol to give colorless needles (mp. 236–237°C, yield 156 mg). UV $\lambda^{95\%\ EtOH}_{max}$ nm (log $\varepsilon$) : 271 (3.38), 280 (3.29). UV $\lambda^{0.1N\ NaOH}_{max}$ nm (log $\varepsilon$) : 254 (4.13), 285 (3.48). IR $\nu^{KBr}_{max}$ $cm^{-1}$ : 3500, 3220, 1615, 1520, 1470, 1460, 1430, 1335, 1230, 1160, 1120. The compound was acetylated and crystallized from ethanol to give colorless needles (mp. 169.0–170.2°C) *Anal.* Calcd. for $C_{27}H_{32}O_{12}$ (as tetraacetate) : C, 59.12 ; H, 5.84. Found : C, 59.02 ; H, 5.84.

### II. 3. 8 Isolation of compound-H (1-(4-Hydroxy-3, 5-dimethoxyphenyl)-2-(4-hydroxy-3-methoxyphenyl)-propane-1, 3-diol)

The combined fraction from AS•D•5 to AS•D•7 which was free from compound-G, was further separated by a silica gel column (benzene-methanol 12 : 1), giving a fraction which revealed a spot at Rf value 0.22 on TLC (GT-I). The fraction was chromatographed on two different silica gel columns (benzene-methanol 14 : 1, chloroform-methanol 50 : 1) to give needles. The compound was recrystallized from methanol to give colorless needles (mp. 195.5–196.7°C, yield 24 mg). UV $\lambda^{MeOH}_{max}$ nm (log $\varepsilon$) : 279 (3.50). UV $\lambda^{0.1N\ NaOH}_{max}$ nm (log $\varepsilon$) : 280 (3.60). IR $\nu^{KBr}_{max}$ $cm^{-1}$ : 3450, 1620, 1520, 1470, 1435, 1330, 1280, 1225, 1155, 1130, 1070, 1030. HRMS m/e : 332.1260 (Calcd. for $C_{18}H_{20}O_6$ ($M^+-H_2O$) : 332.1261), 302.1139 (Calcd. for $C_{17}H_{18}O_5$ (m/e 322–HCHO) : 302.1155).

### II. 3. 9 Isolation of compound-I (2, 3-Dihydro-7-methoxy-2-(4-hydroxy-3, 5-dimethoxyphenyl)-3-hydroxymethyl-5-(2-formylvinyl)-benzofuran)

ES•E•9 fraction (3. 1 g) was separated by a cellulose column by gradient development to give three fractions (ES•E•9•A–ES•E•9•C). Elution began with a solvent mixture of xylene-dimethylformamide (15 : 1), and then the proportion of dimethylformamide increased gradually. ES•E•9•B fraction composed mainly of a compund which gave a spot at Rf value 0.32 on TLC (GT-I). The fraction was further chromatographed on three different silica gel columns (dichloroethane-methanol 50 : 1, dichloroethane-ethyl acetate 4 : 1 and 3 : 1), giving a yellow syrup (yield 123 mg). UV $\lambda^{95\%\ EtOH}_{max}$ nm (log $\varepsilon$) : 228 (4.18), 281 (3.70), 338 (3.81). UV $\lambda^{0.1N\ NaOH}_{max}$ nm (log $\varepsilon$) : 283 (3.92), 342 (3. 95). IR $\nu^{KBr}_{max}$ $cm^{-1}$ : 3400, 1670, 1600, 1520, 1470, 1430, 1390, 1330, 1225, 1110, 1040. *Anal.* Calcd. for $C_{25}H_{26}O_9$ (as diacetate) : C, 63.82 ; H, 5.57. Found : C, 62.91 ; H, 5.51.

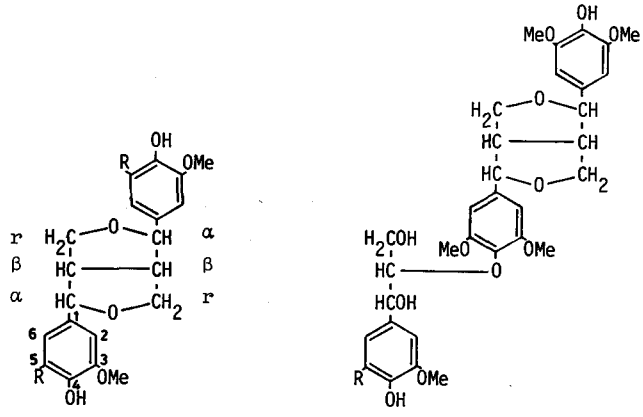
### II. 3. 10 Isolation of compound-J (2, 3-Dihydro-7-methoxy-2-(4-hydroxy-3, 5-dimethoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol)

ES•E•11 fraction was separated by three different silica gel columns (benzene-methanol 20 : 1, benzene-ethyl acetate 3 : 2 and 1 : 1), giving a fraction which revealed a spot at Rf value 0.32 on TLC (GT-I). The fraction was chromatographed on a silica gel column (benzene-ethyl acetate 1 : 1) to give colorless syrup (yield 21 mg). UV $\lambda^{95\%\ EtOH}_{max}$ nm (log $\varepsilon$) : 232 (4.13), 280 (3.62). UV $\lambda^{0.1N\ NaOH}_{max}$ nm (log $\varepsilon$) : 254 (3.99), 282 (3.78). *Anal.* Calcd. for $C_{27}H_{32}O_{10}$ (as triacetate) : C, 62.78 ; 6.24. Found : C, 62.19 ; H, 6.04.

## III. Results and discussion

### III. 1 Compound-A (D, L-Syringaresinol) (I)

Compound-A colored pink by spraying a solution of diazotized sulfanilic acid. This color reaction and UV spectra patterns of compound-A are typical for syringylpropane derivatives. The mass spectrum of compound-A revealed the parent peak at m/e 418 (94%), and the other main fragment peaks were m/e 251, 235, 210, 193, 181 (100%), 167 (97%), 161 and 154. Each signal in the $^1$H-NMR spectrum of compound-A was assigned as follows: $\delta^{CDCl_3}$ (ppm) 2.90–3.20 (2H, m, $H_\beta$), 3.85 (12H, s, OMe), 3.72–4.40 (4H, m, $H_\gamma$), 4.69 (2H, d, $H_\alpha$ $J_{\alpha\beta}$=4Hz), 5.53 (2H, s, ph-OH), 6.53 (4H, s, Ar-H). Each signal in the $^{13}$C-NMR spectrum of compound-A was also assigned as follows: $\delta^{(CD_3)_2CO\text{-}D_2O(9:1)}$ (ppm) 52.5 ($C_\beta$), 54.3 (OMe), 69.5 ($C_\gamma$), 83.8 ($C_\alpha$), 101.2 ($C_{2,6}$), 129.4 ($C_1$), 132.2 ($C_4$), 144.4 ($C_{3,5}$). This compound has no optical activity. All these data indicate that compound-A is D, L-syringaresinol (I).



I R=OMe
IV R=H

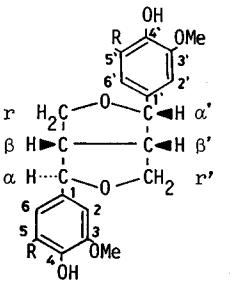
II R=H
III R=OMe

Previously, NIMZ and GABER[31] isolated this compound from the mild hydrolysis products of beech wood lignin. Later, it was also isolated from dioxane-water hydrolysis[37], hydrogenolysis[38] and acidolysis[39,40] products of other hardwood lignins. Further, two different trilignols (II, III) containing syringaresinol moiety have been isolated by SAKAKIBARA *et al.* from both hydrolysis[35,36] and hydrogenolysis[41] products of ash (*Fraxinus mandshurica* RUPR.) wood lignin.

Therefore, syringaresinol structural unit considerably exists in hardwood lignin. On the other hand, pinoresinol (IV) could not be detected in the products of hydrolysis with dioxane-water, even in trace amounts. This result is accordance with the report obtained by $^{13}$C-NMR studies[42] that the pinoresinol unit may exist only in small amounts in softwood lignin.

### III. 2 Compound-B (D, L-Episyringaresinol) (V)

Compound-B colored pink by spraying a solution of diazotized sulfanilic acid. The color reaction with diazotized sulfanilic acid, UV and IR spectra of compound-B are very similar to those of syringaresinol. The mass spectrum of compound-B revealed the parent peak at m/e 418 (64%), and the other main fragment peaks were m/e 210, 193, 181 (100%), 167 (88%), 161 and 154. Each signal in the $^1$H-NMR spectrum of compound-B was assigned as follows: $\delta^{CDCl_3}$ (ppm) 2.75–3.56 (2H, m, $H_{\beta,\beta'}$), 3.86 (12H, s, OMe), 3.56–4.24 (4H, m, $H_{\gamma,\gamma'}$), 4.41 (1H, d, $H_{\alpha'}$ $J_{\alpha'\beta'}$=7Hz), 4.82 (1H, d, $H_\alpha$ $J_{\alpha\beta}$=4Hz), 5.54 (2H, s, ph-OH), 6.56 (4H, s, Ar-H.). Each signal in the $^{13}$C-NMR spectrum of compound-B was also assigned as follows: $\delta^{CDCl_3}$ (ppm) 49.3 ($C_{\beta'}$), 53.7 ($C_\beta$), 55.6 (OMe), 68.7 ($C_{\gamma'}$), 70.3 ($C_\gamma$), 81.7 ($C_{\alpha'}$), 87.3 ($C_\alpha$), 102.7 ($C_{2,2',6,6'}$), 129.3 ($C_{1'}$), 132.2 ($C_1$), 133.9 ($C_{4'}$), 134.4 ($C_4$), 147.0 ($C_{3,3',5,5'}$). This compound has no optical activity. All these data indicate that compound-B is D, L-episyringaresinol (V).

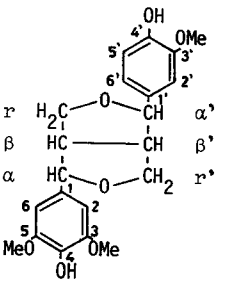


V R=OMe
VI R=H

Previously, this compound was isolated by hydrolysis with dioxane-water[34] and acidolysis[39,40] of hardwood lignins. It is probable that episyringaresinol unit may exist originally in hardwood lignin, since it had been found that epipinoresinol (VI) existed in the intermediates of the enzymatic dehydrogenation products of coniferyl alcohol. On the other hand, from the results of model experiments[34,43] it was shown that a part of syringaresinol was epimerized to give episyringaresinol under the both hydrolysis conditions. Thus the question is still open to debate.

### III. 3 Compound-C (D, L-Medioresinol) (VII)

Compound-C colored pink by spraying a solution of diazotized sulfanilic acid. The mass spectrum of the acetylated compound-C revealed the parent peak at m/e 472, and the other main fragment peaks were m/e 430 (68%), 388, 357, 210, 205, 193, 181, 167, 151, 137 and 43 (100%). Each signal in the $^1$H-NMR spectrum of the acetate was assigned as follows: $\delta^{CDCl_3}$ (ppm) 2.32 (3H, s, ph-OAc on guaiacyl moiety), 2.35 (3H, s, ph-OAc on syringyl moiety), 2.94–3.23 (2H, m, $H_{\beta,\beta'}$), 3.84 (9H, s, OMe), 3.76–4.44 (4H, m, $H_{\gamma,\gamma'}$), 4.78 (1H. d, $H_\alpha$ $J_{\alpha\beta}$=4Hz), 4.82 (1H d, $H_{\alpha'}$ $J_{\alpha'\beta'}$=4Hz), 6.60 (2H, s, Ar-H on syringyl nucleus), 6.81–7.08 (3H, m, Ar-H on guaiacyl nucleus). Each signal in the $^{13}$C-NMR spectrum of the acetate was also assigned as follows: $\delta^{CDCl_3}$ (ppm) 19.6 (Me in OAc), 54.0 ($C_{\beta,\beta'}$), 55.7 (OMe), 71.7 ($C_{\gamma,\gamma'}$), 85.2 ($C_{\alpha,\alpha'}$), 102.3 ($C_{2,6}$), 110.5 ($C_{2'}$), 118.3 ($C_{6'}$), 123.3 ($C_{5'}$) ,128.9 ($C_4$), 139.6 ($C_{1'}$), 140.2 ($C_1$), 140.7 ($C_{4'}$), 151.6 ($C_{3'}$), 152.7 ($C_{3,5}$), 169.0 (CO in OAc). This compound has no optical activity. These data indicate that compound-C is D, L-medioresinol (VII). Its melting point (184.5–185.5°C as diacetate), however, is considerably higher than the value reported early (155–156°C as diacetate)[34].

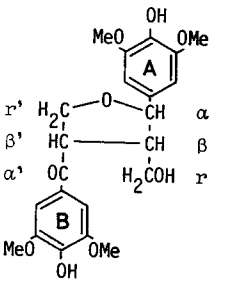


VII

Earlier, OMORI and SAKAKIBARA[34] isolated this compound from dioxane-water hydrolysis products of ash (*Fraxinus mandshurica* RUPR.) wood lignin. Further, very recently it has been obtained by hydrogenolysis of the same wood lignin[41]. On the other hand, (+)-medioresinol has been isolated from the extractives of the bark of *Liriodendron tulipifera* L.[44].

### III. 4 Compound-D (3-Hydroxymethyl-2-(4-hydroxy-3, 5-dimethoxyphenyl)-4-(4-hydroxy-3, 5-dimethoxybenzoyl)-tetrahydrofuran) (VIII)

Compound-D colored pink and brown by spraying solutions of diazotized sulfanilic acid and 2, 4-dinitrophenylhydrazine, respectively. The absorption band at 1660 $cm^{-1}$ in the IR spectrum and the color reaction with 2, 4-dinitrophenylhydrazine suggest that this compound has carbonyl in side chain. The mass spectrum of compound-D revealed the parent peak at m/e 434, and the other main fragment peaks were m/e 416 (98%), 400, 386, 367, 319, 262, 208, 181 (57%), 167, 155 (100%), 137 and 123. Each signal in the $^1$H-NMR spectrum of the acetylated compound-D was assigned as follows: $\delta^{CDCl_3}$ (ppm) 1.95 (3H, s, alc-OAc), 2.34 (3H, s, ph-OAc on A-ring), 2.37 (3H, s, ph-OAc on B-ring), 2.94–3.26 (1H, m, $H_\beta$), 3.85 (6H, s, OMe on A-ring), 3.90 (6H, s, OMe on B-ring), 3.80–4.50 (5H, m, $H_{\beta',\gamma,\gamma'}$), 4.64 (1H, d, $H_\alpha$ $J_{\alpha\beta}$=9Hz), 6.70 (2H, s, Ar–H on A-ring), 7.21 (2H, s, Ar–H on B-ring). This compound has no optical activity. All these data indicate that compound-D is 3-hydroxymethyl-2-(4-hydroxy-3, 5-dimethoxyphenyl)-4-(4-hydroxy-3, 5-dimethoxybenzoyl)-tetrahydrofuran (VIII).




VIII

Earlier, $\alpha$-carbonyl content of about 0.07 per methoxyl in spruce milled wood lignin was reported[45]. Later, this compound was isolated by OMORI and SAKAKIBARA[35] from the mild hydrolysis products of ash (*Fraxinus mandshurica* RUPR.) wood lignin, representing structural units containing $\alpha$-carbonyl in lignin. This structural unit may be formed by the $C_\beta$–$C_\beta$ coupling of sinapyl alcohol radicals followed by one molecular water addition and disproportionation.

### III. 5 Compound-E (6-Oxo-2-(4-hydroxy-3, 5-dimethoxyphenyl)-3, 7-dioxabicyclo-[3, 3, 0]-octane) (IX)

Compound-E colored pink and brown by spraying solutions of diazotized sulfanilic acid and 2, 4-dinitrophenylhydrazine, repectively. The absorption band at 1765 $cm^{-1}$ in the IR spectrum of this compound suggests that compound-E contains $\gamma$-lactone in the molecule. The mass spectrum of compound-E (Fig. 2) revealed the parent peak at m/e 280 (100%), and the other main fragment peaks were m/e 265, 249, 210, 182 (49%) and 167 (63%). The mass spectrum of the acetate revealed the parent peak at m/e 322, and the other main fragment peaks were m/e 280 (100%), 265, 249, 210, 182, 167, 165 and 43. In these mass spectra, the peaks at m/e 210 are presumed to be sinapyl alcohol ion. The $^1$H-NMR spectrum of compound-E (Fig. 3) was assigned as follows: $\delta^{CDCl_3}$ (ppm) 2.96–3.21 (1H, m, $H_\beta$), 3.29–3.58 (1H, m, $H_{\beta'}$), 3.89 (6H, s, OMe), 4.05–4.31 (2H, m, $H_{\gamma'}$), 4.32–4.49 (2H, m, $H_\gamma$),

4.56 (1H, d, $H_\alpha$ $J_{\alpha\beta}$=7Hz), 5.53 (1H, s, ph-OH), 6.55 (2H, s, Ar-H). The $^1$H-NMR spectrum of the acetate was also assigned as follows: $\delta^{(CD_3)_2CO}$ (ppm) 2.25 (3H, s, ph-OAc), 3.32-3.80 (2H, m, $H_{\beta,\beta'}$), 3.81 (6H, s, OMe)., 3.93-4.63 (4H, m, $H_{\gamma,\gamma'}$), 4.78 (1H, d, $H_\alpha J_{\alpha\beta}$=6Hz), 6.80 (2H, s, Ar-H). In the $^1$H-NMR spectrum of the acetate, irradiation of the signal region corresponding to $\beta$-proton ($\delta$ 3.22 ppm) caused the doublet at $\delta$ 4.78 ppm to a singlet and the multiplet at $\delta$ 4.30-4.63 ppm to simple one. Further, irradiation of the signal region corresponding to $\beta'$-proton ($\delta$ 3.57 ppm) caused the multiplet at $\delta$ 3.93-4.38 ppm to collapse to somewhat simple one. All these data indicate that compound-E is 6-oxo-2-(4-hydroxy-3, 5-dimethoxy-

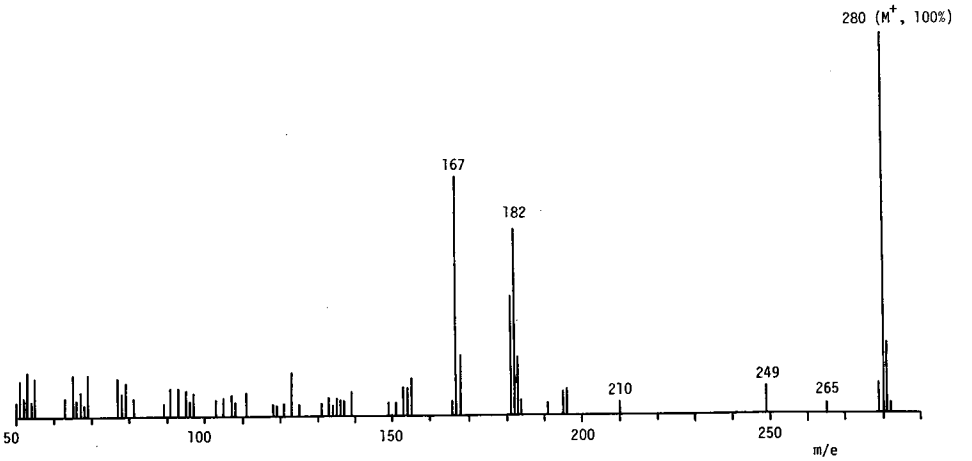



**Fig. 2.** Mass spectrum of 6-oxo-2-(4-hydroxy-3, 5-dimethoxyphenyl)-3, 7-dioxabicyclo-[3, 3, 0]-octane (IX) (Compound-E).

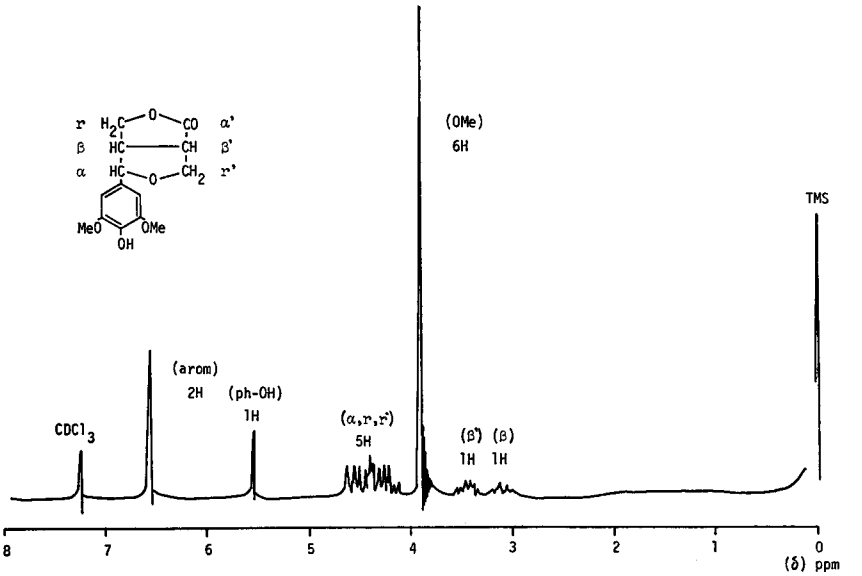
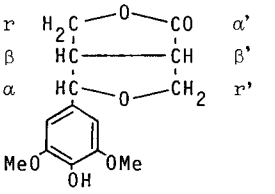



**Fig. 3.** $^1$H-NMR spectrum of 6-oxo-2-(4-hydroxy-3, 5-dimethoxyphenyl)-3, 7-dioxabicyclo-[3, 3, 0]-octane (IX) (Compound-E).

phenyl)-3, 7-dioxabicyclo-[3, 3, 0]-octane (IX).



IX

Earlier, FREUDENBERG and GEIGER[46] isolated two different dilignols containing $\gamma$-lactone from the enzymatic dehydrogenation products of coniferyl alcohol, suggesting the existence of ester linkage in lignin. In fact, very recently YASUDA and SAKAKIBARA[47] isolated two different trilignols containing $\gamma$-lactone from the hydrogenolysis products of larch (*Larix leptolepis* GOLD.) compression wood lignin. These $\gamma$-lactone may be formed by the coupling of coniferyl alcohol radical $R_\beta$ and ferulic acid radical $R_\beta$. The $\gamma$-lactone in compound-E, however, may be formed by another mechanism (Fig. 4 and 5) involving elimination of an aromatic ring. In both proposed mechanisms, compound-E (IX) may be derived from the structural unit of 3-hydroxymethyl-2-(4-hydroxy-3, 5-dimethoxyphenyl)-4-(4-hydroxy-3, 5-dimethoxybenzoyl)-tetrahydrofuran (VIII). In the case of mechanism-I, as shown in Fig. 4, lignol IX may be formed by ring closure after splitting a diarylpropane. This mechanism may represent a different example for the formation of diarylpropane units in lignin from the one proposed so far[21,32]. In the alternative mechanism-II (Fig. 5), the nucleophilic attack to carbonyl carbon followed by cyclization during the hydrolysis reaction may postulated. The mechanism of the formation of lignol IX is, however, still open to debate.

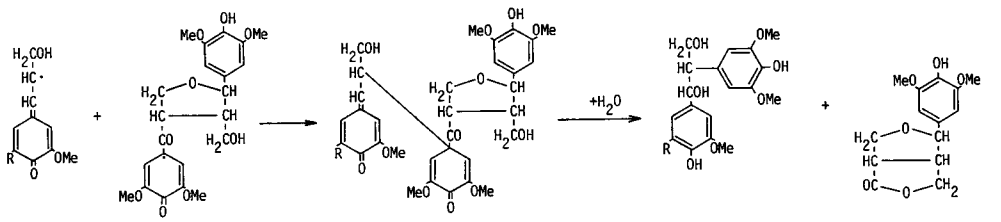


**Fig. 4.** A proposed mechanism for the formation of 6-oxo-2-(4-hydroxy-3, 5-dimethoxyphenyl)-3, 7-dioxabicyclo-[3, 3, 0]-octane (IX) Mechanism-I.

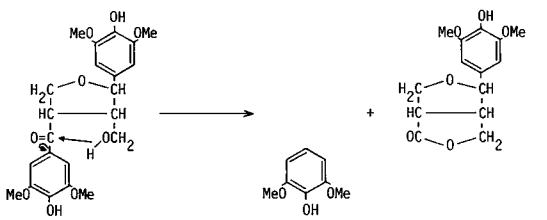
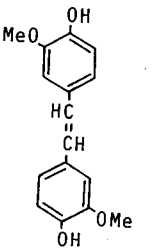


**Fig. 5.** Alternative mechanism for the formation of 6-oxo-2-(4-hydroxy-3, 5-dimethoxyphenyl)-3, 7-dioxabicyclo-[3, 3, 0]-octane (IX) Mechanism-II.

### III. 6 Compound-F (4, 4′-Dihydroxy-3, 3′-dimethoxy-stilbene) (X)

Compound-F colored red and red-brown by spraying solutions of diazotized sulfanilic acid and ferric chloride, respectively. In the IR spectrum of compound-F, the absorption band at 955 $cm^{-1}$ suggests that this compound has vinyl configuration. The mass spectrum of compound-F revealed the parent peak at m/e 272

(100%). Each signal in the $^1$H-NMR spectrum of the acetylated compound-F was assigned as follows: $\delta^{CDCl_3}$ (ppm) 2.32 (6H, s, ph-OAc), 3.88 (6H, s, OMe), 6.95–7.18 (8H, m, aromatic and olefinic side chain protons). All these data indicate that compound-F is 4, 4′-dihydroxy-3, 3′-dimethoxy-stilbene (X).

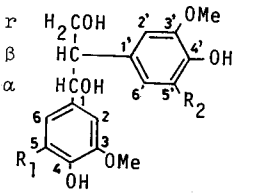


X

Under the present hydrolysis conditions, a part of 1, 2-bis-(4-hydroxy-3-methoxyphenyl)-propane-1, 3-diol structural units in lignin may suffer elimination of $\gamma$-hydroxymethyl group to form this stilbene. Such a reaction occurs extensively during the acidolysis of lignin[20].

### III. 7 Compound-G (1, 2-Bis-(4-hydroxy-3, 5-dimethoxyphenyl)-propane-1, 3-diol) (XI)

Compound-G colored red and gray by spraying solutions of diazotized sulfanilic acid and ferric chloride, respectively. The mass spectrum of compound-G revealed no parent peak at expected position as a result of rapid splitting of a water molecule by electron beam impact. The phenomenon is observed in some compounds involving hydroxyl at $\alpha$-carbon. The other main fragment peaks were m/e 362, 344, 332 (100%), 180 (75%), 167 and 165. The mass spectrum of the acetate revealed the parent peak at m/e 548. Each signal in the $^1$H-NMR spectrum of the acetylated compound-G was assigned as follows: $\delta^{CDCl_3}$ (ppm) 2.02 (6H, s, alc-OAc), 2.32 (6H, s, ph-OAc), 3.36 (1H, m, $H_\beta$), 3.74 (12H, s, OMe), 4.32 (2H, m, $H_\gamma$), 6.05 (1H, d, $H_\alpha$ $J_{\alpha\beta}$=7Hz), 6.34 (4H, s, Ar-H). Each signal in the $^{13}$C-NMR spectrum of the acetate was also assigned as follows: $\delta^{CDCl_3}$ (ppm) 20.3 (Me in OAc), 49.8 ($C_\beta$), 55.7 (OMe), 63.3 ($C_\gamma$), 74.9 ($C_\alpha$), 104.3 ($C_{2',6'}$), 106.3 ($C_{2,6}$), 129.0 ($C_{4,4'}$), 135.8 ($C_1$), 136.9 ($C_{1'}$), 153.0 ($C_{3,3',5,5'}$), 169.0 (CO in ph-OAc), 170.2 (CO in $\alpha$-alc-OAc), 171.3 (CO in $\gamma$-alc-OAc). All these data indicate that compound-G is 1, 2-bis-(4-hydroxy-3, 5-dimethoxyphenyl)-propane-1, 3-diol (XI). Further, its configuration was identified to be *erythro* form by the signals corresponding to aliphatic acetoxyls in the $^1$H-NMR spectrum.



XI $R_1=R_2=OMe$
XII $R_1=R_2=H$
XIII $R_1=H, R_2=OMe$

Earlier, NIMZ[32,33] isolated three different 1, 2-diaryl-propane-1, 3-diols (XI, XII, XIII) from the mild hydrolysis products of beech wood lignin. Later, two of them (XI, XII) were isolated by OMORI and SAKAKIBARA[35,48] from the mild hydrolysis products of ash wood lignin. Further, NIMZ[28] isolated tri- and tetralignol containing $C_\beta$–O–$C_4$ and $C_\beta$–$C_1$ linkages from hydrolysis products of spruce lignin. SANO and SAKAKIBARA[30] isolated a different trilignol containing $C_\beta$–$C_5$ and $C_\beta$–$C_1$ linkages from spruce hydrolysis products. Moreover, many di- and trilignols containing $C_\beta$–$C_1$ linkage have been isolated by other degradation procedures such as hydrogenolysis[10], treatment with thioacetic acid[15,17], acidolysis[19] and degradation with metallic sodium in liquid ammonia[49]. Thus, diarylpropane derivatives have been isolated extensively and in good yields, suggesting the predominant existence of its

structural units in lignin. In fact, a relative frequence for $C_\beta$–$C_1$ linkage in beech wood lignin was estimated at about 15% of total linkages[17,18].

### III. 8 Compound-H (1-(4-Hydroxy-3, 5-dimethoxyphenyl)-2-(4-hydroxy-3-methoxyphenyl)-propane-1, 3-diol) (XIV)

Compound-H colored orange and gray by spraying solutions of diazotized sulfanilic acid and ferric chloride, respectively. The mass spectrum of compound-H (Fig. 6) revealed no parent peak at the expected position, as in the case of compound-G. The other main fragment peaks were m/e 332, 314, 302 (100%), 299,

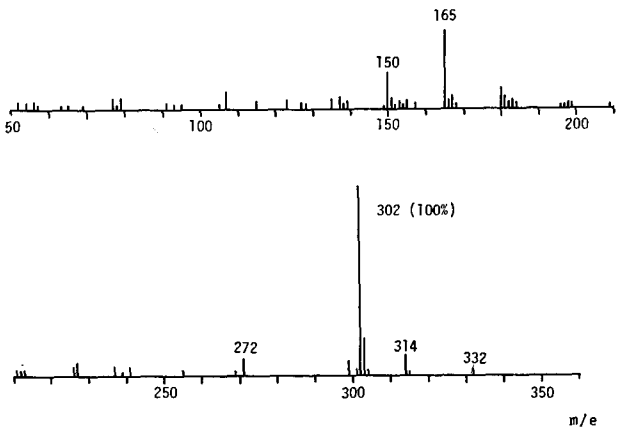



**Fig. 6.** Mass spectrum of 1-(4-hydroxy-3, 5-dimethoxyphenyl)-2-(4-hydroxy-3-methoxyphenyl)-propane-1, 3-diol (XIV) (Compound-H).

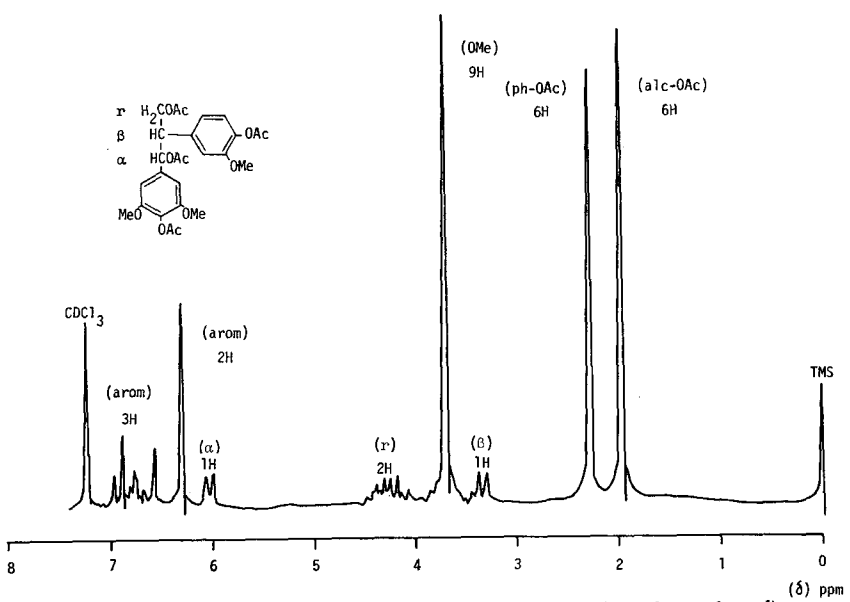
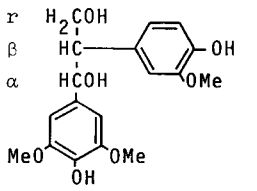



**Fig. 7.** $^1$H-NMR spectrum of 1-(4-hydroxy-3, 5-dimethoxyphenyl)-2-(4-hydroxy-3-methoxyphenyl)-propane-1, 3-diol (XIV) (Compound-H) tetraacetate.

272, 180, 167, 165, 150, 137 and 105. The mass sepctrum of the acetylated compound-H revealed the parent peak at m/e 518, and the other main fragment peaks were m/e 476, 416, 374, 302, 267, 239, 225, 209, 197, 195, 183 (100%), 167, 153 and 150. Each signal in the $^1$H-NMR spectrum of the acetate (Fig. 7) was assigned as follows: $\delta^{CDCl_3}$ (ppm) 1.97 (6H, s, alc-OAc), 2.28 (6H, s, ph-OAc), 3.36 (1H, m, $H_\beta$), 3.69 (9H, s, OMe), 4.29 (2H, m, $H_\gamma$), 6.04 (1H, d, $H_\alpha$ $J_{\alpha\beta}$=7Hz), 6.30 (2H, s, Ar-H on syringyl nucleus), 6.50-7.00 (3H, m, Ar-H on guaiacyl nucleus). Irradiation of the signal corresponding to $\beta$-proton ($\delta$ 3.36 ppm) caused the doublet at $\delta$ 6.04 ppm to a singlet and the multiplet at $\delta$ 4.29 ppm to somewhat simple one. Previously, NIMZ[32,33] isolated three different diarylpropanes (XI, XII, XIII) from the mild hydrolysis products of beech wood lignin and investigated the signals of side chain protons in the $^1$H-NMR spectra (as tetraacetate). The $\alpha$-proton signal adjacent to syringyl nucleus (XI) located on slightly higher field ($\delta$ 6.05 ppm) than those of others (XII and XIII, $\delta$ 6.12 ppm). The chemical shift of $\alpha$-proton of the acetylated compound-H was $\delta$ 6.04 ppm. Further, the characteristic peak at m/e 267 in the mass spectrum of the acetylated compound-H also suggested that the $\alpha$-carbon of compound-H was adjacent to syringyl nucleus. Moreover, the melting point of compound-H (195.5-196.7°C) was slightly higher than that of 1-(4-hydroxy-3-methoxyphenyl)-2-(4-hydroxy-3, 5-dimethoxyphenyl)-propane-1, 3-diol (XIII) (192-193°C)[33]. Recently, *threo* and *erythro* isomers of 1-(4-hydroxy-3, 5-dimethoxyphenyl)-2-(4-hydroxy-3-methoxyphenyl)-propane-1, 3-diol were synthesized by NAKATSUBO and HIGUCHI[50]. In the $^1$H-NMR spectra of those acetylated isomers, a remarkable difference between *threo*- and *erythro* forms is observed: the chemical shift of $\alpha$-acetoxyl protons in *thero* form was clearly different from that of $\gamma$-acetoxyl protons, while in *erythro* from those signals almost overlapped. In the case of compound-H, the signals corresponding to $\alpha$- and $\gamma$-acetoxyl protons appeared as a singlet indicating *erythro* form. All these data indicate that compound-H is *erythro*-1-(4-hydroxy-3, 5-dimethoxyphenyl)-2-(4-hydroxy-3-methoxyphenyl)-propane-1, 3-diol (XIV).



XIV

The isolation of dilignol XIV demonstrates that all expected diarylpropane structures composed of both guaiacyl and syringyl units exist in harwood lignin.

### III. 9 Compound-I (2, 3-Dihydro-7-methoxy-2-(4-hydroxy-3, 5-dimethoxyphenyl)-3-hydroxymethyl-5-(2-formylvinyl)-benzofuran) (XV)

Compound-I colored orange, brown and red-violet by spraying solutions of diazotized sulfanilic acid, 2, 4-dinitrophenylhydrazine and phloroglucinol-HCl, respectively. The red-violet color with phloroglucinol-HCl suggests that this compound contains $\alpha$, $\beta$-unsaturated aldehyde as a side chain. The absorption maximum at 338 nm of the UV spectrum in neutral solution and the absorption band at 1670 $cm^{-1}$ of the IR spectrum support the result of the color reaction with phloroglucinol-HCl. The mass spectrum of compound-I (Fig. 8) revealed the parent peak at m/e 386, and the other main fragment peaks were m/e 368 (100%), 356, 353, 342, 337,

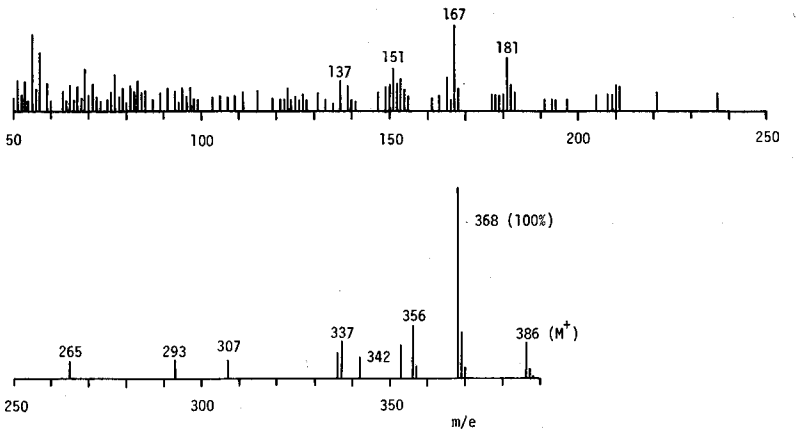



**Fig. 8.** Mass spectrum of 2, 3-dihydro-7-methoxy-2-(4-hydroxy-3, 5-dimethoxyphenyl)-3-hydroxymethyl-5-(2-formylvinyl)-benzofuran (XV) (Compound-I).

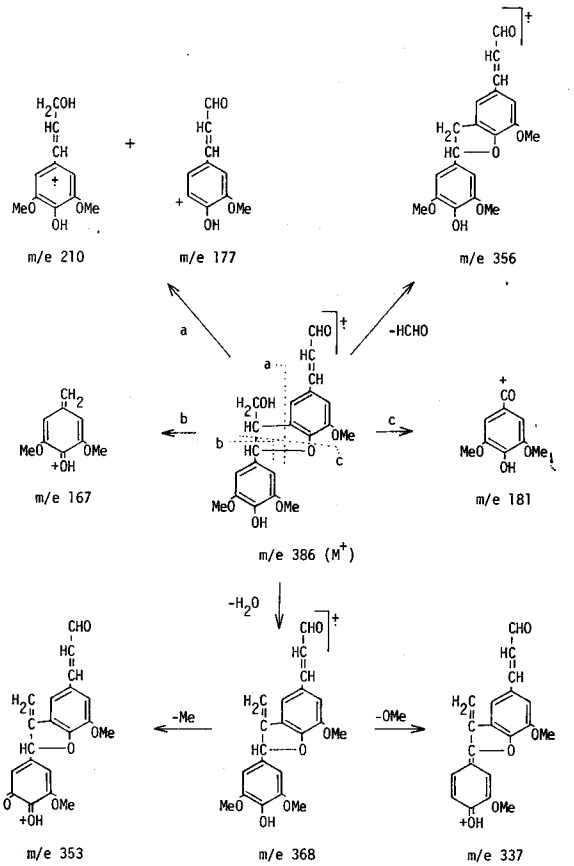



**Fig. 9.** A possible fragmentation of 2, 3-dihydro-7-methoxy-2-(4-hydroxy-3, 5-dimethoxyphenyl)-3-hydroxymethyl-5-(2-formylvinyl)-benzofuran (XV) (Compound-I).

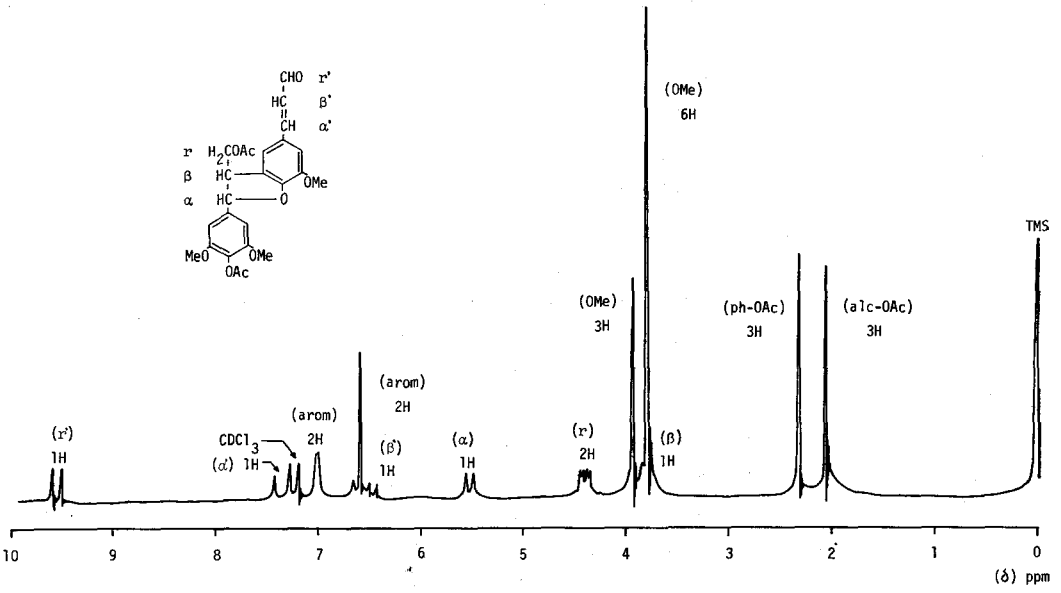
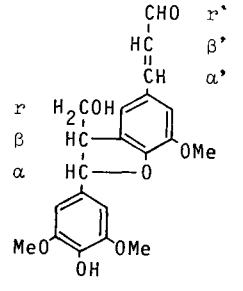



**Fig. 10.** $^1$H-NMR spectrum of 2, 3-dihydro-7-methoxy-2-(4-hydroxy-3, 5-dimethoxyphenyl)-3-hydroxymethyl-5-(2-formylvinyl)-benzofuran (XV) (Compound-I) diacetate.

237, 221, 210, 181, 167, 151 and 137. A possible fragmentation is illustrated in Fig. 9. The mass spectrum of the acetylated compound-I revealed the parent peak at m/e 470. The $^1$H-NMR spectrum of the acetate is shown in Fig. 10. Each signal was assigned as follows: $\delta^{CDCl_3}$ (ppm) 2.06 (3H, s, alc-OAc), 2.32 (3H, s, ph-OAc), 3.78 (6H, s, OMe on syringyl moiety), 3.79 (1H, m, $H_\beta$), 3.92 (3H, s, OMe on guaiacyl moiety), 4.40 (2H, m, $H_\gamma$), 5.52 (1H, d, $H_\alpha$ $J_{\alpha\beta}$=7.1Hz), 6.55 (1H,dd, $H_{\beta'}$ $J_{\alpha'\beta'}$= 15Hz and $J_{\beta'\gamma'}$=7.5Hz), 6.58 (2H, s, Ar-H on syringyl nucleus), 7.02 (2H, d, Ar-H on guaiacyl nucleus $J_{meta}$=3Hz), 7.34 (1H, d, $H_{\alpha'}$), 9.54 (1H, d, $H_{\gamma'}$). The signal of the doublet at $\delta$ 5.52 ppm in the $^1$H-NMR spectrum of the acetate clearly shows that this compound has a phenylcoumaran structure which is one of main structural units in lignin. And then, the signals of methoxyls and aromatic protons may lead to the conclusion that compound-I is constituted of both guaiacyl and syringyl units. All these data indicate that compound-I is 2, 3-dihydro-7-methoxy-2-(4-hydroxy-3, 5-dimethoxyphenyl)-3-hydroxymethyl-5-(2-formylvinyl)-benzofuran (XV).




XV

Earlier, FREUDENBERG and LEHMANN[51] isolated two aldehydic dilignols from the enzymatic dehydrogenation products of coniferyl alcohol. One of them, guaiacylglycerol-$\beta$-coniferyl aldehyde ether was isolated by NIMZ from the mild hydrolysis products of spruce lignin[27]. In the present study, another dilignol of phenylcoumaran type with a cinnamic aldehyde side chain was isolated. 1-(4-Hydroxy-3, 5-dimethoxy-5-propylphenyl)-ethane was isolated by NIMZ[17]

as phenylcoumaran composed of both guaiacyl and syringyl units from the degradation products of beech wood lignin by treating with thioacetic acid.

The structural units with cinnamic aldehyde side chain provide the well known typical lignin color reaction with phloroglucinol-HCl.

### III. 10 Compound-J (2, 3-Dihydro-7-methoxy-2-(4-hydroxy-3, 5-dimethoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol) (XVI)

Compound-J colored orange by spraying a solution of diazotized sulfanilic acid. The mass spectrum of compound-J (Fig. 11) revealed the parent peak at m/e 390, and the other main fragment peaks were m/e 372 (100%), 360 (62%), 357, 342, 328, 210, 181, 167 (58%), 151 and 137. A possible fragmentation is illustrated in Fig. 12. The mass spectrum of the acetylated compound-J revealed the parent peak at m/e 516, suggesting that this compound has three hydroxyl groups. The $^1$H-NMR spectrum of the acetate is shown in Fig. 13. Each signal was assigned as follows: $\delta^{CDCl_3}$ (ppm) 1.94 (2H, m, $H_{\beta'}$), 2.10 (6H, s, alc-OAc), 2.36 (3H, s, ph-OAc), 2.69 (2H, t, $H_{\alpha'}$ $J_{\alpha'\beta'}=7$Hz), 3.79 (1H, m, $H_\beta$), 3.83 (6H, s, OMe on syringyl moiety), 3.93 (3H, s, OMe on guaiacyl moiety), 4. 12 (2H, t, $H_{\gamma'}$ $J_{\beta'\gamma'}=6.8$Hz), 4.42 (2H, m, $H_\gamma$), 5.52 (1H, d, $H_\alpha$ $J_{\alpha\beta}=6$Hz), 6.65 (2H, s, Ar-H), 6.67 (2H, s, Ar-H). From the signal of the double at $\delta$ 5.52 ppm in the $^1$H-NMR spectrum, it is clear that this compound has a phenylcoumaran structure. All these data indicate that compound-J is 2,3-dihydro-7-methoxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol (XVI). This compound has no optical activity, suggesting that it originates from lignin.

Hitherto any dilignol containing $\omega$-hydroxypropyl group at the side chain has not been isolated from the mild hydrolysis products of lignin and also from the intermediates of the enzymatic dehydrogenation products of coniferyl alcohol. Earlier, BRINK *et al.*[52,53] detected dihydroconiferyl alcohol in the nitrobenzene

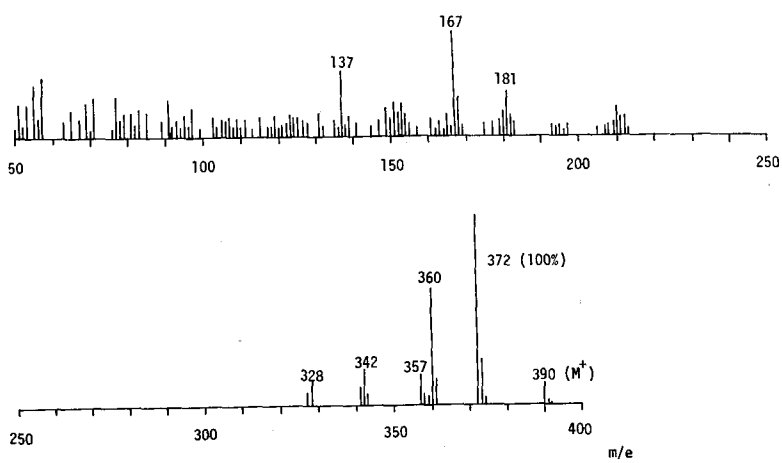



**Fig. 11.** Mass spectrum of 2, 3-dihydro-7-methoxy-2-(4-hydroxy-3, 5-dimethoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol (XVI) (Compound-J).

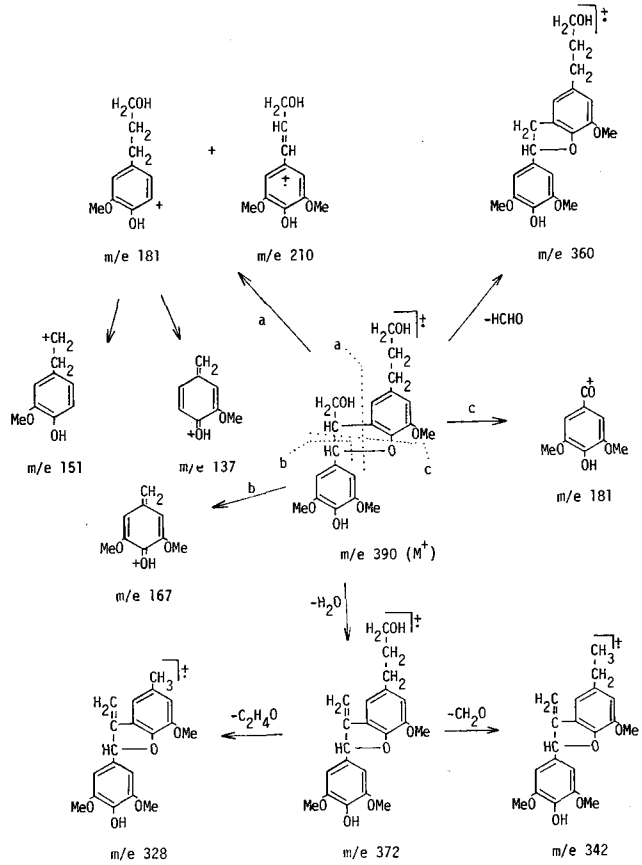



**Fig. 12.** A possible fragmentation of 2, 3-dihydro-7-methoxy-2-(4-hydroxy-3, 5-dimethoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol (XVI) (Compound-J).

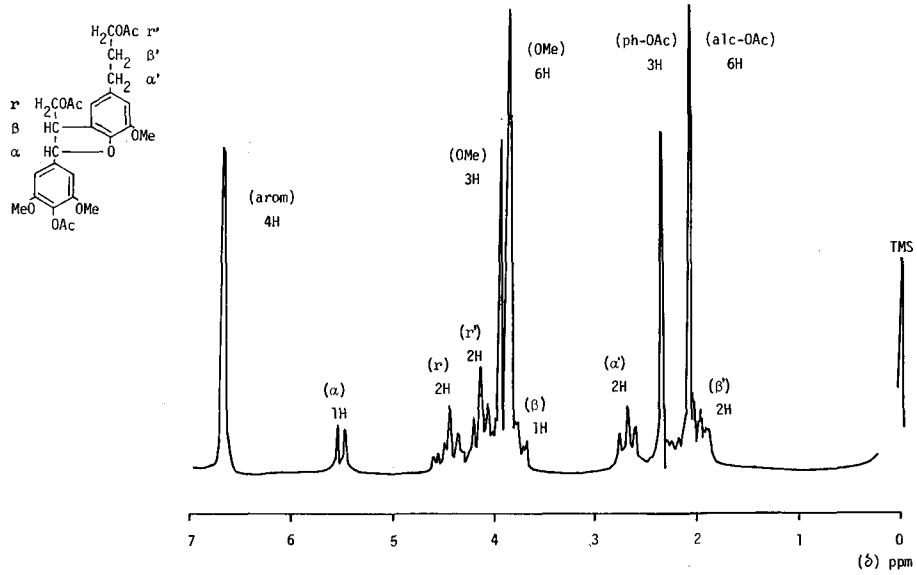
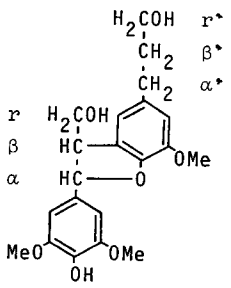



**Fig. 13.** $^1$H-NMR spectrum of 2, 3-dihydro-7-methoxy-2-(4-hydroxy-3, 5-dimethoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol (XVI) (Compound-J) triacetate.

oxidation products from white fir wood. The compound might be formed after secondary oxido-reduction reaction, and the reduced side chain remained because of its resistance against the oxidation. TANAKA[54] obtained aryl ethanes from protolignin by alkali cooking. A model experiment showed that $\beta$-hydroxy-$\beta$-aryl-propionic acid would give aryl ethane as a result of hydrogen substitution of benzylic hydroxyl. Detailed mechanism is, however, open to debate. On the other hand, KLEMOLA[55] detected dihydrosinapyl alcohol in the steam hydrolysis of birch wood. The possibility of relation to oxido-reduction or CANNIZZARO reaction was discussed, but any resonable structure of precursor could not be found. Ultimately, it was concluded that a small amount of dihydrosinapyl alcohol unit (0.1% of lignin) might exist in birch wood lignin.




XVI

In the NMR spectra of milled wood ligins[56,57] and the enzymatic dehydrogenation products of coniferyl alcohol[58], the presence of the highly shielded aliphatic signals was observed, even in small amounts, and it belongs to one of unknown parts of lignin. The saturated side chain in dilignol XVI may elucidate those signals. The formation mechanism is, however, unknown. It is very probable that disproportionation or specific reduction may occur during the lignin formation.

Recently, MANNERS and SWAN[59], POPOFF and THEANDER[60,61] and HIGUCHI *et al.*[62,63] isolated several glycosides of phenylcoumaran and arylglycerol-$\beta$-aryl ether type dilignols which contain $\omega$-hydroxypropyl group as side chain from extractives of needles. Further, very recently TAKEHARA and SASAYA[64] isolated those aglycones and dihydrodehydrodiconiferyl alcohol from larch extractives. Those compounds, however, possess optical activity.

## IV. Conclusion

In this study, ten dilignols were isolated from the mild hydrolysis products of hardwood (*Quercus mongolica* FISCH. var. *grosseserrata* REHD. et WILS.) lignin, and their chemical structures were elucidated. Among them four compounds (IX, XIV, XV, XVI) were new dilignols.

6-Oxo-2-(4-hydroxy-3, 5-dimethoxyphenyl)-3, 7-dioxabicyclo-[3, 3, 0]-octane (IX, compound-E) has a $\gamma$-lactone. Considering the elimination of an aromatic ring, it may be derived from the structural unit of 3-hydroxymethyl-2-(4-hydroxy-3, 5-dimethoxyphenyl)-4-(4-hydroxy-3, 5-dimethoxybenzoyl)-tetrahydrofuran (VIII) which was also isolated in this study. And two different mechanisms for the formation of dilignol IX are proposed (Fig. 4 and 5). In the case of mechanism-I (Fig. 4), a diarylpropane is formed accompanied with dilignol IX. This mechanism may represent an example for the formation of diarylpropane structural units in lignin. On the other hand, in the alternative mechanism-II (Fig. 5), the nucleophilic attack to carbonyl carbon followed by cyclization may be postulated. The mechanism of the formation of dilignol IX is still open to debate as yet.

1-(4-Hydroxy-3, 5-dimethoxyphenyl)-2-(4-hydroxy-3-methoxyphenyl)-propane-1, 3-diol (XIV, compound-H) is one of diarylpropanes which are composed of both guaiacyl and syringyl units. The isolation of dilignol XIV demonstrates that all kinds of diarylpropane structures constituted of both guaiacyl and syringyl units exist actually in hardwood lignin.

2, 3-Dihydro-7-methoxy-2-(4-hydroxy-3, 5-dimethoxyphenyl)-3-hydroxymethyl-5-(2-formylvinyl)-benzofuran (XV, compound-I) has a cinnamic aldehyde side chain. Earlier, FREUDENBERG and LEHMANN[51] isolated two aldehydic dilignols from the enzymatic dehydrogenation products of coniferyl alcohol. One of them, guaiacylglycerol-$\beta$-coniferyl aldehyde ether was isolated by NIMZ[27] from the mild hydrolysis products of spruce lignin. Another dilignol, the phenylcoumaran type has not been isolated. Its syringyl derivative, however, was isolated in present work. The structural units with the cinnamic aldehyde side chain show the well known lignin color reaction with phloroglucinol-HCl.

2, 3-Dihydro-7-methoxy-2-(4-hydroxy-3, 5-dimethoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol (XVI, compound-J) has $\omega$-hydroxypropyl group at the side chain. As yet, any dilignol with $\omega$-hydroxypropyl side chain has not been isolated from the mild hydrolysis products of protolignins and also from the enzymatic dehydrogenation products of coniferyl alcohol. Dilignol XVI has no optical activity, indicating that it originates from lignin. Further, dihydrosinapyl alcohol has been detected in the steam hydrolysis mixture of birch wood[55]. From these facts, it seems that small amounts of dihydrocinnamic alcohol units may exist in lignin. In the $^1$H-NMR spectra of the acetylated protolignin[56], the presence of highly shielded aliphatic protons was indicated, and they belong to one of unknown parts of lignin. The saturated side chain in dilignol XVI may elucidate those protons.

The results obtained from the present study almost confirm the radical coupling mechanism proposed by FREUDENBERG. Further, the isolation of dilignol XVI also indicates that the extensive disproportionation or specific enzymatic reduction may occur during the lignin formation.

It should be also noted that linkage pattern between various hardwood lignins would not be different, judged from the results of the hydrolysis of two harwood species, Yachidamo (*Fraxinus mandshurica* RUPR.) and Mizunara (*Quercus mongolica* FISCH. var. *grosseserrata* REHD. et WILS.).

Earlier, SANO and SAKAKIBARA subjected some model compounds to hydrolysis under the same reaction conditions, and found that arylglycerol-$\beta$-aryl ethers were cleaved by homolysis[65~68]. Formed radicals coupled again to give some lignols which might obscure the presence of the primary hydrolysis products. However, the primary hydrolysis products are predominant, and the homolysis products would be negligible for the following reasons: 1) Under these hydrolysis conditions, benzyl ethers are almost quantitatively cleaved to give various lignols. But, arylglycerol-$\beta$-aryl ether type structure etherified at phenolic hydroxyl which is predominant linkage in lignin molecule cannot be cleaved[69]. Only a minor part of $\beta$-O-4 type units with free phenolic hydroxyl would suffer homolysis, but these units in lignin molecule are small amounts and occur only as terminal ones. The

facts mean that the cleavage except benzylic ether would not occur in lignin molecule practically. 2) Even if another possibility of the homolysis of the primary products from benzylic ether is not deniable, the amounts of the homolysis products would be too small to detect them, as indicated by the yields of any homolysis lignols from model compounds. Thus, pinoresinol[65], phenylcoumarans[65~68] and biphenyl[68] which are homolysis products from model compounds could not be detected even in traces in the reaction mixture from protolignins. The results indicate that the various lignols which have been isolated so far are not from homolysis products but from primary hydrolysis products after cleavage from benzylic ether.

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## 摘　　要

本研究は広葉樹プロトリグニンの温和な加水分解物から，主として未知2量体を単離することにより，広葉樹リグニン構成単位間結合様式に関して新たな知見をえる目的で行われた。その結果，ミズナラ (*Quercus mongolica* FISCH. var. *grosseserrata* REHD. et WILS.) リグニンから10種の2量体が単離，同定されたが，そのうち4種 ((IX, XIV, XV, XVI) は新化合物である。

6-Oxo-2-(4-hydroxy-3, 5-dimethoxyphenyl)-3, 7-dioxabicyclo-[3, 3, 0]-octane (IX, compound-E) はプロパン側鎖において γ-lactone を有している。以前，FREUDENBERG と GEIGER[46] は，coniferyl alcohol の酵素的脱水素重合中間体より γ-lactone を有する2種の2量体 (pinoresinolide, lignenolide) を単離し，リグニン分子中にエステル結合が存在する可能性を指摘した。事実，最近，保多，榊原[47] はカラマツ (*Larix leptolepis* GOLD.) 圧縮アテ材リグニンの水素化分解物より2種の γ-lactone を有する3量体を単離し，FREUDENBERG の提案を実証している。しかし本研究でえられた γ-lactone (IX) については異なる形成機構が考慮された。すなわち，その形成に 3-hydroxymethyl-2-(4-hydroxy-3, 5-dimethoxyphenyl)-4-(4-hydroxy-3, 5-dimethoxybenzoyl)-tetrahydrofuran (VIII) を原構造とする2種の機構 (Fig. 4 および Fig. 5) が考慮された。機構 -I は 1, 2-bis-diarylpropane-1, 3-diol 構造形成に付随する脱離側鎖に関する従来の説とは異なる反応例を与えるものである。一方，機構 -II は2次的な芳香核の脱離による pyrogallol-1, 3-dimethyl ether の形成と側鎖の閉環である。

1-(4-Hydroxy-3, 5-dimethoxyphenyl)-2-(4-hydroxy-3-methoxyphenyl)-propane-1, 3-diol (XIV, compound-H) は，グアヤシル，シリンギル両基から成る 1, 2-bis-diarylpropane-1, 3-diol である。この化合物の異性体は，すでに NIMZ[33] によりブナ材の加水分解物から単離されているが本研究で従来まで未単離であったもう一つの同族体 (XIV) がえられたわけである。

2, 3-Dihydro-7-methoxy-2-(4-hydroxy-3, 5-dimethoxyphenyl)-3-hydroxymethyl-5-(2-formyl-vinyl)-benzofuran (XV, compound-I) は cinnamic aldehyde 側鎖を有するフェニールクマランである。以前，FREUDENBERG と LEHMANN[51] は2種の cinnamic aldehyde 単位を有する化合物を coniferyl alcohol の酵素的脱水素重合中間体より単離した。それらの1種，すなわち，guaiacyl-glycerol-β-coniferyl aldehyde ether は，後に NIMZ[27] によりトウヒリグニン加水分解物からえられている。しかしもう1種，すなわち，フェニールクマラン型の化合物はこれまでプロトリグニンの加水分解からはえられていない。一方，本研究でえられた化合物はグァヤシル，シリンギル共重合体であるが，cinnamic aldehyde 型フェニールクマラン構造がプロトリグニン中に存在することを実証するものである。さらに，これらの cinnamic aldehyde 側鎖がリグニンの特異的な呈色反応であるフロログルシノール・塩酸反応に与える基として意義深い。

2, 3-Dihydro-7-methoxy-2-(4-hydroxy-3, 5-dimethoxyhenyl)-3-hydroxymethyl-5-benzofuran-propanol (XVI, compound-J) は ω-hydroxypropyl 基を有している。この様な dihydrocinnamic alcohol 単位を有する dilignol 類は，最近，植物抽出成分から単離されているが，化合物 (XVI) が光学的に不活性であることは極めて重要である。すなわち，抽出成分からえられている類似化合物はすべて光学的に活性であり，このことは化合物 (XVI) がリグニンに由来する確率が高いことを示すものである。またカバ材リグニン加水分解物から dihydrosinapyl alcohol が検出された事実も，プロトリグニン中に dihydrocinnamic alcohol 単位がオリジナルに存在することを支持している[55]。さらに，化合物 (XVI) の ω-hydroxypropyl 側鎖は，従来まで由来が

不明であったプロトリグニンのNMRスペクトル中の高度に遮蔽されたシグナルの由来を説明するものである。化合物(XVI)の単離により，リグニン形成時に広範な不均衡化反応(disproportionation)が起こるのか，あるいは特異的な還元機構が存在するのかという点において，リグニン生発生的見地から興味がもたれている。

他の6種の2量体は，すでにNIMZ，榊原らによりプロトリグニンの温和な加水分解物から単離されているものである。

以上，本研究でえられた結果はFREUDENBERGのリグニン生合成理論を逸脱することはないが，それを中心として広範な付随的反応の可能性を示唆するものである。またヤチダモ材における同様の分解物から考えて，広葉樹においては樹種間の相違はリグニン分子中の結合型に本質的な違いをもたらさないものと思われる。