Acidolysis of Bamboo Lignin II

Isolation and Identification of Acidolysis Products*

Fumiaki NAKATSUBO**, Mitsuhiko TANAHASHI**
and Takayoshi HIGUCHI**

Abstract——Guaiacyl, syringyl and *p*-hydroxyphenyl monomers which are expected by cleavage of the β-ether bonds in arylglycerol-β aryl ether structures have been isolated and identified in the acidolysis products of a bamboo MWL by various methods such as IR and NMR spectrometry. ω-Hydroxysyringylacetone was the predominant product in monomeric fraction followed by ω-hydroxyguaiacylacetone. DL-Syringaresinol and DL-episyringaresinol were isolated from dimeric fraction and identified by melting point, UV, IR, NMR and mass spectrometry. These findings in addition to previous results indicate that the bamboo MWL is a mixed polymer of guaiacyl and syringyl propanes and a small amount of *p*-hydroxyphenylpropane connected through similar linkages found in spruce MWL.

Introduction

In a previous paper[1], acidolysis monomers of a bamboo MWL have been identified as trimethylsilyl (TMS) derivatives and determined quantitatively by gas-liquid chromatography and mass spectrometry. ω-Hydroxysyringylacetone, 2-hydroxy-1-(4-hydroxyphenyl)-1-propanone, 1-hydroxy-1-(4-hydroxyphenyl)-2-propanone and *p*-hydroxyphenylacetone were found as new additional compounds. A similar experimental result on the analysis of TMS derivatives of the acidolysis monomers of spruce MWL has been reported recently by Lundquist and Kirk[2].

In the present investigation acidolysis products of a bamboo MWL have been fractionated by gel filtration on Sephadex and individual compounds in both monomeric and dimeric fractions have been identified by various methods such as IR, NMR and mass spectrometry.

Experimental

Preparation of Milled Wood Lignin (MWL)

Milled wood lignin of a bamboo (*Phyllostachys pubesens*) was prepared as previously[1] and purified by Björkman's standard method[3].

Acidolysis

The acidolysis was performed according to Lundquist[4]. The bamboo MWL (18 g) was dissolved in 1.8 *l* of dioxane-water (9 : 1) containing 0.2 *N* HCl and heated at reflux temperature for 4 hrs bubbling nitrogen through the solution. The reaction mixture was adjusted at about pH 3 with 0.4 *N* $NaHCO_3$ and extracted with chloroform, and polymeric materials in the extract (19.2 g) were removed by silicagel column chromatography (50 mm × 60 cm) eluted with benzene-ethyl acetate (3 : 1). The eluate was evaporated into dryness and the residue (10.4 g) was fractionated by gel filtration on Sephadex G-25 M (40 mm × 162 cm, 600 g). The elution was

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** Division of Lignin Chemistry.

accomplished with dioxane-water (1 : 1, flow rate, 20 ml/hr). An aliquot of the eluate collected in 5 ml fractions was used for the measurement of UV spectrum at 280 mμ as ethanol solution and the elution curve, (optical density at 280 mμ against elution volume) was obtained.

Isolation of Compounds in Fraction C (3.5 g)

The fraction C was extracted with chloroform-dioxane (1 : 1), the organic layer was dried over anhydrous Na_2SO_4 and the solvent was evaporated. The residues were dissolved in chloroform and white insoluble crystals (600 mg) which were identified to be *p*-coumaric acid by IR and NMR spectra were filtered off and the soluble portion was subjected to silicagel column chromatography (30 mm × 115 cm, solvent, chloroform) and the eluate was divided to 5 ml fractions by a fraction collector. The compounds in each fraction were tested by silicagel TLC (PF_{254}) using a UV lamp and the fractions giving the same single quenching spot were combined, whereas the fractions containing more than two compounds were further separated by silicagel preparative thin layer chromatography (PTLC, 20 cm × 20 cm, PF_{254}), and respective quenching bands on the TLC plate were scratched off and extracted with chloroform containing 10 % methanol. The solvent was evaporated and the residue was weighed and crystallized with suitable solvents. However, by single PTLC procedure compounds were sometimes difficult to be separated and in such cases comparatively pure fraction was scratched off, extracted and subjected further to a separate PTLC. The eluates of the Fraction C were analyzed as follows, and individual compounds obtained were identified by mixed melting point, IR and NMR spectrometry. The mixed melting point, IR and NMR spectra of the compounds were completely identical with those of authentic compounds.

Tubes 1-29 (96.5 mg)

By PTLC developed with chloroform vanilloyl methyl ketone (44 mg), vanillin (15 mg), *p*-hydroxybenzaldehyde (13 mg) and vanillic acid (10 mg) were isolated and identified.

Vanillin, m.p. 81-82°C colorless needles from *n*-hexane-benzene. ν (KBr) 1672 cm^{-1}; δ (ppm, $CDCl_3$) 3.92 (3H, s), 7.03 (1H, d, J=8), 7.43 (1H, d, J=2), 7.51 (1H, dd, J=8, 2), 9.68 (1H, s).

Vanilloyl methyl ketone[5], m.p. 68-69°C yellow needles from pet. ether. ν (KBr) 1720, 1665 cm^{-1}; δ (ppm, $CDCl_3$) 2.42 (3H, s), 3.93 (3H, s), 6.33 (1H, broad), 6.98 (1H, d, J=8), 7.12 (1H, d, J=2), 7.05 (1H, dd, J=8, 2).

Vanillic acid, m.p. 210-211°C colorless needles from water. ν (KBr) 1680-1710, 1600, 1525, 1300, 1115 cm^{-1}; δ (ppm, $CDCl_3/CD_3OD$, 9 : 1) 2.68 (1H, broad), 3.48 (3H, s), 6.87 (1H, d, J=8), 7.53 (1H, d, J=2), 7.62 (1H, dd, J=8, 2).

p-Hydroxybenzaldehyde, m.p. 115-116°C needles from water. ν (KBr) 1672, 1600, 1295, 1160 cm^{-1}; δ (ppm, $CDCl_3$) 6.92 (2H, d, J=8), 7.77 (2H, d, J=9), 9.84 (1H, s).

Tubes 30-50 (225 mg)

By PTLC using benzene as solvent vanilloyl methyl ketone with higher Rf value (48 mg) was first isolated and the residual materials (150 mg) were further separated by PTLC (solvent, isopropyl ether saturated with water), and syringaldehyde (20 mg) and syringoyl methyl ketone (100 mg) were isolated and identitied as deseribed previously.

Syringoyl methyl ketone[5], m.p. 80-81°C yellow needles from pet. ether. ν (KBr) 1720, 1660, 1520, 1340, 1130 cm^{-1}; δ (ppm, $CDCl_3$) 2.50 (3H, s), 3.92 (6H, s), 6.44 (1H, broad), 7.23 (2H, s).

Syringaldehyde, m.p. 109-110°C needles from pet. ether. ν (KBr) 1675, 1600, 1335, 1100 cm^{-1}; δ (ppm, $CDCl_3$) 3.90 (6H, s), 6.05 (1H, broad), 6.97 (2H, s), 9.47 (1H, s).

Tubes 51-74 (314 mg)

The fractions were subjected to PTLC, developed five times with benzene and then with chloroform. Sinapaldehyde (50 mg) was isolated and from the residual materials (186 mg) syringylacetone (50 mg) was separated by further developing with isopropyl ether saturated with water. The residues (100 mg) were again separated by PTLC developed 4 times with chloroform into guaiacylacetone (38 mg) and the residue (21 mg). Further separation of the last residue by PTLC developed 7 times with *n*-hexane-ether-pet. ether (1 : 1 : 2) guaiacylacetone (5 mg) and coniferylaldehyde (10 mg) were isolated and identfiied.

Sinapaldehyde, m.p. 190°C yellow needles from benzene. δ (ppm, $CDCl_3$) 3.90 (6H, s), 6.00 (1H, broad), 6.60 (1H, dd, J=16, 8), 6.83 (2H, s), 7.40 (1H, d, J=16), 9.69 (1H, d, J=8). Characteristic red purple coloration with phloroglucinol-HCl.

Syringylacetone[5], m.p. 69–70°C colorless needles from chloroform. ν (KBr) 1720, 1615, 1520, 1110 cm^{-1}; δ (ppm, $CDCl_3$) 2.12 (3H, s), 3.55 (2H, s), 3.80 (6H, s), 6.40 (2H, s).

Guaiacylacetone[5], yellow liquid. δ (ppm, $CDCl_3$) 2.13 (3H, s) 3.58 (2H, s), 3.83 (3H, s), 6.50–7.00 (3H, m).

Coniferylaldehyde, m.p. 80–82°C yellow needles from benzene. ν (KBr) 1685, 1650, 1520, 1435, 1295, 1140, 1030 cm^{-1}; δ (ppm, $CDCl_3$) 3.80 (3H, s), 6.50 (1H, dd, J=16,8), 6.70 (1H, d, J=8), 7.04 (1H, d, J=2), 7.11 (1H, dd, J=8,2), 7.43 (1H, d, J=16), 9.64 (1H, d, J=8). Characteristic red purple coloration with phloroglucinol-HCl.

Tubes 75–184 (118 mg)

The fractions were separated into two major quenching bands (I and II), each of them was accompanied with a weak band of a little lower Rf value than that of the major compound, by PTLC developed 3 times with isopropyl ether. The compound with higher Rf value (I) was isolated and purified, but complete separation from the accompanied compound could not be accomplished because of newly formation of the latter during purification. The NMR spectrum showed that the compound (I) was a mixture of 2-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone and 1-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-2-propanone which gave deep blue color with quinonemonochlorimide reagent[6], with 4 : 1 ratio. However, when the compound (I) was acetylated with pyridine-acetic anhydride and subjected to PTLC developed 3 times with isopropyl ether-*n*-hexane (1 : 1) the acetate of the former compound (68 mg) was obtained as colorless crystals and identified.

2-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone diacetate, m.p. 120–121°C colorless needles from chloroform-pet. ether. ν (KBr) 1778, 1744, 1720, 1605, 1275, 1255, 1212, 1200, 1180, 1108 cm^{-1}; δ (ppm, $CDCl_3$) 1.46 (3H, d, J=7), 2.07 (3H, s), 2.26 (3H, s), 7.52 (1H, dd, J=8, 2), 7.60 (1H, d, J=2).

Similarily complete separation of the compound (II) with lower Rf value from the accompanied compound was not successful and isolated major compound contained a small amount of the accompanied compound which gave deep blue color with quinonemonochlorimide reagent[6]. Both compounds gave red color in chlorination and subsequent spraying with 3% Na_2SO_3, and the NMR spectrum indicated that the compound II was a mixture of 2-hydroxy-1-(4-hydroxy-3, 5-dimethoxyphenyl)-1-propanone and 1-hydroxy-1-(4-hydroxy-3, 5-dimethoxyphenyl)-2-propanone with 20 : 1 ratio. The purification of the former compound was accomplished by PTLC of its acetate at the same condition used for the acetate of the compound I, and obtained as colorless crystals (70 mg).

2-Hydroxy-1-(4-hydroxy-3, 5-dimethoxyphenyl)-1-propanone diacetate, m.p. 131–132°C colorless needles from chloroform-pet. ether. ν (KBr) 1778, 1748, 1711, 1604, 1253, 1210, 1115, 1129 cm^{-1}; δ (ppm, $CDCl_3$) 1.46 (3H, d, J=7), 2.08 (3H, s), 2.29 (3H, s), 3.83 (6H, s), 5.94 (1H, q, J=7),

7.22 (2H, s).

Tubes 185-259 (160 mg)

The fractions were purified by PTLC developed 2 times with benzene-ethyl acetate (4 : 1), and ω-hydroxyguaiacylacetone (80 mg) was obtained as crystal and identified as described previously.

Tubes 260-310 (112 mg)

The fractions gave ω-hydroxyguaiacylacetone (90 mg) by the same PTLC procedure as in the former fractions, and the compound was identified.

ω-Hydroxyguaiacylacetone[1], m.p. 81-82°C colorless needles from chloroform. ν (KBr) 1745, 1620, 1535, 1295, 1155 cm^{-1}; δ (ppm, $CDCl_3$) 3.62 (2H, s), 3.83 (3H, s), 4.18 (2H, s), 6.97-6.47 (3H, m).

Tubes 311-380 (350 mg)

The fractions were separated into ω-hydroxysyringylacetone (250 mg), *p*-hydroxybenzoic acid (5 mg), syringic acid (7 mg) and *p*-coumaric acid (26 mg), which were identified by mixed melting point, IR and NMR spectrometry, by PTLC developed 5 times with chloroform containing 2 % methanol.

ω-Hydroxysyringylacetone[1], m.p. 104-105°C needles from chloroform. ν (KBr) 1720, 1613, 1515, 1205, 1120, 1155 cm^{-1}; δ (ppm, $CDCl_3$) 3.58 (2H, s), 3.82 (6H, s), 4.25 (2H, s), 5.52 (1H, broad), 6.42 (2H, s).

p-Hydroxybenzoic acid, m.p. 213-214°C crystallized from water. ν (KBr) 1710-1670, 1600, 1520, 1255 cm^{-1}.

Syringic acid, m.p. 204-205°C crystallized from water. ν (KBr) 1710, 1610, 1525, 1380 cm^{-1}.

p-Coumaric acid, m.p. 211-213°C crystallized from methanol-chloroform. ν (KBr) 1710-1660, 1635, 1605, 1455, 1320, 1220, 1257, 830 cm^{-1}; δ (ppm, $CDCl_3$) 6.20 (1H, d, J=16), 6.78 (2H, d, J=8), 7.35 (2H, d, J=8), 7.39 (1H, d, J=16).

Isolation of Compounds in Fraction B (2.7 g)

The fractions were subjected to silicagel column chromatography (30 mm×90 cm) developed with chloroform-benzene-ethyl acetate (1 : 1 : 1), benzene-ethyl acetate (2 : 1) and with benzene-ethyl acetate (1 : 1), successively. The eluate was collected in 5 ml fractions and each fraction was tested by TLC using a UV lamp. The fractions containing similar compounds were combined and the following fractions were finally obtained. Fractions I (117 mg), II (144.3 mg), III (188 mg), IV (138 mg) and V (115 mg). Further fractions gave polymeric materials and were not investigated.

Fractions I-III were subjected to PTLC with isopropyl ether-chloroform-benzene (1 : 1 : 1) as solvent. However, compounds on the PTLC plate sometimes gave tailing and undivided bands and individual compounds could not be separated. Further separation gave relatively pure compounds, but their amounts decreased less than 2 mg and were not enough for identification.

Fraction IV (138 mg)

The fraction was subjected to PTLC developed 7 times using isopropyl ether-chloroform-benzene (1 : 1 : 1) as solvent and 8 quenching bands were separated. The compounds extracted from the bands 1-4 from the top of the plate were trace in amount, and bands 5, 6, 7 and 8 gave 5.5, 18, 80 and 20 mg, respectively.

Fraction IV-band 6 (18 mg)

The fraction gave a single quenching spot on TLC and then it was acetylated with pyridine-acetic anhydride and purified by PTLC developed 2 times with chloroform. The acetate

extracted from the PTLC plate gave colorless oil (5 mg). It was crystallized from methanol and identified to be DL-episyringaresinol diacetate by melting point, UV, IR, NMR and mass spectrometry. The IR spectrum was identical to that of syringaresinol diacetate, but the NMR spectrum differed from that of syringaresinol. The proton at α carbon (δ 4.83 ppm) gave different chemical shift from that of the proton at α' carbon (δ 4.45 ppm) as found for epipinoresinol[7], and the spectrum was in good agreement with the configuration of episyringaresinol.

DL-Episyringaresinol diacetate, m.p. 178–179°C ν (KBr) 1770, 1606, 1465, 1375, 1200, 1130, 1075 cm^{-1}; δ (ppm, $CDCl_3$) 2.26 (6H, s), 2.70–3.00 (1H, m), 3.15–3.50 (1H, m), 3.65–4.25 (4H, m), 3.79 (12H, s), 4.45 (1H, d, J=7), 4.83 (1H, d, J=5), 6.61 (4H, s); $[\alpha]_D^{25}$ 0° (C=1.0, chloroform); UV_{max}^{EtOH} (log ε) 237 (4.18), 272 (3.47), 280 (sh. 3.26); MS 502 (M^+).

Fraction IV-band 7 (80 mg)

The fraction was subjected to PTLC developed 5 times using isopropyl ether-chloroform-benzene (1 : 2 : 1) and separated into three bands (7a, 21 mg, 7b, 9 mg, 7c, 29.2 mg). The compound of 7a was crystallized from methanol. Colorless crystals (7 mg). Identified to be DL-syringaresinol by various methods. The UV, NMR and IR spectra were identical to those of authentic compound donated by Dr. J. M. Harkin, and also to the data by Terazawa et al[8] and Omori et al[9], respectively.

Syringaresinol, m.p. 166–167°C ν (KBr) 1775, 1605, 1468, 1372, 1200, 1132, 1075 cm^{-1}; δ (ppm, $CDCl_3$) 3.07 (2H, m), 3.75–4.00 (4H, m), 4.10–4.90 (2H, m), 4.75 (2H, d, J=5), 6.60 (4H, s); $[\alpha]_D^{25}$ 0° (C=1.5, chloroform); UV_{max}^{EtOH} (log ε) 270 (3.54), 277 (sh. 3.46); MS 418 (M^+).

Faction IV-band 8 (20 mg)

The fraction gave a tailing and undivided band with low Rf value and could not be identified.

Method of Analysis

HITACHI model 124 double beam spectrophotometer and JASCO model IR-S were used for UV and IR spectra, respectively. NMR spectra were taken by the use of an JEOL Minimer (MH-60) and a R22 HITACHI high resolution NMR spectrometer with TMS internal standard, respectively. Mass spectrometry was conducted by the use of a SHIMAZU-LKB 9000 mass-spectrometer.

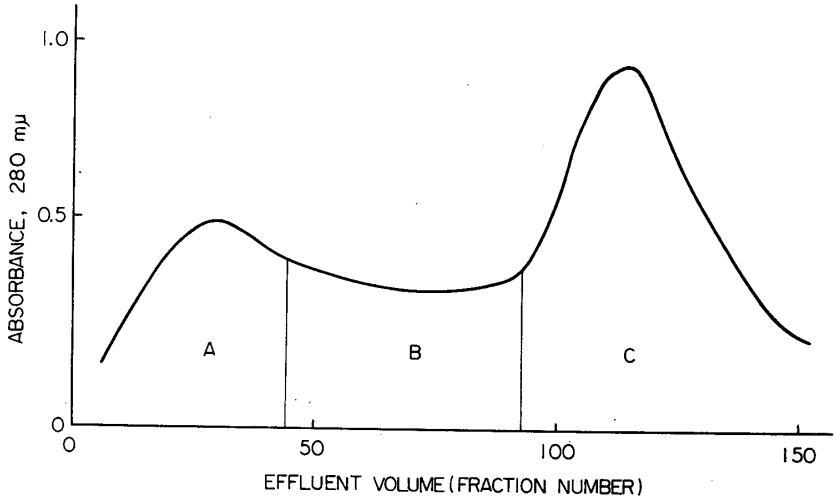

Fig. 1. Gel filtration of acidolysis products of bamboo MWL.

Results and Discussion

A recent work by Lundquist[4] has established that low molecular weight phenols liberated in the acidolysis of Norway spruce are separated by gel filtration on Sephadex and that the phenols are due to primarily to cleavage of the β-ether bonds in arylglycerol-β-aryl ether structure in the lignin.

Separation of the acidolysis products of a bamboo MWL on Sephadex column is shown in Fig. 1. The elution curve which was arbitrarily divided into A, B and C toward low molecular

Compounds in Fraction C (3.5g)

- Tubes 1-29 (96.5mg)
  - Vanillin (15mg)
  - Vanillic acid (10mg)
  - Vanilloyl methyl ketone (44mg)
  - p-Hydroxybezaldehyde (13mg)
- Tubes 30-50 (225mg)
  - Vanilloyl methyl ketone (48mg)
  - Syringaldehyde (20mg)
  - Syringoyl methyl ketone (100mg)
- Tubes 51-74 (314mg)
  - Sinapaldehyde (50mg)
  - Syringyl acetone (50mg)
  - Guaiacyl acetone (43mg)
  - Coniferyl aldehyde (10mg)
- Tubes 75-184 (118mg)
  - 2-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone (48mg)
  - 2-Hydroxy-1-(4-hydroxy-3.5-dimethoxyphenyl)-1-propanone (50mg)
- Tubes 185-259 (160mg)
  - ω-Hydroxyguaiacyl acetone (80mg)
- Tubes 260-310 (112mg)
  - ω-Hydroxyguaiacyl acetone (90mg)
- Tubes 311-380 (350mg)
  - ω-Hydroxysyringyl acetone (250mg)
  - p-Hydroxybenzoic acid (5mg)
  - Syringic acid (7mg)
  - p-Coumaric acid (26mg)

Total yield of monomers (1.559g), 8.7% of lignin
Yield of P-Coumaric acid (626mg), 3.5%

Compounds in Fraction B (2.7g)

- DL-Episyringaresinol (5mg)
- DL-Syringaresinol (7mg)

Fig. 2. Isolation of compounds in fractions B and C.

fractions was apparently similar to that of Norway spruce found by Lundquist. However, the curve between fractions 45 and 91 (B) which was assumed to be composed of dimeric compounds showed a plateau but not such a peak in monomeric fraction (C), and the same pattern of the curve was found through five separate experiments. Thus it may be conceivable that different pattern of the elution curve between spruce and bamboo is ascribed to different chemical structure of both lignins in acidolysis but not to the experimental conditions used. It was found, however, that only 26 % of the fraction C subjected to silicagel column chromatography was eluted with chloroform as identifiable monomeric compounds. The result may therefore explain the different pattern of the elution curve between both lignins as such that some of the dimeric compounds are contained in the uneluted remainder of the fraction C on the column. Further investigations are in progress for the uneluted fraction concerning this problem.

Isolation and identification of individual compounds in the eluate of the fraction C were carried out by PTLC as described in the Experimental. Seven guaiacyl and syringyl monomers and 3 *p*-hydroxyphenyl monomers were identified as summarized in Fig. 2.

These compounds, except *p*-coumaric acid, should have been liberated by cleavage of the β-ether bonds in guaiacyl, syringyl and *p*-hydroxyphenylglycerol-β-aryl ether structures, whereas *p*-coumaric acid should be produced by splitting of the esterified acid which may link at γ-carbon of the side chain of C_6-C_3 units[10].

Lundquist[4] established that 2-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone and 1-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-2-propanone formed an equilibrium mixture on acidolysis and that the complete separation of both compounds on silicagel column was impossible. It was the case in the present investigation, and 2-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone extracted from the corresponding band on the PTLC plate showed to be a mixture with its 2-propanone, and the NMR spectrum indicated that the amount of terminal methyl protons of the 1-propanone (δ 1.44 ppm) was 4 times greater than that of 2-propanone (δ 2.06 ppm). Similarily 2-hydroxy-1-(4-hydroxy-3, 5-dimethoxyphenyl)-1-propanone extracted was a mixture with its 2-propanone, and the ratio of both compounds corresponded to 20 : 1 jugging from the ratio of integrated terminal methyl protons (1-propanone, δ 1.40 ppm ; 2-propanone, δ 2.04 ppm).

ω-Hydroxysyringylacetone which was identified as TMS derivative previously[1] was first obtained as crystals in the acidolysis monomers of the bamboo MWL, and it was the most abundant phenol followed by ω-hydroxyguaiacylacetone. However, ω-hydroxy-1-(4-hydroxyphenyl)-2-propanone and its derived monomers which were also detected as TMS derivatives by gas chromatography and mass spectrometry[1] could not be isolated in the present investigation. As reason for lacking *p*-hydroxyphenylmonomers the following may be supposed. 1) The amount of participated *p*-hydroxyphenylpropane components in the bamboo MWL is considerably low. 2) *p*-Hydroxyphenylpropanes of which aromatic nuclei are not substituted at both C_3 and C_5, are connected mainly through those carbons by C-C linkages each other and thus *p*-hydroxyphenyl monomers are not formed appreciably in the acidolysis of the bamboo MWL.

The first explanation may be more probable, because a recent investigation[11] on permanganate oxidation of methylated DHP of *p*-coumaryl alcohol showed that the ratio of isophutalic acid to *p*-anisic acid was almost the same to that of isohemipinic acid to veratric acid from methylated DHP of coniferyl alcohol. Concerning this problem investigations on the difference in reactivity of coniferyl and *p*-coumaryl alcohols in their enzymic dehydrogenation are in progress.

In the acidolysis of guaiacylglycerol-β-guaiacyl ether, syringylglycerol-β-guaiacyl ether, *p*-hydroxyphenylglycerol-β-guaiacyl ether and guaiacylglycerol-α-guaiacylpropyl-β-guaiacyl diether

at the present experimental condition, the β-ether bond of these model compounds cleaved completely within 30 min and produced corresponding ω-hydroxyphenylacetones, guaiacol (and guaiacylpropane in the last model[12]). It is thus conceivable that the β-ether bonds in the bamboo MWL should be cleaved completely in the present experimental condition.

Thus the following reason is suggested concerning low content of the acidolysis monomers. 1) Considerable parts of the arylglycerol moiety carring β-aryl ether is linked other with phenylpropane units through C-C linkages and do not produce the monomers and/or 2) During progress of the β-ether cleavage the formation of the C-C linkages such as C_α-C_5 and C_α-C_6 occurs concomitantly and the product thus formed can not be cleaved any more[13]. The recent investigation[12] on a bamboo MWL has indicated that the total amount of β-0-4 linkage is 0.54/C_6-C_3 among which 0.30 is found as condensed type which can not produce acidalysis monomers, and that secondary condensation reaction through C-C linkages also occurred considerably during acidolysis.

The amount of dimeric fraction was considerably low and the gradient elution from silicagel column with benzene-ethyl acetate was not successful. PTLC was used finally for isolation of this fraction, but repeated procedure of PTLC lost substantial amounts of the compounds and did not give enough amount for identification in many cases.

Two dimeric compounds, DL-syringaresinol and DL-episyringaresinol were only isolated from the fraction B and identified by UV, IR, NMR and mass spectrometry. The former compound has been isolated as a hydrolysis product of hardwood lignins[9,14] and from the extractives of *Fraxinus mandshurica*[8]. However, the latter compound has not hitherto been isolated as lignin degradation product although it is known in the dehydrogenation products of sinapyl alcohol[15].

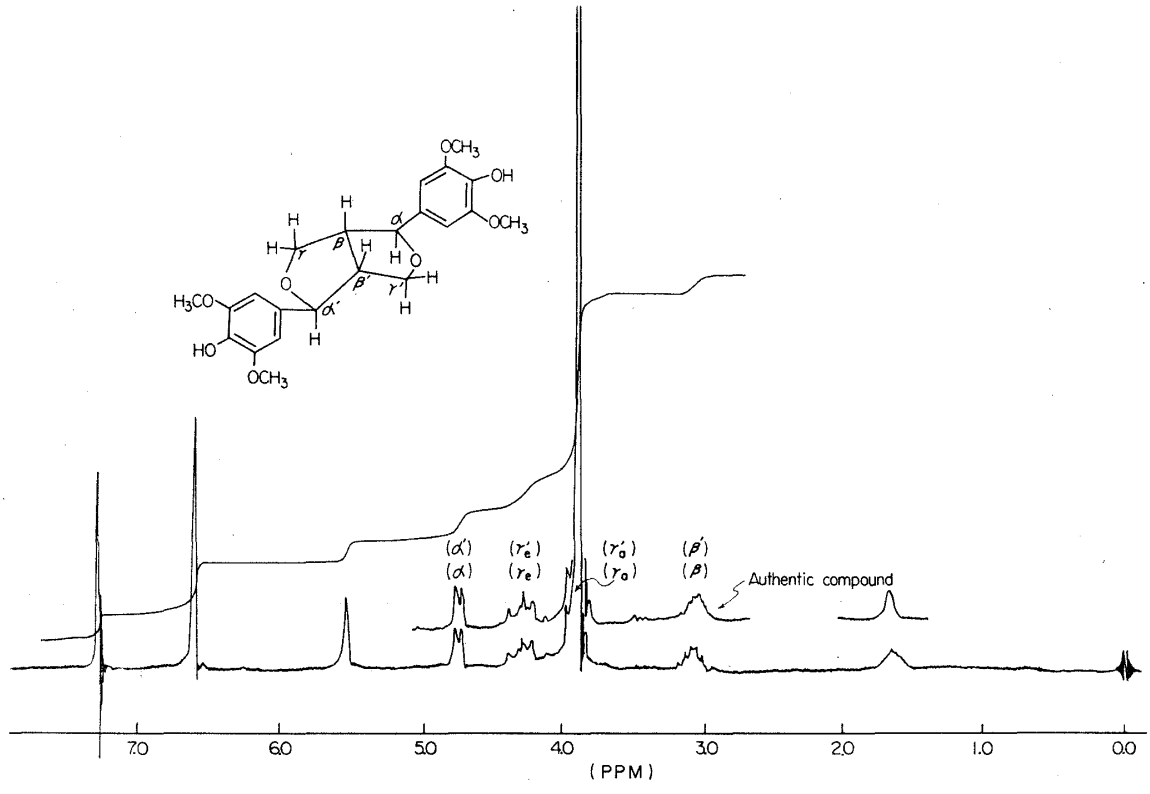

Fig. 3. NMR spectrum of DL-syringaresinol.

Then it is not clear at present whether or not the compound was formed by isomerization of DL-syringaresinol during acidolysis.

As shown in Fig. 3, NMR spectrum of DL-syringaresinol was identical to those reported previously[8,9]. The spectrum indicated that the chemical shift of the protons linked at α, β and γ carbons corresponds completely to that of the protons at α', β' and γ' carbons, reflecting its configuration, whereas the NMR spectrum of DL-episyringaresinol clearly differs from that of syringaresinol and the protons linked at α, β and γ carbons gave different chemical shift to that at α', β' and γ' carbons, respectively as found in epipinoresinol[7] (Fig. 4).

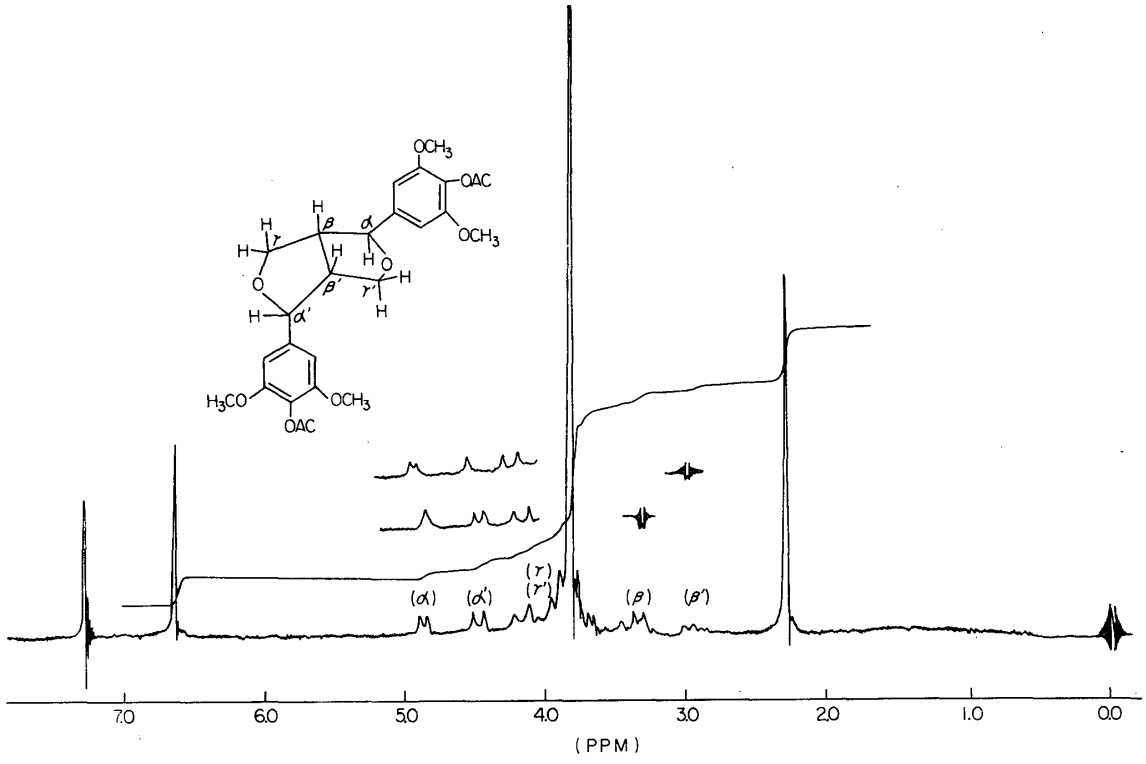

Fig. 4. NMR spectrum of DL-episyringaresinol diacetate.

Thus the present investigation confirmed previous results[1] on the analysis of the acidolysis products by gas chromatography and mass spectrometry, and that the bamboo MWL is a mixed polymer of guaiacyl and syringyl propanes and a small amount of *p*-hydroxyphenylpropane connected through similar linkages found in spruce lignin.

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