

Structural Elucidation of Bamboo Lignin by Acidolysis and Ozonolysis I.*

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Abstract—Bamboo lignin was completely degraded into the oligomers composed of monomeric to pentameric products by acidolysis and mild ozonolysis. Model experiment showed that the phenylcoumarone moiety which is known to be produced from the phenylcoumarane structure in lignin in acidolysis is cleaved almost quantitatively to 5-acetyl derivatives and *p*-hydroxybenzoic acid derivatives by the mild ozonolysis (-70°C). From the monomer fraction of the ozonolysis products of the lignin residue by acidolysis *p*-hydroxybenzoic acid derivatives (XXI, XVII), methyl *p*-hydroxybenzoate derivatives (XXII, XXVI, XXVIII), *p*-cresol derivatives (XVIII, XXIII), *p*-hydroxybenzaldehyde derivatives (XX, XXV, XXVII), *p*-hydroxybenzylmethyl ether derivatives (XIX, XXIV) and dimethyloxalate (XXIX) were obtained, respectively.

Introduction

Many degradation reactions¹⁾ have been applied for the structural elucidation of lignin. However, lignin which is composed of a variety of ether and C-C linkages could not be completely degraded to low molecular weight compounds without any secondary condensations during reactions. Therefore the structure of lignin has been formulated based on the information of low molecular weight degradation products of a small portion of lignin²⁾. To obtain full information of lignin structure, lignin must be completely degraded to identifiable low molecular weight compounds without secondary reactions. Arylglycerol- α - and β -arylether bonds in lignin are cleaved in acidolysis, and the low molecular weight fraction obtained has been well studied³⁻¹³⁾. However, the high molecular weight fraction, "acidolysis residue" which comprises about 50 % of original lignin³⁾, has scarcely been studied.

In the present investigation, in order to clarify the structure of the high molecular weight fraction, ozone degradation reaction was tested under a mild condition (-70°C). Ozonolysis of lignin which involved the cleavage of aromatic rings under a drastic condition had been done with intention of bleaching of pulp and degradation of isolated lignin¹⁴⁻¹⁹⁾. Although lignin would be completely degraded to the low molecular weight

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compounds by these reactions, it is impossible to use such a drastic condition for elucidation of chemical structure of lignin.

Thus, in the present experiment, the acidolysis residue was subjected to ozonolysis at a low temperature (-70°C) which cleaved only the double bonds of phenylcoumarone and stilbene derived from the phenylcoumarane and 1,2-diarylpropane moieties of lignin in acidolysis.

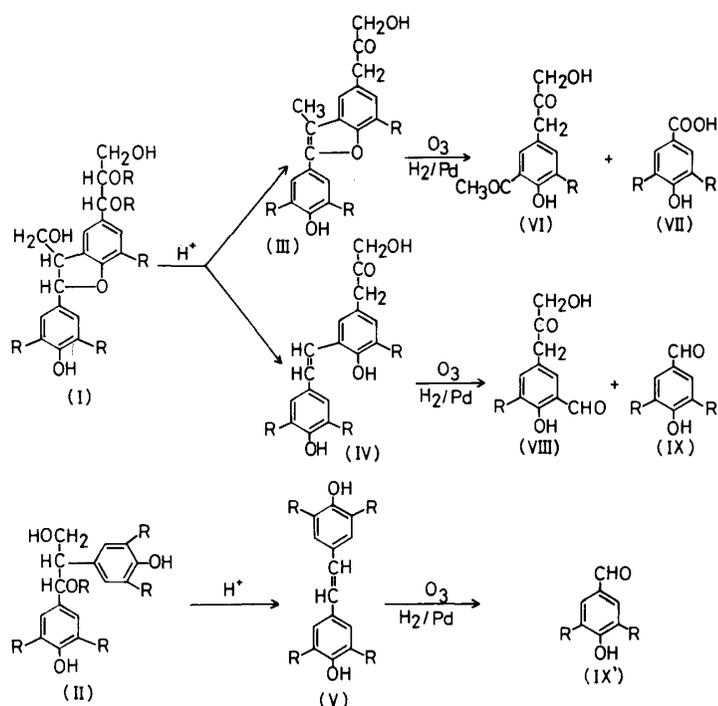


Fig. 1. Possible reaction mechanisms in acidolysis and ozonolysis of lignin.

Fig. 1 shows the reaction processes of the compounds of the structures (I) and (II), which are known to be abundant in lignin. Arylglycerol- α - and β -ether bonds of the phenylcoumarane (I) are cleaved in acidolysis and the side chain of the guaiacylglycerol moiety is converted to the Hibbert's ketone type structure²⁰⁾ and further, the phenylcoumarane ring is converted to the phenylcoumarone (III) by dehydration or to the stilbene (IV) by elimination of formaldehyde⁶⁾. Similarly the 1,2-diarylpropane (II) is converted to the stilbene (V)^{7,8)}. Thus, when these acidolysis products are subjected to ozonolysis and subsequent catalytic hydrogenation both 5-acetyl compound (VI) and *p*-hydroxybenzoic acid (VII) are expected to be produced from the phenylcoumarone (III), and 5-formyl compound (VIII) with *p*-hydroxybenzaldehyde (IX) from the stilbene (IV), respectively. While the stilbene (V) from the compound (II) gives two *p*-hydroxybenzaldehydes (IX', IX'').

Thus, arylglycerol- α - and β -arylether, phenylcoumarane and 1,2-diarylpropane

moieties of lignin may be cleaved by acidolysis and the following ozonolysis, resulting in fairly low molecular weight compounds.

Experimental

Syntheses

Dehydro-di-isoeugenol (XI) was prepared from isoeugenol (X) according to the procedure described by Leopold²¹⁾.

Dihydro-dehydro-di-isoeugenol (XII). To a solution of dehydro-di-isoeugenol (7.4 g, 22.7 mmol) in 80 ml of methanol, 1.5 g of 5% palladium-carbon was added as catalyst, and the solution was stirred for 24 hours at room temperature under hydrogen. The catalyst was removed by filtration and the filtrate was evaporated to dryness under a reduced pressure. The oil remained was crystallized from ether-hexane (1:1), and the crystals were filtered, washed with ether-hexane and dried *in vacuo*. Yield: 6.35 g (85.3%). Analytical data were in good agreement with the structure of the expected compounds.

3-Methyl-5-propyl-7-methoxy-2-(4-hydroxy-5-methoxyphenyl) coumarone: Phenylcoumarone (XIII) was prepared according to the procedure described by A. Spetz²²⁾. Dihydro-dehydro-di-isoeugenol (XII) (6.56 g, 20 mmol) was heated in a metal bath with 0.704 g (22 mmol) of sublimed sulfur. Reaction temperature was raised from 100°C to 250°C for one hour, and the reaction mixture was then distilled *in vacuo* (1 mmHg) at 220~240°C. Yellow oil obtained was crystallized and further purified by alumina column chromatography. Yield; 4.79 g (73.5%). NMR (CCl₄) δ : 0.93, 3 H, t, $J=6.5$; 1.64, 2 H, m, $J=6.5$; 2.31, 3 H, s; 2.58, 2 H, d, $J=6.5$; 3.87, 3 H, s; 3.94, 3 H, s; 5.61, 1 H, s; 6.48, 1 H, d, $J=1.5$; 6.76, 1 H, d, $J=1.5$; 6.88, 1 H, d, $J=9.0$; 7.21, 1 H, dd, $J=9.0, J=1.5$; 7.25, 1 H, s: M.p. 86~87°C. (literature 86.5~87.5°C).

Other model compounds. Creosol, vanillyl methyl ether, methyl vanillate, *p*-hydroxybenzyl methyl ether and methyl *p*-hydroxybenzoate were synthesized and used as authentic compounds. Other chemicals used were commercial compounds.

Ozonolysis of phenylcoumarone (XIII)

A solution of phenylcoumarone (XIII) (150 mg, 0.42 mmol) in 15 ml of methanol-dichloroethane (4:1) was cooled at -70°C in a dry ice-methanol bath. Into the solution, ozone which was produced by a generator (secondary voltage: 40 V) from dry oxygen (flow rate: 150 l/hr.) was introduced for 30 min. When the reaction was completed, nitrogen was introduced for several minutes and excess ozone was removed. Ozonide thus obtained was subjected to catalytic hydrogenation with 60 mg of 5% palladium-carbon. The catalyst was filtered and the filtrate was evaporated. As degradation products, 5-acetyl guaiacylpropane (XVI), vanillic acid (XVII) and a small portion of

their ester (XV) were identified. The ester (XV) was hydrolyzed with sodium hydroxide and the desired compounds (XVI) and (XVII) were obtained quantitatively. These products were separated by preparative thin layer chromatography and identified by NMR, IR, and Mass spectrometry.

5-Acetyl guaiacylpropane (XVI): Yield 72 mg (82%). NMR (CDCl_3) δ : 0.92, 3 H, t, $J=7.0$; 1.60, 2 H, m, $J=7.0$, $J=8.0$; 2.51, 2 H, t, $J=8.0$; 2.58, 3 H, s; 3.87, 3 H, s; 6.89, 1 H, d, $J=2.0$; 7.11, 1 H, d, $J=2.0$. IR (NaCl): 3040, 2965, 1655, 1470, 1385, 1340, 1278, 1190, 1150, 1065, 978, 800, 768, 655 cm^{-1} . MS (m/e): 208 (M^+), 193, 179, 161, 43.

Semicarbazone of 5-acetyl guacylpropane ($\text{C}_3\text{H}_7\text{-C}_6\text{H}_2(\text{OH})(\text{OCH}_3)\text{C}(\text{CH}_3)=\text{NNH-CONH}_2$): IR (KBr); 3580, 3510, 3440, 3120, 3000, 1685, 1560, 1460, 1308 cm^{-1} . M.p. 190~192°C.

Vanillic acid (XVII): Yield 53 mg (75%).

Vanillic acid-guaiacylpropane ester (XV): Yield 15 mg (9.9%). NMR (CDCl_3) δ : 0.96, 3 H, t, $J=7.3$; 1.64, 2 H, m, $J=7.3$; 2.46, 3 H, s; 2.60, 2 H, t, $J=7.3$; 3.80, 3 H, s; 3.96, 3 H, s; 6.19, 1 H, broad; 6.98, 1 H, d, $J=2.0$; 7.02, 1 H, d, $J=8.0$; 7.22, 1 H, d, $J=2.0$; 7.71, 1 H, d, $J=2.0$; 7.86, 1 H, dd, $J=8.0$, $J=2.0$. IR (KBr): 3450, 3000, 1730, 1690, 1603, 1522, 1440, 1293, 1210, 1190 cm^{-1} . MS (m/e): 358 (M^+), 208, 151, 123. M.p. 135~137°C.

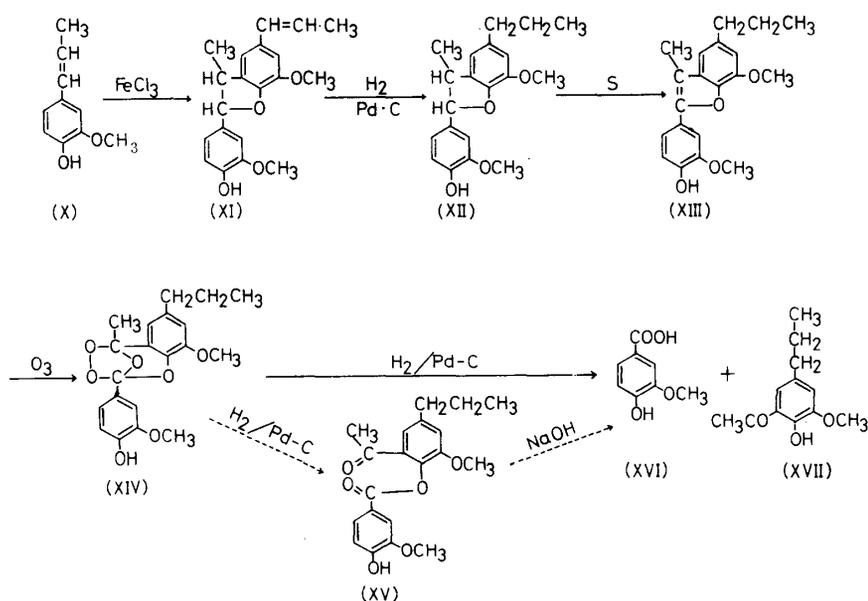


Fig. 2. Synthesis and ozonolysis of phenylcoumarone model compound.

MWL was prepared according to the standard method of Björkman²³.

Acidolysis

Bamboo *MWL* (20.0 g) was dissolved in 1 l of a solution of dioxane-water (9:1)

containing 0.2 N HCl. The solution was refluxed for 4 hours under the nitrogen atmosphere, and after cooling, the reaction mixture was added dropwisely into 10 l of water with stirring. The solution was adjusted to pH 6 with saturated NaHCO₃ solution and the precipitated lignin, "acidolysis residue", was collected by centrifugation and dried over P₂O₅ *in vacuo*. Yield 10.1 g (50.5 %).

The aqueous solution was extracted with chloroform and the chloroform solution was dried over anhydrous Na₂SO₄. The solvent was evaporated and the residue was dried over P₂O₅ *in vacuo*. Yield 7.8 g (39 %).

Mild acidolysis

The same condition and procedure as in acidolysis were used except that the reaction was carried out at 50°C for 24 hours.

Ozonolysis of acidolysis residue

The acidolysis residue (20 g) was dissolved in methanol-dichloroethane (4:1) and ozonolysis was carried out as the same condition for the model compound. The reaction process was followed by ultraviolet absorption spectrum and the reaction was stopped after 5 hours. The reaction mixture was treated as in the model compound, and the ozonide was reduced by catalytic hydrogenation with 1 g of paradium-carbon. Yield 22.95 g.

Gel filtration

The distribution of molecular weight of MWL, lignin residue in mild acidolysis, acidolysis residue, low molecular weight acidolysis products and ozonolysis products were measured with Sephadex LH-20 and G-50, respectively. Sephadex G-50 was used for the measurement of higher molecular weight fractions with dimethylsulfoxide as solvent, and Sephadex LH-20 was used for low molecular weight fractions with dimethylformamide as solvent. The column sizes were 13φ×320 mm and 10φ×400 mm, respectively. Samples (3 mg each) were dissolved in 0.2 ml of the respective solvents and applied to the columns. The elution rates for the respective fractions were 0.5 ml/2 min. and 0.5 ml/5 min., and each fraction was diluted with the solvents to a suitable concentration to measure the UV absorption at 280 mμ.

Isolation

Ozonolysis products 22.0 g were separated to five fractions by gel filtration with Sephadex LH-20 by using methanol as solvent. Amount of gel; 270 g. Column; 3φ×140 cm.

The monomer fraction (3.4 g) was separated by silicagel column chromatography by the use of chloroform and chloroform-methanol as solvents, subsequently. Then each fraction was purified by preparative thin layer chromatography, and the isolated compounds were identified by NMR, IR, TLC and MS in comparison with those of the authentic samples.

Methods of analysis

NMR spectra were recorded on a R-22 HITACHI high resolution NMR spectrometer with TMS internal standard. Mass spectrometry was conducted by using a SHIMAZU-LKB 9000 gas chromatograph-mass spectrometer. A HITACHI model 124 double beam UV spectrometer and a JASCO model IR-S were used for UV and IR spectra, respectively.

Results and discussion*Ozonolysis of phenylcoumarone (XIII)*

Phenylcoumarone (XIII) was degraded with ozone at -70°C in methanol-dichloroethane (4:1) and the reaction was followed by TLC and UV spectra in methanol intermittently (Fig. 3). A strong absorption at $320\text{ m}\mu$ of the phenylcoumarone decreased with the progress of the reaction, and the absorption disappeared after 30 min. ozonolysis. It was found at this point by TLC that the starting compound was



Fig. 3. UV spectral changes in ozonolysis of phenylcoumarone (XIII).

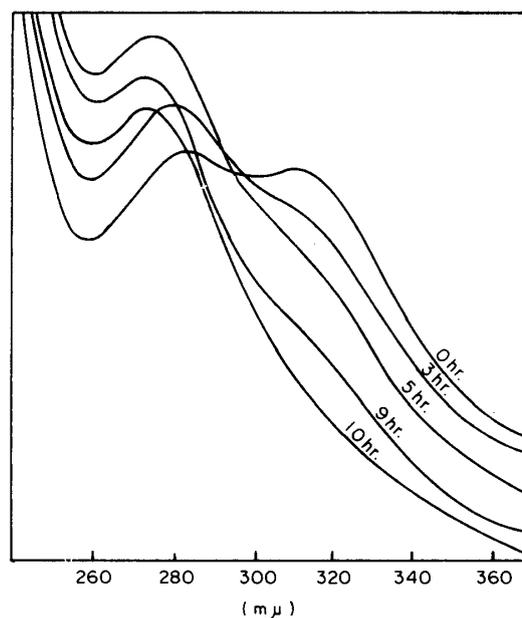


Fig. 4. UV spectral changes of the ozonolysis products of the acidolysis residue of bamboo lignin.

completely converted to its ozonide. Then the reaction was stopped and nitrogen was bubbled to remove the remaining ozone. The ozonide (XIV) was reduced by catalytic hydrogenation and 5-acetyl guaiacylpropane (XVI) and vanillic acid (XVII) were obtained almost quantitatively (Fig. 2). After about one hour ozonolysis, the solution was saturated with ozone and gave a blue color. The absorption at $280\text{ m}\mu$ then gradually decreased indicating the beginning of the degradation of aromatic ring by ozone. Methyl glyoxalate which is suggested as one of ring cleaved compounds was obtained from the reaction mixture of 5 hours ozonolysis.

The model experiments showed that the double bond in the phenylcoumarone is easily cleaved, whereas aromatic ring is stable under these conditions but it is degraded when the concentration of ozone is further raised after cleavage of the double bond. Thus the reactivities for ozone between double bond of the phenylcoumarone and the aromatic ring is remarkably different, and then only the double bond of the phenylcoumarone could be cleaved preferentially by ozonolysis.

Ozonolysis of acidolysis residue of bamboo MWL

Ozonolysis of the acidolysis residue was carried out under the same condition as in the model compound. The reaction was followed by UV spectrum (Fig. 4). As shown in Fig. 4, the acidolysis residue has a strong absorption at $320\text{ m}\mu$ owing to phenylcoumarone and stilbene, and the absorption gradually decreased when ozone was bubbled into the solution. After 5 hours ozonolysis the absorption spectrum at $320\text{ m}\mu$ remained only as a small shoulder, and then the reaction was stopped and ozonides formed were reduced by catalytic hydrogenation. The absorption shoulder at $320\text{ m}\mu$ did not appreciably decrease for several hours when ozonolysis reaction was further continued. However, the absorption at $280\text{ m}\mu$ began to decrease gradually and after about 10 hours, the absorption at $320\text{ m}\mu$ completely disappeared with considerable decrease of the maximum absorption at $280\text{ m}\mu$, indicating the degradation of aromatic rings of lignin.

From these results, it is assumed that the absorption at $320\text{ m}\mu$ which still remained after 5 to 9 hours ozonolysis would not be ascribed to the double bonds of phenylcoumarone and stilbene produced by acidolysis, but to that of biphenyl ring which is originally contained in lignin²⁴.

Although the end point of the reaction was detected clearly by the disappearance of the absorption at $320\text{ m}\mu$ for the model experiment, the detection of the exact end point of the ozonolysis was rather difficult for lignin. However as described previously, the difference of the reactivity between double bonds and aromatic rings for ozone was so large and careful observation enabled the detection the end point of the reaction.

Gel filtration

Fig. 5 and 6 show the gel filtration patterns of bamboo MWL (1), lignin residue

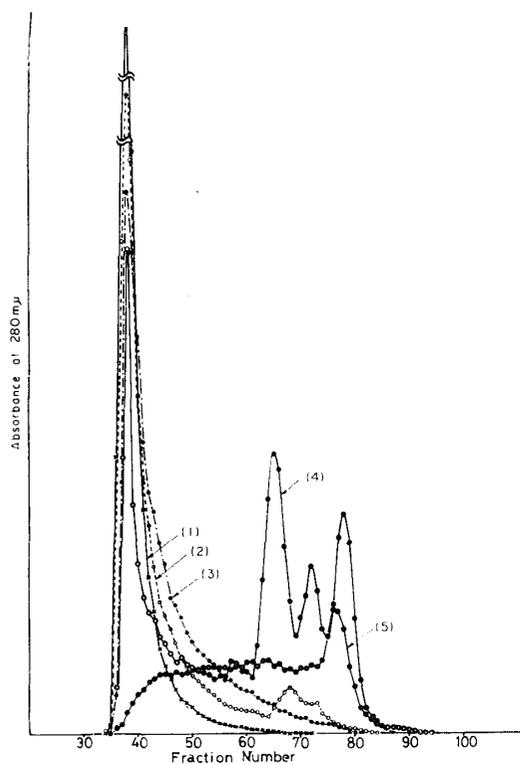


Fig. 5. Elution curves obtained on gel filtration (Sephadex LH-20) of bamboo MWL (1), lignin residue of mild acidolysis (2), acidolysis residue (3), low molecular weight acidolysis products (4) and ozonolysis products of acidolysis residue (5). Eluent: DMF.

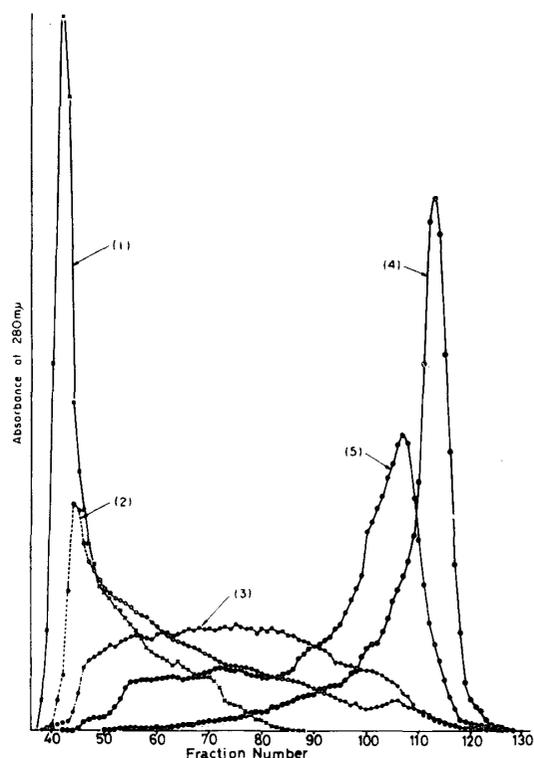


Fig. 6. Elution curves obtained on gel filtration (Sephadex G-50) of bamboo MWL (1), lignin residue of mild acidolysis (2), acidolysis residue (3), low molecular weight acidolysis products (4) and ozonolysis products of acidolysis residue (5). Eluent: DMSO.

in mild acidolysis (2), acidolysis residue (3), low molecular weight acidolysis products (4) and ozonolysis products (5) with Sephadex LH-20 and G-50, respectively. LH-20 and G-50 were effective for the separation of low and high molecular weight fractions, respectively. An average molecular weight of about 10,000 has been known for MWL (1). The molecular weight of the fraction (2) which was obtained through degradation of only α -ether bonds of MWL (1) by mild acidolysis⁵⁾ decreased a little, but low molecular weight fraction was scarcely obtained. By acidolysis, both α - and β -aryl ethers in lignin (1) were cleaved, and acidolysis residue (3) and the low molecular weight fraction (4) were obtained with the ratio of about 5:4^{3~5)}. The weight of the fraction (4) which was almost composed of monomers, dimers and trimers corresponded to about a half that of MWL. The compounds contained in fraction (4) have been well studied^{3~13)}, and Hibbert's ketones with guaiacyl, syringyl and *p*-hydroxyphenyl groups were mainly identified²⁵⁾. Although the molecular weight of the acidolysis residue (3) was considerably lower than that of original lignin (1),

it was still so large that the structure of this condensed fraction could hardly be studied. Therefore the structure of the acidolysis residue has not hitherto been elucidated. The acidolysis residue (3) was further degraded by ozonolysis to the fraction (5). As shown in Figs. 5 and 6, the ozonolysis products (5) almost consist of the low molecular weight fractions from monomers to pentamers. Thus, MWL (1) was completely degraded to the fractions (4) and (5) which were composed of the low molecular weight compounds by acidolysis and subsequent ozonolysis.

Isolation of ozonolysis products

The ozonolysis products were separated to the five fractions with Sephadex LH-20, and last fraction was found to correspond to monomers. The monomer fraction was separated by silicagel column. Each fraction was further purified for some times with preparative thin layer chromatography, and the compounds (XVIII)-(XXVIII) were obtained (Fig. 7). The reaction process of the fraction of these compounds is shown in Fig. 8. First, ozone is added to the double bond of the phenylcoumarone to produce an ozonide, a part of which is reacted with methanol used as solvent. The ozonide and the peroxide are reduced to carbonyl and carboxymethyl groups, respectively, by subsequent catalytic hydrogenation. Similarly, aldehyde, methyl and methoxymethyl groups are given from the stilbene produced from 1,2-diarylpropane and a small part of phenylcoumarane moieties of lignin. Dimetyloxalate which may correspond to methylglyoxalate from guaiacylcoumarone (XIII) is suggested to be given from syringyl ring as shown in Fig. 9.

The amount of *p*-hydroxyphenyl unit was largest in monomers isolated, and the ratio of *p*-hydroxyphenyl:guaiacyl:syringyl units was about 9:3:1. The possibility of the demethoxylation of lignin during ozonolysis has been pointed out¹⁵⁾. However

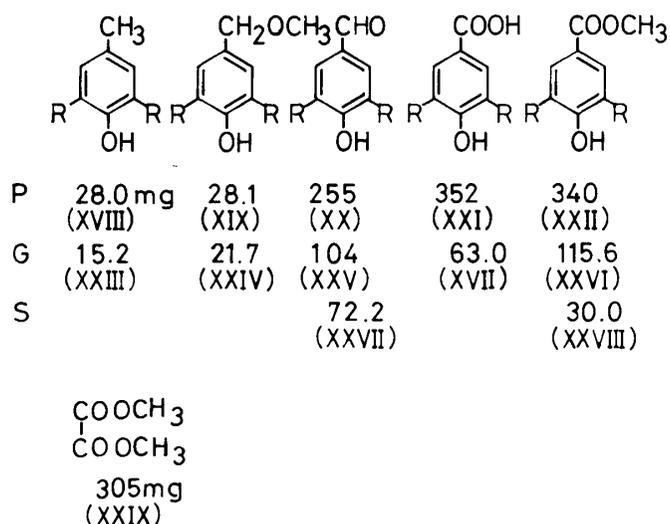


Fig. 7. Ozonolysis products of acidolysis residue of bamboo lignin.

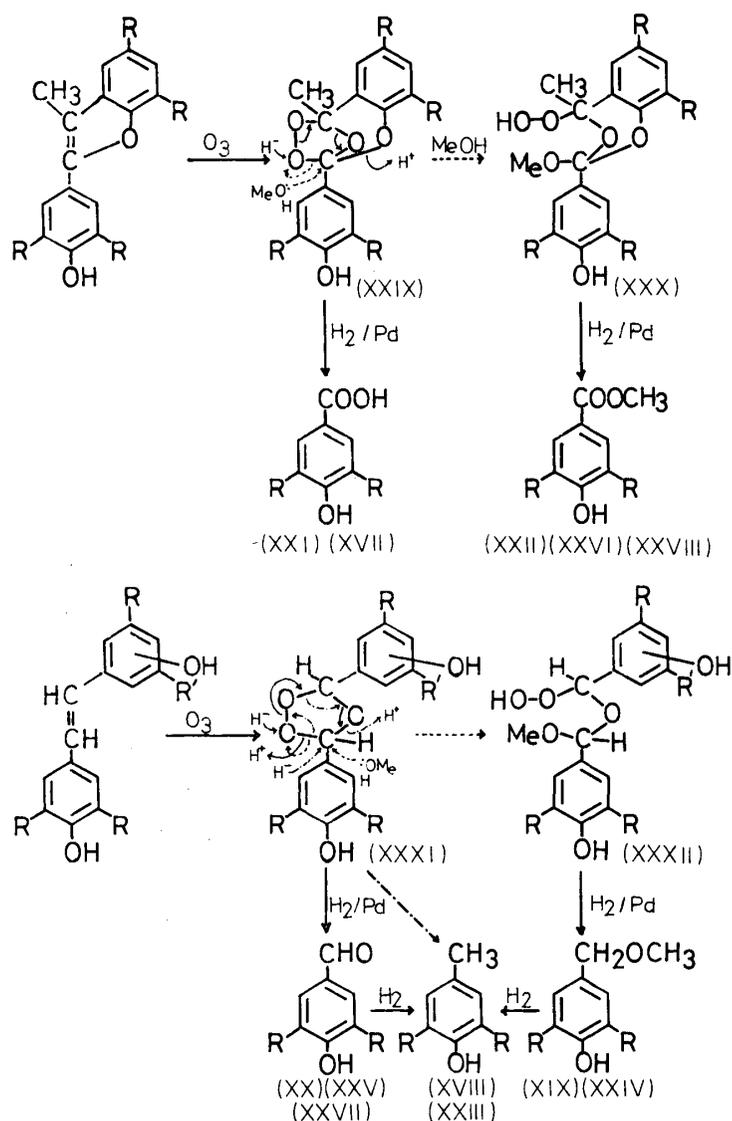


Fig. 8. Possible reaction mechanisms of phenylcoumarone and stilbene.

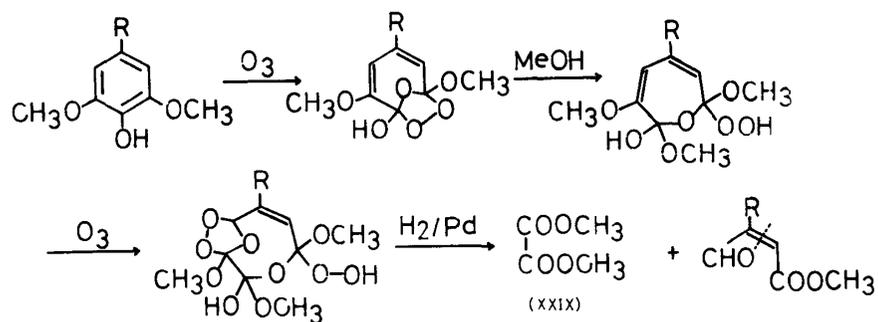


Fig. 9. Possible reaction mechanism in ozonolysis of syringyl ring.

in view of the experiment of the model compound the demethoxylation would not occur under the conditions in the present investigation. On the other hand, the reactivities of *p*-hydroxyphenyl, guaiacyl and syringyl rings for ozone were quite different, and syringyl ring was easily degraded, guaiacyl ring followed, and *p*-hydroxyphenyl ring was the most stable²⁵). Thus, the difference of the reactivity might be slightly responsible for the ratio of the ozonolysis products. The results suggest that many *p*-hydroxyphenyl units are contained in the acidolysis residue, whereas in the low molecular weight acidolysis products many syringyl units are contained. In other word, bamboo lignin might be built up with both condensed and uncondensed parts, and the former includes mainly *p*-hydroxyphenyl units and the latter mainly syringyl and guaiacyl units. Further investigations in this problem are in progress.

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