ENZYMIC DEHYDROGENATION OF *P*-COUMARYL ALCOHOL AND SYNTHESES OF OLIGOLIGNOLS

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CONTENTS

PREFACE

Lignin, one of the most important constitutional compōnents of vascular plants comprises about 20-30% of woods and 15-20% of grasses in dry weight and its abundance is the next of natural organic materials to cellulose on the earth. Lignin is being important economically related to the recent serious problem derived from the shortage of fossilized resources. Lignin, which widely occurs in vascular plants above pteridophyte, functions as a binding and an encrusting materials for cell wall composed of cellulose and hemicelluloses giving rigidity to the wall to resist the external rigorous contitions, gravity, wind, rain and attack of wood-decay fungi. It is also considered that lignin protects from the water-leaking by the lining cell walls of the conductive tissue by which water is transported smoothly from root to metabolic tissues, leaves, flowers and cambium etc.

The difficulty in the elucidation of chemical structure of lignin over one hundred years since the term "Lignin" was proposed by F.Schulze in 1865 should be ascribed to its complexity; lignin has neither regularity, optical activity nor crystallinity which is made impossible to determine by X-ray analysis as in other natural polymers, cellulose, protein, DNA , RNA etc. Therefore, it is inevitable fate that lignin structure is described only as a statistical combination pattern of probable substructures and not as definite one. However, current knowledge of lignin structure which has been obtained as the result of continuous and passionate investigations by a great number of researchers over one hundred years, is probably close to the

truth, qualitatively and quantitatively.

The history of the structural studies of lignin seems to be divided into three periods: The first is the period of about seventy years from the proposal of "encrusting material" by Payen in 1838 to the "Coniferyl alcohol theory" by Klason in 1907. At the end of this period, Klason advanced the idea that lignin is chemically related to coniferyl alcohol and proposed that the coniferyl alcohol units might be linked together by a continuous condensation between alcoholic and phenolic hydroxyl groups. Although he could not solve the problem "how does coniferyl alcohol link each other", his basic idea on coniferyl alcohol undoubtedly greatly influenced the thinking of later lignin chemists.

We had to wait for the solution of the problem until forty years later, the second period when the "Dehydrogenation theory" that lignin is formed by dehydrogenation of phenolic,α,β-unsaturated C_6C_3 progenitors of the coniferyl alcohol type was proposed by H.Erdtman in 1933 and K.Freudenberg in 1942.

The third period of lignin research history is about forty years from 1942 to today and in this period experimental results justifying the dehydrogenation theory have been obtained. The validity of the dehydrogenation theory has been sufficiently established through the enzymic dehydrogenation experiments of p-hydroxycinnamyl alcohols, the structural determination of the products obtained by the various degradation methods, spectral and functional analysis of lignins, and structural simulation of lignin by computer; the structures of softwood- and hardwood lignins can be illustrated.

On the other hand, in the history of lignin studies the chemistry of pulping can not be neglected. In 1874, the sulfite pulping method was first industrialized by E.Ekman in Sweden. This remarkably stimulated the structural studies of lignin and the lignin study from pulping aspect was started from softwood lignin because only softwood was used at that time for pulping. With the exhausting of softwood, hardwoods and grasses had gradually been used for pulping and the lignin studies was shifted to hardwood and grass lignins from softwood lignin, and now the lignins can be divided into three groups, softwood, hardwood and grass lignins by the plant sources.

Softwood (gymnosperm) lignin is a dehydrogenation polymer of coniferyl alcohol. Hardwood (angiosperm) lignin is a mixed dehydrogenation polymer of coniferyl and sinapyl alcohols and grass lignin is composed of a mixed dehydrogenation polymer of coniferyl, sinapyl and p-coumaryl alcohols, and in grass lignin, 5-10% of p-coumaric acid is esterified to the C_γ-hydroxyl groups of the side chains in the lignin polymer.

It is considered that enzymic dehydrogenation study is basically most important in the structural elucidation of lignin and this has been established by the dehydrogenation studies of p-hydroxycinnamyl alcohols. The dehydrogenation of coniferyl and sinapyl alcohols by the mushroom laccase was first carried out by K.Freudenberg.

The dehydrogenation of p-coumaryl alcohol discussed in this thesis has been studied in comparison with that of coniferyl alcohol by K.Freudenberg. The elementary analysis and hydrogen absorption by the formed DHP (Dehydrogenation polymer) of p-

coumaryl alcohol showed that this DHP is similar to that from coniferyl alcohol. However, p-coumaryl alcohol having no methoxyl groups at the ortho-position of phenolic group has a possibility to react preferentially at this positions to give much more condensed type, "double condensation" proposed by D.E.Bland. However, Yamasaki et al. recently found the same condensation pattern as in coniferyl alcohol DHP found by K.Freudenberg by the various degradation studies of the p-coumaryl alcohol DHPs. However, the dehydrogenation studies of p-coumaryl alcohol studied so far are only concerned with DHP polymer and not with dimer formation.

In the Chapter I, the dimer formation of p-coumaryl alcohol with hydrogen peroxide/peroxidase system is mainly discussed. In the Section I-1, the isolation and the structural determination of the dimeric compounds of p-coumaryl alcohol obtained by the dehydrogenation are described. In the Section I-2, the formation mechanism and the stereochemistry of the arylglycerols which have been isolated from the degradation products of natural lignins are investigated in connection with the dehydrogenation of p-coumaryl alcohol. The stereochemistry of the phenylcoumaran substructures, dehydrodiconiferyl alcohol, and dehydrodi-p-coumaryl alcohol, and the analysis of dimeric compounds described in the Section I-1 by gas chromatography and NMR spectrometry are discussed in the Section I-3 and I-4, respectively compared with those of coniferyl alcohol. The threo isomers of p-hydroxyphenylglycerol-β-p-coumaryl ether and arylglycerols obtained in the investigations described in Section I-1 and I-2, respectively, were found to predominate over erythro isomers. This erythro/

threo determining step, the water addition step to quinonemethide which is an important intermediate after radical formation, and subsequent coupling step involved in lignin polymerization will be discussed in the Section I-5.

The isolation and structural identification of the products in the dehydrogenation and lignin degradation have been the most important works in the structural studies of lignin. However, it is generally considered in the chemistry of natural products that the final proof of the structure of compounds might be obtained by the synthesis. The structures of lignin oligomers isolated can not be determined by X-ray analysis because these compounds occur as the noncrystalline mixture of the diastereomers. Nevertheless, in lignin chemistry the structures have been scarcely proved by the synthesis because, unfortunately, the general synthetic method for these oligomers has not yet been established. The general synthetic method for the oligolignols, involving β-hydroxy ester intermediates, and the reactions focused on the common unit of lignin substructures, p-oxyphenylpropane-1,3-dioxy structure, is described in the Chapter II. These synthetic methods might be useful for the synthetic determination of the dehydrogenation products and degradation products of lignins. The synthesized oligomers can be conveniently used as the lignin model compounds in the vaious lignin reactions such as pulping, chemical utilization and biodegradation of lignin. It is believed that this synthetic method would be increasingly important for the future lignin chemistry.

The arylglycerol-β-aryl ether substructure is the most important interphenylpropane unit in lignins: 30-50% or more

of the phenylpropane units are found to occur as this substructures in lignin. For this reason, guaiacylglycerol-β-guaiacyl ether has been generally used as a lignin model compound. In the Section II-1, a new method by a convergent synthesis of the compound is described. And the convergent synthetic method involving β-hydroxy ester intermediate established in the Section II-1 was further applied to the synthesis of guaiacylglycerol-β-coniferyl and β-coniferyl aldehyde ethers in the Section II-2. 1,2-Diaryl-propane-1,3-diol substructure is also quite common in lignins and its general synthetic method involving β-hydroxy ester intermediate is discussed in the Section II-3. In the Section II-4, the general synthetic method via β-hydroxy ester intermediate of phenylcoumaran, the synthesis of which has not yet been reported, is described. Finally, in the Section II-5 and II-6, the application of the general synthetic method established in the preceding Sections to the synthesis of the trilignols composed of phenylcoumaran, β-O-4 and β-1 substructures is described.

CHAPTER I

ENZYMIC DEHYDROGENATION OF P-COUMARYL ALCOHOL

I-1 Structure of dimeric compound

I-1-1 Introduction

It is believed that the grass lignin is a polymer composed of p-hydroxyphenyl, guaiacyl and syringyl propane units and is characterized by the occurrence of larger amount of p-hydroxyphenyl unit than in hard- and soft-wood lignins. Although some of p-hydroxyphenyl unit (about 10%) have been ascribed to the esterified p-coumaric acid[1)], major portions of the unit should be due to the lignin polymer. Thus, elucidation of enzymic dehydrogenation of p-coumaryl alcohol (1) is conceivable to provide useful informations on the chemical structure of p-hydroxyphenyl component of the grass lignin.

In this Section, the chemical structures of the four dimeric compounds, p-coumarylresinol (2), dehydrodi-p-coumaryl alcohol (3), p-hydroxyphenylglycerol-β-p-coumaryl ether (4) and 2-(4-hydroxyphenyl)-3-hydroxymethyl-4-(α,4-dihydroxybenzyl)-tetrahydrofuran (monoepoxylignan)(5) obtained by enzymic dehydrogenation of p-coumaryl alcohol are described.

I-1-2 Isolation and identification of dimeric compounds

A flow sheet of the dehydrogenation procedures of p-coumaryl alcohol, which were described in detail in the experimental section, is shown in Figure I. The ethyl acetate soluble portion was applied onto silica gel column chromatography and the column was eluted with a mixture of benzene and ethyl acetate. p-Coumarylresinol (2), dehydrodi-p-coumaryl alcohol (3), p-hydroxyphenylglycerol-β-p-coumaryl ether (4) and monoepoxylignan (5)

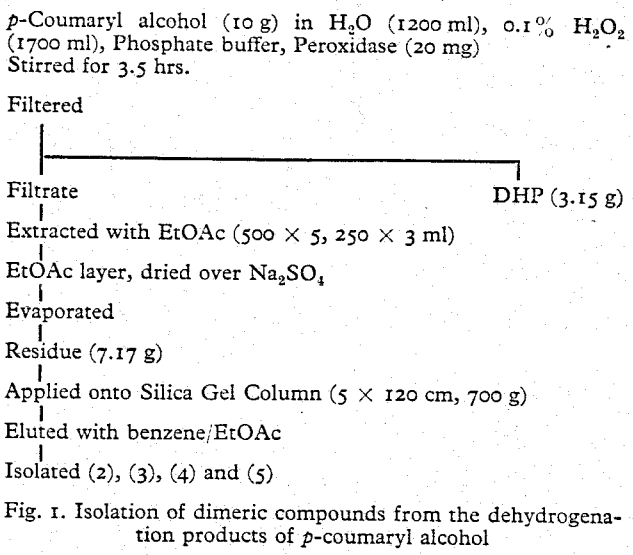

Fig. 1. Isolation of dimeric compounds from the dehydrogenation products of *p*-coumaryl alcohol

were obtained respectively. The compounds (2-4) were corresponding to the three dimeric ones obtained in the dehydrogenation of coniferyl alcohol by Freudenberg et al.[2] However, the compound (5) was a new dimer whose formation was interested in view of coupling mechanism of the phenoxy radical of p-coumaryl alcohol as discussed later.

The NMR spectrum of p-coumarylresinol diacetate is shown in Figure 2. The chemical shifts and the coupling modes of the protons attached to the tetrahydrofuran ring were approximately identical to those of the rings of pinoresinol[3], sesamine[4] and syringaresinol[5]. The results indicated that the chemical shifts and the coupling modes of the protons attached to the tetrahydrofuran rings were scarcely influenced by the substituent groups on the aromatic rings. The signals of the equatorial protons on the carbons gave a quartet at δ 4.24 whose coupling constants were JHe, βH = 7.0 and JHe, Ha = 9.2, whereas axial protons gave a quartet at δ3.89 as well, and the coupling constants were JHa, βH = 3.8 and JHe, Ha = 9.2, respectively. On the other hand, the protons of He and He' were of cis configuration and the Ha and Ha' were trans to the adjacent βH and β'H protons,

respectively. In conclusion, the protons with cis configuration on the tetrahydrofuran ring gave larger coupling constants than those with trans configuration. This fact gave an information for the possible configuration of monoepoxylignan (5) as explained later.

The NMR spectrum of dehydrodi-p-coumaryl alcohol triacetate, which corresponded to that of dehydrodiconiferyl alcohol, was shown in Figure 3. The geometric configuration associated with the double bond in the side chain was trans because of $J\alpha'H$, $\beta'H$ = 16 in the NMR spectrum and 968 cm^{-1} in the IR spectrum, respectively. Thus, the results indicated that the configuration of the side chain of p-coumaryl alcohol was not altered during dehydrogenation. The γ-CH_2 protons gave a quartet signal each other because of their nonequivalency. Designating the two protons as Ha and Hb tentatively as given in Fig.3, the Ha gave a quartet at δ4.44 having JHa, Hb = 10 and JHa,βH =5.8 , respectively, and the Hb gave a quartet as well at δ4.26 having JHa,Hb=10 and JHb,Hβ=7.5, respectively. It is understandable that the nonequivalency is probably due to β-asymmetric carbon and not to the inhibition for the rotation of Cβ-Cγ bond as described by Ludwig et al.[5] This was supported by the following fact that the γ-CH_2 protons of p-hydroxyphenylglycerol-β-p-coumaryl ether (4) which has no such effect for the Cβ - Cγ bond gave quartets as well resulting in the nonequivalency as described later. For the protons attached to C-α and C-β of the coumaran ring, Ludwig et al. gave trans configuration based on only the fact that the configuration of C-α and C-β protons of dehydrodiisoeugenol was trans. However, in the NMR spectrum of dehydrodiisoeugenol

(synthesized and its NMR spectrum was measured using the same instrument in our laboratory), the proton of α-CH gave a doublet at δ5.12(J=9.0) which was markedly different from those of the former two coumarans, dehydrodi-p-coumaryl alcohol (3) and dehydrodiconiferyl alcohol, as respect to the chemical shifts and coupling constants. Consequently, it is doubtful from the NMR spectra whether these coumarans have the same configuration, although trans configuration is conceivable in terms of the reaction mechanism. The conclusive evidence for the trans configuration of dehydrodi-p-coumaryl alcohol (3) and dehydrodiconiferyl alcohol will be presented in Section I-3.

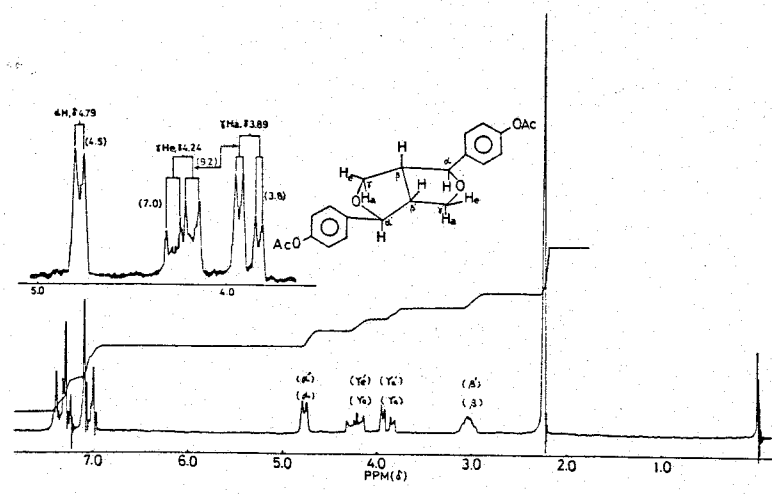

Fig. 2. NMR spectrum of *p*-coumarylresinol diacetate

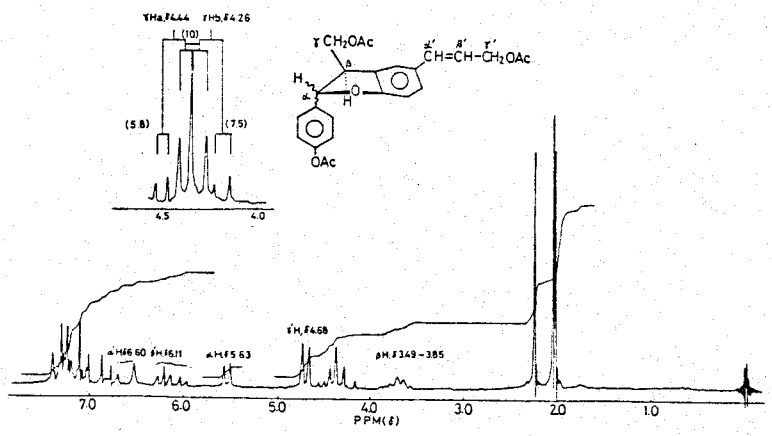

Fig. 3. NMR spectrum of dehydro-di-*p*-coumaryl alcohol triacetate

p-Hydroxyphenylglycerol-β-p-coumaryl ether (4) was isolated as a mixture of threo and erythro isomers which did not crystallize. The NMR spectrum of the acetate is shown in Fig. 4. The NMR spectrum indicated that the mixture consisted mainly of the threo isomer indicating a relatively clear doublet peak of α-CH proton. The spectrum further gave the following information: 1) the configuration associated with the double bond of the side chain is trans because of Jα'H,β'H=16. This was supported by the peak of 965 cm^{-1} in its IR spectrum, 2) the γ-CH_2 protons are nonequivalent and the nonequivalency is more remarkable than in the case of coumaran. Designating the two protons as Ha and Hb as in the case of coumaran, the Ha gave a quartet at δ 4.01 having JHa, Hb = 12 and JHa, βH = 6.3 and the Hb gave a quartet as well at δ 4.27 having JHa, Hb = 12 and JHb, βH = 4.2, respectively. This nonequivalency seems to be ascribed to the same reason as in coumaran.

The NMR spectrum of monoepoxylignan tetraacetate whose signals were determined by decoupling method is shown in Figure 5. A doublet peak at δ5.73 was assigned to α-methine proton which shifted from δ4.95 by acetylation. On the other hand, a doublet peak at δ4.55 was assigned to α'-methine proton attached to the ether bond because of the retention of original chemical shift after acetylation. Irradiation of the peaks corresponding to β-proton (δ2.45-2.85) and β'-proton (δ1.80-2.20) caused two doublet peaks at δ5.73 and δ4.55 to collapse to respective broad singlets, and the peaks of γ-CH_2

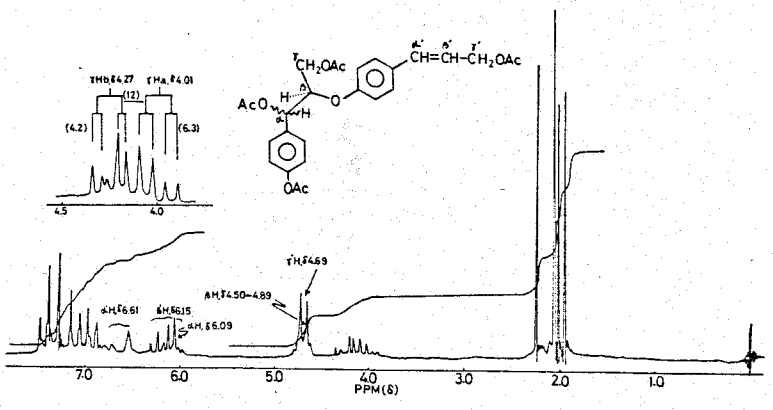

Fig. 4. NMR spectrum of *p*-hydroxyphenyl-glycerol-*β*-*p*-coumaryl ether tetraacetate

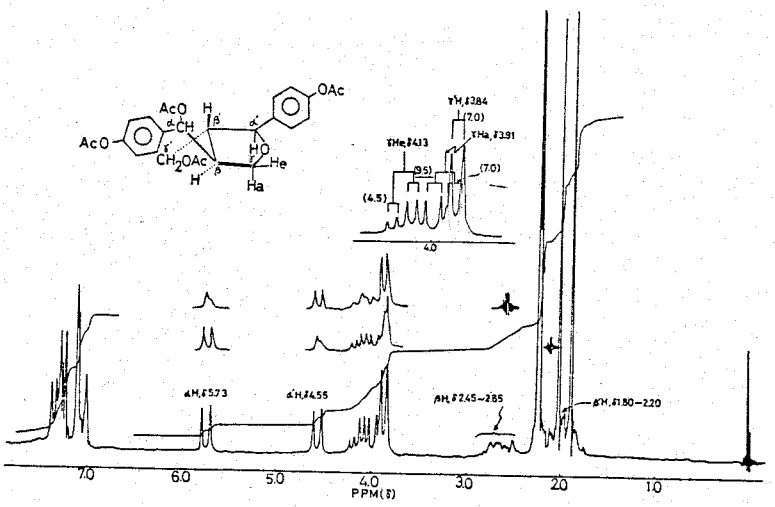

Fig. 5. NMR spectrum of monoepoxylignan tetraacetate

protons at δ3.80-4.20 to broad peaks as shown in Figure 5. The results indicated the existence of spin-spin coupling between them, and these NMR data gave information for the structure of the compound (5). The configuration of Cβ and Cβ' was assumed to be trans which differed from the case of resinol (2) by the following facts. The γ-He proton on the tetrahydrofuran ring gave a quartet having JHe, Ha = 9.5 and JHe, βH = 4.5, and γ-Ha proton gave also the same pattern having JHa, He = 9.5 and JHa, βH = 7.0. That is, JHa, βH (7.0) was larger than JHe, βH (4.5). Consequently, the Ha proton was cis relative to βH proton and He proton was trans to βH proton, respectively. If this interpretation is correct, the configuration of Cβ and Cβ' must be trans

This conclusion is supported by the reaction mechanism in formation of (5). At the C β and C β' coupling in dehydrogenation of p-hydroxycinnamyl alcohols, two modes, racemoid and mesoid types are probable as shown in Figure 6. In racemoid coupling, when one quinonemethide is attacked by the hydroxyl group attached to the C γ to form a tetrahydrofuran ring, the other quinonemethide and the γ'-hydroxyl group is favorably located for ring closure so that the ring closure proceeds smoothly to produce resinol (2).

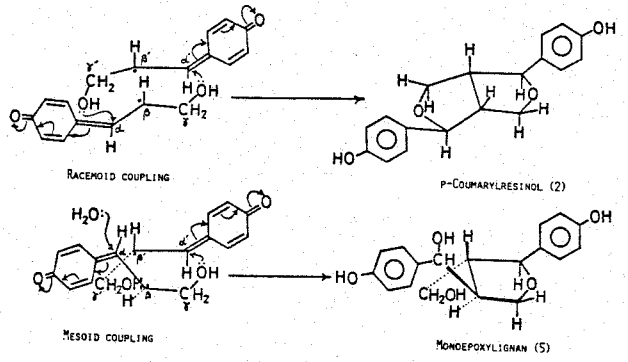

Fig. 6. Formation mechanism of *p*-coumarylresinol (2) and monoepoxylignan (5)

On the other hand, in mesoid coupling, when ring closure of one tetrahydrofuran proceeds, the other quinonemethide is no longer located to be attacked by the γ'-hydroxyl group because of the trans configuration of Cβ and Cβ'. Consequently, the quinonemethide is attacked by the water in medium to produce monoepoxylignan (5). Thus, the monoepoxylignan which was first isolated in the present investigation seemed to be produced by the mesoid type coupling.

Recently, Sarkanen *et al.*[5] described that *racemoid* β-β' coupling mode appears to be exclusive for *trans* isomer, while both *racemoid* and *mesoid* β-β' couplings occur in *cis* isomer. Thus, it may be assumed that the monoepoxylignan (5) is produced by the *mesoid* coupling mode of a trace amount of *cis* *p*-coumaryl alcohol contaminated in *trans* isomer. But the monoepoxylignan (5) was identified in the dehydrogenation products by gas chromatography even when the contaminated *cis* isomer was completely removed by recrystallization of the *trans* *p*-coumaryl alcohol. Therefore, the monoepoxylignan (5) obtained in the present investigation should be formed from *trans* *p*-coumaryl alcohol. The ratio of the two coupling modes will be described in Section I-4.

As described above, the dimeric compounds obtained by dehydrogenation of *p*-coumaryl alcohol corresponded to the case of coniferyl alcohol. The main reaction site was β-position of the side chain indicating that the most reactive radical was β-radical in the four resonance structures. Therefore, at the early stage of lignification in the grass, *p*-coumaryl alcohol will take the same dehydrogenation behavior as coniferyl alcohol and will not react preferentially at C-3 and C-5 positions of the alcohol.

I-1-3 Experimental

trans-p-Coumaryl alcohol (1) was prepared by the method of Freudenberg et al.[8] and recrystallized from ethyl acetate, mp 123-124°C (Lit. 124°C); NMR δ (ppm: CD_3COCD_3). 4.19 (2H, dd, J= 5.0, 1.0), 6.14 (1H, dt, J=16.0, 5.0), 5.85(1H, dt, J=16.0, 1.0), 6.50 (2H, d, J=8.5), 7.75 (2H, d, J=8.5)

Dehydrogenation of (1) and isolation of dehydro Dimers

The compound (1) (log, 0.066 mol) was dissolved in 2000 ml of H_2O with warming and the solution was cooled to 10°C in an ice bath. To 1200 ml of a phosphate buffer solution (0.05 mol, pH=6.0) containing 20 mg of horseradish peroxidase, the solution of (1) and 1700 ml (0.05 mol) of 0.1% H_2O_2 were added dropwise over 90 min at 25°C under stirring. Stirring was continued for additional two hours, and then the mixture was saturated with NaCl and extracted with EtOAc (EtOAc-Extractives 7.17g). (1), p-coumaryl aldehyde, the dimeric compounds (2-4) and (5) were isolated by column chromatography on sillica gel with benzene and EtOAc, whose ratio was changed 2:1 to 1:2 gradually. The compounds were purified by preparative tlc of silica gel with benzene-EtOAc and methanol-chloroform, respectively.

p-Coumarylresinol (2)

was obtained as crude crystals after evaporation of the corresponding fraction (crude crystals 2.4g) and recrystallized from n-hexane/acetone; mp 232-233°C (needles) Anal. Calcd. for $C_{18}H_{18}O_4$: C, 72.46; H, 6.08. Found: C, 72.48; H, 6.24. IR(KBr); 3500, 3380, 1615, 1603, 1523, 1455, 1400, 1275, 1235, 1172 1028,

950, 828 cm^{-1}. NMR δ(ppm: $CDCl_3$) 2.26 (6H,s), 2.95-3.20 (2H, m), 3.89 (2H, dd, J=9.2, 3.8), 4.24 (2H, dd, J=9.2, 7.0), 4.79 (2H, d, J=4.5), 6.95-7.40 (8H, m). MS(70 eV); 298 (27), 297 (6), 281 (4), 280 (4), 268 (4), 267 (15) 204 (9), 191 (9), 179(13), 176 (9), 175 (31), 166 (11), 161 (11), 160 (13), 147 (18), 145 (8), 135 (13), 134 (11), 122 (27), 121 (100), 120(24), 107 (53), 94 (16), 77 (13), 65 (12), 55 (11), 54 (11), 39 (11). UV λ_{max}^{EtOH}nm(log ε):276.5 (3.56), 282 (shoulder),

Dehydrodi-p-coumaryl alcohol (3)

was obtained as crude crystals after evaporation of the corresponding fraction (crude crystals 2.4g and recrystallized from n-hexane/acetone; mp 232-233°C (needles) Anal. Calcd. for $C_{18}H_{18}O_4$: C, 72.46; H, 6.08. Found: C, 72.01; H, 6.24 IR (KBr); 3560, 3300, 1615, 1603, 1490, 1388, 1241, 1160, 1088, 1020, 990, 968, 800, 820 cm^{-1}. NMR δ(ppm:$CDCl_3$) 2.02 (3H,s), 2.04(3H,s), 2.24 (3H,s), 3.49-3.85 (1H,m), 4.26 (1H, dd, J=10.0, 7.5), 4.44 (1H, dd, J=6.0), 6.11 (1H, dt, J=16.0, 6.5), 6.60 (1H, dt, J= 16.0, 0.8), 6.25-7.40 (7H, m). MS (70 eV); 298 (100), 280 (79), 279 (43), 268 (31), 267 (17), 263 (11), 251 (7), 250(11), 249 (8), 239 (11), 237 (32), 236 (10), 225 (17), 224 (16), 223 (24), 212 (8), 207 (10), 194 (8), 181 (10), 165 (12), 152 (9), 145 (9), 144 (8), 133 (8), 132 (13), 131 (20), 127 (8), 121 (22), 120 (11), 115 (14), 107 (41) 91 (11), 77 (16), 65 (8), 55 (9). UV λ_{max}^{EtOH}nm(log ε) :267.5 (4.04), 303 (shoulder).

p-Hydroxyphenylglycerol-β-p-coumaryl ether (4)

was obtained as a colorless foaming material (600 mg) after the purification of the combined corresponding fractions by preparative tlc with cyclohexane/EtOAc(2:3). IR (KBr); 3400, 1607,

1515, 1455, 1237, 1175, 1087, 1035, 965, 837 cm^{-1}. NMR δ(ppm: $CDCL_3$) 1.92 (3H, s), 1.99 (3H,s), 2.03 (3H,s), 2.23(3H,s), 4.01 (1H, dd, J-12.0, 6.3), 4.27 (1H, dd J-12.0, 4.2), 4.50-4.89 (1H, m), 4.69 (2H, dd, J=6.5, 0.8), 6.09(1H, d, J-6.5), 6.15 (1H, dt, J=16.0, 6.4), 6.61 (1H, dt, J=16.0, 0.8), 6.75-7.55 (8H, m). MS (75 eV); 316.12768 (M^+, $C_{18}H_{20}O_5$ requires; 316.13106) (10), 298 (3), 268 (11), 225 (3), 177 (15), 176 (100), 174 (9), 150 (32), 149 (13), 134 (14), 133 (64), 132 (14), 131 (21), 123 (55), 115 (9), 108 (12), 107 (74), 103 (14), 95 (19), 94 (33), 93 (11), 91 (21), 77 (33), 65 (17), 55 (13). UV λ_{max}^{EtOH}nm(log ε):263(4.41).

Monoepoxylignan (5)

was purified by preparative tlc with methylene chloride after acetylation of the corresponding fraction, and obtained as a colorless foaming material (94mg).

IR (KBr); 3000, 1785, 1765, 1755, 1615, 1520, 1375, 1230, 1200, 1165, 1010, 900, 830 cm^{-1}. NMR δ(ppm:$CDCl_3$) 1.88 (3H, s), 2.00 (3H,s), 2.22 (6H, s), 1.80-2.22 (1H, m), 2.45 to 2.85 (1H, m), 3.84 (2H, d, J=7.0), 3.91 (1H, dd, J=9.5, 7.0), 4.13 (1H, dd, J=9.5, 4.5), 4.55 (1H, d, J=7.7), 5.73 (1H, d, J=8.5), 7.00-7.45 (8H, m). MS (75 eV); 484.17249 (M^+, $C_{26}H_{28}O_9$ requires; 484.17334) (0.06), 424 (4), 384 (5), 365 (14), 364 (39), 322 (23) 305 (8), 280 (8), 263 (6), 218 (10), 217 (34), 200 (9), 192 (18), 175 (21), 158(100), 149 (11), 145 (11), 133 (18), 121 (41), 107 (25), 60(22), 43(87). UV λ_{max}^{EtOH}nm(log ε): 260(3.05), 268(shoulder) 281(shoulder).

HITACHI model 124 double beam spectrometer and JASCO model IR-S were used for UV and IR spectra, respectively. NMR spectra were taken by the use of R-22 HITACHI high resolution NMR spectrometer (90 MHz) with TMS internal standard. Chemical shifts and coupling constants were given in δ-values and Hz, respectively. Mass spectra were taken by the use of SHIMADZU-LKB 9000 gas chromatograph-mass spectrometer and JEOL-JMS-ol-SG double beam mass spectrometer, and relative abundance of each peak was designated in parentheses. These instruments were used for further investigations in the following Sections.

I-1-4 Summary

p-Coumaryl alcohol (1) was dehydrogenated with peroxidase and H_2O_2. Four dimeric compounds, p-coumarylresinol (2), dehydrodi-p-coumaryl alcohol (3), p-hydroxyphenylglycerol-β-p-coumaryl ether (4) and 2-(4-hydroxyphenyl)-3-hydroxymethyl-4-(α,4-dihydroxybenzyl)-tetrahydrofuran [monoepoxylignan (5)] were isolated and identified. The nonequivalency and assignment of the γ-methylene protons of these dimeric compounds on the NMR spectra were discussed and their configurations were determined. The compound (4) was obtained as mainly threo form which contained a small amount of erythro isomer. Cβ- and Cβ'-hydrogens on the tetrahydrofuran rings of (2) and (5) were determined to be of cis and trans configurations respectively and their possible formation by racemoid and mesoid couplings in the enzymic dehydrogenation were discussed.

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I-2 Enzymic formation of arylglycerols from p-hydroxycinnamyl alcohols

I-2-1 Introduction

Since the finding of guaiacyl- and syringylglycerols in the mild hydrolysis products of conifer- and hardwood lignins[1,2], the origin of both compounds has been ascribed to hydrolysis of arylglycerol moiety of guaiacyl- and syringylglycerol-β-aryl-propane ether units, respectively, and the occurrence of free arylglycerol side chains in lignin molecules has been doubted.

However, guaiacylglycerol was recently isolated from the degradation products of spruce lignin with sodium in liquid ammonia[3,4], and the occurrence of arylglycerol unit as such has been suggested. The present investigation describes the formation and possible incorporation of arylglycerols into dehydrogenation polymers in enzymic dehydrogenation of p-hydroxycinnamyl alcohols as lignin precursor.

I-2-2 Isolation and identification of arylglycerols

A flow sheet of separation procedures for the dehydrogenation products of p-hydroxycinnamyl alcohols by peroxidase/H_2O_2 and fungal laccase/O_2, which were described in detail in the experimental section, is shown in Figure 1.

Dehydrodiconiferyl alcohol, dl-pinoresinol, guaiacylglycerol-β-coniferyl ether, coniferyl aldehyde, syringaresinol, sinapaldehyde, dehydrodi-p-coumaryl alcohol, dl-p-coumarylresinol, p-hydroxyphenylglycerol-β-p-coumaryl ether and p-coumaryl aldehyde which are all expected from the coupling and oxidation of phenoxy radicals of the respective p-hydroxycinnamyl alcohols

were isolated from the ethyl acetate soluble fractions of the dehydrogenation products and identified.

Furthermore, guaiacylglycerol, syringylglycerol and p-hydroxyphenylglycerol which have not been reported in the dehydrogenation products of the corresponding p-hydroxycinnamyl alcohols were isolated for the first time from the water soluble -and ethylacetate insoluble fractions of the dehydrogenation

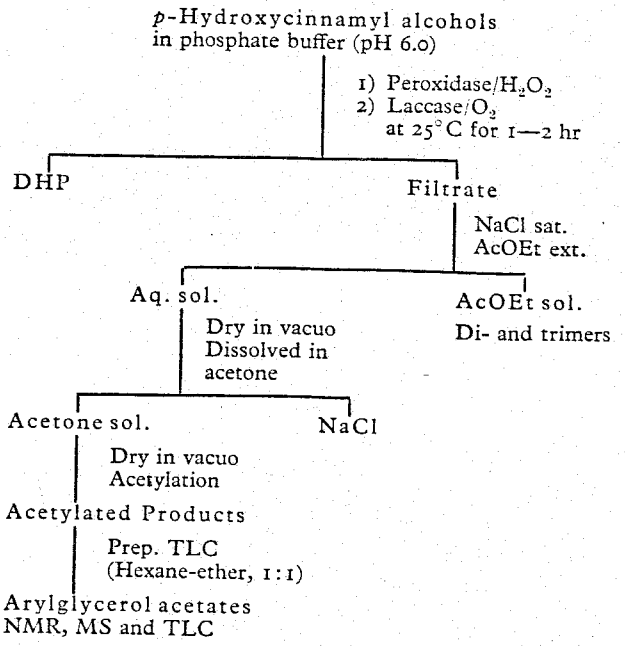

Fig. 1. Preparation and separation of dehydrogenation products of *p*-hydroxycinnamyl alcohols

products and identified by GC-MS and NMR spectrometries. The mass spectra of guaiacyl-, syringyl- and p-hydroxyphenylglycerol tetraacetates are shown in Figures 2,3 and 4. These arylglycerols were obtained as a mixture of threo and erythro isomers which were separated by tlc and glc (Figs. 5,6,7). Table 1 shows the ratio of both isomers estimated from the peak area on gas chromatogram. The amounts of threo isomers were 1-4 times higher than

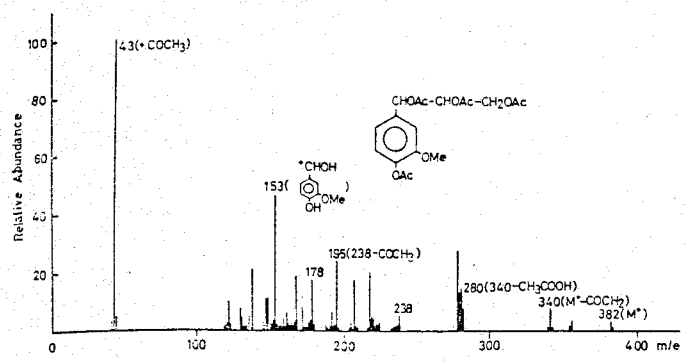

Fig. 2. Mass spectrum of guaiacylglycerol tetraacetate

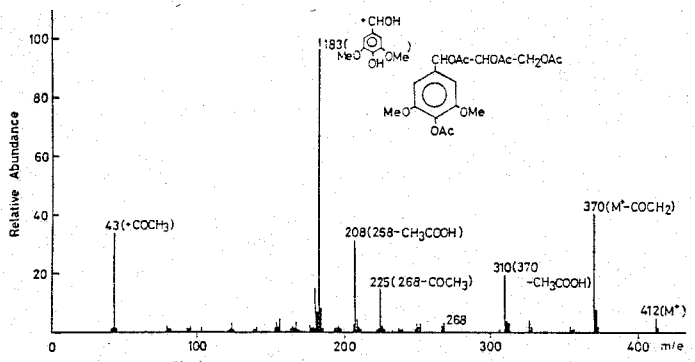

Fig. 3. Mass spectrum of syringylglycerol tetraacetate

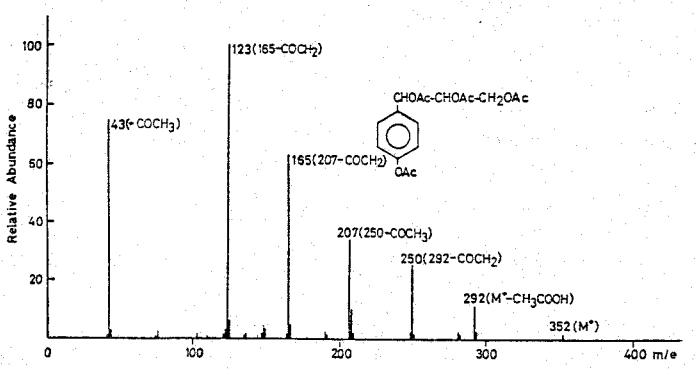

Fig. 4. Mass spectrum of *p*-hydroxyphenylglycerol tetraacetate

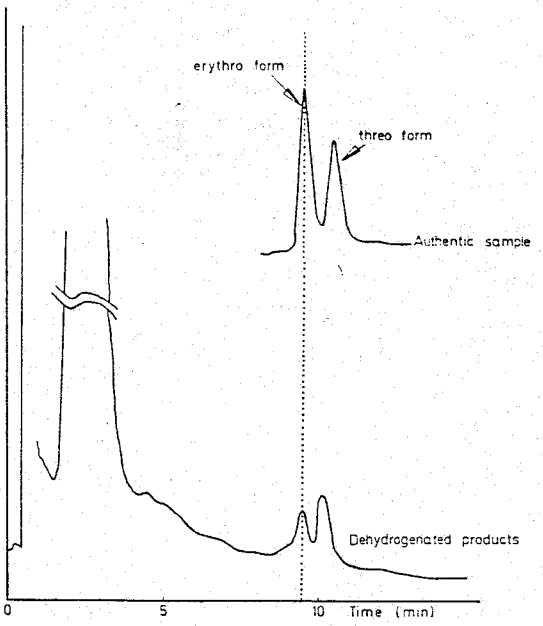

Fig. 5. Gas chromatogram of guaiacylglycerol tetraacetate 2% OV-17 on chromosorb AW, 2 m, 218°

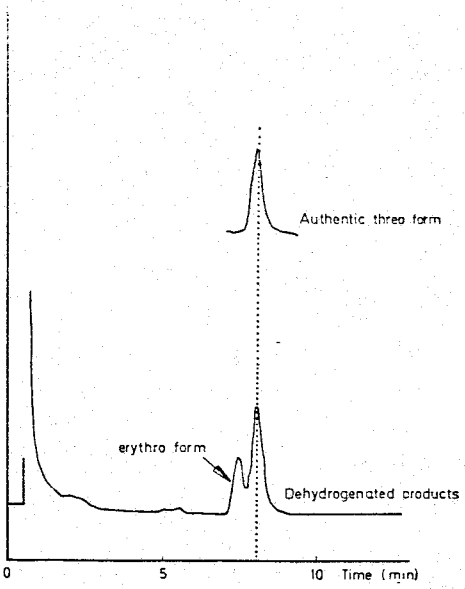

Fig. 6. Gas chromatogram of syringylglycerol tetraacetate 2% OV—17 on chromosorb AW, 2 m, 240°

those of erythro isomers, and the results, especially the case of guaiacylglycerol, were in good agreement with the results in the hydrolysis and sodium-liquid ammonia degradation of lignin[3,4].

The yield of dehydrogenation products of p-hydroxycinnamyl alcohols obtained by the mediation of both enzymes are shown in Table 2, and 0.03-0.6% of p-hydroxycinnamyl alcohols were found to be converted to the corresponding arylglycerols.

Nimz[1,2] has isolated and identified guaiacylglycerol,

guaiacylglycerol-β-guaiacyl-glycerol ether and syringylglycerol by hydrolysis of finely-powdered spruce and beech woods with percolating water at 100°C. Subsequently Omori and Sakakibara[5] further isolated syringylglycerol-β-syringylglycerol ether as well as guaiacyl- and syringylglycerols from the hydrolysis products of Fraxinus wood meal.

Table 1
Ratio of peak area of erythro- and threo isomers of arylglycerol tetraacetates

Compound	erythro %	threo %
Guaiacylglycerol tetraacetate .	45	55
Syringylglycerol tetraacetate .	26	74
p-Hydroxyphenylglycerol tetraacetate	19	81

Table 2
Yields of dehydrogenation products of *p*-hydroxycinnamyl alcohols

Substrate	DHP %	Ethylacetate sol. %	Water sol. %	Arylglycerol acetate mg	Arylglycerol acetate %***
Coniferyl 1)*	66.1	36.9	1.5	6.0	0.06
alcohol 2)**	60.3	41.9	2.8	13.0	0.20
Sinapyl 1)	10.7	79.6	14.0	9.6	0.60
alcohol 2)	13.6	81.1	6.3	8.0	0.50
p-Coumaryl 1)	30.0	70.0	1.2	5.0	0.03
alcohol 2)**** . . .	4.3	108.9	0.6	3.4	0.20

*1) peroxidase/H_2O_2, **2) laccase/O_2, *** value as acetyl-free arylglycerol, **** laccase of low activity was used

The formation of these arylglycerol compounds in hydrolysis has been explained by direct nucleophilic displacement of the β-ether moiety of arylglycerol-β-ether units in lignin molecules under mildly acidic conditions[6]. However, the mild hydrolysis of guaiacylglycerol-β-guaiacyl ether did not give any guaiacylglycerol, whereas moderately strong acid hydrolysis gave exclusively Hibbert's ketones[7]. Yamaguchi[3] found that guaiacylglyceryl, in the degradation of spruce lignin with sodium in liquid ammonia, is not derived from guaiacylglycerol-β-aryl ether units.

In consideration of these results and the present investigation it is concluded that the arylglycerols obtained as degradation products of lignins are ascribed to arylglycerol units which were formed by the coupling of β-radicals of p-hydroxy-

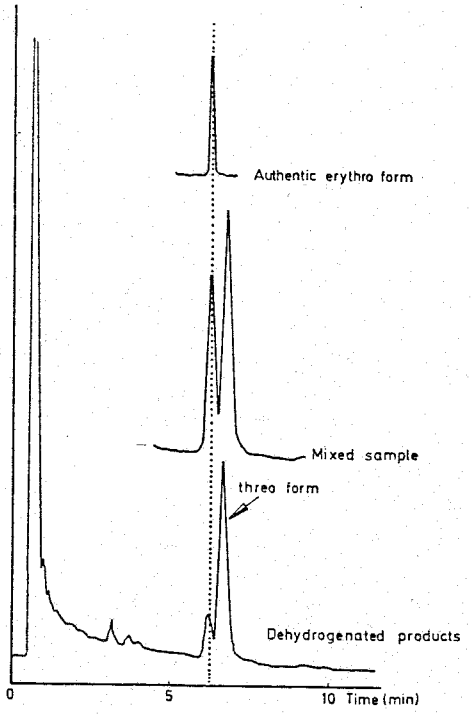

Fig. 7. Gas chromatogram of *p*-hydroxyphenylglycerol tetra-acetate 2% OV—17 on chromosorb AW, 2 m, 218°

cinnamyl alcohols with phenoxy radicals of arylglycerols during enzymic dehydrogenation, and that lignins contain arylglycerol units with free glycerol side chain as original structure.

In the present investigation only arylglycerols which are very soluble in water were estimated. Since a considerable portion of the arylglycerol is supposed to be incorporated into arylglycerol-β-arylglycerol ether substructures in polymers during dehydrogenation, total amount of the arylglycerols formed should be higher than those estimated.

Investigation on the mechanism of arylglycerol formation during enzymic dehydrogenation of p-hydroxycinnamyl alcohols is currently in progress.

I-2-3 Experimental

Syntheses of compounds

Coniferyl, sinapyl and p-coumaryl alcohols were prepared by the method of Freudenberg[8]. Melting points, IR and NMR spectra were identical with those of authentic compounds.

Guaiacylglycerol and p-hydroxyphenylglycerol were synthesized by the method of Adler[9] and Yamaguchi[10], respectively and the analytical data of their acetates were in good accordance with their chemical structures.

Guaiacylglycerol tetraacetate, NMR δ (ppm: $CDCl_3$), 1.70 to 2.15 (9H, alcoholic $COCH_3$), 2.20 (3H, s, phenolic $COCH_3$), 3.82 (3H, s, OCH_3), 3.50-4.40 (2H, m, Cγ-H_2), 5.10-5.50 (1H, m, Cβ-H), 5.80-6.00 (1H, m, Cα-H), 6.94 (2H, s, arom.); MS (70 eV), 382 (M^+), 340 (M^+ - $COCH_2$), 280 (340-CH_3COOH), 238 (280-$COCH_2$), 195 (238-$COCH_3$), 178 (238-CH_3COOH), 153 ($^+CHOHC_6H_3(OCH_3)OH$), 43 ($^+COCH_3$); metastable ions, 302.62 (382 340), 230.59 (230 280), 202.30 (280 238), 133.13 (238 178). p-Hydroxyphenylglycerol (erythro), M. p. 148-151°. p-Hydroxyphenylglycerol tetraacetate, NMR δ (ppm: $CDCl_3$), 1.91 (3H, s, γ-$COCH_3$), 1.96 (3H, s, β-$COCH_3$), 2.07 (3H, s, α-$COCH_3$), 2.22 (3H, s, phenolic $COCH_3$), 4.17 (2H, d, J=5.0, Cγ-H_2), 5.31 (1H, dt, J=6.0, 5.0, Cβ-H), 5.98 (1H, d, J=6.0, Cα-H), 7.07 (2H, d, J=8.0, arom.), 7.38 (2H, d, J=8.0, arom.); MS (70 eV), 352 (M^+), 292 (M^+-CH_3COOH), 250 (292-$COCH_2$), 207 (250-$COCH_3$), 165 (207-$COCH_3$), 123 (165-$COCH_2$), 43 ($^+COCH_3$).

For the synthesis of syringylglycerol the same method was preliminarily adopted, but aromatic hydrogen of syringyl group was substituted with bromine at bromination step of side chain

and 2-bromosyringylglycerol which was identified by nmr and mass spectrometries was finally obtained. Then syringylglycerol was synthesized by an alternative method[11], that is acetate of ethylsinapate was oxidized to the corresponding diol with OsO_4, and 2,3-dihydroxysinapate thus obtained was reduced to syringylglycerol (threo) with $LiAlH_4$ after acetylation with acetic anhydride in pyridine and subsequent methylation of the acetate with diazomethane.

Syringylglycerol tetraacetate, NMR δ(ppm: $CDCl_3$), 1.97 (3H, s, γ-$COCH_3$), 2.04 (3H, s, β-$COCH_3$), 2.22 (3H, s, α-$COCH_3$), 2.33 (3H, s, phenolic $COCH_3$), 3.04 (1H, dd, J=12.0, 6.5, Cγ-HaHb), 3.82 (6H, s, OCH_3), 4.27 (1H, dd, J=2.0, 4.0, Cγ-HaHb), 5.37 (1H, m, Cβ-H), 5.87 (1H, d, J=7.5, Cα-H), 6.01 (2H, s, arom.); MS (70 eV), 412 (M^+), 370 (M^+ -$COCH_2$), 310 (370-CH_3COOH), 268 (310-$COCH_2$), 225 (268-$COCH_3$), 208 (268-CH_3COOH), 183 (+$CHOHC_6H_2(OCH_3)_2OH$), 43 (+$COCH_3$); metastable ions, 332.28 (412 370), 259.73 (370 310), 231.69 (310 268).

Dehydrogenation of p-hydroxycinnamyl alcohols

Dehydrogenation of the alcohols was carried out at about 25° with horseradish peroxidase (Sigma Chemical Co.) and a fungal laccase (Coriolus versicolor) donated by Dr. K. Konishi, Sankyo Co. Ltd., respectively. 1) The horseradish peroxidase, 2-5 mg was dissolved in suitable amounts of a phosphate buffer (0.05 M) pH 6.0 in a 3 necked flask equipped with two addition funnels. Each of p-hydroxycinnamyl alcohols (1-5 g, 0.5% solution) and an equimolar amount of H_2O_2 (0.1% solution) were added dropwise into the enzyme solution from the respective funnels

for 30 min with aeration and stirring, and then the reaction mixture was treated in a similar way.

Dehydrogenation polymer (DHP) of each of the alcohols was filtered off, and the filtrate was saturated with NaCl and extracted with ethylacetate. The etylacetate soluble fraction was concentrated and the products were chromatographed on silicic acid column using mixture solutions of benzene-ethyl acetate (2:1) 1:2) as solvents. The products isolated were purified by preparative tlc of silicagel (PF_{254}) with benzene-ethyl acetate (2:1) and methanol-chloroform (1:9), respectively.

p-Coumaryl aldehyde, p-coumarylresinol, dehydrodi-p-coumaryl alcohol, p-hydroxyphenylglycerol-β-p-coumaryl ether and 2-(4-hydroxyphenyl)-3-hydroxymethyl-4-(α,4-dihydroxybenzyl)-tetrahydrofuran which are reported separately were obtained from the dehydrogenation products of p-coumaryl alcohol.

The ethylacetate soluble fraction of the dehydrogenation products of coniferyl alcohol gave coniferyl aldehyde, dl-pinoresinol, dehydrodiconiferyl alcohol and guaiacylglycerol-β-coniferyl ether, the analytical data of which were identical with those of authentic compounds.

Coniferyl aldehyde, NMR δ (ppm: $CDCl_3$), 3.92 (3H, s, OCH_3), 6.56 (1H, dd, J=16.0, 7.8, Cβ-H), 6.88-7.16 (3H, m, arom.), 7.38 (1H, d, J=16.0, Cα-H), 9.62 (1H, d, J=7.8, Cγ-H). Red purple coloration with phloroglucinol-HCl.

Pinoresinol, NMR δ(ppm: CD_3COCD_3), 2.90-3.20 (2H, m, Cββ´ -H), 3.82 (2H, dd, J=9.0, 4.0, Cγγ´ -Ha), 3.84-(6H, s, OCH_3), 4.11 (2H, dd, J=9.0, 7.0 Cγγ´-He), 4.68 (2H, d, J=4.2, Cαα´ -H), 6.77-7.00 (6H, m, arom.). Dehydrodiconiferyl alcohol, NMR δ

(ppm: $CDCl_3$), 3.35-3.70 (1H, m, Cβ-H), 3.76 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 4.19 (2H, d, J=5.5, Cγ'-H_2), 5.49 (1H, d, J=6.0, Cα-H), 6.12 (1H, dt, J=15.5, 5.0 Cβ'-H), 6.49 (1H, d, J=15.5, Cα'=H), 6.70-7.00 (5H, m, arom.).

Guaiacylglycerol-β-coniferyl ether tetraacetate, NMR δ (ppm: $CDCl_3$), 2.00 (3H, s, erythro Cγ-OAc), 2.03 (3H, s, threo Cγ-OAc), 2.07 (3H, s, Cγ'-OAc), 2.14 (3H, s, Cα-OAc), 2.28 (3H, s, phenolic OAc), 3.81 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 3.98-4.44 (2H, m, Cγ-H_2), 4.51-4.78 (1H, m, Cβ-H), 4.69 (2H, d, J= 5.9, Cγ'-H_2), 6.02-6.13 (1H, m, Cα-H), 5.85-6.33 (1H, m, Cβ'-H), 6.57 (1H, d, J=16.0, Cα'-H), 6.73-7.11 (6H, m, arom.).

Sinapaldehyde and syringaresinol were also obtained from the ethyl acetate soluble fraction of the dehydrogenation products of sinapyl alcohol and the analytical data were identical with those of authentic compounds.

Sinapaldehyde, NMR δ(ppm: $CDCl_3$), 3.94 (6H, s, OCH_3) 6.59 (1H, dd, J=16.0, 7.8, Cβ-H), 6.82 (2H, s, arom.), 7.40 (1H, d, J=16.0, Cα-H), 9.67 (1H, d, J=7.8, Cγ-H). Purple coloration with phloroglucinol-HCl. Syringaresinol, NMR δ(ppm: $CDCl_3$), 2.95-3.15 (2H, m, Cββ'-H), 3.75 to 4.00 (2H, m, Cγγ'-He), 4.75 (2H, d, J=5.0, Cαα'-H), 6.60 (4H, s, arom.).

The water soluble fraction separated from ethyl acetate soluble fraction was evaporated to dryness in vacuo and dissolved in acetone. A small amount of the acetone soluble products was subjected to tlc on silicagel (solvent, ethylacetate-benzene (2:1)), and arylglycerols were detected with the aid of UV light and quinone-monochloroimide spray. Major portion was acetylated and the acetates were subjected to preparative tlc

(PF_{254}) with n-hexane-ether (1:1). The corresponding band to authentic acetate which was found under UV light was extracted with acetone, and the product was further purified by second preparative tlc with n-hexane-methylene chloride (1:2). The purified product giving a single spot on the tlc plate was subjected to nmr and mass spectrometries. Guaiacylglycerol, syringylglycerol and p-hydroxyphenylglycerol were isolated from the water soluble fractions of the dehydrogenation products of the respective p-hydroxycinnamyl alcohols, and the analytical data of their acetates were completely identical with those of authentic compounds.

I-2-4 Summary

Guaiacyl-, syringyl- and p-hydroxyphenylglycerols were isolated from the enzymic dehydrogenation products of the corresponding p-hydroxycinnamyl alcohols and identified. These arylglycerols were a mixture of threo and erythro isomers and the amount of the former was 1-4 times higher than that of the latter. Possible occurrence of free arylglycerol side chains in lignin molecules were discussed.

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I-3 Configuration of phenylcoumarans

I-3-1 Introduction

In Section I-1, isolation and identification of the four dimers obtained by dehydrogenation of p-coumaryl alcohol were described. These dimers are p-coumarylresinol, dehydrodi-p-coumaryl alcohol, p-hydroxyphenylglycerol-β-p-coumaryl ether and monoepoxylignan, a new dimer. But the configuration of the coumaran ring of dehydrodi-p-coumaryl aocohol (3) has remained unknown.

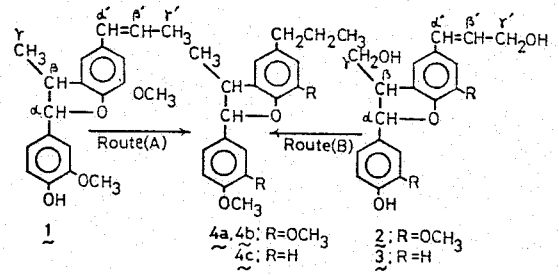
G. A. Erdtman et al.[1] determined that the configuration of the coumaran portion of dehydrodiisoeugenol (±) is to be trans on the fact that the compound gave erythro-β-methyl malic acid by treating with ozone in acetic acid. C. H. Ludwig et al.[2] subsequently reported that on the NMR spectrum of dehydrodiconiferyl alcohol triacetate, the γ-methylene peak reflected the nonequivalence and that the α-methine peak (δ5.61) gave the relatively large coupling constant of 7.2 cps. From these results, they suggested that the methylene acetoxyl group attached to the

β-carbon is cis to the aromatic ring on the α-carbon of the coumaran ring. But Ludwig later described in "Lignins"[3] without any reliable evidence that the configuration of dehydrodiconiferyl alcohol is trans.

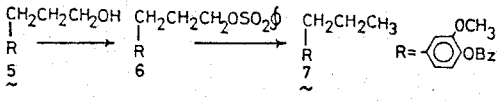
In the present Section, the trans coniguration of the coumaran portions of both dehydrodiconiferyl alcohol (2) and dehydrodi-p-coumaryl alcohol (3) is discussed.

I-3-2 Configuration of dehydrodiconiferyl alcohol

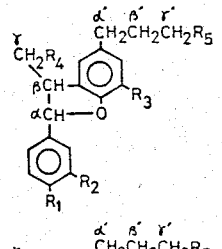
Since the configuration of dehydrodiisoeugenol (1) was determined to be trans, the configuration of dehydrodiconiferyl alcohol (2) is considered to be determined by comparing the spectral property of (1) to that of (2) after the γ, γ′-hydroxymethyl groups of the latter are reduced to the methyl groups.



Thus, comparison of the two coumarans (4a) and (4b) which are synthesized through both (A) and (B) routes should make possible to determine the configuration of (2). The model experiment was then carried out for reduction of the γ, γ′-hydroxymethyl groups to methyl groups. Dihydroconiferyl alcohol benzyl ether (5) used as a model compound was synthesized in



almost quantitative yield from eugenol by benzylation and subsequent hydroboration. The compound (5) was sulfonated with benzene sulfonyl chloride in pyridine and the sulfonate (6) was reduced with lithium aluminum hydride to the expected compound (7) in an 85% overall yield. Thus, the hydroxymethyl groups were reduced to methyl groups, and the synthesis of (4b) was undertaken. First, the compound (4a) was synthesized through the route (A) by methylation of (1) with diazomethane followed by catalytic reduction with Pd-carbon in methanol, and the product was crystallized from methanol. Alternatively, the compound (4b) was synthesized through the route (B) according to the same method used in the case of model compound (5). That is, the



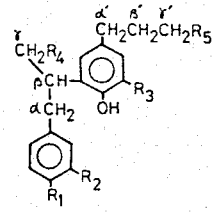
8 ; $R_1=R_2=R_3=OCH_3, R_4=R_5=OH$

9 ; $R_1=R_2=R_3=OCH_3, R_4=R_5=OSO_2\phi$

11 ; $R_1=OCH_3, R_2=R_3=H, R_4=R_5=OH$

13 ; $R_1=OCH_3, R_2=R_3=H, R_4=R_5=OSO_2\phi$

14 ; $R_1=OCH_3, R_2=R_3=H, R_4=OSO_2\phi, R_5=H$



10 ; $R_1=OCH_3, R_2=R_3=H, R_4=R_5=OH$

12 ; $R_1=OCH_3, R_2=R_3=H$ $R_4=OH, R_5=H$

compound (2) was reduced with Pd-carbon in methanol and the dihydro compound thus obtained was methylated with diazomethane to the compound (8). On the NMR spectrum of (8), both the chemical shift and coupling constant of α-methine proton were almost the same as those of the compound (2) suggesting that the chemical shift of α-methine proton was little affected by the substituent groups of the aromatic rings. Since the configuration of the coumaran portion seems to be unchanged during reaction steps, the compound (8) must hold the same configuration as (2). The compound (8) was sulfonated with benzene sulfonyl chloride in pyridine at 5°C and the sulfonate (9) obtained was immediately reduced with lithium aluminum hydride without crystatallization. After reduction for 30 minutes at room temperature, the product (4b) was extracted with ether, purified by preparative tlc and crystallized from methanol.

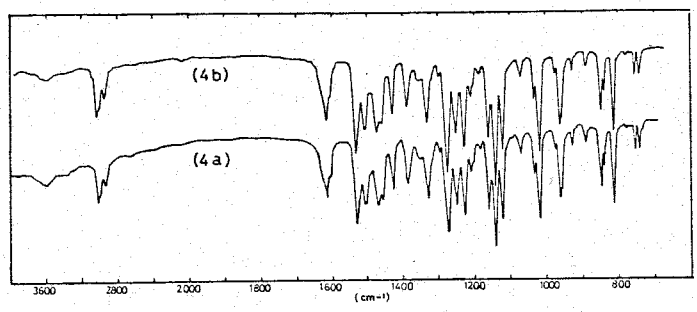


Fig. 1. IR spectra of 4a and 4b

It was concluded that these two coumarans (4a) and (4b) synthesized through both (A) and (B) routes, were identical by the following facts. 1) The mixed melting point showed no depression. 2) All the spectral data of NMR, IR (Fig. 1), UV and mass were completely identical between the two compounds. Since the configuration of the compound (1) is trans,

both the compounds (4a) and (4b) must be trans. Consequently, dehydrodiconiferyl alcohol (2), which is the starting material of (4b) must be of trans configuration.

I-3-3 Configuration of dehydrodi-p-coumaryl alcohol

The reduction of the γ,γ'-hydroxymethyl groups of dehydrodi-p-coumaryl alcohol (3) to methyl groups was tried in the same way. However, the coumaran ring of (3) was sensitive to both the catalytic and hydride reductions, and a ring cleavage compound was easily produced. That is, when the catalytic hydrogenation of (3) was carried out in methanol, the ring cleavage compound (10) was obtained in over 80% yield. It is known that the activity of catalytic hydrogenation reagent increases in a polar acidic solvent than in a neutral nonpolar solvent[4]. Therefore, the hydrogenation of (3) was carried out in a mixed solution of methanol/dioxane (1:2) to avoid the ring opening as much as possible and the dihydrocompound (11) was obtained quantitatively. These experiments suggested that the α-position of the coumaran (3) was less stable for nucleophilic attack than that of (2). This is a characteristic property of the coumaran (2) in comparison with (3). But in contrast with such instability of the coumaran ring, γ-sulfonyl group was stable for the hydride attack and the reduction of the γ-sulfonyl group without fission of the coumaran ring was very difficult. Under relatively drastic condition (using about 20 eq. of LAH at room temperature), the ring fission compound (12) was produced as a main product, whereas under the milder condition the starting sulfonate (13) was

recovered, and the hydride reduction gave many products containing a trace amount of the desired compound (4c). From these facts, the synthesis of (4c) by the one step reduction seemed to be difficultly feasible so that the monosulfonate (14) was synthesized and then it was reduced to the compound (4c)(about 34% yield). Easy opening of the coumaran ring to the compound (13) under such reduction condition suggests that the configuration of the coumaran ring is alterable to the more stable trans form by recyclization of the ring fission compound. But, once the ring fission occurs, α-methine compound must be altered to α-methylene one which is no longer capable of cyclization. Therefore, it should be assumed that the compound (4c) obtained without any ring fission through sulfonation and subsequent reduction holds the same configuration with (3).

Table 1
Chemical Shifts (δ, ppm) and Coupling Constants (Hz) of Protons in 4a, 4b and 4c

	α-CH-	β-CH-	γ-CH_3	α′-CH_2-	β′-CH_2	γ′-CH_3
4c	5.12, d, J = 9.0	3.20 — 3.60, m	1.34, d, J = 7.0	2.54, t, J = 8.0	1.65, m	0.93, t, J = 7.0
4a, 4b	5.05, d, J = 9.5	3.20 — 3.73, m	1.34, d, J = 7.0	2.51, t, J = 8.0	1.60, m	0.93, t, J = 7.0

d = doublet, t = triplet, m = multiplet

On NMR spectra of both compounds, (4a) and (4c), the chemical shifts and coupling constants of the corresponding protons of side chains were completely identical with each other. For manifesting the similarity, only signals of the side chains are listed on Table 1. These NMR data suggest the following facts. 1) γ-CH_3 protons (δ1.34, J=7.0) of (4a) and (4c) gave the same chemical shifts and coupling constants. If the configuration of (4c) is cis, two limited conformations will be conceivable, in which

γ-CH_3 group lies on the same plane with A ring (a) or vertical to the A ring (b) as shown in Figure 2. However, the preferred conformation of both cases seems to be (b), because in the

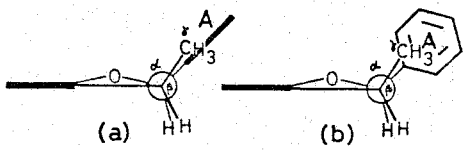


Fig. 2. Two limited conformations of cis-coumaran

case of (a), a strong steric repulsion exists between γ-CH_3 group and A ring. Consequently, the peak of γ-CH_3 group of cis compound would shift to the higher field by the shielding effect of A ring than in the trans compound. However, the signal of γ-CH_3 protons of (4c) gave almost the same chemical shift and coupling constant as (4a) which has trans configuration. Taking in consideration quite a slight difference in the chemical shifts and coupling constants of the side chain protons by the substituent group on the aromatic ring, the γ-CH_3 group of (4c) does not seem to be influenced by such an effect of A ring. Consequently, the compound (4c) must have the same trans configuration as (4a). On the basis of the above results, it is concluded that dehydrodi-p-coumaryl alcohol (3) has the same trans configuration with dehydrodiisoeugenol (1). 2) The signals of α-methine protons of both (2) and (3) at δ 5.49 (J=6.0) and δ 5.54 (J=5.8) were shifted to δ 5.05 (J=9.5) and δ 5.12 (J=9.0), respectively, by conversion of the γ-hydroxymethyl groups to methyl groups. This indicates that α-methine protons are influenced by the deshielding effect of γ-hydroxymethyl groups, especially due to the lone pairs of hydroxyl groups.

I-3-4 Experimental

Dihydroconiferyl alcohol benzyl ether (5)

Hydroboration was carried out by a modified method of the method of Brown and Rao[5]. The solution containing sodium borohydride (498 mg, 13.15 mM) and aluminum chloride (589 mg, 4.42 mM) in 50 ml of anhydrous diglyme in a 500 ml three necked flask was flushed with nitrogen and maintained under a slightly static pressure of the gas with stirring. Eugenol benzyl ether (6.67 g, 26.3 mM) which was synthesized from eugenol in the usual way, was dissolved in 20 ml of anhydrous diglyme and added into the solution over a period of 0.5 hour. The reaction was allowed to proceed without external heating for 3 hours, and then heated at 90°C for an hour, with swirling. The hot reaction mixture was hydrolyzed by the slow addition of 2 ml of water (hydrogen evolved) and then 10 ml of 3N sodium hydroxide, yielding a homogeneous solution. The separatory funnel was replaced by a water-cooled condenser and 11.7 ml of 35% aqueous hydrogen peroxide was added slowly, at a rate sufficient to maintain a gentle reflux. After the addition of the hydrogen peroxide, stirring was continued for additional an hour, and the flask was then cooled and diluted with ice-water. The products were extracted with ether after acidifying with 10% HCl solution. The organic layer was washed with water and dried over anhydrous sodium sulfate, and the solvent was evaporated *in vacuo*. A colorless oily substance which was crystallized from *n*-hexane was obtained (6.83g, 95.5%). NMR δ (ppm: $CDCl_3$) 1.69-2.00 (2H, m), 2.48-2.73 (2H, m), 3.62 (2H, t, J=6.5), 5.09 (2H, s), 6.50-6.89 (3H, m), 7.22-7.51

(5H, m).

Guaiacylpropane benzyl ether (7)

To the stirred solution of the alcohol (5)(381 mg, 1.4 mM) dissolved in anhydrous pyridine, benzene sulfonyl chloride (0.2 ml, 1.57 mM) was added at 0°C, and the reaction mixture was placed at 5°C in a refrigerator for 24 hours. The mixture was then poured onto ice and extracted with several portions of ether. The ether solutions were combined, washed with cold dilute HCl and bicarbonate solutions, and dried over anhydrous sodium sulfate, and then the ether was evaporated to dryness *in vacuo*. The colorless oily sulfonate which gave one spot on silica gel tlc plate developed with ether/*n*-hexane (1:1), was obtained. The sulfonate (6) dissolved in 2 ml of anhydrous ether was added dropwise to 114 mg of lithium aluminum hydride in 10 ml of ether at room temperature with stirring and the stirring was continued for an hour. The reaction mixture was cooled and a small portion of water was added to decompose the excess lithium aluminum hydride and then extracted with ether after acidification with 10% HCl solution. The ether solution was washed with water and dried over anhydrous sodium sulfate and then the ether was evaporated to dryness *in vacuo* to yield a colorless oily substance. The product which was purified with silica gel tlc (developed with *n*-hexane) gave 256 mg of guaiacylpropane benzyl ether (7)(85% yield). NMR δ(ppm: $CDCl_3$) 0.89 (3H, t, J=7.0), 1.25-1.85 (2H, m), 2.25 (2H, t, J=7.0), 3.74 (3H, s), 4.92 (2H,s), 6.40-6.70 (3H, m), 7.10 to 7.40 (5H, m).

Compound (4a), Synthesis through the route (A)

Dehydrodiisoeugenol (1) was synthesized by the method of B. Leopold[6] and recrystallized from a small amount of alcohol, mp 133°C (ref. mp 132-133°C).

To the stirred solution of (1)(652 mg, 2mM) dissolved in 10 ml of methanol, diazomethane ether solution was added at room temperature. After methylation was completed (checked by tlc), the reaction mixture was evaporated in vacuo to yield colorless needles (646 mg, 95% yield). The methylated dehydrodiisoeugenol (510 mg, 1.5 mM) was dissolved in the methanol containing 500 mg of 5%-Pd carbon and hydrogenated until hydrogen uptake ceased. The mixture was filtered and the filtrate was evaporated in vacuo. A slightly yellow oily substance which was crystallized from methanol was obtained. Recrystallization of the compound from methanol afforded colorless needles, mp 91-92°C, IR (KBr); Fig. 1, NMR δ (ppm: $CDCl_3$) 0.93 (3H, t, J=7.0), 1.34 (3H, d, J=7.0), 1.40-1.85 (2H, m), 2.51 (2H, t, J=8.0), 3.20-3.75 (2H, m), 3.83 (9H, s), 5.05 (1H, d, J=9.5), 6.50-7.05 (5H, m). Mass fragmentation pattern of this compound was completely identical with that of (4b). UV λ_{max}^{EtOH} nm(log ε): 280(4.63)

Compound (4b), Synthesis through the route (B)

Dehydrodiconiferyl alcohol (2) was synthesized by the method of K. Freudenberg et al.[7] and recrystallized from methylene chloride/pet. ether, mp 155-156°C (ref. 156-157°C).

Fifty mg of (2) dissolved in 2 ml of methanol containing 50 mg of 5%-Pd carbon was hydrogenated until hydrogen uptake ceased. The mixture was filtered and the filtrate was evaporated.

The product was dissolved in methanol again and methylated with diazomethane ether solution in the usual way. A colorless oily substance (8, 46.7 mg, 90% yield) was obtained by means of preparative TLC with 2.5% methanol/chloroform used as developer.

Reduction of compound (8) to (4b)

To the stirred solution of the compound (8) (37.4 mg, 0.1 mM) dissolved in 2 ml of anhydrous pyridine, benzene sulfonyl chloride (70.6 mg, 0.4 mM) was added at 0°C and the reaction mixture was placed in a refrigerator for 24 hours. Then, the reaction mixture was treated in the same way as for the model compound (5). The sulfonate (9), which gave one spot on the silica gel tlc developed with methylene chloride, was reduced with 20 mg of lithium aluminum hydride in 5 ml of anhydrous ether solution at room temperature for an hour. The reduced products were purified by tlc developed with ether/n-hexane (1:2). A colorless oily substance (about 10 mg, 29.2 % overall yield) was obtained and crystallized from methanol, mp 92-93°C, IR (KBr); (Fig. 1). NMR was completely agreement with that of (4a). MS (75 eV); 342.18229 (M^+, $C_{21}H_{26}O_4$, requires; 342.18310) (100), 340 (2.0), 328 (2.0), 327 (9.2), 313 (7.0), 311 (2.5), 299 (3.2), 295 (3.7), 283 (2.0), 281 (1.7), 268 (1.6), 267 (3.2), 253 (2.0), 239 (1.9), 225 (1.8), 223 (1.9), 191 (2.9), 181 (1.9), 178 (1.9), 176 (4.7), 175 (8.3), 171 (2.5), 165 (4.5), 164 (3.1), 163 (4.5), 161 (3.4), 156 (2.6), 153 (2.0), 152 (3.5), 151 (9.8), 150 (2.0), 147 (2.3), 145 (2.2), 141 (2.0), 137 (2.2), 131 (2.0), 115 (4.2), 107 (2.3), 105 (2.2), 104 (2.0), 103 (2.8), 91 (5.1), 77 (3.9), 65 (2.2), 55 (2.0), 51 (1.9), 43 (3.2), 41

(3.8). UV λ_{max}^{EtOH}nm(log ε): 280(4.63)

Monosulfonate (14)

Dehydrodi-p-coumaryl alcohol (3) was synthesized by the method described in Section I-1[8], mp 155-157°C.

A hundred thirty mg of (3) dissolved in 6 ml of dioxane/methanol (2:1) containing 50 mg of 5%-Pd carbon was hydrogenated until hydrogen uptake ceased. The mixture was filtered and the filtrate was evaporated in vacuo. The dihydro compound was dissolved in methanol and methylated with diazomethane ether solution in the usual way (checked by tlc developed with 5% methanol/chloroform). After evaporation of the solvent, a colorless oily substance (11) was obtained almost quantitatively.

To the stirred solution of the compound (11) (120 mg, 0.38 mM) dissolved in 2 ml of anhydrous pyridine, benzene sulfonyl chloride (135 mg, 0.76 mM) was added at 0°C and the reaction mixture was placed at 5°C for 24 hours. Then, the reaction mixture was treated in the same way described above and the sulfonate (13) (170 mg) which gave one spot on the silica gel tlc plate developed with n-hexane/ether (2:1) was obtained. The sulfonate (13) was dissolved in 6 ml of anhydrous THF at 0°C and lithium aluminum hydride (24.5 mg) was added. After stirring for 1.5 hours, the reaction mixture was worked up in the same way as described above and the product was purified by the preparative tlc developed with n-hexan/ether (4:1) to obtain monosulfonate (14) (50.5 mg). The NMR date of this compound (14) were as follows; NMR δ (ppm: $CDCl_3$) 9.17 (3H, t, J=7.0), 1.20-1.80 (2H, m), 2.52 (2H, t, J=8.0), 3.50-3.90 (1H, m), 3.78 (3H,

s), 4.29 (1H, broad d, J=7.0, γ-H), 4.30 (1H, broad d, J=6.5, γ-H), 5.39 (1H, d, J=6.0), 6.60-7.35 (7H, m), 7.45-8.00 (5H, m).

Compound (4c)

Monosulfonate (14) (50 mg) was dissolved in 1 ml of anhydrous ether and lithium aluminum hydride (5 mg) was added to the solution at 0°C. The reaction mixture was stirred for an hour at 0°C and then for two hours at room temperature. The expected compound (4c) (11 mg) was purified by the preparative tlc developed with n-hexane and its structure was established by the NMR and mass spectra. NMR δ (ppm: $CDCl_3$) 0.93 (3H, t, J=7.0), 1.34 (3H, d, J=7.0), 1.65 (2H, m), 2.54 (2H, t, J=8.0), 3.20-3.60 (1H, m), 3.83 (3H, s), 5.12 (1H, d, J=9.0), 6.73-7.09 (5H, m), 7.41 (2H, d, J=9.0). MS (75eV); 282.16272 (M^+, $C_{19}H_{22}O_2$, requires; 282.16197) (100), 281 (3.1), 280 (4.2), 268 (2.4), 267 (10.6), 254 (7.0), 253 (24), 251 (2.9), 239 (3.9), 238 (1.5), 235 (2.7), 224 (1.7), 223 (4.4), 221 (1.4), 209 (1.3), 205 (2.2), 195 (1.5), 178 (1.4), 165 (2.0), 161 (3.1), 150 (2.4), 146 (3.8), 145 (15.4), 141 (4.0), 135 (2.3), 134 (3.8), 133 (3.1), 131 (1.7), 127 (5.6), 125 (2.8), 122 (1.9), 121 (12.5), 120 (2.5), 119 (3.2), 117 (2.2), 115 (4.2), 105 (1.7), 104 (1.4), 91 (3.8), 77 (2.4), 57 (2.1), 56 (1.8), 55 (1.3), 44 (1.7), 43 (2.3), 41 (2.3).

I-3-5 Summary

Dehydrodiconiferyl alcohol (2) was reduced with H_2/Pd-carbon and the reduction product was methylated with diazomethane. The

methylated product was then sulfonated with benzene sulfonyl chloride and the product was reduced with lithium aluminum hydride to γ, γ'-methyl derivative (4b), the structure of which was the same to dihydrodehydrodiisoeugenol methyl ether (4a). By comparison of the spectral data of both compounds, the configuration of dehydrodiconiferyl alcohol (2) was determined to be trans. Furthermore, γ,γ'-methyl derivative (4c) of dehydrodi-p-coumaryl alcohol (3) was synthesized by the same method used in the case of (2). On the NMR spectrum, the chemical shifts and coupling constants of protons of the side chain of (4c) were closely identical with those of (4a). These results indicated that the configuration of dehydrodi-p-coumaryl alcohol (3) was trans.

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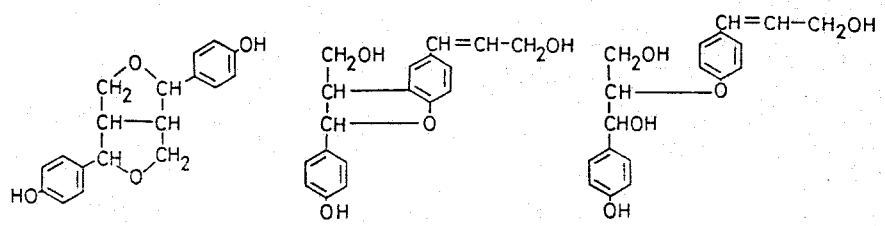
I-4. Analysis of dilignols by gas chromatography and NMR spectrometry

I-4-1. Introduction

In 1951, Freudenberg et al.[1] reported that p-coumaryl alcohol produced a very similar dehydrogenation polymer (DHP) to that of coniferyl alcohol based on the hydrogen uptake by both DHP's and their elementary analysis. Later, Bland et al.[2] reported that an artificial lignin prepared from p-coumaric acid on potato parenchyma and Sphagnum MWL were highly condensed polymers containing double condensations at C-3 and C-5 of the p-hydroxyphenyl ring, and suggested the different reactivity between p-coumaryl and coniferyl alcohols on dehydrogenation. Recently, Yamasaki et al.[3] reported that no difference of condensation pattern between p-coumaryl and coniferyl alcohols occurred in their DHP formation from the yield of the condensed and non-condensed type compounds obtained by permanganate and hydrogen peroxide oxidation of both the methylated DHP's.

It seems that this problem is solved more clearly from the yield of the dilignols of both the alcohols. In previous Section I-1, the isolation and identification of the four dilignols of p-coumaryl alcohol, p-coumarylresinol (1), dehydrodi-p-coumaryl alcohol (2), p-hydroxyphenylglycerol-β-p-coumaryl ether (3) and monoepoxylignan (4)[4], and the trans configuration of the coumaran ring of the dilignol (2)[5] were described. In this Section, configuration of the dilignol (3) and the yields of these dilignols determined by gas chromatography and NMR spectrometry are described.



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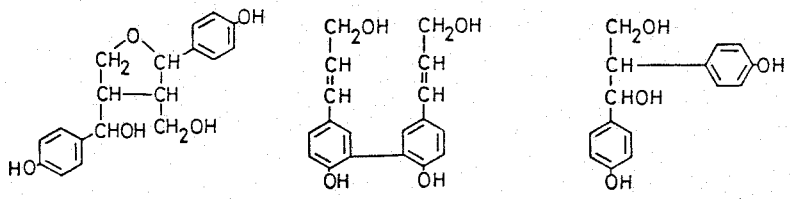

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I-4-2. Configuration of p-hydroxyphenylglycerol-β-p-coumaryl ether

Recently guaiacylglycerol-β-guaiacyl ether, the model compound of arylglycerol-β-aryl ether structure in lignin was synthesized in high yield by the condensation reaction between benzyl vanillin and ethyl 2-methoxyphenoxy acetate and subsequent reduction with 5% Pd-C and lithium aluminum hydride. The ratio of two isomers (erythro/threo) was about 3:1[6]. These configurations were determined by comparison with the results by Miksche et al[7]. In NMR spectra of these acetates, the chemical shifts and coupling constants of α-methine protons were δ 6.12 (1H, d, J=5.0, erythro isomer) and δ 6.17 (1H, d, J=6.2, threo isomer), respectively and a doublet peak of α-CH of erythro isomer appeared in higher field and gave a smaller coupling constant than that of threo isomer. On the other hand, NMR spectrum of the dihydro acetyl derivative of the dilignol (3) gave two doublet peaks at δ 6.09 (1H, d, J=5.0) and δ 6.13 (1H, d, J=6.2)

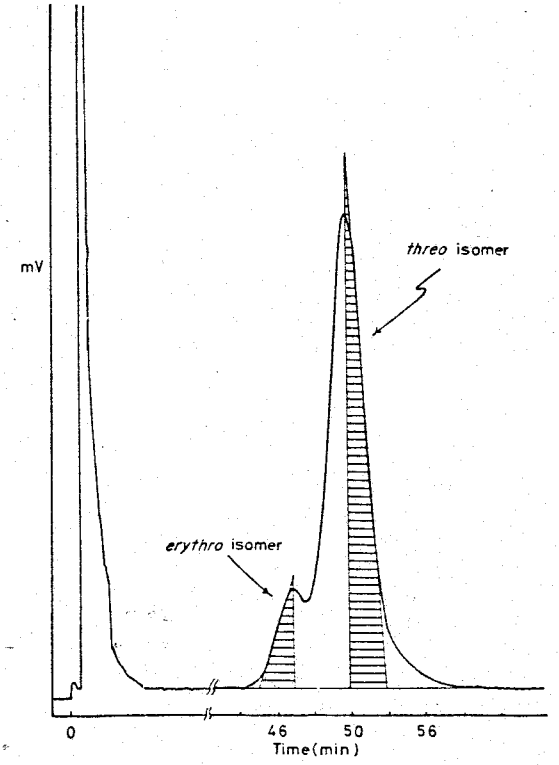

Fig. 1. Gas chromatogram of TMS derivatives of dihydro-dilignol (3). Column: 2% OV-17 on chromosorb AW, 2m-glass column, 200°C. Carrier gas: helium, 28 ml/min.

whose ratio was about 1 : 5, and this result was also supported by gas chromatography as shown in Fig. 1. The retention times of TMS derivatives of erythro and threo dihydrodilignols (3) were 47.1 and 49.8 min., respectively and the ratio of the peak areas was about 1 : 4.7. Thus, it was concluded that the dilignol (3) was a mixture consisting of erythro and threo isomer whose ratio was 1 : 4.7.

I-4-3. Analysis of dilignols

A dilignol fraction was converted to its hydro-dilignol fraction by catalytic hydrogenation with 5%Pd/C and H_2 in

dioxane/ethanol (2:1);this catalytic hydrogenation was indispensable from the following two reasons.

First, the peak area of the propenol dilignols, e.g., dilignol (2) and (3) etc., on gas chromatogram is not proportional to the amounts of the compounds injected, but only when the propenol side chains are reduced to the propanol side chains, the peak area of the dilignols is almost proportional to their amounts.

Second, the 5-5'-dilignol (5) seems to be stable when its propenol side chains are converted to the propanol side chains by reductions, as found for the coniferyl 5-5'-dilignol[8].

Furthermore, since the formation of the ring cleavage compound has been reported by catalytic hydrogenation of the dilignol (2) in methanol, the mixed solvent, dioxane/methanol (2:1) which avoided the ring fission had to be used[5].

Figure 2 shows the gas chromatogram of TMS derivatives of the hydro-dilignol fraction. Six of ten peaks were identified by comparison with the retention times and mass fragmentation pattern of authentic dilignols. The amounts of the dilignols are caluculated from the peak areas, and summarized in Table 1. In the Table, numbers in column (A) represent the retention times of each peak, and,those in (B), (C) and (D), peak areas, ratio of the peak areas and product distribution of three main dilignols, respectively. In the last column (F) are reproduced the product distribution of three main dilignols of coniferyl alcohol which was reported earlier[8].

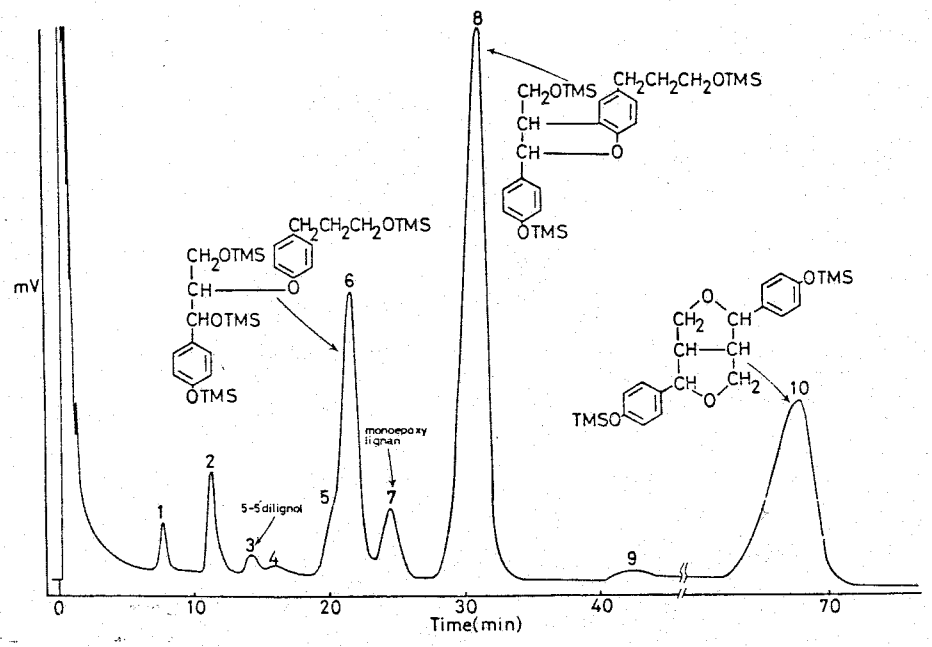

Fig. 2. Gas chromatogram of TMS derivatives of hydro-dilignol fraction obtained by dehydrogenation of p-coumaryl alcohol with H_2O_2 and peroxidase system. Column: 2% OV-17 on chromosorb AW, 2m, 220°C.
Peak 3: tetrahydro dilignol (19), Peak 5: dihydro erythro dilignol (3), Peak 6: dihydro threo dilignol (3), Peak 7: monoepoxylignan (4), Peak 8: dihydro dilignol (2), Peak 10: dilignol (1).

The NMR spectrum of the hydro-dilignol fraction is shown in Fig. 3. α-Methine protons of the three main dilignols (1), (2) and (3) give the peak at δ 4.68 (2H, d, J=4.0), δ 5.50 (1H,

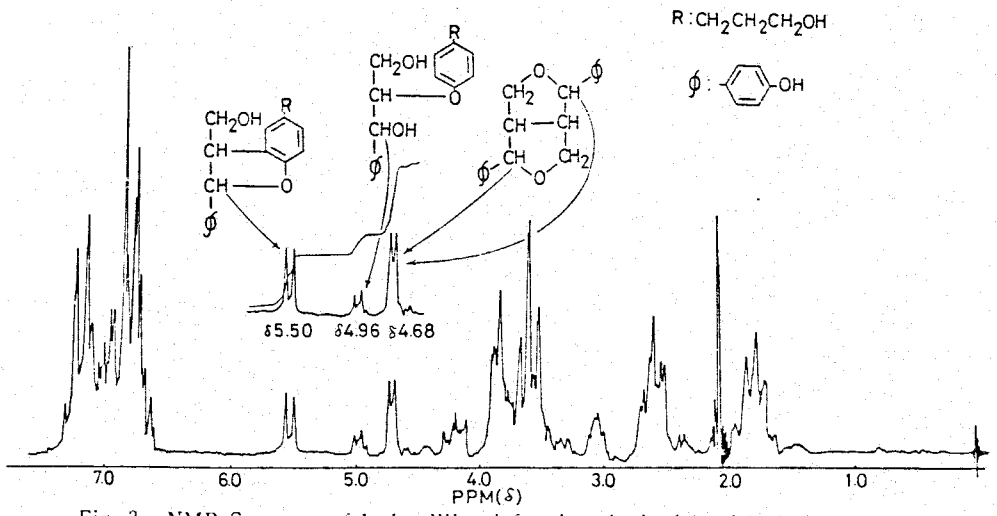

Fig. 3. NMR Spectrum of hydro-dilignol fraction obtained by dehydrogenation of *p*-coumaryl alcohol.

m) and δ 4.69 (1H, d, J=6.0), respectively and these peaks do not interfere with other peaks. Therefore, the product distribution of these dilignols may be determined by the integration curve of their α-methine peaks. The result is given in the column (E) of the Table 1.

Table 1. Estimation of dilignois by gas chromatography and NMR spectrometry.

Peak number*	A(min.)	B(cm²)	C(%)		D(%)	E(%)	F(%)
1	7.7	1.7	1.0				
2	11.2	5.0	3.0				
3	14.2	1.0	0.6				
4	15.8	0.5	0.3				
5	20.0	30.3	18.0		20	20	19
6	21.5						
7	24.4	5.2	3.0	1.0			
8	31.0	76.3	45.0		49	48	54
9	42.0	1.4	0.8				
10	67.5	48.3	28.3	9.4	31	32	27

A: retention time, B: peak area, C: ratio of the peak area, D: ratio of three main dilignols, E: ratio of three main diligonls obtained by NMR analysis, F: ratio of three main dilignols of coniferyl alcohol[9].

* peak number corresponds to those of the compounds in Fig. 2.

These data lead to the following conclusions. TMS derivative of synthetic 1,2-diarylpropane-1,3-diol (6) gives a peak at 5.8 min. on gas chromatogram, but the hydro-dilignol fraction did not give any peak at the same retention time (Fig. 2). Furthermore, p,p'-dihydroxystilbene which was synthesized from the dilignol (6) by alkali degradation could not be found in the alkali degradation products of DHP and dilignol fraction. Therefore, the dilignol (6) seems to be formed at a later stage of dehydrogenation. Only 0.6% of 5-5'-dilignol (5) was detected by gas chromatography, and then the double condensation at C-3 and C-5 reported by Bland et al.[2] may not be possible at this dehydrogenation stage and also at DHP's stage[3]. The ratio of the p-coumarylresinol (1) and monoepoxylignan (4) which were

formed on the racemoid and mesoid couplings at C-β and C-β' carbons, respectively, was 9.4:1. Thus, it is expected that coniferyl and sinapyl alcohols give the corresponding monoepoxylignans with the same ratio on dehydrogenation. Investigation on this point, is now in progress. The ratio of the amounts of the three main dilignols (1), (2) and (3) was 31:49:20, respectively by gas chromatography, and the same result was obtained by NMR analysis as shown in column (E) of Table 1. The ratio of the three coniferyl dilignols corresponding to the dilignols (1), (2) and (3) formed on dehydrogenation of coniferyl alcohol[9] is shown in column (F) of Table 1. The most reactive radical of the four resonance radicals of both alcohols is β-radical because the three main dilignols are not formed without any participation of β-radical of the side chain, and the second reactive one is the radical at 5-position of aromatic ring because the yields of the coumaran type dilignols are larger than that of β-ether type dilignols.

Both p-hydroxycinnamyl alcohols, p-coumaryl and coniferyl alcohols have a comparable reactivity on enzymic dehydrogenation as described above, but a typical difference manifests in the amounts of the coumarans. That is, the percentage of dehydrodiconiferyl alcohol (54%) is larger than that of dehydrodi-p-coumaryl alcohol, dilignol (2) (49%), indicating the radical activating effect of the methoxyl group at 3-position of aromatic ring.

I-4-4. Experimental

Preparation of authentic dilignols (1), (2), (3), (4), (5) and (6)

Dilignols (1), (2), (3) and (4) were prepared by dehydrogenation of p-coumaryl alcohol with peroxidase and H_2O_2 system as described in Section I-1[4], and dilignols (2) and (3) were hydrogenated with 5% Pd-C and hydrogen in a mixed solvent of dioxane/ethanol (2:1).

Tetrahydro-5-5'-dilignol (5) was obtained by dehydrogenation of dihydro-p-coumaryl alcohol with peroxidase and H_2O_2 system. The spot of this compound on a silica gel TLC plate (Merck Kieselgel PF_{254}) showed a sky blue color under UV lamp (TOSHIBA Fl-3-S-type), and easily isolated by preparative TLC. NMR δ (ppm: $CDCl_3$) of tetra acetate: 1.99 (6H, s,γ,γ'-acetyl groups), 2.01 (6H, s, phenolic acetyl groups), 1.80-2.30 (4H, m, β,β'-methylene protons), 2.69 (4H, t, J=6.5,α,α'-methylene protons), 4.01 (4H, t, J=6.5,γ,γ'-methylene protons), 6.90 7.30 (8H, m, aromatic protons). It is characteristic of 5-5'-dilignol that the phenolic acetyl protons give the peaks at almost the same field with γ,γ'-primary alcoholic acetyl protons by the shiel ing effects of each aromatic ring . MS (70 eV):320 (M^+, 100), 256 (66.7), 239 (57.2), 211 (35), 197 (33.4).

1,2-Diarylpropane-1,3-diol (6) was synthesized by condensation of benzyl p-hydroxybenzaldehyde and benzyl p-hydroxybenzoic acid methyl ester and subsequent hydrogenation with 5% Pd-C and reduction with lithium aluminum hydride. This synthetic method and the determination of configuration of the products are described in Section II-4. NMR δ(ppm: CD_3COCD_3): 2.70 3.20 (1H, m, β-methine proton), 3.50-4.20 (2H, m, γ-methylene protons), 4.89 (1H, d, J=9.0, α-methine proton of threo isomer), 5.02 (1H,

d, J=5.5, α-methine proton of erythro isomer), 6.55-7.10 (8H, m, aromatic protons).

Dehydrogenation of p-coumaryl alcohol

Dehydrogenation of p-coumaryl alcohol was carried out by the method described in Section I-1[4], and the dilignols were extracted with ethyl acetate and then hydrogenated with 5% Pd-C and hydrogen in dioxane/ethanol (2:1). A colorless foaming product, hydro-dilignol fraction (about 5mg) was dissolved in pyridine (0.1 ml) and then hexamethyl disilazane (0.1 ml) and trimethylchlorosilane (0.05 ml) were added, successively. The reaction mixture was shaken vigorously for 1 min. and after 5 min. keeping at room temperature, it was evaporated to dryness in a vacuum desiccator containing P_2O_5. The residue was dissolved in CCl_4 (0.5 ml) and analyzed by gas chromatograph-mass spectrometer. The amounts of dilignols were calculated from the peak area on the chromatogram by using calibration curves prepared previously for the respective authentic compounds. Alternatively the hydro-dilignol fraction was analyzed by NMR spectrometer.

NIHONDENSHI J.G.C-750 gas chromatograph with a flame ionization detector and a SHIMADZU-LKB 9000 gas chromatograph-mass spectrometer were used for analysis of the trimethylsilyl ethers of the hydro-dilignol fraction at the following condition. Stainless steel column (2 m, 3 mm ID) packed with 2% OV-17 on chromosorb AW. Column temperature: 220°C. Injector temperature: 250°C. Carrier gas: helium, 2 kg/cm^2. Mass spectra were taken by the use of a glass column at the same condition, and relative

abundance of each peak was designated in parentheses. NMR spectra were taken by the use of an R-22 HITACHI high resolution NMR spectrometer (90 MHz) with TMS internal standard. Chemical shifts and coupling constants were given in δ-value and Hz, respectively.

I-4-5. Summary

p-Coumaryl alcohol was dehydrogenated with peroxidase and H_2O_2 system. Five dilignols, p-coumarylresinol (1), dehydrodi-p-coumaryl alcohol (2), p-hydroxyphenylglycerol-β-p-coumaryl ether (3), monoepoxylignan (4) and 5-5'-dilignol (5) were identified and determined by both gas chromatography and NMR spectrometry, and the ratio of the amounts of the three main dilignols (1, 2 and 3) was 31:49:20. The dilignol (5) was detected in a trace (0.6%), and 1,2-diarylpropane-1,3-diol (6) could not be found. The ratio of the racemoid and mesoid couplings at C-β and C-β' carbons was about 9.4:1, and the dilignol (3) was a mixture consisting of erythro and threo isomers (1:4.7) whose ratio was determined by gas chromatography. From these results, it was concluded that coniferyl and p-coumaryl alcohols had almost the same reactivity on enzymic dehydrogenation.

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I-5. Reactivity of quinonemethide

I-5-1. Introduction

It is well known that quinonemethide intermediates play an important role in the polymerization of lignins. Subsequent to the coupling reactions of mesomeric radicals of p-hydroxycinnamyl alcohols, ionic reactions occur between quinonemethides and various nucleophiles. Thus, investigations on the reactivity of quinonemethide are indispensable to understand the mechanism of the polymerization of lignins, as has been discussed by Freudenberg et al.[1] and Adler.[2] Stereochemistry of the products is especially interesting when one chiral center is introduced by the attack of water to quinonemethides as in the formation of β-O-4 dilignols. Sarkanen reported that threo β-O-4 dilignol is formed more than erythro isomer on the dehydrogenation of isoeugenol.[3] Our investigations also showed that threo isomers are produced more than erythro counterparts on the dehydrogenation

of p-coumaryl, sinapyl and coniferyl alcohols.[5]

As quinonemethide intermediates are almost a planar molecule, it is considered that water attacks from both sides of the compound with equal probability, giving almost 1.0 in the ratio of erythro to threo isomers. Sarkanen suggested that the predominant formation of the threo isomer is ascribed to steric reasons.[3]

In this Section, based on the reaction of the quinonemethide derived from guaiacylglycerol-β-guaiacyl ether with various nucleophiles, factors which have an effect on the ratio of both isomers are discussed.

I-5-2. Reaction of quinonemethide with various nucleophiles

Quinonemethide (3) was prepared by the method of B. Johansson et al.[6] as shown in Fig.1. Guaiacylglycerol-β-guaiacyl ether (1) synthesized by the method of Nakatsubo et al.[7] was converted to its bromide (2) with hydrogen bromide at -60°C in chloroform. The chloroform solution of the bromide (2) was treated with a saturated sodium bicarbonate solution, and a yellow quinonemethide solution which is stable at 5°C was obtained. The chloroform solution of the quinonemethide (Q.M.) was used for the following reactions.

The UV spectrum of this quinonemethide showed the maximum peak at 301 nm(ε=15150) as shown in Fig.2, and the reaction rate with nucleophiles could be followed by the decreasing rate of the absorption at the maximum spectrometrically. The configuration of this reaction products was determined by the analysis

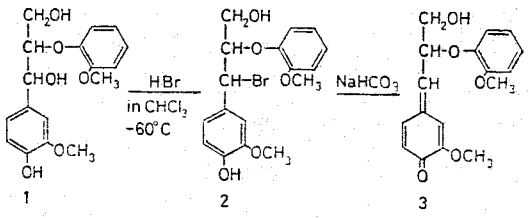

Fig. 1. Synthetic route of quinonemethide.

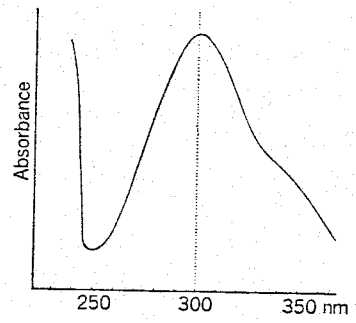

Fig. 2. UV Spectrum of quinonemethide in $CHCl_3$.

of NMR spectra. On the NMR spectrum of triacetyl guaiacylglycerol-β-guaiacyl ether (1), the α-methine doublet peak of erythro isomer appears at higher field (δ6.12, J=5.0) than that of threo counterpart at δ 6.17(J=6.2). Moreover, the α-methine doublet peak of erythro α-acetyl derivative of the compound (1) which is synthesized by the reaction of Q.M. (3) with acetic acid, appears at δ 6.02(J=6.0) and that of threo counterpart at δ 6.11 (J=8.0) as shown in Fig.3. As β-protons which couple with these α-protons give spectra in the field significantly higher than α-methine ones, α-protons give parallel lines which are of the same height and do not interfere with other peaks. From these considerations, the ratio of erythro to threo isomers (E/T) can be determined by the measurement of the height or integration curve of both side peaks among three peaks. As α-methine peaks are only important for the determination of the E/T ratio, the peaks

of the products, which were obtained by the reactions between Q.M. (3) and aliphatic carboxylic acids, such as formic, propionic, isobutyric and trimethyl acetic acids, are given in Fig.3.

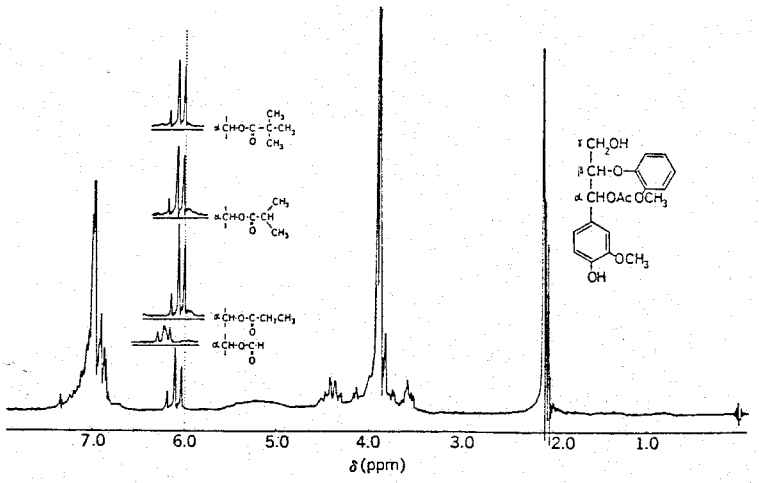

Fig. 3. NMR Spectrum of α-acetyl guaiacylglycerol-β-guaiacyl ether.

These NMR data indicated that the more bulky nucleophiles give the more erythro isomers. The relative reaction times and E/T ratios by the differences of various nucleophiles are summarized in Table 1.

Table 1. Reaction of quinonemethide and nucleophiles.

ROH	Relative reaction times	*Erythro* / *Threo*
HOH		1.1
CH_3CH_2OH	2.1×10^5	
CH_3OH	1.3×10^5	
$(CH_3)_3CCOOH$		3.9
$(CH_3)_2CHCOOH$	3.2×10^4	3.8
CH_3CH_2COOH	1.0×10^4	3.2
CH_3COOH	1.8×10^3	2.6
HCOOH	1	1.6

These reactions were conducted quantitatively, and all reactions were carried out in chloroform solution of Q.M.(3).

The relative reaction times which correspond to the decrease in maximum absorption of Q.M. (3) caused by reaction of the nucleophiles are also listed in Table 1. The configuration of guaiacylglycerol-β-guaiacyl ether (1) was derermined by NMR spectrometry after acetylation as described above, but the stereochemistry of the α-alkoxy derivatives obtained by reactions of methyl and ethyl alcohols has not been determined. Water reacted with the Q.M. in chloroform only in the presence of catalytic amounts of acid (e.g. HCl) because of two-phase reaction, and the reaction with trimethyl acetic acid was very sluggish because of the steric hindrance, hence the rates of these two reactions were not listed in Table 1.

The data in Table 1 clearly show that the rate of the reactions is proportional to the acidity of nucleophiles because acids act as substrates for the Q.M. and also as acid catalyst. On the other hand, the more the steric hindrance of nucleophiles increases, the more the rate decreases, but the formation of erythro isomers remarkably increases. For example, the mixture consisting of erythro (80%) and threo (20%) isomers was obtained by reaction of trimethyl acetic acid. Consequently, the E/T ratio was remarkably influenced by the steric hindrance of nucleophiles. This result was also supported by the fact that the reaction with water which does not give such a steric hindrance gave a mixture consisting of almost equal amount of the isomers (E/T ratio was about 1.0).

Thus, three limited conformations of the transition state in which the quinonemethide group takes trans orientation for each of γ-hydroxymethyl group (A), β-hydrogen (B) and β-phenoxy

group (C), respectively, are conceivable as shown in Fig. 4. For each conformation, erythro or threo isomer is formed by the attack of a nucleophile from the right or left side of the planar quinonemethide group, respectively. In these conformations, (B) may not participate in the reaction because of the unstability due to a large steric hindrance existing between Q.M. group and γ-hydroxymethyl or β-phenyoxy group. If the reaction proceeds via (C)-conformation, threo isomer may be preferentially produced, because nucleophiles attack from the same side of β-hydrogen, but not from the side of γ-hydroxymethyl group for the steric hindrance. By a similar steric factor, erythro isomar may be formed predomiantly via (A)-conformation, which favors erythro isomer.

However, it has been found that threo isomer predominates on enzymic dehydrogenations of p-hydroxycinnamyl alcohols [4)5)] and isoeugenol,[3)] and the difference between the reactions should be ascribed to the properties of solvent used.

All the reactions described above have been carried out in chloroform solution, whereas enzymic dehydrogenation have been conducted in aqueous solution. Thus, the reaction of Q.M. in aqueous solution was subsequently tested. The chloroform solution of Q.M. was evaporated in vacuo at 10°C under nitrogen stream, and the residue was dissolved in dioxane. All the reactions discussed below were carried out using dioxane solution of Q.M.(3).

When the Q.M. dioxane solution was added dropwise into water, a bright yellow color of Q.M. disappeared after 15 min in dioxane/water (1:9) and 4 hours in dioxane/water (1:1), respectively. Guaiacylglycerol-β-guaiacyl ether which was quantitatively

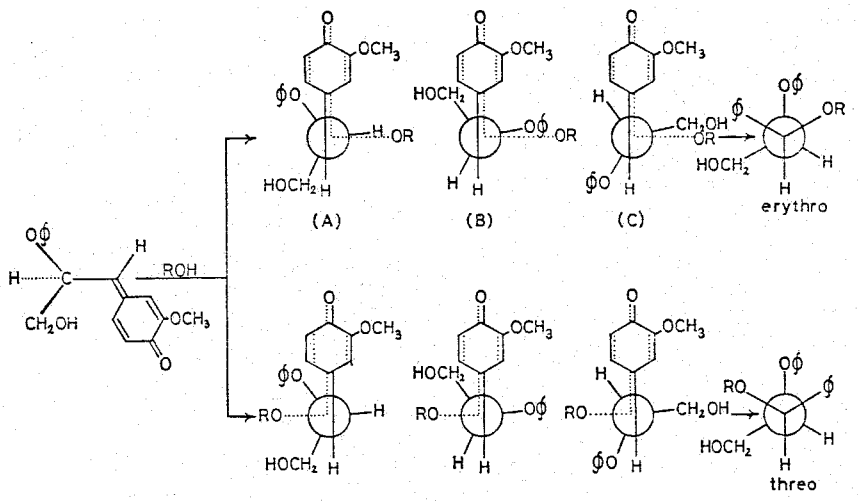

Fig. 4. Possible conformations of quinonemethide in transition state.

obtained, was acetylated with Ac_2O/pyridine for determination of the configuration by NMR spectometry. Surprisingly, the E/T ratio was about 0.5 in dioxane/water (1:9) and 0.4 in dioxane/water (1:1), respectively, and these values did not change when phosphate buffer (pH=6.0, 0.05 M) was used instead of water, or when reaction temperature was changed (20°C and 50°C). These results suggest that the E/T ratio is determined only by the difference of solvent. As the reason for variations of E/T ratio by the difference of solvent, the following three views might be considered; 1) the difference of the conformation on the transition state in the respective solvents, 2) stability of the products and 3) others.

If the reaction occurs via (C)-conformation in water, the more threo isomer must be produced by the reaction with acetic acid in water because acetic acid has larger steric hindrance than that of water. On the basis of this assumption, the reaction was carried out in the equimolar solution of water and acetic acid. Q.M. (0.16 mM) dissolved in dioxane (1 ml) was added dropwise at 20°C into a mixture of water (1.98 g, 0.11 M)

and acetic acid (6.60 g, 0.11 M) under stirring, and almost equimolar mixture of α-hydroxy and α-acetyl derivatives which were formed by the attack of water and acetic acid to the Q.M., respectively, was obtained. Unexpectedly, the E/T ratios of α-hydroxy and α-acetyl derivatives were about 0.66 and 1.0, respectively. Therefore, the Q.M. reaction in water does not proceed via (C)-conformation, and the first view is ruled out. However, it is noteworthy that the formation of threo isomer increases by the exchange of chloroform for water in both reactions of water and acetic acid.

As the next step, the isomerization reaction of a crystalline erythro isomer of guaiacylglycerol-β-guaiacyl ether (1) was carried out in order to determine the thermodynamic stability of threo and erythro isomers. Erythro guaiacylglycerol-β-guaiacyl ether (1) was dissolved in dioxane/water (9:1) containing 0.2 N HCl and heated at 50°C for 1, 12 and 24 hours (condition of mild acidolysis[8]). Reaction product in each case gave one spot which has the same Rf value with the starting compound, on silica gel TSC plate developed with 5% methanol/chloroform. After acetylation, the E/T ratio in respective reactions were determined to be about 9.0, 1.0 and 1.0, showing that the isomerization was completed in 12 hours. These results indicate that thermodynamic stabilities are not different between erythro and threo isomers. Thus, the E/T ratio on the reaction of Q.M. in water is not determined by the product development control,[9] and the second view must be ruled out.

Finally, it was proved from the results described above that the attack of water in aqueous solution is not controlled

by steric factor, thermodynamic stability of the products and salt effect of buffer. However, water actually approachs preferentially to the almost planar Q.M. molecule from the favorable side for the formation of threo isomer, suggesting the formation of some attracting force, such as a hydrogen bonding between two molecules on the transition state of the reaction. Among five oxygens of the Q.M. molecule, ketonic, methoxyl and γ-CH_2OH groups should be ruled out because threo isomer predominates on the dehydrogenation of isoeugenol which has γ-CH_3 group, instead of γ-CH_2OH. Therefore, a hydrogen bonding formed with oxygen of β-phenoxy group might be important for the control of the E/T ratio.

Thus, it is concluded that water attacks preferentially from the same left side with β-phenoxy or β-hydroxyl (in the case of the formation of arylglycerols) group of quinonemethide via (A)-conformation by forming a hydrogen bonding between a hydrogen of water and oxygen of β-phenoxy or β-hydroxyl group, resulting in predominant threo isomer on enzymic dehydrogenation. The results also suggest that such a hydrogen bonding factor participates in the polymerization of lignins.

I-5-3 Experimental

Chloroform and dioxane solutions of quinonemethide (3)

To a solution of guaiacylglycerol-β-guaiacyl ether (1) (320 mg, 1mM) in 50 ml of chloroform, HBr was blown at -60°C for 30 min under stirring. The chloroform solution which turned pale yellow, was transferred into a 100 ml

separating funnel and shaken twice with 20 ml of saturated $NaHCO_3$ solution. A bright yellow chloroform solution which contains the corresponding quinonemethide was separated and dried over anhydrous Na_2SO_4. This quinonemethide solution was evaporated *in vacuo* at 10°C under nitrogen and dissolved in 10 ml of dioxane.

Reactions of quinonemethide (3) with aliphatic carboxylic acids

A chloroform solution of the quinonemethide prepared by the method described above was added to the solution of each carboxylic acid dissolved in chloroform under stirring. After confirmation of the absence of quinonemethide by the disappearance of yellow color or UV-maximum at 301 nm the reaction mixture was washed with a saturated $NaHCO_3$ solution to remove an excess carboxylic acid and dried over Na_2SO_4. The chloroform solution was evaporated *in vacuo*, and a colorless substance was used for NMR spectrometry.

Isomerization of guaiacylglycerol-β-guaiacyl ether (1)

An *erythro* guaiacylglycerol-β-guaiacyl ether (1) (32 mg, 0.1 mM) was dissolved in 3 ml of a solution of dioxane-water (9:1) containing 0.2 N HCl and heated at 50°C under nitrogen stream for definite hours. The product was extracted with chloroform and the solvent was evaporated *in vacuo*. A colorless substance formed was acetylated with 1 ml of Ac_2O/pyridine (1:1). Acetylated compound was extracted with chloroform and used for NMR spectrometry after purification using a preparative TLC developed with chloroform.

I-5-4. Summary

It was found that the rate of the reactions between quinonemethide and various nucleophiles (aliphatic carboxylic acids, water and alcohols) depends on the acidity and the steric factor of nucleophiles. That is, a nucleophile with stronger acidity reacts faster than that with lesser acidity, and a bulky nucleophile reacts slower than a smaller one. Erythro isomers are produced more than threo ones in chloroform solution, whereas in water threo isomers tend to be produced more than erythro ones. The ratio of erythro to threo isomers (0.5) in the reaction of quinonemethide with water in dioxane/water (1:9) was similar to that of the isomers obtained by enzymic dehydrogenation of p-hydroxycinnamyl alcohols. These results suggest that water attacks predominantly from the same side with β-phenoxy group of quinonemethide by forming a hydrogen bonding between water and oxygen atom of β-phenoxy group, resulting in predominant threo isomer.

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CHAPTER II

SYNTHESES OF A LIGNIN MODEL COMPOUND AND OLIGOLIGNOLS

II-1 Synthesis of guaiacylglycerol-β-guaiacyl ether

II-1-1 Introduction

Arylglycerol-β-aryl ether structure is one of the most important interphenylpropane linkage in lignins and it has been reported that the structure comprises about 30 to 50% of the phenylpropane units.[1,2] Therefore, guaiacylglycerol-β-guaicyl ether (1) has been used as an important model compound for various reactions of lignin such as pulping processes.

This compound (1) had been synthesized by Adler *et al*[3], Kratzl *et al*.[4] and Miksche *et al*.[5] However, the synthetic method by these investigators required many reaction steps in a linear synthesis and the overall yield of the product was low. The method proposed by Miksche *et al*.[5] requires by far the most steps although both the *erythro* (75%) and *threo* (10%) isomers of the β-hydroxy ester were obtained as crystals by the reduction of β-keto ester, and then the final compound (1) was also obtained as crystal by this method.

Since the compound (1) has been in increasing use for the reaction and chemical elucidation of lignin, the synthetic method by which the compound (1) is obtained in high yield by shorter reaction steps will be required.

A new method by a convergent synthesis of the β-hydroxy ester (6), one of the intermediate in the method by Miksche *et al*. is described in this Section.

II-1-2 A new convergent synthesis of the compound

The compound (6) which is a β-hydroxy ester is expected to be cleaved to the compound (4) and (5) by a retro aldol condensation type reaction as shown in Fig.1. Thus, it is assumed that the compound (6) can be synthesized through the reverse reaction from the compound (4) which is obtained from commercially available ethyl chloroacetate (2) and benzyl vanillin (5). Along the above described synthetic route, following experiments were carried out.

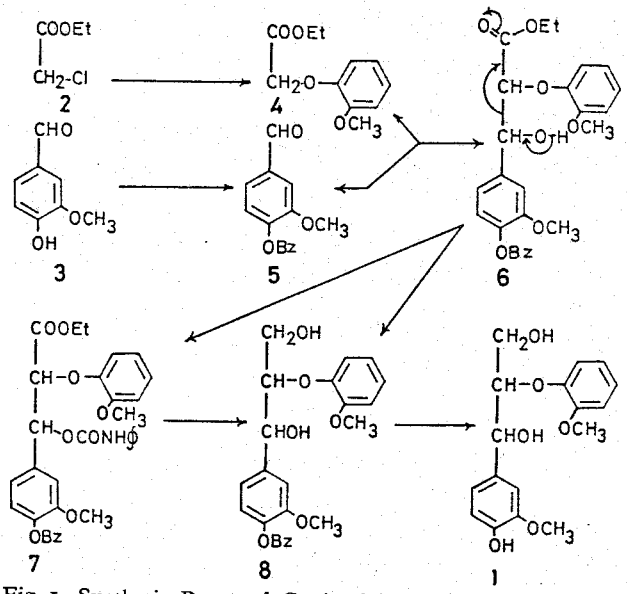


Fig. 1. Synthetic Route of Guaiacylglycerol-β-guaiacyl Ether

The compound (4) was synthesized in quantitative yield with stirring of ethyl chloroacetate (2) and guaiacol in acetone at room temperature in the presence of potassium iodide and potassium carbonate. In this case, the absence of porassium iodide decreased the reaction rate. The compound (4) which was determined by NMR and IR spectra was obtained pure as an oil by distillation under reduced pressure in ca. 70% yield. A singlet peak appeared at δ4.66 in NMR and the IR spectrum showed the presence of the carbonyl band at 1780 cm^{-1}.

It is assumed that even if the compound (6) could be synthe-

sized by the condensation of (4) and (5) under the drastic-condition, the compound would be converted immediately to an α,β - unsaturated ester. On the other hand, under the mild condition by the use of the same base, the condensation does not proceed. In fact, this was reported earlier by Freudenberg et al.[6] in a convergent synthesis of the β-hydroxy ester. That is, when the condensation was carried out at the reflux temperature of ether using sodium as the base, the α,β-unsaturated ester was obtained in high yield, while the β-hydroxy ester was obtained in a low yield at a low temperature (0°C). The low yield of the β-hydroxy ester suggests that the self-condensation of the α-phenoxy acetate proceeded under their reaction condition in addition to the conversion of the β-hydroxy ester to the α,β-unsaturated ester, although the reason for this low yield was not described in their paper. It was indicated in the present investigation that the enolate anion of the compound (4) was liable to self-condensation. Therefore, this reaction step should be carried out at low temperature. Furthermore, as the α-hydrogens of the ester are less acidic than those of aldehyde and ketone groups, by far the stronger bases should be used for synthesis of α-carbanion of ester.

In consideration of these facts, the condensation reaction which is the key step in this synthetic route must be carried out under the mild condition at very low temperature, and under such condition the carbanion of the compound (4) must be synthesized in high yield. Thus, it is assumed that lithium diisopropyl amide satisfies such conditions. Actually, Cregge et al.[7] reported the alkylation of α-position of the ester in a high yield using

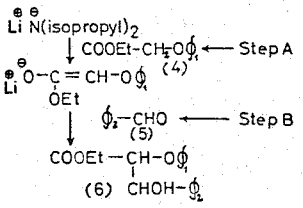
this reagent. But such an example as the condensation reaction between α-phenoxy ester and aldehyde has not so far been reported.

In the present investigation, the reaction sequence was considered to be divided into two steps, synthesis of carbanion (Step A) and condensation between the carbanion and the aldehyde (Step B) as shown in Table 1. The ratio of geometrical isomers

Table 1
Effect of Reaction Conditions on the Yield of Erythro and Threo Isomers

Condition	Solvent	Temp. (°C)		Yield %	erythro/ threo
		Step A	Step B		
I	Et_2O	—30	—70	30	0.8
II	Et_2O	—70	—70	70	0.8
III	THF	—70	—70	85	3.5
IV	THF	—70	—70	77	3.0
V	THF	—70	—70	90	3.5
VI	THF	—74	—74	95	3.5

Condition I—V: methyl lithium/ether solution was used as the base
Condition VI: n-butyl lithium/n-hexane solution was used as the base
Condition IV: 1.0 eq. of hexametapol was added
Condition V: 1.2 eq. of the compound (4) was added



in the reaction mixture and the yield of the β-hydroxy ester (6) from the aldehyde were determined by the NMR spectra. The α-protons of the β-hydroxy ester (6) and aldehydic proton of benzyl vanillin gave doublets at δ4.48(threo) and δ4.71(erythro) and a singlet at δ9.98, respectively, and these peaks did not interfere with the other peaks. The results were summarized in Tablel. Under the condition I-V, ether solution of methyl lithium was used, and n-hexane solution of n-butyl lithium was used under the condition VI. From these results, the following points are indicated.

First, when the step A was carried out at -30°C, the yield of the compound (6) was only 30% and the self-condensation product of the compound (4) was found, but no unreacted (4). The results indicated that the self-condensation of the compound (4) proceeded at such a temperature. In fact, it has been reported that by Cregge et al.[7] that the self-condensation of methyl acetate is found under -78°C, which is avoided by using t-butyl ester . Thus, the self-condensation is supposed to be avoided by using the ester with a large steric hindrance. However, this is not available for the present investigation, and then the reaction must be carried out under the temperature as low as possible. Thus, the temperature was kept at lower than -70°C, and the self-condensation was avoided.

Second, the ratio of threo and erythro isomers depended on the solvent used. The ratio (erythro/threo) was about 0.8 and 3.0 in ether and in tetrahydrofuran, respectively and value somewhat varied with changes in experimental condition. It seemed that the ratio was proportional to the yield of the β-hydroxy ester (6), and in tetrahydrofuran the increase in the yield of compound (6) paralleled that of the erythro isomer.

There is no definite explanation for this result at present. However, it seems that the results are due to the differences of the transition state of the condensation reaction in both solvents. In consideration of the experimental conditions described above, the reaction was carried out under the optimized condition, that is n-butyl lithium in hexane solution was used as base in tetrahydrofuran at -74°C. Under this condition, the mixture of erythro (75%) and threo (25%) isomers.in 95% yield could be obtained in

which only erythro isomer was crystallized in 51% yield. Since the mother liquor consisting of the mixture of isomers did not crystallize, the compound was converted to its carbamate (7) which easily crystallized from ether in 70% yield. Although the acetate and carboethoxy derivatives of the compound (6) were also prepared, these derivatives did not crystallize. At this step, about forty five grams of the β-hydroxy ester (6) was easily obtained by one reaction. The β-hydroxy ester (6) was reduced with lithium aluminum hydride to the compound (8) which was converted in almost quantitative yield to the final compound (1) by catalytic hydrogenation. The erythro isomer of the compound (1) was crystallized from ethyl acetate. Melting points of the compound (1) and its triacetate were 94-95°C and 107°C, respectively, which were identical with those obtained by Miksche et al.[7] The carbamate (7) was also treated as described above and the mixture of stereoisomers of the compound (1) was obtained in almost quantitative yield as a colorless foaming substance which gave one spot on TLC developed with 5%-methanol/chloroform. The overall yield of the final compound (1) from benzyl vanillin was about 72%.

II-1-3 Experimental

Benzyl vanillin (5) was prepared from vanillin and benzyl chloride in the presence of KOH in ethanol by the usual way and recrystallized from n-hexane/ethanol. Mp 60-61°C. Anal.Calcd. for $C_{15}H_{14}O_3$:C,74.36; H,5.83. Found: C,73.99; H,5.82.

Ethyl 2-methoxyphenoxy acetate (4)

Ethyl chloroacetate (122.56 g, 1M), guaiacol (137 g, 1.1M),

K_2CO_3 (300 g, 2.17 M) and KI (83 g, 0.5 M) were stirred in 1.6 l of acetone at room temperature for 170 hours. Inorganic salts were filtered and washed with acetone. The combined filtrate was evaporated *in vacuo* to dryness and the residue was partitioned with ether and saturated NaCl solution. The ether layer was washed with 10% KOH solution (100 ml x 3) and saturated NaCl solution containing dilute HCl successively and then the aqueous layer was neutralized. The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. A pale yellow oily substance (190 g, 90% yield) which gave a colorless oily substance by distillation under the reduced pressure (114°C/0.8 mmHg). IR(film); 1780 cm^{-1}. NMR δ(ppm: $CDCl_3$) 1.26 (3H, t, J=7.0), 3.86 (3H, s), 4.24 (2H, q, J=7.0), 4.66 (2H, s), 6.78-7.00 (3H, m). Anal. Calcd. for $C_{11}H_{14}O_4$: C, 62.84; H, 6.71. Found: C, 62.72; H, 6.97. UV λ_{max}^{EtOH} nm(log ε): 272(4.40).

β-Hydroxy ester (6)

n-Butyl lithium in *n*-hexane solution was obtained commercially (Merck, 20% solution) and titrated in the usual way[8] before using. Diisopropyl amine was dried over sodium metal and distilled. Tetrahydrofuran was refluxed with calcium chloride for 48 hours and distilled, and the distillate was redistilled from lithium aluminum hydride under nitrogen. Benzyl vanillin was dried over P_2O_5 for 2 days before using. All glasswares were dried at 110°C in oven for 2 days.

To the stirred solution which contained 16.3 ml (0.12 M) of diisopropyl amine in 100 ml of anhydrous THF, 62.5 ml (0.12 M) of *n*-butyl lithium hexane solution (1.92 N) was added at 4° C over a period of 15 min. under nitrogen gas, and the stirring

was continued for additional 30 min. at the same temperature. After cooling the reaction mixture to -74°C, 21 g (0.1 M) of ethyl 2-methoxyphenoxy acetate dissolved in 100 ml of anhydrous THF was added at the same temperature over a period of 2.5 hours. The reaction mixture became pale yellow color. The stirring was continued for additional 4 hours at -74°C, and the resulting pale yellow reaction mixture was transferred into a 2 l separatory funnel and 100 ml of saturated NaCl solution was added. After neutralization with 10% HCl solution, the reaction mixture was extracted with 500 ml of ether and the aqueous layer was then extracted twice with 40 ml of ether. The ether solution was combined and washed with 50 ml of saturated NaCl solution and dried over Na_2SO_4 and evaporated *in vacuo*. A pale yellow viscous oily substance (48 g) was obtained. After crystallization from *n*-hexane/ethanol, the product gave crystalline *erythro* isomer of β-hydroxy ester (23 g, 51%). The mother liquor was evaporated and applied onto silica gel column chromatography (silica gel 300 g, 5 x 4 cm) and eluted with $CHCl_3$. A pale yellow substance (20 g) of the mixture (erythro/threo=about 1:1) of the β-hydroxy ester which gave one spot on silica gel TLC developed with $CHCl_3$(44%) was obtained. This isomeric mixture was converted to its carbamate. The total yield of the β-hydroxy ester was 95%. Crystalline *erythro* isomer was recrystallized from *n*-hexane/ethanol.

Mp 80.5-81°C. *Anal*. Calcd. for $C_{26}H_{28}O_7$: C, 69.01; H, 6.24. Found: C, 68.84; H, 6.39. IR (KBr): 1765 cm^{-1}. NMR δ(ppm: $CDCl_3$) of *erythro* isomer: 1.11 (3H, t, J=7.0), 3.86 (3H, s), 3.92 (3H, s), 4.17 (2H, q, J=7.0), 4.71 (1H, d, J=5.3), 5.18 (2H, s), 5.16

(1H, d, J=5.3), 6.61-7.17 (7H, m), 7.22-7.50 (5H, m). Threo isomer: 0.99 (3H, t, J=7.0), 3.99 (2H, q, J=7.0), 4.48 (1H, d, J=7.0). MS (70 eV): 452 (0.07), 434 (0.06), 343 (0.14), 312 (0.3), 242 (8), 210 (24), 137 (22.6), 124 (8), 123 (10), 122 (8), 109 (7), 91 (100), 77 (10), 65 (8). UV λ_{max}^{EtOH} nm(log ε):276.5 (3.74).

Carbamate (7) of the isomeric β-hydroxy esters (6)

To a stirred solution of the isomeric β-hydroxy ester (20 g, 0.0449 M) dissolved in 50 ml of anhydrous pyridine, 8.02 g (0.0674 M) of phenylisocyanate was added and the mixture was heated at 100°C for 5 hours, and then anhydrous methanol (20 ml) was added and boiled for another 10 min. to destroy excess phenyl isocyanate. The reaction mixture was cooled and transferred into a I l separatory funnel and 300 ml of $CHCl_3$, 100 ml of saturated NaCl solution containing a small amount of $NaHCO_3$ and dried over Na_2SO_4 and evaporated in vacuo. A brown oily substance which gave a pale brown crystal (18 g, 70% yield) from ether was obtained. After recrystallization from ethanol it gave colorless needles. Mp 124-125°C. Anal. Calcd. for $C_{33}H_{33}O_8N$: C, 69.34; H, 5.82; N, 2.45. Found: C, 69.13; H, 5.74; N, 2.48. IR(KBr): 3446, 3386, 1752, 1745, 1733, 1603, 1555, 1540, 1517, 1268 cm^{-1}. NMR δ(ppm: $CDCl_3$) of erythro isomer: 1.16 (3H, t, J=7.0), 1.83 1.83(1H, broad s), 3.75(3H,s), 3.92(3H,s), 4.18(2H,q,J=7.0), 5.03(1H,d,J=6.2), 5.15(2H,s), 6.22(1H,d,J=6.2), 6.73-6.84(17H, m). Threo isomer: 1.16(3H,t,J=7.0), 1.83(1H, broad s), 3.73(3H, s), 3.89(3H,s), 4.18(2H,q,J=7.0), 4.86(1H,d,J=4.5), 5.13(2H,s), 6.28(1H,d,J=4.5), 6.73-6.84(17H,m).MS(70eV): 442(0.54), 435(14.2),

343(29.3), 312(23.7), 242(8.9), 210(34.1), 137(17.9), 123(14.3), 119(67.1), 109(10.7), 91(100), 77(13.2), 64(15), 44(15.7). UV λ_{max}^{EtOH}nm(log ε): 275(3.82).

Reduction of the compound (6) to the compound (1) with LAH and Pd-C/H_2

To a stirred solution containing 1.14g (0.03M) of LAH in 70 ml of anhydrous THF, 4.52g (0.01M) of the compound (6) dissolved in 30 ml of THF was added dropwise over a period of 50 min. at 50°C under nitrogen and the stirring was continued for additional 3 hours. The reaction mixture was cooled to 0°C and 3 ml of water dissolved in 16 ml of THF was added dropwise for decomposition of excess LAH. The lithium complex was decomposed by adding dry ice, and the resulting inorganic lithium salt was filtered and washed with ethyl acetate. The filtrate and the washings were combined and dried over Na_2SO_4, and the solvent was evaporated in vacuo. A colorless substance (4.2g) which gave one spot on the TLC plate developed with 5% methanol/chloroform was obtained. The colorless substance was dissolved in 60 ml of ethanol containing 1g of 5% Pd-C and stirred under hydrogen for 5.5 hours. After confirmation of disappearance of the starting material by TLC, palladium charcol was filtered off and washed with ethanol. The solvent was evaporated in vacuo to afford a colorless foaming substance (3.1 g) which gave the colorless crystal (2.8 g, 91% yield) from ethyl acetate. Mp 94-95°C (acetate: 107°C). Anal. Calcd. for $C_{17}H_{20}O_6$: C,63.74;H,6.29. Found: C,63.81;H,6.35. IR (KBr): 3600, 1600, 1518, 1285, 1265, 1128, 1020 cm^{-1}. NMR δ(ppm: $CDCl_3$): 3.45-3.90(2H,m), 3.88(6H,s), 3.99-4.28(1H,m), 4.97(1H,d, J=4.5), 6.75-7.00(7H,m). MS(70eV): 320(0.11), 272(0.9), 167(0.6),

153(10.7), 151(10.6), 150(100), 137(10), 125(26.8), 121(9.6), 109 (25.2), 93(10.07), 91(7.5), 82(9.6), 78(7.7), 65(8.1). UV λ_{max}^{EtOH} nm(log ε): 277(3.62).

Reduction of the carbamate (7) to the compound (1) with LAH and Pd-C/H_2

To a stirred solution containing 2.28 g (0.06M) of LAH dissolved in 140 ml of anhydrous THF, 10.58 g (0.02M) of the carbamate (7) dissolved in 70 ml of THF was added dropwise over a period of 90 min. at 50°C under nitrogen and the stirring was continued for additional 60 min. The reaction mixture was cooled to 0°C and 6ml of water dissolved in 32 ml of THF was added dropwise for decomposition of excess LAH. The aluminum complex was decomposed by adding dry ice, and the resulting inorganic lithium salt was filtered and washed with ethyl acetate. The filtrate and the washings were combined, washed with 10% HCl solution (50x2 ml) and brine (50x2 ml), and then dried over Na_2SO_4. The solvent was evaporated in vacuo. A colorless substance (7.5 g) which gave one spot on the TLC plate developed with 5% methanol/chloroform was obtained. The colorless substance was dissolved in 100 ml of ethanol containing 2 g of 5% Pd-C and stirred under hydrogen for 5 hours. After confirmation of disappearance of the starting material by TLC, palladium charcol was filtered off and washed with ethanol and the solvent was evaporated in vacuo to give a colorless foaming substance, the compound (1)(5.8 g, 90.6%) which was the mixture consisting of erythro and threo isomers. NMRδ(ppm;$CDCl_3$) of triacetyl erythro isomer: 2.02(3H,s), 2.10(3H, s), 2.30(6H,s), 3.80(3H,s), 3.83(3H,s), 4.00-4.50(2H,m), 4.50-4.80

6.12(1H,d,J=5.0), 6.70-7.15(7H,m). Threo isomer: 1.99(3H,s), 2.06 (3H,s), 2.30(3H,s), 3.83(6H,s), 3.90-4.40(2H,m), 4.50-4.85(1H,m), 6.17(1H,d,J=6.2), 6.70-7.15(7H,m).

II-1-3 Summary

Guaiacylglycerol-β-guaiacyl ether (1), the model compound of arylglycerol-β-aryl ether structure in lignin was synthesized in high yield through five reaction steps from vanillin. The key step of this synthetic method was the condensation reaction between ethyl 2-methoxyphenoxy acetate (4) and benzyl vanillin (5). At this step, lithium diisopropyl amide was used as the base, and β-hydroxy ester (6) was obtained in 95% yield as an oily substance consisted of two isomers, from which only erythro isomer was obtained as crystal in 51% yield. The residual oily substance was converted to its carbamate (7) and crystallized in 70% yield. The crystalline β-hydroxy ester (6) and the carbamate (7) were then converted to the final compound (1) by the lithium aluminum hydride reduction and subsequent hydrogenation with Pd-C/H_2. The overall yield of the guaiacylglycerol-β-guaiacyl ether (1) from benzyl vanillin was about 72%.

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II-2 Syntheses of guaiacylglycerol-β-coniferyl and coniferyl aldehyde ethers

II-2-1 Introduction

The arylglycerol-β-aryl ether substructure is the most important in lignins. It has been reported that 30-50% or more of the phenylpropane units are found in these structures[1,2]. For this reason, guaiacylglycerol-β-guaiacyl ether has been used as a lignin model compound for studying various lignin reactions, such as in pulping, in chemical utilization and in biodegradation. However, this compound is not truly representative of the lignin structure, because the β-aryl ether residues in lignins contain C_3-side chains. To study the effect of chemical changes on functioanl groups in the side chains of β-aryl ether substructure, it is desirable to use structural models containing allyl alcoholic or allyl aldehyde type side chains, which do occur in lignins. Guaiacylglycerol-β-coniferyl (5) and β-coniferyl aldehyde (4) ethers, therefore, are suitable model compounds. These compounds (4) and (5) have been isolated in low yield as lignin hydrolysis products[3,4], and as products formed by the oxidative coupling of coniferyl alcohol[5,6]. However, the separation and purification of the two ethers are difficult because many other products

are formed in both hydrolysis and dehydrogenation, and compounds are obtained as a mixture of erythro and threo isomers which cannot be purified by crystallization.

In this Section, one in a series of synthetic studies of lignin model compounds, is described the novel synthetic method for preparing guaiacylglycerol-β-coniferyl (5) and β-coniferyl aldehyde (4) ethers.

II-2-2 A new high yield syntheses of the compounds

The synthetic method for the target ethers is analogous to that used to prepare guaiacylglycerol-β-guaiacyl ether[7] described in Section II-1. For the present syntheses (Fig.1), coniferyl

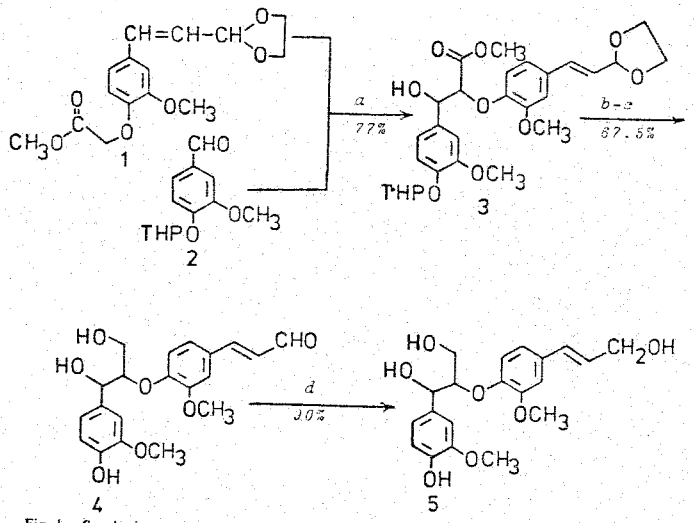

Fig. 1. Synthetic route of guaiacylglycerol-β-coniferyl (5) and β-coniferyl aldehyde (4) ethers.
[a]Lithium diisopropyl amide/THF/−78°C [b]$LiAlH_4$/THF/50°C [c]1N-HCl/THF/0°C
[d]$NaBH_4$/MeOH/0°C

aldehyde is used as the starting materials instead of guaiacol.

The condensation of compound (1) with (2) by the use of lithium diisopropyl amide (LDA) gives the expected compound (3), with the small amounts of the starting materials and polar impurities. Purification by silica gel TLC (PF-254 Merck), developed with

ethyl acetate/n-hexane (1:1), gives the pure compound (3). The use of silica gel chromatography (Wako gel C-100) for large-scale preparation, resulted in a partial deacetalization. Thus, the purification is carried out after the subsequent LAH reduction or at the stage of compound (4). The structure of compound (3) is substantiated by IR, which shows the absorption of the ester group at 1760 cm^{-1}, and by NMR spectra. The compound (3) is a mixture consisting of the erythro and threo isomers; the ratio was found to be about 3.5 : 1.0 by the NMR spectrum, which shows clearly distinguishable peaks of the ester protons at δ3.55(s, threo) and δ3.68(s, erythro) and of the β-methine proton at δ4.48(d,J=6.0, threo) and δ4.66(d,J=5.3, erythro). The erythro isomer might be expected to predominate from the reaction mechanism involving a six-membered transition state intermediate.

Compound (3) was subjected sequentially to lithium aluminum hydride reduction in THF at 50°C (75%), and to hydrolysis with 1N-HCl /THF (1 : 2) at 0°C (90%) to afford the expected compound (4). The yield of compound (4) from the starting material (1) is about 52%. Compound (4) was converted to the final compound (5) by sodium borohydride reduction in methanol at 0°C (90%). The structures of compound (4) and (5) are supported by UV,IR,MS and NMR spectra, as described in the Experimental section. The NMR spectra of the acetyl derivatives are shown in Figs.2 and 3. It is noteworthy that the peaks of the α-methine, γ-methylene and α,γ -alcoholic acetyl are clearly distinguishable between the erythro and threo isomers in the NMR spectra. The assignment of these protons is based on the presumed reaction mechanism, which gives predominantly the erythro isomer, and also by comparison with the NMR spectra of compound consisting of erythro/threo

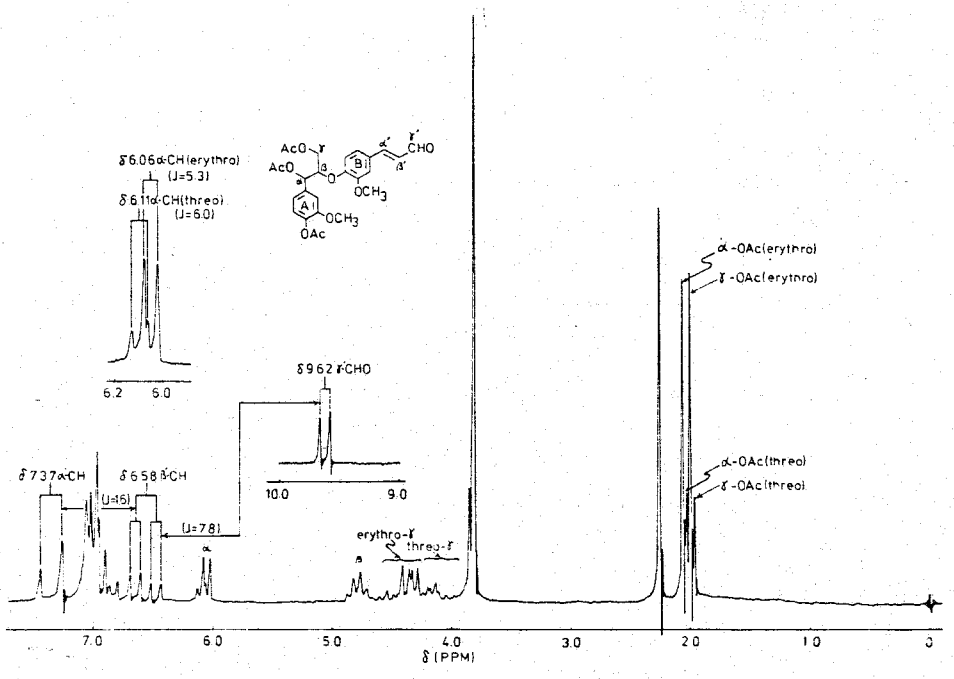

Fig. 2. NMR spectrum of acetylated guaiacylglycerol-β-coniferyl aldehyde ether (4)

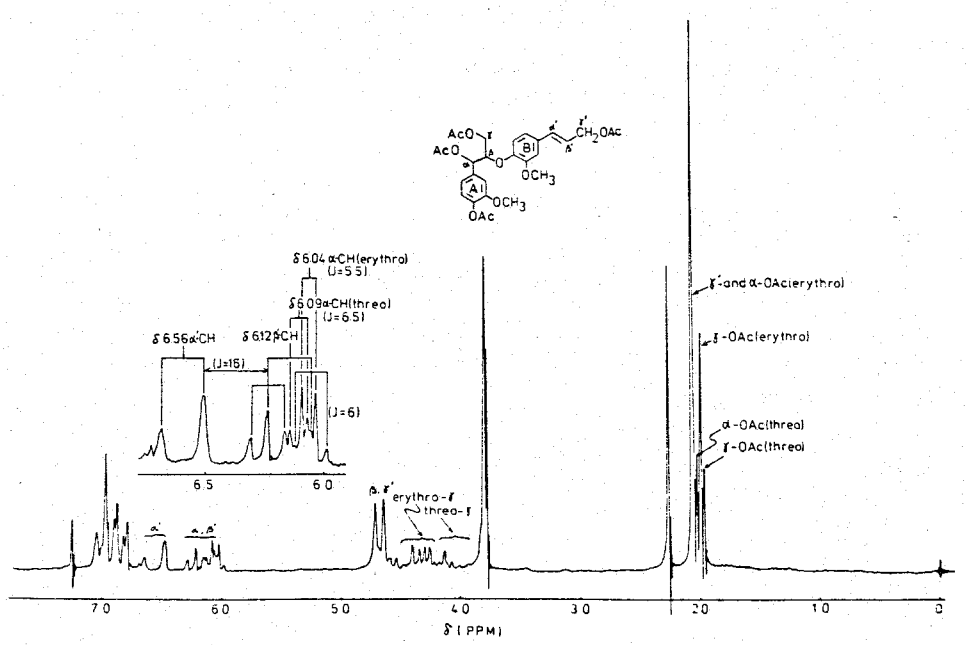

Fig. 3. NMR spectrum of acetylated guaiacylglycerol-β-coniferyl ether (5)

(about 1.0 : 1.1 ratio) obtained by the oxidative coupling of coniferyl alcohol. α-Methine protons in erythro isomers, having a smaller coupling constant, appear at higher magnetic fields than in threo isomers. γ-Methylene and also γ-alcoholic acetyl protons of erythro isomers appear at lower magnetic fields than these in the threo isomers.

II-2-3 Experimental

Compounds (1) and (2)

These compounds were prepared by the method reported previously[8,9], and stored in a refrigerator to avoid partial decomposition of the acetals at room temperature.

Compound (3)

To a stirred solution of lithium diisopropyl amide (5.6mM) in 20 ml of anhydrous THF, 1.37 g (4.66mM) of compound (1) in 15 ml of THF was added dropwise over a period of about 40 min. at -78°C under nitrogen. The reaction mixture gradually turned to orange-red color. The mixture was stirred for an additional 30 min., and 1.1 g (4.66mM) of compound (2) in 15 ml of THF was added dropwise over a period of 30 min. at the same temperature. The orange-red color of the solution gradually disappeared during the addition of compound (2) and finally turned to pale yellow. After stirring for an additional 30 min., the reaction mixture was neutralized by the addition of finely-powdered dry-ice. The solution was partitioned between ethyl acetate and brine. The ethyl acetate layer was washed with brine, dried over Na_2SO_4 and evaporated *in vacuo* to give a yellow-colored oil (2.5 g) which was used for the subsequent LAH reduction without purification. An aliquot of the product obtained above was purified by silica gel TLC developed with ethyl acetate/*n*-hexane (1 : 1) for the NMR and IR measurements.

IR(CH_2Cl_2): 1760 cm^{-1}. NMR δ(ppm:$CDCl_3$): 1.4-2.0(6H,m,-CH_2- of THP), 3.4-3.7(2H,m,-CH_2-O- of THP), 3.55(s, *threo*) and 3.63

(s, erythro)(3H, two singlets, CH_3- of methyl ester), 3.79 and 3.80(6H, two singlets, CH_3- of methoxyl), 3.7-4.1(4H,m, $-CH_2$- of acetal), 4.48(d,J=6.0, threo) and 4.66(d,J=5.3, erythro)(1H, tow doublets, β-CH-), 5.0-5.2(1H,m, α-CH-), 5.25-5,45(1H,m,-O-CH-O- of THP), 5.32(1H,d,J=5.7, -O-CH-O- of acetal), 5.59(1H,dd, J=15.5, 5.7, β'=CH-), 6.59(1H,d,J=15.5, α'-CH=), 6.6-7.1(6H,m, aromatic).

Compound (4)

To a stirred solution of LAH (497.8 mg, 13.1mM) suspended in 20 ml of anhydrous THF, 2.3 g of the crude yellow oil obtained above in 20 ml of THF was added dropwise over a period of 50 min. at 50°C under nitrogen. After stirring for an additional 30 min., the reaction mixture was cooled to 0°C and about 1 ml of water in 5 ml of THF was added dropwise to the solution to decompose the excess hydride. After the addition of dry-ice to the solution, the reaction mixture was partitioned between ethyl acetate and water. The aqueous layer was extracted twice with ethyl acetate. The combined ethyl acetate solution was washed with brine, dried over Na_2SO_4 and evaporated in vacuo to give a slightly yellow glass (2.3 g). An aliquot of the product was purified by silica gel TLC developed twice with ethyl acetate/n-hexane (3 : 2) for NMR analysis.

NMR δ(ppm:$CDCl_3$):1.9-2.0(6H,m,-CH_2- of THP), 3.4-3.7(2H,m, -CH_2-O- of THP), 3.78(6H,s, CH_3- of methoxyl), 3.80-4.10(4H,m, -CH_2-O- of acetal), 4.80-5.00(1H,m, α-CH), 5.2-5.4(1H,m, -O-CH-O- of THP), 5.32(1H,d,J=6, -O-CH-O- of acetal), 5.96(1H,dd,J=16, 6, β'=CH-), 6.60(1H,d,J=16, α'-CH=), 6.15-7.10(6H,m, aromatic).

The product obtained above was purified on a silica gel column (Wakogel C-100, 40 g, 2.6x10 cm) eluted with ethyl acetate/n-hexane (1 : 1) to give the expected diol product (1.36 g, 58% overall yield from compound (1)) containing some partly hydrolyzed aldehyde; this mixture was used for the subsequent reaction as follows.

To a stirred solution of the diol derivative (1.36 g, 2.71 mM) obtained above in 15 ml of THF, 7.5 ml of 1N-HCl was added dropwise at 0°C under nitrogen. After stirring for 2 hours, the solution was partitioned between ethyl acetate and brine. The ethyl acetate solution was washed with brine until the washings became neutral, and then dried over Na_2SO_4. Evaporation of the solvent *in vacuo* afforded a yellow glass which was purified by TLC developed twice with 3% methanol/methylene chloride to give the expected compound (4), pure by TLC, as a slightly yellow glass (913 mg, 90%).

UV λ_{max}^{MeOH}nm(log ε): 230(4.14), 336.5(4.27). IR(KBr): 3500, 1665, 1625, 1595, 1520, 1510, 1465, 1450, 1425, 1275, 1220, 1130, 1020, 965. NMR δ(ppm:d_6-acetone): 3.40-3.80(2H,m,$-CH_2-$), 3.82(3H,s,CH_3O- of A-ring), 3.88(*erythro*) and 3.92(*threo*)(3H,two singlets, CH_3O- of B-ring), 4.51(1H, broad q,J=4.5, β-CH), 4.91(1H, broad d,J=5.5, α-CH), 6.60(dd,J=16, 7.5, *erythro*) and 6.62(dd, J=16, 7.5, *threo*)(1H, β'=CH-), 6.63-7.35(6H,m,aromatic), 7.50(*erythro*) and 7.52(threo)(1H, two doublets, J=16, α'-CH=), 9.57(d,J=7.5, *erythro*) and 9.58(d,J=7.5, *threo*)(1H, γ'-CHO). MS(70eV): 374(M^+, 0.9), 356(4.2), 338(4.2), 326(33.2), 297(7.3), 265(7.3), 237(3.9), 204(48.8), 178(100), 161(29.3), 151(38), 147(38), 137(61), 135(46.3), 124(23.4), 119(24.4), 107(41), 91(35.6), 89(32.7), 77(52.7), 65(34.1), 51(40.5).

Compound (5)

To a stirred solution of compound (4) (194.2 mg, 0.52mM) in 4 ml of methanol, 9.5 mg (0.26mM) of $NaBH_4$ was added at 0°C under nitrogen. After 30 min., the product was partitioned between ethyl acetate and brine. The ethyl acetate solution was dried over Na_2SO_4 and evaporated *in vacuo* to give a colorless glass which was purified by TLC developed with 5% methanol/methylene chloride to afford the pure compound (5) (176 mg, 90%) as a colorless glass.

UV λ_{max}^{MeOH}nm(log ε): 265.6(4.22). IR(KBr): 2500, 1605, 1520, 1270, 1155, 1130, 1085, 1028, 965, 855 cm^{-1}. NMR δ(ppm:d_6-acetone): 3.82(3H,s, CH_3O- of A-ring), 3.85(CH_3O- of *erythro* B-ring), and 3.89(CH_3O- of *threo* B-ring)(3H, two singlets), 4.23(2H,broad d, J=5.0, γ'-CH_2, in one drop of D_2O), 4.43(1H,q,J=5, β-CH, in one drop of D_2O), 4.94(1H, groad d,J=5, α-CH, in one drop of D_2O), 6.23(*erythro*) and 6.24(*threo*)(1H, two dt,J=16, 5.0, β'=CH-), 6.53 (1H, broad d, J=16, α'-CH=), 6.65-7.15(6H,m, aromatic). MS(70eV): 376(M^+, 3.7), 358(3.7), 340(1.8), 328(12.9), 326(5.2), 206(48), 180(60), 162(23), 151(37), 137(100), 131(25), 124(48), 119(38), 91(49), 77(33), 65(33).

Acetates of compounds (4) and (5)

About 50 mg of compound (4) or (5) dissolved in 2 ml of THF was treated with 2 ml of acetic anhydride/pyridine(1:1) overnight at room temperature. The reaction mixture was evaporated *in vacuo* and acetic anhydride/pyridine were removed azeotropically by evaporation with benzene. The products were purified by TLC (ethyl acetate/n-hexane=1:1) for the NMR measurements.

NMR spectrum of acetyl compound (4)(Fig.2) δ(ppm:$CDCl_3$): 1.98(threo) and 2.02(erythro) (3H, two singlets, γ'-OAc), 2.04(threo) and 2.09(erythro) (3H, two singlets, α'-OAc), 2.26(3H, phenolic-OAc), 3.83(CH_3O- of A-ring and erythro B-ring) and 3.87 (CH_3O- of threo B-ring)(6H, two singlets), about 3.95-4.25(threo), and 4.26-4.70(erythro)(2H,m, γ-CH_2), 4.80(1H, broad q, J=5, β-CH), 6.06(d,J=5.3, erythro) and 6.11(d,J=6.0, threo)(1H,α-CH), 6.58(1H,dd,J=16, 7.8, β'=CH-), 6.78-7.20(6H,m, aromatic), 7.37(1H,d, J=16, α'-CH=), 9.62(1H,d,J=7.8, γ'-CHO).

NMR spectrum of acetyl compound (5)(Fig.3) δ(ppm:$CDCl_3$): 1.96(threo) and 2.00(erythro)(3H, two singlets, γ-OAc), 2.02(threo, -OAc) and 2.08(erythro,α-OAc and γ'-OAc)(6H, two singlets, methoxyl), about 3.9-4.2(threo) and 4.2-4.6(erythro)(2H,m, γ-CH_2), 4.5-4.8(iH,m,β-CH), 4.68(2H, broad d,J=6, γ'-CH_2), 6.04(d,J=5.5, erythro) and 6.09(d,J=6.5, threo)(1H, two doublets, α-CH), 6.12 (1H, dt,J=16, 6, β'=CH-), 6.56(1H, broad d J=16, α'-CH=), 7.65-7.10(6H,m, aromatic).

II-2-4 Summary

A new synthetic method for the preparation of guaiacylglycerol-β-coniferyl and β-coniferyl aldehyde ethers, representing the most important lignin substructure is described as one in a series of synthetic studies of lignin model compounds.

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II-3 Syntheses of 1,2-diarylpropane-1,3-diols and determination of their configurations

II-3-1 Introduction

1,2-Bis-(4-hydroxy-3-methoxyphenyl)-1,3-diol which is one of the typical structural mode in lignin was synthesized by Lundquist et al[1]. The key step of their synthetic method is benzoin condensation of benzyl vanillins, γ-hydroxymethyl group is derived by aldol condensation with formaldehyde, and the 1,3-diol compound is obtained as a colorless foaming substance consisting of threo and erythro isomers. However, it is difficult to synthesize the 1,3-diol compounds containing different aryl groups by this method. Although the syntheses of many asymmetric benzoins were reported[2], it has not been known whether the condensation reaction between each of vanillin, syringaldehyde and p-hydroxybenzaldehyde occurs or not. If the reaction would occur, it is

still unknown whether the only desired asymmetric benzoin of the possible benzoins is synthesized and isolable.
Therefore, their synthetic method does not seem to be a general one for the synthesis of 1,2-diarylpropane-1,3-diols. Since some 1,2-diarylpropane-1,3-diol compounds containing different aryl groups have been isolated[3], a new synthetic method which is applied for any combination between different aryl groups should be required for identification and studies of their chemical properties.

In this Section, the synthesis of 1-(4-hydroxy-3,5-dimethoxyphenyl)-2-(4-hydroxy-3-methoxyphenyl)-propane-1,3-diol(1) by general synthetic method of 1,2-diarylpropane-1,3-diol is described.

II-3-2 A new general sythetic method of the compound

The key step of this synthetic method is the formation of α,β -carbon-carbon bond by condensation of methyl benzylhomovanillate (3) with benzyl syringaldehyde (4). Methyl benzylhomovanillate (3) was synthesized in over 80% yield by oxidative rearrangement, treating benzyl vanilloylmethylketone (2) with thallium (III) nitrate and 70% perchloric acid in methanol at room-temperature. This synthetic method reported by Mckillop et al.[4],

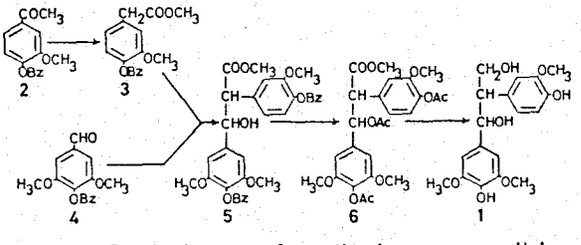

Fig. 1. Synthetic route of 1,2-diarylpropane-1,3-diol

is superior to the method reported earlier[5]. However, the method can not be employed for syringyl analogue, because by the Friedel-Crafts reaction between methyl ketonium ion and 2,6-dimethoxyphenol, 3-acetyl-2,6-dimethoxyphenol is mainly obtained instead of expected methyl ketone derivative. p-Hydroxyphenyl analogue was also synthesized by this method. β-Hydroxy ester (5) was synthesized from the homoacid ester (3) in about 70% yield using lithium diisopropyl amide as base which was also used on the synthesis of guaiacylglycerol-β-guaiacyl ether, the model compound of arylglycerol-β-aryl ether in lignin as described in Section II-1[6]. The reaction was carried out in anhydrous THF below -70°C and unreacted starting compounds were removed by silica gel column chromatography with chloroform as eluent. The eluate gave colorless needles in about 18% yield (5a, erythro isomer, Rf=0.18, developed with ether/n-hexane=1:1 on silica gel plate) and colorless prisms from the mother liquor in about 54% yield (5b, threo isomer, Rf=0.09, developed with the same solvent and plate). The ratio of the amounts of (5a) and (5b) is about 1:3 which obtained on the synthesis of guaiacylglycerol-β-guaiacyl ether as described in Section II-1[6]. Thus, the condensation reactions on the syntheses of both guaiacylglycerol-β-guaiacyl ether and 1,2-diarylpropane-1,3-diol (1) in THF below -70°C using lithium diisopropyl amide as base, seem to assume the six-membered ring transition states including lithium cation, in which α-phenyl groups and β-phenyl (or phenoxy) groups take the trans and cis configurations with ratio of about 3:1 due to the steric hindrance.

The β-hydroxy esters (5a) and (5b) were converted to the final compounds (1a) and (1b) by subsequent experiments described

below. Preliminarily, the compound (5a) was reduced with lithium aluminum hydride and hydrogenated with 5% Pd-C and hydrogen, but the desired end product (1a) could not be obtained because of the formation of by-products on the catalytic hydrogenation in ethanol. Therefore,the synthesis of the diol (1) was carried out along the route shown in Fig.1. The compounds (5a) and (5b) were hydrogenated with 5% Pd-C and hydrogen in dioxane/ethanol (1:1) and acetylated subsequently with acetic anhydride and pyridine, and triacetyl esters (6a) and (6b) were obtained in over 95% yield, respectively. The triacetyl esters were then converted to the 1,2-diarylpropane-1,3-diols,(1a, erythro isomer) and (1b, threo isomer) in about 80% yield, by reduction with LAH in THF at 50°C. The structures of these compounds were determined by NMR, MS, IR, UV spectra and elementary analysis.

The NMR spectra of the tetraacetates of these erythro (1a) and threo (1b) compounds are shown in Fig.2 and 3, respectively. Each peak was determined by the decoupling method, and the results are visualized in the same Figs. The aromatic protons of both the compounds give sufficiently separated peaks, and these spectra show the same pattern except for the difference of the acetyl proton region (at δ1.90 to δ2.30). That is, the peak of α-acetoxyl group of the acetyl compound of (1a) gives almost the same chemical shift to that of γ-acetoxyl group, and therefore, the acetoxyl protons of erythro isomer (Fig.2) gives almost two peaks, while threo isomer (Fig.3) three peaks. From these characteristics, three 1,2-diarylpropane-1,3-diols reported by Nimz[3] were determined to be all erythro isomers.

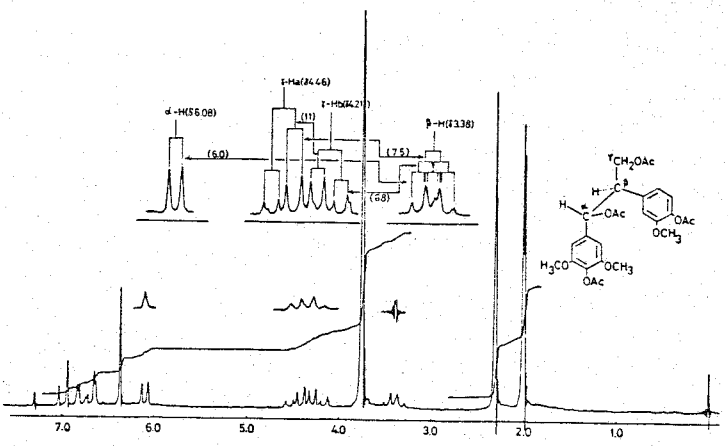

Fig. 2. NMR spectrum of tetraacetate of erythro-1,2-diaryl-propane-1,3-diol (1a)

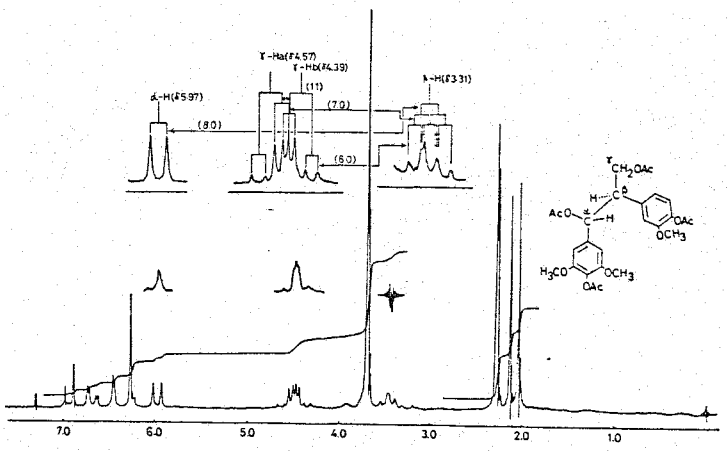

Fig. 3. NMR spectrum of tetraacetate of threo-1,2-diaryl-propane-1,3-diol (1b)

II-3-3 Determination of the configuration by NMR spectrometry

Since guaiacylglycerol-β-guaiacyl ether (7) and 1,3-propanediol (1) have a same 1,3-dihydroxy structure, the reagent which reacts with the 1,3-dihydroxyl groups of the β-aryl ether (7) is supposed to react with those of the compound (1). Thus, the configurations of these derivatives might be determined by the same method. The configuration of the β-aryl ether (7) was determined by NMR spectrometry first. Guaiacylglycerol-β-guaiacyl ether (7) was synthesized through long tedious reaction steps by Adler and Eriksoo[7], Kratzl et al.[8] and Miksche et al.[9] The high yield

synthesis of this compound by a convergent route with only five reaction steps was described in Section II-1. The configuration of the compound (7) was determined by Gierer et al.[10] based on the fact that the borate complex of the threo isomer moved faster than that of the erythro isomer in paper electrophoresis.

It seems that if conformation of the compound (7) is fixed through the six-membered ring structure by cyclic ester formation between the 1,3-dihydroxyl groups of the compound (7) and phenyl boronic acid, its configuration and also conformation will be determined by NMR spectrometry. The phenylboronate (8) could be synthesized in quantitative yield by refluxing equimolar amounts of the compound (7) and phenylboronic acid in benzene for 4 hours without any acidic catalyst. The phenylboronate (8) thus obtained was stable and purified by preparative TLC, and the phenylboronate group could be removed easily by treating the ester with 1,3-propanediol in a suitable solvent at room temperature. Moreover, the phenylboronate obtained from a mixture of erythro (7a) and threo (7b) isomers which could not be separated on the silica gel TLC plate developed with various solvent systems, gave separable spots on the silica gel plate developed with 1.5% methanol/chloroform, and each isomer could be obtained as pure substance, respectively.

NMR spectra of these phenylboronates (8a) and (8b) are shown in Figs. 4 and 5. On NMR analysis, the following two points have to be noted. These six-membered ring compounds may prefer the stable chair forms, and then the coupling constant (J) between β- and γ-axial protons may be almost 10 cps ($\nu\beta H, \gamma\text{-Ha} = 180^{\circ}$) when the β-proton takes axial orientation.

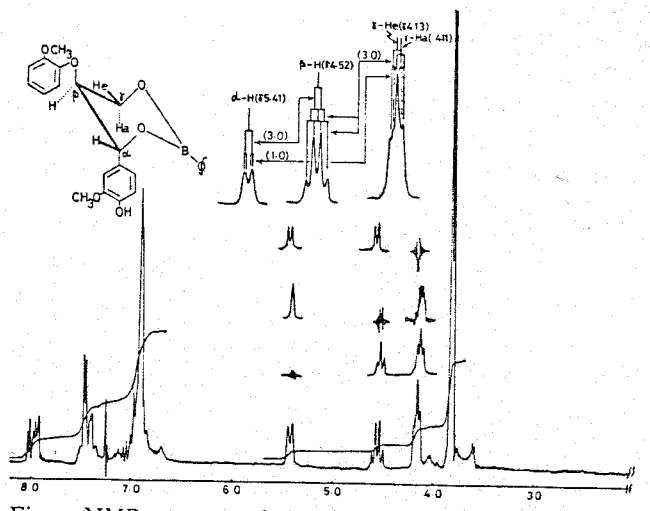

Fig. 4. NMR spectrum of phenylboronate of erythro-guaiacyl-glycerol-β-guaiacyl ether (8a)

Respective peaks of the phenylboronate (8a) were assigned by the decoupling method as shown in Fig.4.

The β-proton gives a quartet at δ4.52 whose coupling constants are JβH,αH=JβH,γHe=JβH,γHa=3.0 cps, indicating equatorial orientation. Furthermore, the quartet peak of the β-protone changed to triplet, and in addition γ-proton peak gave surprisingly a triplet when a doublet peak of α-proton, at δ5.41, was irradiated. Such results indicated the occurrence of a long range coupling between α- and γ-equatorial protons. Thus, the equatorial orient-

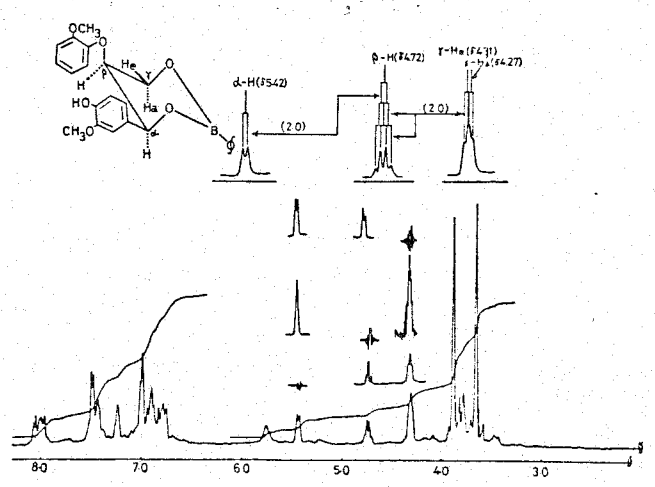

Fig. 5. NMR spectrum of phenylboronate of threo-guaiacyl-glycerol-β-guaiacyl ether (8b)

ation of α-proton was determined, and the erythro configuration and its conformation of the compound (8a) were determined as shown in Fig.4. NMR spectrum of the compound (8b) gave the same pattern as that of the compound (8a). But a long range coupling between α- and γ-He protons was not found in this case suggesting the axial orientation of α-proton. Furthermore, the small coupling constants, $J_{\beta H,\alpha H}=J_{\beta H,\gamma Ha}=J_{\beta H,\gamma He}=2.0$ suggest equatorial orientation of the β-proton. Such configuration is also supported by the high-field shift of one of the methoxyl groups, which is attributable to the β-orientations of both α-phenyl and β-phenoxy groups. Therefore, the compound (8b) is unequivocally threo isomer and takes the conformation shown in Fig.5. Thus, the configuration of guaiacylglycerol-β-guaiacyl ether (7) was determined by NMR spectrometry of the only one phenylboronate isomer,

Similarly, the stereochemistry of the 1,2-diarylpropane-1,3-diol (1) is discussed. The phenylboronates of the compound (1a) and (1b) were synthesized by the same method, and these NMR spectra are shown in Figs. 6 and 7, respectively. Both NMR spectra did not show any long range coupling between α- and γ-He protons reflecting the axially oriented α-protons. Large coupling constans, $J_{\beta H,\alpha H}=J_{\beta H,\gamma Ha}=10$ cps on the NMR spectrum of the compound (9b) shown in Fig.7, suggests that the dihedral angles between these protons are 180°, respectively, corresponding trans configuration, and then the low-field shift of the γ-Ha peak is attributable to the deshielding effect by the β-equatorial phenyl group. These results indicate that the compound (9b) is threo isomer, and takes the conformation shown in Fig.7. By contrast, a small coupling constant between α- and β-protons of the com-

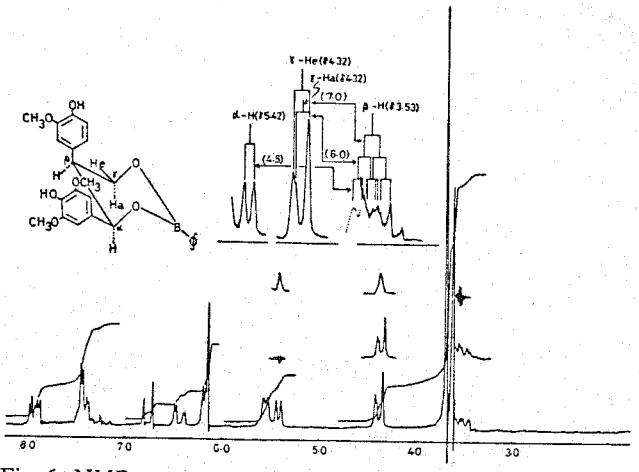

Fig. 6. NMR spectrum of phenylboronate of erythro-1,2-diarylpropane-1,3-diol (9a)

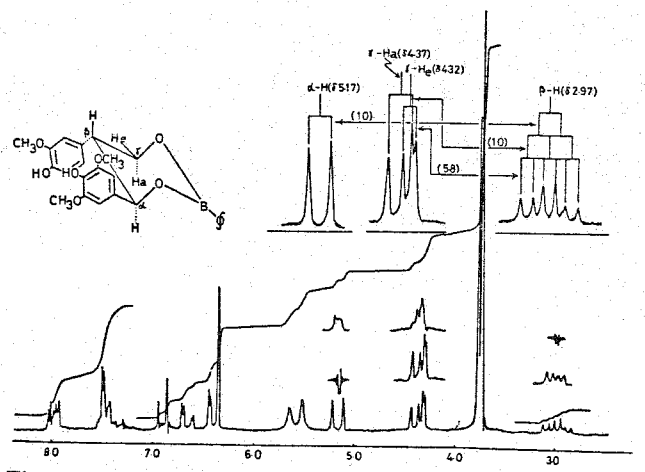

Fig. 7. NMR spectrum of threo-1,2-diarylpropane-1,3-diol (9b)

pound (9a), 4.3 cps shown in Fig.6, corresponds to about 45° of the dihedral angle reflecting axial α-proton and equatorial β-proton. Consequently, the configuration of the compound (1a) was determined to be erythro.

II-3-4 Experimental

Benzyl vanilloylmethyl ketone (2) was prepared by the benzylation of vanilloylmethyl ketone which was synthesized by the method reported earlier.[11] Mp 86°C (Lit.[12] 86-86.5°C).

Benzyl p-hydroxyacetophenone was prepared by the benzyl-

ation of commercially obtained p-hydroxyacetophenone. Mp 90.5-91°C.

Benzylsyringaldehyde was prepared by the benzylation of commercially available syringaldehyde. Mp 62.5°C.

Synthesis of homoacid methyl ester by oxidative rearrangement with thallium (III) nitrate

Methyl benzylhomovanillate (3) and methyl p-hydroxyphenyl-homobenzoate were synthesized by the modified method reported by McKillop et al.[4] The methyl ketone derivative (0.01M) was added to a solution of 0.01M of thallium (III) nitrate[13] in 25 ml of methanol containing 5 ml of 70% perchloric acid, and the mixture was stirred at room temperature for 4 hours. After confirmation of the disapperance of the starting methyl ketones by TLC, the methanol was evaporated in vacuo, and the residue was partitioned between $CHCl_3$ and brine. The chloroform solution was washed with a saturated $NaHCO_3$ solution and brine, successively and then dried over Na_2SO_4, and the solvent was evaporated in vacuo. A pale brown oily substance was purified with silica gel column chromatography (1.5x9 cm) eluted with ether/n-hexane(2:1), the eluent was evaporated in vacuo, and the crude crystal was re-crystallized from ethanol.

Methyl benzylhomovanillate (3): Mp 64.5-65°C. Anal. Calcd. for $C_{17}H_{18}O_4$: C,71.31;H,6.34. Found: C,71.33;H,6.45. IR(KBr): 1765 cm^{-1} NMR δ(ppm:$CDCl_3$): 3.43(2H,s), 3.62(3H,s), 3.82(3H,s), 4.99(2H,s), 6.55-6.85(3H,m), 7.10-7.50(5H,m). UV λ_{max}^{EtOH} nm(logε):275 (3.32), 282(3.27).

Methyl benzyl-p-hydroxyhomobenzoate: Mp 56-57.5. Anal. Calcd. for $C_{16}H_{16}O_3$:C,74.98;H,6.29. Found:C,74.44;H,6.39. IR(KBr):1744 cm^{-1}

NMR δ(ppm:$CDCl_3$): 3.42(2H,s), 3.58(3H,s), 4.98(2H,s), 6.83(2H, d,J=8.0), 7.13(2H,d,J=8.0), 7.10-7.40(5H,m). UV λ_{max}^{EtOH}nm(log ε): 275(3.32), 282(3.27).

β-Hydroxy ester (5)

A commercial n-butyl lithium in n-hexane (Merck 20% solution) was titrated in the usual way[14] before use. Diisopropyl amine was dried over sodium metal and distilled. Tetrahydrofuran was distilled from potassium metal and benzophenone. Methyl benzylhomovanillate (3) and benzyl syringaldehyde (4) were dried in a vacuum desiccator containing P_2O_5 for 2 days.

To a stirred solution which contained 4.13 ml (0.03M) of diisopropyl amine in 100 ml of THF, 15.63 ml (0.03M) of n-butyl lithium in n-hexane (1.92N) was added at 4°C over a period of 15 min. under nitrogen, and the stirring was continued for additional 30 min. at the same temperature. After cooling the reaction mixture to -73°C, 5.72g (0.02M) of methyl benzylhomovanillate (3) dissolved in 50 ml of THF was added dropwise over a period of 1 hour, and then 5.44g (0.02M) of benzyl syringaldehyde dissolved in 50 ml of THF was added dropwise at the same temperature over a period of 1.5 hour. The stirring was continued for additional 5 hours at -74°C, and the resulting pale yellow reaction mixture was transferred into a 1000 ml of separating funnel and 100 ml of brine was added. After neutralization with a 10% HCl solution, the reaction mixture was extracted with 200 ml of ether and the aqueous layer was then extracted twice with 50 ml of ether. The ether solution was combined and washed with 50 ml of brine and dried over Na_2SO_4 and evaporated in vacuo. A pale yellow

viscous substance (12.6g) was obtained. The products were purified with silica gel column chromatography (Silica gel 300g, 5x33cm) eluted with $CHCl_3$, and colorless crystals of the erythro compound (5a, 2g, 18%) and the threo compound (5b, 6g, 54% yield) were obtained in 72% total yield, respectively. These compounds (5a) and (5b) which were recrystallized from ethanol gave colorless needles and colorless prisms, respectively.

Erythro β-hydroxy ester (5a): Mp 126-127°C. Anal. Calcd. for $C_{33}H_{34}O_8$: C,70.95;H,6.14. Found: C,71.14;H,6.16. IR(KBr): 1737, 1595, 1255, 1130 cm^{-1} NMR δ(ppm:$CDCl_3$): 2.69(1H,d,J=2.0), 3.57(3H,s), 3.77(6H,s), 3.87(3H,s), 4.99(2H,s), 4.03(2H,s), 4.11 (1H,d,J=2), 6.50(2H,s), 6.75-6.90(3H,m), 7.20-7,50(10H,m). MS(70eV): 286(9.8), 272(6.9), 227(2.9), 195(5.9), 163(15.5), 91(100), 77(7.8), 65(8.8). UV λ_{max}^{EtOH}nm(log ε): 277.5(3.7i).

Threo β-hydroxy ester (5b): Mp 131-132°C. Anal. Calcd. for $C_{33}H_{34}O_8$:C,70.95;H,6.14. Found: C,71.08;H,6.27. IR(KBr): 1740, 1597, 1270, 1130 cm^{-1} NMR δ(ppm:$CDCl_3$): 3.17(1H,d,J=3.5), 3.63(6H,s), 3.76(6H,s), 4.93(2H,s), 4.99(1H,d,J=3.5), 5.07(2H,s), 6.26 (2H,s), 6.53(1H, dd, J=8, 2), 6.59(1H,d,J=2), 6.71(1H,d,J=8), 7.20-7.45(10H,m). MS(70eV): showed the same fragmentation pattern as the erythro isomer. UV λ_{max}^{EtOH} nm(log ε):278(3.58).

Triacetyl esters (6a) and (6b)

β-Hydroxy ester (5a, 837 mg, 1.5mM) was dissolved in 20 ml of dioxane/ethanol (1:1) containing 450 mg of 5% Pd-C and stirred under hydrogen for 5 hours. After confirmation of the disappearance of the starting material by silica gel TLC developed with $CHCl_3$, palladium charcol was filtered off and washed with ethanol.

The solvent was evaporated in vacuo, and the residue was acetylated by dissolving in 10 ml of pyridine/acetic anhydride(1:1) at room temperature for 20 hours. The reaction mixture was diluted with 50 ml of ether, and transferred into a 100 ml of separating funnel and washed with 10% HCl solution (10x2 ml), brine(10 ml) and a saturated $NaHCO_3$ solution(10x2 ml), successively. The ether solution was dried over Na_2SO_4 and evaporated in vacuo. The colorless crystal(6a, 750 mg, 99% yield) which was recrystallized from ethanol was obtained. Triacetyl ester (6b) was also synthesized by the same method described above from β-hydroxy ester (5b) in 95% overall yield.

Erythro triacetyl ester (6a): Mp 161-161.5°C. Anal.Calcd. for $C_{25}H_{28}O_{11}$: C,59.52;H,5.59. Found:C,59.44;H,5.69. IR(KBr): 1773, 1755, 1613, 1255, 1205, 1140 cm^{-1} NMR δ(ppm:$CDCl_3$): 1.85 (3H,s), 2.29(3H,s), 2.31(3H,s), 3.57(3H,s), 3.80(6H,s), 3.83(3H, s), 4.04(1H,d.J=9), 6.42(1H,d,J=9), 6.39(2H,s), 6.90-7.10(3H,m). MS(70eV): 504(M^+, 5.7), 462(15.7), 413(2), 402(2.1), 360(15.5), 302(4), 280(3.1), 268(14.3), 238(25.7), 225(64.3), 196(28.5), 183(100). UV λ_{max}^{EtOH}nm(log ε):274(3.69), 279(3.68).

Threo triacetyl ester (6b): Mp 164°C. Anal.Calcd. for $C_{25}H_{28}O_{11}$: C,59.52;H,5.59. Found:C,59.01;H,5.69. IR(KBr): 1775, 1753, 1610, 1205, 1132, 1032 cm^{-1} NMR δ(ppm:$CDCl_3$): 2.10(3H,s), 2.24(3H,s), 2.25(3H,s), 3.67(6H,s), 3.69(3H,s), 3.76(3H,s), 3.93 (1H,d,J=10), 6.14(1H,d,J=10), 6.62(1H,d,J=2), 6.74(1H,dd,J=8,2), 6.90(1H,d,J=8). MS(70eV): showed the same fragmentation pattern as the erythro isomer. UV λ_{max}^{EtOH}nm(log ε):274(3.60), 279(3.58).

1,2-Diarylpropane-1,3-diols (1a) and (1b)

To a stirred solution containing 228 mg (6mM) of LAH in 20 ml of anhydrous THF, 504 mg (1mM) of the triacetyl ester (6a) dissolved in 10 ml of anhydrous THF was added dropwise at 50°C under nitrogen for a period of 30 min. and the stirring was continued for additional 2 hours. The reaction mixture was cooled to 0°C and 1 ml of water dissolved in 4 ml of THF was added dropwise for decomposition of excess LAH. The aluminum complex was decomposed by adding of dry ice, and the resulting inorganic salts was filtered off and washed with ethyl acetate. The filtrate and the washings were combined and dried over Na_2SO_4, and the solvent was evaporated in vacuo. A colorless crystal (298 mg, 85% yield) which gave one spot on the silica gel TLC developed with 2.5% methanol/chloroform was obtained and recrystallized from ethyl acetate. 1,2-Diarylpropane-1,3-diol (1b) was also synthesized by the method described above from the triacetyl ester (6b) in 80% yield.

Erythro 1,2-diarylpropane-1,3-diol (1a): Mp 195-198°C. Anal. Calcd. for $C_{18}H_{22}O_7$:C,61.70;H,6.33. Found:C,60.67;H,6.51. IR(KBr): 3560, 2910, 1615, 1520, 1463, 1280, 1225, 1125 cm^{-1} NMR δ(ppm: $CDCl_3$)(Acetate Fig. 2): 1.99(3H,s), 2.00(3H,s), 2.28(3H,s), 2.29 (3H,s), 3.38(1H,m), 3.73(3H,s), 3.74(6H,s), 4.21(1H,dd,J=11,6.8), 4.46(1H,dd,J=11,7.5), 6.08(1H,d,J=6.0), 6.36(2H,s), 6.63(1H,d,J= 2), 6.77(1H,dd,J=8,2), 6.97(1H,d,J=8). MS(70eV): 350(M^+, 0.5), 332(0.6), 330(0.8), 313(4.8), 302(100), 229(6.5), 167(27), 150 (45.7). UV λ_{max}^{EtOH}nm(log ε): 279(3.64).

Threo 1,2-diarylproapne-1,3-diol (1b): Mp 160.5°C. Anal. Calcd. for $C_{18}H_{22}O_7$:C,61.70;H,6.33. Found:C,61.67;H,6.50. IR(KBr): 3400, 2900, 1625, 1520, 1465, 1435, 1235, 1130, 1028 cm^{-1} NMR δ (ppm:$CDCl_3$)(Acetate, Fig.3): 2.04(3H,s), 2.13(3H,s), 2.27(3H,s),

2.28(3H,s), 3.31(1H,m), 3.68(3H,s), 3.70(6H,s), 4.39(1H,dd,J=11, 6.0), 4.57(1H,dd,J=11, 7.0), 5.97(1H,d,J=8), 6.27(2H,s), 6.46(1H,d,J=2), 6.68(1H,dd,J=8, 2), 6.93(1H,d,J=8). UV λ_{max}^{EtOH}nm(log ε): 279(3.50).

Phenylboronates (8a), (8b), (9a) and (9b)

1,3-Propanediol derivatives (7a),(7b),(1a) and (1b) (0.1mM, each) and phenylboronic acid (0,1mM) in a mixed solution of anhydrous dioxane (1 ml) and anhydrous benzene (20 ml) was refluxed for 4 hours collecting the water in a Dean-Stark head. After confirmation of the dissapearance of the starting compouud by silica gel TLC, the solvent was removed, and then boronate formed was purified by preparative TLC developed with 1.5% methanol/chloroform, and each phenylboronate was used for NMR spectrometry without any crystallization.

Erythro phenylboronate (8a): NMR δ(ppm:$CDCl_3$) (Fig.4): 3.74(3H,s), 3.76(3H,s), 4.11(1H,d,J=3,γ-Ha), 4.13(1H,dd,J=3, 1, γ-He), 4.52(1H,q,J=3,β-H), 5.41(1H,dd,J=1, 3, α-H), 6.70-7.10(7H,m, aromatic), 7.20-7.50(3H,m, =B-Ø, m,p -protons), 7.85-8.05 (2H,m, =B-Ø, o-protons).

Threo phenylboronate (8b): NMRδ(ppm:$CDCl_3$)(Fig.5): 3.64(3H, s), 3.84(3H,s), 4.27(1H,d,J=2, γ-Ha), 4.31(1H,d,J=2, γ-He), 4.72 (1H,q,J=2, β-H), 5.42(1H,d,J=2, α-H), 6.70-7.20(7H,m,aromatic), 7.25-7.55(3H,m, =B-Ø, m,p-protons), 7.75-8.10(2H,m, =B-Ø, o-protons).

Erythro phenylboronate (9a): NMR δ(ppm:$CDCl_3$)(Fig.6): 3.53 (1H,ddd,J=7, 6, 4.5, β-H), 3.62(3H,s), 3.69(6H,s), 4.319(1H,d,J=6, γ-Ha), 4.321(1H,d,J=7, γ-He), 5.42(1H,d,J=4.5, α-H), 6.16(2H,

s, aromatic), 6.19(1H,d,J=2, aromatic), 6.44(1H,dd,J=8, 2, aromatic), 6.77(1H,d,J=8, aromatic),7.30-7.50(3H,m, =B-Ø, m,p-protons), 7.80-8.00(2H,m, =B-Ø, o-protons).

Threo phenylboronate (9b): NMR δ(ppm:$CDCl_3$)(Fig.7): 2.97(1H,dt,J=10, 6, β-H), 4.32(1H,d,J=6, γ-He), 4.37(1H,d,J=10, γ-Ha), 5.17(1H,d,J=10, α-H), 6.36(2H,s, aromatic), 6.44(1H,d,J=2, aromatic), 6.66(1H,dd,J=8, 2, aromatic), 6.91(1H,d,J=8, aromatic), 7.20-7.50 (3H,m, =B-Ø. m,p-protons), 7.80-8.00(2H,m, =B-Ø, o-protons).

II-3-5 Summary

1-(4-Hydroxy-3,5-dimethoxyphenyl)-2-(4-hydroxy-3-methoxyphenyl)-propane-1,3-diol (1), one of the main structural units in hardwood lignin was synthesized. The key step of this synthetic method is the condensation reaction between methyl benzylhomovanillate (3) and benzylsyringaldehyde (4). At this step, lithium diisopropyl amide was used as base, and threo and erythro isomers of β-hydroxy ester (5) whose ratio was about 3:1 were obtained as crystal, respectively. These β-hydroxy esters (5) were converted to the final compounds (1) by hydrogenation with Pd-C and hydrogen, and subsequent acetylation and reduction with lithium aluminum hydride. The configurations of these 1,2-diarylpropane-1,3-diols were established by NMR analysis of their phenylboronates. The coupling constants between α- and β-protons were 10 cps (threo) and 4.3 cps (erythro), respectively, and the values supported these configurations.

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II-4 Syntheses of phenylcoumarans

II-4-1 Introduction

Phenylcoumaran structure, one of the main constitutional units, in lignin is present in about 14% per C_9-unit[1] and has been investigated in relation to lignin reactions such as pulping, chemical utilization and biodegradation of lignin. Besides, the phenylcoumaran derivatives have been isolated from plant extractives[2] recently, and this has promoted interest in the studies of biogenetic differences between optically inactive lignin and optically active lignans. To elucidate such problems, the most suitable phenylcoumaran compounds must be used in each experiment. Nevertheless, only two phenylcoumaran compounds, dehydrodiconiferyl alcohol and dehydrodiisoeugenol, both obtained by oxidative couplings of coniferyl alcohol and isoeugenol are available, and the general synthetic method of phenylcoumaran is not established yet. In the previous Sections, the syntheses of guaiacylglycerol-β-guaiacyl ether [3] (Section II-1) and 1,2-diarylpropane-1,3-diols[4] (Section II-3) were described in a series of the synthetic studies of lignin model compounds.

In this Section, the syntheses of dehydrodiconiferyl alcohol and its derivatives, and the first general synthetic method of phenylcoumaran are described.

II-4-2 A new general synthetic method of phenylcoumarans

An "irrational" synthetic method for the phenylcoumarans was reported by E.Schmid *et al.* earlier[5] who demonstrated that

on heating in N,N-diethylaniline at 225°C 2-(1-arylally)-phenols are transformed into trans 2-aryl-3-methyl coumarans by an abnormal Claisen rearrangement. However, 3-hydroxymethyl coumarans can not be obtained by their method without any modification.

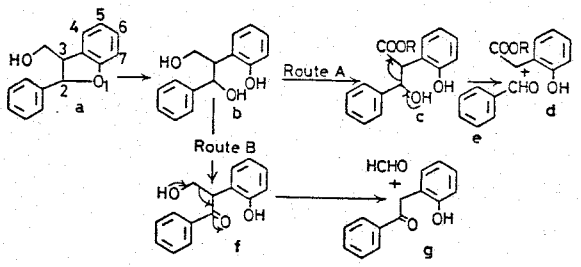

Fig.1 Synthetic pathway of phenylcoumaran

In consideration of a "rational" synthetic method affording 3-hydroxymethyl coumarans, the expected coumaran (a) is thought to be synthetically equivalent to 1,2-diarylpropane-1,3-diol system (b) which could be obtained by the two synthetic routes. First, if the terminal hydroxymethyl group is assumed to be equivalent to the ester group, the compound (b) can be converted anti-synthetically into β-hydroxy ester (c) which could be synthesized by an aldol condendation between ortho-hydroxyphenyl acetate derivative (d) and an aldehyde (c) (route A). On the other hand, if the benzylcarbinol group is assumed to be equivalent to the ketone group, the resultant β-hydroxy ketone (f) might be synthesized by an aldol condensation of the deoxybenzoin (g) with formaldehyde (route B). In the present Section, the former possibility via a β-hydroxy ester intermediate is discussed.

II-4-3 Synthesis of dehydrodiconiferyl alcohol

The synthetic route of dehydrodiconiferyl alcohol (12) in

Fig.2 is divided into three main steps, i.e., the introduction of a two carbon side cahin to C_5-position of vanillin derivative (compound 5), the formation of the phenylcoumaran ring, which is a key step in the present synthetic method (compound 9), and finally the side chain extention.

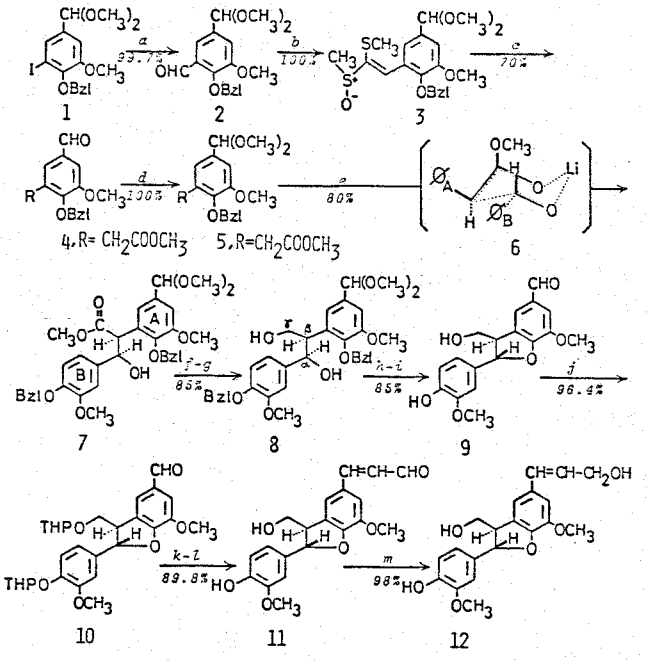

Fig.2 Synthetic route of dehydrodiconiferyl alcohol (12)
[a] n-BuLi/DMF/Et_2O/-35°C. [b] FAMSO/NaH/DMF/r.t. [c] HCl/MeOH/5°C. [d] $CH(OCH_3)_3$/p-TsOH/MeOH/reflux. [e] Lithium diisopropylamide/Benzyl vanillin/THF/-70°C. [f] Me_3SiCl/Et_3N/THF/r.t. [g] $LiAlH_4$/THF/50°C. [h] H_2/10%Pd-C/MeOH/r.t. [i] BF_3-Et_2O/CH_2Cl_2/r.t. [j] Dihydropyran/p-TsOH/CH_2Cl_2/0°C. [k] $Ø_3PCH_2CH(-OCH_2-)_2Br$/t-BuOK/t-BuOH-THF/reflux. [l] 10%HCl/THF/r.t. [m] $NaBH_4$/MeCH/0°C.

The first target molecule (5) is assumed to be obtained by the Claisen rearrangement of O-allylvanillin, and subsequent oxidative split-off of the carbon-carbon double bond of 5-allyl-benzylvanillin derivative. The oxidation of 5-allyl-benzylvanillin acetal gave 5-(2,3-dihydroxypropyl) and 5-formyl vanillin derivatives in low yield even if the mildest condition (OsO_4/$NaIO_4$) and resulted in deprotection of aldehyde at C_1-position.

Therefore, the compound (5) was finally obtained by the following method (Fig.2).

The compound (1) was prepared by Freudenberg et al. earlier,[6] but the intermediate, 5-iodovanillin dimethylacetal, was unstable and decomposed to the starting material without any acid catalysis. Thus, this compound was prepared in over 90% crystal-isolation yield form 5-iodovanillin by the benzylation and subsequent acetalization. The compound (1) was treated with n-butyl lithium in ether and subsequent N,N-dimethylformamide to give the aldehyde (2) in the quantitative yield. In this case, when 1,3-dioxolans were used instead of dimethylacetal as protcting group, the cleavage of the acetal occurred. It is assumed that this acetal is fairly unstable because of the eledtron -donating effect of benzyloxy group at p-position and in addition, coordination of lithium cation to the two fixed oxygens of dioxolan having a cyclic structure. It is considered that the coordination acts as a driving force for the cleavage of the acetal, but in the case of dimethyl-acetal with free rotation, lithium cation can not coordinate so well. When THF was used as solvent instead of ether, the yield was low with the formation of by-products, which was due to that 5-lithio derivative is soluble and unstable in THF. The use of the dimethylacetal as protcting group and ether as solvent are therefore essential.

Condensation of the aldehyde (2) with a commercially available methyl methylthiomethyl sulfoxide (FAMSO)[7] in the presence of sodium hydride in DMF and the subsequent acid-catalyzed methanolysis of the resultant 1-methylsulfinyl-1-methylthioethylenyl derivative (3) led to the formation of the compound (4) in 70% yield.

This methanolysis was condiserably sensitive to the concentration of acid, and the best yield was obtained under the reaction condition of about 30% hydrogen chloride in methanol at 5°C for 60 hours. The aldehyde (4) was converted to the first target molecule (5) in the quantitative yield by the acetalization.

The next conversion of the compound (5) to the compound (9) is the most significant and interesting step in the synthetic route. Condensation of the compound (5) with benzylvanillin in the presence of lithium diisopropylamide (LDA) in THF at -70°C afforded the expected β-hydroxyester (7) in 80% yields. The ester gave colorless crystals from ethyl acetate and n-hexane and was found to be only a threo isomer, but not a mixture of erythro and threo isomers generally obtained by aldol condensation reactions. The ratios of erythro and threo isomers corresponding to the β-hydroxyester (7) in the syntheses of guaiacylglycerol-β-guaiacyl ether and 1,2-diarylpropane-1,3-diol were found to be 3:1[3] and 1:3[4], respectively. Such condensations conceivably proceed via a six-membered transition state, in which the trans diequatorial orientation of the bulky functional groups (ϕ_A and ϕ_B) is more favorable than cis orientation for each other because of a steric repulsion in a transition state (6). In the present case, however, the only trans diequatorial orientation might take place because of the extraordinary steric repulsion with an additional steric hindrance of a benzyloxy group substituted on A-ring, and brought a highly stereoselective reaction to give only threo isomer of β-hydroxyester (7). This interpretation is supported by NMR of the ester (7); the methylene protons of the benzyl group attached to the B-ring appear as a

singlet at δ4.97, whereas those of A-ring give an AB type at δ 4.34 (d, J=11) and δ4.84 (d, J=11), respectively, due to a free rotational hindrance.

It was difficult to obtain the expected diol (8) in high yields by the hydride reduction under various conditions, e.g., reduction with lithium alumin hydride at -50°, 0° and 50°C and with sodium borohydride in the presence of $CuCl_2$ in THF at reflux temperature, etc. An α-deoxy derivative of the diol (8) was always obtained as a main product under these conditions. However, the difficulty was finally solved by trimethylsilylation of the ester (7) with trimethylsilyl chloride and triethyl amine at room temperature and subsequent reduction with lithium aluminum hydride at 50°C afforded the expected diol (8) in 85% yield after a purification by silica gel TLC developed with ethyl acetate/n-hexane (1:3).

The diol (8), a key intermediate (b) in Fig.1, thus obtained was unexpectedly easily converted to the phenylcoumaran (9) in 85% yield by catalytic hydrogenation with 10% palladium on carbon and subsequent treatment with catalytic amounts of boron trifluoride etherate in methylene chloride. On addition of boron trifluoride etherate to the hydrogenated compound suspended in methylene chloride, previously dried over alumina, the reaction mixture turned yellow in a moment suggesting instantaneous formation of a quinonemethide-like intermediate in this cyclization.

The phenylcoumaran (9) was unequivocally identified comparing with those obtained by the oxidation of dehydrodiconiferyl alcohol ($OsO_4/NaIO_4$ in ether and water, about 60% yield) and

also by biodegradation of dehydrodiconiferyl alcohol with Fusarium solani M-13-1[8]. Since the trans configuration of dehydrodiconiferyl alcohol was determined earlier[9], the phenylcoumaran (9) must be trans.

Finally, the sidechain extention of phenylcoumaran (9) to dehydrodiconiferyl alcohol (12) was achieved. In general, malonic acid derivatives have been used as a two-carbon source, but the condensation of a ditetrahydropyranyl ether derivative (10) with these reagents did not proceed smoothly. And Wittig reaction was found to be suitable for this purpose.

The Wittig reaction of the compound (10) with 3.0 mol. equiv. of 1,3-dioxan-2-ylmethyl triphenylphosphonium bromide[10] in the presence of potassium t-butoxide in THF at reflux temperature and subsequent acid hydrolysis afforded the aldehyde (11) in about 90% overall yield . The aldehyde was unequivocally identified comparing with those obtained by oxidative coupling of coniferyl alcohol[11] and also by biodegradation of dehydrodiconiferyl alcohol[8]. The aldehyde (11) was finally converted to dehydrodiconiferyl alcohol (12) in quantitative yield by sodium borohydride reduction.

II-4-4. Syntheses of dehydrodiconiferyl alcohol derivatives

To prove that the synthetic method thus established is a general one, we tried the syntheses of various dehydrodiconiferyl alcohol derivatives which are difficult to obtain in high yield by oxidative couplings of p-hydroxycinnamyl alcohols. When benzylsyringaldehyde was used instead of benzylvanillin at the

stage of condensation with the compound (5) in Fig. 2 and the subsequent reactions were followed, the final compound (13), 2-(4-hydroxy-3,5-dimethoxy) phenyl-3-hydroxymethyl-5 (3-hydroxy) propenyl-7-methoxy coumaran was obtained in almost the same yield in the respective steps as in the case of the compound (12). The compound (13) seems to be important in hardwood lignin which is known to be a copolymer of coniferyl and sinapyl alcohols. Its dihydro derivative was actually isolated from hydrolysis products of Mizunara (Quercus mongolica) wood[12].

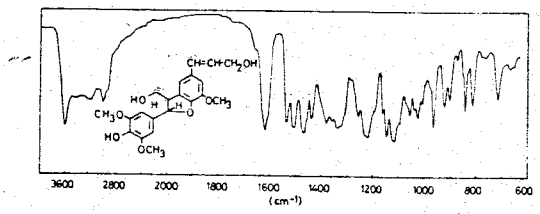

Fig. 3 IR spectrum of a dehydrodiconiferyl alcohol derivative (13)

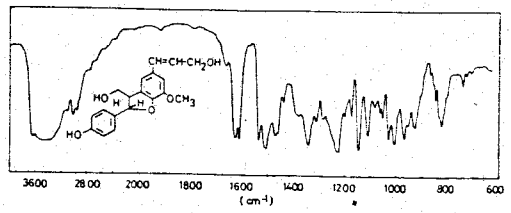

Fig. 4 IR spectrum of a dehydrodiconiferyl alcohol derivative (14)

When p-hydroxybenzaldehyde benzyl ether was used at the stage of the condensation, the phenylcoumaran (14), 2-(p-hydroxy) phenyl-3-hydroxymethyl-5(3-hydroxy) propenyl-7-methoxy coumaran was obtained. The IR spectra of these compounds are shown in Fig. 3 and 4, respectively.

Another possible rout (B) shown in Fig. 1 and also the conversions of the phenylcoumaran (9) to trimeric models which consist of phenylcoumaran and each of arylglycerol-β-aryl ether, and 1,2-diarylpropane-1,3-diol moieties, are in progress.

II-4-5. Experimental

5-Iodobenzylvanillin dimethylacetal (1)

This compound was prepared from 5-iodovanillin by the benzylation (benzyl chloride/K_2CO_3/KI in DMF at 70°C) and then acetalization (trimethylorthoformate/p-TsOH in methanol at room temperature) in over 90% yield. mp. 43°C (MeOH).

5-Formyl benzylvanillin dimethylacetal (2)

To a stirred solution of 5-iodobenzylvanillin dimethylacetal (10.35g, 25mM) in 50 ml of ether (dried over sodium metal), n-butyl lithium (1.37 N) in n-hexane solution (20.07 ml, 27.5 mM) was added dropwise at -70°C under nitrogen over a period of 20 min. On addition of about one-half of n-butyl lithium, white precipitates (5-lithio benzylvanillin dimethylacetal) appeared. After the addition of total n-butyl lithium, the temperature was raised to -35°C, and 8.1 ml of DMF (105 mM, dried over molecular sieves 3A) was added over a period of 5 min. to give a pale yellow clear solution which was partitioned between ether and water. The aqueous layer was extracted with ether. The combined ether solution was washed with brine three times and dried over Na_2SO_4. The evaporation of the ether *in vacuo* gave a slightly yellow oil which was crystallized from n-hexane to give colorless crystals (7.88 g, 99.7%).

Mp. 55.5-56°C (n-hexane). Anal. Calcd. for $C_{18}H_{20}O_5$: C, 68.34; H, 6.37, Found: C, 68.17; H, 6.24. IR(KBr): 1705 cm^{-1} NMR δ(ppm:$CDCl_3$): 3.31(6H,s), 3.92(3H,s), 5.13(2H,s), 5.28(1H,s), 7.20-7.45(7H,m). MS(70eV): 316(M^+, 2.6), 285(18.3), 257(4.4), 241 (1.4), 224(6.6), 193(14), 179(2.8), 166(18.8), 152(2.2), 136(3.4), 135(3.4), 91(100), 65(17.5). UV λ_{max}^{MeOH}nm(log ε): 320(3.45), 261.3 (3.83).

5-(1-Methylsulfinyl-1-methylthioethylenyl) benzylvanillin dimethylacetal (3) and 5-carbomethoxymethylbenzylvanillin dimethyl acetal (4)

To a stirred solution containing methyl thiomethyl sulfoxide (FAMSO)(1.25 g, 11mM) and 5-formyl benzylvanillin dimethylacetal (2) (3.16g, 10mM) in 30 ml of DMF, 254 mg of sodium hydride(50% in mineral oil), which was washed with n-hexane at least three times before use, was added at room temperature under nitrogen, and then the stirring was continued for one hour at the same temperature. The reaction mixture was poured onto ice/water, and the product was extracted with ether. The ether extracts were washed with brine at least three times , dried over Na_2SO_4 and evaporated in vacuo to give a pale yellow oil (4.17g). The oil contained a small amount of impurity which is less polar and gives a higher Rf value than the starting compound on silica gel TLC developed with ethyl acetate/n-hexane (1:2). The product could be used for the following methanolysis without purification because the 5-carbomethoxymethylbenzylvanillin was easily crystallized from methanol.

IR(KBr): 1054 cm^{-1} NMR δ(ppm:$CDCl_3$): 2.14(3H,s), 2.65(3H,s), 3.34(6H,s), 3.90(3H,s), 5.01(2H,s), 5.32(1H,s), 7.01(1H,d,J-1.5), 7.10-7.45(5H,m), 7.66(1H,d,J=1.5), 7.82(1H.s). MS(70eV): 422(M^+, 2), 406(2.3), 391(1), 290(1), 375(2), 360(3), 359(3), 343(2), 327 (3), 315(7), 299(3), 295(3), 285(4), 269(34), 268(34), 252(35), 237(85), 223(100), 222(56), 207(28), 137(24), 91(74), 75(39).

Into the stirred solution of the slightly-yellow oil (3)(13.7g, 32.5mM) dissolved in 100 ml of methanol, hydrogen chloride generated from 60 ml of conc.H_2SO_4 and 40 ml of conc.HCl solution

was bubbled at -20°C over a period of one hour, and then the solution was stirred for 60 hours at 5°C. The slightly red reaction mixture resulted was evaporated *in vacuo* to about 50 ml at 30°C and partitioned between ethyl acetate and brine. The ethyl acetate solution was dried over Na_2SO_4 and evaporated *in vacuo* to give a pale yellow oil which was crystallized from methanol to give colorless crystals (8.14g, 70%).

Mp 82.5-83°C (ether). *Anal*. Calcd. for $C_{18}H_{18}O_5$: C, 68.87 ; H, 5.77, Found: C,68.64;H,5.78. IR(KBr): 1742, 1704 cm^{-1} NMR δ(ppm:$CDCl_3$): 3.60(3H,s), 3.62(2H,s), 3.93(3H,s), 5.13(2H.s), 7.28 (1H,d,J=2), 7.32(5H,broad s), 7.39(1H,d,J=2), 9.82(1H,s). MS m/e (%): 314 (M^+, 7), 282 (4), 245 (3), 240 (3.5), 223 (2), 222 (2), 192 (11), 164 (5), 163 (7), 135 (4), 134 (4), 133 (4), 121 (4), 107 (5), 105 (4), 91 (100), 65 (31). UV λ_{max}^{MeOH}nm(log ε): 273.2(4.06), 308.8(3.78).

5-Carbomethoxymethylbenzylvanillin dimethylacetal (5)

The reaction mixture containing the aldehyde (4)(628 mg, 2 mM), trimethylorthoformate (1 ml) and *p*-TsOH·H_2O(60 mg) in methanol (10 ml) was refluxed for 2 hours under nitrogen. After cooling the reaction mixture, excess amounts of $NaHCO_3$ (about 500 mg) was added and then filtered off and washed with ethyl acetate. The combined solution of the filtrate and washings was evaporated. The products were partitioned between ether and a saturated $NaHCO_3$ solution. The ether layer was washed with brine, dried over Na_2SO_4 and evaporated *in vacuo* to dryness to give a colorless oil (720 mg. quantitative yield). The oil gives one spot on TLC developed with methylene chloride and can be used for the subsequent reaction without any purification, but must be dried in a vacuum

desiccator containing P_2O_5 overnight before use.

IR(KBr): 1746 cm^{-1} NMR δ(ppm:$CDCl_3$): 3.33(6H,s), 3.59(5H,s), 3.89(3H,s), 5.02(2H,s), 5.32(1H,s), 6.87(1H,d,J=2), 6.97(1H,d,J=2), 7.15-7.45(5H,m). MS(70eV): 360(M^+, 3.5), 329(5.5), 314(3), 297(2.5), 282(2), 269(5), 265(2), 254(2), 238(20), 222(5.5), 209(6), 207(7), 192(5), 179(8), 178(18), 165(4.5), 163(5.5), 149(4.5), 135(4), 121(4), 105(3.5), 91(100), 75(17), 65(27).

β-Hydroxy ester (7)

This compound was prepared by the modified methods previously reported.[3,4]

To a stirred solution of lithium diisopropylamide (2.4mM), prepared by the usual method in anhydrous THF (freshly distilled from benzophenone and potassium metal before use), 720 mg (2mM) of the ester (5) dissolved in 5 ml of anhydrous THF was added dropwise over a period of 40 min. at -70°C under nitrogen. The stirring was continued for additional 40 min. at the same temperature and then 484 mg of benzylvanillin (2mM) dissolved in 5 ml of anhydrous THF was added dropwise over a period of 40 min. After the stirring for one hour, a pale yellow reaction mixture was partitioned between ethyl acetate and brine. The organic layer was washed with brine until the washings became to neutral, dried over Na_2SO_4 and evaporated *in vacuo* to give a slightly yellow oil which was crystallized from ethyl acetate/*n*-hexane to give colorless crystals (963 mg, 80%).

Mp 105°C (ether). *Anal.*Calcd. for $C_{35}H_{38}O_9$: C, 69.75 ; H,6.36. Found: C,69.45 ; H,6.36. IR(KBr): 1737 cm^{-1} NMR δ(ppm:$CDCl_3$): 3.23(6H,s), 3.57(3H,s), 3.62(3H,s), 3.76(3H,s), 4.34(1H,d,J=11),

4.40(1H,d,J=9), 4.48(1H,d,J=11), 4.97(2H,d), 5.05-5.25(1H,m), 5.20(1H,s), 6.45-6.70(3H,m), 6.80(1H,d,J=2), 6.87(1H,d,J=2); 7.15-7.40(10H,m). MS(70eV): 360(1), 329(1.2), 314(0.5), 297(0.6), 296 (1.5), 242(1.5), 238(3), 222(1.2), 209(2), 207(2), 194(1), 191(1), 179(3.5), 178(4), 165(1.5), 163(2.3), 151(1.5), 150(1.5), 133(2), 121(2.3), 105(7), 91(100), 75(34), 65(77). UV λ_{max}^{MeOH}nm(log ε):280.2 (3.70).

Diol (8)

To a stirred solution of the β-hydroxy ester (7) (301 mg, 0.5 mM) dissolved in 5 ml of anhydrous THF, triethylamine (0.418ml, 3mM) and then trimethylsilyl chloride (0.19ml, 1.5mM) were added at 0°C under nitrogen. After the stirring for 30 min. at room temperature, the solution of TMS derivative was added dropwise into a suspension of lithium aluminum hydride (76 mg, 2mM) in 10 ml of anhydrous THF at 50°C over a period of 30 min. and the stirring was continued for 30 min. The reaction mixture was partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate. The combined ethyl acetate extracts were washed with brine, dried over Na_2SO_4 and evaporated *in vacuo* to give a colorless oil which was purified by silica gel TLC developed with 2% methanol in methylene chloride to give a pure expected diol as colorless glass (244mg, 85%).

NMR δ(ppm:$CDCl_3$): 3.24(6H,s), 3.59(3H,s), 3.78(3H,s), 3.45-4.15(3H,m), 4,34(1H,d,J=11), 4.81(1H,d,J=11), 4.97(1h,d,J=8), 4. 98(2H,s), 5.22(1H,s), 6.54(2H,broad s), 6.69(1H, broad s), 6.80 (2H,s), 7.15-7.40(10H,m).

Phenylcoumaran (9)

The diol (8)(250 mg, 0.44mM) and 10% Pd-C(100 mg) were suspended in 8 ml of methanol and stirred under hydrogen for 30 min. at room temperature and then Pd-C was filtered off and washed with methanol. The filtrate and the washings were combined and evaporated in vacuo to give a colorless glass in the quantitative yield. To a stirred suspension of the product thus obtained in 10 ml of methylene chloride (dried over alumina, Alumina Woelm B, Akt.1), one drop of boron trifluoride etherate was added at room temperature under nitrogen, and the stirring was continued for one hour. The reaction mixture was washed with brine, dried over Na_2SO_4 and then evaporated in vacuo to give a colorless glass (162 mg, 98%).

Mp 135-136°C (MeOH). Anal.Calcd. for $C_{18}H_{18}O_6$:C,65.44 ; H, 5.49, Found: C,65.18 ; H,5.60. IR(KBr): 1685 cm^{-1} NMR δ(ppm:$CDCl_3$): 3.55-4.10(3H,m), 3.84(3H,s), 3.92(3H,s), 5.67(1H,d,J=7), 6.84(3H, s), 7.29-7.40(2H,m), 9.7(1H,s). MS(70eV): 330(M^+, 47.5), 312(100), 300(59.6), 297(97.5), 280(34.5), 269(16.4), 252(24.8), 239(35.6), 223(15.1), 211(21.2), 197(16.6), 183(14.5), 181(15.5), 178(13), 169(12.5), 152(13.4), 137(25). UV λ_{max}^{MeOH}nm(logε): 235.1(4.26), 289.4(4.14), 303(4.13).

Ditetrahydropyranyl ether (10)

To a stirred solution of the phenylcoumaran (9)(330 mg, 1mM) and dihydropyran (1 ml, about 10mM) in 10 ml of methylene chloride, 3 mg of p-TsOH·H_2O was added at 0°C under nitrogen. After the stirring for 30 min., three drops of triethylamine was added and then the reaction mixture was washed with a saturated $NaHCO_3$

solution and brine, dried over Na_2SO_4 and evaporated *in vacuo* to give a colorless oil which was purified by silica gel TLC developed with ethyl acetate/*n*-hexane (1:2) to afford the pure ditetrahydropyranyl ether (10)(480 mg. 96.4%).

NMR δ(ppm:$CDCl_3$): 3.83(3H,s), 3.94(3H,s), 4.63(1H, broad t), 5.36(1H, broad t), 5.66(1H,m), 6.78-7.18(3H,m), 9.73(1H,s).

Aldehyde of dehydrodiconiferyl alcohol(11)

To a stirred suspension of the ditetrahydropyranyl ether (10) (480 mg, 0.964mM) and 1,3-dioxan-2-ylmethyl triphenylphosphonium bromide (1.24g, 2.9mM) in anhydrous THF (20 ml), 325 mg (2.9mM) of potassium *t*-butoxide dissolved in 6 ml of anhydrous *t*-butanol and 4 ml of THF was added dropwise over a period of 30 min. at reflux temperature under nitrogen and the stirring was continued for one hour. The reaction mixture was partitioned between ethyl acetate and brine, and then the ethyl acetate layer was dried over Na_2SO_4 and evaporated *in vacuo* to give a yellow oil which was purified by TLC developed with ethyl acetate/n-hexane (1:2) to afford a slightly yellow oil which gives a strong magenta coloration with phloroglucinol-hydrochloric acid reagent. The product obtained was dissolved in 10 ml of each of THF and 10% hydrochloric acid solution at room temperature under nitrogen and stirred for 5 hours. After the disappearance of the starting material, the reaction mixture was diluted with ethyl acetate, washed with brine three times, dried over Na_2SO_4 and evaporated *in vacuo* to give an oil which was crystallized from methylene chloride/*n*-hexane to give slightly yellow crystal (320 mg, 89.8%).

Mp 165.5-166.5°C (MeOH). *Anal*. Calcd. for $C_{20}H_{20}O_6$: C, 67.40; H,5.66, Found:C, 67.32;H,5.65. IR(KBr): 1670 cm^{-1} NMR δ(ppm:$CDCl_3$):

3.50-4.05(3H,m), 3.84(3H,s), 3.91(3H,s), 5.59(1H,d,J=6.5), 6.51 (1H,dd,J=15, 7.5), 6.82(3H,s), 6.96(1H,d,J=1.5), 7.06(1H,d,J=1.5), 7.33(1H,d,J=15), 9.51(1H,d,J=7.5). MS(70eV): 356(M^+, 60.3), 338(100), 326(27.6), 323(60.3), 306(22.6), 295(17), 277(12.1), 177(9. 6), 165(12.1), 151(11.1), 137(22.9). UV λ_{max}^{MeOH}nm(log ε): 229.7(4. 29), 294(3.85, sh), 342.6(4.39).

Dehydrodiconiferyl alcohol (12)

To a stirred solution of the aldehyde (11)(178 mg, 0.5mM) dissolved in 3 ml of methanol and 2 ml of THF, 12 mg of sodium borohydride (0.32mM) was added at 0°C under nitrogen. The stirring was continued for 30 min. The reaction mixture was partitioned between ethyl acetate and brine, and then the ethyl acetate layer was dried over Na_2SO_4 and evaporated in vacuo to give colorless crystals (175 mg, 98%).

Mp 165-166°C (ethyl acetate). Anal.Calcd. for $C_{20}H_{22}O_6$: C, 67.02 ; H,6.19, Found: C,66.73 ; H,6.03. IR(KBr): agreed with that reported earlier. NMR δ(ppm:d_6-acetone): 3.54(1H, broad t, J=6.5), 3.75-4.00(2H.m), 3.81(3H,s), 3.86(3H,s), 4.18(2H,d,J=5, in the presence of one drop of D_2O), 5.57(1H,d,J=6.3), 6.22(1H,dd, J=15.5, 5.0), 6.56(1H,d,J=15.5), 6.80-7.10(5H,m). MS(70eV): 358(M^+, 30), 340(95.5), 322(100), 307(75.5), 291(40.9), 279(27), 275 (23.9), 263(20.8), 247(23.9), 235(24.5), 165(20.9), 162(24.9), 151(24.5), 137(39.8). UV λ_{max}^{MeOH}nm(log ε): 278 (4.29).

Dehydrodiconiferyl alcohol derivative (13)

Mp 158-159°C (ethyl acetate). Anal. Calcd. for $C_{21}H_{24}O_7$: C, 64.93; H,6.23, Found: C,64.38;H,6.10. IR(KBr): Fig.3. NMR δ(

(ppm:d_6-acetone): 3.55(1H, broad t J=6), 3.65-3.95(2H,m), 3.76 (6H,s), 3.83(3H,s), 4.14(2H,d,J=5, in the presence of one drop of D_2O), 5.49(1H,d,J=5.2), 6.13(1H,dt,J=15.5, 5), 6.46(1H,d,J=15.5), 6.64(2H,s), 6.86(2H, braod q), 7.08(1H, braod s). MS(70eV): 388 (M^+, 38.9), 370(84.0), 354(98.3), 353(83.8), 340(78.3), 323(85.7), 321(100), 309(4), 293(3.8), 279(3.8), 265(3.2), 252(84), 235(5), 219(3.3), 191(2.9), 181(3.9), 167(74.3), 163(87.4), 149(84.6). UV λ_{max}^{MeOH}nm(log ε): 273(4.34).

Dehydrodiconifreyl alcohol derivative (14)

Mp 155-157°C (ethyl acetate). Anal.Calcd. for $C_{19}H_{20}O_5$: C, 69.50 ; H,6.14, Found: C,68.89 ; H,6.17. IR(KBr): Fig.4. NMR δ(ppm:d_6-acetone): 3.47(1H, braod t,J=6), 3.65-3.90(2H,m), 4.16(2H, d,J=5, in the presence of one drop of D_2O), 5.5(1H,d,J=6), 6.12 (1H,dt,J=15.5, 5), 6.43(1H,d,J=15.5), 6.71(2H,d,J=8.5), 6.84(2H, braod q), 7.13(2H,d,J=8.5). MS(70eV): 328(M^+,82), 310(100), 298(47.2), 294(30), 282(50), 280(38.8), 267(27.2), 253(19.2), 237(18), 221(16.8), 219(16.8), 207(16.8), 181(18.8), 165(25.2), 152(16.8), 131(28.8), 121(36), 115(28), 107(56.4), 91(19.8), 77(27.3), 65(18.8), 55(28.2). UV λ_{max}^{MeOH}nm(log ε):274.5(4.31).

II-4-6 Summary

The first general synthetic method of phenylcoumarans was established starting from vanillin. The present synthetic method must be important not only in the fields of lignin chemistry but also extractives, e.g., lignans.

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II-5 Syntheses of trilignols composed of phenylcoumaran and β-O-4 structures

II-5-1 Introduction

The general synthetic method of phenylcoumaran was described in previous Section[1]. In this Section, the syntheses of the trilignols composed of phenylcoumaran and β-O-4 structures are described. The trimer is generally difficult to obtain by oxidative couplings of p-hydroxycinnamyl alcohols. The syntheses of the compounds (7) and (8) should be important for the following reasons: 1) Demonstration for the applicability of β-ether synthetic method previouly described[2] (Section II-1) to the syntheses of the trimeric lignin model compounds. 2) Acidolysis product due to the phenylcoumaran attached to the β-O-4 component has been actually isolated[3] and such a structure is considered to play a fairly important role in lignins. 3) The compounds (7) and (8) must be useful for the studies of lignin reactions such as pulping, chemical utilization and biodegradation of lignin. The lignin monomers and dimers exist as the bonded units to consist lignin polymer, and not as individual entities for each other. Nevertheless, the lignin model compounds used so far are only monomers and dimers. It is reasonable to consider that the chemical change arising in one structural unit causes the different chemical reactivity in another, as in the case of neighbouring participation of functional group in organic chemistry. For example, when a benzylcarbinol group of the structural unit (a) in lignin molecule is oxidized to keto group, the structure (b) which expressed

as a resonance hybrid (c) is formed. Accordingly the ether bond between R" and oxygen must be unstable than in the case of (a), and which results in easy decomposition to $R"^{\oplus}$ and the structure (d) as shown in Fig.1. Actually, syringaldehyde has been obtained from 3,4,5-trimethoxybenzaldehyde by the selective demethylation with sodium p-thiocresolate in 90% yield.[4] For such a experiment, trimeric compounds could be used conveniently.

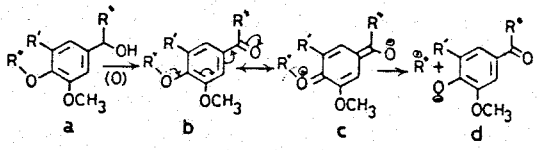


Fig. 1 Effects of neighbouring groups in lignin molecule

II-5-2 First syntheses of trilignols

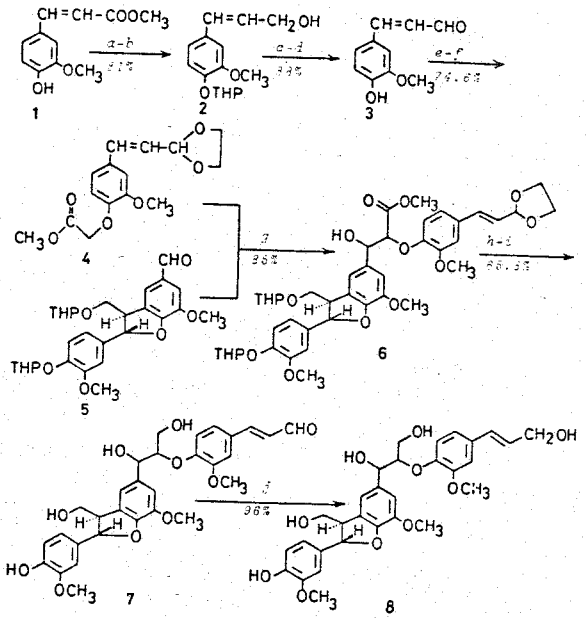
The synthetic route to trilignol (8) shown in Fig.2 is divided into three main steps, i.e., the synthesis of α-phenoxy acetate derivative (1 ⟶ 4), the synthesis of β-hydroxy ester by an aldol condensation (4 + 5 ⟶ 6) and finally the transformation of functional groups in the side chain (6 ⟶ 8).

In the first step, coniferyl alcohol was used instead of coniferyl aldehyde (3). That is, coniferyl alcohol was converted to α-phenoxy acetate derivative with methyl monochloroacetate and base by the usual method, and then the hydroxyl group was protected with tetrahydropyranyl (THP) ether. The subsequent reactions were followed in the reasonable yields except that the final step, the acid-catalyzed hydrolysis of THP ether, resulted in low yield. In general, coniferyl aldehyde is more stable than coniferyl alcohol under acidic condition and reduced to the alcohol with sodium borohydride in a high yield. Coniferyl aldehyde was

then used as the starting material in the present syntheses.

Although many synthetic methods for coniferyl aldehyde has been reported so far[5], the present method is the easiest and gives the best yield. Because, the oxidation of an allylic alcohol with active MnO_2[6] generally gives an aldehyde in a high yield in non-polar solvent, especially, carbon tetrachloride which can dissolve the THP ether of methyl ferulate. The overall yield from methyl ferulate was about 70%. This synthetic method will be reported in detail elsewhere together with the syntheses of other lignin monomer, p-hydroxycinnamaldehyde and sinap aldehyde[7].

The coniferyl aldehyde (3) was converted to the α-phenoxy acetate derivative with methyl monochloroacetate and potassium carbonate and then the aldehyde group of the derivative was protected with dioxolan to give the compound (4) in 74.6% overall yield. When dimethyl acetal which could be easily obtained by the reaction with methyl orthoformate and catalytic amounts of p-toluenesulfonic acid was used as a preliminary, the subsequent condensation reaction gave β-hydroxy ester in only 67.5%. And it was shown that dimethyl acetal is more labile than dioxolan and partly decomposed to aldehyde during drying in a desiccator with P_2O_5 before use. Dioxolan derivative (4) gave the β-hydroxy ester (6) by the condensation with phenylcoumaran (5)[1] as a colorless glass in 85% yield after purification by silica gel TLC developed twice with ethyl acetate/n-hexane (1:1). On the NMR spectrum of the β-hydroxy ester (6), respective protons attached to the side chain appear as fairly isolated peaks for each other as assigned in the experimental section, nevertheless the β-hydroxy ester (6) has six asymmetric carbons. It is considered on the basis of



[a]Dihydropyran/p-TsOH/CH_2Cl_2/0°C. [b]$LiAlH_4$/THF/-25°C. [c]MnO_2/CCl_4/r.t. [d]1N-HCl/THF/r.t. [e]$ClCH_2COOCH_3$/K_2CO_3/KI/acetone/reflux. [f]Ethylene glycol/p-TsOH/benzene/reflux. [g]Lithium diisopropylamide/THF/below -70°C. [h]$LiAlH_4$/THF/50-60°C. [i]pyridinium p-toluenesulfonate/ethanol/50°C. [j]$NaBH_4$/methanol/0°C.

Fig. 2 Synthetic route of trimeric lignin model composed of phenylcoumaran and β-O-4 structures

the discussion of six-membered transition state reported preciously[1] that the erythro form is predominant in the configuration between α' and β'-carbons although no experimental evidence has been obtained.

The β-hydroxy ester (6) was reduced with lithium aluminum hydride to 1,3-propane diol derivative whcih was then subjected to the hydrolysis of THP ether in ethanol with a tenth equivalent of pyridinium p-toluenesulfonate[8] at 50°C, and the trimeric aldehyde (7) was obtained in 65.8% overall yield. The trimeric aldehyde gave an strong magenta coloration with phloroglucinol-HCl reagent gradually turning blue with 2,6-dichloro-quinone chloroimide/1N-NaOH solution and orange-yellow with alcoholic 2,4-dinitrophenylhydrazine/HCl solution, respectively. The NMR spectrum is shown in Fig.3. Finally, sodium borohydride reduction of the

trimeric aldehyde (7) obtained gave the target molecule, trilignol (8) composed phenylcoumaran and β-O-4 sturctures in 96% yield. The NMR spectrum of this compound is shown in Fig.4. The trilignol (8) was fairly susceptible to air oxidation and gave the trimeric aldehyde (7) in about 20% on standing at room temperature for a month. This observation reasonably explains that freshly cut and thoroughly extracted sections of spruce wood gave a strong coloration for coniferyl alcohol groups, and that the reaction was

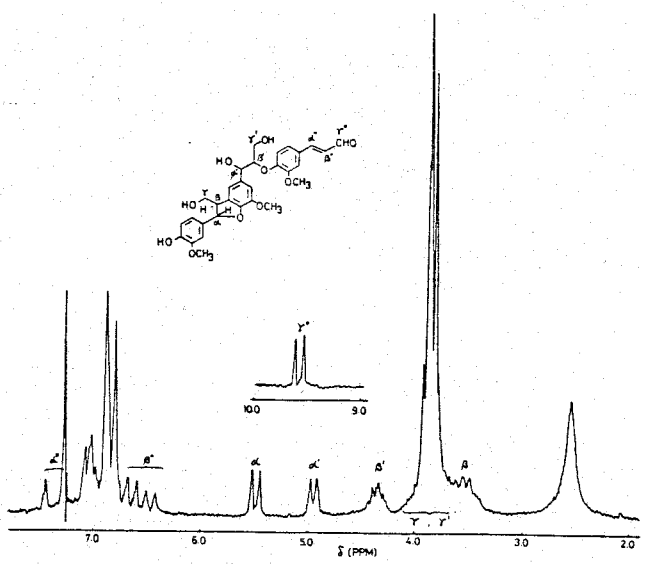


Fig. 3 NMR spectrum of trimeric aldehyde (7)

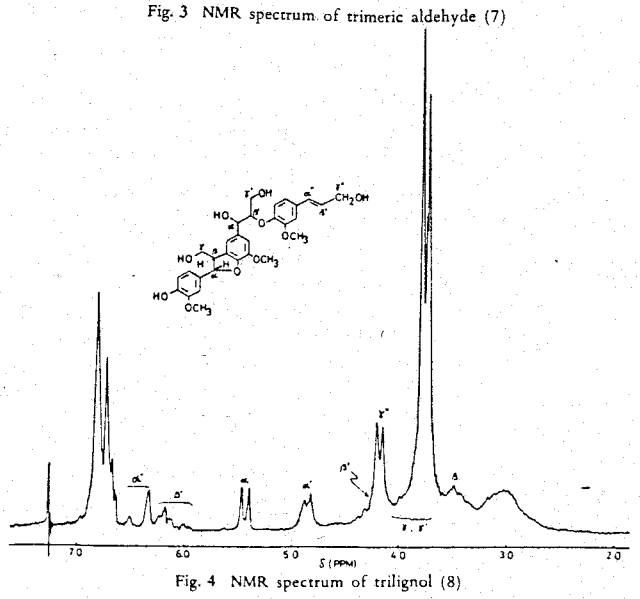


Fig. 4 NMR spectrum of trilignol (8)

negative for sections stored in air for six months[9].

Here, it was confirmed that our synthetic method of β-ether lignin model can be applied to the synthesis of trilignol. It is suspected that the other methods hitherto reported may give unsatisfactory results in the synthesis of the trilignol (8).

II-5-3 Experimental

Coniferyl aldehyde (3)

This synthetic method will be reported in detail elsewhere together with the syntheses of other lignin monomers, p-hydroxy cinnamaldehyde and sinap aldehyde[7].

α-Phenoxy acetate (4)

The reaction mixture containing coniferyl aldehyde (1.7g, 9.5mM), methyl monochloroacetate (1.0 ml. 11.5mM), potassium iodide (2g, 12mM) and potassium carbonate (1.66g, 12mM) suspended in acetone (30 ml) was refluxed for 5 hours with vigorous stirring and then cooled to room temperature. The inorganic salts were filtered off and washed with acetone. The combined filtrate and washings were evaporated *in vacuo* to dryness. The products were partitioned between ethyl acetate and brine. The ethyl acetate layer was dried over Na_2SO_4 and the evaporation of the ethyl acetate *in vacuo* gave a slightly yellow oil which was crystallized from ether to give colorless crystals (2.13g, 91.4%).

Mp 108.5-109.5°C (ether). IR(KBr): 1740, 1670 cm^{-1}. NMR δ(ppm: $CDCl_3$): 3.79(3H,s), 3.92(3H,s), 4.72(2H,s), 6.56(1H,dd,J=16, 8), 6.78(1H,d,J=8), 7.16-6.98(2H,m), 7 36(1H,d.J=16), 9.59(1H,d,J=8).

The suspension containing the aldehyde obtained above (1.22g,

5mM), ethylene glycol (1.4 ml, 25mM) and p-TsOH·H_2O (50 mg) in dry benzene (100 ml) was refluxed for 1.5 hours with vigorous stirring. During the reaction, the water formed was collected azeotropically in a Dean-Stark head. The reaction mixture was cooled, neutralized by the addition of a solid $NaHCO_3$ (about 500 mg) and washed with brine at least three times. The benzene solution was dried over Na_2SO_4 and evaporated *in vacuo*. The resultant slightly yellow syrup was crystallized from methanol to give slightly yellow needles (1.2g, 81.6%).

Mp 76-77°C (methanol). IR(KBr): 1769 cm^{-1}. NMR δ(ppm:$CDCl_3$): 3.78(3H,s), 3.88(3H,s), 3.87-4.09(4H,m), 4.68(2H,s), 5.39(1H,d.J=6), 6.03(1H,dd,J=16, 6), 6.69(1H,d,J=16), 6.67-7.02(3H,m). MS(70 eV): 298(M^+, 85.6), 222(100), 149(95.2).

β-Hydroxy ester (6)

To a stirred solution of lithium diisopropylamide(0.92mM), prepared by the usual method in 10 ml of anhydrous THF (freshly distilled from benzophenone and potassium metal before use), 265 mg (0.92 mM) of α-phenoxy acetate (4) dissolved in 3 ml of anhydrous THF was added dropwise over a period of 30 min. below -70°C under nitrogen. The stirring was continued for an additional 30 min. at the same temperature and then 227 mg (0.46 mg) of phenylcoumaran (5)[1] dissolved in 3 ml of anhydrous THF was added dropwise over a period of 50 min. After the stirring for 30 min., the pale yellow reaction mixture was partitioned between ethyl acetate and brine. The organic layer was washed with brine until the washings became neutral, dried over Na_2SO_4 and evaporated *in vacuo* to give a slightly yellow syrup which was purified by a silica gel

TLC developed twice with ethyl acetate/n-hexane(1;1) and then the pure β-hydroxy ester (6) was obtained as a colorless gaiss (305.3 mg, 84.4% based on the startng phenylcoumaran).

IR(KBr): 1755 cm^{-1}. NMR δ(ppm:$CDCl_3$): 3.70(3H,s), 3.84(3H,s), 3.86 and 3.88(6H, two singlet), 3.85-4.15(4H,m,-O-CH_2CH_2-O-), 4.63(1H, braod t, -O-CH-O- of -O-THP), 4.69(1H,d,J=5.5, β'-CH-), 5.12(1H,m, α'-CH-), 5.37(1H, braod t, -O-CH-O- of pehnolic -O-THP), 5.41(1H,d,J=6, γ"-CH-), 5.58(1H,m, α-CH-), 6.01(1H,dd,J=16, 6, β"=CH-), 6.69(1H,d,J=16, α"-CH=), 6.65-7.20(8H,m). MS(70eV): 413(14.5), 329(7.2), 328(7.2), 312(58), 300(47.1), 294(65.2), 280(10.4), 273(7.2), 235(13), 222(66.7), 149(62.3), 137(29), 121(14.5), 102(20.3), 85(68.8), 84(62.5), 83(34.8), 55(100). UV λ_{max}^{MeOH}nm(log ε): 269(4.31), 300(3.85, sh)..

Trimeric aldehyde (7)

To a stirred solution of lithium aluminum hydride (64.6 mg, 1.7mM) suspended in anhydrous THF (10 ml), 273 mg (0.34mM) of β-hydroxy ester (6) in anhydrous THF (10 ml) was added dropwise over a period of 40 min. at 50-60°C under nitrogen. The stirring was continued for an additional 30 min. After decomposition of excess hydride by the addition of water-THF mixture at 0°C, the reaction mixture was partitioned between ethyl acetate and water. The ethyl acetate layer was washed with brine, dried over Na_2SO_4 and evaporated in vacuo to give a colorless oil which was purified by silica gel TLC developed twice with ethyl acetate/n-hexane (1:1) to give a pure diol compound (243.7 mg, 94%).

NMR δ(ppm:$CDCl_3$): 3.85(3H,s), 3.91 and 3.93(6H, two singlet), 3.85-4.15(4H,m,-O-CH_2CH_2-O-), 4.66(1H,m,-O-CH-O- of γ-O-THP), 4.77 (1H,m, α'-CH-), 5.41(1H,m, -O-CH-O- of phenolic THP), 5.44(1H,d,

J=6, γ"-CH-), 5.60(1H, broad t, α-CH-), 6.10(1H,dd,J=16, 6, β"=CH-), 6.76(1H,d.J=16, α"-CH=), 6.80-7.20(8H,m).

The diol compound obtained above (235 mg, 0.31mM) and pyridinium p-toluenesulfonate (7.8 mg, 0.031 mM) was dissolved in 10 ml of ethanol and heated at 50°C for about 16 hours. The reaction mixture was partitioned between ethyl acetate and brine. The ethyl acetate layer was dried over Na_2SO_4, evaporated in vacuo to give a yellow oil which was purified by silica gel TLC developed with 7% methanol/methylene chloride to yield a pure trimeric aldehyde (7) (120 mg, 70%) as a slightly yellow glass.

Anal.Calcd. for $C_{30}H_{32}O_{10}$: C, 63.15 ; H,6.01, Found: C, 63.57 ; H,5.97. IR(KBr): 1666. NMR δ(ppm:$CDCl_3$/CD_3OD): 3.56(1H, collapsed t, β-CH-), 3.82(3H,s), 3.87(3H,s), 3.88(3H,s), 4.34(1H,m, β'-CH-), 4.94(1H,d,J=5.2, α'-CH-), 5.49(1H,d,J=6.5, α-CH-), 6.56 (1H,dd,J=15.8, 7.3, β"=CH-), 6.73-7,18(8H,m), 7.36(1H,d,J=15.8, α"-CH=), 9.56(1H,d,J=7.3, γ"-CHO)(Fig.3). MS(70eV): 552(M^+, 3.5), 534(3.7), 522(9.3), 516(1.7), 504(5.8), 486(1.5), 474(0.9), 356(10), 344(13), 340(21.9), 328(57.8), 312(56.9), 300(28.1), 204(32.5), 192(17.2), 178(100), 161(29.7), 147(26.6), 137(36.9), 107 (18.8). UV λ_{max}^{MeOH}nm(log ε): 236.2(4.41), 288.2(4.18), 336.5(4.32).

Trilignol (8)

To a stirred solution of the trimeric aldehyde (7) (47 mg, 0.085 mM) dissolved in 3 ml of methanol, 3.2 mg (0.085mM) of $NaBH_4$ was added at 0°C under nitrogen. The stirring was continued for 10 min. The reaction mixture was partitioned between ethyl acetate and brine and then the ethyl acetate layer was dried over Na_2SO_4. Evaporation of the solvent in vacuo gave a colorless oil which

afforded a pure trilignol (8)(45 mg, 96%) by a silica gel TLC developed twice with 7% methanol/methylene chloride.

Anal.Calcl.for $C_{30}H_{34}O_{10}\cdot H_2O$: C,62.92 ; H,6.34, Found:C,63.61 ; H,6.40. NMR δ(ppm:$CDCl_3/CD_3OC$): 3.50(1H,m. β-CH-), 3.74-3.76 (6H, two singlets), 3.80(3H,s), 4.18(2H,d,J=4.8, γ"-CH_2-), 4.86(1H, colapsed d, J=4.6, α'-CH-), 5.43(1H,d,J=6, α-CH-), 6.10(1H, colapsed dt, J=16, 4.8, β"=CH-), 6.41(1H, broad d,J=16, α"-CH=), 6.61-7.11(8H,m)(Fig.4). MS(70eV):554(M^+, 2.1), 536(2.3), 524(2.5), 518(2.5), 506(6.2), 356(12.9), 344(9.2), 340(12.9), 328(25.9), 312(14.8), 300(17.9), 206(100), 180(30.2), 137(39.4). UV λ_{max}^{MeOH}nm (log ε): 267.1(4.23), 285(4.06,sh), 300(3.76,sh).

II-5-3 Summary

A trilignol (8) composed of phenylcoumaran and β-O-4 structures was synthesized in high yield in a series of the synthetic studies of lignin model compounds. This synthetic method should be important for the studies of lignin reactions such as pulping chemical utilization and biodegradation of lignin.

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II-6 Synthesis of a trilignol composed of phenylcoumaran and β-1 structures

II-6-1 Introduction

A trilignol (5) composed of phenylcoumaran and β-1 structures has been isolated from the lignin hydrolysis products of Ezomatsu (Picea jezoensis) as an optically inactive compound[1]. Such a structural unit is considered to play an important role in lignin, and its synthesis is believed to be useful for the studies of lignin reaction such as pulping , chemical utilization and especially biodegradation of lignin.

An optically active trilignol (5) was recently isolated[2] from the heart wood extractives of Japanese larch (Larix leptolepis Gord.), which is interested in relation to study of biogenetic differences between optically inactive lignin and optically active lignans.

Thus, the trilignol (5) is really attractive compound in such fields, but the synthetic method has not yet been reported. The synthesis of a trimeric lignin model compound composed of phenylcoumaran and β-O-4 structures was described in Section II-5[3]. In this Section, the synthesis of the trilignol (5) is described

starting from methyl homovanillate benzyl ether (1) and phenylcoumaran (2)[4], in a series of the synthetic studies on lignin model compounds.

II-6-2 A high yield synthesis of the compound

The synthesis of trilignol (5) may be easily understood based on the synthetic method of 1,2-diarylpropane-1,3-diol previously reported[5]; the compound might be synthesized from homobenzoic acid derivative (1) and benzaldehyde derivative (2) as shown in Fig.1. Lithium enolate prepared from methylhomovanillate benzyl ether (1) and lithium diisopropyl amide (LDA) reacted successfully with the phenylcoumaran (2) in THF below -70°C to give the expected β-hydroxy ester (3) in 87% yield. The compound (3) whcih contains six asymmetric carbons may occur theoretically as a mixture of thirty two diastereomers. However, the phenylcoumaran ring is fixed trans and then the compound (3) presently prepared is considered to consist of sixteen diastereomers. The β-hydroxy ester (3) gave two spots with Rf-value 0.45 and 0.35, respectively, on a silica gel TLC plate developed with ethyl acetate/n-hexane (1:1). Since these two spots become a single spot after acid hydrolysis of THP ethers, the separation of these two compounds is not needed at this stage. The NMR spectrum of the compound (3) is too complicated to be assigned, but the spectrum supported the existence of phenylcoumaran moiety as shown by the acetal protons of alcoholic and phenolic THPs at δ4.00-4.20 (1H,m) and δ5.33(1H, m), respectively, α-methine proton of phenylcoumaran at δ5.51 (1H,m) and furthermore, homovanillate moiety by the benzylic me-

thylene and α'-methine protons at δ4.94-5.17(3H,m) and aromatic protons of benzyl ether at δ7.17-7.39(5H,m). IR spectrum also showed the existence of the ester group at 1745 cm^{-1}. These data and the reaction mechanism support that the structure exactly corresponds to the expected β-hydroxy ester.

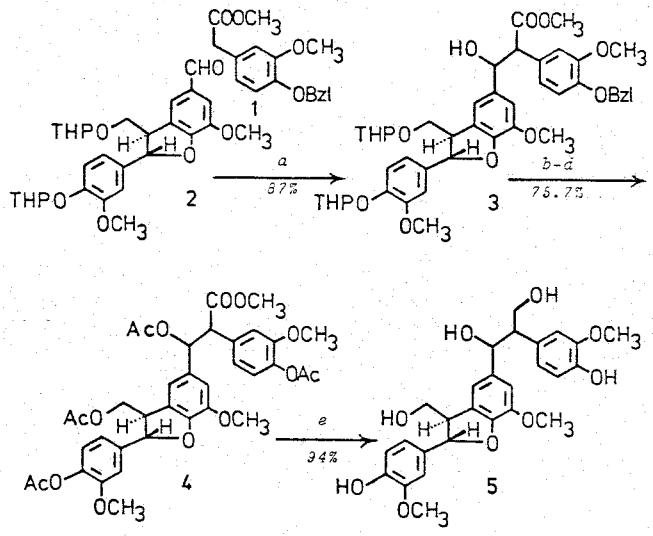

Fig. 1 Synthetic route of a trimeric lignin model composed of phenylcoumaran and β-1 structures
[a] Lithium diisopropyl amide/THF/ below −70°C. [b] AcOH-H_2O (9:1)/50°C (78.3%). [c] H_2/10% Pd-C/MeOH/r.t. (97.4%). [d] Ac_2O/Pyridine/EtOAc/50°C (99.2%). [e] Lithium aluminium hydride/THF/50°C.

The conversion of the β-hydroxy ester (3) to trilignol (5) might be accomplished by the two methods, i.e., 1) LAH reduction to diol derivative and the subsequent removal of the protecting groups and 2) the removal of the protecting groups and the subsequent LAH reduction. The former method which had been used for the synthesis of trilignol composed of phenylcoumaran and β-O-4 structures was examined first[3]. The LAH reduction of the β-hydroxy ester (3) in THF at 50°C gave three spots on a silica gel TLC plate developed with ethyl acetate/n-hexane(1:1), in which the lowest spot gave the expected diol derivative in only 40% yield after the purification by TLC. The other two compounds were found

to be reduction products from the compound (1) and (2) in 45% and 50 % yields, respectively. The formation of these two by-produets are due to the retro-aldol condensation of the β-hydroxy ester under the LAH reduction condition. This retro-aldol condensation was avoided to some extent by the trimethylsilylation of the β-hydroxy ester before LAH reduction[4] and the yield was improved to 66.6 % by this treatment. A similar result had been also obtained in the synthesis of the dimeric 1,2-diarylpropane-1.3-diols[5]. For the complete elimination of such an unexpected reaction, however, the second method shown in Fig.1 was carried out.

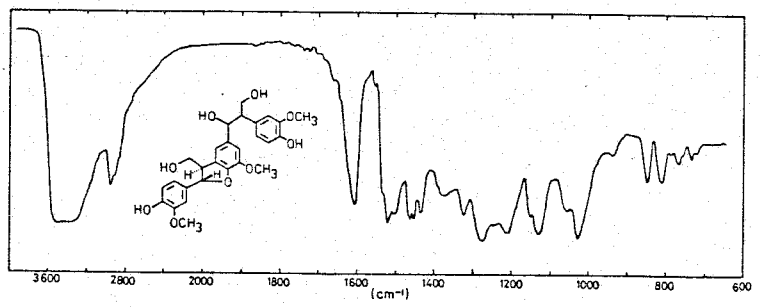

Fig. 2 IR spectrum of a trilignol (5)

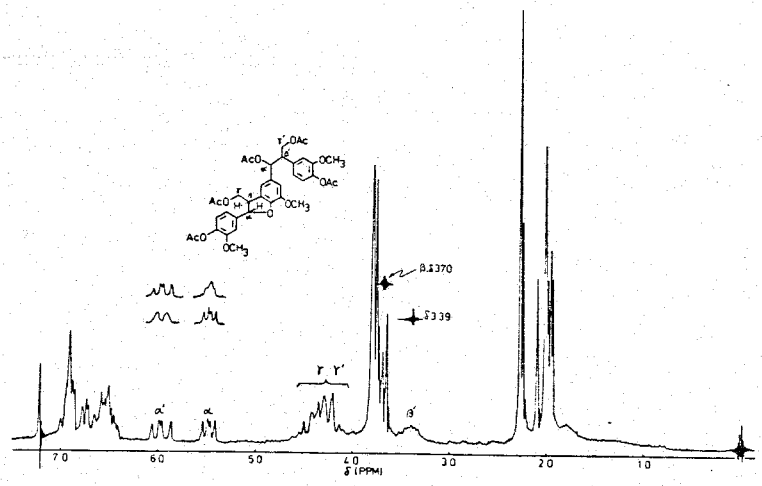

Fig. 3 NMR spectrum of a pentaacetyl trilignol (5)

β-Hydroxy ester (3) was dissolved in a solution of acetic acid/H_2O(9:1) and heated at 50°C for 30 min. under nitrogen. The expected triol derivative whcih was obtained in 78.3% yield was

subjected to the catalytic hydrogenation on 10% palladium carbon to afford the tetraol derivative in 97.6% yield. The acetylation of the tetraol gave the tetraacetyl ester (4) in 99.2% yield. The LAH reduction of the tetraacetyl ester (4) at 50°C in THF gave target molecule, trilignol (5) in 94% yield expectedly. The trilignol (5) is slightly hygroscopic and its elementary analysis corrected for the addition of one mole of water agreed very closely with the calculated value. The spectroscopic data, UV, IR (Fig.2) and Mass were completely identical with those of the lignin hydrolysis product from Ezomatsu (Picea jezoensis) isolated by Sano et al.[1] The structure of the isolated trilignol was, therefore, confirmed by this synthetic method except for the stereochemistry of the side chain.

NMR analysis of the trilignol (5) is difficult because of overlapping of the signals of hydroxyl groups and the protons of the side chains, and then, the NMR spectrum of the acetylated compound was analyzed as shown in Fig.3.Respective peaks of the protons attached to the side chains could be easily assigned by the decoupling method as shown in Fig.3. Although β-proton of the compound could not be found because of overlapping with methoxyl groups, two doublet peaks of α-methine protons at δ5.46 and 5.51 gave a broad triplet by the irradiation at δ3.70 assigned generally as the β-methine proton of phenylcoumaran. On the other hand, two doublet peaks of α'-methine proton at δ5.90 and 6.03 gave a broad doublet by the irradiation of the β'-proton at δ3.40. It was found that the NMR spectrum is somewhat different from that of the isolated compound. The difference between the synthetic and isolated trilignols seems to be ascribed to the different

ratio of the diastereomers. Trilignol containing four asymmetric carbons in which two asymmetric carbons of phenylcoumaran ring are fixed trans, is considered to be a mixture of four diastereomers, α(S)-β(R)-α"(R)-β"(R), α(S)-β(R)-α"(S)-β"(S), α(S)-β(R)-α"(S)-β"(R), α(S)-β(R)-α"(R)-β"(S), respectively. However, threo isomers, α(S)-β(R)-α"(R)-β"(R) and α(S)-β(R)-α"(S)-β"(S) seems to be predominant than erythro isomers, α(S)-β(R)-α"(S)-β"(R) and α(S)-β(R)-α"(R)-β"(S) based on the reaction mechanism via six-membered transition state containing lithium at the step of β-hydroxy ester synthesis. However, no evidence supporting this reasoning has been obtained by this NMR spectrum.

It should be noted that α- and α'-methine protons appear as two doublets with almost the same ratio which is reasonably understood to correspond to threo isomers rather than erythro isomers. To elucidate such a problem, NMR analysis of the phenyl boronate and other six-membered cyclic ketal derivatives of the trilignol (5) is in progress.

II-6-3 Experimental

β-Hydroxy ester (3)

To a stirred solution of lithium diisopropylamide (0.7mM) prepared by the usual method, in 10 ml of anhydrous THF (freshly distilled from benzophenone and potassium metal before use), 200.2 mg (0.7mM) of methyl homovanillate benzyl ether (2) dissolved in 3 ml of anhydrous THF was added dropwise over a period of 30 min. below -70°C under nitrogen. The stirring was continued for additioanl 30 min. at the same temperature and then 233.7 mg (0.469mM)

of phenylcoumaran (1) dissolved in 3 ml of anhydrous THF was added dropwise over a period 30 min. After the stirring for 30 min., the pale yellow reaction mixture was neutralized by the addition of dry ice, and partitioned between ethyl acetate and brine. The ethyl acetate layer was dried over Na_2SO_4 and evaporated *in vacuo* to give a slightly yellow syrup which was purified by a silica gel TLC plate developed with ethyl acetate/*n*-hexane(2:3), and then pure β-hydroxy ester (3) was obtained as a colorless glass (320 mg, 87% based on the starting phenylcoumaran).

IR(KBr): 1745 cm^{-1}. NMR δ(ppm:$CDCl_3$): 3.54, 3.72, 3.80, 3.82 and 3.84(12H, five singlets), 4.00-4.20(1H,m, -O-CH-O- of γ-O-THP), 4.94-5.17(3H,m, $-O-CH_2-Ø$ and α'-CH-), 5.33(1H,m, -O-CH-O- of phenolic -O-THP), 5.51(1H,m, α-CH-), 6.28-7.11(8H,m), 7.17-7.39(5H, m, aromatic protons of benzyl ether).

Tetraacetyl ester (4)

β-Hydroxy ester (131.8 mg, 0.168mM) dissolved in 2ml of acetic acid/H_2O (9:1) was heated at 50°C for 30 min. under nitrogen. The reaction mixture was evaporated *in vacuo* below 55°C to give a colorless syrup which was purified by a silica gel TLC plate developed with ethyl acetate/n-hexane (1:1). The trihydroxyester was obtained as a colorless glass (81 mg, 78.3%).

IR(KBr): 1739 cm^{-1}. NMR δ(ppm:$CDCl_3$): 3.52, 3.54, 3.72, 3.79, 3.80 and 3.82(12H, six singlets), 4.94-5.18(3H,m, $-O-CH_2-Ø$ and α'-CH-), 5.37-5.54(1H, broad t, α-CH-), 6.22-6/94(8H,m), 7.17-7.44(5H,m, aromatic protons of benzyl ether).

Trihydroxy ester (79 mg, 0.128mM) obtained above was dissolved in 10 ml of methanol and 40 mg of 10% Pd-C was suspended to

the solution. The reaction mixture was stirred under hydrogen for 30 min. at room temperature, and filtered, and then Pd-C was washed with methanol. The combined filtrate and washings was evaporated *in vacuo* to give a colorless syrup (66 mg) which was acetylated at 50°C for 2 hours by the treatment of 0.2 ml of acetic anhydride/pyridine (1:1) in 3 ml of ethyl acetate. The reaction mixture was evaporated *in vacuo* to give a colorless syrup which was purified by a silica gel TLC plate developed with ethyl acetate/*n*-hexane(1:1). The tetraacetyl ester (5) was obtained as a colorless glass (85.8 mg, 96.6% overall yield from trihydroxy ester).

IR(KBr): 1745, 1770 cm^{-1}. NMR δ(ppm:$CDCl_3$): 1.81, 1.97, 2.02 2.04 and 2.06(6H, five singlets, alcoholic acetates), 2.23 and 2.28(6H, two singlets, phenolic acetates), 3.52, 3.67, 3.70, 3.73, 3.81, 3.83 and 3.89(12H, nine singlets, methyl ester and methoxyl), 3.67(1H,m, β-CH-), 3.39(1H,m, β'-CH-), 4.00-4.44(2H,m, γ-CH_2-), 5.25-5.57(1H, collapsed t, α-CH-), 6.11 and 6.33(1H, two doublets, J=10, α'-CH-), 6.33-7.06(8H,m, aromatic).

Trilignol (5)

To a stirred solution of lithium aluminum hydride (47.12 mg, 1.24mM) suspended in 10 ml of anhydrous THF, 85.8 mg (0,124mM) of tetraacetyl ester (4) dissolved in 5 ml of anhydrous THF was added dropwise at 50°C over a period of 30 min. under nitrogen. After decomposition of excess hydride by the addition of water-THF mixture at 0°C, the reaction mixture was neutralized by the addition of dry ice, and then partitioned between ethyl acetate and water. The ethyl acetate layer was washed with brine, dried

over Na_2SO_4 and evaporated in vacuo to give a colorless glass which was purified by a silica gel TLC plate developed twice with 7% methanol/methylene chloride. Trilignol (5) was thus obtained as a colorless glass (60 mg, 94%).

Anal. Calcd. for $C_{27}H_{30}O_9 \cdot H_2O$: C, 62.78 ; H, 6.24, Found: C, 62.75 ; H, 6.24. IR(KBr): Fig.2. NMR δ(ppm:$CDCl_3$)(Acetate): 1.96, 1.99, 2.00, 2.01 and 2.11(9H, five singlets, alcoholic acetates), 2.26 and 2.29(6H, two singlets, phenolic acetates), 3.40(1H,m, β'-CH-), about 3.70 (1H,m, β-CH-), 3.66, 3.70, 3.74 3.77 and 3.81(9H, five singlets, methoxyl), 4.07-4.60(4H,m, γ- and γ'-CH_2-), 5.46(d,J=6.8) and 5.51(d,J=6.8) (1H, α-CH-), 5.90 (d,J=8.3) and 6.03(d,J=7.4)(1H, α'-CH-), 6.39-7.09(8H,m, aromatic). MS(70ev): 480(1.9), 462(8.6), 450(41.4), 432(89.3), 420(100), 150 (55.4), 137(96.2). UV λ_{max}^{MeOH}nm(log ε): 281.3(3.88).

II-6-4 Summary

A trilignol (5) composed of phenylcoumaran and β-1 structures, a main substructure compound in lignin, was synthesized in high yields in a series of the synthetic studies of lignin model compounds. This synthetic method should be important for the studies of lignin reactions such as pulping, chemical utilization and biodegradation of lignins.

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CHAPTER III

CONCLUSION

Enzimic dehydrogenation of p-coumaryl alcohol

p-Coumaryl alcohol was dehydrogenated with peroxidase/H_2O_2 to give four dimeric compounds, p-coumarylresinol, dehydrodi-p-coumaryl alcohol, p-hydroxyphenylglycerol-β-p-coumaryl ether and 2-(4-hydroxyphenyl)-3-hydroxymethyl-4-(α,4-dihydroxybenzyl)-tetrahydrofuran (monoepoxylignan). These dimers corresponded to those from coniferyl alcohol except for monoepoxylignan resulted in the mesoid β-β' coupling which was first identified in the present experiment (Section I-1). Furthermore, p-hydroxyphenylglycerol was isolated from aqueous solution of dehydrogenation mixture of p-coumaryl alcohol after extraction with ethyl acetate, and identified by GC-MS spectrometry compared with the synthetic compound. Both guaiacylglycerol and syringylglycerol were also isolated from the enzymic dehydrogenation products of the corresponding p-hydroxycinnamyl alcohols and identified. These arylglycerols were a mixture of the threo and erythro isomers and the amount of the former was 1-4 times higher than that of the latter. Arylglycerol was suggested to be formed from the corresponding p-hydroxycinnamyl alcohols with peroxidase/H_2O_2 and incorporated into arylglycerol-β-arylglycerol ether substructure in lignin polymer during dehydrogenation (Section I-2).

The direct chemical proof on the configuration of phenylcoumaran ring has not yet been reported and then the stereochemistry of the both rings of dehydrodi-p-coumaryl alcohol (Section I-1) and dehydrodiconiferyl alcohol obtained by the dehydrogenation of coniferyl alcohol was investigated chemically in the

Section I-3. The γ-methyl deriyative of dihydrodehydrodiconiferyl alcohol synthesized by the reduction of the double bond in the side chain and also of γ-hydroxymethyl to methyl group was completely identical with methyl dihydrodehydrodiisoeugenol whose trans configuration had been determined by ozonolysis. The γ-methyl derivative of dihydrodehydrodi-p-coumaryl alcohol was synthesized subsequently by the same method and its trans configuration was determined by the comparison of its NMR spectrum with that of methyl dihydrodehydrodiisoeugenol.

Four dilignols, p-coumarylresinol, dehydrodi-p-coumaryl alcohol, p-hydroxyphenylglycerol-β-p-coumaryl ether and monoepoxylignan isolated and identified in Section I-1 were analyzed by both gas chromatography and NMR spectrometry, and the ratio of the amounts of former three dilignols was found to be 31 : 49 : 20. Furthermore, following four points were clarified by gas chromatographic analysis; 1) 5-5' dilignol was trace (0.6%), 2) 1,2-di-p-hydroxyphenylpropane-1,3-diol which is considered to be formed by β-1 coupling could not be found. 3) the ratio of the racemoid and mesoid couplings at C-β and C-β' carbons was about 9.4 : 1. 4) p-hydroxyphenylglycerol-β-p-coumaryl ether was a mixture of erythro and threo isomers (1 : 4.7). These results indicated that coniferyl and p-coumaryl alcohols have almost the same reactivity on enzymic dehydrogenation (Section I-4).

As described in Section I-2 and I-4, the water addition to quinonemethide intermediate which is formed by radical coupling during enzymic dehydrogenation participates in the formation of arylglycerols and p-hydroxyphenylglycerol-β-p-coumaryl ether. Both compounds occurred as diasereomeric mixture and threo isomers are predominant than erythro counterparts. This is different from

the expectation that the attack of nucleophile, water in this case, to quinonemethide may occur with the same probability from the both sides of planner molecule to afford 1 : 1 mixture. It was found in Section I-5 that the rate of the reaction between quinonemethide and various nucleophiles and also the stereochemistry of the products depend on the acidity and the steric factor of the nucleophiles. It was suggested that water attacks predominantly from the same side with β-hydroxyl or β-phenoxy groups of quinonemethide by forming a hydrogen bonding between water and oxygen atom of β-hydroxyl or β-phenoxy groups, resulting in threo isomer.

The present study on the dehydrogenation of p-coumaryl alcohol described in Chapter I might be better to be extended in connection with the structural studies of grass lignins and also of compression wood lignin which contains much more p-hydroxyphenyl unit compared with normal wood lignin. The remarkable difference of reactivity between coniferyl and p-coumaryl alcohols is the possible contribution by condensation via C_5-position of the latter alcohol; it is expected that the prolonged dehydrogenation of p-coumaryl alcohol and aging of the DHP in acid condition promote the formation of C-C linkage via C_5.

Syntheses of oligolignols

Considering the main substructure units in lignins, such as β-O-4(1), β-5(2), β-1(3) and β-β' (4), the common structural unit in these substructures is thought to be p-oxyphenylpropane-1,3-dioxy structure (5) as shown by broad lines in Fig.1 which is equivalent to p-hydroxyphenylpropane-1,3-dihydroxy system (6) from the synthetic point of view. Therefore, the synthesis of lignols and lignin model compounds would be understood as a problem how to prepare

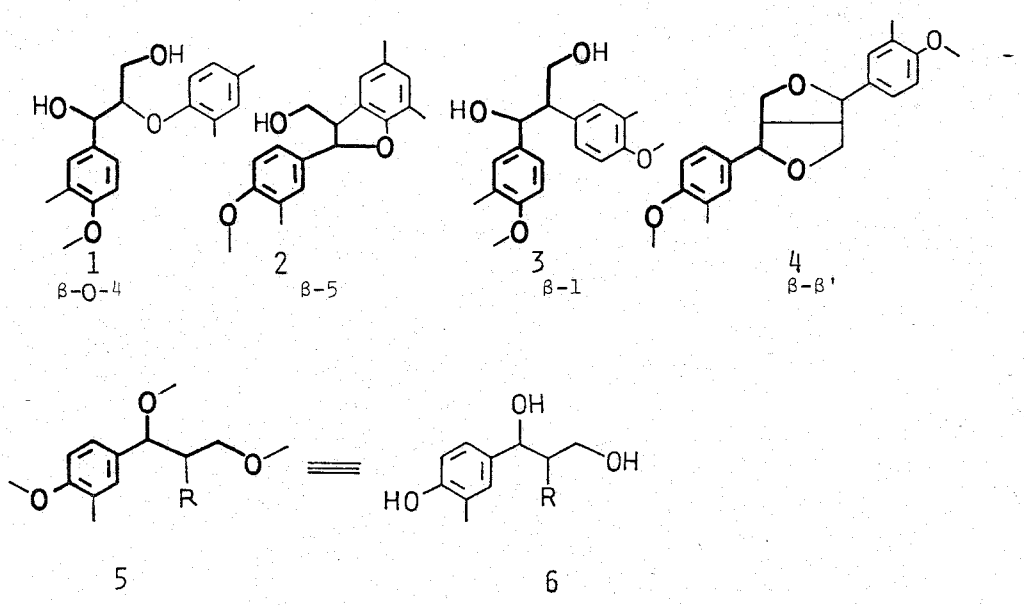


Fig.1 Common structural unit in lignin substructures

this system successfully. In consideration of a "rational" synthetic method affording this system, p-hydroxyphenylpropane-1,3-diol (6) would be prepared by the three synthetic routes depending on the key intermediates as shown in Fig.2. As the benzylcarbinol group is presumed to be equivalent to the ketone group, the β-hydroxy ketone (7) might be synthesized by an aldol con-

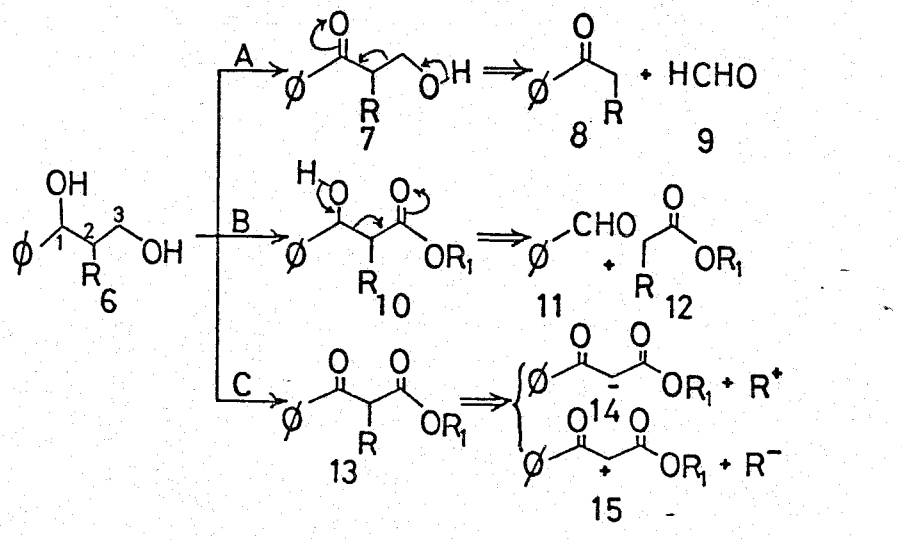


Fig.2 Synthetic methods of p-hydroxyphenylpropane-1,3-diol (6)

densation of the acetophenone derivative (8) and formaldehyde (9) (method A). On the other hand, presuming that the terminal hydroxymethyl group is equivalent to the ester group, this system would be converted into β-hydroxy ester (10) which could be synthesized anti-synthetically by an aldol condensation between acetate (12) and benzaldehyde derivative (11) (method B). The third method C involves β-keto ester (13) as key intermediate which is derived from the consideration that the terminal hydroxymethyl and benzylcarbinol groups are thought to be equivalent to ester and keto

Compounds / Methods	β-O-4	β-5	β-1	β-β'
A	O		O	
B	O	O	O	O
C	O			O

A(β-O-4): E.Adler,et al., Sv. Papperstidn.,55,245(1952)
E.Adler,et al., Acta Chem. Scand.,9,341(1955)
K.Freudenberg,et al., Chem. Ber.,88,626(1955)
K.Kratzl,et al., Monatsh.,90,771(1959)
H.Nima, Chem. Ber., 100,2633(1967)
G.E.Miksche, Acta Chem. Scand.,27,1355(1973)
S.Hosoya,et al.,Mokuzai Gakkaishi,26,118(1980)
B(β-O-4): K.Freudenberg,et al., Ann.,584,40(1953)
K.Lundquist,et al.,Acta Chem. Scand.,29,1355(1973)
F.Nakatsubo,et al.,Holzforschung,29,165(1975)
K.Lundquist,et al.,Acta Chem. Scand.,31,725(1977)
F.Nakatsubo,et al.,Wood Research,66,23(1980)
F.Nakatsubo,et al., Mokuzai Gakkaishi,26,31(1980)
T.Katayama,et al., ibid,26(1980)
H.Namba,et al., ibid,26(1980)
Y.Kamaya,et al., ibid, 26(1980)
C(β-O-4): I.A.Pearl,et al.,J.Org. Chem.,27,2111(1962)
G.E.Miksche,et al., Acta Chem. Scand.,20,1038(1966)
B(β-5) : F.Nakatsubo,et al.,Mokuzai Gakkaishi,25,735(1979)
A(β-1) : K.Lundquist,et al., Terahedron Letters,25,2131(1965)
B(β-1) : F.Nakatsubo,et al., Holzforschung,29,193(1975)
F.Nakatsubo,et al., Mokuzai Gakkaishi,26,107(1980)
B(β-β') : H.Fujimoto,et al., ibid,26(1980)
C(β-β') : K.Kratzl,et al.,Monatsh.,94,434(1963)

Table 1. Synthetic methods of lignin related compounds reported hitherto and hereafter

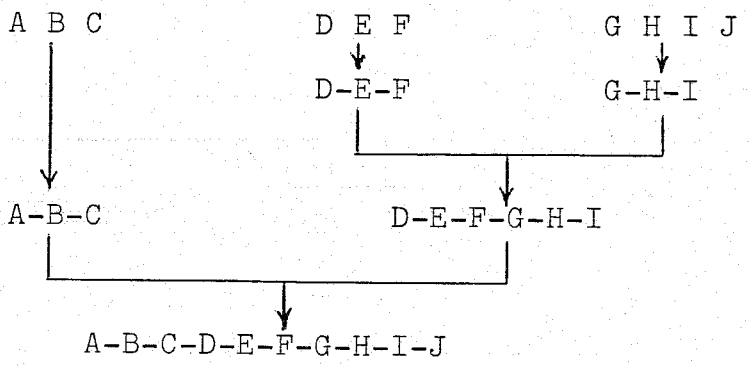
groups, respectively. The β-keto ester intermediate (13) could be used as carbanion source (14) treating with base and also as carbonium ion source (15) after halogenation in carbon-carbon bond formation. The synthetic method of oligolignols and lignin model compounds reported hitherto and hereafter are summarized on Table 1 based on this basic idea. The circles on the table indicate the methods reported already. For example, A(β-O-4) shows the synthetic method of guaiacylglycerol-β-guaiacyl ether by E.Adler *et al.* and K.Kratzl *et al.*, B(β-O-4) shows the convergent synthetic method by F.Nakatsubo *et al.*, and C(β-O-4) shows the method by I.A.Pearl *et al.*, and G.E.Miksche *et al.* Among these three methods A, B and C, the method B seems to be especially attractive because of the convergent synthesis involving direct α,β-bond formation of lignin substructures.

In general, two major synthetic ways are conceivable to polyatomic molecules: In the "linear" method the molecule comprised of the units A,B,C,D,.....M is synthesized commencing with unit A, adding B, subsequently adding C to the resultant A-B, and so on:

$$A \xrightarrow{B} A\text{-}B \xrightarrow{C} A\text{-}B\text{-}C \xrightarrow{D} A\text{-}B\text{-}C\text{-}D \longrightarrow \text{etc.}$$

Since organic reactions scarcely give a 100% yield it is clear that with the linear method a long sequence of reactions will require a large amount of starting material A. Adding a similar amount of unit B may increase the yield of A-B but soon the amount of A-B-C-D becomes larger compared to the added units E,F,G and the yield may decrease alarmingly resulting in very little end product.

For this reason the second "convergent" method is generally preferable: the units A-B-C, D-E-F, G-H-I-J which are separately built up by the linear method would be linked together subsequently. This strategy enables wokers to keep reasonable amounts of the units A-B-C and D-E-F separately: when approximately equal weights of the both units are used the resulting A-B-C-D-E-F is probably obtained in good yield. Another advantage of the convergent method is that even if a batch of A-B-C is inadvertently lost it does not bring a catastrophic result because these subunits will be obtained by the comparative short steps different from the case of that the material A-.....J becomes increasingly precious as single units in the linear method. In general, for short syntheses the linear method may be used whereas for long syntheses a combination of the linear and convergent methods may be preferable.



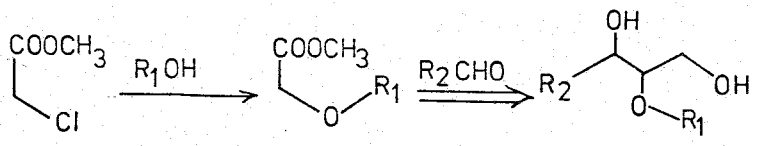
Consequently, although the "linear" method A and C in Fig.2 could be used for the syntheses of comparatively smaller molecules, i.e., monomeric and dimeric compounds, the syntheses of other higher lignols, trimeric and tetrameric compounds etc. would be achieved only by the convergent method B which make possible the synthesis of any kind of oligolignols.

In Chapter II, based on the above discussion the generality

of this convergent synthetic method B was confirmed experimentally. In Section II-1, the high yield convergent synthesis of guaiacylglycerol-β-guaiacyl ether which has been used as the most popular model for the β-O-4 substructure in lignins was achieved via five steps involving key condensation between ethyl 2-methoxyphenoxy acetate and benzylvanillin in the presence of lithium diisopropyl amide. The overall yield of the expected compound from benzylvanillin was about 72% which is the best yield recorded so far. This basic idea was further applied for the synthesis of dilignols, guaiacylglycerol-β-coniferyl and β-coniferyl aldehyde ethers which are difficult to obtain by the dehydrogenation of coniferyl alcohol and the first high yield synthesis of these compounds was accomplished as described in Section II-2. In Section II-3, a new general synthesis involving β-hydroxy ester key intermediate of 1,2-diarylpropane-1,3-diols containing different aryl groups was established in high yield and their configurations were determined by NMR analysis of their phenyl boronate derived from the 1,3-propanediol system of the lignols. The first synthetic method of dehydrodiconiferyl alcohol is described in Section II-4 and the method was applied successfully for the syntheses of different types of phenylcoumarans which have been isolated from the lignin degradation products in low yield but hardly from the dehydrogenation of p-hydroxycinnamyl alcohols. The usefulness and validity of the above synthetic methodology was further proved by its application for the syntheses of trilignols composed of phenylcoumaran, β-O-4 and β-1 substructures as described in Section II-5 and II-6, respectively.

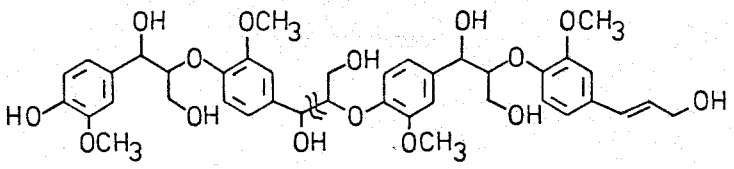
Arylglycerol-β-aryl ether (β-O-4) structure is the most

important substructure in lignins, and 30-50% or more of the phenylpropane units have been found as this structure. It is interesting to know that the present convergent synthetic method could be applied for the syntheses of another higher oligolignols by the β-O-4 bond formation between oligomers which have been synthesized so far: The principle of the present synthetic method is the direct α,β-bond formation by the use of commercially available methyl monochloroacetate, "joint reagent", as two-carbon source between a phenol (R_1OH) and a benzaldehyde derivative (R_2-CHO) as follow:



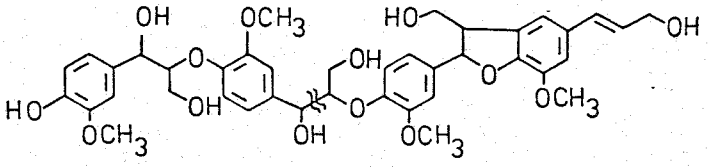
R_1(monomer) + R_2(monomer) ⟶ Dimer
R_1(monomer) + R_2(dimer) ⟶ Trimer
R_1(dimer) + R_2(monomer) ⟶ Trimer
R_1(dimer) + R_2(dimer) ⟶ Tetramer
R_1(trimer) + R_2(dimer) ⟶ Pentamer
R_1(trimer) + R_2(trimer) ⟶ Hexamer

R_2= G-p(β-O-4)-G-CHO + R_1= G-p(β-O-4)-G-p



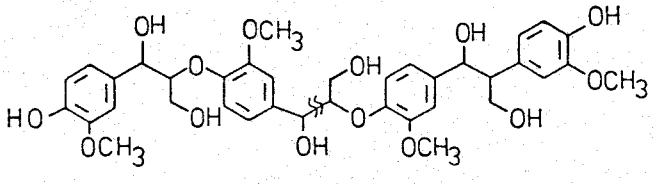
G-p(β-O-4)-G-p(β-O-4)-G-p(β-O-4)-G-p **(16)**

R_2= G-p(β-O-4)-G-CHO + R_1= G-p(β-5)-G-p



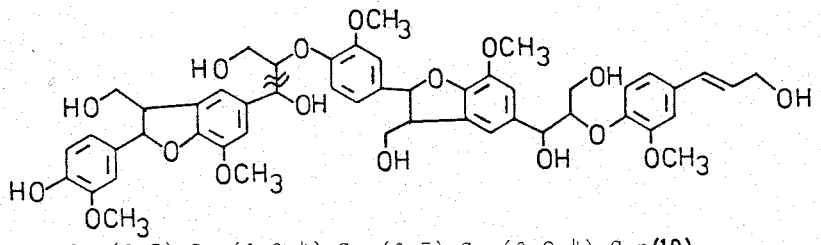
G-p(β-O-4)-G-p(β-O-4)-G-p(β-5)-G-p **(17)**

R_2= G-p(β-O-4)-G-CHO + R_1= G-p(β-1)-G



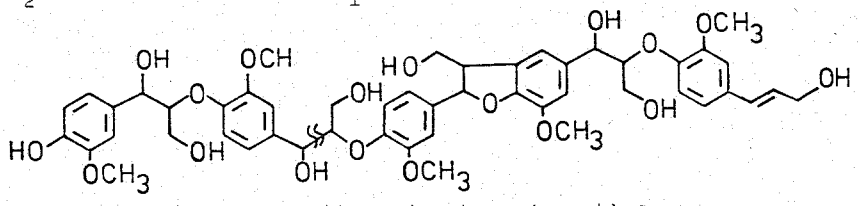
G-p(β-O-4)-G-p(β-O-4)-G-p(β-1)-G **(18)**

R_2= G-p(β-5)-G-CHO + R_1= G-p(β-5)-G-p(β-O-4)-G-p



G-p(β-5)-G-p(β-O-4)-G-p(β-5)-G-p(β-O-4)-G-p **(19)**

R_2= G-p(β-O-4)-G-CHO + R_1= G-p(β-5)-G-p(β-O-4)-G-p



G-p(β-O-4)-G-p(β-O-4)-G-p(β-5)-G-p(β-O-4)-G-p **(20)**

If a dimeric benzaldehyde derivative, G-p(β-O-4)-G-CHO and a dimeric phenol, G-p(β-O-4)-G-p synthesized in Section II-2 were used as the starting materials, finally the tetrameric lignol (16) composed of only β-O-4 linkage would be obtained as end product. Similarly if a dimeric benzaldehyde, G-p(β-5)-G-CHO and a trimeric phenol, G-p(β-5)-p(β-O-4)-G-p synthesized in Section II-4 and II-5, respectively were used, a pentameric lignol (19) would be prepared and so on.

Thus, the convergent synthetic method B of oligolignols involving β-hydroxy ester key intermediate prepared by the use of lithium diisopropyl amide has satisfactorily established, and the method would be applied successfully for the syntheses of higher oligolignols, tetramers, pentamers and hexamer etc., only in consideration of protecting groups. It seems that the present synthetic strategy would be increasingly important for the future lignin chemistry toward the complete utilization of lignins, fourth period in lignin history after the end of third period in 1970.

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