Enzymic Dehydrogenation of *p*-Coumaryl Alcohol. III. Analysis of Dilignols by Gas Chromatography and NMR Spectrometry*

Fumiaki NAKATSUBO** and Takayoshi HIGUCHI**

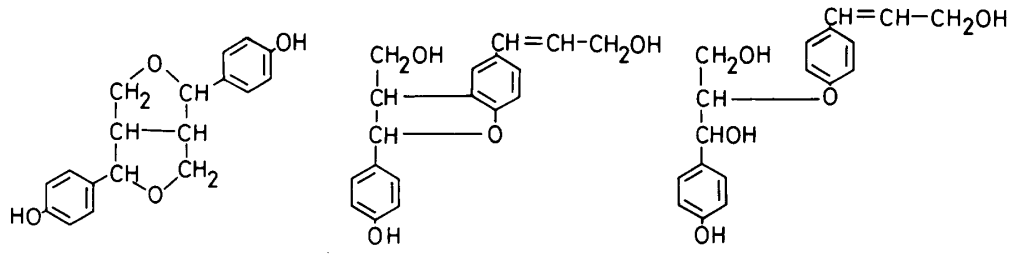
Abstract——*p*-Coumaryl alcohol was dehydrogenated with peroxidase and H_2O_2 system. Five dilignols, *p*-coumarylresinol (I), dehydrodi-*p*-coumaryl alcohol (II), *p*-hydroxyphenylglycerol-*β*-*p*-coumaryl ether (III), monoepoxylignan (IV) and 5-5′-dilignol (V) were identified and determined by both gas chromatography and NMR spectrometry, and the ratio of the amounts of the three main dilignols (I, II and III) was 31 : 49 : 20. The dilignol (V) was trace in amount (0.6 %), and 1, 2-diarylpropane-1, 3-diol (VI) 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 (III) 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.

Introduction

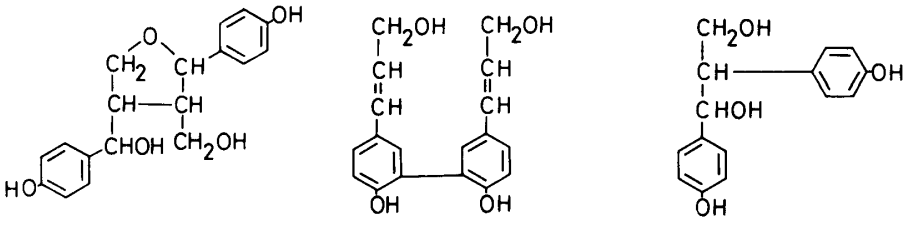
In 1951, Freudenberg *et al.*[1] reported that *p*-coumaryl alcohol produced a very similar dehydrogenation polymer (DHP) to that of coniferyl alcohol based on the hydrogen uptake by the both DHP's and their elementary analysis. Later, Bland *et al.*[2] reported that an artificial lignin prepared from *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 occured in their DHP formation from the yield of the condensed and noncondensed 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 the previous paper, the isolation and identification of the four dilignols of *p*-coumaryl alcohol, *p*-coumarylresinol (I), dehydrodi-*p*-coumaryl alcohol (II), *p*-hydroxyphenylglycerol-*β*-*p*-coumaryl ether (III) and monoepoxylignan (IV)[4], and

* Partly presented at the 23th Meeting of Japan Wood Research Society, Kyoto, April 1973.
** Division of Lignin Chemistry.
This paper is dedicated to Prof. Dr. Karl Kratzl, Organisch-Chemischen Institut der Universität Wien for his 60th birthday.



I II III



IV V VI

the *trans* configuration of the coumarane ring of the dilignol (II)[5] were reported. In this paper, configuration of the dilignol (III) and the yields of these dilignols determined by gas chromatography and NMR spectrometry are reported.

Experimental

Preparation of authentic dilignols (I), (II), (III), (IV), (V) *and* (VI)

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

Tetrahydro-5-5′-dilignol (V) 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 F1-3-S-type), and easily isolated by preparative TLC. NMR δ (ppm: $CDCl_3$) of tetra acetate: 1.99 (6 H, s, γ, γ'-acetyl groups), 2.01 (6 H, s, phenolic acetyl groups), 1.80~2.30 (4 H, m, β, β'-methylene protons), 2.69 (4 H, t, J=6.5, α, α'-methylene protons), 4.01 (4 H, t, J=6.5, γ, γ'-methylene protons), 6.90~7.30 (8 H, 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 shielding effects of each aromatic rings. MS (70 eV) : 320 (M^+, 100), 256 (66.7), 239 (57.2), 211 (35), 197 (33.4).

1, 2-Diarylpropane-1, 3-diol (VI) was synthesized by condensation of benzyl *p*-hy-

droxybenzaldehyde 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 will be reported elsewhere. NMR δ (ppm : CD_3COCD_3) : 2.70~3.20 (1 H, m, β-methine proton), 3.50~4.20 (2 H, m, γ-methylene protons), 4.89 (1 H, d, J=9.0, α-methine proton of *threo* isomer), 5.02 (1 H, d, J=5.5, α-methine proton of *erythro* isomer), 6.55~7.10 (8 H, m, aromatic protons).

Dehydrogenation of p-coumaryl alcohol

Dehydrogenation of *p*-coumaryl alcohol was carried out by the method reported previously[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 5 mg) 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 diligonls 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 SHIMAZU-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 a 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.

Results and Discussion

Configuration of the dilignol (III)

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 compari-

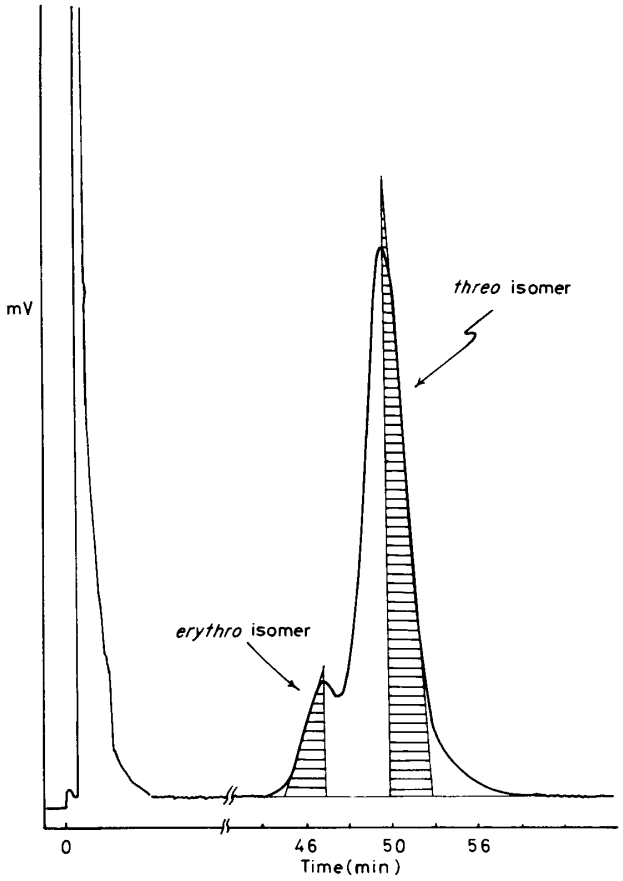


Fig. 1. Gas chromatogram of TMS derivatives of dihydro-dilignol (III). Column: 2% OV-17 on chromosorb AW, 2 m-glass column, 200°C. Carrier gas: helium, 28 ml/min.

son with the results reported 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 (1 H, d, J=5.0, *erythro* isomer) and δ 6.17 (1 H, 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 (III) gave two doublet peaks at δ 6.09 (1 H, d, J=5.0) and δ 6.13 (1 H, d, J=6.2) 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 (III) 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 (III) was a mixture consisting of *erythro* and *threo* isomer whose ratio was 1 : 4.7.

Analysis of the dilignols by gas chromatography and NMR spectrometry

A dilignol fraction was converted to its hydro-dilignol fraction by catalytic hydrogenation with 5% Pd-C and hydrogen 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 (II) and (III) *etc.*, on gas chromatogram are 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 are almost proportional to their amounts.

Second, the 5-5′-dilignol (V) is seemed 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 opened compound has been reported by catalytic hydrogenation of the dilignol (II) in methanol, the mixed solvent, dioxane/methanol (2 : 1) which avoided the ring opening had to be used[5].

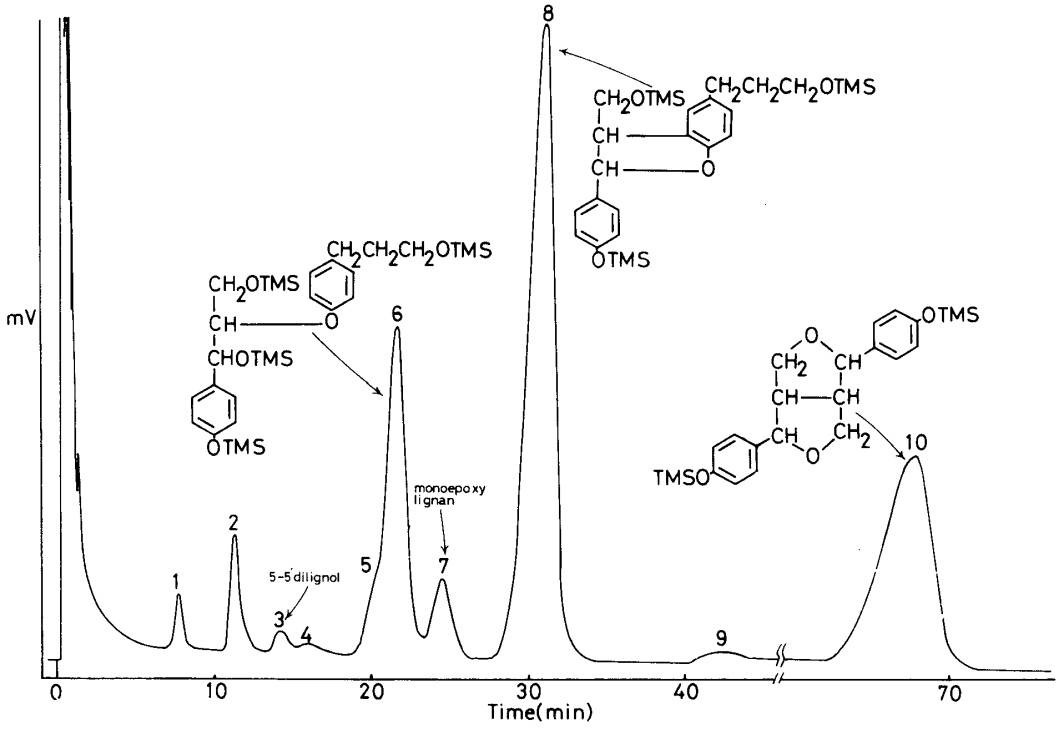


Fig. 2. Gas chromatogram of TMS derivatives of hydro-dilignol fraction obtained by dehydrogenation of *p*-coumaryl alcohol with H_2O_2 and peroxidase system. Column : 2 % OV-17 on chromosorb AW, 2 m, 220°C. Peak 3 : tetrahydro dilignol (V), Peak 5 : dihydro *erythro* dilignol (III), Peak 6 : dihydro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

Figure 2 shows the gas chromatogram of TMS derivatives of the hydro-dilignol fraction. Six of ten peaks were identified by comparison of the retention times and mass fragmentation patterns of authentic dilignols. The amounts of the dilignols are calculated from the peak areas, and summarized in Table 1. In the Table, column (A) is the retention times of each peak, and (B), (C) and (D) are peak areas, ratio of

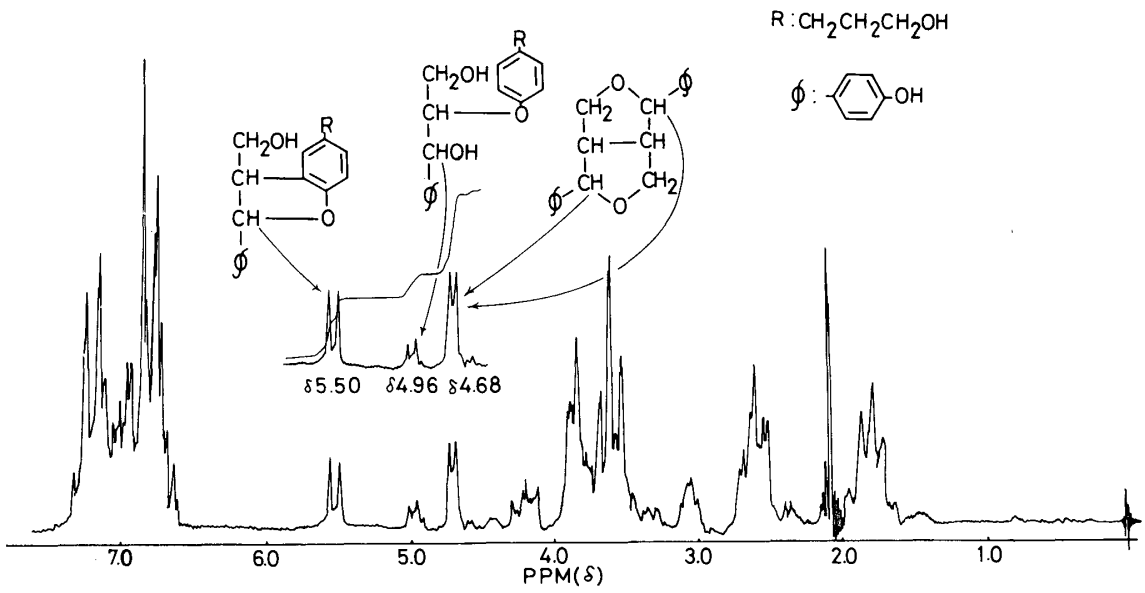


Fig. 3. NMR Spectrum of hydro-dilignol fraction obtained by dehydrogenation of *p*-coumaryl alcohol.

the peak areas and ratio of the amounts of three main dilignols, respectively. Column (F) shows the ratio of the amounts of three main dilignols of coniferyl alcohol which was reported earlier[9].

The NMR spectrum of the hydro-dilignol fraction is shown in Fig. 3. α-Methine protons of the three main dilignols (I), (II) and (III) give the peak at δ 4.68 (2 H, d, J=4.0), δ 5.50 (1 H, m) and δ 4.69 (1 H, d, J=6.0), respectively and these peaks are not interfered with other peaks. Therefore, the ratio of the amounts of these dilignols

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.

will be determined by the integration curve of their α-methine peaks. The result is given in the column (E) of the Table 1.

The following results were obtained from these data. TMS derivative of synthetic 1, 2-diarylpropane-1, 3-diol (VI) 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 (VI) by alkali degradation could not be found in the alkali degradation products of DHP and dilignol fraction. Therefore, the dilignol (VI) seems to be formed at a later dehydrogenation stage. Only 0.6 % of 5-5'-dilignol (V) 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 (I) and monoepoxylignan (IV) which were formed on the racemoid and mesoid coupling 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 progressing. The ratio of the amounts of the three main dilignols (I), (II) and (III) 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 (I), (II) and (III) 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 the 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 coumarane type dilignols are larger than that of β-ether type dilignols.

Both *p*-hydroxy cinnamyl alcohols, *p*-coumaryl and coniferyl alcohols have a similar reactivity on enzymic dehydrogenation as described above, but a typical difference appears on the amounts of the coumaranes. That is, the percentage of dehydrodiconiferyl alcohol (54 %) is larger than that of dehydrodi-*p*-coumaryl alcohol, dilignol (II) (49 %), indicating the radical activating effect of the methoxyl group at 3-position of aromatic ring.

Acknowledgement

This investigation was supported in part by a Scientific Research Fund of the Ministry of Education.

References

1) K. FREUDENBERG and G. GEHRKE, Chem. Ber., **84**, 443 (1951).

2) D. E. BLAND and A. F. LOGAN, Biochem. J., **95**, 515 (1965).
3) T. YAMASAKI, K. HATA and T. HIGUCHI, Mokuzai Gakkaishi, **18**, 361 (1972).
4) F. NAKATSUBO and T. HIGUCHI, Holzforschung in press.
5) F. NAKATSUBO and T. HIGUCHI, Holzforschung in press.
6) F. NAKATSUBO, K. SATO and T. HIGUCHI, Holzforschung in press.
7) G. E. MIKSCHE, J. GRATZL and M. FRIED-MATZKA, Acta Chem. Scand, **20**, 1038 (1966).
8) K. FREUDENBERG and KLAUS-CHRISTIAN RENNER, Chem. Ber., **98**, 1979 (1965).
9) K. V. SARKANEN and C. H. LUDWIG, Lignins, 140 (1971), John Wiley Sons, Inc., New York.