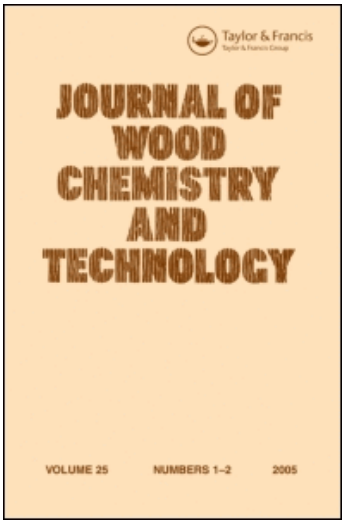

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Application of the DFRC (Derivatization Followed by Reductive Cleavage) Method to Dilignols

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Abstract: The DFRC method was applied to dimeric model compounds of lignin; dehydrodiconiferyl alcohol, syringaresinol, pinoresinol, syringylglycerol-β-sinapyl ether, and guiacylglycerol-β-coniferyl ether. Dehydrodiconiferyl alcohol yielded no coniferyl alcohol, but syringaresinol and pinoresinol gave small amounts of corresponding *p*-hydroxycinnamyl alcohol. In the case of syringaresinol, an acetylated bromo dihydro-(Compound 1, 23%) and tetrahydronaphthalene (Compound 2, 52%) were obtained after acetyl bromide treatment, the first step in the DFRC method. Further, a small amount of sinapyl alcohol diacetate was obtained from compounds 1 and 2 by zinc and successive acetylation treatments. Compound 2 yielded a larger amount of sinapyl alcohol than compound 1.

Keywords: DFRC method, lignin dimmers, β-aryl ethers, syringaresinol, pinoresinol, dehydroiconiferyl alcohol

INTRODUCTION

The DFRC method, which involves three steps: acetyl bromide treatment, zinc treatment, and acetylation, was developed as a simple method for a selective

We thank Dr. Shingo Kawai, Shizuoka University, for giving us guiacylglycerol-β-guiacyl ether. We also thank Dr. Siaw Onwona-Agyeman, Gifu University, for helping in the preparation of this manuscript. Finally, we thank the staff of the Division of Instrumental Analysis, Life Science Research Center, Gifu University, for their help in ^{1}H-NMR, ^{13}C-NMR, and GC-MS analysis.

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cleavage of α- and β-aryl ethers in lignins by Lu and Ralph.[1] There are reports on the application of DFRC to β-aryl ether models[2] and lignins obtained from plants.[3] In these reports, it was mentioned that the DFRC method gave a higher yield of monolignols to be used for the quantitative determination of β-aryl ether linkage compared with conventional methods. Furthermore, there is also a report on the structural analysis of dimers isolated from DFRC-degraded loblolly pine wood.[4] However, there are no reports on the application of DFRC to resinol and coumaran substructure model dimers to the best of our knowledge. Knowledge of the behavior of resinol and coumarane substructures in the DFRC method would be necessary in the structural analyses of lignins and especially dehydrogenation polymers (DHP), which usually contain large amounts of syringaresinol substructure, when DHP is synthesized from sinapyl alcohol, or coumaran structures, when DHP is synthesized from coniferyl alcohol. It is against this backdrop that we applied the DFRC method to five enzymatically synthesized dilignols, that is guiacylglycerol-β-coniferyl ether, syringylglycerol-β-sinapyl ether, pinoresinol, dehydrodiconiferyl alcohol, syringaresinol, and two model compounds, guiacylglycerol-β-guiacyl ether (GG model) and syringylglycerol-β-syringyl ether (SS model).

EXPERIMENTAL

Reagent grade acetyl bromide and acetic acid were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Reagent grade chloroform and ethyl acetate were purchased from Nacalai Tesque and used without further purification. Reagent grade 1,4-Dioxane was obtained from Kishida Chemical Co. Ltd (Osaka, Japan). Zinc powder (purity of 99.998%) was obtained from Aldrich (Milwaukee, WI, USA). Horseradish peroxidase (HRP4) was purchased from Biozyme Laboratories Ltd. (CA, USA). Preparative TLC plates of silica gel 60 F_{254} (0.5 and 2 mm thickness) were obtained from Merck Japan Limited (Tokyo, Japan).

Pinoresinol and dehydrodiconiferyl alcohol were synthesized from coniferyl alcohol by an enzymatic dehydrogenation process with HRP-H_2O_2 as follows. One mmol of monolignol and 1 mg of HRP were dissolved in 200 mL of water in a round-bottom flask. The reaction was started by adding 510 μL of 3% aqueous H_2O_2. After 5 h, the reaction mixture was extracted with ethyl acetate. The ethyl acetate layer was evaporated to dryness. Finally, pinoresinol and dehydrodiconiferyl alcohol were purified with preparative TLC (ethyl acetate:hexane, 2:1, two runs).

Syringaresinol was synthesized from sinapyl alcohol in the same way as just described. One mmol of sinapyl alcohol was dissolved in 200 mL of water. The reaction was started by adding 510 μL of 3% aqueous H_2O_2. After extracting with ethyl acetate, the ethyl acetate layer was evaporated. The syringaresinol was crystallized easily from ethyl acetate and *n*-hexane.

Syringylglycerol-β-sinapyl ether and guaiacylglycerol-β-coniferyl ether were synthesized from sinapyl alcohol and coniferyl alcohol, respectively, in accordance with our previous report[5] (dehydrogenation of sinapyl alcohol in 1,4-dioxane-water solution with $FeCl_3$ as an oxidant).

We followed the DFRC method as described by Lu and Ralph.[6] Five to fifteen mg of each lignin dimer were dissolved in 3 mL of acetyl bromide reagent (acetyl bromide:acetic acid, 1:4). The reaction was conducted at 15 or 40°C for 3 h, after which the solvent was evaporated under reduced pressure at 45°C to dryness. The sample was then dried *in vacuo*. Next, the reductive cleavage step was conducted as follows: the dried samples were dissolved in 3 mL of acidic solvent (dioxane:acetic acid:water, 5:4:1) and then 50 mg of zinc powder was added. The reaction was conducted at 15°C. After 30 min, the solid materials were filtered, and the solution was separated between saturated aqueous ammonium chloride solution and chloroform, into which 0.2 mg of tetracosane was added as an internal standard. The chloroform solution was evaporated to dryness and the residue was acetylated overnight with 1 mL of pyridine and 1 mL of acetic anhydride. The reaction solution was evaporated to dryness with addition of toluene at 40°C. The sample was dissolved in chloroform, and the solution was subjected to GC-MS analysis: column, DB-5ms (J&W) 0.25 mm × 30 m; film thickness 1.0 μm; carrier gas, He (1 mL/min); injector temperature, 250°C. The initial column temperature was 150°C, held for 1 min, ramped at 7°C/min to 300°C and held for 20 min. Mass spectra were collected on GC Mate II or JMS-K9 (JEOL, Japan) with an ionization energy of 70 eV. A four-point calibration curve was used to determine the quantity of sinapyl and coniferyl alcohols: both sinapyl and coniferyl alcohols were dissolved in pyridine at various concentrations. A constant volume of tetracosane was added in each solution as an internal standard. By adding acetic acid anhydride, acetylation was conducted. After evaporating the solution, samples were dissolved in one mL of chloroform and used for making the calibration curve. The concentration range for sinapyl alcohol and coniferyl alcohol were 0.099–1.50 and 0.09–1.56 mM, respectively. The concentration of tetracosane was 0.58 mM.

The spectral data (UV, ^{1}H-NMR, ^{13}C-NMR, MS, precise MS) of compounds that were obtained by acetyl bromide treatment for syringaresinol are as follows:

Compound 1: UV (CH_3OH); $\lambda_{max} = 283$ nm ($\varepsilon = 1.4 \times 10^4$ dm^3/(mol cm)): ^{1}HNMR ($CDCl_3$); 6.64 (s, 1H, α'), 6.58 (s, 1H, aromatic-H (2′)), 6.33 (s, 2H, aromatic-H (2,6)), 4.44 (s, 1H, α), 4.21–4.24 (dd, 1H, γ, $J = 10.98$ and 5.26 Hz), 4.08–4.14 (d, 2H, γ', $J = 17.16$ and 10.30), 3.91–3.95 (dd, 1H, γ, $J = 10.98$ and 8.01 Hz), 3.84 (s, 3H, Ar-OC$\underline{H}_3$ (3′ or 5′)), 3.72 (s, 6H, Ar-OC$\underline{H}_3$ (3 and 5)), 3.54 (s, 3H, Ar-OC$\underline{H}_3$ (3′ or 5′)), 3.01 (m, 1 H, β), 2.32 (s, 3H, phenolic- OCOC$\underline{H}_3$), 2.28 (s, 3H, phenolic-OCOC$\underline{H}_3$), 2.02 (s, 3H, alcoholic-OCOC$\underline{H}_3$): ^{13}C NMR ($CDCl_3$, 77.0 ppm as internal standard); 170.8 (alcoholic-Ac, -O$\underline{C}$OCH$_3$), 168.6, 168.2 (phenolic-Ac, -O$\underline{C}$OCH$_3$), 151.8 (3,5), 151.5, 151.2 (3′ and 5′), 141.3 (1), 134.2 (β'),

133.5 (4′), 130.6 (1), 130.6 (α′), 127.4 (4), 120.7 (6′), 106.4 (2′), 104.5 (2 and 6), 65.0 (γ), 61.2 (−OCH_3 (3′ or 5′)), 56.2 (−OCH_3 (3, 5 and 3′ or 5′)), 43.0 (β), 39.4 (α), 36.0 (γ′), 20.9, 20.5 (−OCO$\underline{C}H_3$): MS m/z (rel. int.); 608 (5), 606 (3), 566 (17), 564 (20), 548, 546, 527 (3), 524 (2), 522 (3), 506 (7), 504 (12), 485 (85), 464 (6), 462 (6), 443 (53), 425 (46), 383 (100), 351 (18), 337 (9), 323 (9), 307 (9), 291 (6), 277 (6), 265 (4), 246 (8), 229 (11), 212 (16), 167 (34), 82 (9), 80 (10): precise mass; calculated for $C_{28}H_{31}O_{10}$ ^{81}Br, 608.10801; found 608.10528.

Compound 2: ^{1}HNMR ($CDCl_3$); 6.70 (s, 1H, aromatic-H (6′)), 6.58 (s, 2H, aromatic-H (2,6)), 5.56 (d, 1H, α′, $J = 2.29$ Hz), 4.49–4.52 (dd, 1H, γ1, $J = 12.13$ and 2.52 Hz), 4.41–4.45 (dd, 1H, γ′1, $J = 10.98$ and 5.03 Hz), 4.07–4.11 (dd, 1H, γ′2, $J = 10.99$ and 2.06 Hz), 4.02–4.04 (d, 2H, α and γ2, J (α) = 9.8 Hz), 3.86 (s, 3H, Ar-OC$\underline{H}_3$ (5′)), 3.76 (s, 6H, Ar −OC$\underline{H}_3$ (3 and 5)), 3.01 (s, 3H, Ar-OC$\underline{H}_3$ (3′)), 2.43–2.45 (m, 1H, β), 2.2–2.4 (m, 1H, β′), 2.31 (s, 3H, phenolic−OCOC$\underline{H}_3$) 2.29 (s, 3H, phenolic-OCOC$\underline{H}_3$), 2.12 (s, 3H, alcoholic-OCOC$\underline{H}_3$) 2.10 (s, 3H, alcoholic-OCOC$\underline{H}_3$): ^{13}C NMR ($CDCl_3$, 77.0 ppm as internal standard); 171.0, 170.6 (alcoholic-Ac,-O$\underline{C}OCH_3$), 168.7, 168.5 (phenolic-Ac, −O$\underline{C}OCH_3$), 152.0 (3,5), 151.4, 151.3 (3′, 5′), 145.0 (1), 135.1 (1′), 134.0 (4′), 127.0 (4), 125.2 (2′), 106.9 (6′), 105.6 (2 and 6), 64.8 (γ′), 61.3 (γ), 59.7 (−OCH_3 (3′)), 56.3 (−OCH_3 (3 and 5), 56.3 (−OCH_3 (5′), 55.6 (α′), 43.8 (α), 41.2 (β), 38.8 (β′), 21.0, 21.0, 20.5, 20.5 (−OCO$\underline{C}H_3$): MS m/z (rel. int.); 668 (1), 666 (1), 626 (11), 624 (8), 606, 608, 586 (8), 584, 582, 566 (4), 564 (4), 544 (20), 526, 502 (13), 485 (46), 466 (20), 463, 460, 442 (66), 424 (44), 411 (17), 399 (6), 394 (8), 382 (100), 369 (48), 351 (61), 337 (31), 323 (19), 307 (14), 293 (11), 288 (9), 277 (10), 261 (10), 245 (16), 233 (12), 217 (16), 206 (10), 167(43), 82 (75), 80 (71): precise mass; calculated for $C_{30}H_{35}O_{12}$ ^{81}Br, 668.12914; found 668.13083.

^{1}H-NMR, ^{13}C-NMR, and 2D NMR (^{1}H-^{1}H COSY, NOESY, ^{13}C-^{1}H HMQC and HMBC) were conducted on a Varian Inova 400 or 500 with $CDCl_3$ as the solvent and tetramethylsilane as an internal standard. UV absorption spectra were obtained on a Jasco V-550 UV/Vis spectrometer.

RESULTS AND DISCUSSION

GC-MS chromatograms of products obtained by the DFRC treatment of dehydrodiconiferyl alcohol, pinoresinol, and syringaresinol are shown in Figure 1. It was shown that each corresponding monolignol was included in the degraded products from syringaresinol and pinoresinol but not from dehydrodiconiferyl alcohol. The identification of sinapyl and coniferyl alcohols were based on their retention times and mass spectra. The yields of acetylated *p*-hydroxycinnamyl alcohols produced by DFRC treatment are summarized in Table 1. Very small quantities of acetylated monolignols were obtained by DFRC treatment for each corresponding monolignol. Further, small

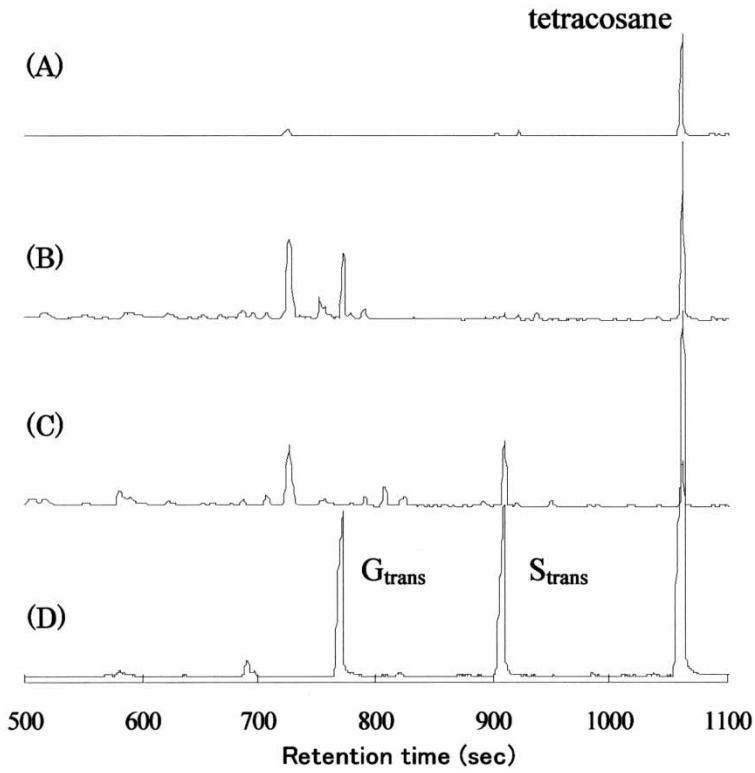

Figure 1. Total ion chromatogram of DFRC products from dehydrodiconiferyl alcohol. (A) pinoresinol; (B) syringaresinol; (C) mixture of sinapyl alcohol, coniferyl alcohol, and tetracosane; and (D) G_{trans}, trans-coniferyl alcohol and S_{trans}, trans-sinapyl alcohol.

Table 1. Yields (in mol% on starting materials) of monolignol diacetates by DFRC treatment for each compound

Starting materials	Coniferyl alcohol diacetate	Sinapyl alcohol diacetate
Sinapyl alcohol	n.d.	trace
Coniferyl alcohol	trace	n.d.
Dehydrodiconiferyl alcohol	n.d.	n.d.
Syringaresinol	n.d.	4 ± 1
Pinoresinol	5 ± 1	n.d.
Syringylglycerol-β-sinapyl ether	n.d.	43 ± 2
Guiacylglycerol-β-coniferyl ether	68 ± 4	n.d.
Syringylglycerol-β-syringyl ether	n.d.	65 ± 5
Guiacylglycerol-β-guiacyl ether	77 ± 4	n.d.

n.d.: Not detectable at testing limit. trace: Too low to quantify.

amounts of acetylated monolignols were obtained from each resinol at yield of 4–5 mol%. These yields were lower compared with those from guiacylglycerol-β-coniferyl ether or syringylglycerol-β-sinapyl ether, for which the acetylated monolignols were obtained at yields of 68 and 43 mol%, respectively. Further, 77 and 65 mol% of acetylated monolignols were obtained from the GG model and SS model, respectively, although these values were smaller than those reported by Lu and Ralph (ca. 90 mol%).[1] By taking these results into consideration it may not be necessary to take a precaution when treating samples known to contain less amounts of resinol. However, this becomes a problem during the quantitative determination of β-aryl ether in DHPs, especially if the DHP was synthesized from sinapyl alcohol, which is likely to contain large amounts of syringaresinol substructures. There is the need for a correction factor to subtract the quantity of cinnamyl alcohols produced from the corresponding resinols, when the precise quantitative determination of β-aryl ether is needed or when samples containing much resinol are treated.

We attempted to obtain the intermediate products in the acetyl bromide step to confirm the products that give the acetylated monolignol by zinc treatment during the DFRC procedure. Two compounds (compound 1 and 2) were obtained as the main products from syringaresinol by acetyl bromide treatment. The yields of these compounds collected by preparative TLC (chloroform, three runs) are summarized in Table 2. The structure of compound 1 established on the basis of the spectral data that were described in the experimental section, DEPT, HMQC, and HMBC, is shown in Figure 2 with major correlations in HMBC spectrum. The DEPT and ^{13}C-NMR spectra indicated that compound 1 has seven CH_3, two CH_2, six CH, and thirteen quaternary carbons. Further, an MS and a HMBC spectrum, respectively, showed that compound 1 has one bromine, and that the bromine is attached to the γ position. Furthermore, λ_{max} and ε of compound 1 were 283 nm and $1.4 \times 10^4\,dm^3/(mol\ cm)$, respectively, in methanol, whereas the corresponding values for syringaresinol acetate were 271 nm and $2.2 \times 10^3\,dm^3/(mol\ cm)$, respectively, in methanol. These values derived for compound 1 were very similar to those of sinapyl alcohol: λ_{max} and ε of sinapyl alcohol are 276 nm and $1.4 \times 10^4\,dm^3/(mol\ cm)$, respectively.[7] This indicates that compound 1 has a structure

Table 2. Yields (in mol%) of compounds 1 and 2 by acetyl bromide treatment of syringaresinol

	Yield
Compound 1	23
Compound 2	52

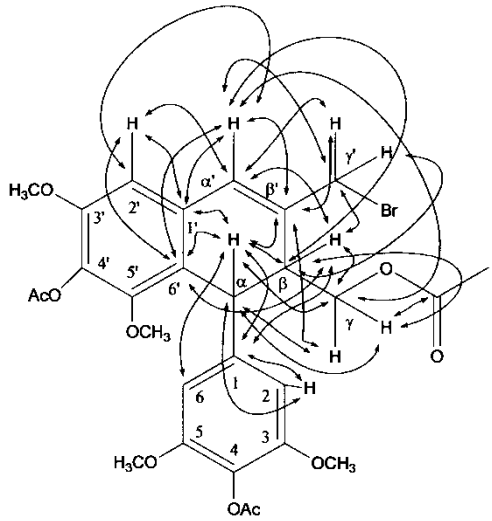

Figure 2. Estimated structure of compound 1 with major correlations in HMBC spectrum.

similar to that of sinapyl alcohol from the viewpoint of mesomeric effect. Further, from the result of ^{1}H-^{1}H cosy spectrum, no correlation was observed between H_α and H_β. Thus, it was considered that compound 1 has a trans configuration.

The structure of compound 2, estimated on the basis of similar data, is shown in Figure 3. From the MS spectrum, the molecular ion peak of compound 2 was larger than that of compound 1 by 60. Further, another

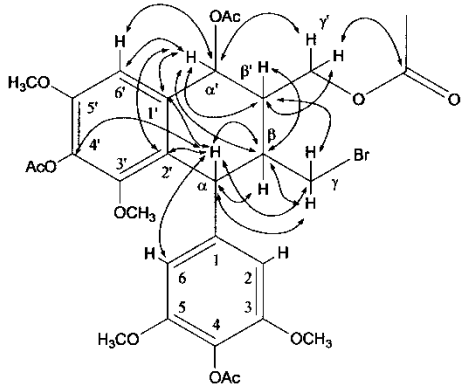

Figure 3. Estimated structure of compound 2 with major correlations in HMBC spectrum.

peak of alcoholic acetate appeared in the ^{1}H-NMR spectrum. Thus, we inferred that compound 2 was made by adding acetic acid to compound 1. However, the results of HMBC indicate that the position of the bromine differed between compounds 1 and 2, if the acetoxyl group of the benzyl position in compound 2 was considered to be added to double bond. In addition, when the acetyl bromide treatment was conducted on compounds 1 and 2, respectively, each compound was yielded without any changes. Furthermore, when acetylated syringaresinol was added to the acetyl bromide-acetic acid solution, compound 2 was obtained, but compound 1 was not. These results would indicate that compounds 1 and 2 were made via different pathways. In other words, the initially acetylated syringaresinol would yield compound 2, whereas compound 1 would be produced via an initial reaction different from acetylation of phenolic hydroxides.

A proposed pathway to the formation of compounds 1 and 2 is shown in Figure 4. It was considered that the ring-opening reaction of tetrahydrofurofuran ring occurred and then the acetylation of alcoholic hydroxide would occur. In a report by Ciminale et al.,[8] the reaction of stilbenes with an aminium salt yielded a mixture of indane and/or tetrahydronaphthalene derivatives. From their reports, the mechanism for production of dihydronaphthalene and tetrahydronaphthalene structures in our study was considered to occur via a carbocation. We also inferred that there is a bifurcation in this butadiene structure. In the case of an internal nucleophilic attack of the benzene nucleus to the α position of another sinapyl alcohol substructure, a

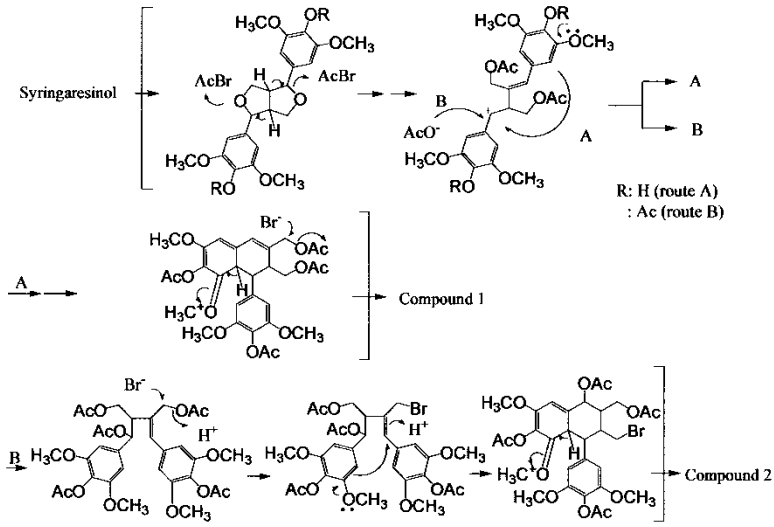

Figure 4. Proposed pathway for the production of compounds 1 and 2 from syringaresinol by AcBr treatment.

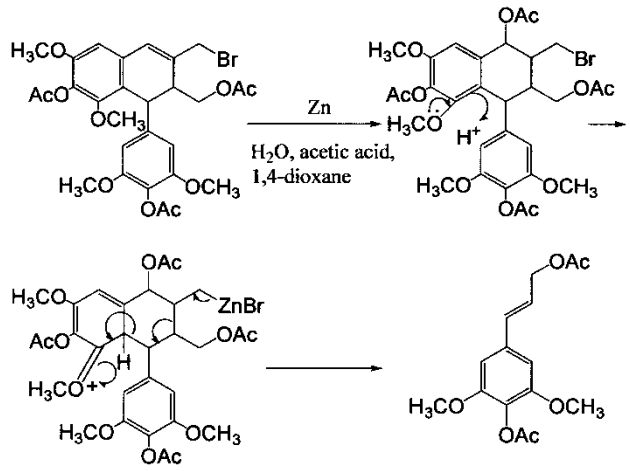

Figure 5. Proposed mechanism for the production of acetylated synapyl alcohol by zinc treatment of compound 1.

1,3-butadiene structure would afford compound 1, accompanied by proton release and rebuilding of the benzene ring (route A in Figure 4). In the case of addition of acetic acid on the α position, a 1,3-butadiene structure would become compound 2 (route B). The bromo groups of both compounds 1 and 2 would be substituted for the acetoxyl group of the γ position adjacent to double bond that is conjugated with the benzene ring. Also, this pathway

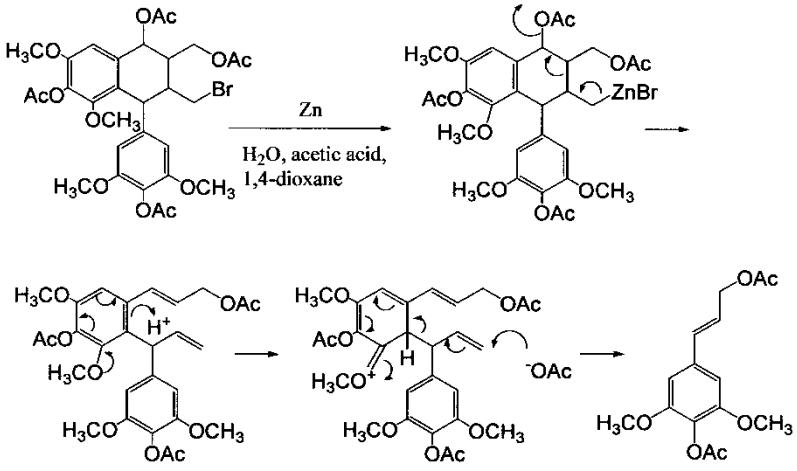

Figure 6. Proposed mechanism for the production of acetylated synapyl alcohol by zinc treatment of compound 2.

Table 3. Yields (in mol% on starting material) of monolignol diacetates by zinc treatment for each compound

Starting materials	Coniferyl alcohol diacetate	Sinapyl alcohol diacetate
Syringaresinol	n.d.	n.d.
Pinoresinol	n.d.	n.d.
Compound 1	n.d.	2.4
Compound 2	n.d.	4.0

n.d.: Not detectable at testing limit.

is consistent with the fact that syringaresinol acetate afforded no compound 1 by acetyl bromide treatment.

The proposed schemes of production of acetylated sinapyl alcohol from compound 1 or 2 are shown in Figures 5 and 6, respectively. This ring-opening reaction, affording acetylated sinapyl alcohol, is obviously not the main reaction, because many dimeric compounds were found by GC-MS analysis (data not shown). The details of this reaction are still under consideration by investigating other products from compounds 1 and 2 by zinc treatment.

The yields of sinapyl alcohol from compound 1 or 2 by zinc treatment were 2.4 and 4.0 mol%, respectively (Table 3). These results suggest that the vast majority of acetylated sinapyl alcohol produced from syringaresinol at 4 mol% was generated from compounds 1 and 2. Thus, the quantitative determination of compounds 1 and 2 would be useful in determining a correction factor for the quantitative determination of β-aryl ether to subtract the amount of sinapyl alcohol from syringaresinol.

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