Tech Bull Fac Agr Kagawa Univ, Vol 46, No 2, 117～125 1994

NMR OF DIMETHOXYLARICIRESINOL AND DIMETHOXYSECOISOLARICIRESINOL, AND BENZYL ETHER REDUCTION OF FUROFURAN LIGNANS IN *ZANTHOXYLUM SCHINIFOLIUM*

Takeshi KATAYAMA and Takeshi MASAOKA

In lignan biosynthesis of *Forsythia intermedia*, following pathway was reported; coniferyl alcohol → (+)−pinoresinol → (+)−lariciresinol → (−)−secoisolariciresinol. Interestingly the sign of specific rotation of some lignans such as (−)−pinoresinol and (−)−epipinoresinol in *Zanthoxylum* plants is opposite to that in *Forsythia* plants, but secoisolariciresinol has the same (−) sign. According to the analogy of the above pathway, (+)−secoisolariciresinol should be formed from (−)−pinoresinol. Syringaresinol isolated from *Zanthoxylum* plants is almost racemate. In order to clarify these subjects, reaction of syringaresinol as well as pinoresinol in *Zanthoxylum* plants was investigated. (±)−Dimethoxylariciresinol and (±)−dimethoxysecoisolariciresinol were prepared by catalytic reduction of (±)−syringaresinol with 10% palladium charcoal in a mixture of methanol and acetic acid, and their structures were characterized by 400 MHz nuclear magnetic resonance spectroscopy. Those three lignans were separated by reversed phase HPLC which was used for the following enzyme assay. (±)−Syringaresinol was incubated with cell−free extracts from *Zanthoxylum schinifolium* in the presence of NADPH under the same conditions as (±)−pinoresinol. Neither dimethoxylariciresinol nor dimethoxysecoisolariciresinol was detected from the reaction solution, although the same cell−free extracts transformed (±)−pinoresinol to (+)−larisiresinol.

Keywords : lignan biosynthesis, syringaresinol, dimethoxylariciresinol, dimethoxysecoisolariciresinol, *Zanthoxylum*

Introduction

Lignans are dimeric phenylpropanoids connected at the 8−8' position[1] and widely distributed in vascular plants. Lignan branch in phenylpropanoide metabolism had been a mistery. The differences in the biosynthesis between lignans and lignins, racemic polymer, have been unknown. However, recently an initial stage of lignan biosynthesis was clarified in enzymatic level using *Forsythia* plants: stereoselective coupling of coniferyl alcohol occurs to give (+)−pinoresinol followed by its stereospecific reduction to (+)−lariciresinol then to (−)−secoisolariciresinol[2,3,4,5,6]. Lignans are normally isolated as optically active natural products. However, some lignans are not always found optically pure, and have been isolated from different plant species with widely varying specific rotations. The reason is unknown. In *Zanthoxylum* plants the sign of specific rotation of some lignans such as (−)−pinoresinol and (−)−epipinoresinol is opposite to that in *Forsythia* plants, but secoisolariciresinol has the same

(−) sign[7]. According to the analogy of the above pathway, (+)−secoisolariciresinol should be formed from (−)−pinoresinol. To investigate these points, we started the research on biosynthesis of *Zanthoxylum* lignans[8].

On the other hand, syringaresinol isolated in *Z. ailanthoides* was almost racemate (−9.6°)[7] and racemate (0°) in *Z. inerm*[9]. There have been no report on the isolation of dimethoxysecoisolariciresinol as a natural product. In order to clarify these subjects, reaction of syringaresinol as well as pinoresinol in *Zanthoxylum* plants was investigated. Some stereoisomers of pinoresinol were used as a substrate of a reaction with the cell−free extracts of *F. intermedia* to investigate stereospecificity of the pinoresinol reductase[5], but syringaresinol has not been checked.

This paper describes the spectrometric characterization of dimethoxylariciresinol (dihydrosyringaresinol) and dimethoxysecoisolariciresinol and then the reaction of syringaresinol with cell-free extracts from *Zanthoxylum schinifolium*, which is closely related species to *Z. ailanthoides* and easily available.

Results and Discussion

Preparation and NMR of dimethoxylariciresinol(dihydrosyringaresinol)and dimethoxysecoisolariciresinol

Weinges[10]reported the preparation of some substituted-furan lignans (e. g. lariciresinol and dimethoxylariciresinol) by catalytic hydrogenation of furofuran lignans (e. g. pinoresinol and syringaresinol) with a palladium catalyst in ethyl acetate. Sakakibara and others[11] reported the 90 MHz NMR data of triacetate of dimethoxylariciresinol prepared by the above method. But spectrometric characterization of dimethoxysecoisolariciresinol have been not reported. In this paper dimethoxylariciresinol and dimethoxysecoisolariciresinol were obtained by the catalytic reduction of syringaresinol with 10% palladium charcoal in a mixture of methanol and acetic acid (Fig. 1). Those compounds were characterized by electron impact mass spectrometry (EI−MS) and ^{1}H NMR and ^{1}H−^{1}H COSY NMR spectroscopy.

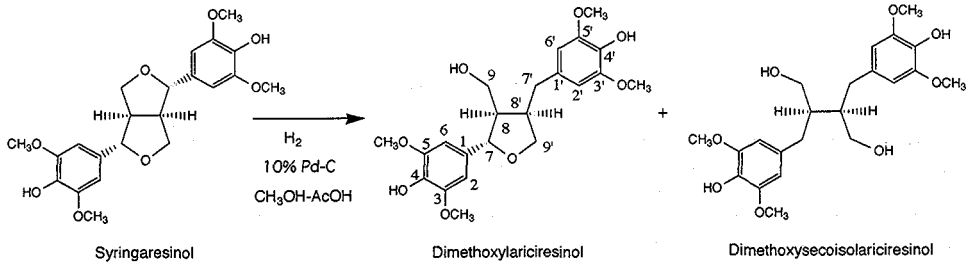

Fig. 1 Preparation of dimethoxylariciresinol and dimethoxysecoisolariciresinol by catalytic reduction of syringaresinol

^{1}H NMR of dimethoxylariciresinol were assigned as follows by the aid of its ^{1}H−^{1}H COSY NMR (Fig. 2). ^{1}H NMR ($CDCl_3$) δ: 2.43 (1H, quintet, C_8−H), 2.54 (1H, dd, J=13.5Hz, J=11.0Hz, C_7−H_a), 2.73 (1H, m, C_8−H), 2.93 (1H, dd, J=13.4Hz, J=4.9Hz, C_7−H_b), 3.77 (1H, dd, J=8.4Hz, J=6.0Hz, C_9−H_a), 3.80 (1H, dd, C_9−H_a), 3.88 (6H, s, OCH_3), 3.89 (6H, s, OCH_3), 3.94 (1H, dd,

J=10.7Hz, J=6.3Hz, C_9-H_b), 4.06 (1H, dd, J=8.5Hz, J=6.6Hz, C_9-H_b), 4.79 (1H, J=6.6Hz, C_7-H), 5.40 (1H, s, Ar−OH), 5.47 (1H, s, Ar−OH), 6.42 (2H, s, C_2 and C_6-H), 6.57 (2H, s, C_2 and C_6-H). Fig. 2 shows each ^1H signal has the cross peaks with the following ^1H signals in the parentheses δ: 2.43 (3.80, 3.94, 4.79), 2.54 (2.73, 2.93), 2.73 (2.54, 2.93, 3.77, 4.06), 2.93 (2.54, 2.73), 3.77 (2.73, 4.06), 3.80 (2.43, 3.94), 3.94 (2.43, 3.80), 4.06 (2.73, 3.77), 4.79 (2.43).

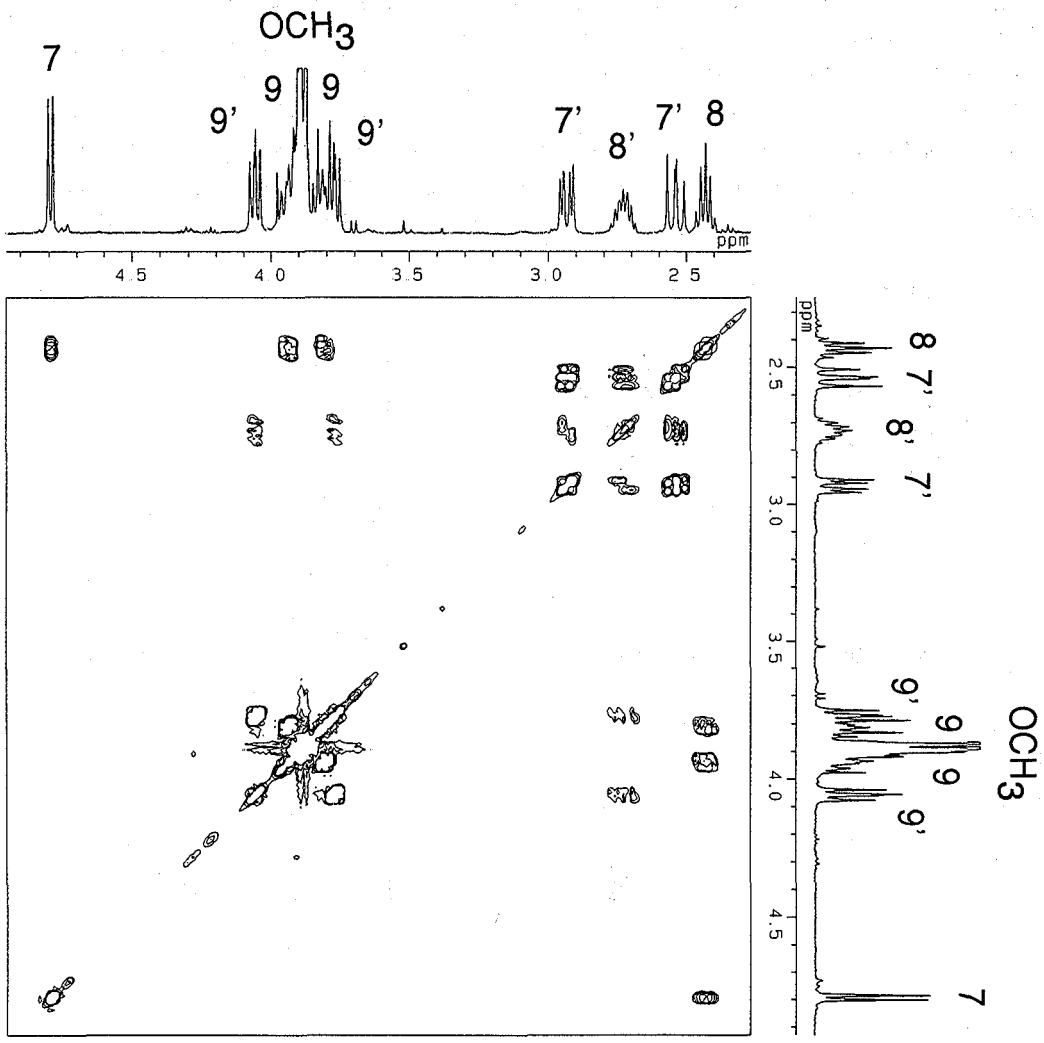

Fig. 2 ^1H-^1H COSY NMR spectrum of dimethoxylariciresinol

^{13}C NMR spectrum of dimethoxylariciresinol was assigned as follows with the aid of $^{13}C-^1H$ COSY spectrum. ^{13}C NMR ($CDCl_3$) δ: 33.86 (C_7), 42.44 (C_8), 52.68 (C_8), 56.32 (2C, OCH_3), 56.34 (2C, OCH_3), 60.92 (C_9), 72.87 (C_9), 82.97 (C_7), 102.43 (2C, C_2 and C_6), 105.22 (2C, C_2 and C_6), 131.44 (C_1), 133.09 (C_1), 133.95 (C_4), 134.01 (C_4), 147.06 (4C, C_3, C_5, C_3, and C_5) $^{13}C-^1H$ COSY ($CDCl_3$) δ: each ^{13}C signal has the cross peaks with following ^1H signals in the parentheses: 33.86 (2.54 C_7-H_a; 2.93 C_7-H_b), 42.44 (2.73 C_8-H), 52.68 (2.43 C_8-H), 56.32 and 56.34 (3.88

and 3.89; two singlets of OCH_3), 60.92 (3.80 C_9-H_a; 3.94 C_9-H_b), 72.87 (3.77 C_9-H_a; 4.06 C_9-H_b), 82.97 (4.79 C_7-H), 102.43 and 105.22 [6.57 ($C_{2,6}-H$) and 6.42 ($C_{2,6}-H$), respectively].

EI-MS of dimethoxysecoisolariciresinol showed a molecular ion peak at m/z 422, and following fragment peaks, $[M-H_2O]^+$ at 404, $[ArCH_3]^+$ at 168, and $[ArCH_2]^+$ at 167. ^1H NMR spectra of dimethoxysecoisolariciresinol were assigned as follows with the aid of $^1H-^1H$ COSY spectrum shown in Fig. 3. ^1H NMR ($CDCl_3$) δ: 1.86 (2H, m, C_8-H and C_8-H), 2.66 (2H, dd, J=13.7Hz, J=6.9Hz, C_7-H_a and C_7-H_a), 2.75 (2H, dd, J=13.8Hz, J=8.0Hz, C_7-H_b and C_7-H_b), 3.59 (2H, dd, J=11.3Hz, J=4.5Hz, C_9-H_a and C_9-H_a), 3.83 (12H, s, four OCH_3), 3.84 (2H, dd, J=11.3Hz, J=2.2Hz, C_9-H_b and C_9-H_b), 5.39 (2H, s, two ArOH), 6.33 (4H, s, four Ar-H). [the right half of the double doublet (3.83-3.84) was overlapped with the singlet of OCH_3] Fig. 3 shows each ^1H signal has the cross peaks with the following ^1H signals in the parentheses δ: 1.86 (2.66, 2.75, 3.59, 3.84), 2.66 (1.86, 2.75), 2.75 (1.86, 2.66), 3.59 (1.86, 3.84), 3.84 (1.86, 3.59).

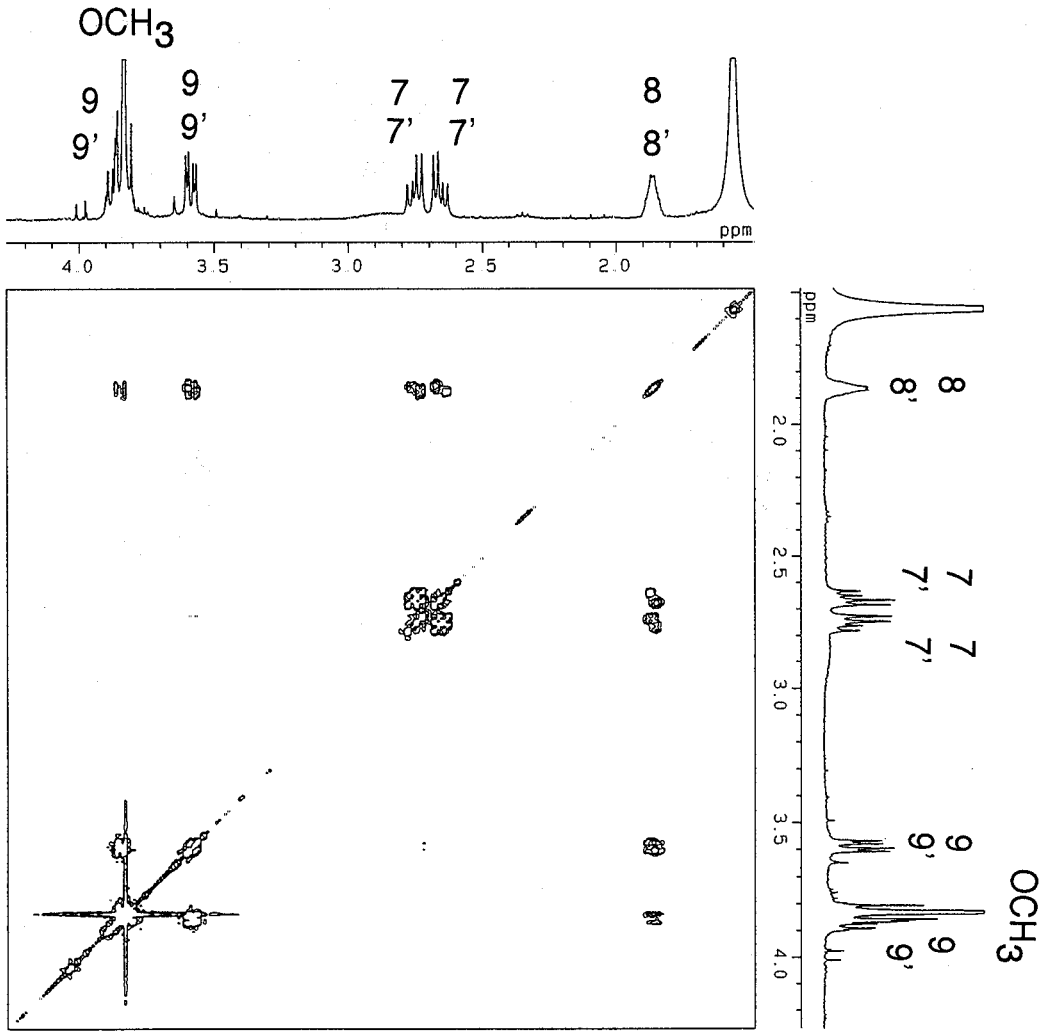

Fig. 3 $^1H-^1H$ COSY NMR spectrum of dimethoxysecoisolariciresinol

Incubation of syringaresinol with the cell-free extracts of Zanthoxylum schinifolium

Separation of the three lignans by reversed phase HPLC was investigated in order to analyze the enzymatic products from syringaresinol. Fig. 4 (A) shows good separation of the lignans; retention time of (±)−dimethoxylariciresinol, (±)−dimethoxysecoisolariciresinol and (±)−syringaresinol are 9.79, 7.75, and 16.84, respectively, with acetonitrile : water (3% acetic acid) = 25:75 at 1.0 ml/min.

Fig. 4 shows C_{18}−HPLC chromatograms of reaction products (B) and three control solutions, without NADPH (C), without substrate (D), and boiled enzyme prepration (E). Fig. 4 (B) shows neither dimethoxylariciresinol nor dimethoxysecoisolariciresinol was detected even under this high sensitivity.

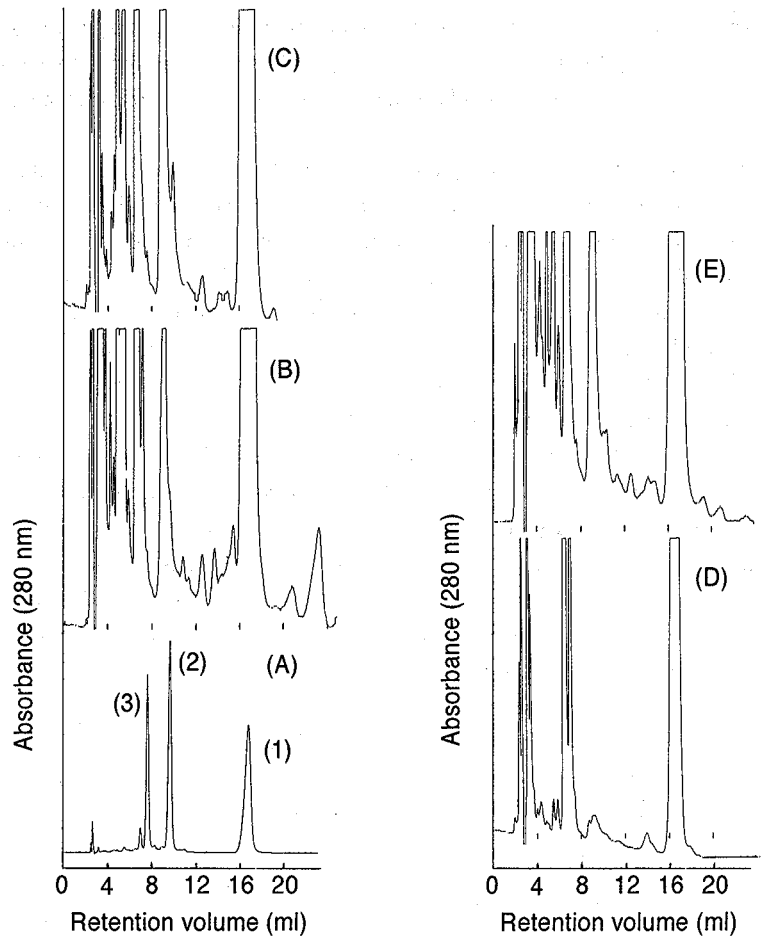

Fig 4 C_{18}-HPLC chromatograms of (A) three lignans, syringaresinol (1), dimethoxylariciresinol (2), and dimethoxysecoisolariciresinol (3);(B) a solution obtained following incubation of (±)-syringaresinol with cell-free extracts of *Zanthoxylum schinifolium* in the presence of NADPH;(C) a control solution without NADPH; (D) a control solution without substrate syringaresinol;(E) a control solution incubated with boiled cell-free extracts.

The authors *et al.*[8] very recently found that cell-free extracts of both *Z. ailanthoides* and *Z. schinifolium* transformed (±)−pinoresinol to (+)−lariciresinol in the presence of NADPH. The results are important to explain the presence of (−)−secoisolariciresinol in *Z. ailanthoides*, because the absolute configuration of (+)−lariciresinol is same as that of (−)−secoisolariciresinol. On the other hand, the fact that (±)−syringaresinol is stable for the reduction could be related to its presence as racemate in *Zanthoxylum* plants and to the specificity of the pinoresinol reduction.

Experimental

Preparation of compounds

Ethyl sinapate: Potassium ethyl malonate was prepared by partial hydrolysis of diethyl malonate with potassium hydroxide[12]. Monoethyl malonate was prepared by treating potassium ethyl malonate with concentrated hydrochloric acid[12] and used as syrup without purification by distillation.

To a stirred solution of monoethyl malonate (6.21 g, 47 mmol) and syringaldehyde (4.28 g, 23.5 mmol) in dry pyridine (9.43 ml) was added aniline (0.86 ml) and piperidine (0.86 ml). The solution was stirred for 21 hours at 55℃. The reaction solution was cooled to room temperature and diluted by the addition of water. The mixture was acidified to pH 2 with 2N HCl. The whole was extracted three times with ethyl acetate. The combined ethyl acetate solution was washed with saturated $NaHCO_3$, and then saturated NaCl solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to afford crude crystals (5.54 g) of ethyl sinapate which was used next reaction without further purification.

Ethyl 4−acetoxy−3, 5−dimethoxycinnamate: To a stirred solution of ethyl sinapate (5.18 g, 20.5 mmol) in dry pyridine (9.6 ml) was added acetic anhydride (9.90 ml, 105.0 mmol) at room temperature. After stirring for 13 hours at the same temperature, the reaction solution was cooled to 0℃ and acidified to pH 3 by the addition of 6 N HCl. The whole was extracted with ethyl acetate, and the extracts were combined, washed with saturated NaCl solution, dried over anhydrous Na_2SO_4, and evaporated to dryness *in vacuo*. The resulting crude crystals were purified by silica gel column chromatography (30cm×2cm i. d., ethyl acetate−*n*−hexane=1:3) to give ethyl 4−acetoxy−3, 5−dimethoxycinnamate (5.32 g, 88.2%).

Sinapyl alcohol: Ether pre-dried with anhydrous $CaCl_2$ and molecular sieves (4A/16) was refluxed over potassium and benzophenone and distilled. To a stirred suspension of $LiAlH_4$ (2.88 g, 75.9 mmol) in anhydrous ether (70 ml) was added dropwise a solution of ethyl 4−acetoxy−3−methoxycinnamate (2.79 g, 9.48 mmol) in anhydrous ether (70 ml) over a period of 60 min at −25℃ under nitrogen. The stirring was continued for additional 60 min. Ethyl acetate was added to destroy excess $LiAlH_4$. The whole was transferred into a beaker and neutralized (pH 7−8) by the addition of dry ice. The whole was shaken with ethyl acetate and water; note that water was added carefully until the lithium salts precipitation were of sufficient size to be directly removed by filtration, thus avoiding emulsification problems. Following filtration, the lithium salts were washed with ethyl acetate. The ethyl acetate solubles were combined, washed with saturated NaCl solution, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness to afford yellow syrup. This was purified by a light-shielded silica gel column

(30cm×2cm i. d.) eluted with ethyl acetate−*n*−hexane=2:1. Fractions containing sinapyl alcohol were combined, evaporated to dryness *in vacuo* and cooled to −30℃ to give crystals of sinapyl alcohol. EI−MS (%): 210 (100, M^+), 192 (12.3), 182 (29.9), 181 (19.6), 167 (80.5), 154 (27.3), 149 (40.8), 135 (8.9), 123 (9.8), 121 (17.3), 106 (13.4), 91 (17.3), 77 (22.1), 65 (12.8).

(±)−Syringaresinol. A solution of sinapyl alcohol (498 mg, 2.37 mmol) in a minimum amount of acetone was added dropwie to a potassium phosphate (K−Pi) buffer solution (48 ml) with stirring. To this solution was added a solution of horseraddish peroxidase in K−Pi buffer (24 ml) and then a solution of 0.5% of hydrogen peroxide (47 ml) over a period of 14 min at room temperature. Following stirring for 4 hours, the reaction solution was saturated with NaCl to stop the enzymatic reaction. The reaction solution was extracted four times with ethyl acetate. The combined ethyl acetate solution was combined, washed with saturated NaCl solution, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was purified by silica gel column (30cm×2cm i. d., EtOAc−*n*−hexane=2:1) to give syringaresinol (302 mg, 62%). ^1H NMR ($CDCl_3$) δ: 3.10 (2H, m, C_8−H), 3.87−3.93 (2H, m, $C_{9'}$−H_b), 3.90 (12H, s, OCH_3), 4.25−4.32 (2H, m, $C_{9'}$−H_a), 4.74 (2H, d, J=4.4 Hz, C_7−H), 5.51 (2H, s, Ar−OH), 6.59 (4H, s, $C_{2,6}$−H and $C_{2',6'}$−H). ^{13}C NMR ($CDCl_3$) δ: 54.37 ($C_{8,8'}$), 56.70 (OCH_3), 71.82 ($C_{9'}$), 86.10 ($C_{7,7'}$), 102.72 ($C_{2,6}$ and $C_{2',6'}$), 132.12 ($C_{1,1'}$), 134.32 ($C_{4,4'}$), 147.18 ($C_{3,5}$ and $C_{3',5'}$). EI−MS *m/z* (%): 418 (45.5, M^+), 235 (2.3), 210 (9.4), 205 (5.4), 193 (26.7), 183 (11.4), 182 (49.4), 181 (100), 180 (16.0), 168 (16.4), 167 (92.8), 161 (28.4), 154 (21.4), 139 (10.4), 123 (12.7).

Dimethoxylariciresinol and dimethoxysecoisolariciresinol. Syringaresinol (74.3 mg, 0.18 mmol) was dissolved in a mixture of methanol−acetic acid (=1:1, 2.0 ml). To the solution, 10% palladium charcoal (75.7 mg) was added. The mixture was stirred for 7 hours under hydrogen. The reaction was followed by TLC. The reaction mixture was filtered and the catalyst was washed with methanol. The filtrate and the washings were combined and evaporated to dryness *in vacuo*. The residue was purified by TLC (5% methanol in dichloromethane) to give 18.3 mg (24.5%) of dimethoxylariciresinol and 1.6 mg (2.1%) of dimethoxysecoisolariciresinol. Recovered syringaresinol was 40.2 mg.

Dimethoxylariciresinol. EI−MS *m/z* (%): 421 (15.1, M^++1), 420 (59.9, M^+), 402 (3.8, M^+-H_2O), 371 (3.0), 266 (4.0), 249 (6.4), 235 (8.7), 221 (9.6), 205 (11.9), 210 (13.7), 194 (13,7), 183 (22.3), 181 (35.9, $ArCO^+$), 168 (61.1), 167 (100, $ArCH_2^+$), 155 (10.1), 154 (11.6), 153 (11.8), 137 (8.6), 123 (20.0).

Dimethoxysecoisolariciresinol. EI−MS *m/z* (%): 422 (16.2, M^+), 404 (4.8), 187 (3.1), 168 (100), 167 (95.7), 153 (5.8), 137 (5.4), 123 (8.0), 122 (6.4).

Chromatography and Spectrometry

Analytical TLC was performed by using precoated plates with Merck silica gel 60 F_{254} (0.25 mm thickness). Preparative TLC was conducted by using precoated plates with Merck silica gel 60 F_{254} (0.5 mm and 0.2 mm thickness) and plates coated with Merck silica gel 60 PF_{254} (2.0 mm thickness). Column chromatography was performed on the FMI high performance low to medium pressure chromatograph equipped with a column of Merck silica gel 60 (230−400 ASTM mesh). HPLC was performed by using a Jasco Gulliver PU−980 HPLC pump system with a Jasco UV−970 UV/VIS detector. Peak area was calculated by using a Shimadzu Chromatopac C−R3A. The column was a Chemco Pak Finesil C_{18}−5 (4.6 mm i. d. × 15 cm) with a precolumn (4.6 mm i. d. × 5.0 cm).

^{1}H and ^{13}C NMR spectra were recorded on a JEOL α−400 NMR spectrometer (400 MHz) with tetramethylsilane as an internal standard. Electron impact mass spectra (EI−MS) were taken by a JEOL JMS DX−300 mass spectrometer with a direct inlet system at an ionizing voltage of 70 eV; relative intensity of each peak was designated in parentheses.

Plant material

Zanthoxylum schinifolium was obtained at Kamiyama forest, Kagawa University and maintained in Faculty of Agriculture, Kagawa University March 1993.

Enzyme extraction.

All steps were carried out at 4℃. Young *Z. schinifolium* shoots (8−12 cm) were defoliated with the resulting stems (5 g) washed with tap and distilled water, sectioned (2−5 mm), frozen with liquid nitrogen, and pulverized with a mortar and a pestle. The resulting powder was homogenized with polyvinylpolypyrrolidone (PVPP, 20% w/w), acid washed sea sand (5 g), and K−Pi buffer (0.1 M, pH 7.0, 11 ml) containing 10 mM dithiothreitol (DTT). The homogenate was filtered through four layers of cheesecloth, with the filtrate centrifuged (15000 g, 20 min). The supernatant was filtered with filter paper (Toyo), with an aliquot (2.5 ml) applied to a PD−10 column (Pharmacia, Sephadex G−25M) equilibrated with K−Pi buffer (0.1 M, pH 7.0); the excluded fraction (3.5 ml) was used as the enzyme preparation. Protein contents were determined using the method of Bradford[13].

Incubation of (±)−syringaresinol with Z. schinifolium

Each assay mixture (250 μl) consisted of (±)−syringaresinol (5.2 mM, 20 μl), NADPH (50 mM, 20 μl), K−Pi buffer (10 μl) and the cell-free preparation (200 μl). NADPH and (±)−syringaresinol were previously dissolved in K−Pi buffer and in a mixture of methanol−K−Pi buffer (=1:1), respectively. The reaction was initiated by addition of the enzyme preparation. Assays were conducted in quintuplicate. After 1 hour incubation at 30℃, acetic acid (20 μl) was added with the whole then shaken vigorously with ethyl acetate (500 μl). The resulting emulsion was centrifuged and the ethyl acetate solubles were removed. An aqueous solution was extracted further with ethyl acetate (500 μl). The ethyl acetate solubles from 5 assays were combined and evaporated to dryness *in vacuo*. The resulting residues were reconstituted in methanol (300 μl), filtered, with an aliquot (10 μl) subjected to C_{18}−HPLC (UV 280 nm).

Control experiments were carried out as above except that NADPH and the substrate were individually omitted. In the control using denatured enzyme, the cell-free preparation was boiled at 96℃ for 10 min, cooled to 30℃, and incubated with (±)−syringaresinol and NADPH as before.

Acknowledgement

The authors thank Professor Shigeyuki Yoshida, Faculty of Agriculture, Kagawa University, for help and advise for collecting and transplanting *Zanthoxylum schinifolium*. This research was supported in part by a grant-in-aid for Scientific Research (No. 05660191) from the Ministry of Education, Japan.

References

(1) WHITING, D. A.: *Natural Products Reports*, 2, 191−212 (1985)

(2) DAVIN, L. B., BEDGAR, D. L., KATAYAMA T., and LEWIS, N. G.: *Phytochemistry*, 31, 3869−3874 (1992).

(3) KATAYAMA, T., DAVIN, L. B., and LEWIS, N. G: *Phytochemistry*, 31, 3875−3881 (1992).

(4) LEWIS, N. G., DAVIN, L. B., KATAYAMA, T., and BEDGAR, D. L.: *Bull. Liaison Groupe Polyphenols*, 16, 98−103 (1992).

(5) KATAYAMA, T., DAVIN, L. B., CHU, A., and LEWIS, N. G.: *Phytochemistry*, 33, 581−591 (1993).

(6) KATAYAMA, T., DAVIN, L. B., BEDGAR, D. L., and LEWIS, N. G: Proceedings of Seventh International Symposium on Wood and Pulping Chemistry, Volume 2, 984−992, Beijing (1993).

(7) ISHII, H., ISHIKAWA, T, MIHARA, M., and AKAIKE, M: *Yakugaku Zasshi*, 103, 279−292 (1983)

(8) KATAYAMA, T., YAMADA, H., MASAOKA, T., and Futaoka, T.: Abstracts of the 44th Annual Meeting of the Japan Wood Research Society, p. 236, Nara (1994)

(9) ISHII, H., OHIDA, H., and HAGINIWA, J.: *Yakugaku Zasshi*, 92, 118−128 (1972).

(10) WEINGES, K: *Chem. Ber.*, 94, 2522−2533 (1961)

(11) SAKAKIBARA, A., SUDO, K., KISHI, M., AOYAMA, M., and HWANG, B. H.: *Mokuzai Gakkaishi*, 26, 628−632 (1980)

(12) STRUBE, R. E.: *Org. Synthesis*, Coll. Vol. 4, 417−419 (1963)

(13) BRADFORD, M. M: *Anal. Chem.*, 72, 248−252 (1976)

(received May 31, 1994)

ジメトキシラリシレジノールとジメトキシセコイソラリシレジノールのNMRおよび*Zanthoxylum schinifolium*によるフロフラン型リグナンのベンジルエーテルの還元

片山　健至・正岡　武

レンギョウ属*Forsythia intermedia*におけるリグナン生合成は，コニフェリルアルコール→（＋）−ピノレジノール→（＋）−ラリシレジノール→（−）−セコイソラリシレジノールであることが報告された．サンショウ属において見出されているリグナンの比旋光度の符号は，（−）−ピノレジノールや（−）−エピピノレジノールなどレンギョウ属の符号と反対のものが多いが，一方，セコイソラリシレジノールは同じで（−）体である．上記の経路から類推すると，（−）−ピノレジノールからは（＋）−セコイソラリシレジノールが生ずるはずである．また，単離されたシリンガレジノールはほぼラセミ体である．これらのことを解明するために，レンギョウ属で見出されたベンジルエーテルの酵素的還元を，ピノレジノールに加えてシリンガレジノールについても検討した．まず，（±）−シリンガレジノールを接触還元して（±）−ジメトキシラリシレジノールと（±）−ジメトキシセコイソラリシレジノールを合成し，2次元NMR等で解析した．そして，それらの逆相HPLCでの分離条件を決定した．（±）−シリンガレジノールを*Zanthoxylum schinifolium*の若枝からの無細胞抽出液とNADPHとともにインキュベートした．（±）−ピノレジノールが（＋）−ラリシレジノールに還元されているが，（±）−シリンガレジノールの反応液からジメトキシラリシレジノールとジメトキシセコイソラリシレジノールはともに検出されなかった．