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Isolation and enzymatic formation of lignans of *Daphne genkwa* and *Daphne odora*

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Abstract Four lignans – pinoresinol, lariciresinol, secoisolariciresinol, matairesinol – were isolated from each of *Daphne odora* and *Daphne genkwa* (Thymelaeaceae). Matairesinol isolated from both plants was optically pure (>99% e.e.) and dextrorotatory. Pinoresinol and lariciresinol isolated from the plants were not optically pure, and their enantiomeric compositions ranged from 88% to 95% e.e. in favor of (−)-enantiomers. As for secoisolariciresinol, the one from *D. odora* was optically pure [(+)-enantiomer, >99% e.e.], and that from *D. genkwa* was 97% e.e. in favor of the (+)-enantiomer. Lignan-synthesizing enzyme activity was detected from a Thymelaeaceae plant for the first time; cell-free extracts from *D. genkwa* catalyzed the formation of (−)-lariciresinol (23% e.e.) from racemic (±)-pinoresinols. The stereochemistry of the enzymatic reaction is discussed in relation to the stereochemical features of the isolated lignans.

Key words Lignan · *Daphne odora* · *Daphne genkwa* · Thymelaeaceae · Pinoresinol reductase

Introduction

Studies of lignan biosynthesis, mostly with *Forsythia* plants, have demonstrated the following enzymatic conversion: coniferyl alcohol → pinoresinol (furofuran lignan) → lariciresinol (furan lignan) → secoisolariciresinol (dibenzylbutane lignan) → matairesinol (dibenzylbutyrolactone lignan).[1–13] Most of these reactions were also demonstrated enzymatically or by feeding experiments with some other plant species,[14–19] suggesting that the conversion occurs generally in plants. The stereochemistry of the upstream lignans (i.e., furofuran and furan lignans) isolated from many plant species were found to be rather complicated, suggesting there is stereochemical diversity in the upstream steps of the lignan biosynthesis.[20] Thus, most of furofuran and furan lignans are not optically pure; that is, they are composed of both (+)- and (−)-enantiomers. In addition, predominant enantiomers of these lignans vary among plant species. On the other hand, all the dibenzylbutyrolactone lignans, the enantiomeric compositions of which have so far been examined by chiral high-performance liquid chromatography (HPLC), were found to be optically pure (>99% e.e.).[20] Most of the dibenzylbutyrolactone lignans are levorotatory and have the same absolute configuration at C_8 and $C_{8'}$ with respect to the carbon skeleton.[20] However, a literature survey[20] indicated that this class of lignan isolated from Thymelaeaceae plants were dextrorotatory except for one example.[21] These results indicated that the stereochemical mechanisms of lignan biosynthesis in Thymelaeaceae plants were different from those in other plant species. To determine the general stereochemical mechanisms of lignan biosynthesis, elucidating lignan biosynthetic mechanisms in Thymelaeaceae plants is of particular interest.

In this context, we surveyed lignans in a Thymelaeaceae plant, *Wikstroemia sikokiana*, that is endemic in southwest Japan; it is called ganpi in Japanese.[22,23] We also characterized stereochemistry of several lignans isolated from the plant.[22,23] Thus, all the dibenzylbutyrolactone lignans (matairesinol, kusunokinin, methyltrachelogenin, wikstromol) obtained from *W. sikokiana* were found to be dextrorotatory and optically pure.[22,23] We did not succeed in extracting activities of lignan-synthesizing enzymes from this species despite several trials. Hence, we decided to use other Thymelaeaceae plants for biochemical work. Because two

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dibenzylbutyrolactone lignans, (+)-matairesinol and (+)-nortrachelogenin [= (+)-wikstromol],[24] and a furan lignan, (−)-lariciresinol,[25] were isolated from another Thymelaeaceae plant, *Daphne odora*, and because this plant and *Daphne genkwa* are common and are easily available in Japan, we employed the two *Daphne* plants for our study of lignan biosynthesis. We report herein isolation of lignans from the two *Daphne* plants, their stereochemical characterization, and a *D. genkwa* enzyme preparation that catalyzes selective conversion of (−)-enantiomer in racemic pinoresinol to (−)-lariciresinol.

Experimental

Instruments and chromatography

^{1}H-nuclear magnetic resonance (NMR) spectra were obtained with a JNM-LA400MK FT-NMR system (JEOL) with tetramethylsilane as an internal standard. Chemical shifts and coupling constants (J) were expressed as δ values and in Hz, respectively. Gas chromatography-mass spectrometry (GC-MS) was conducted as previously described.[10] Electron impact-mass spectrometry (EI-MS), HPLC, and chiral HPLC were done as previously described.[10,22,23] Silica gel column chromatography employed Kieselgel 60 (Merck, 70–230 mesh). Silica gel thin-layer chromatography (TLC) employed Kieselgel 60 F_{254} (Merck, 20 × 20 cm, 0.5 and 0.25 mm).

Preparation of compounds and chemicals

(±)-Pinoresinols,[10] (±)-lariciresinols,[26] (±)-secoisolariciresinols,[10] (±)-matairesinols,[27] (±)-[9,9,9′,9′-$^2H_4$]pinoresinols,[23] (±)-[9,9,9′,9′-$^2H_4$]lariciresinols,[23] (±)-[9,9,9′,9′-$^2H_4$]secoisolariciresinols,[23] and [9,9-$^2H_2$, OC^2H_3]coniferyl alcohol[10] were prepared previously. All the chemicals used were of reagent grade unless otherwise stated.

Plant material

Daphne odora Thunb. and *Daphne genkwa* Sieb. et Zucc. were obtained from a local nursery and were maintained in the experimental forest of Wood Research Institute, Kyoto University. They were used for lignan isolation and as enzyme sources.

Isolation of lignans

Stems and leaves of *D. odora* (fresh weight 114.88 g) and *D. genkwa* (fresh weight 132.62 g) were freeze-dried, pulverized using a Wiley mill, and extracted with hot MeOH. The MeOH extracts thus obtained were incubated individually with β-glucosidase (Sigma G-0395; about 5000 U/g MeOH extracts) in 0.1 M NaOAc buffer (pH 5.0) at 37°C for 24 h and extracted with CH_2Cl_2. The CH_2Cl_2 extracts (*D. odora*, 1.36 g; *D. genkwa*, 2.36 g) were submitted individually to purification by a combination of column chromatography, TLC, and reversed-phase HPLC to afford pure lignans, which were identified by spectrometric analysis. Four lignans (pinoresinol, lariciresinol, secoisolariciresinol, matairesinol) were isolated from each *Daphne* plant. Spectral data of the lignans isolated from *D. genkwa* are as follows.

Pinoresinol (41.3 mg): ^{1}H-NMR ($CDCl_3$): 3.10 (2H, m, C_8H and $C_{8'}H$), 3.86 (2H, m, C_9H and $C_{9'}H$), 3.88 (6H, s, OCH_3 × 2), 4.24 (2H, dd, J = 6.8, J = 9.0, C_9H and $C_{9'}H$), 4.73 (2H, d, J = 4.4, C_7H and $C_{7'}H$), 6.80–6.89 (6H, m, aromatic H); MS m/z (%): 358(M^+, 77.6), 327 (10.1), 221 (7.0), 205 (20.2), 180 (11.7), 163 (36.9), 152 (29.3), 151 (100), 150 (32.1), 137 (55.4), 131 (35.4), 124 (14.8); high-resolution MS m/z (M^+): Calculated for $C_{20}H_{22}O_6$: 358.1416, found: 358.1408.

Lariciresinol (25.0 mg): ^{1}H-NMR ($CDCl_3$): 2.36–2.43 (1H, m, C_8H), 2.53 (1H, dd, J = 10.7, J = 13.4, $C_{7'}H$), 2.68–2.77 (1H, m, $C_{8'}H$), 2.90 (1H, dd, J = 5.1, J = 13.4, $C_{7'}H$), 3.74 (1H, dd, J = 6.2, J = 8.4, $C_{9'}H$), 3.76 (1H, dd, J = 6.6, J = 10.7, C_9H), 3.85 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 3.90 (1H, dd, J = 7.3, J = 10.8, C_9H), 4.04 (1H, dd, J = 6.6, J = 8.5, $C_{9'}H$), 4.78 (1H, d, J = 6.6, C_7H), 6.68–6.87 (6H, m, aromatic H); MS m/z (%): 360 (M^+, 100), 236 (20.8), 221 (16.0), 219 (14.5), 206 (11.9), 205 (13.0), 194 (39.3), 191 (13.3), 190 (13.3), 180 (24.6), 175 (22.0), 164 (12.8), 153 (31.3), 151 (43.2), 137 (93.5), 131 (11.7), 124 (12.4), 122 (12.4); high-resolution MS m/z (M^+): calculated for $C_{20}H_{24}O_6$: 360.1573, found: 360.1577.

Secoisolariciresinol (0.6 mg): ^{1}H-NMR ($CDCl_3$): 1.84 (2H, m, C_8H and $C_{8'}H$), 2.64 (2H, dd, J = 6.7, J = 13.8, C_7H and $C_{7'}H$), 2.74 (2H, dd, J = 8.2, J = 13.8, C_7H and $C_{7'}H$), 3.56 (2H, dd, J = 4.5, J = 11.3, C_9H and $C_{9'}H$), 3.81 (6H, s, OCH_3 × 2), about 3.82 (2H, C_9H and $C_{9'}H$), 6.55 (2H, d, J = 2.0, aromatic H), 6.62 (2H, dd, J = 1.7, J = 8.1, aromatic H), 6.80 (2H, d, J = 8.0, aromatic H); MS m/z (%): 362 (M^+, 29.2), 344 (23.3), 189 (13.0), 137 (100), 122 (9.2); high-resolution MS m/z (M^+): calculated for $C_{20}H_{26}O_6$: 362.1730, found: 362.1746.

Matairesinol (1.8 mg): ^{1}H-NMR ($CDCl_3$): 2.42–2.63 (4H, m, $C_{7'}H$ × 2, C_8H and $C_{8'}H$), 2.87 (1H, dd, J = 6.8, J = 13.9, C_7H), 2.94 (1H, dd, J = 5.4, J = 14.1, C_7H), 3.80 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 3.88 (1H, dd, J = 7.2, J = 9.2, $C_{9'}H$), 4.14 (1H, dd, J = 7.2, J = 9.2, $C_{9'}H$), 6.40 (1H, d, J = 2.0, aromatic H), 6.50 (1H, dd, J = 1.7, J = 8.1, aromatic H), 6.58–6.60 (2H, m, aromatic H), 6.79 (1H, d, J = 8.1, aromatic H), 6.81 (1H, d, J = 7.6, aromatic H) ; MS m/z (%): 358 (M^+, 57.5), 221 (8.7), 164 (8.2), 138 (35.4), 137 (100), 122 (9.7); high-resolution MS m/z (M^+): calculated for $C_{20}H_{22}O_6$: 358.1416, found: 358.1419.

The lignans pinoresinol (20.6 mg), lariciresinol (6.3 mg), secoisolariciresinol (0.3 mg), and matairesinol (1.7 mg) isolated from *D. odora* gave spectral data consistent with those of synthesized authentic samples as well as those isolated from *D. genkwa* in all respects.

Preparation of cell-free extracts

Stems of *D. genkwa* (8.03 g) were washed with tap and distilled water, frozen (liquid N_2), and pulverized with a Waring blender. All the subsequent procedures were conducted at about 4°C or on an ice bath. The powder so obtained was further ground for a few minutes in the presence of polyclar AT (1.6 g), acid-washed sea sand (1.6 g), and a 0.1 M potassium phosphate buffer (pH 8.0) containing 10 mM dithiothreitol (60 ml). The slurry thus obtained was filtered through four layers of gauze, and the filtrate (50 ml) was centrifuged (10 000 *g*, 20 min). The supernatant (48.5 ml) was filtered through a Whatman GF/A glass fiber filter. Solid ammonium sulfate was added to the filtrate up to 70% saturation. The precipitate obtained after centrifugation (14 000 *g*, 15 min) was redissolved in 0.1 M potassium phosphate buffer (pH 8.0) containing 10 mM dithiothreitol (4.0 ml); the solution (5.0 ml) so obtained was applied to a Sephadex G-25 column [Pharmacia, particle size 100–300 (coarse)] preequilibrated in 0.1 M potassium phosphate buffer (pH 8.0) containing 10 mM dithiothreitol. The protein fraction (15.4 ml) excluded from the gel was collected. Solid ammonium sulfate was added to the fraction to 70% saturation. The precipitate formed was collected by centrifugation (14 000 *g*, 15 min) and was redissolved in 2.0 ml 0.1 M potassium phosphate buffer (pH 7.0). The resulting solution was applied to a Sephadex G-25 column [Pharmacia, particle size 100–300 (coarse)] preequilibrated in 0.1 M potassium phosphate buffer (pH 7.0). The protein fraction (5.0 ml) excluded from the gel was collected and used as the cell-free preparation. GC-MS analysis showed that the EtOAc extract of the cell-free preparation did not contain any detectable amounts of the lignans pinoresinol, lariciresinol, secoisolariciresinol, or matairesinol. The protein content of the enzyme preparation was measured by the method of Bradford[28] using bovine serum albumin as a standard.

Incubation of [9,9-$^2H_2$, OC^2H_3]coniferyl alcohol and (±)-[9,9,9′,9′-$^2H_4$]pinoresinols with cell-free extracts

The assay mixture (625 μl) consisted of 50 μl of the substrate solution {[9,9-$^2H_2$, OC^2H_3]coniferyl alcohol (25 mM in 0.1 M potassium phosphate buffer, pH 7.0) or (±)-[9,9,9′,9′-$^2H_4$]pinoresinols (2.8 mM in MeOH)}, 50 μl of NADPH (50 mM in 0.1 M potassium phosphate buffer, pH 7.0), 25 μl of H_2O_2 (10 mM in 0.1 M potassium phosphate buffer, pH 7.0), 100 μl of 0.1 M potassium phosphate buffer (pH 7.0), and the enzyme preparation (400 μl). After 1 h of incubation at 30°C, the reaction mixture was extracted with EtOAc containing unlabeled racemic (±)-pinoresinols and (±)-lariciresinols as internal standards. EtOAc solubles were dried and submitted to quantitative analysis of formed lignans by GC-MS and purification of the lignans by reversed-phase HPLC.

Determination of enantiomeric composition

Lignans isolated from *Daphne* plants were subjected to chiral HPLC analysis. Lignans that gave only a single peak corresponding to one enantiomer on the chiral HPLC chromatogram were expressed as optically pure or >99% e.e. Enantiomeric compositions of lignans with peaks corresponding to both (+)- and (−)-enantiomers in the chiral HPLC analysis were determined by GC-MS using deuterium-labeled racemic lignans as internal standards, as described previously.[16,23] Enantiomeric compositions of enzymatically formed deuterium-labeled lignans were determined as above[16,23] but with unlabeled racemic lignans as internal standards.

Results and discussion

Four lignans – pinoresinol, lariciresinol, secoisolariciresinol, matairesinol (Fig. 1) – were isolated from each of *Daphne odora* and *Daphne genkwa* and identified by comparing their ^{1}H-NMR and mass spectra with those of authentic samples. Figure 2 shows chiral HPLC chromatograms of the lignans, revealing that matairesinol samples isolated from both plants as well as secoisolariciresinol from *D. odora* were optically pure (>99% e.e.) and dextrorotatory (Figs. 1, 2); the other lignans were found to be composed of both enantiomers (Fig. 2). The enantiomeric compositions of pinoresinol and lariciresinol from both species were in the range of 88%–95% e.e. in favor of (−)-enantiomers (Figs. 1, 2), and that of secoisolariciresinol from *D. genkwa* was 97% e.e. in favor of (+)-enantiomer (Figs. 1, 2). The results accord well with the general features of the enantiomeric compositions of lignans.[20] First, pinoresinol and lariciresinol

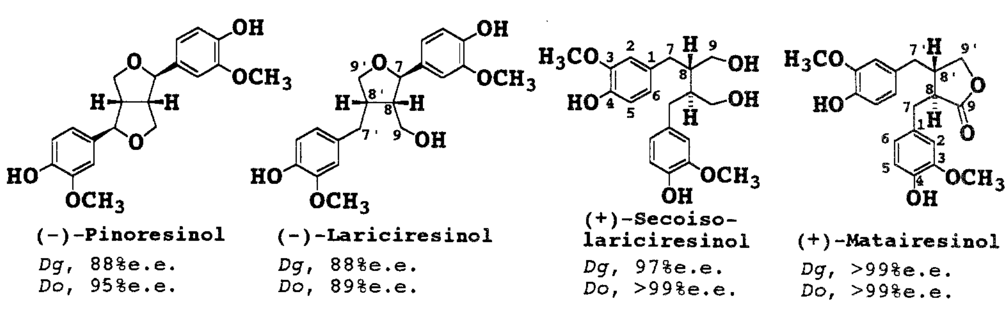


Fig. 1. Structures and enantiomeric compositions of lignans isolated from *Daphne genkwa* (*Dg*) and *Daphne odora* (*Do*). Note that only the predominant enantiomers are shown

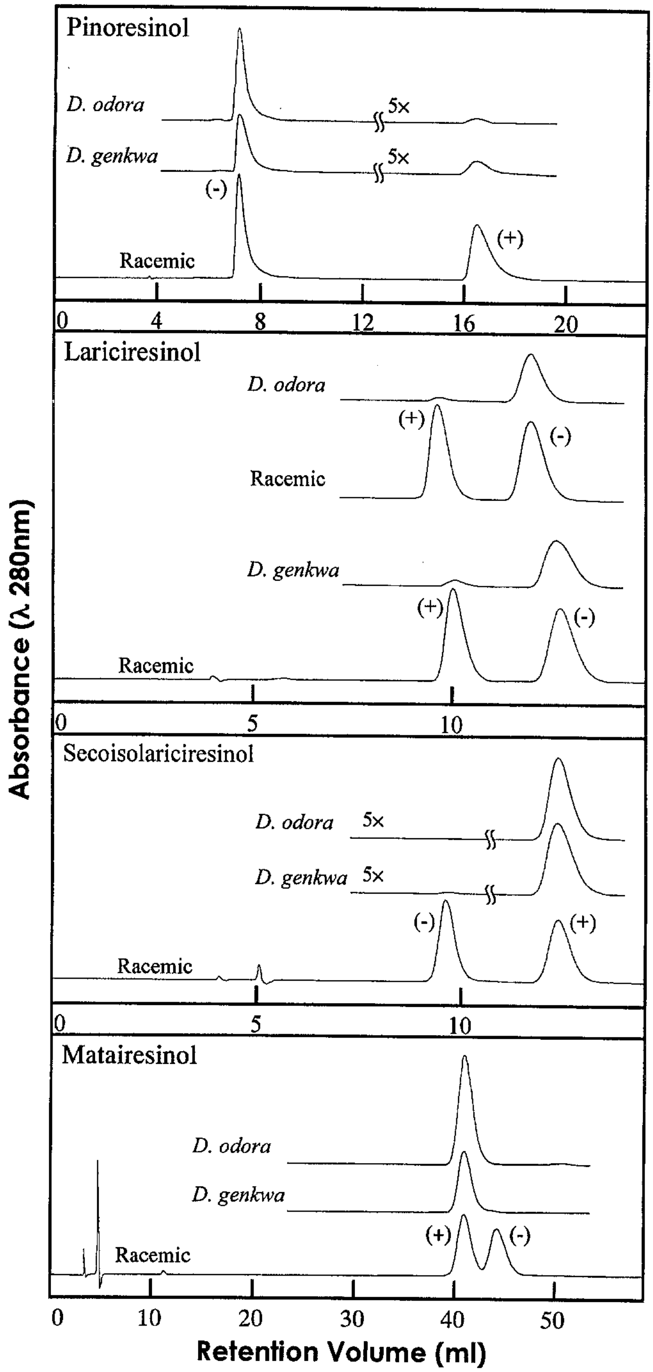


Fig. 2. Chiral high-performance liquid chromatograms of lignans isolated from *D. genkwa* and *D. odora*. *D. genkwa* and *D. odora*: lignans isolated from *D. genkwa* and *D. odora*: respectively; *Racemic*: racemic authentic sample; (+), (−): dextrorotatory and levorotatory enantiomers, respectively. The elution details are as follows: Chiralcel OD column (Daicel Chemical, 250 × 4.6 mm) with EtOH at 0.4 ml/min for pinoresinol, Chiralcel OC column (Daicel Chemical, 250 × 4.6 mm) with EtOH-*n*-hexane (80:20) at 0.5 ml/min for lariciresinol, Chiralcel OD column with EtOH-1% AcOH in *n*-hexane (30:70) at 0.9 ml/min for secoisolariciresinol, and Chiralcel OD column with EtOH-1% AcOH in *n*-hexane (15:85) at 0.9 ml/min for matairesinol

were not optically pure, whereas a dibenzylbutyrolactone lignan, matairesinol, was optically pure.[20] Second, the finding that matairesinol from both species was dextrorotatory is characteristic of dibenzylbutyrolactone lignans of Thymelaeaceae plants.[20,22,23] To our knowledge, isolation of the four lignans from *D. genkwa* or of pinoresinol and secoisolariciresinol from *D. odora* has not been reported before now.

Next, before enzyme assay was carried out, feeding experiments with deuterium-labeled substrates were conducted to confirm that the metabolism of lignan biosynthesis was active. When [9,9-$^2H_2$,OC^2H_3]coniferyl alcohol, [9,9,9′,9′-$^2H_4$]pinoresinol, [9,9,9′,9′-$^2H_4$]lariciresinol, and [9,9,9′,9′-$^2H_4$]secoisolariciresinol were administered individually to shoots of *D. genkwa*, the following transformation was observed by GC-MS analysis of methanol extracts obtained following the administration (data not shown): [9,9-$^2H_2$,OC^2H_3]coniferyl alcohol → pinoresinol-d_{10} and lariciresinol-d_{10}, [9,9,9′,9′-$^2H_4$]pinoresinol → lariciresinol-d_4, [9,9,9′,9′-$^2H_4$]lariciresinol → pinoresinol-d_4, [9,9,9′,9′-$^2H_4$]secoisolariciresinol → lariciresinol-d_4, similarly, when [9,9-$^2H_2$,OC^2H_3]coniferyl alcohol was administered to *D. odora* shoots, formation of pinoresinol-d_{10}, lariciresinol-d_{10}, and secoisolariciresinol-d_{10} were observed (data not shown). These results confirmed that lignan biosynthesis was occurring in the shoots of *D. genkwa* and *D. odora*.

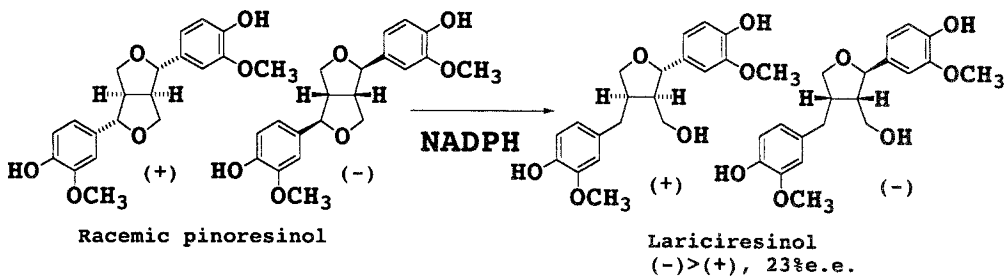
With the data indicating active lignan biosynthesis now in hand, we assayed the lignan synthesizing enzyme activities. Pinoresinol-d_{10} and lariciresinol-d_{10} as well as small amounts of secoisolariciresinol-d_{10}, were formed following incubation of [9,9-$^2H_2$,OC^2H_3]coniferyl alcohol with cell-free extracts of *D. genkwa* in the presence of H_2O_2 and NADPH (Table 1). The incubation of racemic (±)-[9,9,9′,9′-$^2H_4$]pinoresinols with the cell-free extracts also yielded lariciresinol-d_4 (Table 1, Fig. 3). The enantiomeric composition was found to be 23% e.e. in favor of (−)-enantiomer, which was determined as previously reported.[16,23] Briefly, the formed lariciresinol-d_4 was purified together with unlabeled racemic (±)-lariciresinols by reversed-phase HPLC followed by chiral HPLC to afford (+)- and (−)-enantiomers individually. Each enantiomer was then submitted to GC-MS. Figure 4 shows mass chromatograms of the molecular ions of the enzymatically formed lariciresinol-d_4 and unlabeled internal standard lariciresinol trimethylsilyl (TMS) ethers. Based on a comparison of the peak ratios of the deuterium-labeled one and the unlabeled one, the enantiomeric composition of the enzymatically formed (−)-lariciresinol-d_4 (23% e.e.) was determined (Fig. 3). The results indicate selective reduction of (−)-pinoresinol over (+)-enantiomer to afford (−)-lariciresinol with retention of stereochemistry at C_8 and $C_{8'}$ by pinoresinol reductase, which was consistent with the fact that the predominant enantiomer of pinoresinol recovered after the incubation was dextrorotatory (27% e.e.). In addition, secoisolariciresinol was not detected following incubation of pinoresinol, indicating that the predominance of the (−)-enantiomer of lariciresinol in the enzymatic reduction of pinoresinol was due to the stereochemical nature of lariciresinol formation but not selective reduction of

Table 1. Enzymatic formation of lignans

Assay[a]	Pinoresinol formation[b]	Lariciresinol formation[b]
Formation of pinoresinol-d_{10} and lariciresinol-d_{10} from [9,9-$^2H_2$, OC^2H_3]coniferyl alcohol		
Complete	0.34	2.07
Control with denatured enzyme[c]	8.55	0.00
Formation of lariciresinol-d_4 from (±)-[9,9,9′,9′-$^2H_4$]pinoresinols		
Complete		7.03
Control with denatured enzyme[c]		0.00

[a] All assays employed NADPH and H_2O_2 as cofactors
[b] Expressed in nmol h^{-1} mg^{-1} protein
[c] Denatured by boiling at 100°C for 5 min

Fig. 3. Formation of lignans with *Daphne genkwa* enzyme



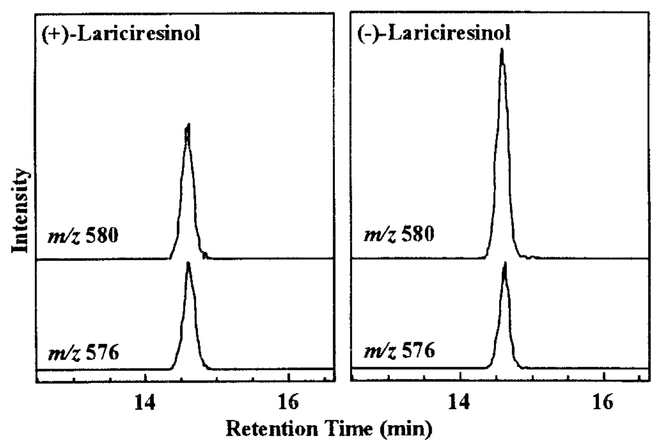


Fig. 4. Mass chromatograms of molecular ions of the trimethylsilyl (TMS) ethers of lariciresinols. *m/z 580*: mass chromatograms of the molecular ions of TMS ethers of lariciresinol-d_4 formed from the incubation of (±)-[9,9,9′,9′-$^2H_4$]pinoresinols with the *D. genkwa* enzyme preparation. *m/z 576*: mass chromatograms of the molecular ions of TMS ethers of the unlabeled lariciresinols

(+)-lariciresinol, giving rise to (−)-secoisolariciresinol accompanied by an accumulation of (−)-lariciresinol.

Several examples of enzymatic reduction of benzyl positions of pinoresinol and lariciresinol giving rise to lariciresinol and secoisolariciresinol, respectively, have been reported with *Forsythia intermedia*,[7,12] *Forsythia koreana*,[10] and *Zanthoxylum ailanthoides*[19] as enzyme sources. These studies reported selective reduction of (+)-pinoresinol or (+)-lariciresinol to afford (+)-lariciresinol or (−)-secoisolariciresinol (or both), respectively. It should be noted that both the substrate and product lignans have the same absolute configurations at C_8 and $C_{8'}$, which is opposite to that of (−)-lariciresinol formed by the *D. genkwa* enzyme. The enzyme of *Forsythia intermedia* was purified and designated pinoresinol/lariciresinol reductase.[12]

On the other hand, we reported selective formation of both (+)- and (−)-enantiomers of secoisolariciresinol by two *Arctium lappa* enzyme preparations.[15,16] Thus, like the *Forsythia* and *Zanthoxylum* reductases, a crude *A. lappa* seed enzyme catalyzed selective formation of (−)-secoisolariciresinol from coniferyl alcohol,[16] probably via pinoresinol and lariciresinol (Suzuki, Umezawa, Shimada, in preparation). In sharp contrast, an enzyme from petioles of the same species catalyzed selective formation of the opposite enantiomer, (+)-secoisolariciresinol, under the same condition.[15] The results indicated that two isoforms catalyzing the same chemical reaction but with opposite stereochemical properties were involved in lignan biosynthesis even in a single plant species. This accords well with the recent findings by Fujita et al. regarding two *Thuja plicata* recombinant pinoresinol/lariciresinol reductases.[29] Thus, one of the recombinant reductases converted (−)-enantiomer of pinoresinol selectively to (−)-lariciresinol, like the *D. genkwa* reductase, whereas the other reduced (+)-pinoresinol selectively to (+)-lariciresinol.[29]

These previous results of enzymatic reduction of pinoresinol and lariciresinol strongly suggested that the reduction step in lignan biosynthesis was rather complicated in terms of stereochemistry, probably controlled spatially and temporally by the differential expression of different isozymes having opposite stereochemical properties. This view is in harmony with the fact that the enantiomeric composition of pinoresinol and lariciresinol varies dramatically among plant species.[20]

Although the complexity of the expression of pinoresinol reductase isozymes might be valid for lignan biosynthesis in *D. genkwa*, selective formation of the (−)-enantiomer of lariciresinol by the *D. genkwa* enzyme preparation was of interest in relation to the stereochemistry of lignans occurring in *D. genkwa*: the predominant (−)-enantiomer of the enzymatically formed lariciresinol was not only the same as that of the lignan isolated from the plant, it also has the same absolute configurations at C_8 and $C_{8'}$ as those of the predominant enantiomers of the other lignans (−)-pinoresinol, (+)-secoisolariciresinol, and (+)-matairesinol isolated from *D. genkwa* and *D. odora*.[20] This suggests that the presently detected pinoresinol reductase plays an important role in the in vivo formation of predominant enantiomers of the *D. genkwa* lignans, although final conclusions await molecular biological evidence by, for example, Northern hybridization using the gene of the enzyme. In addition, it is of interest to examine whether *D. genkwa* has another pinoresinol reductase dedicated to the selective reduction of (+)-pinoresinol.

Conclusions

Lignans isolated from *D. genkwa* and *D. odora* in this study exhibited stereochemical features that are observed generally in naturally occurring lignans and specific to Thymelaeaceae lignans. Activity of a lignan-synthesizing enzyme, pinoresinol reductase, was detected in a Thymelaeaceae plant for the first time, and the stereochemical properties of the enzymatic reaction was found to accord well with the stereochemistry of *D. genkwa* lignans, suggesting the physiological importance of the enzyme in the in vivo synthesis of lignans in the plant.

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