

NOTE

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Stereochemistry and biosynthesis of (+)-lyoniresinol, a syringyl tetrahydronaphthalene lignan in *Lyonia ovalifolia* var. *elliptica* I: isolation and stereochemistry of syringyl lignans and predicted precursors to (+)-lyoniresinol from wood

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Abstract Steps leading to the biosynthesis of syringyl lignans and tetrahydronaphthalene and naphthalene lignans, especially the formation of the C2-C7' linkage, have not been elucidated. Lyoniresinol is a typical syringyl lignan, as well as a tetrahydronaphthalene lignan found in *Lyonia ovalifolia* var. *elliptica*. To demonstrate the biosynthetic pathway for (+)-lyoniresinol, three putative biosynthetic intermediates of lyoniresinol, syringaresinol, 5,5'-dimethoxyariciresinol, and 5,5'-dimethoxysecoisolariciresinol, were isolated from wood. The identity of the putative intermediates was confirmed by spectroscopic analyses, as well as by comparison of spectral and chromatographic data with those of authentic samples previously synthesized. The stereochemistry (enantiomeric composition and absolute configuration) of the isolated lignans were determined as (±)-syringaresinol, (8*S*,8'*S*)-(-)-5,5'-dimethoxyariciresinol [46% enantiomeric excess (e.e.)], (8*S*,8'*S*)-(+)-5,5'-dimethoxysecoisolariciresinol (91% e.e.), and (8*R*,8'*R*)-(+)-lyoniresinol (42% e.e.). The absolute configurations of (+)- and (-)-5,5'-dimethoxyariciresinols, and (+)- and (-)-5,5'-dimethoxysecoisolariciresinols were determined by their synthesis (catalytic reduction) from (8*R*,8'*R*)-(+)- and (8*S*,8'*S*)-(-)-syringaresinols and by subsequent chiral high-performance liquid chromatography analysis.

Key words Lignan · *Lyonia ovalifolia* var. *elliptica* · 5,5'-Dimethoxyariciresinol · 5,5'-Dimethoxysecoisolariciresinol · Lyoniresinol

Introduction

Lignans and neolignans are typically dimeric phenylpropanoids that are widely distributed in higher plants. Lignans are connected through the C8-C8' linkage and neolignans are connected through linkages other than the 8-8' linkage; for example 3-3' (or 5-5'), 8-*O*-4', and 8-3' (8-5') linkages. Most of the lignans and neolignans in plants are optically active and display important physiological functions in plant defense and human health. There are many structural types of lignans due to the diversity of structures forming aromatic rings and side chains. The typical lignans that have oxygen at the 9,9'-C position are considered to be formed from monolignol, coniferyl alcohol (CA), and sinapyl alcohol (SA). Dehydrogenative dimerization of CA, SA, and a pair of CA and SA yield guaiacyl, syringyl, and guaiacyl-syringyl lignans and neolignans, respectively. Lignans are grouped, according to the side chain types, into furanofurans (or furofurans), tetrahydrofurans, dibenzylbutanes, dibenzylbutyrolactones, aryltetrahydronaphthalenes, aryl-naphthalenes, and others.

Lyoniresinol (LYR) is a typical syringyl lignan, as well as a tetrahydronaphthalene lignan. Haq et al.¹ reported that lyoniresinol is an effective inhibitor of tyrosinase, a key enzyme for melanin biosynthesis in plants and animals. As a result, lyoniresinol could be useful clinically for treatment of some dermatological disorders associated with melanin hyperpigmentation. Lyoniside and LYR are the major constituents isolated from the wood of *Lyonia ovalifolia* var. *elliptica*.²⁻⁴ Although LYR was also isolated from some other plants, the LYR from *L. ovalifolia* has the highest specific rotation (Table 1). This tree is native to Japan and is distributed in Shikoku, Kyushu, and southern Tohoku to the western region of Honshu. The medium-sized tree is deciduous with oblong-ovate or elliptical leaves and twisted bark.

The biosynthetic pathway leading to LYR has not been completely elucidated. In general, biosynthetic studies of syringyl lignans are behind those of guaiacyl lignans. Mechanisms for biosynthesis of tetrahydronaphthalene

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lignans such as lyoniresinol and even isolariciresinol, especially the formation of the C2-C7' linkage, are also poorly documented. We have initiated a study to provide details of the biosynthetic pathway in *Lyonia ovalifolia* var. *elliptica* leading to the formation of LYR. We have also investigated whether expected biosynthetic intermediates of LYR are present in wood or other tissues of this plant. Spectral and stereochemical analyses were conducted to confirm the identity of the isolated syringyl lignans.

The biosynthetic pathway of coniferyl alcohol \rightarrow (+)-pinoresinol \rightarrow (+)-lariciresinol \rightarrow (-)-secoisolariciresinol, has been established in *Forsythia* plants.⁵⁻¹⁰ Dehydrogenation of CA by an oxidase or a laccase and subsequent

stereoselective coupling of the resulting 8-radicals by the action of dirigent protein produces (+)-pinoresinol (a furofuran type). (+)-Pinoresinol then undergoes a highly enantiospecific reduction by an NADPH-dependant reductase to yield (+)-lariciresinol (a tetrahydrofuran type) and then (-)-secoisolariciresinol (a dibenzylbutane type). Secoisolariciresinol is subsequently dehydrogenated to form (-)-matairesinol in *Forsythia*. Further metabolism of (-)-matairesinol presumably generates podophyllotoxin through C2-C7' bond formation and other reactions in *Podophyllum peltatum*.^{11,12} By analogy to the above pathway and by the fact that chemical transformation of lariciresinol with acid catalysis to isolariciresinol proceeds without oxidation-reduction,¹³ the biosynthetic pathway of (+)-LYR was assumed to be as shown in Fig. 1. In this model, a dehydrogenative dimer of sinapyl alcohol, syringaresinol (SYR) is reduced to give 5,5'-dimethoxyylariciresinol (DMLR), which would be reduced further to give 5,5'-dimethoxysecoisolariciresinol (DMSLR). This compound might be further transformed into lyoniresinol through C2-C7' bond formation. Furthermore, DMLR could be cyclized to form LYR. In this study, we isolated lyoniresinol and the predicted intermediates (SYR, DMLR, and DMSLR) and investigated the stereochemistry (absolute configuration and enantiomeric composition) of these compounds.

Table 1. Specific rotation of lyoniresinol and lyoniside isolated to date from different plant species

Plant sources	Specific rotation $[\alpha]_D$		References
	Lyoniresinol	Lyoniside	
<i>Lyonia ovalifolia</i> var. <i>elliptica</i>	+68.4°	+35.1°	2,3,4
<i>Trippetaleia paniculata</i>	+60.1°	+28.5°	14
<i>Cinnamomum cassia</i>	+58.1°	–	15
<i>Sorbus scopulina</i>	–	+41.5°	16
<i>Sorbus decora</i>	–	+41.0°	17
<i>Lyonia ovalifolia</i> var. <i>ovalifolia</i>	–	+33.9°	3
<i>Justicia tranquebariensis</i>	+7.23 ^{oa}	–	18
<i>Quercus robur</i>	0°	–	19
<i>Ulmus thomasii</i>	0°	–	20
<i>Cinnamosma</i> <i>madagascariensis</i>	0°	–	21
<i>Hakea saligna</i>	0°	–	22
<i>Phellodendron</i> <i>amurense</i>	0°	–	23
<i>Pygeum acuminatum</i>	–5.2°	–	24

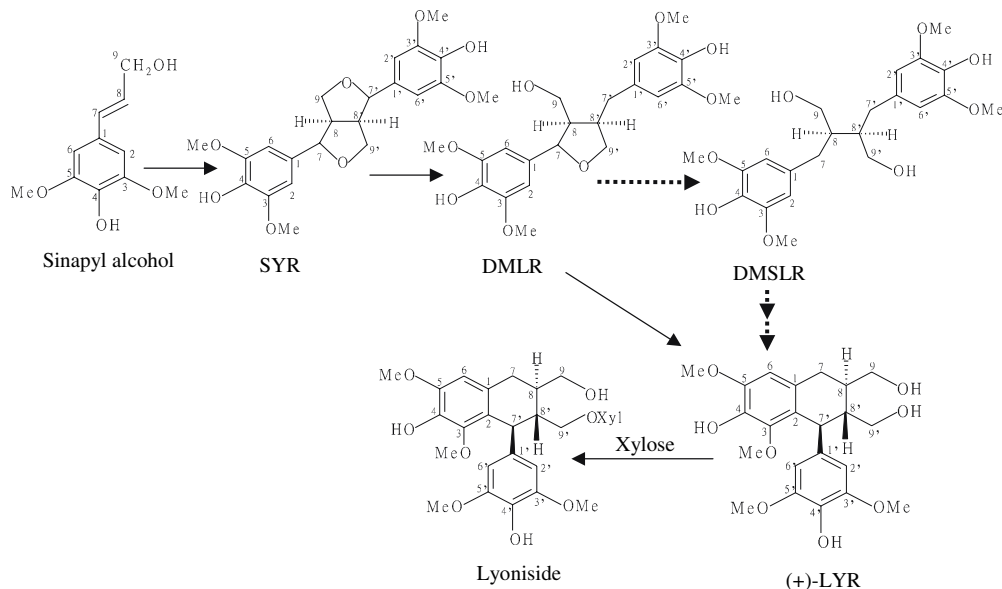
^a Diacetate form

Experimental

General

All reagents and solvents were reagent grade. Open column chromatography and medium-pressure column chromatography (FMI pump system) were performed with silica gel (Wakogel C-200, particle size 75–150 μm ; Wako Pure

Fig. 1. Assumed biosynthetic pathway for (+)-lyoniresinol (LYR) and lyoniside. SYR, Syringaresinol; DMLR, dimethoxyylariciresinol; DMSLR, dimethoxysecoisolariciresinol



Chemical Industries, Osaka, and Merck silica gel 60, 230–400 ASTM mesh, respectively). Analytical thin-layer chromatography (TLC) and preparative TLC were accomplished by using plates precoated with Merck silica gel 60 F₂₅₄ (0.25 mm and 0.5 mm thickness, respectively). ¹H, ¹³C, and two-dimensional (2D) nuclear magnetic resonance (NMR) spectra (400 MHz) were recorded on a Jeol JNM-Alpha 400 FT-NMR spectrometer using tetramethylsilane as internal standard. Electron-impact mass spectra (EI-MS) were acquired on a Jeol JMS-SX102A mass spectrometer with a direct inlet system at an ionizing voltage of 70 eV. Infrared (IR) spectra were measured on a Jasco FT/IR-670 Plus Fourier transform infrared spectrometer. Analytical high-performance liquid chromatography (HPLC) was carried out on a Jasco PU-2089 equipped with a Jasco UV-2075 Plus Intelligent UV/Vis detector and a Shimadzu chromatopac C-R7A plus using a reversed-phase column (TSKgel ODS-80Ts). Chiral column HPLC used the same HPLC system as above with Diacel Chiralcel OC and OD columns to determine the enantiomeric composition of isolated lignans. Sign of optical rotation was determined with a Jasco OR-990 chiral detector attached with an integrator (Shimadzu, chromatopac C-R3A). All melting points were uncorrected.

Preparation of lignans

Sinapyl alcohol. SA was prepared by the reduction of ethyl sinapate with a solution (1.5 M, 3.5 ml) of diisobutylaluminum hydride (DIBAL-H, Aldrich) in toluene at 0°C.²⁵

(±)-*Syringaresinol*. (±)-SYR was prepared by dehydrogenative dimerization of SA in a solution of K-Pi buffer (0.1 M, pH 7.0) with horseradish peroxidase (Sigma, 60 units/mg) and 0.5% H₂O₂ solution at room temperature.^{26,27} The product was obtained as pale yellow needles; mp 167°–168°C. ¹H NMR (CDCl₃): δ 3.10 (2H, m, 8,8'-H), 3.91 (12H, s, 3,3',5,5'-OCH₃), 3.88–3.92 (2H, overlapping, 9,9'-Ha), 4.29 (2H, dd, *J* = 9.15 and 6.95 Hz, 9,9'-Hb), 4.72 (2H, d, *J* = 4.39 Hz, 7,7'-H), 5.49 (2H, s, 4,4'-Ar-OH), 6.58 (4H, s, 2,6,2',6'-Ar-H). ¹³C NMR (CDCl₃): δ 54.4 (2C, 8,8'-C), 56.4 (4C, 3,3',5,5'-OCH₃), 71.8 (2C, 9,9'-C), 86.1 (2C, 7,7'-C), 102.7 (4C, 2,2',6,6'-C), 132.1 (2C, C-1,1'), 134.3 (2C, 4,4'-C), 147.2 (4C, 3,3',5,5'-C). IR (KBr): ν_{\max} cm⁻¹ 3328 (OH), 2932, 1681, 1656, 1622, 1518, 1458, 1227, 1112. EI-MS *m/z* (rel. int.): 418 [M⁺] (22), 387 (<3), 221 (4), 205 (4), 193 (5), 191 (7), 183 (14), 182 (100), 167 (57), 154 (21), 139 (29), 123 (25).

(±)-5,5'-*Dimethoxylariciresinol* and (±)-5,5'-*dimethoxysecoisolariciresinol*. To a stirred solution of (±)-SYR (45.9 mg, 0.109 mmol) in MeOH (7.6 ml), 20% palladium hydroxide on carbon (wet) (Aldrich, 51.8 mg) was added. The mixture was stirred under H₂ gas. After 1 h of stirring, TLC analysis of the reaction mixture showed that progress of the reaction was not satisfactory. Therefore, a small amount of acetic acid (AcOH, 0.6 ml) was added to the reaction mixture to accelerate the reaction. Stirring was continued for an additional 2.4 h. The reaction mixture was

then filtered and the catalyst was washed with MeOH. The filtrate and washings were combined and evaporated to dryness in vacuo. The residue was purified by TLC (EtOAc/*n*-hexane, 3:1) to give (±)-DMLR (13.0 mg, 28.2% yield) and (±)-DMSLR (7.4 mg, 16% yield). Both were isolated as colorless crystals. DMLR: mp 85°C; ¹H NMR (CDCl₃): δ 2.43 (1H, quin, 8-H), 2.54 (1H, dd, *J* = 13.42 and 10.98 Hz, 7'-Ha), 2.73 (1H, m, 8'-H), 2.93 (1H, dd, *J* = 13.29 and 5.00 Hz, 7'-Hb), 3.77 (1H, dd, *J* = 8.54 and 6.10 Hz, 9'-Ha), 3.79–3.84 (1H, overlapping, 9-Ha), 3.87 (6H, s, 3',5'-OCH₃), 3.89 (6H, s, 3,5-OCH₃), 3.94 (1H, dd, *J* = 10.61 and 7.20 Hz, 9-Hb), 4.06 (1H, dd, *J* = 8.54 and 6.59 Hz, 9'-Hb), 4.79 (1H, d, *J* = 6.59 Hz, 7-H), 5.41 (1H, s, 4'-Ar-OH), 5.48 (1H, s, 4-Ar-OH), 6.42 (2H, s, 2',6'-Ar-H), 6.57 (2H, s, 2,6-Ar-H). ¹³C NMR (CDCl₃): δ 33.9 (7'-C), 42.4 (8'-C), 52.7 (8-C), 56.3 (2C, OCH₃), 56.4 (2C, OCH₃), 61.0 (9-C), 72.9 (9'-C), 83.0 (7-C), 102.5 (2C, 2,6-C), 105.2 (2C, 2',6'-C), 131.5 (1-C), 133.1 (1'-C), 134.0 (4'-C), 134.1 (4-C), 147.1 (4C, 3,5, 3',5'-C). IR (KBr): ν_{\max} cm⁻¹ 3421 (OH), 2939, 1614, 1519, 1459, 1428, 1329, 1217, 1115. EI-MS *m/z* (rel. int.): 420 [M⁺] (20), 235 (3), 221 (6), 205 (5), 191 (6), 182 (25), 181 (29), 168 (25), 167 (100), 154 (12), 137 (10), 123 (27). DMSLR: mp 178°C; ¹H NMR (CDCl₃): δ 1.86 (2H, m, 8,8'-H), 2.65 (2H, dd, *J* = 13.66 and 6.83 Hz, 7,7'-Ha), 2.75 (2H, dd, *J* = 13.78 and 7.93 Hz, 7,7'-Hb), 3.58 (2H, dd, *J* = 11.22 and 4.39 Hz, 9,9'-Ha), 3.83 (12H, s, OCH₃), 3.82–3.87 (2H, overlapping, 9,9'-Hb), 5.40 (2H, s, 4, 4'-Ar-OH), 6.33 (4H, s, Ar-H). ¹³C NMR (CDCl₃): δ 36.5 (2C, 8,8'-C), 43.7 (2C, 7,7'-C), 56.3 (4C, OCH₃), 61.0 (2C, 9,9'-C), 105.6 (4C, 2,2',6,6'-C), 131.6 (2C, 1,1'-C), 132.9 (2C, 4,4'-C), 146.9 (4C, 3,3',5,5'-C). IR (KBr): ν_{\max} cm⁻¹ 3420 (OH), 2938, 1615, 1519, 1459, 1429, 1331, 1220, 1116. EI-MS *m/z* (rel. int.): 422 [M⁺] (14), 404 (3), 221 (6), 181 (4), 168 (51), 167 (100), 153 (7), 147 (11), 137 (7), 123 (12), 122 (16).

(±)-*Lyoniresinol*. To (±)-DMLR (2.4 mg, 0.0057 mmol) in a reaction flask, 2% AcOH solution (2.0 ml) was added at 70°C.¹³ The mixture was stirred for ca. 1.3 h at the same temperature. The reaction mixture was extracted three times with EtOAc. The combined EtOAc solution was washed once with a saturated NaCl solution, dried over anhydrous Na₂SO₄, and evaporated to dryness in vacuo to give (±)-LYR as colorless crystals (2.12 mg, yield 88%); mp 170°–171°C. ¹H NMR (CDCl₃): δ 1.78 (1H, m, 8-H), 1.93 (1H, m, 8'-H), 2.61 (1H, dd, *J* = 15.36 and 4.39 Hz, 7-Hb), 2.70 (1H, dd, *J* = 15.06 and 11.77 Hz, 7-Ha), 3.30 (3H, s, 3-OCH₃), 3.60 (1H, dd, *J* = 10.98 and 6.10 Hz, 9'-Hb), 3.66 (1H, dd, *J* = 10.86 and 6.71 Hz, 9-Hb), 3.77–3.82 [1H, overlapping (dd), *J*₂ = 3.90 Hz, 9-Ha], 3.81 (6H, s, 3',5'-OCH₃), 3.81–3.85 [1H, overlapping (dd), *J*₂ = 4.14 Hz, 9'-Ha], 3.88 (3H, s, 5-OCH₃), 4.03 (1H, d, *J* = 7.56 Hz, 7'-H), 5.30 (1H, s, 4-Ar-OH), 5.35 (1H, s, 4'-Ar-OH), 6.35 (2H, s, 2',6'-Ar-H), 6.46 (1H, s, 6-Ar-H). ¹³C NMR (acetone-*d*₆): δ 33.9 (7'-C), 41.2 (8-C), 42.6 (7-C), 49.3 (8'-C), 56.4 (3-OCH₃), 56.8 (2C, 3',5'-OCH₃), 59.4 (5-OCH₃), 63.8 (9'-C), 66.5 (9-C), 107.1 (6-C), 107.2 (2',6'-C), 126.4 (1-C), 129.8 (2-C), 134.9 (4'-C), 138.6 (1'-C), 139.3 (5-C), 147.1 (3-C), 147.7 (4-C), 148.3 (2C, 3',5'-C). IR (KBr): ν_{\max} cm⁻¹ 3436 (OH), 2938, 1615, 1518, 1503, 1458, 1425, 1322, 1218, 1115. EI-MS *m/z* (rel. int.): 420

[M⁺] (100), 402 (7), 371 (19), 249 (18), 248 (15), 217 (34), 210 (18), 205 (39), 184 (27), 183 (52), 167 (49).

Determination of absolute configuration of synthesized lignans

Absolute configurations of (+)- and (-)-SYRs are known. Synthetic (±)-SYR (11.8 mg) was separated to (8*R*,8'*R*)-(+)- and (8*S*,8'*S*)-(-)-enantiomers by preparative chiral HPLC. Both the enantiomers were transformed individually to DMLR and DMSLR by catalytic reduction with 20% palladium hydroxide on carbon under the same condition as above. Sign of optical rotation of the resulting (8*R*,8'*R*)-DMLR and (8*R*,8'*R*)-DMSLR, and (8*S*,8'*S*)-DMLR and (8*S*,8'*S*)-DMSLR, respectively, were determined by chiral HPLC with a chiral detector.

Plant material

Trunks and large branches of *Lyonia ovalifolia* var. *elliptica* were collected at Kamiyama Forest of Kagawa University, Sanuki, Kagawa, in September 2003.

Extraction and isolation

The air-dried trunks and branches from which bark was removed were chopped into small pieces, with a metallic chopper, and then ground in a Wiley mill and sieved (40–80 mesh). The resulting wood powder (3.0 kg) was extracted in Erlenmeyer flasks with cold MeOH for 10 days at room temperature. The whole extract was combined and concentrated in vacuo to make the volume about one tenth of the starting one, to which five times volume of milli-Q water was added and the solution was centrifuged (1100 g) at 20°C for 20 min. The supernatant was extracted three times with EtOAc to give the organic and aqueous phases. The combined organic phase (the EtOAc solution) was washed once with saturated NaCl solution. The organic phase was evaporated to dryness in vacuo to give the crude extract (26.2 g) of the wood. The aqueous phases were also combined and evaporated to dryness by freeze drying.

The crude extract was chromatographed over silica gel using *n*-hexane with gradually increasing proportions of EtOAc to 100% EtOAc and finally with MeOH as eluent giving fractions 1, 2, 3, 4, 5, 6, and 7. Fractions 4, 5, 6, and 7 were chosen for further analysis to isolate the lignans by comparing the retention times (reversed-phase HPLC analysis) of authentic lignans with those of the four selected fractions. Preparative TLC separation of the four fractions was repeated. Fraction 5 was separated by preparative TLC (dichloromethane/MeOH, 91:9) to give four bands (bands 5–1, 5–2, 5–3, and 5–4). The bands 5–1 and 5–2 were identified as SYR and DMLR, respectively. The latter was also obtained as band 6–2, one of six bands given by preparative TLC (dichloromethane/MeOH, 97:3) of fraction 6. Fraction 7 gave four bands (bands 7–1 to band 7–4) by preparative TLC (dichloromethane/MeOH, 90:10). Further separation of band 7–1 by preparative TLC

(dichloromethane/MeOH, 91:9) gave four subfractions (f-7-1-1 to f-7-1-4), where subfraction f-7-1-1 was identified as SYR and the bands 7–2 and 7–3 were identified as DMSLR and LYR, respectively.

Enantiomeric composition of the isolated lignans was determined by chiral HPLC. Each of the lignans was dissolved in a limited amount of MeOH. The MeOH solutions were subjected to the chiral column, with conditions (column, solvent, flow rate) of: SYR, Chiralcel OD (Diacel), EtOH/*n*-hexane 50:50, 0.8 ml/min; DMLR, Chiralcel OC (Diacel), MeOH, 0.8 ml/min; DMSLR, Chiralcel OD, EtOH/*n*-hexane 30:70, 0.5 ml/min; LYR, Chiralcel OC, EtOH/*n*-hexane 80:20, 0.5 ml/min.

Results and discussion

Absolute configuration

The absolute configurations of (+)-SYR, (-)-SYR, (+)-LYR, and (-)-LYR are (8*R*, 8'*R*), (8*S*, 8'*S*), (8*R*, 8'*R*), and (8*S*, 8'*S*), respectively (Fig. 2),^{28–30} whereas those of DMLR and DMSLR have been unknown. Figure 3 showed that the catalytic reduction of (8*R*,8'*R*)-(+)-SYR with 20% palladium hydroxide on carbon provided (+)-DMLR and (-)-DMSLR, respectively, and that of (-)-SYR gave (-)-DMLR and (+)-DMSLR, respectively. The upward and downward peaks given by the chiral detector show (+)- and (-)-enantiomers, respectively. Therefore, (+)-DMLR, (-)-DMSLR, (-)-DMLR, and (+)-DMSLR were determined as (8*R*, 8'*R*), (8*R*, 8'*R*), (8*S*, 8'*S*), and (8*S*, 8'*S*), respectively (Fig. 2).

Isolation and identification of lignans from wood

From the methanol extract of wood from *Lyonia ovalifolia* var. *elliptica*, four syringyl lignans were isolated and identified as SYR, DMLR, DMSLR, and LYR by ¹H NMR and EI-MS spectroscopic and chromatographic data (reversed-phase HPLC, chiral HPLC, and TLC) comparison of the isolated compounds with those of the synthetic standards. SYR, DMLR, and DMSLR were isolated from this plant for the first time. These lignans can be regarded as intermediates of the biosynthetic pathway for (+)-LYR.

SYR was obtained as pale yellow needles (1.4 mg). The EI-MS exhibited a molecular ion [M⁺] peak at *m/z* 418 implying the molecular formula C₂₂H₂₆O₈. It provided half of the expected number of proton signals indicating the presence of a symmetric structure of two phenylpropanoid moieties. All of the ¹H NMR signals were assigned as follows. UV λ_{max}: 280 nm. ¹H NMR (CDCl₃): δ 3.10 (2H, m, 8,8'-H), 3.91 (12H, s, 3,5,3',5'-OCH₃), 3.88–3.92 (2H, overlapping, 9,9'-Ha), 4.28 (2H, dd, *J* = 9.27 and 7.32 Hz, 9,9'-Hb), 4.73 (2H, d, *J* = 4.39 Hz, 7,7'-H), 5.48 (2H, s, 4,4'-Ar-OH), 6.59 (4H, s, 2,6,2',6'-Ar-H). EI-MS *m/z* (rel. int.): 418 [M⁺] (54), 387 (3), 360 (3), 221 (10), 193 (14), 191 (8), 182 (86), 181 (100), 167 (68), 154 (25), 139 (19), 123 (20).

DMLR was obtained as a syrup (less than 0.5 mg). The EI-MS showed a molecular ion [M⁺] peak at *m/z* 420

Fig. 2. Absolute configuration of both enantiomers of the isolated lignans: SYR, DMLR, DMSLR, and LYR. Absolute configuration of DMLR and DMSLR was determined in this study

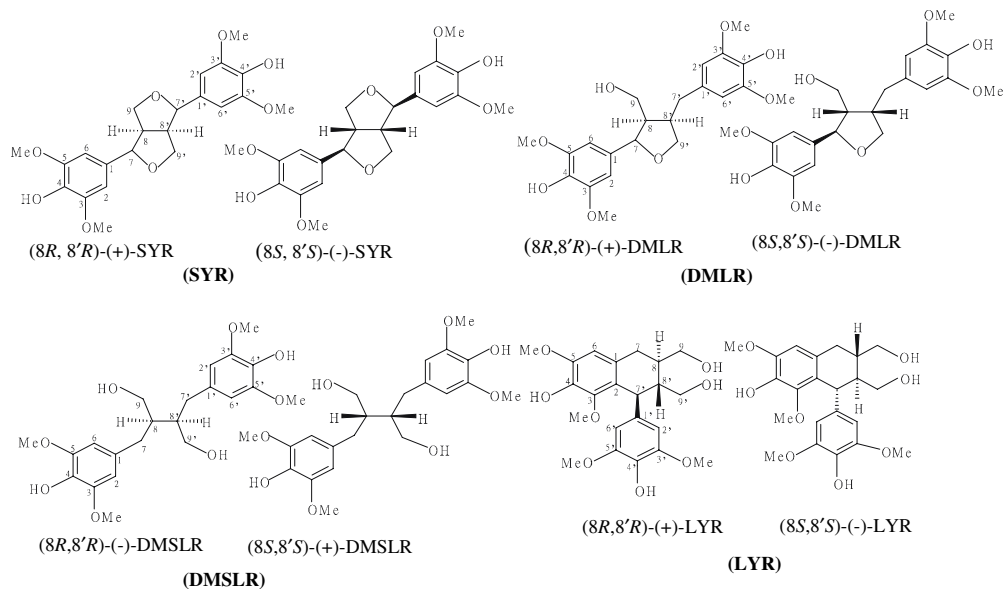
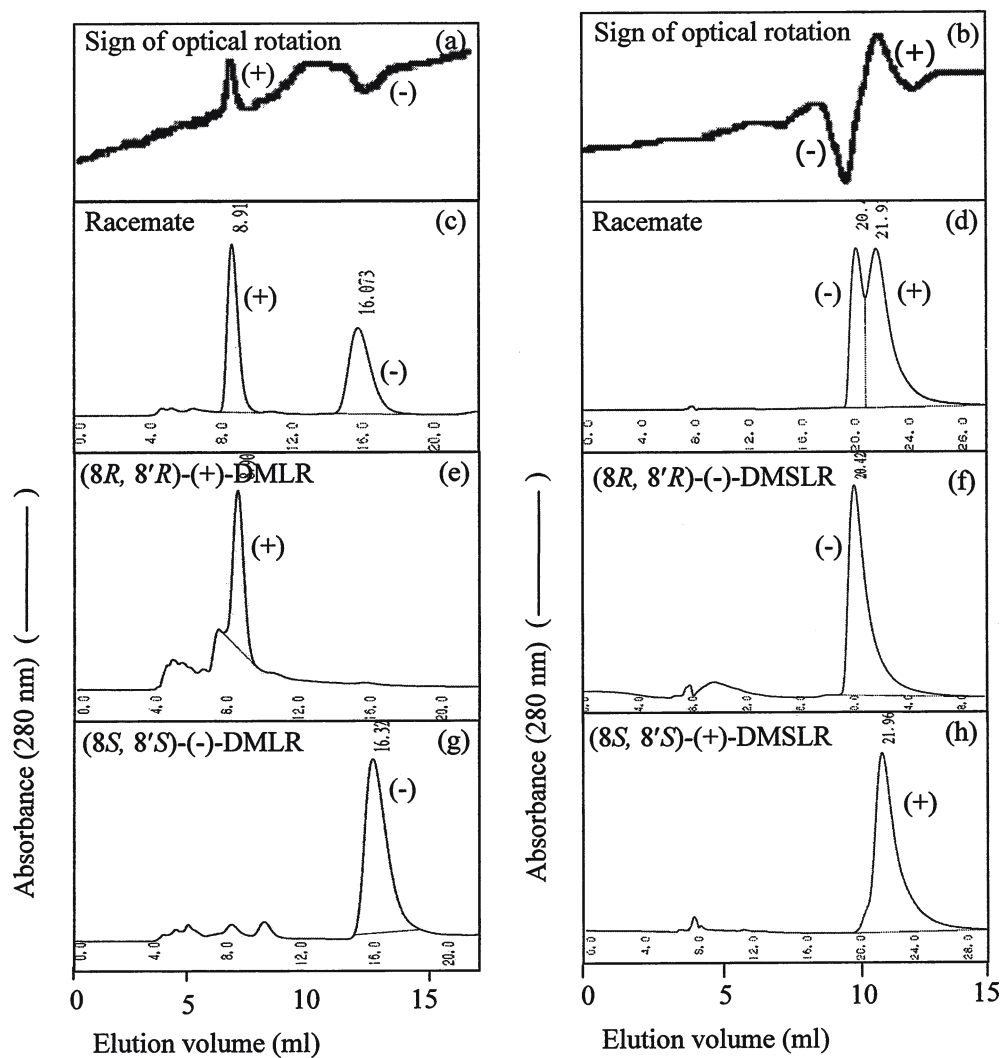


Fig. 3a–h. Determination of absolute configuration of DMLR and DMSLR by their chiral HPLC analysis. **a** Sign of optical rotation (SOR) for DMLR, **b** SOR for DMSLR. Chromatograms for **c** (\pm)-DMLR and **d** (\pm)-DMSLR from synthetic (\pm)-SYR. Chromatograms for **e** (*8R,8'R*)-(+)-DMLR and **f** (*8R,8'R*)-(-)-DMSLR from (*8R,8'R*)-(+)-SYR. Chromatograms for **g** (*8S,8'S*)-(-)-DMLR and **h** (*8S,8'S*)-(+)-DMSLR from (*8S,8'S*)-(-)-SYR



suggesting the molecular formula $C_{22}H_{28}O_8$. The fragment peaks were $[M-H_2O]^+$ at 402, and $[ArCH_2]^+$ at 167. The 1H NMR spectrum showed the following peaks that are identical with those of the synthetic standards, although the 1H NMR spectrum did not show all the signals due to limited quantities of sample. UV λ_{max} : 280 nm. 1H NMR ($CDCl_3$): δ 3.88 (6H, s, 3',5'-OCH₃), 3.89 (6H, s, 3,5-OCH₃), 4.79 (1H, d, $J = 6.59$ Hz, 7-H), 5.39 (1H, s, 4'-Ar-OH), 5.46 (1H, s, 4-Ar-OH), 6.42 (2H, s, 2', 6'-Ar-H), 6.57 (2H, s, 2, 6-Ar-H). Peaks of 8-H and 8'-H, 7'-Ha and 7'-Hb, 9'-Ha and 9'-Hb, and 9-Ha and 9-Hb were under the noise level or overlapping with impurity. EI-MS m/z (rel. int.): 420 [M^+] (28), 402 (32), 235 (15), 221 (16), 205 (25), 191 (15), 182 (40), 181 (62), 168 (39), 167 (100), 154 (21), 137 (32), 123 (28).

DMSLR was obtained as a colorless syrup (1.9 mg). The EI-MS exhibited a molecular ion [M^+] peak at m/z 422 implying the molecular formula $C_{22}H_{30}O_8$ and the following fragment peaks $[M-H_2O]^+$ at 404 (39), and $[ArCH_2]^+$ at 167 (100). All the signals of DMSLR were assigned as follows: UV λ_{max} : 280 nm. 1H NMR ($CDCl_3$): δ 1.87 (2H, m, 8,8'-H), 2.66 (2H, dd, $J = 13.42$ and 6.83 Hz, 7,7'-Ha), 2.75 (2H, dd, $J = 13.54$ and 7.68 Hz, 7,7'-Hb), 3.59 (2H, dd, $J = 11.10$ and 4.27 Hz, 9,9'-Ha), 3.83 (12H, s, 3,5,3',5'-OCH₃), 3.82–3.87 (2H, overlapping, 9,9'-Hb), 5.38 (2H, s, 4,4'-Ar-OH), 6.33 (4H, s, 2,6,2',6'-Ar-H). EI-MS m/z (rel. int.): 422 [M^+] (42), 404 (40), 235 (15), 221 (16), 205 (25), 191 (15), 182 (40), 181

(62), 168 (100), 167 (100), 153 (21), 147 (8), 137 (55), 123 (18), 122 (18).

LYR was obtained as crystalline needles (7.5 mg) from MeOH. The EI-MS exhibited a molecular ion [M^+] peak at m/z 420 implying the molecular formula $C_{22}H_{28}O_8$. It was identified as lyoniresinol from the data assigned as follows: UV λ_{max} : 280 nm. 1H NMR ($CDCl_3$): δ 1.76 (1H, m, 8'-H), 1.93 (1H, m, 8-H), 2.59 (1H, dd, $J = 15.23$ and 4.03 Hz, 7'-Hb), 2.68 (1H, dd, $J = 14.88$ and 12.20 Hz, 7'-Ha), 3.31 (3H, s, 3'-OCH₃), 3.58 (1H, dd, $J = 10.98$ and 5.85 Hz, 9'-Hb), 3.64 (1H, dd, $J = 10.86$ and 6.71 Hz, 9-Hb), 3.75–3.80 [1H, overlapping (dd), $J_2 = 3.90$ Hz, 9-Ha], 3.79–3.82 (1H, overlapping, 9'-Ha), 3.80 (6H, s, 3,5-OCH₃), 3.89 (3H, s, 5'-OCH₃), 4.03 (1H, d, $J = 7.81$ Hz, 7-H), 5.33 (1H, s, 4'-Ar-OH), 5.37 (1H, s, 4-Ar-OH), 6.35 (2H, s, 2,6-Ar-H), 6.45 (1H, s, 6'-Ar-H). EI-MS m/z (rel. int.): 420 [M^+] (46), 402 (100), 400 (19), 371 (13), 302 (13), 249 (9), 248 (14), 217 (25), 205 (30), 184 (11), 183 (22), 167 (54).

Enantiomeric compositions and absolute configurations of the isolated lignans

To elucidate the enantiomeric compositions of the four isolated syringyl lignans (Fig. 2), chiral column HPLC separation into (+)- and (-)-enantiomers was adopted (Fig. 4).

Fig. 4a–l. Chiral separation of lignans. **a** Isolated SYR, **b** SOR for SYR, **c** synthetic authentic (\pm)-SYR, **d** isolated DMLR, **e** SOR for DMLR, **f** synthetic authentic (\pm)-DMLR, **g** isolated DMSLR, **h** SOR for DMSLR, **i** synthetic authentic (\pm)-DMSLR, **j** isolated LYR, **k** SOR for LYR, **l** synthetic authentic (\pm)-LYR

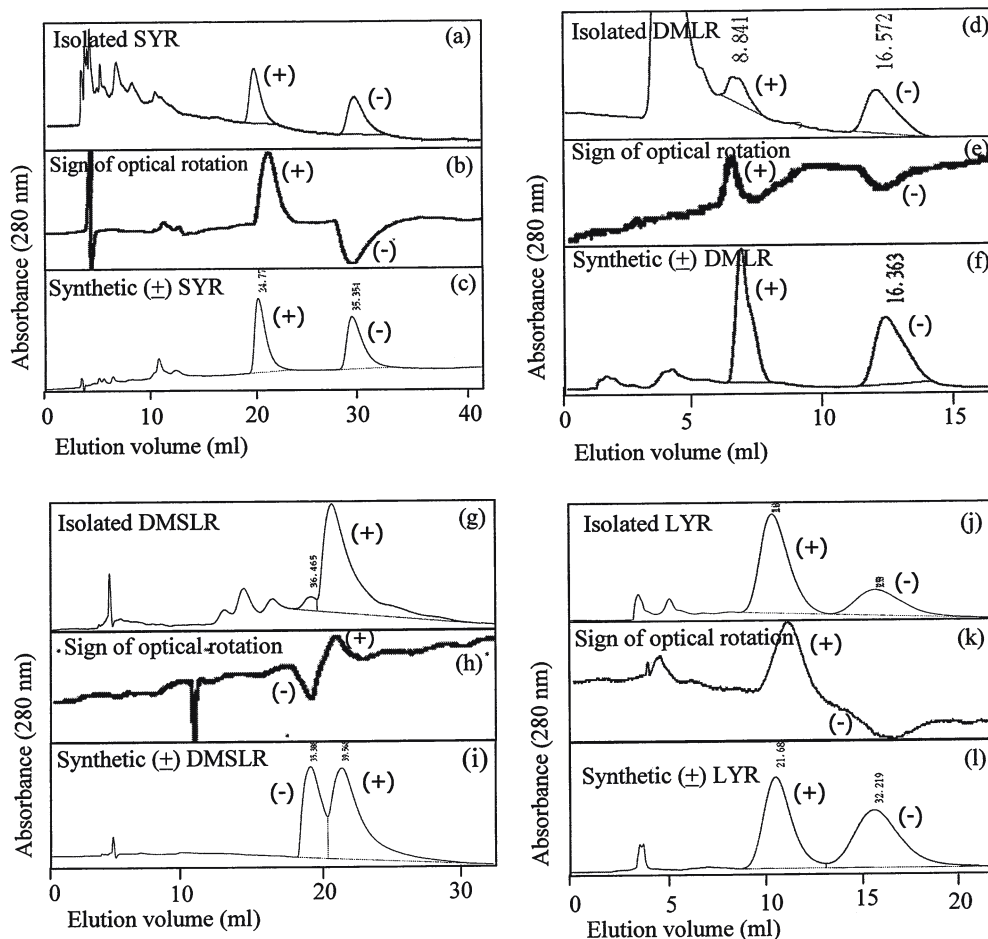


Table 2. Enantiomeric composition of the isolated syringyl lignans and absolute configurations of the preferred enantiomers

Isolated lignans	(+)-form (%)	(-)-form (%)	Enantiomeric excess (% e.e.)
Syringaresinol	49 <i>R</i>	51 <i>S</i>	Almost racemate
5,5'-Dimethoxyariciresinol	27 <i>R</i>	73 <i>S</i>	(-) 46 (8 <i>S</i> , 8' <i>S</i>)
5,5'-Dimethoxysecoisolariciresinol	95 <i>S</i>	5 <i>R</i>	(+) 91 (8 <i>S</i> , 8' <i>S</i>)
Lyoniresinol	71 <i>R</i>	29 <i>S</i>	(+) 42 (8 <i>R</i> , 8' <i>R</i>)

Table 2 shows that because the composition of (+)- and (-)-enantiomers of SYR were almost identical (49% and 51%, respectively), it was a racemate, and that (-)-DMLR (73%), (+)-DMSLR (95%), and (+)-LYR (71%) predominated. Absolute configurations of the four syringyl lignans have already been described above. Therefore, the stereochemistry of the four lignans is (±)-SYR, (8*S*,8'*S*)-(-)-DMLR [46% enantiomeric excess (e.e.)], (8*S*,8'*S*)-(+)-DMSLR (91% e.e.), and (8*R*,8'*R*)-(+)-LYR (42% e.e.) (Fig. 2).

Based on the assumed biosynthetic pathway (Fig. 1) and the above results (isolation and identification of LYR and its putative biosynthetic intermediates from wood, and determination of their enantiomeric compositions and absolute configurations), feeding experiments using radioactive precursors will be performed in our next study to establish the biosynthetic pathway for (+)-LYR.

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