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Stereochemistry and biosynthesis of (+)-lyoniresinol, a syringyl tetrahydronaphthalene lignan in *Lyonia ovalifolia* var. *elliptica* II: feeding experiments with ^{14}C labeled precursors

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Abstract To clarify the biosynthetic pathway for syringyl lignans, especially syringyl tetrahydronaphthalene lignans and formation of the C2-C7′ linkage, production of (+)-lyoniresinol (LYR) and its predicted intermediates [syringaresinol (SYR), 5,5′-dimethoxylariciresinol (DMLR), and 5,5′-dimethoxysecoisolariciresinol (DMSLR)] in *Lyonia ovalifolia* var. *elliptica* was investigated by means of feeding experiments with radiolabeled precursors. Following individual administration of L-[U-^{14}C]phenylalanine (Phe), [8-^{14}C]sinapyl alcohol (SA), and [8,8′-^{14}C]SYR to excised young shoots of *L. ovalifolia* and their subsequent metabolism, free [^{14}C]lignans and [^{14}C]lignan glycosides were extracted with methanol from stems and leaves and were divided into ethyl acetate-soluble fractions (lignans) and aqueous fractions (lignan glycosides), respectively. Using a combination of xylanase, cellulase, and β-glucosidase, the glycosides were hydrolyzed to liberate [^{14}C]lignans as aglycones. L-[U-^{14}C]Phe was incorporated into (+)-[^{14}C]SYR [stem 0.38%, 8% enantiomeric excess (e.e.)], (−)-[^{14}C]SYR (leaves 2.75%, 72% e.e.), (+)-[^{14}C]DMLR (stem 0.07%, 18% e.e. and leaves 0.009%, 58% e.e.), (−)-[^{14}C]DMSLR (stem 0.03%, 46% e.e. and leaves 0.05%, 20% e.e.), (+)-[^{14}C]LYR (leaves 0.013%, 22% e.e.) and glycosides of (+)-[^{14}C]LYR (stem 0.036%, 50% e.e.) in 24 h. Based on the percent incorporation and enantiomeric composition of the lignans, the biosynthetic pathway of (8*R*,8′*R*)-(+)-LYR was proposed as follows: a nonselective dehydrogenative dimerization of sinapyl alcohol yields (±)-SYR, which is reduced with low specificity to give (8*R*,8′*R*)-(+)-DMLR. This is cyclized to directly give (+)-LYR as well as reduced again to (8*R*,8′*R*)-(−)-DMSLR. Although further transformation of (−)-DMSLR also leads to the formation of (+)-LYR, cyclization could be a main pathway for (+)-LYR biosynthesis.

Key words Syringyl lignan · *Lyonia ovalifolia* var. *elliptica* · Biosynthesis · 5,5′-Dimethoxylariciresinol · Lyoniresinol

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Introduction

Lignans are physiologically important for plant defense, particularly for heartwood and seed-forming tissues.[1] Some lignans show important roles in human medicine, such as podophyllotoxin for venereal wart treatment,[2,3] and its semisynthetic derivatives, etoposide, teniposide, and etopophos, in cancer therapies.[4–8] Mammalian lignans such as enterodiol and enterolactone, appear to be effective in preventing the onset of various cancers including breast and prostate cancers.[9–12] As for typical syringyl lignans, liriodendrin has cytotoxic effect;[13] (+)-syringaresinol di-*O*-β-D-glucoside exhibits a prolonging effect on the exercise time to exhaustion in chronically swimming rats;[14] syringaresinol di-*O*-β-D-glucoside shows a significant inhibitory effect on gastric ulcers in restrained cold water-stressed rats.[15]

The pathway for biosynthesis of syringyl lignans, especially those with arylnaphthalene and aryltetrahydronaphthalene (or 4-aryltetralin) skeletons, is largely unknown, although that of guaiacyl lignans has been intensively studied for the past 15 years.[16] Lyoniresinol (LYR), which has recently received much attention due to its antimutagenic and antioxidative activities,[17] is a typical syringyl lignan of the 4-aryltetrahydronaphthalene class. LYR[18] and its xyloside[19] (lyoniside) were first isolated from *Lyonia ovalifolia* var. *elliptica* by Kato and coworkers. In our previous report,[20] we reported the isolation of some syringyl lignans [syringaresinol (SYR), 5,5′-dimethoxylariciresinol (DMLR), and 5,5′-dimethoxysecoisolariciresinol (DMSLR)], which are regarded as the predicted intermediates for LYR biosynthesis according to the analogy to the biosynthetic pathway of guaiacyl lignans in *Forsythia*[21–27]

and podophyllotoxin in *Podophyllum* species,[28,29] together with (+)-LYR from the wood of *L. ovalifolia*. In addition, we determined the stereochemistry (absolute configuration and enantiomeric composition) of these compounds.

In the current study, we performed feeding experiments with excised shoots of the tree using L-[U-^{14}C]phenylalanine (Phe), [8-^{14}C]sinapyl alcohol (SA), and [8,8′-^{14}C]SYR in order to clarify the biosynthetic pathway of LYR. L-Phe is an entry compound into the phenylpropanoid pathway and is transformed into lignans and lignins, as well as some other aromatic secondary metabolites. SA is the immediate precursor of typical syringyl lignans such as SYR[30] and syringyl lignin. SYR is considered to be an entry and pivotal compound in the biosynthetic pathway for different syringyl lignans. Based on the percent incorporation and stereochemistry (absolute configuration and enantiomeric composition) of the three syringyl lignans (SYR, DMLR, and DMSLR) and LYR itself, as well as the results of the previous study,[20] we have elucidated the biosynthetic pathway to (+)-LYR.

Experimental

General

The instrumentation and chromatography were the same as described previously.[20] Radioactivity of the sample was measured in 10 ml of liquid scintillation cocktail consisting of scintiblender II, polyethylene glycol mono-*p*-isooctyl phenyl ether, and toluene (6/40/54; v/v/v) (Nacalai Tesque) using an Aloka LSC-1000 liquid scintillation counter.

Plant material

Young shoots of *Lyonia ovalifolia* var. *elliptica* were collected at Kamiyama forest of Kagawa University, June to August, 2004 and 2005.

Preparation of radiolabeled precursors

L-[U-^{14}C]Phe, [8-^{14}C]SA, and [8,8′-^{14}C]SYR were used as labeled precursors for the feeding experiments. A solution (10 mM) of L-[U-^{14}C]Phe (17.73 MBq/mmol) in potassium phosphate (K-Pi) buffer (0.1 M, pH 7.0) was prepared by diluting L-[U-^{14}C]Phe [Moravek biochemicals, 9.25 MBq, (sp. activity, 14.615 GBq/mmol) (0.0459 mmol)] with unlabeled L-Phe (2.88 mg, 0.017 mmol) as cold carrier. [8-^{14}C]SA prepared as crystals by Lourith et al.[31] was used for the feeding experiment and [8,8′-^{14}C]SYR was synthesized in the following steps.

[8-^{14}C]Sinapic acid. [2-^{14}C]Malonic acid (160.85 mg, 1.55 mmol) was prepared by diluting [2-^{14}C]malonic acid (Moravek Biochemicals, 450 μg, 9.25 MBq, 2.12 GBq/mmol) with unlabeled malonic acid (160.4 mg, 1.54 mmol). The resulting [2-^{14}C]malonic acid and syringaldehyde (420.6 mg, 2.52 mmol) were dissolved in pyridine (5 ml), to which was added piperidine (0.1 ml) and aniline (0.1 ml). The reaction solution was stirred for 23 h at 55°C. After cooling to room temperature, the reaction solution was evaporated and acidified with 2 N HCl solution. The whole was then extracted three times with ethyl acetate (EtOAc). The combined EtOAc solution was washed with sodium bisulfite (20% w/w) solution to remove syringaldehyde and then with saturated NaCl solution. The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo to afford [8-^{14}C]sinapic acid as crude crystals, (468 mg, 80% yield) which was used in the next reaction.

[8-^{14}C]Methyl sinapate. For this reaction, a Soxhlet extractor containing anhydrous $CaSO_4$ was used. Crude sinapic acid (468 mg, 2.08 mmol) was dissolved in distilled methanol (MeOH, 20 ml) in a flask of the extractor, and 80 ml of MeOH was added from the extractor. To the reaction solution in the flask, concentrated H_2SO_4 (0.2 ml) was added. The reaction solution was refluxed with the refluxing MeOH dried continuously. After 3 h, the reaction solution was cooled to room temperature and neutralized (pH 5–6) by the addition of powdered sodium hydrogencarbonate ($NaHCO_3$). The whole was then filtered, and the salts were washed with MeOH. The filtrate and the washings were combined and concentrated in vacuo. The residue was partitioned between dichloromethane (CH_2Cl_2) and 5% saturated $NaHCO_3$ solution, and the aqueous phase was extracted twice with CH_2Cl_2. The CH_2Cl_2 solutions were combined and washed with saturated NaCl solution. The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo to give [8-^{14}C]methyl sinapate (277.4 mg, recovery 60%) as a crude syrup that was used in the next reaction.

[8-^{14}C]SA. A solution of [8-^{14}C]methyl sinapate (crude, 277.4 mg, 1.17 mmol) in toluene (12.5 ml, freshly distilled) was cooled to 0°C in an ice–water bath. To the stirred cold solution, a solution (1.5 M, 3.0 ml) of diisobutylaluminum hydride (DIBAL-H, Aldrich) in toluene was added dropwise with a syringe over 10 min under a nitrogen atmosphere[32] and then stirring was continued for an additional 50 min at the same temperature. The reaction mixture was then carefully quenched with ethanol (3 ml). The solvents were partially removed in vacuo at 40°C. Water (10 ml) was added to the residue, and the aqueous phase, containing a gelatinous precipitate of aluminum salts, was extensively extracted with EtOAc (50 ml × 3). The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo at 35°–38°C under a nitrogen atmosphere to give [8-^{14}C]SA as a syrup (248.6 mg, 89% recovery), which was immediately used in the next reaction.

[8,8′-^{14}C]SYR. A solution of [8-^{14}C]SA (100 mg, 0.47 mol) in 3.0 ml acetone was mixed with a solution of K-Pi buffer (13.0 ml, 0.1 M, pH 7.0) at room temperature.[33,34] To the stirred solution, a solution of horseradish peroxidase (1 mg) (60 units/mg) in the same buffer (0.103 ml) and 0.5% H_2O_2 solution (3.0 ml) was added dropwise successively. Stirring

was continued for 15 min. The reaction mixture was then carefully quenched by the addition of powdered NaCl. The whole was then extracted three times with EtOAc. The EtOAc solutions were combined and washed once with saturated NaCl solution. The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo. The residue was purified by silica gel column chromatography (2 × 30 cm) to give [8,8′-^{14}C]syringaresinol (45.0 mg, 45% yield) as crystals.

Preparation and determination of absolute configuration of 5,5′-dimethoxylariciresinol and 5,5′-dimethoxysecoisolaiciresinol

These procedures are described in a previous report.[20]

Feeding experiments

L-[U-^{14}C]Phe

Eleven young shoots of *L. ovalifolia* var. *elliptica* (13–23 cm) were excised with scissors. A solution (10 mM) of L-[U-^{14}C]Phe (17.7 MBq/mmol) in the K-Pi buffer (140 μl each) was administered to the shoots in an environment-controlled room at 25°C, and the shoots were then allowed to metabolize for 24 h. The stems and leaves were divided and individually treated as follows. Each of them was frozen immediately with liquid nitrogen and lyophilized. The dried stems (7.0 g) and leaves (10.09 g) were chopped into small pieces (1–2 mm) with scissors. The resulting material was extracted five times with hot MeOH. The MeOH solutions were combined and concentrated to about 32 ml (stem) and 65 ml (leaves), to which was added 160 ml and 325 ml of milli-Q water, respectively. The resulting suspension was centrifuged (1100 *g*, 20 min). After an aliquot of the supernatant was subjected to liquid scintillation counting, the remainder was extracted three times with EtOAc. The combined EtOAc solution was washed with saturated NaCl solution and dried over anhydrous Na_2SO_4. Aliquots of the EtOAc solution and the aqueous phase were subjected to liquid scintillation counting. The EtOAc solution was evaporated to dryness in vacuo.

The aqueous phase was lyophilized to give a powder (9.7 g from stems, 12.5 g from leaves), which (each 400 mg) was treated with a mixture of xylanase (*Thermomyces lanuginosus* powder, 2500 U/g, recombinant, expressed in *Aspergillus oryzae*), β-glucosidase (Oriental yeast, from sweet almond, 36.8 U/mg), and cellulase (Wako, from *Trichoderma viride*, 1000 U/mg) in 20 mM sodium acetate buffer (100 ml, pH 5.0) at 50°C under nitrogen for 24 h. The liberated aglycones were extracted three times with EtOAc. The combined EtOAc solutions were dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo to give extracts (4.6 mg from the stems; 15.5 mg from the leaves).

A part of (1.0 mg, 5000 ppm) EtOAc extracts from both stems and leaves were dissolved in MeOH. An aliquout of the soluble extract was applied to reversed-phase high-performance liquid chromatography (HPLC) (TSKgel, ODS-80Ts) and eluted with a gradient solvent system consisting of MeOH/0.3% AcOH in H_2O (30:70) at t = 0 min to 60:40 at t = 40 min (flow rate 0.8 ml/min). The eluate was collected in scintillation vials at every 1 min for liquid scintillation counting.

The remainder of the MeOH-soluble extract was purified by thin-layer chromatography (TLC) (7% MeOH in CH_2Cl_2) to afford [^{14}C]SYR, [^{14}C]DMLR, [^{14}C]DMSLR, and [^{14}C]LYR, which were then reconstituted in a limited amount of MeOH. The MeOH solutions were subjected to chiral column HPLC under the 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 (Diacel), EtOH/*n*-hexane 30:70, 0.5 ml/min; LYR, Chiralcel OC (Diacel), EtOH/*n*-hexane 80:20, 0.5 ml/min. The eluate was collected at 1-min intervals for liquid scintillation counting.

[8-^{14}C]SA

Seven young shoots (14–17 cm) were excised with scissors. A solution (20 mM) of [8-^{14}C]SA (3.59 MBq/mmol) in the K-Pi buffer (0.1 mM, pH 5.0, 140 μl each) was administered to the shoots, which were then allowed to metabolize for 3 h. The methods of extraction, fractionation, and HPLC analysis of the [^{14}C]lignans were similar to those mentioned above except that unlabeled SYR, DMLR, and DMSLR (15 μg of each) were added as radiochemical carrier.

[8,8′-^{14}C]SYR

Twelve young shoots (10–21 cm) were excised with scissors. A solution (5.2 mM) of [8,8′-^{14}C]SYR (17.6 MBq/mmol) in the K-Pi buffer (0.1 mM, pH 5.0, 140 μl each) was administered to the shoots, which were then allowed to metabolize for 24 h. The methods of extraction, fractionation, and HPLC analysis of [^{14}C]lignans were similar to those mentioned above for [8-^{14}C]SA.

Results and discussion

Incorporation of carbon-14 labeled precursors

Following administration of [8-^{14}C]sinapyl alcohol for 3 h or L-[U-^{14}C]Phe or [8,8′-^{14}C]SYR for 24 h to the excised young shoots of *Lyonia ovalifolia*, the stems and leaves were separated and extracted with hot methanol. The methanol extracts were partitioned between the ethyl acetate phase containing free lignans and the aqueous phase containing lignan glycosides. The glycosides were hydrolyzed by a mixture of xylanase, cellulase, and β-glucosidase to liberate lignan aglycones. All the free lignans from the ethyl acetate phases and the lignan aglycones from the aqueous phases were analyzed by both reversed-phase and chiral HPLC.

Reversed-phase radiochromatograms of all the fractions showed that L-[U-^{14}C]Phe (Table 1) was incorporated into

Table 1. Incorporation and enantiomeric composition of radioactive [^{14}C]SYR, [^{14}C]DMLR, [^{14}C]DMSLR, and [^{14}C]LYR obtained from stem and leaves after administration of L-[^{14}C]phenylalanine to excised shoots of *Lyonia ovalifolia* var. *elliptica*

Precursor	Radioactive lignans	Stems			Leaves			Total of C	Isolated lignans from wood[a]	
		A	B	C	A	B	C		A	B
L-[U-^{14}C]Phenylalanine	Syringaresinol									
	Free	54:46	8	0.38	14:86	(–)72	2.75	3.13	49:51	Almost racemate
	Glycosides									
	5,5′-Dimethoxylariciresinol									
	Free	59:41	18	0.07	79:21	58	0.009	0.079	27:73	(–)45.5
	Glycosides									
	5,5′-Dimethoxysecoisolariciresinol									
	Free	27:73	(–)46	0.03	40:60	(–)20	0.05	0.08	95:5	90.7
	Glycosides									
	Lyoniresinol									
	Free				61:39	22	0.013	0.013	71:29	41.8
	Glycosides	75:25	50	0.036				0.036		
	Total of C			0.516			2.822	3.338		

A, Ratio of (+):(–); B, percent enantiomeric excess; C, percent incorporation
[a]Data from Atiar et al.[20]

Table 2. Incorporation and enantiomeric composition of radioactive [^{14}C]SYR, [^{14}C]DMLR, and [^{14}C]LYR obtained from stem and leaves after administration of [8-^{14}C]sinapyl alcohol to excised shoots of *Lyonia ovalifolia* var. *elliptica*

Precursor	Radioactive lignans	Stems			Leaves			Total of C
		A	B	C	A	B	C	
[8-^{14}C]Sinapyl alcohol	Syringaresinol							
	Free	33:67	(–)34	9.12	42:58	(–)16	29	38.12
	Glycosides				14:86	(–)72	0.008	0.008
	5,5′-Dimethoxylariciresinol							
	Free	80:20	60	0.59	90:10	80	0.29	0.88
	Glycosides				55:45	10	0.007	0.007
	Lyoniresinol							
	Free				76:24	52	0.092	0.092
	Glycosides	99:1	98	0.036	80:20	60	0.004	0.04
	Total of C			9.746			29.401	39.147

A, Ratio of (+):(–); B, percent enantiomeric excess; C, percent incorporation

Table 3. Incorporation and enantiomeric composition of radioactive [^{14}C]DMLR and [^{14}C]LYR obtained from stem and leaves after administration of [8,8′-^{14}C]syringaresinol to excised shoots of *Lyonia ovalifolia* var. *elliptica*

Precursor	Radioactive lignans	Stems			Leaves			Total of C
		A	B	C	A	B	C	
[8,8′-^{14}C]Syringaresnol	5,5′-Dimethoxylariciresinol							
	Free	78:22	56	0.33	64:36	28	0.33	0.66
	Glycosides				49:51	(–)2	0.01	0.01
	Lyoniresinol							
	Free	72:28	44	0.04	55:45	10	0.06	0.1
	Glycosides	80:20	60	0.012	71:29	42	0.016	0.028
	Total of C			0.382			0.416	

A, Ratio of (+):(–); B, percent enantiomeric excess; C, percent incorporation

free SYR, DMLR, and DMSLR (and LYR in leaves only) (ethyl acetate fraction), and LYR glycosides in stems only (aqueous fraction). [8-^{14}C]SA (Table 2) was incorporated into free SYR and DMLR (and LYR in leaves only), and glycosides of SYR and DMLR in leaves only, and LYR, and not into either free DMSLR or its glycoside under this condition. [8,8′-^{14}C]SYR (Table 3) was taken prominently into free DMLR and LYR, and glycosides of DMLR in leaves only and LYR, and not into either free DMSLR or its glycoside under this condition.

Comparison between Tables 1, 2, and 3 shows that the percent incorporation values for L-[U-^{14}C]Phe into the lignans were lower than the corresponding values for [8-^{14}C]SA and [8,8′-^{14}C]SYR. This result is consistent with a previous report by this laboratory[30] and is expected because L-[U-^{14}C]Phe, the entry compound into the phenylpropanoid pathway, is incorporated not only into lignans and lignins but also to other classes of aromatic secondary metabolites such as *p*-hydroxycinamate derivatives, flavonoids, condensed tannins, etc., whereas sinapyl alcohol is an immediate precursor of syringyl lignan and syringyl lignin synthesis.

Table 2 indicates that the percent incorporation of [8-^{14}C]SA administration into free SYR in the ethyl acetate fraction is also very high in both stems (9.12%) and leaves (29%) compared with L-[U-^{14}C]Phe administration because SA which is an immediate precursor of syringyl lignans/neolignans and lignin would give most of (±)-SYR by nonspecific peroxidases in the plant.

Enantiomeric composition

Tables 1 and 2 show that [^{14}C]SYRs from L-[U-^{14}C]Phe (in leaf) and [8-^{14}C]SA were levorotatory except [^{14}C]SYR from L-[U-^{14}C]Phe (in stem) that had tiny dextrorotation [8% enantiomeric excess (e.e.)] in stem. [^{14}C]SYR formed by the administration of both L-[U-^{14}C]Phe and [8-^{14}C]SA was levorotatory as a whole, because the percent incorporation from L-[U-^{14}C]Phe in the leaves (2.75%) is higher than that in the stems (0.38%). The tiny enantiomeric composition (8% e.e.) corresponds to the enantiomeric composition of SYR [(+):(–), 49:51, almost racemate] isolated from the wood of *L. ovalifolia*.[20] The enantiomeric excess value of SYR [(–) 33% e.e.] from [^{14}C]SA is not high in the stem (Table 2).

Tables 1–3 show that free radioactive DMLR, DMSLR, and LYR from all administered precursors gave the (8*R*,8′*R*)-enantiomer [(+), (–), and (+)-enantiomers, respectively] predominately in both stems and leaves. All the precursors were also incorporated preferentially into the (8*R*,8′*R*)-(+)-enantiomers of the radioactive aglycones of DMLR and LYR glycosides from both stems and leaves except [8,8′-^{14}C]SYR, which was incorporated into the DMLR aglycone of leaves as a racemate, but with tiny percent incorporation (0.01%) (Table 3).

The preference of (+)-DMLR from all of its precursors indicates that a flux of (+)-SYR induces the formation of (+)-DMLR; however, natural DMLR was found to be in the (–) form (46% e.e.). Table 1 shows that the enantiomeric excess [stems (–) 46% e.e. and leaves (–) 20% e.e.] of radioactive DMSLR (not found from [8-^{14}C]SA and [8,8′-^{14}C]SYR administration, aforementioned) obtained from L-[U-^{14}C]Phe administration is opposite to that of isolated DMSLR [(+) 91% e.e.] from the wood of *L. ovalifolia*.[20] The enantiomeric composition of isolated (+)-LYR (42% e.e.) corresponds to those of radioactive LYR in all feeding experiments (Tables 1–3) indicating the formation of (+)-LYR by a mechanism as proposed in the tree.

Table 2 shows [8-^{14}C]SA was also incorporated into free DMLR, with the (+)-enantiomer dominating in both stems (80%, 60% e.e.) and leaves (90%, 80% e.e.), and LYR with the (+)-enantiomer predominating in leaves (76%, 52% e.e.). The precursor, [8-^{14}C]SA, was also incorporated into the (+)-enantiomer aglycone of LYR in leaves (aqueous phases) (80%, 60% e.e.). DMLR was also converted into an aglycone in leaves (aqueous phases) at a very low level.

Biosynthetic pathway

Based on the percent incorporation and stereochemistry of the [^{14}C]lignans in the feeding experiments, the biosynthetic pathway outlined in Fig. 1 is proposed. Nonselective coupling of SA gives both (8*R*,8′*R*)-(+)-SYR and (8*S*,8′*S*)-(–)-SYR, which are reduced with low specificity to give (8*R*,8′*R*)-(+)-DMLR as a main enantiomer and (8*S*,8′*S*)-(–)-DMLR as a minor enantiomer, respectively. Therefore, the (8*S*,8′*S*)-(–)-enantiomer of SYR remained. The selective formation of (–)-SYR by administration of the radioactive precursors is unlikely because the isolated SYR from the wood was found to be a racemate.[20]

The (8*R*,8′*R*)-(+)-DMLR then leads to (8*R*,8′*R*)-(+)-LYR through a stereospecific cyclization that could be the major pathway. The cyclization is chemically reasonable and this mechanism is partially supported by the absolute configuration of the isolated LYR from the wood.[20]

A low stereospecific reduction of (8*R*,8′*R*)-(+)-DMLR with (8*S*,8′*S*)-(–)-enantiomer might give (8*R*,8′*R*)-(–)-DMSLR with (8*S*,8′*S*)-(+)-enantiomer, part of which is further transformed to (8*R*,8′*R*)-(+)-LYR with (8*S*,8′*S*)-(–)-enantiomer. This proposed mechanism might be a minor pathway for formation of LYR because DMSLR was only found when L-[U-^{14}C]Phe was administered, not when the two other precursors were administered. A large amount of SYR formed from SA could give a certain amount of DMLR, most of which is transformed to LYR (major) and to DMSLR (minor). Therefore, DMLR might not be accumulated. The main enantiomers [(8*R*,8′*R*)] of [^{14}C]DMLR and [^{14}C]DMSLR in the feeding experiments have the same absolute configuration. On the other hand, the main enantiomers [(8*S*,8′*S*)] of DMLR and DMSLR isolated from the wood in the previous study also have the same absolute configuration.[20] However, the former is opposite to the latter. We think the difference might result from the differences of the ratio of (8*R*,8′*R*)-(+)-DMLR to (8*S*,8′*S*)-(–)-DMLR and of the activity of the stereospecific cyclization of the (+)-DMLR between the biosynthetic conditions in nature and those in the feeding experiments. To establish this pathway, we are developing enzyme assays for each step.

Katayama and Ogaki[30] showed the formation of (+)-SYR by feeding experiment using *Liriodendron tulipifera*. The enantiomer is consistent with the isolated (+)-SYR from the inner bark of *L. tulipifera*. We think stereochemistry of the SYR formation in *L. ovalifolia* is different from that in *L. tulipifera*. Although the possibility of a pathway from pinoresinol to syringaresinol by way of medioresinol cannot be disregarded at this time, neither pinoresinol nor medioresinol has been isolated from *L. ovalifolia* yet.

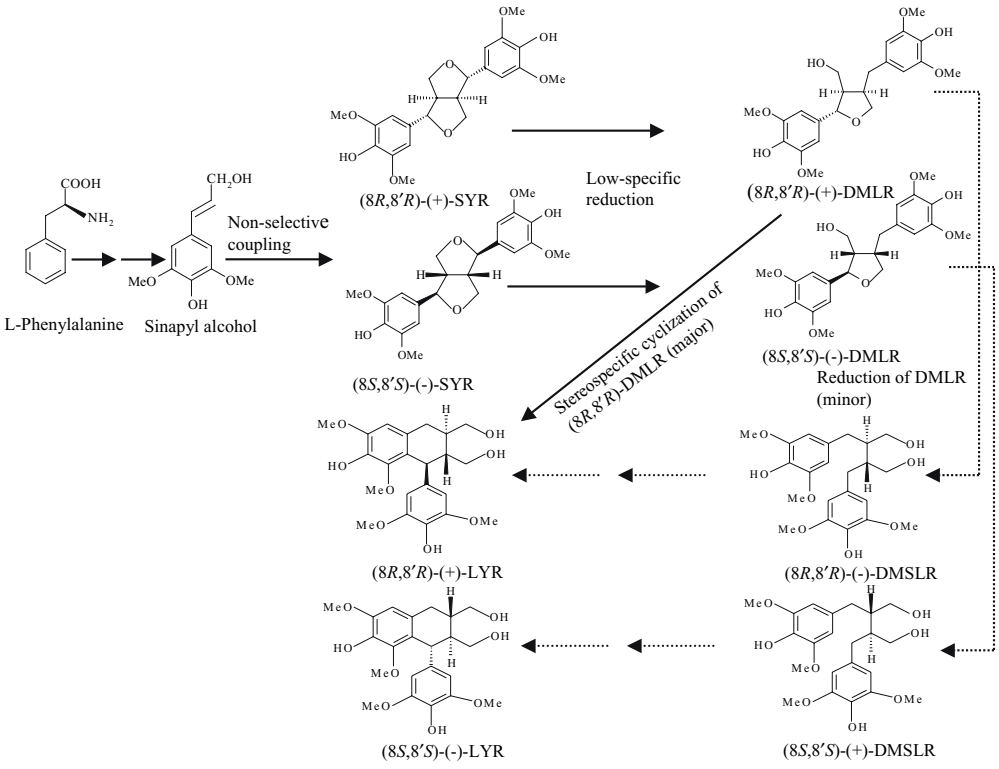

Fig. 1. Biosynthetic pathway for (+)-lyoniresinol (*LYR*) proposed by the feeding experiments. *SYR*, syringaresinol; *DMLR*, 5,5′-dimethoxylariciresinol; *DMSLR*, 5,5′-dimethoxysecoisolariciresinol

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