J Wood Sci (2001) 47:41–47

ORIGINAL ARTICLE

Takeshi Katayama · Atsushi Ogaki

Biosynthesis of (+)-syringaresinol in *Liriodendron tulipifera* I: feeding experiments with L-[U-^{14}C]phenylalanine and [8-^{14}C]sinapyl alcohol

Received: September 17, 1999 / Accepted: March 1, 2000

Abstract To clarify the biosynthesis of syringyl lignans and lignan formation by stereoselective coupling of monolignols, formation of (+)-syringaresinol and (+)-pinoresinol in *Liriodendron tulipifera* were investigated by means of feeding experiments. Following individual administration of L-[U-^{14}C]phenylalanine and [8-^{14}C]sinapyl alcohol to excised shoots of *L. tulipifera* and their subsequent metabolism for 3 h, free [^{14}C]lignans and [^{14}C]lignan glucosides were extracted from both of the stems and leaves with methanol and divided into an ether fraction and an aqueous one, respectively. The glucosides were hydrolyzed by a combination of cellulase and β-glucosidase to liberate [^{14}C]lignans as aglycones. L-[U-^{14}C]Phenylalanine was incorporated into free (+)-[^{14}C]syringaresinol and its glucosides; the (+)-[^{14}C]syringaresinols in the stems and leaves had 52% enantiomeric excess (% e.e.) and 42% e.e., respectively; and the (+)-[^{14}C]syringaresinol aglycones from the glucosides in the stems and leaves had 20% e.e. and 22% e.e., respectively. Furthermore, [8-^{14}C]sinapyl alcohol was incorporated into (+)-[^{14}C]syringaresinol and its glucosides in the stems. These results suggest that the (+)-enantiomer of syringaresinol was enantioselectively formed from two molecules of sinapyl alcohol in *L. tulipifera* followed by transformation into the (+)-syringaresinol glucosides, accompanying the formation of racemic syringaresinol by nonselective coupling and the subsequent transformation of the racemate into their glucosides. L-[U-^{14}C]Phenylalanine was incorporated also into free (+)-[^{14}C]pinoresinol and its glucosides with 12%–42% e.e.

T. Katayama (✉) · A. Ogaki
Faculty of Agriculture, Kagawa University, Miki-cho, Kagawa 761-0795, Japan
Tel. +81-87-891-3083; Fax +81-87-891-3021
e-mail: katayama@ag.kagawa-u.ac.jp

Part of this paper was presented at the 47th Annual Meeting of the Japan Wood Research Society, Kochi, April 1997

Key words Lignan biosynthesis · Syringaresinol · Liriodendrin · Enantioselective coupling · *Liriodendron tulipifera*

Introduction

It had been thought that optically active lignans were biosynthesized by stereochemically controlled dimerization of monolignols and by subsequent stereospecific transformation of the lignan dimers into the other lignans. In fact, it was established that in *Forsythia intermedia* two molecules of coniferyl alcohol regio- and stereoselectively coupled to give (+)-pinoresinol by a combination of a "dirigent" protein and an oxidase; then (+)-pinoresinol underwent stereospecific reduction to first afford (+)-lariciresinol and subsequently (−)-secoisolariciresinol.[1–6] Recently we have found enantioselective formation of (+)-*erythro*- and (−)-*threo*-guaiacylglycerol-β(8-*O*-4′)-coniferyl ether by cell-free extracts of *Eucommia ulmoides*.[7,8]

In contrast to the guaiacyl lignans, little is known about the biosynthesis of syringyl lignans. In analogy with (+)-pinoresinol during guaiacyl lignan biosynthesis, syringaresinol is considered to be a pivotal intermediate and an entry compound in the biosynthetic pathway to various syringyl lignans. On the other hand, syringaresinol has not always been found to be optically pure and has been isolated from various plant species with widely varying $[\alpha]_D$ form.[9,10] Three explanations are possible if syringaresinol is formed by the coupling of two molecules of sinapyl alcohol in the plants. First, two selective enzyme systems (or combinations of an oxidase and two proteins such as the dirigent proteins)[6] catalyzing the formation of (+)- and (−)-enantiomers may present in the plant species. Second, nonselective coupling gives racemic syringaresinol, whose one enantiomer could be metabolized into other compounds, resulting in enrichment of the other enantiomer. Third, a selective enzyme system (or a combination of an oxidase and a protein such as the dirigent protein)[6] may catalyze the

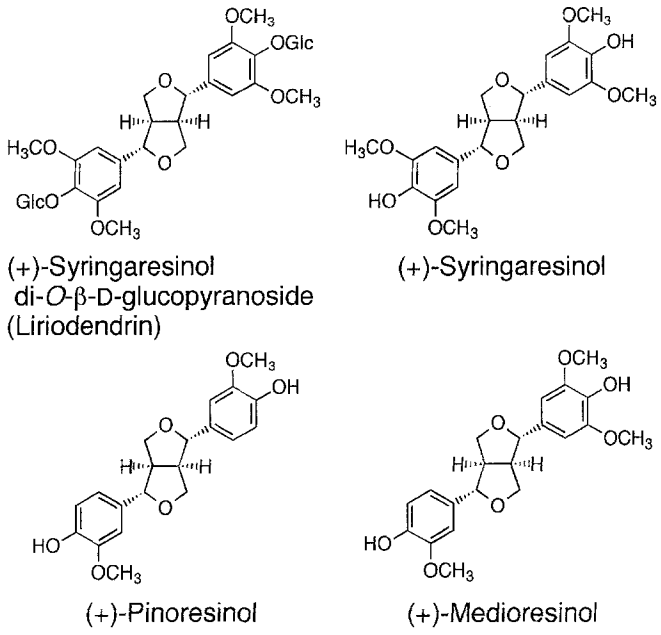

Fig. 1. Structures of lignans in *Liriodendron tulipifera*

formation of a predominant enantiomer accompanying the racemate formation by nonselective coupling.

Liriodendron tulipifera L., a deciduous broad-leafed tree, is native to eastern North America and is widely cultivated as an ornamental material in Japan. From the bark, liriodendrin[11] [(+)-syringaresinol di-*O*-β-D-glucoside] and furofuran lignans such as (+)-syringaresinol,[9,11] (+)-pinoresinol,[9] and (+)-medioresinol[9] were isolated (Fig. 1). The bioactivities of these lignans and those from other plant sources were reported as follows: Liriodendrin has a cytotoxic effect[12]; (+)-syringaresinol di-*O*-β-D-glucoside exhibits a prolonging effect on the exercise time to exhaustion in chronically swimming rats[13]; syringaresinol di-*O*-β-D-glucoside shows a significantly inhibitory effect on gastric ulcers in restrained cold water-stressed rats[14]; and (+)-pinoresinol has good inhibitory effects against cyclic adenosine monophosphate (AMP) phosphodiesterase.[15]

According to Yamaguchi et al.,[10] syringaresinol from this tree has the highest optical purity (+48.9°)[11] of the plant species examined, although syringaresinol isolated from the same tree species by other investigators has lower optical purity (+19.0°).[16] Fujimoto and Higuchi reported in 1977[16] that when several precursors were individually fed to the young shoots of *L. tulipifera*, which were then allowed to metabolize for 72 h, sinapyl alcohol was effectively incorporated into both syringaresinol and liriodendrin, and L-phenylalanine was incorporated into liriodendrin. However, the syringaresinol isolated was levorotatory, an optical rotation opposite to that which occurs naturally,[9,11] and its optical purity was low.[16]

In this paper we report the formation of (+)-syringaresinol and its glucosides following individual administration of L-[U-^{14}C]phenylalanine and [8-^{14}C]sinapyl alcohol to excised shoots of *L. tulipifera* with subsequent metabolism of the shoots for 3 h. The results suggest for the first time that enantioselective coupling of sinapyl alcohol radicals occurs to give (+)-(8*R*, 8′*R*)-syringaresinol in the plant.

Experimental

Materials and instrumentation

Young shoots of *Liriodendron tulipifera* L. cultivated in the University Farm, Kagawa University were collected during July 1995 and 1996. The instrumentation and chromatography materials were the same as described previously.[17]

Preparation of compounds

From commercial L-[U-^{14}C]phenylalanine [Moravek Biochemicals, 1.85 MBq (0.139 μmol)] and unlabeled L-phenylalanine as a cold carrier, a solution (25 mM) of L-[U-^{14}C]phenylalanine (4.9 MBq/mmol) in potassium phosphate (K-Pi) buffer (0.1 M, pH 7.0) was prepared and used for feeding experiments.

[8-^{14}C]Sinapyl alcohol was synthesized in the following four steps.

1. *[8-^{14}C]Sinapic acid*: [2-^{14}C]Malonic acid (160.8 mg, 1.54 mmol) was obtained by diluting [2-^{14}C]malonic acid (ARC, 473 μg, 9.25 MBq, 2.035 GBq/mmol) with unlabeled malonic acid (160.3 mg, 1.54 mmol). The resulting [2-^{14}C]malonic acid and syringaldehyde (280.7 mg, 1.54 mmol) were dissolved in pyridine (3.5 ml), to which was added piperidine (three drops) and aniline (three drops). The temperature was elevated to 55°C, and the reaction solution was stirred for 20 h at the same temperature. After cooling to room temperature, the reaction solution was concentrated to about 1 ml, which was diluted with water (4 ml) and then acidified to pH 2 with 2 N HCl solution. The whole was extracted three times with ethyl acetate (EtOAc). The combined EtOAc solution was washed with sodium bisulfite solution (20% w/w) to remove syringaldehyde and then with saturated NaCl solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo to yield [8-^{14}C]sinapic acid as crude crystals (286.9 mg, yield 76%).
2. *[8-^{14}C]Ethyl sinapate*: A Soxhlet extractor containing anhydrous $CaSO_4$ was used for this reaction. The crude [8-^{14}C]sinapic acid (286.9 mg, 1.17 mmol) was dissolved in 99% ethanol (EtOH, 15 ml) in the flask, and 85 ml of EtOH was added from the extractor. To the reaction solution in the flask was added 2 drops of concentrated H_2SO_4. The solution was refluxed with the refluxing EtOH dried continuously. After 9 h the reaction mixture was cooled to room temperature and neutralized (pH 5) by addition of excess $NaHCO_3$ (400 mg). The whole was filtered, and the salts were washed with EtOH. The filtrate and the washings were combined and concentrated in vacuo. The residue was partitioned between

diethyl ether (Et_2O) and water, and the aqueous layer was extracted twice with Et_2O. The three Et_2O solutions were combined and washed with saturated NaCl solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo to give [8-^{14}C]ethyl sinapate (241.7 mg, yield 82%) as crude syrup.

3. *[8-^{14}C]Ethyl sinapate 4-acetate*: The crude [8-^{14}C]ethyl sinapate (241.7 mg, 0.958 mmol) was dissolved in pyridine (3.0 ml), to which was added 1.05 ml acetic anhydride. The solution was stirred for 22 h at room temperature. The solution was concentrated in vacuo, and the residue was dissolved in ethyl acetate (EtOAc, 25 ml). The solution was washed once with 2 N HCl solution and then saturated NaCl solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo to give pale yellow crude crystals (278.3 mg), which were purified by silica gel column chromatography (EtOAc/*n*-hexane 3:7) to afford [8-^{14}C]ethyl sinapate 4-acetate as white crystals (230.9 mg, yield 82%).
4. *[8-^{14}C]Sinapyl alcohol*: To a stirred suspension of $LiAlH_4$ (300.6 mg, 7.85 mmol) in dry Et_2O (10 ml, freshly distilled over potassium metal and benzophenone) was added dropwise a solution of ethyl sinapate 4-acetate (230.9 mg, 0.783 mmol) in dry Et_2O (10 ml) at −25°C under nitrogen. After stirring for 2 h at the same temperature, EtOAc (12 ml) was added slowly to decompose excess $LiAlH_4$. The mixture was then poured onto Dry Ice in a beaker. The whole was neutralized by the further addition of Dry Ice (up to pH 5). The whole was then partitioned between EtOAc and water, which was added slowly until the lithium salt precipitations were of sufficient size to be directly removed by filtration. Following filtration the lithium salts were washed with EtOAc; the EtOAc solutions were then combined and washed twice with saturated NaCl solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo to give pale yellow crude crystals of [8-^{14}C]sinapyl alcohol. These were purified by light-shielded silica gel column chromatography (EtOAc/*n*-hexane 3:7) to give colorless crystals (125.8 mg, yield 76%), which were recrystallized from dichloromethane/*n*-hexane to afford [8-^{14}C]sinapyl alcohol (3.4 MBq/mmol).

Unlabeled (±)-syringaresinol and (±)-pinoresinol were prepared by the dehydrogenative dimerization of unlabeled sinapyl alcohol and coniferyl alcohol, respectively, using horseradish peroxidase and H_2O_2.[17,18]

Feeding experiments

L-[U-^{14}C]Phenylalanine.

Two young shoots of *L. tulipifera* (15–18 cm) were excised by scissors. A solution (25 mM) of L-[U-^{14}C]phenylalanine (4.9 MBq/mmol) in the K-Pi buffer (140 μl each) was administered to the shoots in an environment-controlled room at 25°C, which were then allowed to metabolize for 3 h. The stems and leaves were divided and individually treated as follows. Both were frozen immediately with liquid nitrogen and lyophilized. The dried stems and leaves were pulverized with a mortar and pestle. The resulting powder was extracted four times with hot MeOH. The MeOH solutions were combined and concentrated to about 10 ml, to which was added 50 ml water. The resulting suspension was centrifuged (850*g*, 20 min). After an aliquot of the supernatant was subjected to liquid scintillation counting, the remainder was extracted three times with Et_2O. The combined Et_2O solution was washed with saturated NaCl solution and dried over anhydrous Na_2SO_4. Aliquots of the Et_2O solution and the aqueous layer were subjected to liquid scintillation counting. The Et_2O solution was evaporated to dryness in vacuo. The aqueous layer was lyophilized to give a powder (228 mg from the stems; 988 mg from the leaves), which (per 1.0 g) was treated with a mixture of β-glucosidase [(200 mg) (Oriental Yeast; from sweet almond, 34 units/mg) or (Sigma; from almond, 14 units/mg)] and cellulase [(700 mg) (Wako; from *Trichoderma viride*, 1000 units/mg)] in 20 mM sodium acetate buffer (200 ml, pH 4.5) at 50°C under nitrogen for 24 h.[19] The liberated aglycones were extracted twice with EtOAc. The combined EtOAc solutions were dried over anhydrous Na_2SO_4 and evaporated in vacuo to afford extracts (16 mg from the stems; 91 mg from the leaves).

The Et_2O and EtOAc extracts from both stems and leaves were dissolved in 350 μl of MeOH. An aliquot (10 μl) of the soluble extract was applied to C_{18}-column reversed-phase high-performance liquid chromatography (HPLC) and eluted with a gradient solvent system consisting of CH_3CN/3% AcOH in H_2O (10:90) at t = 0 min to 40:60 at t = 30 min (flow rate 1.0 ml/min). The eluate was collected in scintillation vials every 30 s for liquid scintillation counting.

The remainder of the MeOH-soluble extract was purified by preparative thin-layer chromatography (TLC) (EtOAc/benzene 2:3, ×2) to afford [^{14}C]syringaresinol and [^{14}C]pinoresinol, which were then dissolved in a limited amount of MeOH. The MeOH solutions were subjected to chiral column HPLC, whose conditions (column, solvent, flow rate) were as follows: syringaresinol,[12] Chiralcel OC (Daicel), MeOH, 1.0 ml/min; pinoresinol,[1,2] Chiralcel OD (Daicel), EtOH/*n*-hexane 50:50, 0.5 ml/min). The eluate was collected at 1-min intervals for liquid scintillation counting.

[8-^{14}C]Sinapyl alcohol

Six young shoots of *L. tulipifera* (15–18 cm) were excised by scissors. A solution (23.8 mM) of [8-^{14}C]sinapyl alcohol (3.4 MBq/mmol) in the K-Pi buffer (140 μl each) was administered to the shoots, which were then allowed to metabolize for 3 h, as described before. The methods of extraction, fractionation, and HPLC analysis of the [^{14}C]lignans were similar to those mentioned above, except that preparative separation of [^{14}C]syringaresinol was done by C_{18} HPLC.

Table 1. Incorporation and enantiomeric composition of syringaresinol and pinoresinol

Precursor	Products and fractions	Stems[a]			Leaves[a]		
		A	B	C	A	B	C
L-Phenylalanine	Pinoresinol						
	Ether	62	24	0.11	56	12	0.18
	Aqueous	69	38	0.16	71	42	0.07
	Syringaresinol						
	Ether	76	52	0.08	71	42	0.12
	Aqueous	60	20	0.21	61	22	0.05
Sinapyl alcohol	Syringaresinol						
	Ether	54	8	4.9	49	−2	1.8
	Aqueous	66	32	1.4	53	5	4.9

Measurements were made following individual administration of L-[U-^{14}C]phenylalanine and [8-^{14}C]sinapyl alcohol to excised shoots of *Liriodendron tulipifera* and subsequent metabolism for 3 h
[a] A represents (+)-enantiomer content (%); B represents % enantiomeric excess; C represents % incorporation

Results and discussion

Incorporation of carbon 14-labeled precursors

Following individual administration of [U-^{14}C] phenylalanine and [8-^{14}C]sinapyl alcohol to the excised shoots of *L. tulipifera* and subsequent metabolism for 3 h, the stems and leaves were divided and extracted with hot methanol. The methanol extracts were partitioned between the ether and aqueous fractions, which contained free lignans and lignan glucosides, respectively. The glucosides were hydrolyzed by a mixture of β-glucosidase and cellulase to liberate lignan aglycones. This hydrolysis condition was based on the following facts: Yamaguchi et al. confirmed that when (±)-syringaresinol di-*O*-β-D-glucoside was incubated with a cellulase (Meiselase) from *T. viride* the ratio of (+)- and (−)-syringaresinols liberated did not change during the hydrolysis[10]; Kuriyama and Murui[19] reported that a combination of cellulase and β-glucosidase catalyzed the hydrolysis of several lignan glycosides from sesame seeds containing fufofuran lignan glucosides similar to the syringaresinol glucosides far more efficiently than β-glucosidase alone, and they optimized the hydrolysis condition.

Reversed-phase radiochromatograms of all the fractions clearly showed that L-[U-^{14}C]phenylalanine was incorporated into free syringaresinol and pinoresinol (ether fraction) and into syringaresinol aglycone and pinoresinol aglycone of their glucosides (aqueous fraction), and that [8-^{14}C]sinapyl alcohol was taken into the free syringaresinol and syringaresinol aglycone of its glucosides. The [^{14}C]syringaresinol and [^{14}C]pinoresinol were apparently detected also by ultraviolet (UV) light (280 nm) without addition of cold carriers.

Incorporation of [8-^{14}C]coniferyl alcohol into syringaresinol and its glucosides was at background level under this condition,[20] although incorporation into almost racemic pinoresinol was observed. These results were consistent with those of Fujimoto and Higuchi.[16]

Table 1 shows that the percent incorporation values for L-[U-^{14}C]phenylalanine into the lignans were lower than the corresponding percent incorporation values for [8-^{14}C]sinapyl alcohol. This result is reasonable because L-phenylalanine, which is an entry compound into the phenylpropanoid pathway, is incorporated into not only lignans but also lignins and other aromatic secondary metabolites such as tannins and *p*-hydroxycinnamate derivatives, whereas coniferyl alcohol and sinapyl alcohol are immediate precursors of lignans and lignins.

Formation of (+)-syringaresinol and (+)-pinoresinol

Enantiomeric compositions of the lignans in Table 1 indicate that L-[U-^{14}C]phenylalanine was incorporated into free syringaresinol, with (+)-enantiomer (a natural major one) predominating in both stems (76%; 52% e.e.) and leaves (71%; 42% e.e.). The precursor was also incorporated preferentially into the (+)-enantiomer aglycone in the aqueous fraction from both stems (60%; 20% e.e.) and leaves (61%; 22% e.e.).

Table 1 also shows that in the stems there was predominant uptake of [8-^{14}C]sinapyl alcohol into (+)-syringaresinol aglycone (66%; 32% e.e.) in the aqueous fraction, with low incorporation of the precursor into free (+)-syringaresinol (54%; 8% e.e.) in the ether fraction. In contrast, in the leaves the precursor was incorporated into (+)-syringaresinol aglycone in the aqueous fraction with low percent e.e. or into free racemic syringaresinol in the ether fraction.

Not only free (+)-[^{14}C]syringaresinol but also (+)-[^{14}C]syringaresinol aglycone from its glucosides were obtained from both precursors. Therefore, the biosynthetic pathways shown in Fig. 2 were suggested. That is, (+)-syringaresinol, in preference to the (−)-enantiomer, was constructed from two molecules of sinapyl alcohol in the tree; then the (+)-enantiomer and the (−)-enantiomer were further transformed into the glucosides. In contrast, (+)-syringaresinol isolated from every fraction contained the (−)-enantiomer. This origin would be ascribed to racemate formation by coupling of sinapyl alcohol by nonspecific peroxidases or oxidases (nonselective coupling)

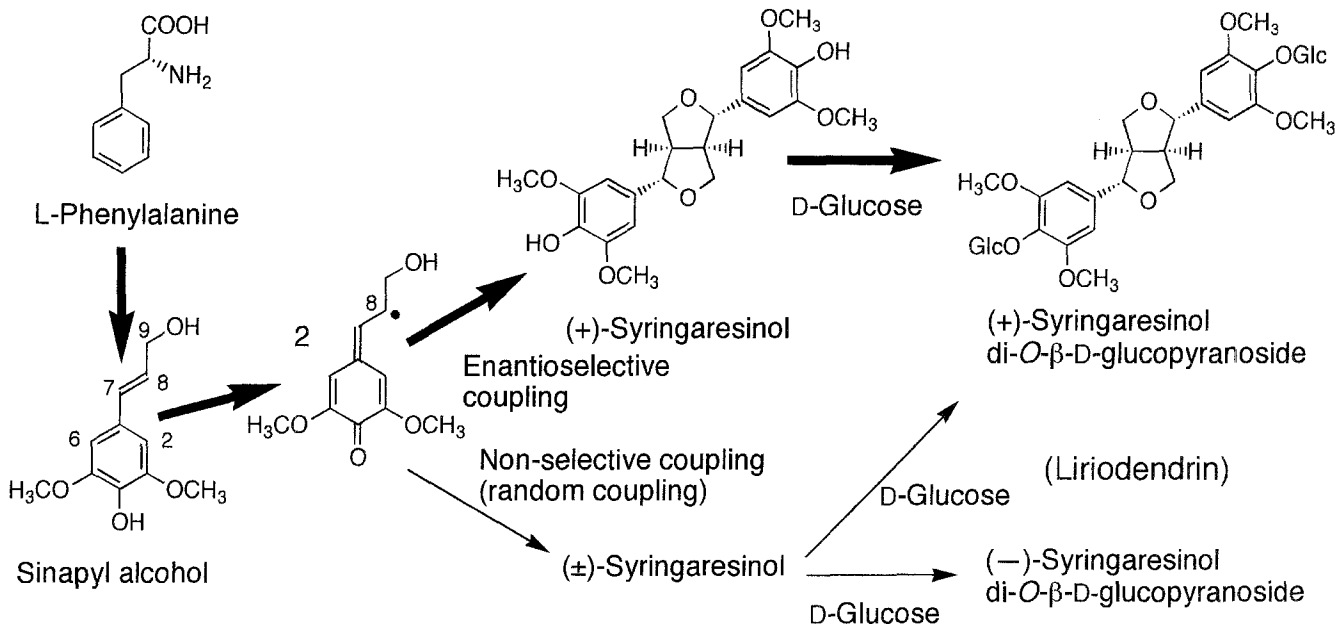

Fig. 2. Proposed biosynthetic pathway to (+)-syringaresinol and liriodendorin in *Liriodendron tulipifera*

(Fig. 2), although the possibility of selective (−)-syringaresinol formation was not neglected completely.

It is unlikely that after formation of racemic syringaresinol by nonselective coupling subsequent transformation of the (−)-enantiomer in the racemate into the glucosides or other compounds would result in enrichment of the (+)-enantiomer. If such transformation had occurred, (−)-syringaresinol would be obtained as aglycone from the aqueous layers. Furthermore, no metabolite derived from the (−)-syringaresinol has been identified in *L. tulipifera* except for its di-*O*-β-D-glucoside as the minor diastereomer in liriodendrin.

Table 1 shows that both (+)- and (−)-syringaresinols were glucosylated, although it remains obscure in this feeding experiment whether the (+)-enantiomer, in preference to the (−)-enantiomer, was transformed to its glucosides or the (+)- and (−)-enantiomers were transformed nonselectively. It is thought that because more (+)-enantiomer was formed, the glucosides of the (+)-enantiomer were present in the aqueous fraction in preference to those of the (−)-enantiomer.

To our knowledge this is the first report to suggest that stereoselective formation of (+)-syringaresinol occurs in plants. Future work must include detection of enzyme activity catalyzing (+)-syringaresinol formation.

The (+)-enantiomer contents of [^{14}C]syringaresinol from L-[U-^{14}C]phenylalanine were higher than those from [8-^{14}C]sinapyl alcohol except in the aqueous fraction from the stem (Table 1). This could be explained as follows: Before an excess of the precursor sinapyl alcohol taken up and sinapyl alcohol transformed from an excess of phenylalanine taken up move to the specific positions in the cell or cell wall where the selective coupling of sinapyl alcohol occurs to afford (+)-syringaresinol, the former sinapyl alcohol may give more racemic syringaresinol than the latter sinapyl alcohol via the action of nonspecific peroxidases or oxidases, because the former would be less properly compartmentalized than the latter.[1]

The enantiomeric compositions of (+)-syringaresinol obtained in these feeding experiments (Table 1) are consistent with the fact that (+)-syringaresinol as the aglycone of liriodendrin predominated (55%–70%) in the inner bark of *L. tulipifera*.[10]

Fujimoto and Higuchi[16] found that following administration of precursors to the excised shoots of *L. tulipifera* and subsequent metabolism of the shoots for 72 h, L-phenylalanine was well incorporated into liriodendrin and sinapyl alcohol into liriodendrin and syringaresinol. However, the syringaresinol isolated was a slightly levorotatory, unnatural form, or almost racemate ($[\alpha]_D$ −2.2°). We think that the 72-h metabolism used might be too long for examination of (+)-syringaresinol formation from sinapyl alcohol, which is the immediate precursor of syringaresinol. Rahman et al. reported that unexpected lignan catabolism occurred during feeding experiments with a forsythia plant with 72 h of metabolism.[21]

In the feeding experiments by Fujimoto and Higuchi, liriodendrins were obtained as slightly levorotatory octaacetate derivatives ($[\alpha]_D$ −0.7° to −4.5°). Although Yamaguchi et al. determined that the di-*O*-β-D-glucoside of an enantiomerically pure (+)-syringaresinol had an $[\alpha]_D$ of +3.3° (close to 0°) and that of the (−)-syringaresinol was −77.5°,[10] the specific rotations for their octaacetates have not been reported; hence the enantiomeric compositions of the syringaresinols in the liriodendrin octaacetates by Fujimoto and Higuchi are unknown. However, the fact that the octaacetates had an $[\alpha]_D$ of −0.7° to −4.5° does not mean that the original syringaresinol aglycones were almost racemic.

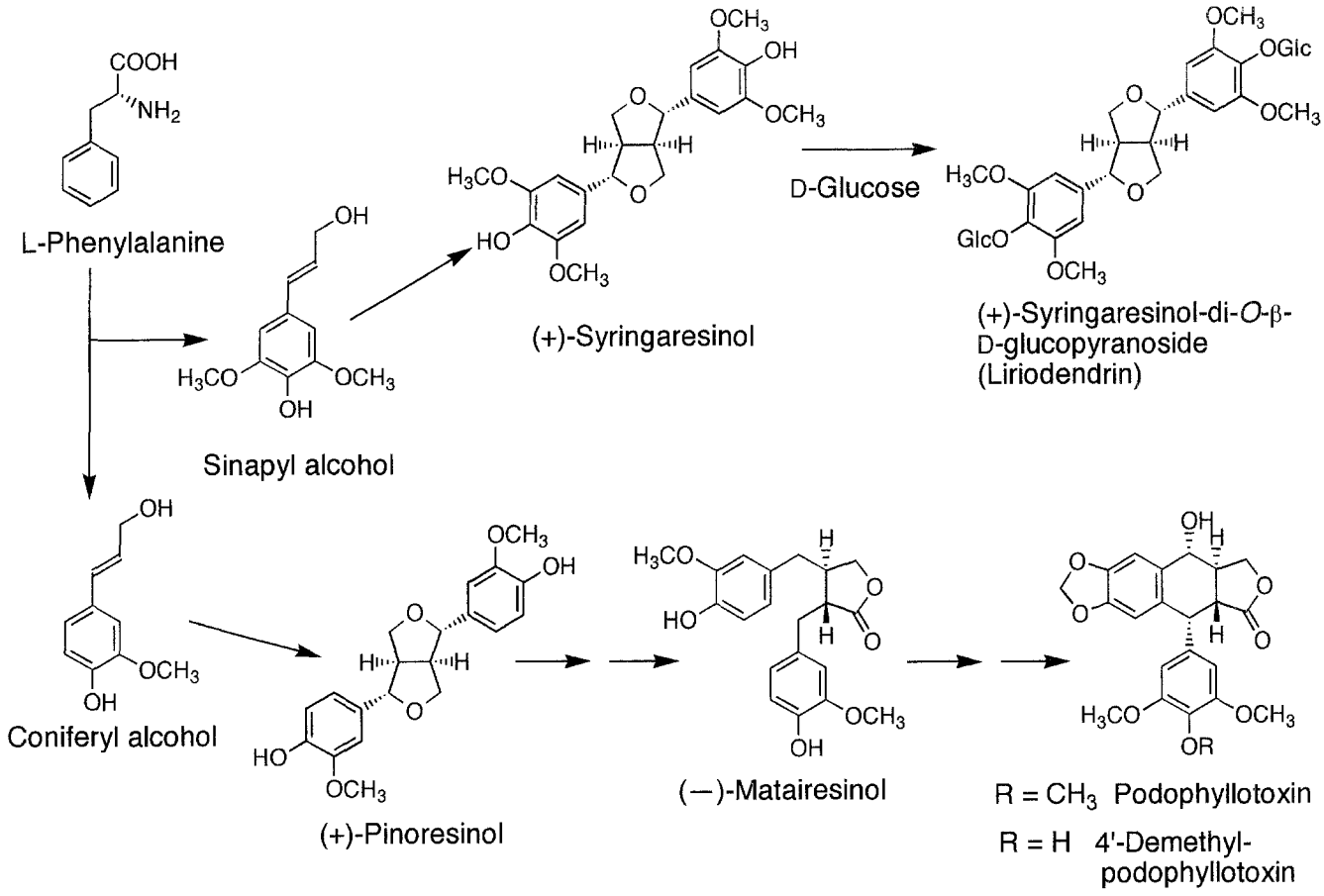

Fig. 3. Comparison of possible biosynthetic pathways for (+)-syringaresinol and liriodendrin in *Liriodendron tulipifera* with those for podophyllotoxin and 4′-demethylpodophyllotoxin in *Podophyllum hexandrum*. (Adapted from Broomhead et al.[22])

It was also found that L-[U-^{14}C]phenylalanine was incorporated into free pinoresinol and pinoresinol glucosides, with the (+)-enantiomer (a naturally occurring one) predominating. Stereoselective coupling of two molecules of coniferyl alcohol derived from the precursor phenylalanine afforded (+)-pinoresinol, which was converted to the glucosides.

Biosynthesis of syringyl lignans

Finally, we discuss the biosynthesis of syringyl lignans or syringyl moieties in lignans. At least two distinct pathways can be proposed for the formation of syringyl or 3,4,5-trisubstituted lignans in vascular plants, as shown in Fig. 3. First, dimerization of two molecules of sinapyl alcohol gives optically active syringaresinol [e.g., (+)-syringaresinol in *L. tulipifera*], which would be transformed into the other syringyl lignans (e.g., liriodendrin in *L. tulipifera*).

A second dimerization of two molecules of coniferyl alcohol gives pinoresinol, which is converted to matairesinol. The pathway from (+)-pinoresinol to (−)-matairesinol has been established using *Forsythia intermedia*.[1-6] Pinoresinol, matairesinol, and their intermediate lignans could be transformed into syringyl and 3,4,5-trimethoxyphenyl nucleus by way of hydroxylation of the aromatic ring followed by methylation. This transformation was deduced from the fact that (−)-matairesinol was incorporated into a 3,4,5-trimethoxyphenyltetrahydronaphthalene lignan (e.g., podophyllotoxin) and syringyltetrahydronaphthalene lignans (e.g., 4-demethylpodophyllotoxin) in *Podophyllum hexandrum*.[22] On the other hand, the possibility of a pathway from pinoresinol to syringaresinol by way of medioresinol cannot be disregarded at this point.

References

1. Umezawa T, Davin LB, Yamamoto E, Kingston DGI, Lewis NG (1990) Lignan biosynthesis in *Forsythia* species. J Chem Soc Chem Commun 1990:1405–1408
2. Davin LB, Bedgar DL, Katayama T, Lewis NG (1992) On the stereoselective synthesis of (+)-pinoresinol in *Forsythia suspensa* from its achiral precursor, coniferyl alcohol. Phytochemistry 31:3869–3874
3. Paré PW, Wang H-B, Davin LB, Lewis NG (1994) (+)-Pinoresinol synthase: a stereoselective oxidase catalysing 8,8′-lignan formation in *Forsythia intermedia*. Tetrahedron Lett 35:4731–4734
4. Katayama T, Davin LB, Lewis NG (1992) An extraordinary accumulation of (−)-pinoresinol in cell-free extracts of *Forsythia intermedia*: evidence for enantiospecific reduction of (+)-pinoresinol. Phytochemistry 31:3875–3881

5. Katayama T, Davin LB, Chu A, Lewis NG (1993) Novel benzylic ether reductions in lignan biogenesis in *Forsythia intermedia*. Phytochemistry 33:581–591
6. Davin LB, Wang H-B, Crowell AL, Bedgar DL, Martin DM, Sarkanen S, Lewis NG (1997) Stereoselective bimolecular phenoxy radical coupling by an auxiliary (dirigent) protein without an active center. Science 275:362–366
7. Katayama T, Kado Y (1998) Formation of optically active neolignans from achiral coniferyl alcohol by cell-free extracts of *Eucommia ulmoides*. J Wood Sci 44:244–246
8. Kado Y, Katayama T (1997) Enantioselective formation of neolignans/lignans by cell-free extracts of *Eucommia ulmoides* (in Japanese). In: Proceedings of the 42nd Lignin Symposium, Sapporo, pp 61–64
9. Fujimoto H, Higuchi T (1977) Lignans from the bark of yellow poplar (*Liriodendron tulipifera* L.). Mokuzai Gakkaishi 23:405–410
10. Yamaguchi H, Nakatsubo F, Katsura Y, Murakami K (1990) Characterization of (+)- and (−)-syringaresinol di-β-D-glucosides. Holzforschung 44:381–385
11. Dickey EE (1958) Liriodendrin, a new lignan diglucoside from the inner bark of yellow poplar (*Liriodendron tulipifera* L.). J Org Chem 23:179–184
12. Jolad SD, Hoffman JJ, Cole JR, Tempesta MS, Bates RB (1980) Cytotoxic agent from *Penstemon deustus* (Scrophulariaceae): Isolation and stereochemistry of liriodendrin, a symmetrically substituted furofuranoid lignan diglucoside. J Org Chem 45:1327–1329
13. Nishibe S, Kinoshita H, Takeda H, Okano G (1990) Phenolic compounds from stem bark of *Acanthopanax senticosus* and their pharmacological effect in chronic swimming stressed rats. Chem Pharm Bull (Tokyo) 38:1763–1765
14. Fujikawa T, Yamaguchi A, Morita I, Takeda H, Nishibe S (1996) Protective effects of *Acanthopanax senticosus* Harms from Hokkaido and its components on gastric ulcer in restrained cold water stressed rats. Biol Pharm Bull 19:1227–1230
15. Nikaido T, Ohmoto T, Kinoshita T, Sankawa U, Nishibe S, Hisada S (1981) Inhibition of cyclic AMP phosphodiesterase. Chem Pharm Bull (Tokyo) 29:3586–3592
16. Fujimoto H, Higuchi T (1977) Biosynthesis of liriodendrin by *Liriodendron tulipifera*. Wood Res (Kyoto) No.62:1–10
17. Katayama T, Masaoka T, Yamada H (1997) Biosynthesis and stereochemistry of lignans in *Zanthoxylum ailanthoides*. I. (+)-Lariciresinol formation by enzymatic reduction of (±)-pinoresinols. Mokuzai Gakkaishi 43:580–588
18. Katayama Y, Fukuzumi T (1978) Enzymatic synthesis of three lignin-related dimers by an improved peroxidase–hydrogen peroxide system (in Japanese). Mokuzai Gakkaishi 24:664–667
19. Kuriyama K, Murui T (1993) Effect of cellulase on hydrolysis of lignan glycosides in sesame seed by β-glucosidase (in Japanese). Nippon Nogeikagaku Kaishi 67:1701–1705
20. Ogaki A, Katayama T (1997) Biosynthesis of furofuran lignans in *Liriodendron tulipifera* (in Japanese). In: Abstracts of the 47th annual meeting of the Japan Wood Research Society, Kochi, p 390
21. Rahman MMA, Dewick PM, Jackson DE, Lucas JA (1990) Biosynthesis of lignans in *Forsythia intermedia*. Phytochemistry 29:1841–1846
22. Broomhead AJ, Rahman MMA, Dewick PM, Jackson DE, Lucas JA (1991) Matairesinol as precursor of *Podophyllum* lignans. Phytochemistry 30:1489–1492