

Stereochemistry of Lignan Biosynthesis in *Wikstroemia sikokiana¹**

Tomoya OKUNISHI*², Toshiaki UMEZAWA*²
and Mikio SHIMADA*²

(Received May 31, 1997)

Keywords: *Wikstroemia sikokiana* lignan, stereochemistry, biosynthesis, lariciresinol, seco-isolariciresinol, kusunokinin

Lignan molecules are generally chiral and naturally occurring lignan specimens are mostly optically active. The sign of optical rotation of a particular lignan can vary with plant sources: (–)-arctigenin was isolated from *Forsythia* plants,^{1–3)} whereas the corresponding (+)-enantiomer was from *Wikstroemia* plant⁴⁾. Hence, stereochemical mechanisms involved in lignan biosynthesis can be different among various plants species.

Many reports have been published on enzyme systems involved in lignan biosynthesis with *Forsythia* plants as enzyme sources^{5–16)}. At present biosynthetic sequences of *Forsythia* lignans have been established, and the stereochemical mechanisms of each enzymatic conversion have been elucidated^{5–18)}.

On the other hand, little studies of the plants which produce opposite enantiomer to those of *Forsythia* lignans have been reported. Recently, Umezawa and Shimada¹⁹⁾ reported isolation of (–)-pinoresinol (74% e.e.), (+)-matairesinol (optically pure) and (+)-wikstromol (optically pure) from *Wikstroemia sikokiana*. These lignans are opposite enantiomer to those from *Forsythia* plants. Importantly, the enantiomeric composition of (–)-pinoresinol isolated from *W. sikokiana* was only 74% e.e. This value was much smaller than that of (+)-pinoresinol obtained from *Forsythia* plants (almost optically pure)³⁾. They concluded that there are two differences in the stereochemical mechanisms of lignan biosynthesis between *W. sikokiana* and *Forsythia* spp.; one is that both plants produce (or accumulate) different enantiomers, and the other is that the step in the metabolic sequence to produce optically pure lignans are different between the plants. However, detailed mechanisms remained to be elucidated.

The present paper reports the further study of lignan biosynthesis in *W. sikokiana*.

*¹ A part of this report was presented at the 46st (in Kumamoto, April, 1996) and 47st (in Kochi, April, 1997) Annual Meeting of the Japan Wood Research Society.

*² Laboratory of Biochemical Control.

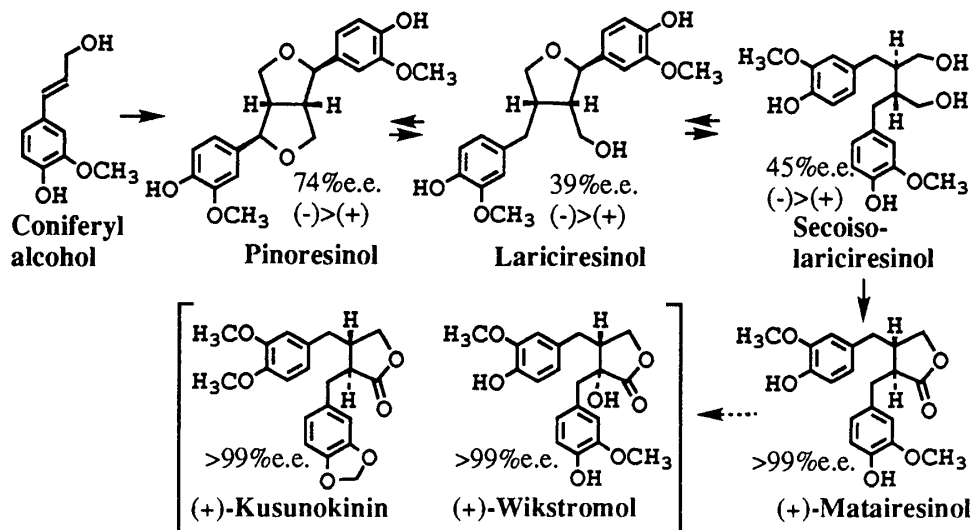


Fig. 1. Lignans isolated from *W. sikokiana* and their proposed biosynthetic pathway.

First, a survey of lignans, especially possible intermediates between pinoresinol and matairesinol, and determination of their enantiomeric compositions were carried out. Then feeding experiments with deuterium-labelled substrates were conducted to establish biosynthetic pathways of *W. sikokiana* lignans.

Six lignans, shown in Fig. 1, were isolated from MeOH extracts of *W. sikokiana* stems. The isolation of (–)-lariciresinol (39% e.e.), (–)-secoisolariciresinol (45% e.e.), and (+)-kusunokinin (optically pure) from this plant was reported for the first time in the present investigation. The predominant enantiomers of the *Wikstroemia* lignans are opposite to those isolated from *Forsythia* plants except for secoisolariciresinol.

Enantiomeric compositions of the lignans from *W. sikokiana* and those from *Forsythia* spp. are also different. Thus, pinoresinol, lariciresinol, and secoisolariciresinol isolated from *W. sikokiana* were not optically pure, whereas those lignans from *Forsythia* spp. were optically pure (or almost optically pure)^{3,7)}. On the other hand, the present and previous investigations^{3,7,12,19)} showed that all the dibenzylbutyrolactone lignans, e.g., matairesinol, isolated from both plants were optically pure.

Next, deuterium-labelled [9,9-²H₂, OC²H₃] coniferyl alcohol as well as four lignans, (±)-[9,9,9',9'-²H₄] pinoresinols, (±)-[9,9,9',9'-²H₄] lariciresinols, (±)-[9,9,9',9'-²H₄] secoisolariciresinols, and (±)-[aromatic-²H] secoisolariciresinols, were administered to young shoots of the plant. Incorporation of [9,9-²H₂, OC²H₃] coniferyl alcohol into pinoresinol, lariciresinol, secoisolariciresinol, and matairesinol was observed. The values of % incorporation into the lignans except for matairesinol were pinoresinol (0.81%), lariciresinol (0.32%), and secoisolariciresinol (0.09%). In addition, the following incorporation was demonstrated: (±)-[9,9,9',9'-²H₄] pinoresinols to lariciresinol and

secoisolariciresinol, ($\pm$)-[9, 9', 9'-²H₄] lariciresinols to pinoresinol and secoisolariciresinol, and ($\pm$)-[9, 9', 9'-²H₄] secoisolariciresinols to pinoresinol and lariciresinol, ($\pm$)-[aromatic-²H] secoisolariciresinols to matairesinol.

Feeding experiments with deuterium-labelled substrates suggested strongly the pathway of lignan biosynthesis in the plant (Fig. 1). This pathway is similar with respect to metabolic sequence to that in *Forsythia* spp., but there are two differences in the stereochemical mechanisms. First, predominant enantiomers of the lignans occurring on these plants are different. Second, there is a difference in the metabolic step to produce optically pure lignans between *W. sikokiana* and *Forsythia* spp. The step is probably the conversion of secoisolariciresinol to matairesinol in *W. sikokiana*, whereas the step is the formation of pinoresinol from coniferyl alcohol in *Forsythia* spp.

In conclusion, a possible biosynthetic pathway for lignans in *W. sikokiana* was proposed. This pathway is similar to that in *Forsythia* spp. The predominant enantiomers of the lignans occurring in both plants were opposite each other, and the step in which an optically pure lignan was produced is different in both plants.

References

- 1) S. KITAGAWA, *et al.*: *Chem. Pharm. Bull.*, **36**, 3667–3670 (1988).
- 2) M.M.A. RAHMAN, *et al.*: *Phytochem.*, **29**, 1971–1980 (1990).
- 3) T. UMEZAWA, *et al.*: “Biotechnology in Pulp and Paper Industry”, M. KUWAHARA; M. SHIMADA, eds., Uni Publ. (Tokyo), p. 507–512 (1992).
- 4) H. SUZUKI, *et al.*: *Phytochem.*, **21**, 1824–1825 (1982).
- 5) T. UMEZAWA, L.B. DAVIN and N.G. LEWIS: *Biochem. Biophys. Res. Commun.*, **171**, 1008–1014 (1990).
- 6) T. UMEZAWA, *et al.*: *J. Chem. Soc. Chem. Commun.*, **1990**, 1405–1408 (1990).
- 7) T. UMEZAWA, L.B. DAVIN and N.G. LEWIS: *J. Biol. Chem.*, **266**, 10210–10217 (1991).
- 8) L.B. DAVIN, *et al.*: *Phytochem.*, **31**, 3869–3874 (1992).
- 9) T. KATAYAMA, L.B. DAVIN and N.G. LEWIS: *ibid.*, **31**, 3875–3881 (1992).
- 10) T. KATAYAMA, *et al.*: *ibid.*, **33**, 581–591 (1993).
- 11) A. CHU, *et al.*: *J. Biol. Chem.*, **268**, 27026–27033 (1993).
- 12) S. OZAWA, L.B. DAVIN and N.G. LEWIS: *Phytochem.*, **32**, 643–652 (1993).
- 13) T. UMEZAWA, *et al.*: *Biosci. Biotech. Biochem.*, **58**, 230–234 (1994).
- 14) P.W. PARÉ, *et al.*: *Tetra. Lett.*, **35**, 4731–4734 (1994).
- 15) A.T. DINKOVA-KOSTOVA, *et al.*: *J. Biol. Chem.*, **271**, 29473–29482 (1996).
- 16) L.B. DAVIN, *et al.*: *Science*, **275**, 362–366 (1997).
- 17) T. UMEZAWA: *Mokuzai Gakkaishi*, **42**, 911–920 (1996).
- 18) T. UMEZAWA: “Biochemistry and Molecular Biology of Wood”, T. HIGUCHI, ed., Springer-Verlag (Berlin), p. 181–194 (1997).
- 19) T. UMEZAWA and M. SHIMADA: *Mokuzai Gakkaishi*, **42**, 180–185 (1996).