Preliminary

Stereochemical Selectivity in Secoisolariciresinol Formation by Cell-free Extracts from *Arctium lappa* L. Ripening Seeds*[1]

Shiro SUZUKI*[2], Toshiaki UMEZAWA*[2] and Mikio SHIMADA*[2]

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Lignans are a class of secondary metabolites found as 8, 8′-linked dimers of two phenylpropane units[1,2]. The enantiomeric compositions of a particular lignan can vary with plant species, and the predominant enantiomer can differ among the plant sources[3–7]. For example, (−)-matairesinol was isolated from *Forsythia intermedia*, whereas the (+)-antipode was isolated from *Wikstroemia sikokiana*[6,11]. In addition, lignans have various biological activities such as antitumor, antimiotic and antiviral, and some of them accumulate in large amounts in heartwood of woody plants[7,8].

The biosynthetic mechanism of lignans has long been marked among phytochemists because of their above features. In 1990, Umezawa *et al.* firstly demonstrated the enantioselective formation of optically pure (−)-secoisolariciresinol from achiral coniferyl alcohol with cell-free extracts from *F. intermedia* in the presence of NAD(P)H and H_2O_2[9]. Since then, many reports have been published on the enzyme systems involved in lignan biosynthesis in *Forsythia* plants as enzyme sources, mostly by Lewis and co-workers[10–20]. As a whole, these studies revealed enzymatic formation of the naturally occurring enantiomers of *Forsythia* lignans and each enzymatic conversion was controlled strictly with respect to stereochemistry.

On the other hand, there are many examples of plants which produce the opposite enantiomers to those of the lignans occurring in *Forsythia* spp.[3–7,21]. Using *Arctium lappa* petioles as an enzyme source, Umezawa and Shimada reported an enzyme activity that catalyzed the formation of the opposite enantiomer of secoisolariciresinol to that formed by *Forsythia* enzyme preparation[22]. In contrast to cell-free extracts from *Forsythia* plants, the enzyme preparation from *A. lappa* petioles catalyzed the enantioselective formation of (+)-secoisolariciresinol (*ca.* 20% enantiomer excess (*e.e.*)) from coniferyl alcohol in the presence of NADPH and H_2O_2. The (+)-enantiomer of secoisolariciresinol was the predominant antipode of secoisolariciresinol isolated from *A. lappa* petioles (78% *e.e.*).

The absolute configuration at C8 position of (+)-secoisolariciresinol is, however, opposite to that of (−)-arctigenin, which is isolated in significant amounts from the seeds[23,24]. Thus, the stereochemical mechanism for producing (+)-secoisolariciresinol and (−)-arctigenin in *A. lappa* awaits further experimental works.

This paper describes the formation of (−)-secoisolariciresinol with cell-free extracts from ripening seeds of *A. lappa*, demonstrating that the different organs of *A. lappa* produced enzymes with the opposite stereochemical selectivity in secoisolariciresinol formation.

A preliminary GC-MS analysis indicated that the production of lignans in *A. lappa* seeds was developmentally regulated. Trace amounts of lignans (pinoresinol, lariciresinol and secoisolariciresinol) were present in both the flower buds and flowers in bloom of *A. lappa*, while the amount was significantly greater in ripening seeds *ca.* 6 days after the start of blooming. Next, cell-free extracts were prepared from ripening seeds of *A. lappa* as described previously[22,25]. When [9, 9-$^2H_2$, OC$^2H_3$]coniferyl alcohol was incubated with the cell-free extracts in the presence of NADPH and H_2O_2,

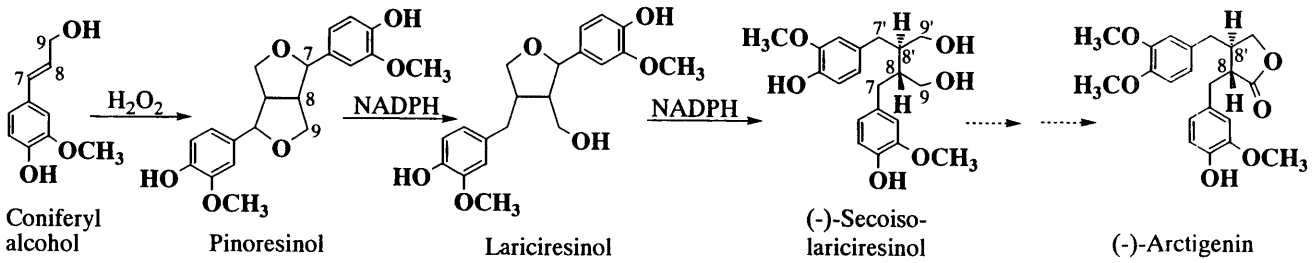

Fig. 1. A possible biosynthetic pathway of lignans in *Arctium lappa* seeds.

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*[2] Laboratory of Biochemical Control.

[$^2H_{10}$]pinoresinol, [$^2H_{10}$]lariciresinol and [$^2H_{10}$]secoisolariciresinol were formed. They were identified by comparing their mass spectra and retention times on GC with those of authentic samples. The enantiomeric compositions of the lignans formed enzymatically from [9, 9-$^2H_2$, OC$^2H_3$]coniferyl alcohol were analyzed by chiral HPLC and GC-MS as described previously[22,25]. The enantiomeric composition of [$^2H_{10}$]secoisolariciresinol was 38% *e.e.* [(−)>(+)].

This result, therefore, strongly suggests that (−)-secoisolariciresinol was enantioselectively formed from coniferyl alcohol via pinoresinol and lariciresinol (Fig. 1). Next we incubated (±)-[9, 9, 9′, 9′-$^2H_4$]pinoresinols and (±)-[9, 9, 9′, 9′-$^2H_4$]lariciresinols individually with the cell-free extracts in the presence of NADPH. The cell-free extracts catalyzed the selective formation of (−)-[$^2H_4$]secoisolariciresinol in both assays. Furthermore, the (−)-enantiomer of secoisolariciresinol which was formed selectively in the enzymatic reaction was also predominant in this lignan isolated from *A. lappa* seeds (65% *e.e.*).

Thus, this is the first report that indicates clearly that two enzyme preparations from different organs of a single plant species catalyzed the selective formation of different enantiomers of lignans, and strongly suggests that there were organ-specific isozymes that catalyzed the secoisolariciresinol formation with different enantioselectivity in *A. lappa*. This view is in good accordance with the study by Fujita *et al.*[26]; they reported the clonings of two putative pinoresinol/lariciresinol reductase cDNAs from the cDNA library of *Thuja plicata*, and *E. coli*-expression of the recombinant proteins. The two putative reductases showed different selectivity in secoisolariciresinol formation in terms of the substrates. Thus, one catalyzed the formation of optically pure (−)-secoisolariciresinol from (+)-pinoresinol, while the other catalyzed the formation of optically pure (+)-secoisolariciresinol from (−)-pinoresinol.

We have recently reported that there is stereochemical diversity in lignan biosynthesis among various plant species[3,27]. The present results go further in suggesting that different stereochemical mechanisms in lignan formation can operate even in different organs of a single plant species.

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