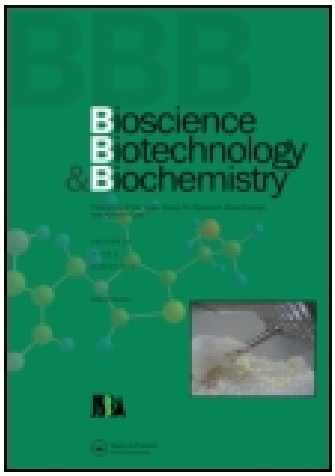

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Formation of the Lignan (+)-Secoisolariciresinol by Cell-free Extracts of Arctium lappa

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Short Communication

Formation of the Lignan (+)-Secoisolariciresinol by Cell-free Extracts of *Arctium lappa*†

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Cell-free extracts of petioles of *Arctium lappa* catalyzed enantioselective formation of (+)-secoisolariciresinol [about 20% enantiomer excess (*e.e.*)] from achiral coniferyl alcohol in the presence of NADPH and H_2O_2. This is the first report of an enzymatic reaction to afford (+)-secoisolariciresinol enantioselectively.

Key words: *Arctium lappa*; (+)-secoisolariciresinol; enantioselective; lignan; biosynthesis

Stereochemical mechanisms in lignan biosynthesis have been receiving widespread interest in two aspects: first, lignans and lignins differ fundamentally in optical activity, although they are closely related in their chemical structures; in general, lignans are optically active,[1] but no optical activity has been reported for lignin specimens.[2,3] Therefore, lignan biosynthesis may involve enantioselective process(es), which sharply contrasts with the non-enantioselective process of lignin polymerization. Hence, study of differences in the stereochemical mechanisms is of importance. Second, optical rotation of a particular lignan varies with plant sources: (−)-matairesinol [(−)-**3**] has been isolated from *Forsythia* plants,[4–7] and (+)-enantiomer [(+)-**3**] from *Wikstroemia sikokiana* (Fig. 1).[8] Therefore, stereochemical machanisms for lignan biosynthesis are different between *Forsythia* and *Wikstroemia* plants. However, no satisfactory explanation has been proposed to account for the stereochemical difference.

In 1990 the first report of an enzymatic reaction involved in formation of an optically pure lignan from an achiral monomer was demonstrated with cell-free extracts of *F. intermedia*.[9] The enzyme preparation catalyzed enantioselective formation of optically pure (−)-secoisolariciresinol [(−)-**2**] from coniferyl alcohol [**1**]. Since then, many reports have been published on enzyme systems involved in lignan biosynthesis with *Forsythia* plants as enzyme sources.[6,10–17] However, no paper has been reported on enzymes catalyzing selective formation of the opposite enantiomer (+)-secoisolariciresinol [(+)-**2**]. This paper describes the first example of enzymatic enantioselective formation of (+)-**2** from **1** using cell-free extracts of *Arctium lappa* (edible burdock, gobo in Japanese).

The cell-free extracts were prepared from petioles of *A. lappa* L. cv. Kobarutogokuwase as described previously.[16] When [9,9-$^2H_2$, OC$^2H_3$]coniferyl alcohol [**1**-d_5]*1 was incubated with the cell-free extracts in the presence of NADPH and H_2O_2 as previously,[16] [$^2H_{10}$]secoisolariciresinol [**2**-d_{10}] was formed, which was identified by comparing the mass spectrum (Me_3Si ether) (Fig. 2) and t_R on GC with those of an unlabelled authentic sample, (±)-secoisolariciresinols [(±)-**2**]. The specific activity forming **2**-d_{10} from **1**-d_5 was 1.53–0.73 nmol h^{-1} mg^{-1} protein. When either cofactor (NADPH or H_2O_2) was omitted or when denatured enzyme (boiled for 5 min) was used, no significant formation of **2**-d_{10} was observed (Table), indicating that the lignan formation was enzymatic.

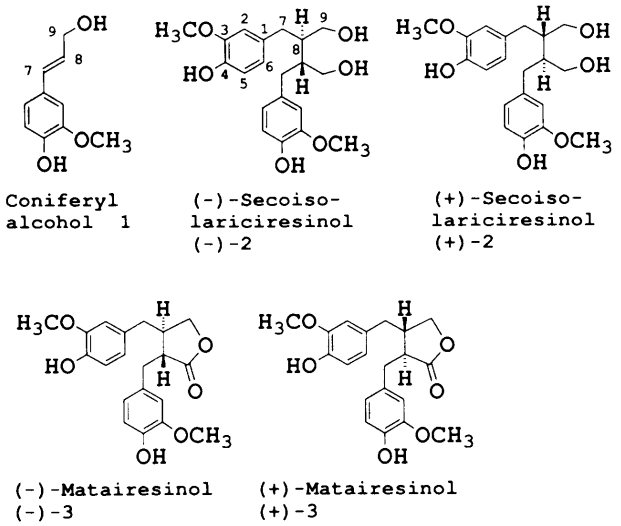

Fig. 1. Chemical Structures of Compounds.

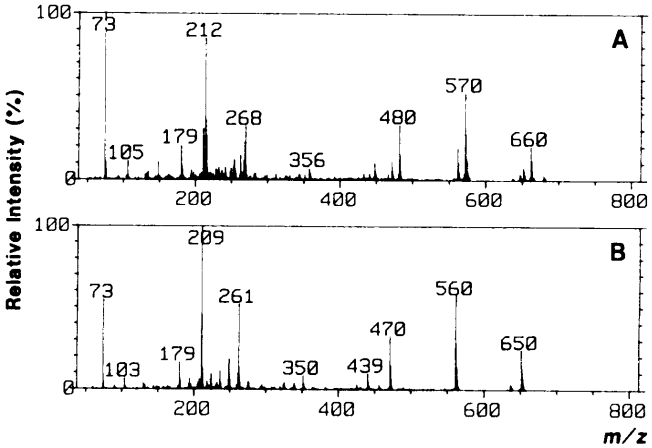

Fig. 2. Mass Spectra of the Me_3Si Ethers of Secoisolariciresinols.

A: [$^2H_{10}$]Secoisolariciresinol [**2**-d_{10}] formed from the incubation of [9,9-$^2H_2$, OC$^2H_3$]coniferyl alcohol [**1**-d_5] with cell-free extracts of *A. lappa* in the presence of NADPH and H_2O_2. B: Chemically synthesized (unlabelled) (±)-secoisolariciresinols [(±)-**2**]. Note that unlabelled (±)-**2** was added as a carrier in the case of A.

† Parts of this work were presented in the 39th Lignin Symposium, October 12, 1994, Fukuoka, Japan; and in the 40th Lignin Symposium, October 12, 1995, Tsukuba, Japan.

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*1 d_2, d_4, d_5, and d_{10} in the compound numbers represent compounds labelled with two, four, five, and ten deuterium atoms, respectively.

Abbreviation: *e.e.*, enantiomer excess.

Table Enzymatic Formation of [$^2H_{10}$]Secoisolariciresinol [**2**-d_{10}] from [9,9-$^2H_2$, OC$^2H_3$]Coniferyl Alcohol [**1**-d_5]

Assay	Cofactor	[$^2H_{10}$]Secoisolariciresinol [**2**-d_{10}] formation[b]
Complete	NADPH/H_2O_2	0.73
Controls[a]	NADPH	0.075
	H_2O_2	0
	None	0
	Denatured enzyme/ NADPH/H_2O_2	0

[a] Control experiments refer to the complete assay with the omission of H_2O_2 or with the denatured enzyme (boiled for 5 min). One other control experiment was done, using the complete assay but the reaction was worked up by adding EtOAc as soon as possible (less than 10 sec) after the start of incubation. In this experiment, no secoisolariciresinol formation was observed.

[b] Expressed in nmol h^{-1} mg^{-1} protein.

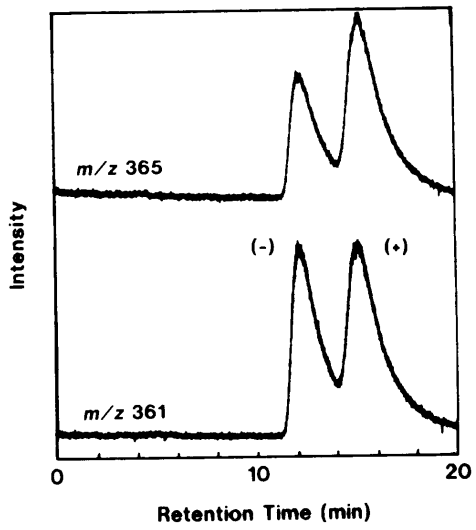

Fig. 3. Chiral LC-Selected Ion Monitoring Chromatograms of the Deprotonated Molecular Ions of Secoisolariciresinols.

m/z 365, chromatogram of the deprotonated molecular ion of [$^2H_4$]secoisolariciresinol [**2**-d_4] formed from the incubation of [9,9-$^2H_2$]coniferyl alcohol [**1**-d_2] with cell-free extracts of *A. lappa* in the presence of NADPH and H_2O_2. *m/z* 361, chromatogram of the deprotonated molecular ion of the unlabelled carrier, (±)-secoisolariciresinols [(±)-**2**]. Column, Chiralcel OD (Daicel Chemical Co., 250 × 4.6 mm); solvent, EtOH *n*-hexane glycerol (300 : 700 : 2) at 0.8 ml/min.

Next, to examine the enantiomeric composition of the secoisolariciresinol [**2**] formed from **1**, [9,9-$^2H_2$]coniferyl alcohol [**1**-d_2] was incubated as above. The formed [$^2H_4$]secoisolariciresinol [**2**-d_4] was recovered and submitted to chiral LC-selected ion monitoring (Frit-fast atom bombardment mass spectrometry, negative ion mode) with an unlabelled carrier (±)-**2** (Fig. 3) as previously described.[16] The chromatograms clearly show that (+)-enantiomer [(+)-**2**-d_4] predominates in the enzymatically formed **2**-d_4 (about 20% *e.e.*).

Furthermore, (+)-secoisolariciresinol [(+)-**2**] (78% *e.e.*) was isolated from the MeOH extracts of *A. lappa* cv. Kobarutogokuwase petioles after β-glucosidase treatment (T. Umezawa and M. Shimada, unpublished results).

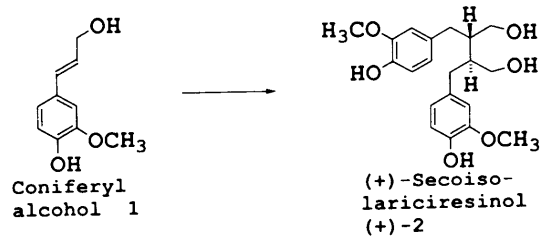

Fig. 4. Formation of (+)-Secoisolariciresinol [(+)-**2**] from Coniferyl Alcohol [**1**] with Cell-free Extracts of *A. lappa*.

Our results with *A. lappa* (Fig. 4) are in sharp contrast to those of *Forsythia* plants; the opposite enantiomer, optically pure (−)-**2**, was isolated from *F. koreana*,[7] and enzyme preparations from *Forsythia* plants are known to catalyze the formation of the naturally occurring (−)-enantiomer [(−)-**2**] from **1**.[9,16] In addition, we have recently reported isolation of (+)-matairesinol [(+)-**3**] from *Wikstroemia sikokiana*,[8] which is the oppoisite enantiomer to the one from *Forsythia* plants[4-7] and *A. lappa* (T. Umezawa and M. Shimada, unpublished results). The previous and present results clearly indicate that there is a diversity in stereochemical mechanisms of lignan biosynthesis in different plants. Research to elucidate detailed mechanisms for the enzymatic formation of **2** from **1** with *A. lappa* is currently under way in our laboratory.

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References

1) D. C. Ayres and J. D. Loike, "Lignans Chemical, Biological and Clinical Properties," Cambridge Univ. Press, Cambridge, 1990.
2) A. Björkman and B. Person, *Sven. Papperstidn.*, **60**, 158–169 (1957).
3) K. Freudenberg, *Science*, **148**, 595–600 (1965).
4) S. Kitagawa, S. Nishibe, R. Benecke, and H. Thieme, *Chem. Pharm. Bull.*, **36**, 3667–3670 (1988).
5) M. M. A. Rahman, P. M. Dewick, D. E. Jackson, and J. A. Lucas, *Phytochemistry*, **29**, 1971–1980 (1990).
6) T. Umezawa, L. B. Davin, and N. G. Lewis, *J. Biol. Chem.*, **266**, 10210–10217 (1991).
7) T. Umezawa, T. Isohata, H. Kuroda, T. Higuchi, and M. Shimada, in "Biotechnology in Pulp and Paper Industry," ed. by M. Kuwahara, M. Shimada, Uni Publ., Tokyo, Japan, 1992, pp. 507–512.
8) T. Umezawa and M. Shimada, *Mokuzai Gakkaishi*, **42**, 180–185 (1996).
9) T. Umezawa, L. B. Davin, and N. G. Lewis, *Biochem. Biophys. Res. Commun.*, **171**, 1008–1014 (1990).
10) T. Umezawa, L. B. Davin, E. Yamamoto, D. G. I. Kingston, and N. G. Lewis, *J. Chem. Soc., Chem. Commun.*, **1990**, 1405–1408.
11) L. B. Davin, D. L. Bedgar, T. Katayama, and N. G. Lewis, *Phytochemistry*, **31**, 3869–3874 (1992).
12) T. Katayama, L. B. Davin, and N. G. Lewis, *Phytochemistry*, **31**, 3875–3881 (1992).
13) T. Katayama, L. B. Davin, A. Chu, and N. G. Lewis, *Phytochemistry*, **33**, 581–591 (1993).
14) A. Chu, A. Dinkova, L. B. Davin, D. L. Bedgar, and N. G. Lewis, *J. Biol. Chem.*, **268**, 27026–27033 (1993).
15) S. Ozawa, L. B. Davin, and N. G. Lewis, *Phytochemistry*, **32**, 643–652 (1993).
16) T. Umezawa, H. Kuroda, T. Isohata, T. Higuchi, and M. Shimada, *Biosci. Biotech. Biochem.*, **58**, 230–234 (1994).
17) P. W. Paré, H.-B. Wang, L. B. Davin, and N. G. Lewis, *Tetrahedron Lett.*, **35**, 4731–4734 (1994).