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Justicidin B 7-hydroxylase, a cytochrome P450 monooxygenase from cell cultures of *Linum perenne* Himmelszelt involved in the biosynthesis of diphyllin

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Abstract

Cell suspension cultures of *Linum perenne* L. Himmelszelt accumulate justicidin B as the main component together with glycosides of 7-hydroxyjusticidin B (diphyllin). A hypothetical biosynthetic pathway for these compounds is suggested. Justicidin B 7-hydroxylase (JusB7H) catalyzes the last step in the biosynthesis of diphyllin by introducing a hydroxyl group in position 7 of justicidin B. This enzyme was characterized from a microsomal fraction prepared from a *Linum perenne* Himmelszelt suspension culture for the first time. The hydroxylase activity was strongly inhibited by cytochrome *c* as well as other cytochrome P450 inhibitors like clotrimazole indicating the involvement of a cytochrome P450-dependent monooxygenase. JusB7H has a pH optimum of 7.4 and a temperature optimum of 26 °C. Justicidin B was the only substrate accepted by JusB7H with an apparent K_m of 3.9 ± 1.3 μM. NADPH is predominantly accepted as the electron donor, but NADH was a weak co-substrate. A synergistic effect of NADPH and NADH was not observed. The apparent K_m for NADPH is 102 ± 10 μM.

Keywords: *Linum perenne*; Linaceae; Lignan; Cytochrome P450; Justicidin B; Diphyllin; Justicidin B 7-hydroxylase

1. Introduction

Lignans, phenolic metabolites widespread in the plant kingdom, are derived by C8–C8′ oxidative dimerization of phenylpropanoids such as caffeyl, coniferyl or sinapyl alcohol (Moss, 2000). Further cyclisation and modifications of the dimers lead to a high structural diversity in this class of compounds. One can divide the lignans into different structural groups like aryltetralin [podophyllotoxin (**16**)] or arylnaphthalene type lignans [justicidins and diphyllin (**9**)] (Fuss, 2003).

Lignans have been of major interest since the early days of medical research as they possess a great variety of biological and pharmacological activities. In the last 15 years more than 120 lignans were reported to have anti-inflammatory, antimicrobial, immunosuppressive, anticancer and antioxidative activity (Saleem et al., 2005). Diphyllin (**9**) derivatives are putative remedies for topical chronic inflammatory disorders such as dermatitis and psoriasis while an acetylapioside derivative of diphyllin is a 5-lipoxygenase inhibitor (Prieto et al., 2002).

Abbreviations: JusB7H, justicidin B 7-hydroxylase; *L. perenne* H, *Linum perenne* Himmelszelt; PLR, pinoresinol–lariciresinol reductase.

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Plant species of different genera like *Haplophyllum* (Puricelli et al., 2002) and *Justicia* (Chen et al., 1996) accumulate arylnaphthalene lignans such as justicidin B (**8**) and diphyllin (**9**). Recently, we have reported on the accumulation of justicidin B (**8**) and glycosides of 7-hydroxyjusticidin B (diphyllin diglycosides) in cell suspension and hairy root cultures of *Linum perenne* H (Hemmati et al., 2007).

The biosynthesis of lignans starts with the coupling of two molecules of *E*-coniferyl alcohol (**1**) with the help of an auxilliary dirigent protein to give pinoresinol (**2**) which was shown for *Forsythia* species (Davin et al., 1990)

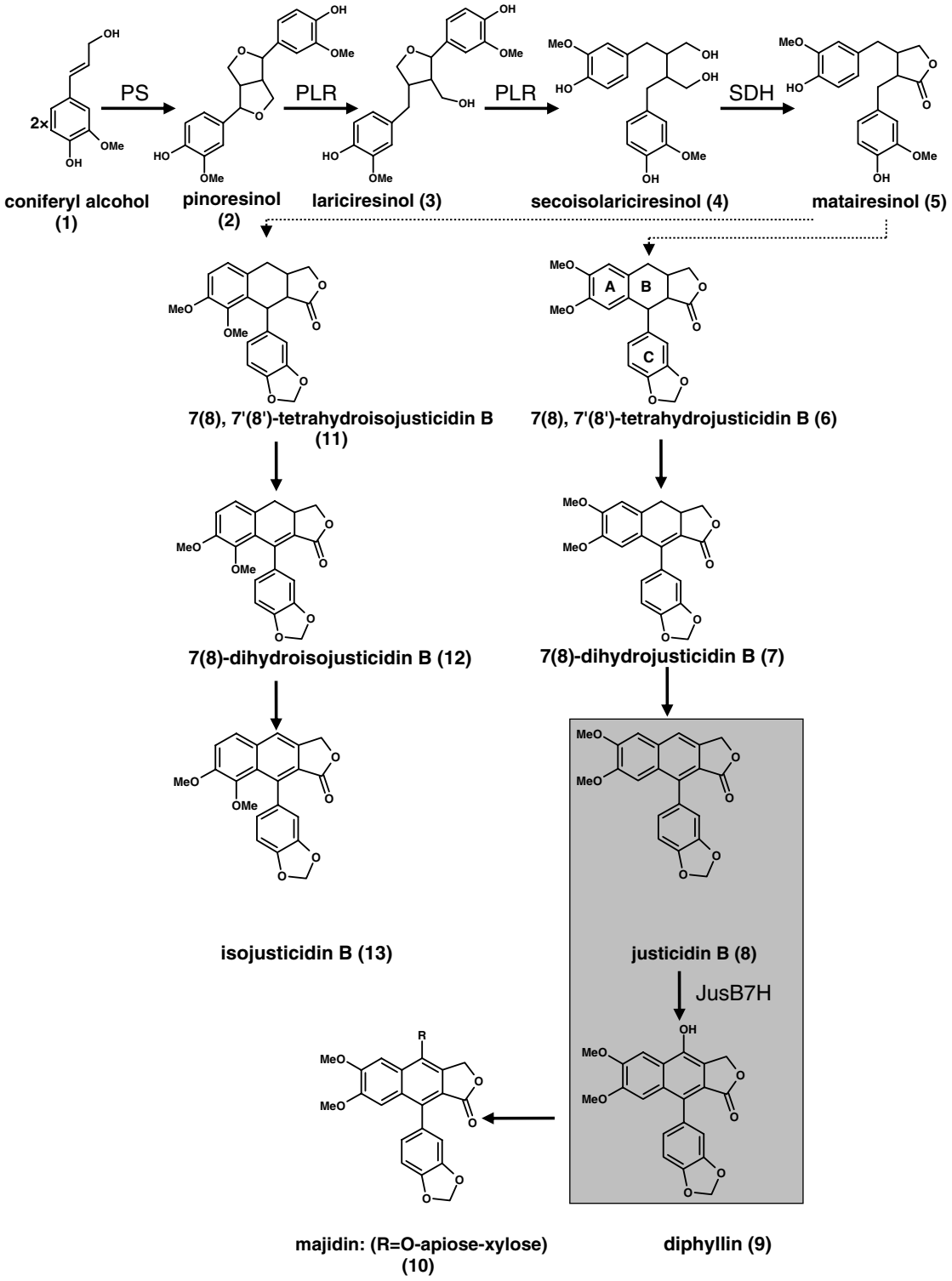

Fig. 1. Hypothetical biosynthetic pathway leading to diphyllin (**9**) and isojusticidin B (**13**). PS: pinoresinol synthase, PLR: pinoresinol–lariciresinol reductase, SDH: secoisolariciresinol dehydrogenase, JusB7H: justicidin B 7-hydroxylase.