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Coniferin dimerisation in lignan biosynthesis in flax cells

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Abstract

[$^{13}C_2$]-Coniferin was provided to a flax (*Linum usitatissimum* L.) cell suspension to monitor subsequent dimerisation by MS and NMR. The label was mainly incorporated into a 8–8′-linked lignan, lariciresinol diglucoside, a 8–5′-linked neolignan, dehydrodiconiferyl alcohol glucoside and a diastereoisomeric mixture of a 8-*O*-4′-linked neolignan, guaiacylglycerol-β-coniferyl alcohol ether glucoside. This latter compound is reported for the first time in flax. The strong and transient increase in these compounds in fed cells was concomitant with the observed peak in coniferin content. These results suggest (i) a rapid metabolisation of coniferin into lignans and neolignans and indicate the capacity of flax cells to operate different types of couplings, and (ii) a continuous synthesis and subsequent metabolisation of coniferin-derived dimers all over the culture period.

Keywords: *Linum usitatissimum*; Linaceae; Flax; LC-MS; MS/MS; NMR; Labelled coniferin; Lignan; Neolignan

1. Introduction

Monolignols are derived from cinnamic acids and supply precursors for various phenylpropanoid compounds such as lignins and lignans. The current view of the monolignol biosynthetic pathway envisages a metabolic grid leading to G and S units, through which the successive hydroxylation and *O*-methylation reactions may occur at different levels of side chain oxidation (Dixon et al., 2001). This general monolignol biosynthetic pathway occurring in most angiosperms is presented in Fig. 1 (adapted from Hoffmann et al., 2004 and from Shadle et al., 2007). The oxidative dimerisation of two coniferyl alcohol units leads to a great variety of secondary metabolites. When this dimerisation involves an oxidative linkage through the C-8 of the propenyl side chains of two coniferyl alcohol moieties, forming 8–8′ bonds, the resulting metabolites are called lignans. The term neolignan is used to define all the other types of linkage (Moss, 2000). Many of the dimers present in vascular plants have linkages other than 8–8′ and, of these, the bulk of the biochemical work has thus far been directed towards elucidating mechanisms of 8–5′, 8-*O*-4′ and 8–2′-linked neolignan formation (Davin and Lewis, 2003).

Lignans and neolignans, found in a wide range of plant species, display numerous pharmacological activities (Pool-Zobel et al., 2000; Arroo et al., 2002). The major lignans from flaxseed (*Linum usitatissimum*), secoisolariciresinol diglucoside and matairesinol, are converted into the "mammalian lignans" enterodiol and enterolactone by intestinal bacteria (Wang et al., 2000). The beneficial effects of these compounds on human health are well recognized (Westcott

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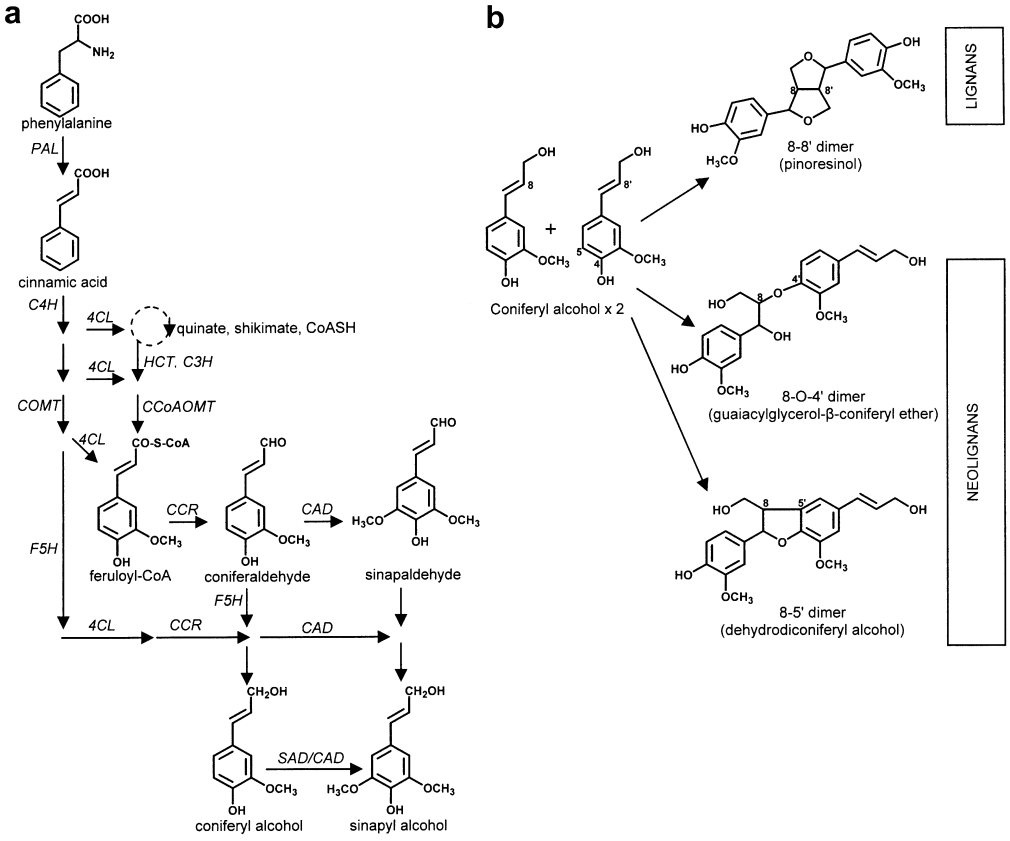

Fig. 1. Monolignol-derived products investigated in this study. (a) Monolignol biosynthetic pathway occurring in most angiosperms (from Hoffmann et al., 2004 and from Shadle et al., 2007). PAL, phenylalanine ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate: CoA ligase; HCT: hydroxycinnamoyltransferase; C3H: *p*-coumarate 3-hydroxylase; CCoAOMT: caffeoyl-CoA *O*-methyltransferase; COMT: caffeic acid *O*-methyltransferase; CCR, cinnamoyl-CoA reductase; F5H: ferulate 5-hydroxylase; CAD, cinnamyl alcohol dehydrogenase; SAD, sinapyl alcohol dehydrogenase. (b) Coniferyl alcohol-derived products: pinoresinol (8-8′-linked lignan) guaicylglycerol-β-coniferyl ether (8-*O*-4′-linked neolignan) and dehydrodiconiferyl alcohol (8-5′-linked phenylcoumaran lignan) resulting from simple dimerisation of two coniferyl alcohols.

and Muir, 2003; McCann et al., 2005) and *in vitro* studies confirm the observed *in vivo* effect (Bylund et al., 2005). In particular, they were shown to reduce the incidence of breast and prostate cancers by modulating steroidal hormone synthesis (Adlercreutz and Mazur, 1997). 8-*O*-4′ neolignans are used as lead compounds for antifungal agents (Apers et al., 2003).

In view of the important pharmacological properties, numerous studies have been carried out in an attempt to get a better knowledge of the biological events linked to the biosynthesis and the accumulation of lignans and neolignans (Seidel et al., 2002; Sicilia et al., 2003). In flax, coniferyl alcohol was clearly evidenced to be the monolignol involved in lignan biosynthesis (Ford et al., 2001). Flax suspension cells are also known to accumulate neolignans (Attoumbre et al., 2006).

In an effort to study the biosynthesis and the accumulation of secondary metabolites, *in vitro* cultures have been established as a very useful tool because this system allows uniformity, accessibility and reduced complexity (Facchini, 2001; Mesnard et al., 2002; Verpoorte et al., 2002). In the present study, a recently established flax (*L. usitatissimum*) cell suspension (Hano et al., 2006; Attoumbre et al., 2006) was used in feeding experiments with ^{13}C-labelled coniferin to investigate monolignol dimerisation into both lignans and neolignans. In order to visualise its dimerisation by NMR and MS, doubly labelled (8,9-$^{13}C_2$)-coniferin was synthesised to offer easier detection (Beejmohun et al., 2006). Coniferin, considered as the putative storage form of coniferyl alcohol (Gross, 1985) was used to facilitate the solubilisation into aqueous culture media (Van Uden et al., 1995).

2. Results and discussion

2.1. Preliminary experiments

2.1.1. Growth kinetics

For the feeding experiments, a minimum concentration of 0.5 g/l (1.46 mM) of labelled coniferin was required due to the sensitivity of the analytical methods used. Such a concentration of an exogeneous precursor might be toxic for the flax suspension cells (Edahiro et al., 2005). Prior to experiments with labelled coniferin, the effect of unlabelled coniferin supplementation on cell growth was therefore