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Pyrone polyketides synthesized by a type III polyketide synthase from *Drosophyllum lusitanicum*

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ABSTRACT

To isolate cDNAs involved in the biosynthesis of acetate-derived naphthoquinones in *Drosophyllum lusitanicum*, an expressed sequence tag analysis was performed. RNA from callus cultures was used to create a cDNA library from which 2004 expressed sequence tags were generated. One cDNA with similarity to known type III polyketide synthases was isolated as full-length sequence and termed DluHKS. The translated polypeptide sequence of DluHKS showed 51–67% identity with other plant type III PKSs. Recombinant DluHKS expressed in *Escherichia coli* accepted acetyl-coenzyme A (CoA) as starter and carried out sequential decarboxylative condensations with malonyl-CoA yielding α-pyrones from three to six acetate units. However, naphthalenes, the expected products, were not isolated. Since the main compound produced by DluHKS is a hexaketide α-pyrone, and the naphthoquinones in *D. lusitanicum* are composed of six acetate units, we propose that the enzyme provides the backbone of these secondary metabolites. An involvement of accessory proteins in this biosynthetic pathway is discussed.

1. Introduction

Drosophyllum lusitanicum Link, the dewy pine, is a carnivorous plant that occurs in the southern part of the Iberian peninsula and northern Morocco. Taxonomically, *D. lusitanicum* belongs to the monotypic family Drosophyllaceae, as shown by recent molecular studies, but is closely related to other families with carnivorous plants, e.g the Droseraceae, Nepenthaceae and Dioncophyllaceae (Meimberg et al., 2000; Heubl et al., 2006). A chemotaxonomic marker of these taxa and related families without carnivory, e.g. the Plumbaginaceae, is the occurrence of acetogenic naphthoquinones. These secondary metabolites serve as defense compounds (Peters et al., 1995; Bringmann et al., 1999) and as antifeedants against insects (Tokunaga et al., 2004a,b), but some have allelopathic effects, as well (Dornbos and Spencer, 1990; Higa et al., 1998). Naphthoquinones are also known to possess useful pharmacological activities. Plumbagin **(8)** (2-methyl-5-hydroxy-1,4-naphthoquinone), for example, was shown to have antimicrobial (Durga et al., 1990; Didry et al., 1994), antimalarial (Likhitwitayawuid et al., 1998), and anticancer (Sugie et al., 1998; Sandur et al., 2006) effects.

The biosynthesis of naphthoquinones can occur *via* three different routes in higher plants. They can be produced from geranylated *p*-hydroxybenzoic acid, as in the case of alkannin and shikonin (Schmid and Zenk, 1971; Inouye et al., 1979), or from *iso*-chorismic acid and α-ketoglutaric acid *via* *o*-succinylbenzoic acid, providing the precursor of vitamin K (Leistner, 1999). A third pathway leading to the naphthoquinones plumbagin **(8)**, droserone and 7-methyljuglone utilizes acetate precursors. First evidence of this pathway had been provided by tracer studies with *Plumbago europaea*, *Drosera* species and *Drosophyllum lusitanicum* (Durand and Zenk, 1971,1974). Recent experiments by Bringmann and co-workers confirmed the acetogenic origin of naphthoquinones in lianas of the Ancistrocladaceae and Dioncophyllaceae families (Bringmann et al., 2000). Based on these experiments, it was postulated that plumbagin **(8)** and biosynthetically related naphthoquinones and tetralones are synthesized by polyketide synthases (PKSs) *via* the acetate-polymalonate pathway (Bringmann and Feineis, 2001).

All PKSs so far isolated from plants belong to the superfamily of type III PKSs (Schröder, 2000; Austin and Noel, 2003), which comprises homodimeric proteins that catalyze the iterative decarboxylative condensation of a starter coenzyme A (CoA) ester with malonyl-CoA **(1)**, or in some cases, methylmalonyl-CoA or ethylmalonyl-CoA (Schröder et al., 1998; Song et al., 2006). The best

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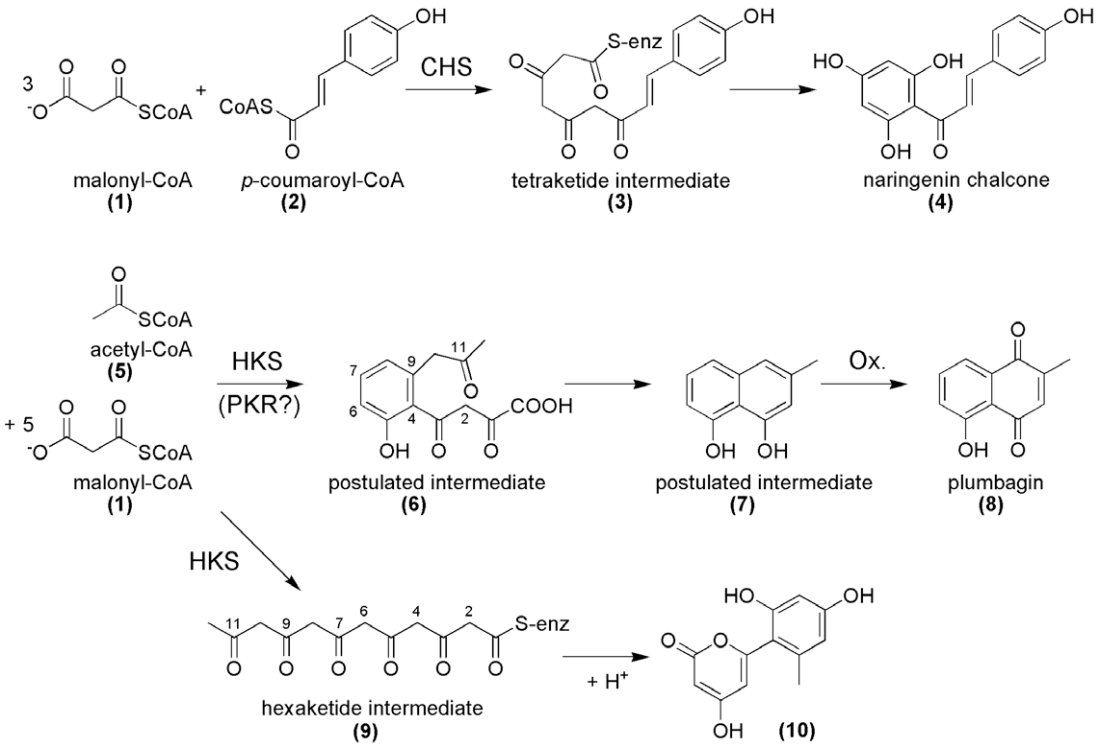

Fig. 1. Postulated mechanism of plumbagin biosynthesis and comparison with the reaction of chalcone synthase (CHS). The PKS involved in plumbagin **(8)** biosynthesis presumably catalyzes the decarboxylative condensation of acetyl-CoA **(5)** with five molecules of malonyl-CoA **(1)**. The oxygen of the third acetate unit is probably removed by a polyketide reductase (PKR) prior to the first cyclization, and one carbon is lost by decarboxylation.

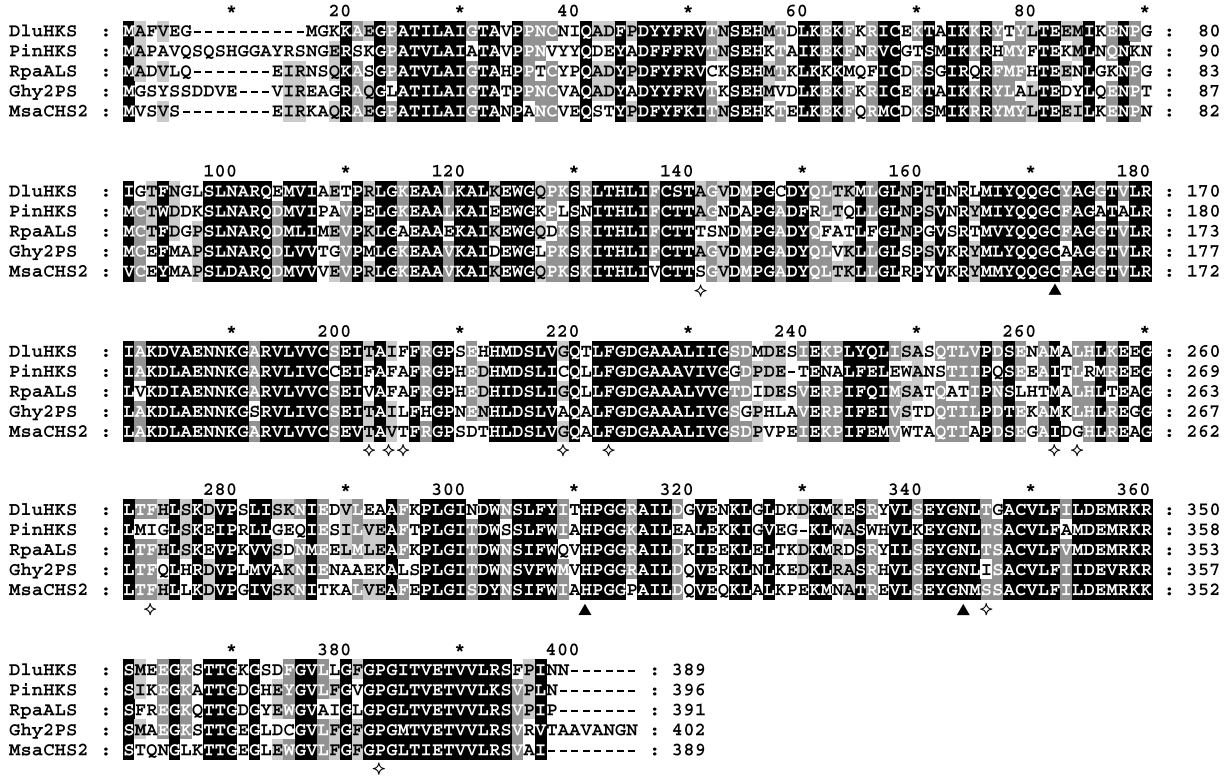

Fig. 2. ClustalW alignment of DluHKS with other type III PKS amino acid sequences. Amino acids of the catalytic Cys-His-Asn triad are marked by triangles. Other amino acids of the active site cavity, which are discussed in the text, are labeled by diamonds. Abbreviations: DluHKS, *Drosophyllum lusitanicum* HKS; Ghy2PS, *Gerbera hybrida* 2-pyrone synthase; MsaCHS2, *Medicago sativa* CHS2; PinHKS, *Plumbago indica* HKS; RpaALS, *Rheum palmatum* aloesone synthase.