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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.

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PHYTOCHEMISTRY

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 carnivoplants, e.g the Droseraceae, Nepenthaceae rous 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-5hydroxy-1,4-naphthoguinone), for example, was shown to have antimicrobial (Durga et al., 1990; Didry et al., 1994), antimalarial

* Corresponding author. Address: Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis, MO 63132, USA. Tel.: +1 314 587 1490; fax: +1 314 587 1590. (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; Inouve et al., 1979), or from iso-chorismic acid and *a*-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.

DluHKS PinHKS RpaALS Ghy2PS MsaCHS2	:::::::::::::::::::::::::::::::::::::::	* NAFVEG NAPAVQSQSHGG NADVLQ MGSYSSDDVE MVSVS	20 MGKK AYRSNGER -EIRNSQK -VIREAGR -EIRKAQR	AEGPATILA SKGPATVLA SGPATVLA AGG <mark>L</mark> ATILA AEGPATILA	* IGTAVPPNC IGTAHPPTC IGTAHPPTC IGTANPANC	40 NIQADEPD YYQDEYADI YPQADYPDI VAQADYAD VEQSTYPDI	* YYFRVINSEH FFFRVINSEH FYFRVCKSEH YYFRVIKSEH FYFKIINSEH	60 MTDIKEKFKRI KTAIKEKFNR MTKIKKKMQFI MVDIKEKFKRI KTEIKEKFQRN	* CEKTAIKKR CGTSMIKKR CDRSGIROR CEKTAIKKR COKSMIKRR	80 YTYLTEEMIK HMYFTEKMLN FMPHTEENLG YLALTEDYLQ YMYLTEEILK	* QNKN KNPG ENPT ENPN	::	80 90 83 87 82
DluHKS PinHKS RpaALS Ghy2PS MsaCHS2	:::::::::::::::::::::::::::::::::::::::	100 IGTENGLSLNAR MCTWDDKSLNAR MCTEDGPSLNAR MCEFMAPSLNAR VCEYMAPSLDAR	* QEMVIAET QDMVIPAV QDMLIMEV QDLVVTGV QDMVVVEV	120 RLGKEAAL ELGKEAAL KLGAEAAE MLGKEAAV RLGKEAAV) KALKEWGQP KAIEEWGKP KAIKEWGQD KAIKEWGQP	* KSRLTHLI LSNITHLI KSRITHLI KSKITHLI KSKITHLI	140 CSTACVDMP CTWTACNDAP CTWTSNDMP CTWTSCVDMP CTWTSCVDMP	* GCDYQLTKMLG GADFRLTQLLG GADYQEATLFG GADYQLVKLLG GADYQLTKLLG	160 UNETINRLM UNESUNRYM UNEGUSRUM USESUKRYM UREYUKRYM	* IYQQGCYAGG IYQQGCFAGA VYQQGCFAGG LYQQGCAAGG MYQQGCFAGG	180 TVLR TALR TVLR TVLR TVLR	:::::::::::::::::::::::::::::::::::::::	170 180 173 177 172
DluHKS PinHKS RpaALS Ghy2PS MsaCHS2	: : : : : : : : : : : : : : : : : : : :	* I AKDVAENNKGA I AKDLAENNKGA LAKDLAENNKGA LAKDLAENNKGA	200 RVLVVCSE RVLIVCCE RVLVVCSE RVLIVCSE RVLVVCSE	TAIFFRGP FAFAFRGP VAFAFRGP TAILFHGP 7TAVTFRGP ♦ ♦ ♦	* 2 SEHHMDSLV HEDHMDSLI HEDHIDSLI NEMHLDSLV SDTHLDSLV	20 GCTLFGDGJ CCLLFGDGJ GCLLFGDGJ ACALFGDGJ GCALFGDGJ ♦ ♦ ♦	* AAALIIGSDM AAAVIVGGDP AAALVVGTDI AAALIVGSGP AAALIVGSDP	240 DESIEKPLYQI DE-TENALFEI DESVERPIFQI HLAVERPIFEI VPEIEKPIFEN	* ISASQTLVP EWANSTIIP MSATCATIP VSTDQTTIP VWTAQTIAP	260 DSENAMALHI QSEEAITLRM NSLHTMALHI DTEKAMKLHI DSEGAIDGHI & &	* REEG TEAG REGG RE <mark>A</mark> G	:	260 269 263 267 262
DluHKS PinHKS RpaALS Ghy2PS MsaCHS2	: : : : : : : : : : : : : : : : : : : :	280 LIFHISKDVPSL IMIGISKEIPRL LIFHISKEVPKV LIFQIHRDVPLM LIFHILKDVPGI ∻	* ISKNIEDV IGEQIESI VSDNMEEL VAKNIENA VSKNITKA	300 EAAFKPLG VEAFTPLG (LEAFKPLG AEKALSPLG VEAFEPLG) I <mark>ndwnslfy</mark> Itdw <mark>s</mark> slfw Itdwnsifw Itdwnsvfw Isdynsifw	* THPGGRA TAHPGGKA QVHPGGRA MVHPGGRA TA <mark>HPGG</mark> PA	320 ILDGVENKLG ILEALEKKIG ILDKIEEKLE ILDQVERKLN ILDQVEQKLA	* LDKDKMKESRY VEG-KLWASWF LTKDKMRDSRY LKEDKLRASRF LKPEKMNATRF	340 VLSEYGNLT VLKEYGNLT ILSEYGNLT VLSEYGNLI VLSEYGNMS	* GACVLFILDE SACVLFAMDE SACVLFVMDE SACVLFILDE SACVLFILDE	360 MRKR MRKR MRKR VRKR MRKK	:	350 358 353 357 352
DluHKS PinHKS RpaALS Ghy2PS MsaCHS2	:::::::::::::::::::::::::::::::::::::::	* SMEECKSTTCKC SIKECKATTCDC SFRECKQTTCDC SMAECKSTTCEC STQNGLKTTCEC	380 SDFGVLLG HEYGVLFG YEwGVAIG LDCGVLFG LEWGVLFG	GPGITVET GPGLTVET GPGLTVET GPGLTVET GPGLTIET	* 4 VVLRSFPIN VVLKSVPIN VVLRSVPIP VVLRSVRVT VVLRSVAI-	00 N AAVANGN	: 389 : 396 : 391 : 402 : 389						

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.

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