

## Characterization of CYP76AH4 clarifies plant phenolic diterpenoid biosynthesis

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Rosemary (*Rosmarinus officinalis*) has long been used as a flavoring agent, and more recently as a nutritional supplement, as well as in cosmetics, due to its antioxidant properties. Carnosic acid (4) and carnosol (5) are the major phenolic diterpenoid constituents of rosemary, are responsible for much of its antioxidant activity, and exhibit a variety of other effects, including acting as anti-HIV and anticancer agents. Moreover, 4 and 5 are likely intermediates in the biosynthesis of more elaborated phenolic diterpenoids, such as the tanshinones (e.g., 6 and 7;) that make up the bioactive lipophilic constituents of the widely used Chinese medicinal herb Danshen (*Salvia miltiorrhiza*). Accordingly, there is significant interest in elucidation of phenolic diterpenoid biosynthesis.

Phenolic diterpenoids such as 2–7 fall within the labdane-related superfamily of natural products, whose biosynthesis is initiated by a sequential pair of cyclization and/or rearrangement reactions. This most often results in the production of an olefin, such as 1, which requires further elaboration to produce bioactive natural products, particularly the incorporation of oxygen catalyzed by cytochromes P450 (CYP). While these heme thiolate mono-oxygenases typically catalyze hydroxylation reactions, members of this enzymatic superfamily are known to catalyze more complex reactions, including aromatization and multiple reaction cycles with certain substrates.

Previous work has identified two diterpene synthases from Danshen that together cyclize the general diterpenoid precursor (*E,E,E*)-geranylgeranyl diphosphate into the tricyclic olefin 1, with initial bicyclization catalyzed by DsCPS, and subsequent cyclization and rearrangement catalyzed by SmKSL. This abietane harbors a planar cyclohexa-1,4-diene ring that is poised for aromatization, and recently was shown to be the precursor of the phenolic diterpenoids 2 and 6 via labeling studies. Herein, via an RNA-Seq approach, we clarify the biosynthesis of phenolic diterpenoids, confining the role of the characterized CYP76AH sub-family members to C12-hydroxylation of the aromatic intermediate 3. The presence of 3, along with 1 and 2, in both rosemary and danshen, is consistent with a role for 3 in biosynthesis of phenolic diterpenes. Thus, the initially formed olefin intermediate 1 first undergoes aromatization to 3 prior to formation of 2. While the conversion of 1 to 3 occurs spontaneously, it seems likely that such aromatization is enzymatically catalyzed in planta, although the relevant enzyme is yet to be determined, providing a target for future investigation.

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