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Unusual chemistries in fungal meroterpenoid biosynthesis

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Meroterpenoids are polyketide and terpenoid hybrid natural products with remarkable biological activities. Recent progress in fungal meroterpenoid biosynthesis has revealed several unusual enzyme reactions and novel enzymes, including unique terpene cyclization reactions by a novel family of membrane-bound terpene cyclases and post-cyclization modification reactions by oxygenases, such as non-heme iron-dependent dioxygenases, flavin adenine dinucleotide-dependent monooxygenases, and cytochrome P450 monooxygenases. They contribute to the structural diversification and increase in complexity of fungal meroterpenoids. Structure-function studies of these enzymes provide strategies for engineering the biosynthetic machinery to create novel molecular scaffolds for drug discovery.

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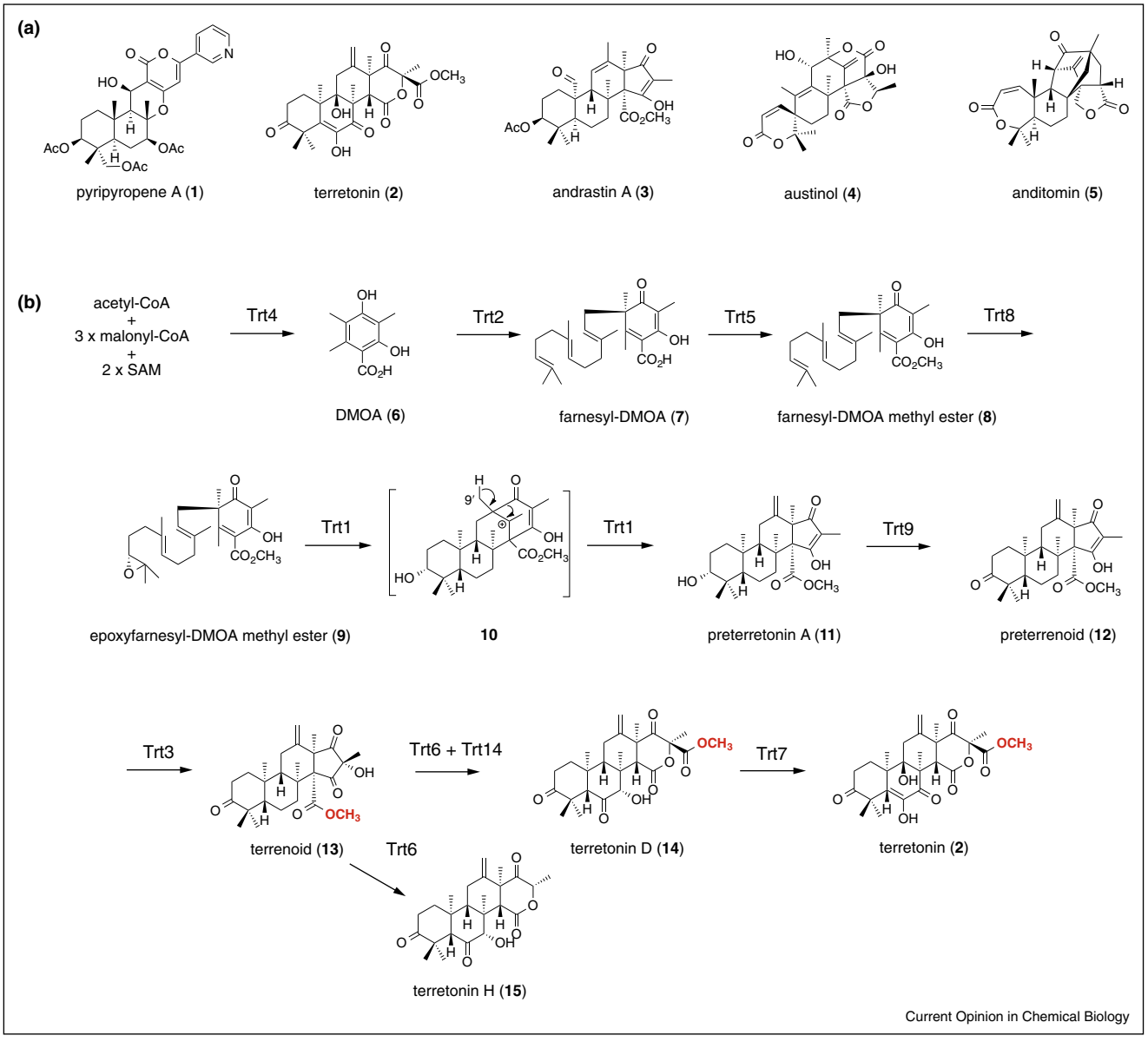
Introduction

Remarkable structural diversity and complex molecular scaffolds are the attractive features of fungal meroterpenoids, polyketide and terpenoid hybrid natural products with important biological activities [1,2••]. The biosynthetic pathways for these complex natural products include a diverse range of fascinating chemistries, and are therefore rich sources of novel enzymes catalyzing unusual enzyme reactions. Indeed, since our first report on the identification and characterization of the biosynthetic gene cluster of pyripyropene A (**1**) from *Aspergillus fumigatus* in 2010 [3], remarkable advances in the studies of fungal meroterpenoid biosynthesis have revealed many interesting enzymes that catalyze quite unique chemical conversions, as well as novel families of enzymes [2••]. This short review summarizes the very recent research progress on the biosyntheses of the fungal meroterpenoids terretonin (**2**) [4–6,7•], andrastin A (**3**) [8], austinol (**4**) [9,10••], and anditomin (**5**) [11••] (Figure 1a). In particular, we will focus on the remarkable terpene cyclization reactions catalyzed by a novel family of membrane-bound terpene cyclases (TPCs) and the post-cyclization modification reactions by oxygenases, including non-heme iron-dependent dioxygenases, flavin adenine dinucleotide-dependent monooxygenases (FMOs), and cytochrome P450 monooxygenases, which significantly contribute to the structural diversification and increase in complexity of fungal meroterpenoids.

Terpene cyclization reactions

The biosynthesis of meroterpenoids starts with the assembly of the polyketide moiety, which is followed by prenylation of the polyketide, stereospecific epoxidation of the olefin of the prenyl chain, and cyclization of the terpenoid moiety to generate the diverse meroterpenoid core scaffolds [1,2••]. For example, the fungal meroterpenoids terretonin (**2**), andrastin A (**3**), austinol (**4**), and anditomin (**5**), are all biosynthesized from a simple aromatic polyketide, 3,5-dimethylorsellinic acid (DMOA) (**6**), but they have different cyclic terpenoid scaffolds [1,2••]. The structural diversity is due to the differences in the mechanisms of the cyclization reactions, which are catalyzed by a novel family of membrane-bound terpene cyclases (TPCs). The cyclization reaction is thus one of the key steps to generate the structural diversity of the fungal meroterpenoids. Remarkably, the stereochemistry of the cyclization reaction, which usually affords a single product, is strictly controlled by each enzyme. These novel TPCs are quite small (ca. 25 kDa) integral membrane proteins with seven transmembrane helices, and share very low sequence similarity to the known TPCs [3,5,11••]. Notably, homologous proteins are widely distributed in the biosynthetic pathways of terpenoid-bearing secondary metabolites from fungi and actinomycetes, including indole diterpenoids [3]. A sequence comparison of these homologous proteins indicated the presence of several conserved motifs, but none of the aspartate-rich motifs (DDXXD or DXDD motifs) normally found among known TPCs [3]. Therefore, the detailed mechanism for the catalysis still remains to be elucidated, but a mutational study of Pyr4, involved in the biosynthesis of pyripyropene A (**1**) in *A. fumigatus*, revealed that two conserved acidic amino acid residues, Glu63 and

Figure 1



(a) Structures of fungal meroterpenoids. **(b)** Biosynthetic pathway of terretonin (**2**) in *Aspergillus terreus*.

Asp218, are essential for the catalytic activity, suggesting that these residues serve as general acids that protonate the terminal epoxide to initiate the sequential ring-forming reaction [3].

Terretonin (**2**), from *Aspergillus terreus*, is one of the DMOA-derived meroterpenoids, with a unique tetracyclic scaffold [4–6,7•]. Interestingly, our recent studies revealed that, in terretonin biosynthesis (Figure 1b), the methylation of the carboxyl group of farnesyl-DMOA (**7**) is essential for the substrate recognition by the novel transmembrane TPC, Trt1 [5]. Without the methylation of the carboxyl group, the cyclization reaction does not occur. Interestingly, this is also the case for the biosyntheses of other DMOA-derived fungal meroterpenoids, including andrastin A (**3**) and austinol (**4**) [5]. The cyclase Trt1 is thought to bind (10'*R*)-epoxyfarnesyl-DMOA methyl ester (**9**) in the '*chair-chair*' conformation, and initiate the sequential ring-forming reaction by protonating the terminal epoxide (Figure 2a) [5]. The sequential C–C bond-forming reaction first produces the 6.6.6.6-fused tetracyclic tertiary carbocationic intermediate (**10**), which then undergoes carbon skeletal rearrangement (D-ring contraction) with proton abstraction from the methyl group at C-9′ (H_a) to generate preterretonin A (**11**) with a 5-membered D-ring. In contrast, in the