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Point of VIEW

Revealing Coupling Patterns in Isoprenoid Alkylation Biocatalysis

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ABSTRACT Diversity of scaffold structure and function is a hallmark of the >50,000 isoprenoid natural products such as taxol. Whereas most members of this class are assembled by iterative head-to-tail enzymatic joining reactions between Δ^2- and Δ^3-isopentenyl diphosphate (IPP) monomers, dimerization of two Δ^2-IPP molecules has now been shown to account for three additional modes of "irregular" coupling patterns at the level of C_{10} monoterpene scaffolds.

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More than 50,000 natural compounds are known that are assembled from the two biological isoprene units, Δ^2- and Δ^3-isopentenyl diphosphate (IPP). The Δ^2 isomer is often referred to as dimethylallyl-pyrophosphate (DMAPP). Isoprenoid natural products are also known historically as terpenes because of their high abundance in turpentine. They include monoterpene (C_{10}) and sesquiterpene (C_{15}) flavor constituents in plants, the triterpene-based sterol superfamily, the C_{40}-based carotenoid pigments, and therapeutic agents such as the diterpene taxol (*1*, *2*) (Figure 1). The isoprenoid origin of many different terpenoid scaffolds was deciphered >100 years ago by the German chemist Wallach (Nobel Prize winner in chemistry in 1910), and the biogenetic isoprene rule was elaborated by the Zurich group headed by Ruzicka (Nobel Prize winner in chemistry in 1939). Still, despite more than a century of biosynthetic focus, new insights continue to emerge about how nature joins the building blocks for isoprenoid chain elongation, five carbons at a time (*3*).

The best-understood pathway, in terms of the mechanism and structure of the enzyme catalysts, involves the head-to-tail (1′–4) joining of Δ^2- and Δ^3-prenyl units by enzymes such as farnesyl-PP synthase, which make the C_{10} geranyl-pyrophosphate (PP) as an intermediate and then acts as the Δ^2 partner in another elongation cycle with a second molecule of Δ^3-IPP. The C—C bond-forming steps utilize the elongating Δ^2 partner as the electrophile, with early S_N1-type dissociation of the C_1–O bond to yield the allyl cationic transition state (*2*) (Figure 2). That delocalized carbocation is regioselectively captured by the π electrons of the Δ^3-(DMAPP) isopentenyl-PP monomer unit acting as the nucleophile. This iterative head-to-tail C—C coupling generates products from C_{10} (one elongation cycle) to C_{110} (21 elongation cycles), with product control probably mediated by enzyme active site volumes that become filled by the elongating isoprenoid product chains.

Yet, this head-to-tail connection is only one of nine patterns known for constructing isoprenoid scaffolds from 3-methyl-1-butyl units, as noted by Thulasiram in a recent paper in *Science* (*4*). Four of those patterns, including the 1′–4 connection noted above, as well as 1′–2 and cyclic 1′–2–3 (cyclopropanes) and 1′–2–3–2′ (cyclobutane), are produced in the first $C_5 + C_5$ to monoterpene (C_{10}) alkylation steps. Four other patterns result from later-stage rearrangements (*e.g.*, head-to-head coupling of farnesyl-PP to squalene *via* presqualene-PP), and the last mode of coupling (4′–4′) is only seen in archaebacterial membrane lipids.

In prior efforts, Poulter and colleagues (*5*) studied the 1′–2–3 joining pattern catalyzed by the enzyme from chrysanthemum that makes the cyclopropenoid C_{10} metabolite chrysanthemyl diphosphate (CPP). In contrast to the head-to-tail joining of a Δ^2-electrophilic partner with a Δ^3-nucleophilic partner, the chrysanthemyl-PP synthase (CPPase) uses two molecules of the Δ^2-IPP to fashion the cyclopropane ring in the C_{10}

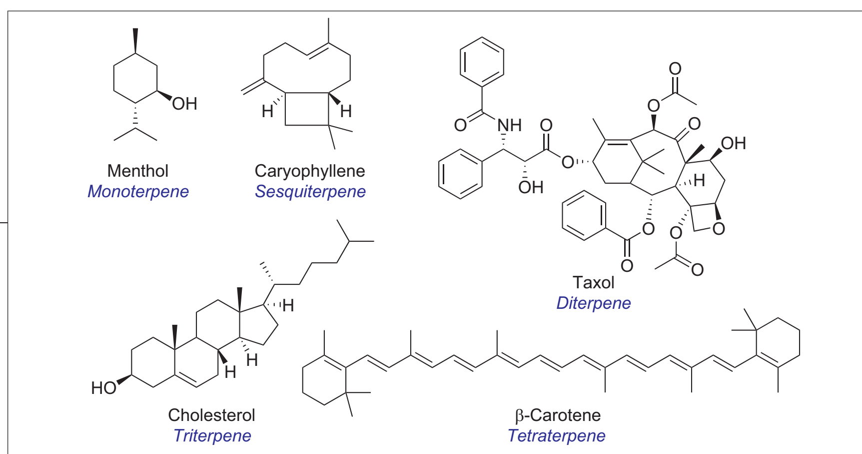

Figure 1. A few examples of isoprenoid natural products.

chrysanthemyl-PP (1′–2–3). Poulter and colleagues continue to make deep insights into how the organic chemistry available to isoprenoid skeletons is directed in nature, as shown in the recent paper in *Science* (*4*) analyzed below.

Now, Thulasiram *et al.* (*4*) report on the construction of 11 chimeras of the farnesyl-PP synthase (FPPase) from sagebrush and CPPase (chrysanthemum) and screening for both the normal chain elongation products (1′–4 joining pattern) and "irregular" products that would reflect the 1′–2–3 cyclopropanation pattern but also perhaps the missing two patterns (1′–2 and 1–2–3–2′) for which no enzymes have yet been characterized. The FPPase/CPPase chimeras produced, after workup, four C_{10} alcohol products. One was the anticipated cyclopropane-containing chrysanthemol, and another was the normal chain elongation product geraniol (*E*-isomer), along with the *Z*-isomer nerol. A surprise product was the 1′–2 connected lavandulol. Chimeras between CPPase-69–FPPase and CPPase-243–FPPase (*e.g.*, the first 243 residues of CPPase fused to the remaining residues of FPPase) also gave the cyclobutane alcohol maconelliol and its double-bond isomer planococcol (see Figure 3). Whereas the 1′–4 chain elongation reaction uses both Δ^2- and Δ^3-building blocks, the other three products arise from only the Δ^2-IPP substrate. Thus, variation of the ratio of Δ^2 to Δ^3 substrates among the chimeras provided altered product ratios.

Stereochemical analysis revealed the formation of single enantiomers by the chimeric enzymes. Absolute stereochemical assignment allowed a unifying model for equivalent orientation of the reacting partners in the synthase active sites, both for the 1′–4 chain elongation and the three irregular outcomes of cyclopropanation, 1′–2 branching and cyclobutanation. In the latter three cases, where two molecules of Δ^2-IPP (DMAPP) are coupled in the enzyme active site, the 1′-carbon of the Δ^2-IPP that will provide the allyl cation electrophile is located on the *Re* face of the 2,3-double bond of the Δ^2-IPP that acts as the nucleophile (Figure 3).

This orientation of the two DMAPPs would lead to the capture of the subsequent allyl cationic transition state *via* a cyclopropyl cation common to all three modes of irregular connection. Proton loss *via* regioselective cleavage of a C—H bond yields the cyclopropane product CPP. Rearrangement of the cyclopropyl cation to an acyclic (presumably more stable) tertiary cation is a precursor to proton loss and olefin formation to account for the 1′–2 connectivity in acyclic lavandulol-PP. Alternatively, the acyclic tertiary cation can be captured intramolecularly by the π electrons of the terminal olefin, yielding a cyclobutyl cation that can be quenched by proton loss to give either of the two observed olefinic cyclobutyl-PP products, macionellyl-PP and planococcyl-PP.

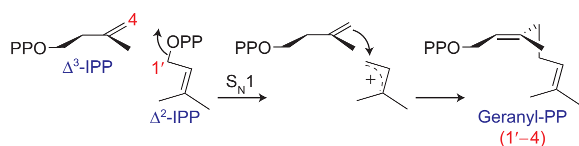

Figure 2. Head-to-tail condensation of a Δ^2-prenyl-PP as electrophile with a Δ^3-IPP monomer as nucleophile to create a 1′–4 linkage during chain elongation. Iteration by prenyl transferase catalysts add additional Δ^3-IPP monomers to the growing Δ^2-prenyl chain.

In addition to the detection of enzymatic catalysis of the 1′–2 and 1′–2–3–2′ joining patterns for the first time, this study shows that minor changes in the native and chimeric enzyme active sites can redirect reaction flux to all the known joining patterns for isoprenoid building blocks. It emphasizes that the allylic cation reaction manifolds can be directed in enzymatic active sites to a range of product scaffold outcomes by control of orientation of intramolecular olefins and strategically placed enzyme bases to catalyze proton abstractions at C—H sites of the bound intermediates. This revelation for C_{10} monoterpene scaffolds appears equally true in enzyme-directed diversification in C_{30} triterpene scaffold manipulations, where cationic intermediates can also be routed to distinct fates (*6*).

Thulasiram *et al.* (*4*) point out that the findings from the chimeric FPPase and CPPase chart a highly plausible path for evolution of the distinct C_{10} monoterpene scaffolds from a common catalytic protein precursor. The enzymatic logic and catalytic machinery for isoprenoid natural products differ dramatically from those of another major natural product class, the polyketides. C—C bond formation in isoprenoids involves allyl cation transition states and olefinic π electrons as the carbon nucleophiles, with no covalent tethering of reaction intermediates in the enzymes' active sites. By contrast, C—C bond formation in polyketides involves covalently tethered thioesters as the electrophiles and decarboxylation of malonyl moieties as carbanion equivalents. There is only one common pattern of chain elongation, head-to-tail, in polyketides, compared with the multiple patterns noted above for isoprenoid unit joining.

The isoprenoid logic and enzymatic machinery may be suited for more rapid evolu-

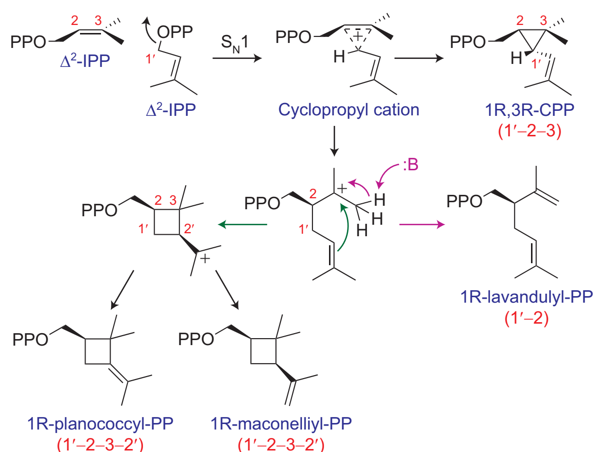

Figure 3. Non-head-to-tail condensation of two molecules of Δ^2-IPP by hybrids of CPPase and FPPase. The lower Δ^2-IPP molecule bound in the active site of the hybrid enzyme acts as electrophile undergoing the assisted S_N1 ionization to the allyl cation to be captured by the π electrons of the upper Δ^2-IPP molecule. The steps leading to the formation of the C—C bonds in the three observed products could be stepwise but are accounted for by an early common cyclopropyl cation transition state (on the way to the chrysanthemyl-PP product). Competing rearrangement of the cyclopropoyl cation to the acyclic tertiary carbocation in turn can yield either lavandulol-PP or the two olefinic isomers of the cyclobutyl-PP products.

tion of diverse structures based on promiscuity of capture and quenching of the cationic intermediates by water, olefins, hydride ions, and methyl migrations. Leakage of intermediates may also be a diversification dividend of this mode of carbon skeleton assembly. The polyketide biosynthetic evolution probably involved more gene shuffling to rearrange protein modules to alter the identity and number of substrate monomers incorporated during chain elongation. The work by Thulasiram *et al.* (*4*) also shows how that may have occurred in isoprene scaffold diversification.

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