Uncovering Novel Carbocation Dynamics in Terpene Biosynthesis

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Terpenes represent one of the largest and most diverse classes of natural products with critical biological functions and applications across medicine and agriculture. The complexity of these molecules arises from enzyme-catalyzed cyclization reactions that manage highly reactive carbocation intermediates. However, the precise mechanisms by which these enzymes control such reactive species to yield specific products remains poorly understood, particularly for rare and complex terpene skeletons.

This team of chemists and biochemists has conducted the first mechanistic study of tetraisoquinene biosynthesis by TiqS, a diterpene synthase from the myxobacterium *Melittangium boletus*. By combining minor metabolite isolation, deuterium labeling, site-directed mutagenesis, and quantum chemical calculations, the researchers uncovered unusual deprotonation pathways and nonstatistical dynamic effects that direct the formation of the unique 5/5/5/5-fused ring system. Their work also demonstrates how the enzyme prevents the formation of secondary carbocations during the complex rearrangement of the carbon skeleton.

This work offers fundamental insights into nature's strategies for terpene cyclization and provides a framework for understanding related transformations in other systems. The findings could facilitate engineering terpene synthases for novel bioactive compounds, especially since the tetraisoquinane scaffold represents a previously unexplored structure with promising biological activities.

Facilities and instrumentation used: AMRIS Facility, University of Florida, 600 MHz NMR spectrometer with a cryogenic probe

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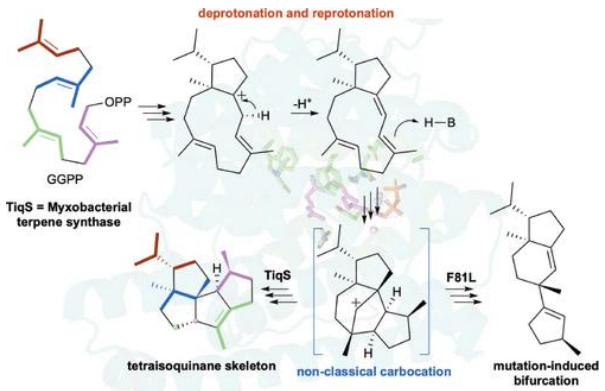

Figure 1 (left): TiqS-catalyzed biosynthesis of tetraisoquinane from GGPP. The myxobacterial terpene synthase transforms GGPP through a series of cyclizations, deprotonation-reprotonation events, and a non-classical carbocation intermediate. The complex reaction pathway showcases how enzymes guide carbocation chemistry to form specific products and can be redirected through strategic mutations to produce alternative scaffolds.

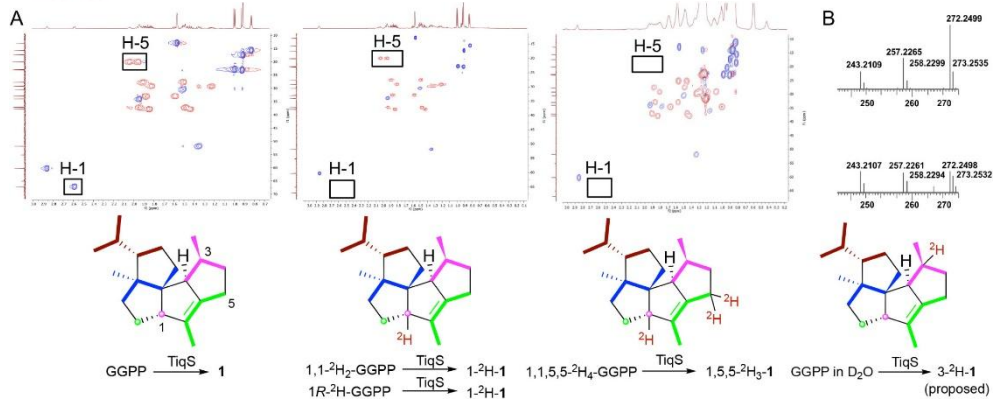

Figure 2 (above): Deuterium-labeled GGPP supports isoprene units and C-1 rearrangement. (A) Incubation of TiqS with unlabeled GGPP, 1,1-$^{2}H_2$-GGPP, 1R-^{2}H-GGPP, and 1,1,5,5-$^{2}H_4$-GGPP gave **1**, 1-^{2}H-**1**, 1-^{2}H-**1**, and 1,5,5-$^{2}H_3$-**1**, respectively, with zoomed-in ^{1}H-^{13}C HSQC spectra shown above each structure. (B) Incubation of TiqS with unlabeled GGPP in D_2O increased the molecular weight of **1** by one mass unit, supporting the proposed mechanism.