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Effect of Isotopically Sensitive Branching on Product Distribution for Pentalenene Synthase: Support for a Mechanism Predicted by Quantum Chemistry

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Supporting Information

ABSTRACT: Mechanistic proposals for the carbocation cascade reaction leading to the tricyclic sesquiterpene pentalenene are assessed in light of the results of isotopically sensitive branching experiments with the H309A mutant of pentalenene synthase. These experimental results support a mechanism for pentalenene formation involving a 7-protoilludyl cation whose intermediacy was first predicted using quantum-chemical calculations.

Pentalenene (1, Scheme 1) is a tricyclic sesquiterpene that is produced in nature from farnesyl diphosphate (FPP) through a cationic cascade reaction promoted by the enzyme pentalenene synthase. The mechanism of this transformation is one of the most highly studied among terpene-forming reactions in part because of the efficient generation of complexity that accompanies conversion of FPP—the universal acyclic, achiral precursor of all sesquiterpenes—into pentalenene, a tricyclic, chiral, stereodense product.

At least two mechanisms have been suggested for the formation of pentalenene from FPP. Path A in Scheme 1 (A → B → D → E → F) represents the earliest and until recently the most commonly accepted mechanistic proposal, involving conversion of the humulenyl cations A and B to a secoillud-6-en-3-yl cation (D) that then undergoes a 1,2-hydride shift and subsequent cyclization to produce the penultimate intermediate, the pentalenyl cation (F). The basic details of this mechanism have been supported by a wide range of experiments with stereospecifically labeled FPP and determination of the precise position and stereochemistry of isotopic labeling in the enzymatically derived pentalenene product. In 2006, Gutta and Tantillo proposed an alternative cyclization mechanism leading from B to F based on quantum-chemical calculations [mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) in the absence of the enzyme active site]. In this mechanism, the 7-protoilludyl cation (C), formed directly from B, would be a mandatory intermediate along the pathway to pentalenene (path B in Scheme 1; A → B → C → F). Although this mechanism invokes an unexpected intermediate (C) followed by an unusual dyotropic rearrangement (C → F), it is completely consistent with all of the reported mechanistic and stereochemical results on the pentalenene synthase reaction. Moreover, the predicted intermediacy of protoilludyl cation C is also consistent with the previously reported formation of the corresponding deprotonation product, Δ⁶-protoilludene (2), as a minor (10–13%) coproduct of pentalenene resulting from the cyclization of FPP by the four pentalenene synthase active-site mutants H309A, H309C, H309S, and H309F. It is also noteworthy that refluxing Δ⁷,13-protoilludene or either epimer of the 7-protoillulid...
alcohol in formic acid gives pentalenene in up to 28% yield, consistent with the intermediacy of a species such as cation C. Although the enzymatic generation of cation C by the pentalenene synthase mutants had previously been thought to result from diversion (path C) of the natural cyclization path A, the quantum-mechanical calculations would place the protoilludyl cation C directly on the natural cyclization path B. More recently, further quantum-mechanical calculations on other possible conformations of intermediate C revealed that C can be converted to F by an alternative stepwise rearrangement, illustrated as path B′ in Scheme 1 (A → B → C → D′ → E′ → F), in which D′ and E′ are geometric isomers of D and E, having Z rather than E C=C double bonds. In fact, path B′ is predicted to have a barrier of only ∼6 kcal/mol for the conversion of C to F by the lowest-energy conformer of C (at the mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) level),9 which is considerably lower than the barrier of nearly 2-fold increase in the ratio if either path B or B′ is followed because of a primary deuteration kinetic isotope effect (KIE) on the deprotonation of cation D by H309A pentalenene synthase, whether by path B or B′, would be expected to be subject to at most a small normal secondary KIE as C6 changes from sp3 toward sp2 hybridization in the transition-state structures for the C → F and C → D′ reactions (these assumptions are supported by our quantum-chemical calculations14,15). In contrast, the diversion of cation D, formed by the previously postulated path A, to give the protoilludyl cation C would be expected to be subject to only a minor secondary KIE (kD/kC, slightly less than 1), since C6 would be changing from sp2 toward sp3 hybridization. Similarly, conversion of D to E along path A should have no significant KIE since H6 is not directly involved in this step. Path A for the cyclization of FPP to pentalenene by way of cation D is therefore excluded by the observation of a decrease in the proportion of 2 due to isotopically sensitive branching of the common intermediate C toward pentalenene, resulting in a net increase in the ratio of the final products 1 and 2.

Incubation of FPP with the purified recombinant pentalenene synthase mutant H309A gave a 60:1 mixture of 1 (81%) and the coproduct 2 (13.4 ± 0.3%), accompanied by minor quantities (<6%) of germacrene A, detected as the derived Cope rearrangement product β-elemene as described previously, consistent with the results of previously reported incubations with H309 mutants.4d,e The assays were carried out in triplicate and analyzed by capillary GC–MS (Figure 1, top) with the identity of each product confirmed by comparison of both the electron-impact mass spectrum and retention index with standards in the MassFinder 4.0 database.12 When [6-2H]FPP was used as the substrate, the distribution of sesquiterpene products was significantly shifted, with the intensity of the protoilludene peak being reduced to only 7.5 ± 0.4% of the total products while the intensity of the pentalenene peak increased to 87% (Figure 1, bottom). This nearly 2-fold increase in the 1:2 ratio (11.6 vs 6.0) as a result of isotopically sensitive branching establishes that the protoilludyl cation C is a common intermediate in the pathways for formation of 1 and 2, as required by either path B or B′ but inconsistent with formation of cation C as a diversion product of path A to pentalenene (assuming that C and D do not rapidly interconvert, i.e., for path A, conversion of D to C is effectively irreversible).

The observed increase in the 1:2 ratio corresponds to a primary KIE of kD/kC = 1.9 on the deprotonation of cation C to yield 2, consistent with previously measured kD/kC values for deprotonation of tertiary carbocations in terpene synthase-promoted reactions (typically ranging from 2–6).11 Quantum-chemical calculations using H2PO4− as a model base predicted a kD/kC of 1.6–1.8.14,15 The conversion of C to F, whether by path B or B′, would be expected to be subject to at most a small normal secondary KIE as C6 changes from sp3 toward sp2 hybridization in the transition-state structures for the C → F and C → D′ reactions (these assumptions are supported by our quantum-chemical calculations14,15). In contrast, the diversion of cation D, formed by the previously postulated path A, to give the protoilludyl cation C would be expected to be subject to only a minor secondary KIE (kD/kC, slightly less than 1), since C6 would be changing from sp2 toward sp3 hybridization. Similarly, conversion of D to E along path A should have no significant KIE since H6 is not directly involved in this step. Path A for the cyclization of FPP to pentalenene by way of cation D is therefore excluded by the observation of a decrease in the proportion of 2 due to isotopically sensitive branching of the common intermediate C toward pentalenene, whether C is further converted to 1 by path B or path B′. The mechanisms proposed on the basis of quantum-chemical calculations on the enzyme-free reaction mechanism are therefore fully consistent with the experimental results described herein. Further experimentation will be necessary to distinguish between the downstream paths B and B′.16

** ASSOCIATED CONTENT **

Supporting Information

Additional details on computations, including the complete Gaussian citation, and description of enzyme assays and product identification. This material is available free of charge via the Internet at http://pubs.acs.org.
Communication

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Notes
The authors declare no competing financial interest.

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REFERENCES

(7) We have recently found that dyotropic rearrangement via a different conformer of C than described in ref 5 can occur through a transition-state structure that is ~12 kcal/mol lower in energy than the one described in ref 5, i.e., corresponding to a barrier for the dyotropic rearrangement step of slightly less than 20 kcal/mol; a full account of this work will be reported in due course.
(9) (a) A full account of the conformational potential energy surface for C and D′ will be published in due course. (b) No minimum corresponding to D has yet been located, likely because of the proximity of the carbocation center to the π bond in such structures.