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Branching Out from the Bisabolyl Cation. Unifying Mechanistic Pathways to Barbatene, Bazzanene, Chamigrene, Chamipinene, Cumacrene, Cuprenene, Dunniene, Isobazzanene, Iso-γ-bisabolene, Isochamigrene, Laurene, Microbiotene, Sesquithujene, Sesquisabinene, Thujopsene, Trichodiene, and Widdradiene Sesquiterpenes

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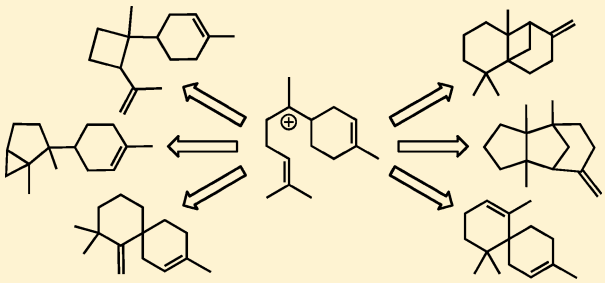
Supporting Information

ABSTRACT: Quantum chemical calculations on the transformation of the bisabolyl cation into an array of sesquiterpenes (iso-γ-bisabolene, trichodiene, cuprenene, laurene, isochamigrene, chamigrene, chamipinene, sesquithujene, sesquisabinene, microbiotene, dunniene, cumacrene, isobazzanene, bazzanene, barbatene, widdradiene, and thujopsene) are described. The bisabolyl cation is the hub of a complicated web of carbocations involved in the construction of diverse and complex molecular architectures present in a large number of Nature's sesquiterpenoids. The results of quantum chemical calculations on the multitude of rearrangements described herein provide reasonable answers to several persistent mechanistic questions in the world of terpene biosynthesis and also provide examples of general reactivity principles for terpene-forming (and other) carbocation rearrangements.



INTRODUCTION

Mechanisms for the construction of the complex hydrocarbon frameworks of terpenoids in Nature[1−4] involve carbocation rearrangements that, on the basis of recent quantum chemical calculations, often involve concerted combinations of bond-making and bond-breaking events, promoted by conformational preorganization leading to orbital alignment, and allowing secondary carbocation intermediates to be avoided.[5−10] The reaction network emanating from the bisabolyl cation (Scheme 1) provides many examples of these general principles. While we have previously described theoretical studies on the transformation of the bisabolyl cation into the acoradiene, amorphadiene, amorphene, bergamotene, bisabolene, cedrene, chamigrene, cuprenene, curcumene, duprezianene, funebrene, sesquithuriferol, trichodiene, santalene, zingiberene, and zizaene classes of sesquiterpene natural products (Scheme 1, green),[8−13] herein we describe theoretical work that expands the web of quantum chemically characterized bisabolyl cation rearrangements to those leading to the barbatene, bazzanene, chamipinene, cumacrene, dunniene, isobazzanene, laurene, microbiotene, sesquithujene, sesquisabinene, thujopsene, and widdradiene sesquiterpenes (Scheme 1, blue). These sesquiterpenes (see Supporting Information for a list of specific stereoisomers examined for each family) are often co-isolated[12−21] and some are purportedly produced in a single enzyme active site,[14,15,17,18] allowing us to probe whether or not the inherent reactivity of their carbocation precursors needs to be overridden by the sesquiterpene synthase enzymes in which they are generated. The network of reactions shown in Scheme 1 is by far the most complex network of interconnected natural product forming reactions interrogated using quantum chemistry and represents a significant portion of the $C_{15}H_{25}^{+}$ potential energy surface.[22] We present herein a unified picture of the inherent reactivity of the carbocation components comprising this network and the implications of such for their enzyme-promoted generation, management, and manipulation.

The mechanisms generally proposed for the enzyme-chaperoned formation of these sesquiterpenes from the bisabolyl cation are shown in green and black in Scheme 2.[1,3,4,14,15,18,23] Alternative pathways that have been examined previously and herein using quantum chemical calculations are shown in orange, red, violet, and blue in Scheme 2.[9−11] For simplicity, hydrocarbon skeletons are shown here without explicit stereochemistry (relative or absolute).[24]

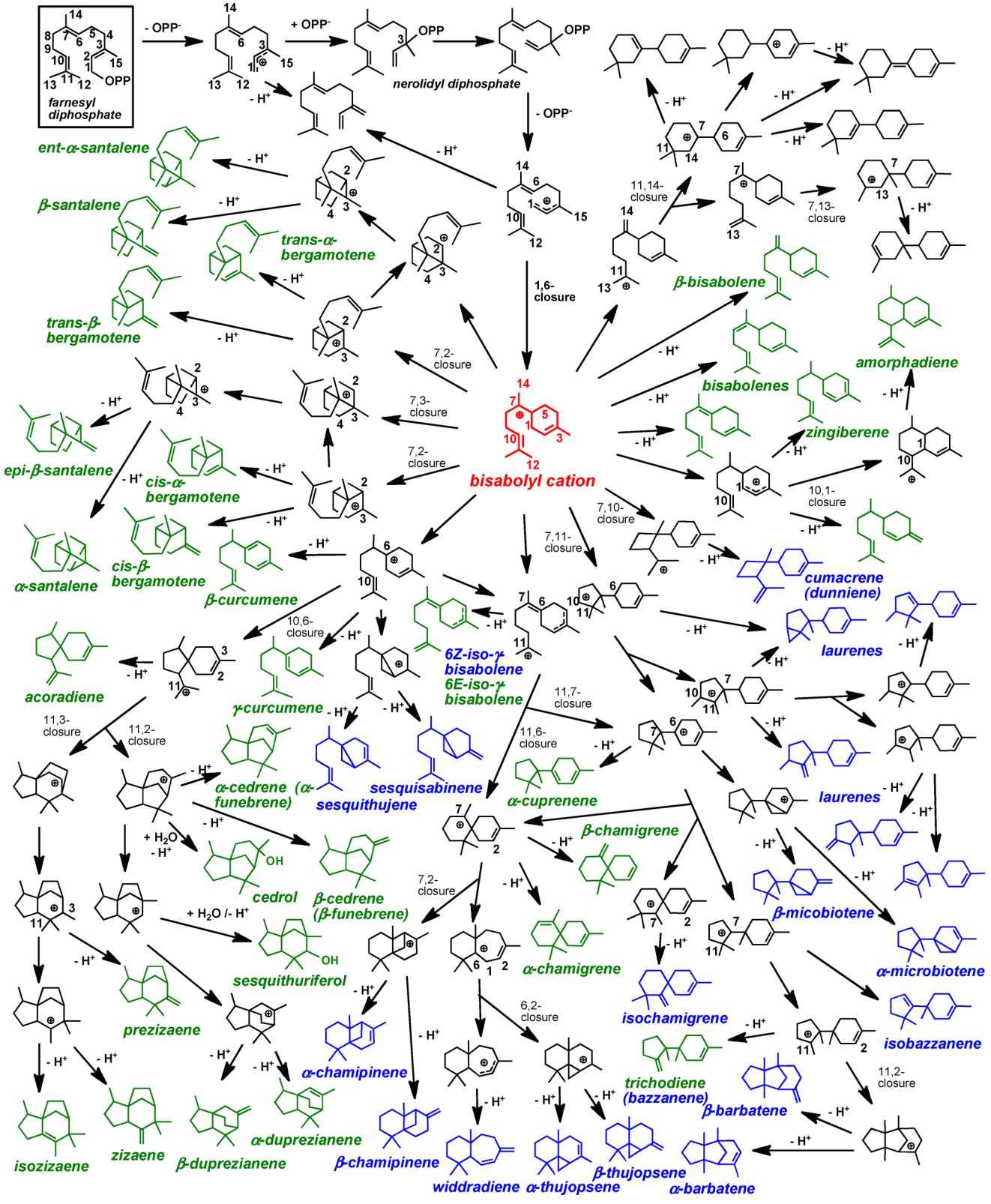
METHODS

All calculations were performed with Gaussian03.[25] Geometries were optimized using the B3LYP/6-31+G(d,p) method.[26] Stationary points were characterized by frequency calculations and reported energies

Received: October 17, 2013
Published: January 23, 2014

   dx.doi.org/10.1021/ja4106489 | J. Am. Chem. Soc. 2014, 136, 2450−2463

Scheme 1



include zero-point energy corrections (unscaled). Intrinsic reaction coordinate (IRC) calculations were used for further characterization of all transition-state structures.[27] We also include mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) energies for comparison, since it is suggested that B3LYP underestimates the relative energies of cyclic structures versus acyclic isomers.[28a] These energies include zero-point energy corrections from B3LYP/6-31+G(d,p) frequency calculations. These methods have been used previously to study other terpene-forming carbocation rearrangement reactions (and results have been compared to the results of other methods).[10] Note that intramolecular π-complexes (see Figures 1−3 and 5) may not persist as minima at other levels of theory, but this does not change our conclusions

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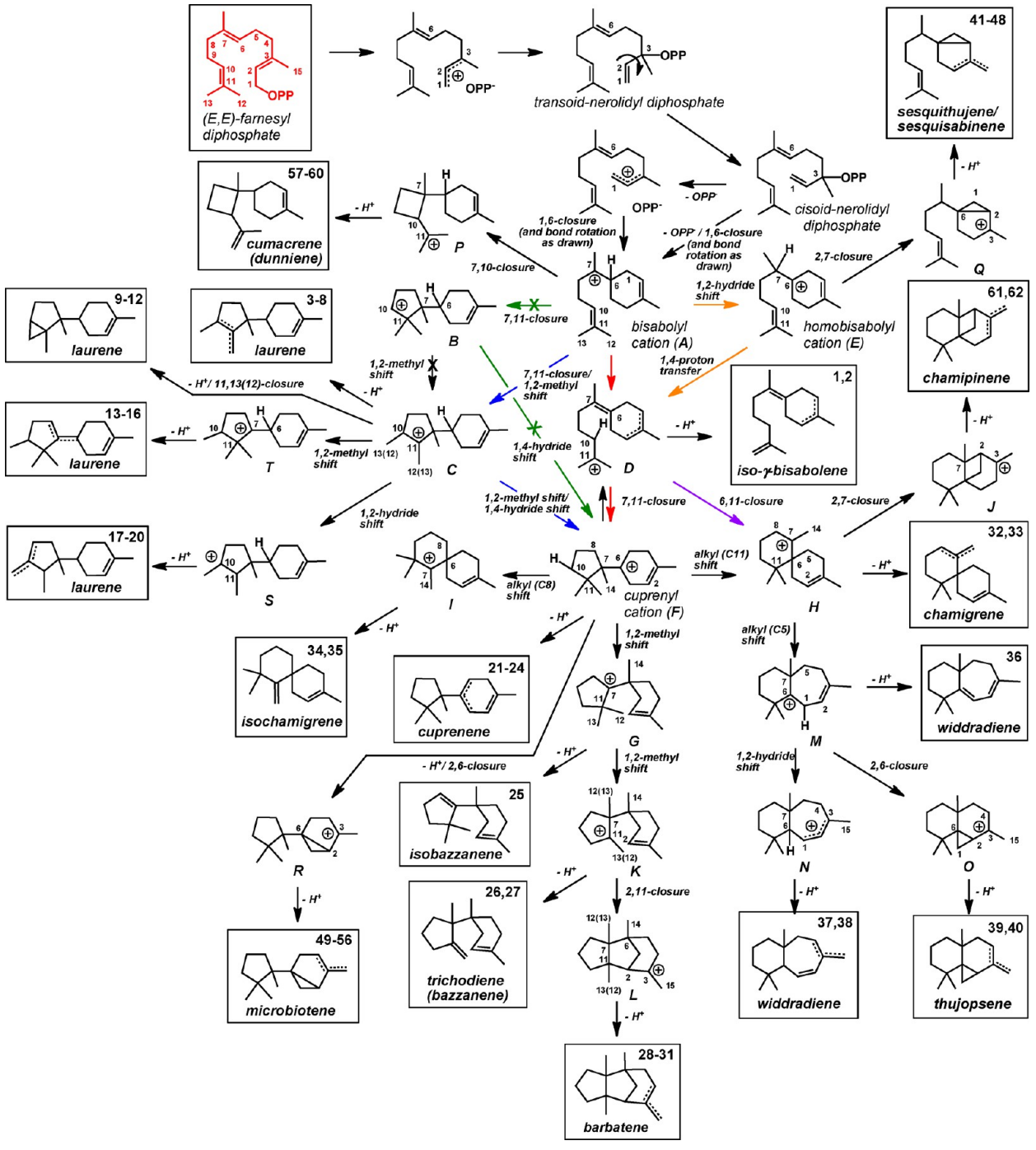
Scheme 2



significantly, as the region of the potential energy surface surrounding such structures is rather flat.[28] The computed structures in this report are all based on the (*R*)-bisabolyl cation to facilitate comparisons between this structure and the stereochemistry of the products; the mirror image reaction pathways have the same energies in the absence of an enzyme. All energies are relative to that of bisabolyl conformer **A1** (Figure 1), the cation that was utilized as a reference in our previous studies.[8−12] Structural drawings were produced using *Ball & Stick*.[29] Atom numbering indicated in the structures in this report refers to that of farnesyl diphosphate (FPP) (Scheme 1). Bold structure numbers are used to label natural products and bold capital letters are used to label carbocations. Numbers following letters for carbocations indicate different conformations or configurations.

RESULTS AND DISCUSSION

The road to Barbatene and Exits to Iso-γ-bisabolene, Cuprenene, Isobazzanene, Bazzanene, and Trichodiene. Transformation of the bisabolyl cation (**A**) to the cuprenyl cation (**F**) is a key transformation in the biosynthesis of most of the sesquiterpenes shown in Scheme 1. Previously, we examined the three routes shown in Scheme 2 (green, blue, and red).[9,11] On the basis of the results of quantum chemical calculations, we suggested that the green pathway via carbocation **B**, which was the widely accepted mechanism, is unlikely; carbocation **B** appears not to be a discrete intermediate en route to the cuprenyl cation. The related pathway shown in blue, which involves a "temporary methyl

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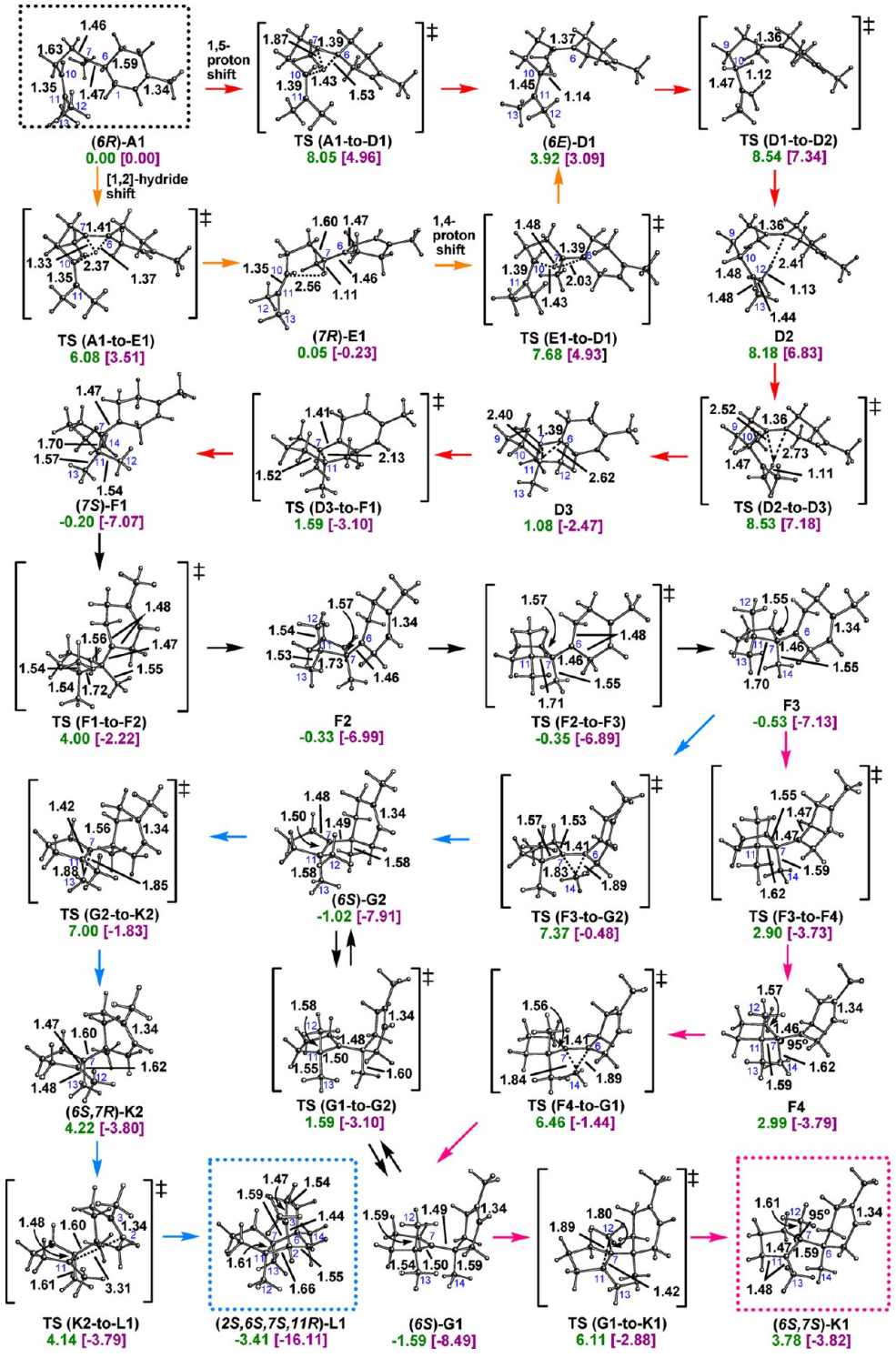

Figure 1. Conversion of bisabolyl cation conformer **A1**[11] to the cations that precede trichodiene (**K1**) and barbatene (**L1**). Computed geometries (distances in Å) and relative energies (kcal/mol) of intermediates and transition-state structures are shown: B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,p) in green and mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) in plum and brackets.[31]

shift" does connect the bisabolyl cation to the cuprenyl cation, but large barriers are involved. In contrast, the pathway shown in red, which involves proton transfer rather than hydride transfer, is predicted to involve only small barriers. The predicted barriers for the related pathway shown in orange are also small. On the basis of computed energetics, we thus favor the two pathways that involve proton transfer over those that involve hydride transfer. Structures involved in these pathways and their computed relative energies are shown at the top of Figure 1. The known sesquiterpene iso-γ-bisabolene (Scheme 2) could be readily derived from these pathways.[30]

We have also previously described the conversion of the cuprenyl cation (**F**) to trichodiene.[11] The pathway from **F** to the immediate precursor (carbocation **K1**) to trichodiene (**26**) is shown at the bottom right of Figure 1. Note that several conformational changes occur along this pathway and no large

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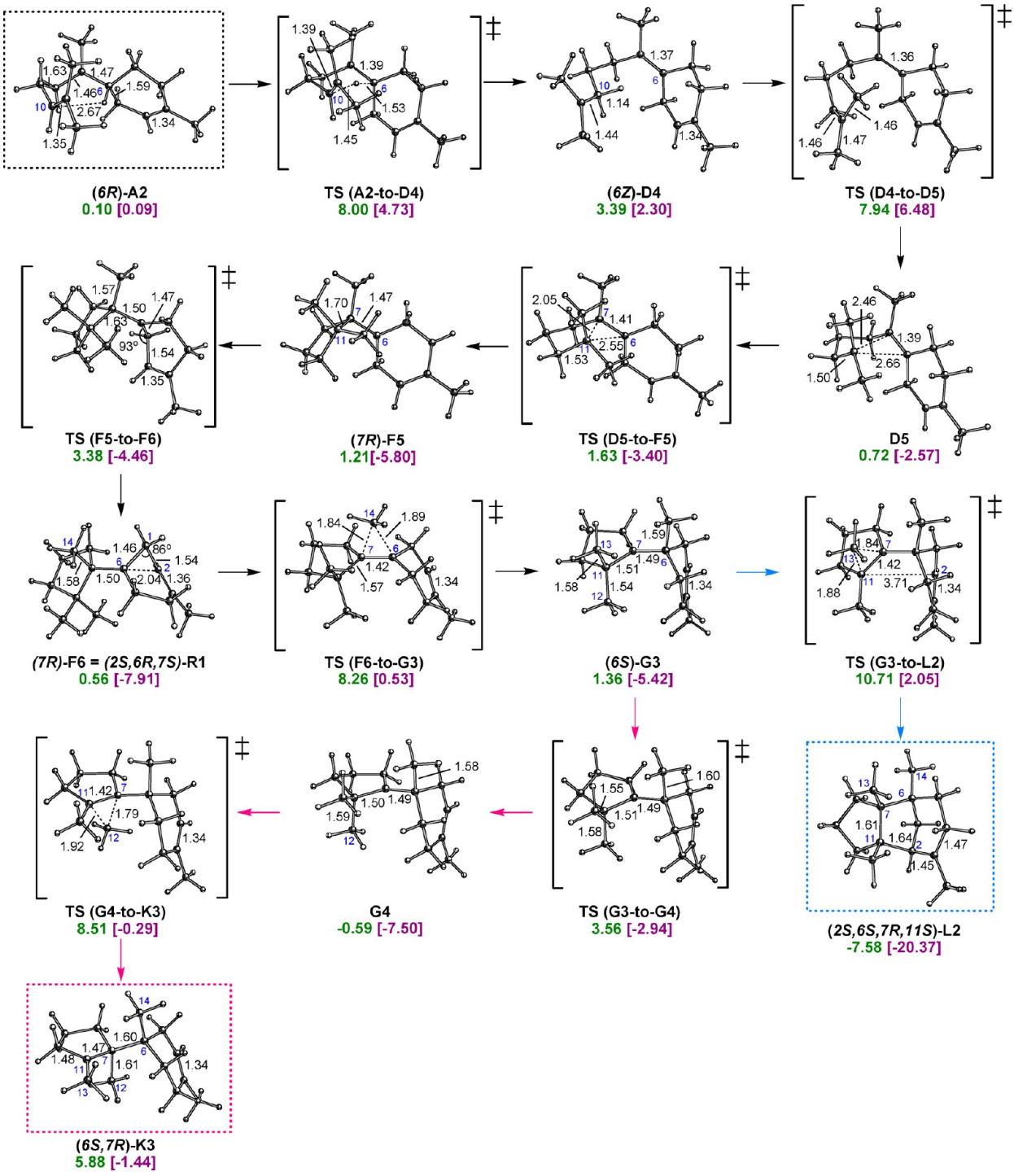

Figure 2. Conversion of bisabolyl cation conformer **A2** to the cations that precede bazzanene (**K3**) and barbatene (**L2**). The pathway shown involves Z isomer of carbocation **D** and R configuration of the cuprenyl cation (**F**). Computed geometries (distances in Å) and relative energies (kcal/mol) of intermediates and transition-state structures are shown: B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,p) in green and mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) in plum and brackets.

barriers are encountered. Note also that deprotonation of the cuprenyl cation (**F**) would produce the cuprenenes (**21** and **23**) and deprotonation of cation **G** would produce isobazzanene (**25**).

Conformer **G2** (Figure 1) can undergo a conformational change, followed by a [1,2]-methyl shift en route to trichodiene, but it can also undergo a [1,2]-methyl shift directly, without a preceding conformational change, that leads to carbocation **K2**. **K2** is a diastereomer of **K1**, differing in the relative stereochemistry of the two stereogenic centers that bear methyl groups (C6 and C7). While deprotonation of cation **K2** would produce bazzanene (**27**), a diastereomer of trichodiene, attack of the cyclohexene π-bond onto the carbocation center (C11) of **K2** (which is predicted to be a barrierless process) leads to carbocation **L**, the immediate precursor to the barbatenes (**28** and **29**). Thus, it is at carbocation **G** that the

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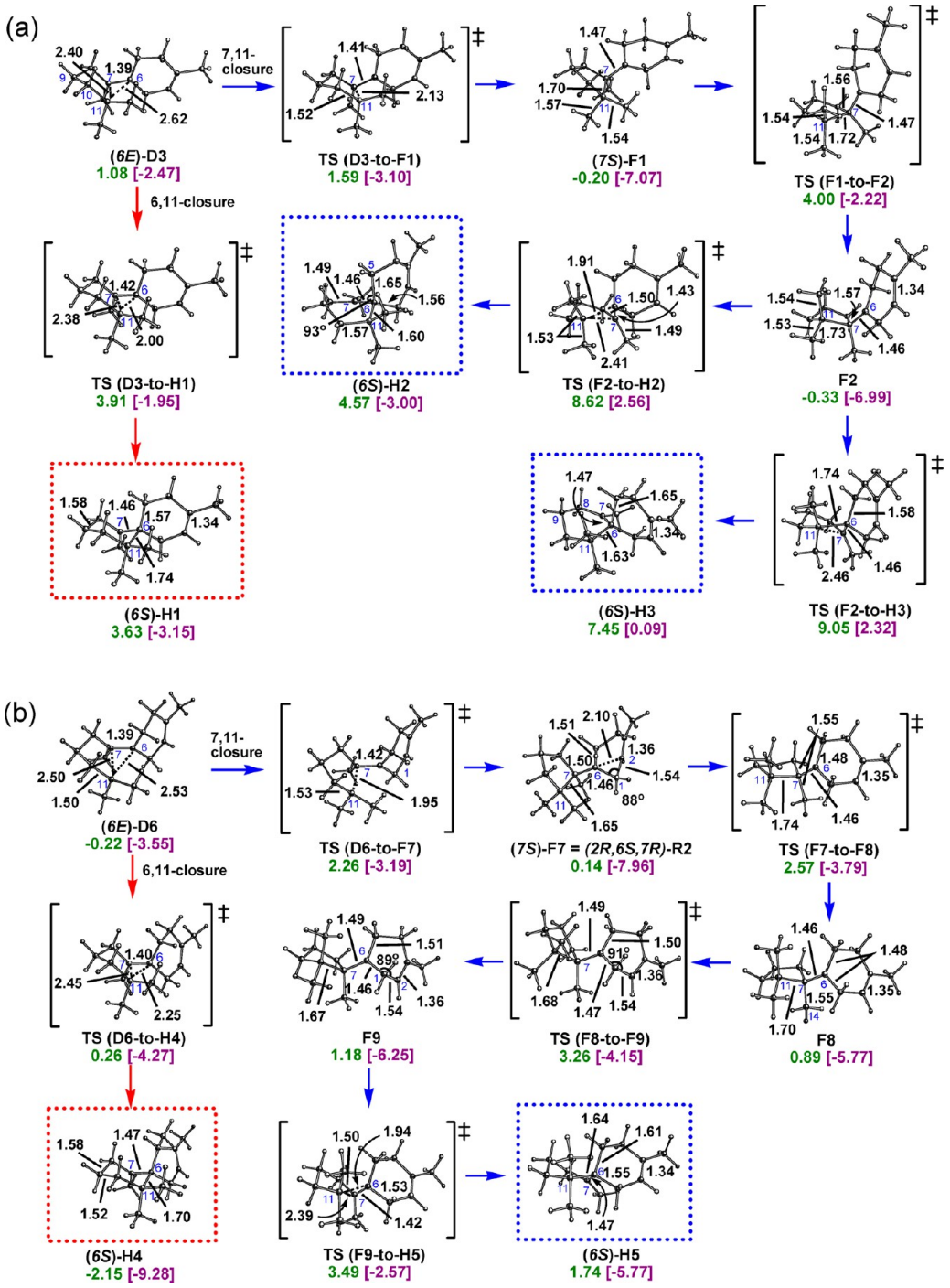

Figure 3. Possible mechanisms for formation of chamigryl cations starting with two different conformers of cation **D** (**D3** in (a) and **D6** in (b)). The pathways shown in (a) and (b) involve different conformers of the cuprenyl cation (**F**). Computed geometries (distances in Å) and energies (kcal/mol) of intermediates and transition-state structures are shown: B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,) in green and mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) in plum and brackets.

pathway diverges toward trichodiene or the barbatenes. Note, however, that if cuprenyl cation conformer **F3** undergoes two methyl shifts *without first changing its conformation*, the barbatenes will be formed. Conversely, if a conformational change occurs for **F3** to form **F4** or for **G2** to form **G1**, trichodiene will be formed instead. Thus, an enzyme that promotes formation of cuprenyl conformer **F3** and whose active site is not large enough or flexible enough to allow for significant conformational changes, could form barbatene to the exclusion of trichodiene.[32] Note, however, that productive bisabolyl cation conformer **A1** cannot be formed directly from NPP (nerolidyl diphosphate). Direct cyclization of NPP would lead to a conformation where the C1−C6 bond is aligned with the formally empty p-orbital at the cationic center. In **A1**, the C5−C6 bond is aligned instead, suggesting that a conformational change (rotation of the cyclohexene ring about C6−C7 bond) after the cyclization of NPP is required, whether or not subsequent conformational changes occur.[33]

If bisabolyl cation conformer **A2** instead of **A1** is formed, similar rearrangements can occur, but with several important

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differences (Figure 2). When starting with **A2**, only a single conformational change of cation **D** is required to prepare for the subsequent cyclization step. In addition, the 6*Z* isomer of iso-γ-bisabolene (**2**) and several cuprenene stereoisomers could be derived from this pathway. Note also that cation **G3** is connected directly to cation **L2**, a diastereomer of **L1** (Figure 1). The direct conversion of **G3** to **L2** differs from the **G2** → **K2** → **L1** conversion described above since it involves a concerted process comprised of two events ([1,2]-methyl shift and cyclization) occurring in an asynchronous manner;[5,6,34] i.e., cation **K** is not a discrete minimum on this route to the barbatenes. Deprotonation of **L2** leads to some barbatene diastereomers that, to our knowledge, have not yet been found in Nature. If cation **G3** can undergo a conformation change to form **G4**, subsequent [1,2]-methyl shift can lead to bazzanene (**27**).

Formation of Laurane Sesquiterpenes. Although the "temporary methyl shift" pathway for formation of the cuprenyl cation (**F**) was found to have relatively high barriers (~20−25 kcal/mol) for both the **A**-to-**C** and **C**-to-**F** conversions (Scheme 2; note that structures resembling carbocation **B** appear along the reaction coordinates for these conversions),[9,11] carbocation **C** could be a precursor to the laurane family of sesquiterpenes, many of which have been isolated from marine red alga *Laurencia* species.[35] We have now examined the production of a variety of conformers of carbocation **C** from a variety of conformers of the bisabolyl cation (**A**; see Supporting Information for details), but all of these pathways are predicted to involve barriers of >20 kcal/mol for formation of **C**. Thus, unless these terpenes are produced by a non-carbocation route, enzymatic intervention in the form of both barrier lowering to allow carbocation **C** to form and suppression of competing proton transfer pathways would seem necessary.

Formation of Chamigrenes. Chamigrenes (Scheme 2) are frequently co-isolated with cuprenenes[14,21] and trichodiene[36] from various terrestrial species. Halogenated chamigrene derivatives have also been isolated along with laurane sequiterpenes from marine red alga *Laurencia* species.[20,37] The generally proposed mechanism for the formation of chamigrene involves ring expansion of the cuprenyl cation (Scheme 2, **F** → **H**).[23] The resultant chamigryl cation (**H**) is also generally invoked in pathways to thujopsene[14,15] and widdradiene (Scheme 2; *vide infra*).[19]

Conformer **F2** (Figure 1) is productive for ring expansion to form **H**. We located two subtly different transition-state structures for ring expansion of **F2** to form two different conformers of **H**, **TS (F2-to-H2)** and **TS (F2-to-H3)** (Figure 3a, right). In both transition-state structures, the forming 6-membered ring has a boatlike conformation. Both transition-state structures are approximately 9−10 kcal/mol higher in energy than **F2** (Figure 3a). Figure 3b shows a ring expansion reaction involving another conformer of **F**, **F8**. The transition-state structure for this ring expansion reaction, which also has a boatlike conformation of the forming ring, is several kcal/mol lower in energy than those connected directly to **F2**. Clearly, strict conformational preorganization is not necessary to access the chamigryl cation (**H**).

In each of the conformers of cation **H** that result from the ring expansion reactions shown in Figure 3, the C5−C6 σ-bond is elongated due to strong hyperconjugation with the cationic center (C8).[38] Consequently, those structures are inherently predisposed toward the subsequent alkyl shift that leads to thujopsene (**39** and **40**) and the widdradienes (**36**−**38**; *vide infra*). Direct deprotonation of conformers of **H** will generate (7*R*)-α-chamigrene (**32**) and (7*R*)-β-chamigrene (**33**).

We also located analogous transition-state structures involving chairlike conformations of the forming 6-membered ring: **TS (D3-to-H1)** in Figure 3a and **TS (D6-to-H4)** in Figure 3b. IRC calculations on these transition-state structures indicated, however, that they actually connect conformers of cation **H** to conformers of cation **D**, the cation that resulted from intramolecular proton transfer of the bisabolyl or homobisabolyl cations (see Scheme 2 and Figure 1). Thus, from some conformations of cation **D**, the chamigryl cation can be accessed directly, without the intermediacy of cuprenyl cation minima.[1] Both pathways with and without cuprenyl cations are accessible energetically (see Figure 4), but the former would require significant conformational changes before ring expansion.[39]

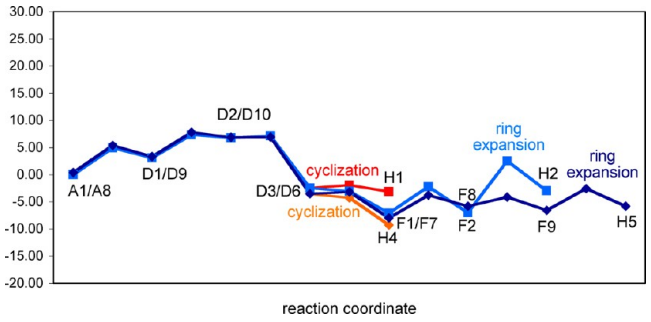

Figure 4. Overall energetics (only mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) values are shown) for the rearrangement of the bisabolyl cation to various conformers of the chamigryl cation via 6,11-cyclization of cation **D** (Scheme 2: **D** → **H**) and via ring expansion of the cuprenyl cation (Scheme 2: **F** → **H**).[40]

Formation of Chamipinene. The proposed mechanism for chamipinene formation involves a ring closure of the spirocyclic chamigryl cation (Scheme 2: **H** → **J**).[19] None of the conformers of cation **H** described above is productive for the expected 2,7-closure to form the chamipinyl cation (**J**), however.[41] We were able to find a productive conformation of the chamigryl cation (**H6**) for the expected ring closure (Figure 5), but the mechanism of its formation from **A** via **D** is predicted to be more complicated than expected. 1,5-Proton transfer converts bisabolyl cation conformer **A3** to cation **D7** (Figure 5), and we expected straightforward conformational changes to convert this cation to a conformer prepared for subsequent ring closure. Instead, the transition-state structure that appears to correspond to a simple conformational change was found to be connected to carbocation **U** via a 1,7-hydride shift (Figure 5; **TS (D7-to-U)**), a very exothermic step. Another transition-state structure (Figure 5; **TS (U-to-D8)**) connects **U** to the productive conformer of cation **D** (Figure 5; **D8**) via a 1,7-hydride shift in the reverse direction, a very endothermic step. This "temporary hydride shift" is reminiscent of the "temporary methyl shift" encountered in our previous studies on trichodiene formation (blue pathway in Scheme 2).[9] Ring closure converts cation **D8** to chamigryl cation conformer **H6** (Figure 5; **TS (D8-to-H6)**).[42] Attack of the cyclohexene π-bond onto the carbocation center (C7) of **H6** leads to carbocation **J**, the immediate precursor to the chamipinenes, through a cyclization that is predicted to be barrierless. In the structure of **J**, the C2−C7 bond is very elongated due to strong

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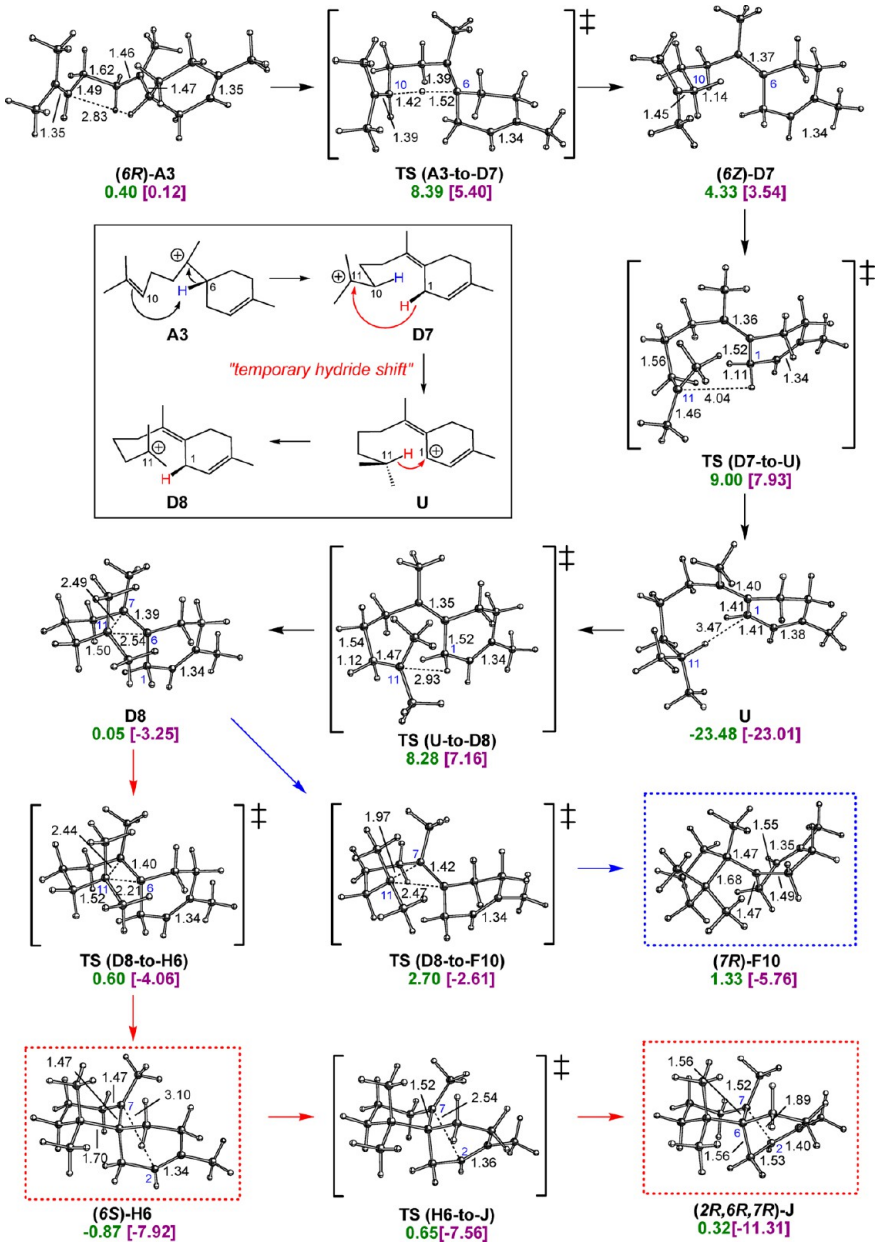

Figure 5. An alternative route to cuprenyl cation (**F**), the chamigryl cation (**H**) and extended route to chamipinyl cation (**J**) that precedes (2*R*,6*R*,7*R*)-$\Delta^{1,6}$-chamipinene (**61**) and (2*R*,6*R*,7*R*)-$\Delta^{3,4}$-chamipinene (**62**) starting with bisabolyl cation conformer **A3**. Computed geometries (distances in Å) and energies (kcal/mol) of intermediates and transition-state structures are shown: B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,p) in green and mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) in plum and brackets.

hyperconjugation with the formal cation at C3, similar to other cyclobutylcarbinyl cations encountered in previous studies on pinenes,[43] bergamotenes,[13] and ylangenes.[7] Note that cation **U** resides in a deep energy well with a barrier of approximately 30 kcal/mol for escape to **D8**. Thus, to form **D8** an enzyme would likely need to suppress the 1,7-hydride transfer leading to **U**. Mechanisms for avoiding such deep energy wells through noncovalent interactions with active site residues and dynamical behavior are under investigation and will be reported in due course.

Formation of Thujopsene and Widdradiene Sesquiterpenes. Pathways typically proposed for formation of thujopsene and widdradiene sesquiterpenes involve ring expansion of the chamigryl cation **H** (Scheme 2).[15,19] Computed pathways from several conformers of cation **H** to cations that precede various diastereomers of widdradienes are shown in Figure 6. Chamigryl cation conformer **H1** (Figure 3a) can be converted to widdraenyl cation **M1** via a ring expansion reaction that does not require a preceding conformational change and has a low predicted barrier (Figure 6, top). Alternatively, chamigryl cation conformer **H2** (Figure 3a) can be converted to widdraenyl cation **M2** with a comparable barrier (Figure 6, bottom). **M1** and **M2** can interconvert via a low barrier conformational change (Figure 6, center). A [1,2]-hydride shift, again with a low barrier, converts **M2** to **N**. **M1** or **M2** could be deprotonated to form widdradiene isomer **36**, while **N** could be deprotonated to form widdradiene isomers **37** and **38**.[44]

Although we located a transition-state structure that looked like it should connect **M1** to a different conformer of **N**, IRC

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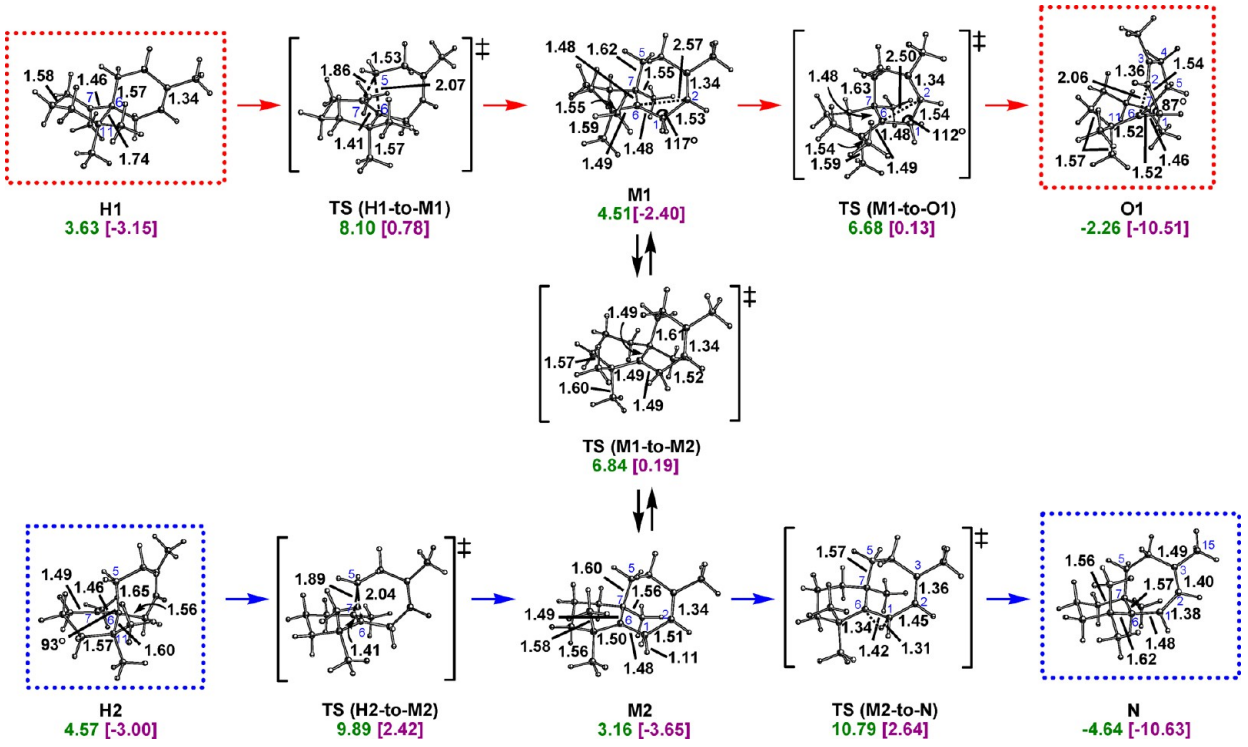


Figure 6. Conversion of conformers of the chamigryl cation (**H**) to the cations that precede (7*S*)-$\Delta^{1,6}$-widdradiene (**36**), (6*S*,7*S*)-$\Delta^{3,4}$-widdradiene (**37**), (6*S*,7*S*)-$\Delta^{3,15}$-widdradiene (**38**), and (2*S*,6*S*,7*S*)-thujopsenes (**39** and **40**). Computed geometries (distances in Å) and energies (kcal/mol) of intermediates and transition-state structures are shown: B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,p) in green and mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) in plum and brackets.[44]

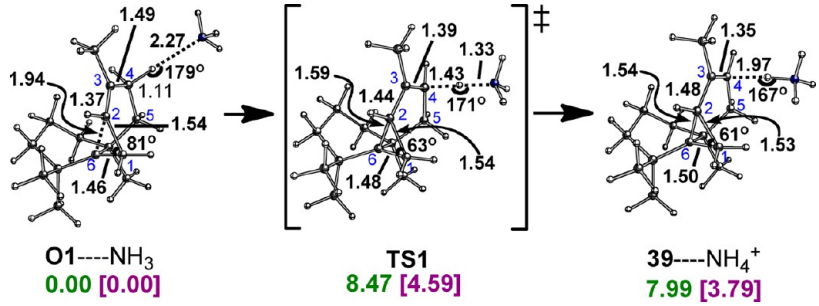


Figure 7. Model of deprotonation of **O1** to form α-thujopsene (**39**).

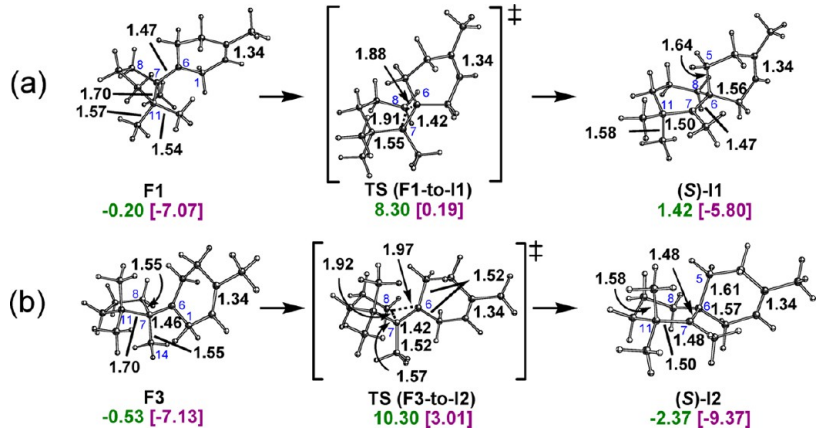


Figure 8. Ring expansion of the cuprenyl cation to the cation that precedes (*S*)-isochamigrene (**34**). Two transition structures, involving (a) a boatlike conformation and (b) a chairlike conformation of the forming 6-membered ring, are shown. Computed geometries (distances in Å) and energies (kcal/mol) of intermediates and transition-state structures are shown: B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,p) in green and mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) in plum and brackets.

dx.doi.org/10.1021/ja4106489 | *J. Am. Chem. Soc.* 2014, 136, 2450−2463

calculations indicated that this transition-state structure actually connects **M1** to thujopsyl cation **O1**. Cation **O1** does not have a full-fledged C2−C6 σ-bond (2.06 Å), but the C2−C1−C6 angle is small (87°) and the C2−C3 distance (1.36 Å) is slightly longer than would be expected for a simple C=C π-bond. Similar hybrids of homoallylic and cyclopropylcarbinyl cations have been found for terpene-forming reactions.[11,45−47] To confirm that deprotonation at C4 of **O1** can indeed lead to C2−C6 ring closure, we examined proton transfer to ammonia (a simple model base used in several previous studies[7,8,43,46,48,61e]). Complexation of cation **O1** by ammonia shortens the C2−C6 distance only slightly (to 1.94 Å), but the C2−C6 σ-bond forms fully (1.54 Å) upon proton transfer from C4 to ammonia (Figure 7). We have observed similar coupled deprotonation/ring closure reactions in studies of other terpenes.[7,8,43,46,48]

Formation of Isochamigrenes. Isochamigrene, which is produced as a byproduct by trichodiene synthase,[18] possesses a spirobicyclic structure distinct from that of the chamigrenes (Scheme 2). In principle, isochamigrene could be formed via an alternative mode of cuprenyl cation ring expansion (C8 rather than C11 shift; Scheme 2).[18] Conformer **F1** of the cuprenyl cation (see Figures 1 and 3a) is productive for the ring expansion leading to isochamigryl cation **I1** via a boatlike transition-state structure, a process that is associated with a predicted barrier of less than 10 kcal/mol (Figure 8a), similar to the barriers computed for the alternative [1,2]-alkyl shifts leading to the chamigryl cation (Figure 3a). Cuprenyl cation conformer **F3** (Figure 1) also is able to undergo ring expansion to isochamigryl cation conformer **I2** (Figure 8b), here via a chairlike transition-state structure. The chairlike transition-state structure is slightly higher in energy than the boat-like transition-state structure due to repulsive interactions between the cyclohexene ring and the forming 6-membered ring.[49]

In the rearrangements just described, the (*S*)-cuprenyl cation is converted to the (*S*)-isochamigryl cation. Interestingly, the (*R*)-isochamigryl cation could also be formed from the (*S*)-cuprenyl cation if the 5- and 6-membered rings in this cation first rotate relative to each other by ∼180°. This would allow C8 to migrate to the other face of the cationic center (C6). This conformational change (see Supporting Information for structures) involves an overall barrier that is comparable to those for ring expansion. Thus, access to either enantiomer of isochamigrene is possible from a single enantiomer of the cuprenyl cation—provided that the active site in which these cations are generated can accommodate the necessary conformational change.[50] This stereochemical flexibility could have some evolutionary utility. Of course, the absolute configuration of the isochamigrene produced could be controlled by restricting the conformational flexibility of the intermediates generated.

Formation of Sesquithujene and Sesquisabinene. The generally proposed mechanism for the formation of sesquithujenes and sesquisabinenes involves ring closure of the homobisabolyl cation (Scheme 2, **E** → **Q**). Ring closure instead of proton transfer (Scheme 2, **E** → **D**) can convert the homobisabolyl cation conformer **E1** (Figure 1) to cation **Q** (Scheme 2 and Figure 9). The transition-state structure for this conversion looked like a transition-state structure for a conformational change, but IRC calculations indicated that this conformational change is coupled to ring closure. Note that the C7−C8 bond in the transition-state structure (Figure 9; **TS (E1-to-Q1)**) is shorter (1.55 Å) than that in **E1** (1.60 Å),

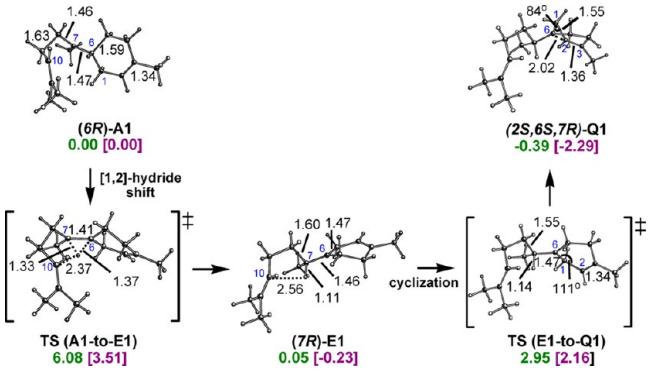


Figure 9. Two-step conversion of the conformer of the bisabolyl cation **A1** via "interior" face hydride shift to the carbocation **Q** that precedes isomers of sesquithujene (**41**) and sesquisabinene (**42**). Computed geometries (distances in Å) and relative energies (kcal/mol) of intermediates and transition-state structures are shown: B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,p) in green and mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) in plum and brackets.

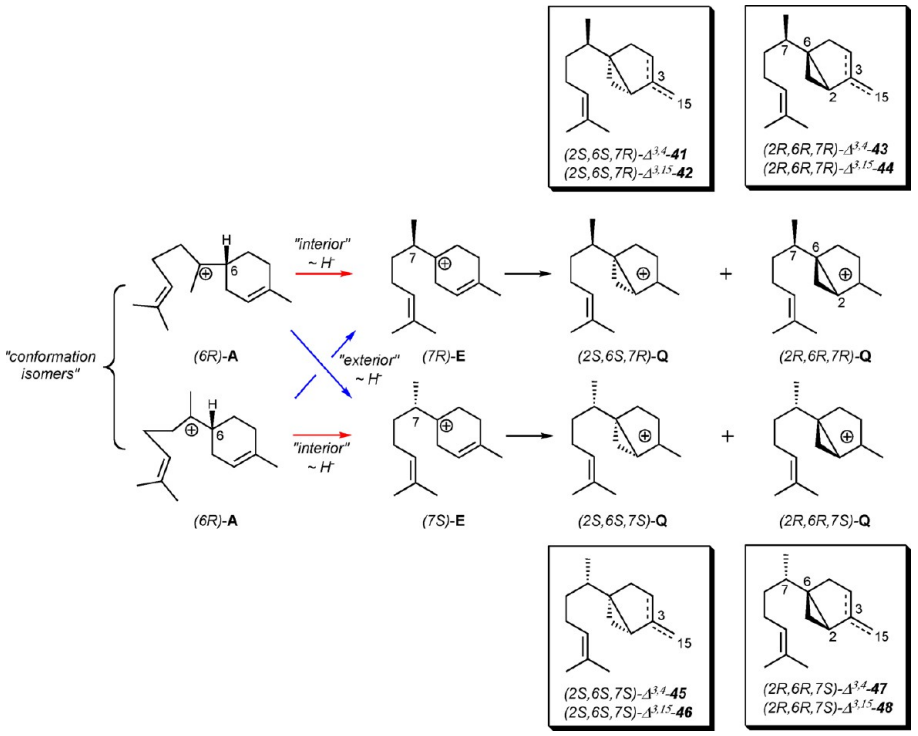
indicating a loss of hyperconjugation with the cationic center (C6) upon rotation of the ring. The C2−C6 distance (2.02 Å), C2−C3 distance (1.36 Å), and C2−C1−C6 angle (84°) in **Q1** again correspond to a homallyl/cyclopropylcarbinyl hybrid structure. Deprotonation of **Q1** with concomitant C2−C6 shortening could generate sesquithujene and sesquisabinene. Related pathways for other conformers of cation **Q**, some which involve formation of **Q** without formation of **E** as a discrete intermediate, are discussed in the Supporting Information. These pathways allow for formation of four epimeric series of sesquithujenes and sesquisabinenes from the same bisabolyl cation stereoisomer (Scheme 3), with the mechanism to form these sesquiterpenes and their stereochemical configurations depending on the conformation and the mode of hydride shift (along "interior" or "outer" face of the bisabolyl cation).

Formation of Microbiotenes. Ring closure of the cuprenyl cation (Scheme 2, **F** → **R**) and deprotonation of the resultant microbiotyl cation **R** could lead to the microbiotenes. We found that isobisabolyl cation conformer **D6** (derived from bisabolyl cation **A8**)[40] is preorganized to undergo a concerted reaction involving two cyclization events that connect **D6** directly to cation **R** (Figure 10). The **D6**-to-**R2** reaction is predicted to have a negligible barrier.[51] Note that cation **R** (equivalent to a delocalized version of cation **F**) is another example of a homoallyl/cyclopropylcarbinyl hybrid (see Figures 6 and 9 for others). Concomitant deprotonation and C2−C6 bond shortening would generate microbiotene isomers **49** and **50** (depending on which proton is removed; see Supporting Information for model deprotonation reactions).

Formation of Dunniene and Cumacrene. The cyclobutane-containing dunniene and cumacrene sesquiterpenes share the same connectivity but have a diastereomeric relationship (Figure 11).[19,52] The formation of these sesquiterpenes is proposed to involve cyclization of the bisabolyl cation (Scheme 2, **A** → **P**).[52] Despite various attempts, we were unable to locate the cyclobutane-containing cation **P** nor a transition-state structure appearing to connect the bisabolyl cation to **P**. Consequently, we suggest that cation **P** is likely not a minimum in the absence of an enzyme. To

dx.doi.org/10.1021/ja4106489 | J. Am. Chem. Soc. 2014, 136, 2450−2463

Scheme 3



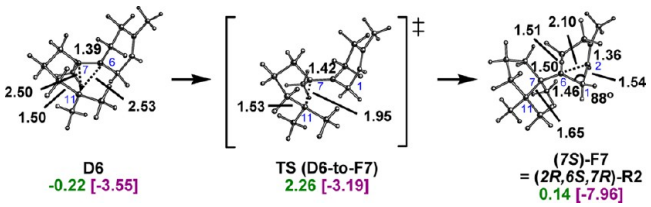


Figure 10. Conversion of the conformer of the homobisabolyl cation **D** to the carbocation **R** (equivalent to a delocalized version of cation **F**) that precedes the microbiotenes (**49**, **50**). Computed geometries (distances in Å) and relative energies (kcal/mol) of intermediates and transition-state structures are shown: B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,p) in green and mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) in plum and brackets.

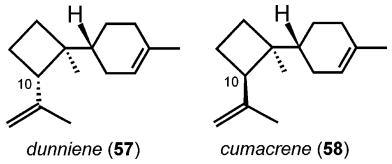


Figure 11. Structures of dunniene and cumacrene.

determine whether or not cation **P** might exist as a minimum in the presence of active site residues, we examined complexes that might involve stabilizing intermolecular C−H···lone pair interactions.[53] Previously we observed that appropriate positioning of lone pair donors can induce dramatic changes to geometries of carbocations and transition-state structures connected to them.[8,54,62e] We were indeed able to locate minima when ammonia (a simple model lone pair donor[7,8,43,46,48]) interacts with isopropyl hydrogens of various conformers and diastereomers of cation **P** (Figure 12),[8,54,55] although the C7−C10 distances in these complexes are again quite long. Note that the C−H bond involved in the C−H···NH_3 interaction is *anti* to the incipient p-orbital at C11 and the forming C7−C10 bond; i.e., all of these are aligned for hyperconjugation, with the isopropyl C−H bond and C7−C10 bond competing for the carbocation center at C11. As shown in Figure 12, proton transfer to the ammonia (via low-energy transition-state structures) leads to dunniene and cumacrene sesquiterpenes with much shorter C7−C10 bonds. These results support the notion that the relative stereochemistry of the sesquiterpenes produced depends on the conformation(s) of the bisabolyl cation that can be realized in an enzyme active site.

IMPLICATIONS

Relevance of Intermediate D. Previously we argued that carbocation **D** is the likely precursor to the cuprenyl cation (**F**), rather than the usually proposed cation **B**.[3,9,56] We also noted that this cation could be the precursor to iso-γ-bisabolene, which previously had been proposed to arise via fragmentation of the cuprenyl cation.[9,19] Our proposal was supported by Dickschat and co-workers' co-isolation of iso-γ-bisabolene and trichodiene.[57] We now also propose that cation **D** can rearrange directly to the chamigryl cation **H**, avoiding a cuprenyl cation (**F**) minimum, provided it can adopt an appropriate conformation. Thus, we suggest that the chamigrenes could form without the formation of a discrete cuprenyl cation precursor.

Relevance of Intermediate C. To our knowledge, carbon skeletons with the stereochemistry of the cation **C** structure that we examined previously as a potential precursor to trichodiene[56,58] are not present in sesquiterpene natural products isolated to date. Nonetheless, a different stereoisomer of cation **C** can readily rearrange to cations that are the likely precursors to all of the known sesquiterpenes with laurane skeletons.[20,59] Unlike most terpene-forming carbocation rearrangements examined to date using quantum chemical

dx.doi.org/10.1021/ja4106489 | J. Am. Chem. Soc. 2014, 136, 2450−2463

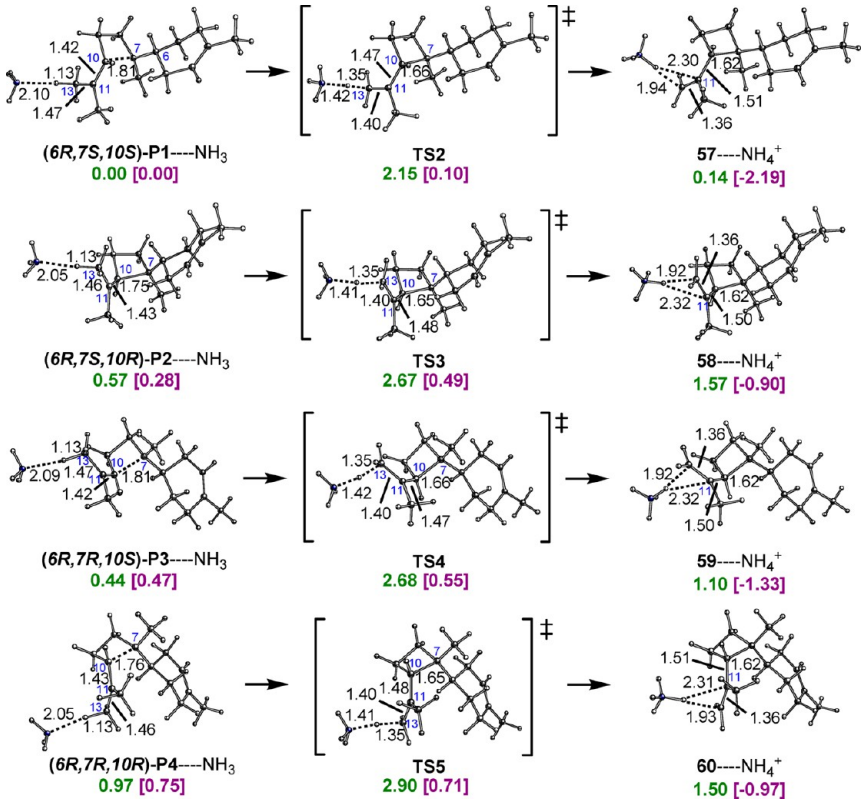


Figure 12. Models for the formation of cumacrenes and dunnienes (**57**−**60**). Computed relative energies are shown in kcal/mol by B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,p) and mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) in green and plum in brackets, respectively.

calculations,[10] formation of **C** would require enzymatic intervention in terms of barrier lowering (rather than just conformational preorganization, shielding carbocations from solvent, and control of deprotonation).

Stereochemistry of Isochamigrene. Although it is generally suggested that (*R*)-isochamigrene is derived from the (*R*)-bisabolyl cation,[60] on the basis of our computed results, we suggest that both (*S*)- and (*R*)-isochamigrene can be produced from the (*R*)-bisabolyl cation and (*S*)-isochamigrene may even be more likely (Figure 8). The conversion of either enantiomer of the bisabolyl cation to either enantiomer of the cuprenyl cation appears to be energetically viable, as is the conversion of either enantiomer of the cuprenyl cation to either enantiomer of the isochamigryl cation. As a result, knowing the absolute configuration of the end product does not allow one to know the absolute stereochemistry of the precursor cations. Which stereoisomeric cation precursors are involved will depend on the nature of the active site of the enzyme in question—is it large enough or flexible enough for certain conformational changes to occur within?—and the reaction dynamics that it allows.

Long "Bonds" and Short "Nonbonds". We describe herein several examples of cyclopropylcarbinyl and cyclobutylcarbinyl cations with exceedingly long (>1.8 Å) C−C bonds. While not nonclassical in terms of bridging/hypercoordination,[12,45,61] these structures stretch usual conceptions of hyperconjugation[38] and cation···π interactions[62] in that the C−C bonds in question are longer than expected for the former, but shorter than expected for the latter, providing further support for the concept of a "carbocation continuum"[63] rather than restrictive definitions of different classes of carbocations involving σ−p and π−p interactions.

Complexity from Simplicity. While a complex network of carbocation conformers (precursors to sesquiterpenes not derived from the bisabolyl cation) was examined in detail previously,[64] the network described herein involves a large number of sesquiterpene outputs with different carbon skeletons, each structurally complex (polycyclic, stereodense), but all arising from a single simple network node, the bisabolyl cation. The results described herein showcase many unexpected interconnections between intervening nodes, the absence of several expected nodes, means for manipulating passage through this network via noncovalent interactions, and means for diverting intermediates toward particular exit channels via positioning of a base for selective deprotonation. The structural and energetic details now available for this portion of the $C_{15}H_{25}^+$ potential energy surface set the stage for future studies on the influence of inherent dynamical effects[65] and collections of active site residues.[66]

■ ASSOCIATED CONTENT

Supporting Information

Coordinates and energies for all computed structures, along with complete ref 25. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We gratefully acknowledge support from UC Davis and the National Science Foundation (CHE-0449845, CHE-0957416, and CHE030089 for supercomputing resources), and we thank Dr. Mike Lodewyk for helpful comments.

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(31) Details of a closely related pathway involving slightly different conformers can be found in the Supporting Information.

(32) An alternative pathway to trichodiene involving other conformers is also possible; see Supporting Information for details.

(33) Previously, we suggested that conformational adjustments of the bisabolyl cation are necessary to form certain sesquiterpenes. See ref 8.

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(39) Formation of chamigryl cations starting with the *Z* isomer of cation **D** were also examined. See Supporting Information for details.

(40) The pathway starting with the **A8** conformer of the bisabolyl cation, which is closely related to the pathway from **A1** (Figure 1), also leads to trichodiene and barbatene. See Supporting Information.

(41) We found that the chamigryl cation **H4** (Figure 3b) is productive for of 3,7-closure. We are examining the rearrangements of the chamigryl cation to other sesquiterpenes, and these results will be reported in due course.

(42) Ring closure of cation **D** leads to form cuprenyl cation with *R* configuration. The *Z* isomer of iso-γ-bisabolene (**2**) and *S* stereoisomers of the cuprenenes (**22** and **24**) could be readily derived from this pathway.

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(47) We were unable to locate any localized tertiary cations with fully formed C2−C6 σ-bonds, but we did locate a conformer with a somewhat shorter C2−C6 distance (1.98 Å vs 2.06 Å in **O1**) and smaller C2−C1−C6 angle (83°). See Supporting Information for details. We also found a route to a conformer of cation **O** that bypasses cation **M**. Chamigryl cation conformer **H6**, which can arise from a conformational change of **H3** (Figure 3a), undergoes a concerted but asynchronous (see refs 5, 6, and 34) ring expansion/cyclization reaction (based on IRC calculations) that connects **H6** directly to **O3** (see Supporting Information for details), but the transition-state structure for the **H6**-to-**O3** reaction is higher in energy than those involved in the alternative stepwise pathways.

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