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Role of Carbocation's Flexibility in Sesquiterpene Biosynthesis: Computational Study of the Formation Mechanism of Terrecyclene

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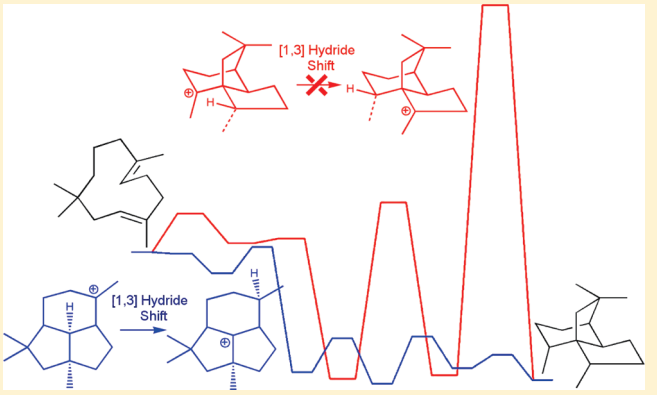
Supporting Information

ABSTRACT: Two mechanisms have been proposed in the literature to explain the formation of the skeleton of terrecyclic acid from farnesyl diphosphate. Both mechanisms satisfy the experimental data obtained using isotopic labeling, but computational results at the mPW1B95/6-31+G(d,p) level of theory allow the differentiation between them. While one of the mechanisms is basically a carbocation cascade, the other one requires several steps that imply high energetic demands. Specifically, there is a [1,3] hydride shift that requires approximately 100 kcal/mol making this mechanisms unlikely. The other mechanism is more plausible, and it suggests the participation of two secondary carbocation as intermediates, but these were not observed as minimums on the potential energy surface analyzed; they only appear as a point near the transition state in the intrinsic reaction coordinate. Both mechanisms proposed a [1,3] hydride shift, but in the less likely mechanism, the rigidity of the intermediate that undergoes the hydride shift greatly increases the energy of the corresponding transition state.



INTRODUCTION

Farnesyl diphosphate (FPP, Scheme 1) can be cyclized by the enzyme humulene synthetase to produce a carbocation with a ring of 11 members (**1**).[1] The sesquiterpene formed by the β elimination of a proton from the humulyl cation (**1**) is known as humulene (**2**). On the other hand, if cation **1** suffers an attack on the 2,3 double bond and the methyl on the formed carbocation's C3 is deprotonated, then β-caryophyllene (**3**) is formed. Both compounds **2** and **3** occur widely found in nature.[2] The humulyl cation is a biogenetic precursor of an important number of sesquiterpenes that are products of later cyclizations (Scheme 1). Since cation **1** has great conformational freedom, many of its biogenetic products are complex tricyclic structures such as sterpurene (**4**), pentalenene (**5**), terrecyclic acid (**6**), quadrone (**7**), silphinene (**8**), and botrydial (**9**).[2]

In 1982, terrecyclic acid (**6**) was isolated for the first time from the mold *Aspergillus terreus*;[3] a few years earlier, quadrone (**7**) was isolated from the same source; both have a similar structure.[4] Both compounds, in addition to having moderate antitumor activity, have a very interesting tricyclic structure, and their biogenetic origin has not been easily determined. Several groups have worked on the elucidation of their biosynthesis. To accomplish this, isotopically marked acetate and mevalonate have been added to *Aspergillus terreus* cultures.[5−9] Two different mechanisms have been proposed to explain the distribution of the labeled atoms. On the first one (Scheme 2),[5,6] it is suggested that the carbon atom with electronic deficiency of the humulyl cation cyclizes onto the 6,7 double bond. This forms a bicyclic cation with one ring with eight members and another one with five (**10**, Scheme 2). Later, the C7 carbon is attacked by the 2,3 double bond to form the C2−C7 bond (**11**). At the same time that C6 is transposed from C7 to C2, the hydrogen atom attached to C2 undergoes a [1,2] hydride shift to position C3 (**12**). Lastly, one of the C12 hydrogen atoms is eliminated so the hydrogen atom at C3 undergoes a [1,3] hydride shift to C7. Compound **13*** (terrecyclene) is the hydrocarbon precursor of **6** and **7** as it has the same carbon skeleton.

The second mechanism uses as starting point the mechanism proposed for the formation of silphinene (**8**),[10,11] which is also proposed for the formation of botrydial (**9**), another sesquiterpene.[12] In the mechanism for the formation of **8**, it has been proposed that cation **1** cyclizes onto the 2,3 double bond to form an intermediate with fused 4- and 9-membered rings (**14**, Scheme 3). In the following step, C1 is transposed from C2 to C3 forming **15**, which suffers the attack of the 6,7 double bond to form the C2−C6 bond (**16**). Cation **16** undergoes [1,3] hydride shift from C2 to C7 forming cation **17**. Then, C7 is transposed from C6 to C2 to form **18**. This is where the biosynthesis of **6** and **8** takes separate paths. In order to form **6** it is proposed that two more consecutive transpositions take place. First, C10 is transposed from C2 to C6 (**19**), and finally, C1 is transposed again from C3 to C2 (**13**).[13−15] In this paper, we perform DFT calculations of both mechanisms in order to

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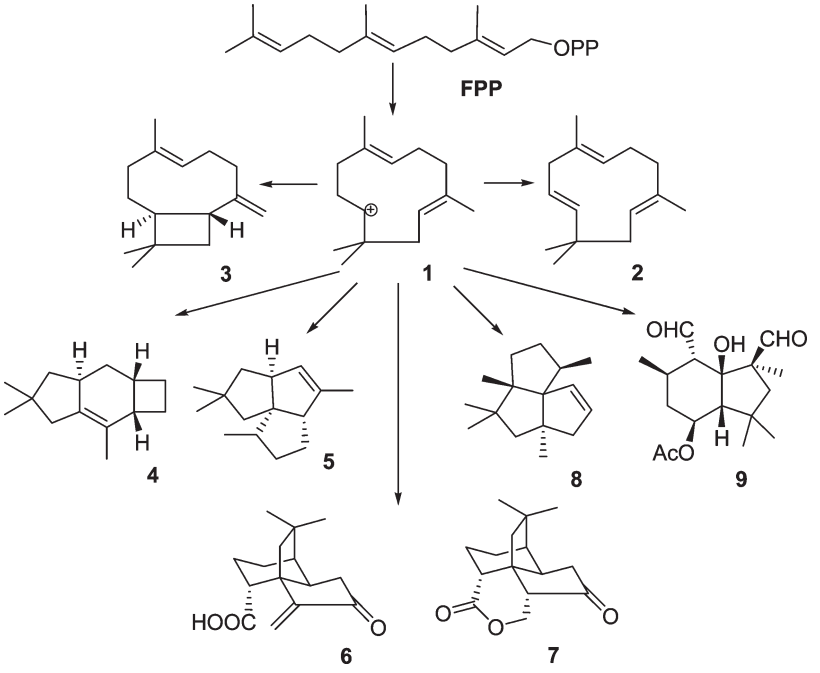
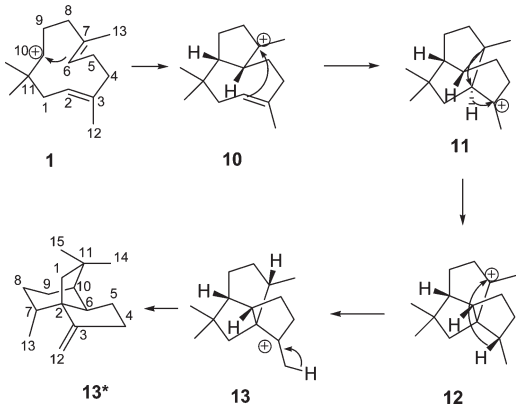
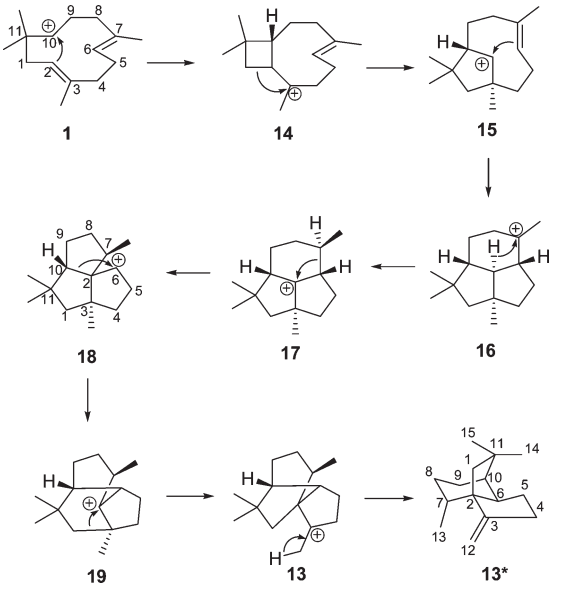
Scheme 1. Some Biogenetic Derivatives of Humulyl Cation 1



Scheme 2. Biogenetic Hypothesis for the Biosynthesis of Terrecyclene Suggested by Hirota et al.[5]



Scheme 3. Biogenetic Hypothesis for the Biosynthesis of Terrecyclene Suggested by Coates et al.[14]



elucidate which of these mechanisms is more likely and to understand the structural and energetic characteristics of the intermediates involved in both paths.

COMPUTATIONAL METHODS

Conformational molecular mechanics calculations were done with the MM3 force field using Tinker 4.[16] All of the quantum chemical calculations were performed with Gaussian 03.[17] Geometries were optimized without geometry constraints using the density functional theory (DFT) hybrid method with mPW1B95 functional.[18] Recent studies in small molecules and in terpenoids have shown that the third-generation mPW1B95 functional produces more reliable thermochemical kinetic data than B3LYP functional.[19,20] The double split valence polarized and diffuse 6-31+G(d,p) basis set was used for geometry optimization and frequency calculations. The 6-31+G(d,p) basis functions were used because the addition of diffuse functions to double split valence basis has been shown to be more important than increasing to a triplet split valence basis when calculating reaction energies and activation energies with DFT.[21] All energies were corrected by zero point energy and were not scaled for comparison purposes. The intrinsic reaction coordinate (IRC) was calculated where the transition state (TS) did not show clearly if it was linked to two specific intermediates.

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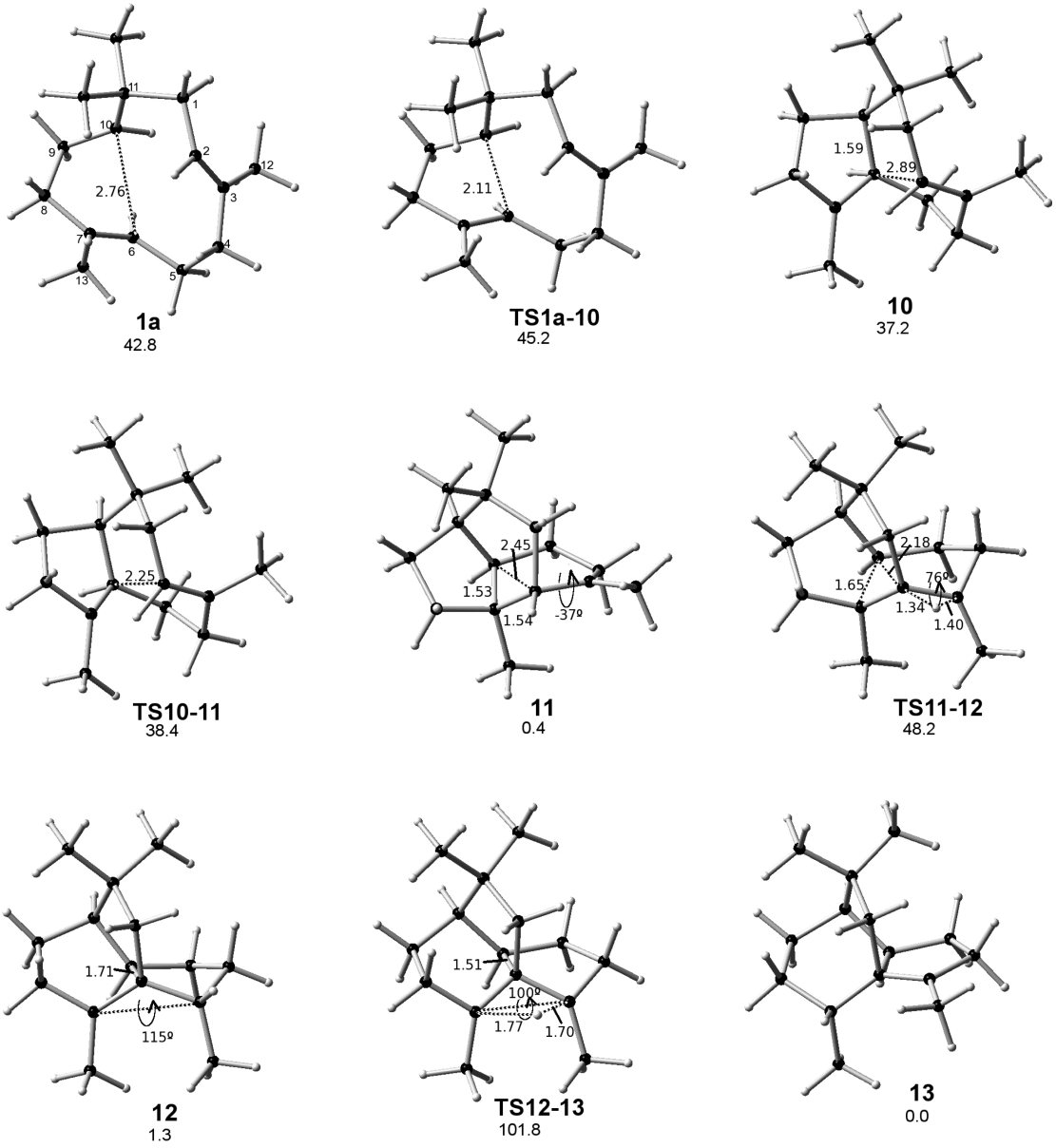

Figure 1. Intermediates and TS optimized at the mPW1B95/6-31+G(d,p) level from the reaction mechanism proposed by Hirota et al. for the formation of terrecyclene.[5] The distances and the dihedral angles H−C2−C3−C12 and C13−C7−C3−H are in angstroms and degrees, respectively.

RESULTS AND ANALYSIS

To model the mechanism proposed by Hirota et al. (Scheme 2), we chose conformer **1a** of cation **1** since this one has the most suitable conformation to follow the proposed mechanism and generates the configuration observed in **6**. Cation **1a** produces bicycle **10** (Figure 1) through a transition state (TS) **TS1a-10** that is reached with an energy of 2.4 kcal/mol. The C10−C6 distance evolves from 2.76 to 1.59 Å and is 2.11 Å at the TS. On the other hand, the C2−C7 distance in cation **10** is of 2.89 Å. This favors the interaction of the two carbon atoms. The activation energy to form **11** is very small, of only 1.3 kcal/mol but produces a strong drop in energy since **11** is 36.8 kcal/mol more stable than **10**.

The tricycle **11** formed through this mechanism is very stable and rigid. The problem of the rigidity of **11** is that it is not possible to find any conformer with a geometry that would be adequate to allow migration of a C2 hydrogen atom that would generate the appropriate configuration (*S*) that intermediate **12** would require. The conformer that has the geometry that allows the closest to what is needed has the C2−H bond practically perpendicular to the plane of carbon C2. The other conformers of **11** would have the opposite configuration. In spite of this, a TS that would correspond to the [1,2] migration of hydrogen from C2 to C3 and that would generate *S* configuration was investigated. This stationary state was located on the potential energy surface associated with this reaction and is shown in Figure 1 as **TS11−12**. This is located at 47.2 kcal/mol in relation to **11**. In this TS, the migration of the hydrogen atom from position 3 to 2 is aided by the concerted formation of the C2−C6 bond, since the C6−C7 bond shows a substantial advance in its rupture increasing the C−C distance to 1.65 Å. Even when the TS exists,

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its energy is too large mainly due to the fact that the conformation of **11** is not the optimum for the migration since the carbon−hydrogen bond that migrates is perpendicular to the p orbital to which it should migrate. As the reaction evolves, a rotation of the C2−C3 bond is observed and this allows the much needed alignment of the acceptor orbital of p character and the C−H bond of the atom that migrates. If the stereoelectronic requirements are not satisfied, the energetic requirements are too large.

Cation **12** suffers a lengthening of the C6−C2 bond to 1.71 Å since this bond participates through hyperconjugation in the stabilization of the atom that suffers the loss of charge at position 7 conferring stability to the molecule. None of the conformations that **12** could adopt (that in reality are very few) has a suitable geometry to allow the [1,3] migration of hydrogen. We searched for the TS for such a migration and we found a TS (**TS12−13**) with a very large energy (101.8 kcal/mol). This very large energy caught our attention because, in general, the activation energy of [1,3] hydride shifts is much lower, at the B3LYP level the activation energy of the [1,3] hydride shift of propyl cation is only 10.5 kcal/mol.[22] When the geometry of this TS is analyzed, it can be observed that the carbon atoms that exchange the hydrogen are very distant so the C−H bond lengths in the TS are quite long at 1.70 and 1.77 Å producing little stability. In addition, the angle between plane C2, C7, C8 and plane C2, C3, C4 in TS is 160°, which keeps away both carbons. During the migration of the hydrogen, C6−C2 bond distance decreases from 1.71 to 1.51 Å since it stops participating in the hyperconjugation process that was happening with C7. At the end of the process, in product **13** the empty orbital is perpendicular to the ring bonds and only a C−C bond (in this case C1−C2) participates in its stabilization. In conclusion, this mechanism implies two transition states of very high energy; one of the transition states has energy of 101.8 kcal/mol so it is not a viable mechanism.

The mechanism proposed by Coates et al. (Scheme 3) starts from a carbocation close in geometry to β-caryophyllene. After an exhaustive search of FPP conformers, we found that some conformations lead directly to caryophyllyl cation instead of humylyl cation. This result shows that the initial formation of a humulyl cation is not strictly needed for the formation of the caryophyllene. It depends on the conformation adopted by FPP, if it cyclizes as humulyl cation or as caryophyllyl cation. Then, the caryophyllyl cation **14a** (Figure 2) was chosen as the starting conformer but it does not have a suitable geometry to produce the configuration observed in product **6**; C10 has to be antiperiplanar to C12. Accordingly, conformer **14a** is transformed by rotation of the C2−C3 bond into conformer **14a′** which is 5.8 kcal/mol more stable. The transannular distance of interaction between C2 and C6 in **14a′** is 3.16 Å and the C1−C2 bond stays lengthened since it participates in the stabilization of the C3 carbon atom. In a theoretical study of the biosynthetic reaction mechanism of formation of presilphiperfolanol, Tantillo et al. suggested that is more likely that **14a** comes directly from the *Z,E*-humulyl cation instead of from the *E,E* cation,[23] but *Z,E,E*-humulene has not been found in any natural source so far. Moreover, the activation energy for the conformational equilibrium between **14a** and **14a′** is practically zero and the conformational change is not dramatic, so the active site of an enzyme would not have to change significantly.

The rotation of the C2−C3 bond of **14a** allows the C6−C7 double bond attack on the *si*-face of the carbocation that leads to the correct configuration of **6**. A TS that will lead to **16** is located at 7.2 kcal/mol, where in addition to the C2−C6 bond formation, an annular expansion of cyclobutane ring due to the migration of bond C2 to C3 occurs. In this way, a tertiary cation is obtained again, and the intermediacy of a secondary one is avoided. The hypothetical intermediate **15** originally proposed by Coates is not produced since it is an unstable secondary cation. Intermediate **16** has to suffer the migration of hydrogen to form **17.** The first mechanism also proposed a [1,3] migration whose TS (**TS12−13**) turned out to have a large energy. However, for this case, the TS for the migration (**TS16−17**) is much lower in energy. The difference is based on the flexibility of **16,** this allows that in **TS16−17** the angle between the plane C2, C3, C6 and the plane C6, C7, C13 is 72°, so the carbon atoms that participate in the migration are closer. The atom that migrates is initially located at 2.08 Å from its destiny and the C−H distances in the TS are 1.21 and 1.44 Å; this means that the hydrogen atom has a larger stabilization effect than the hydrogen in **TS12−13**. The stabilization of **17** is due to the fact that three C−C bonds participate in the stabilization of carbocation through hyperconjugation.

According to what was proposed by Coates et al., once **17** is formed, it will suffer a transposition of the C7−C6 bond to form the hypothetical intermediate **18** (the silphinyl cation). However, all attempts to find this intermediate were not as successful as those for intermediate **15**. Nevertheless, a TS with a similar structure to the hypothetical cation **18** (**TS17−19**) was found and has an energy of 12.9 kcal/mol in relation to intermediate **17**. The imaginary frequency of **TS17−19** only implies the migration of C6−C7. However, when the IRC was reviewed, it was clear that once the migration of C7 happens, the migration of C10 begins to finally form intermediate **19**. This product is at 4.2 kcal/mol from the intermediate that formed it; thus, formally speaking, this process is not a cascade of carbocations. It is not strange that the two intermediates **15** and **18** (proposed in the original mechanism) are not minima on the potential energy surface since both are not very stable secondary carbocations. If the system has the possibility to form a tertiary carbocation, it will form it since this would be more stable. The instability of terpene secondary carbocations like **15** and **18** is almost a rule as was already reported.[19,23−27] Tantillo's group has proposed that secondary carbocations can be stabilized in very specific cases where the carbocations interact intermolecularly with an specific group oriented in a specific way, as interactions that could happen in the active site of an enzyme.[27]

The last step of this mechanisms includes a transposition of bond C1−C3, a migration of 1,2 methylene that occurs with a 3.6 kcal/mol barrier to form **13**, the final cation. From an energetic point of view, it can be concluded that in the mechanism proposed by Coates et al. (Figure 3) none of the energetic barriers is large even when, in reality, it is not a carbocation cascade since intermediate **17** is just a little more stable than the final product **13** by only 0.97 kcal/mol, and the mechanism shows an exothermic pattern that characterizes this type of mechanism. On the other hand, the mechanism proposed by Hirota et al., as already mentioned, is unlikely since **TS11−12** and **TS12−13** are energetic barriers difficult to overcome since there is no adequate conformation that favors the stabilization of this TS based on hyperconjugation. The structural rigidity of **TS11−12** and **TS12−13** would make very difficult even for an enzyme to stabilize these TSs.

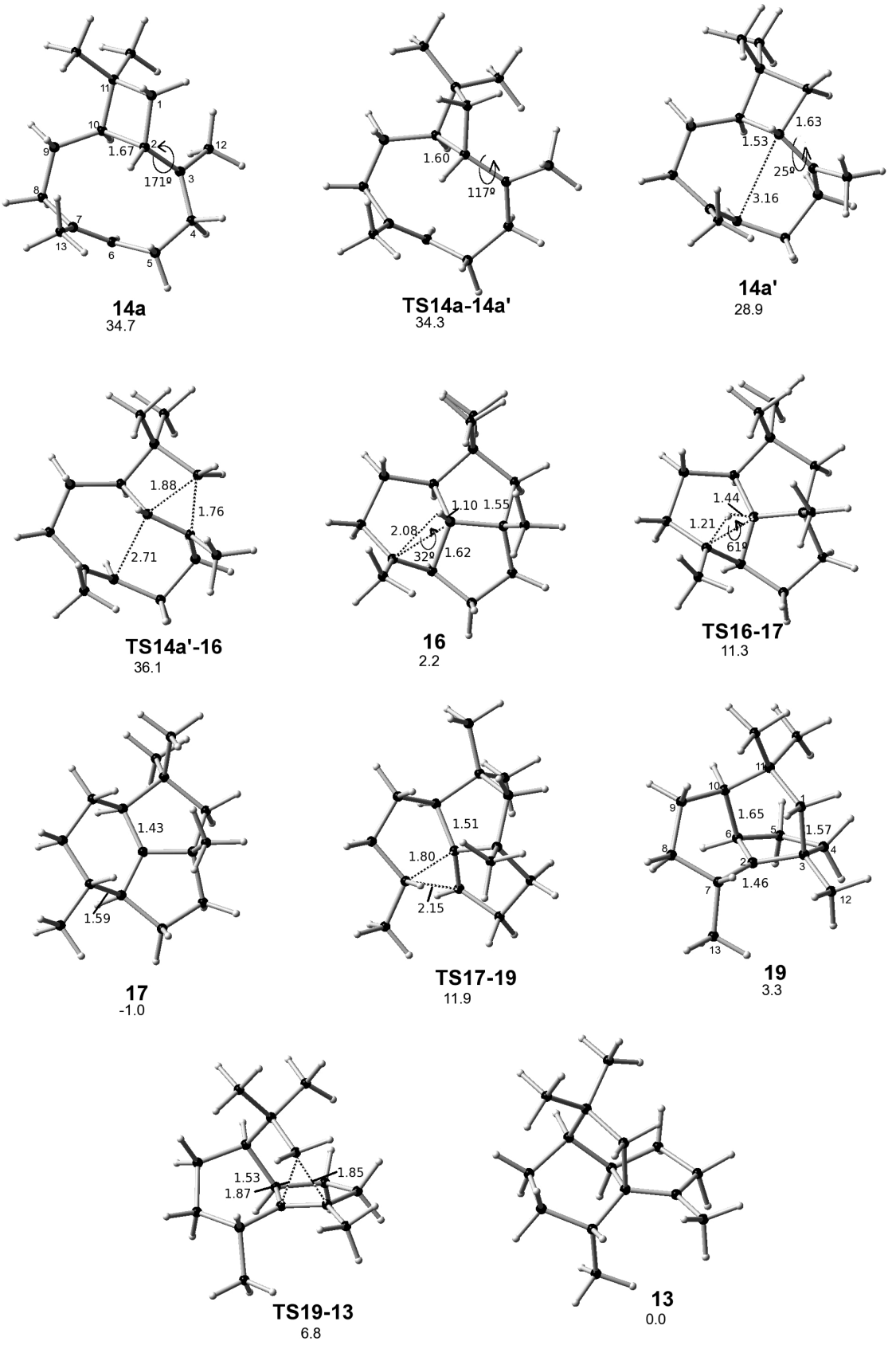

Figure 2. Intermediates and TS optimized at the mPW1B95/6-31+G(d,p) level from the reaction mechanism proposed by Coates et al. for the formation of terrecyclene.[14] The distances and dihedral angles H−C2−C3−C12 and C13−C7−C2−H are in angstroms and degrees, respectively.

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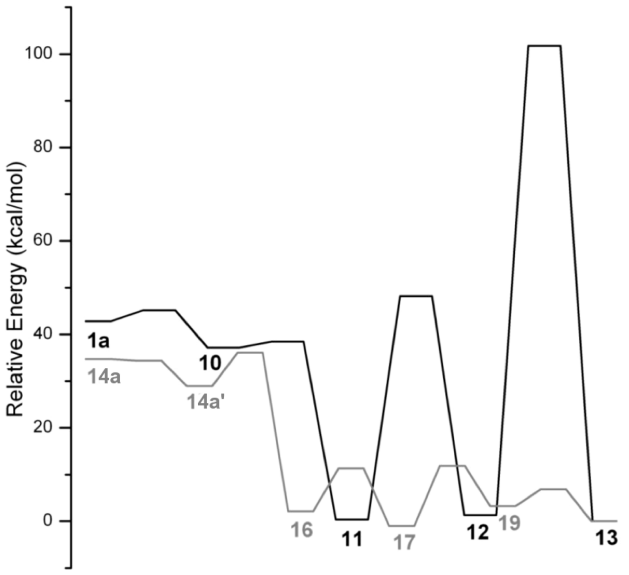

Figure 3. Energetic profile of the reaction mechanisms for the formation of terrecyclene. Hirota (black) and Coates (gray).

CONCLUSIONS

The computational study described above shows that mechanisms proposed by Hirota et al.[5] are unlikely since the last two TSs have very large energy. The mechanism proposed by Coates et al.[14] seems more reasonable even when it needs some adjustments. These modifications are derived from the fact that none of the secondary carbocations suggested by Coates et al. turned out to be minima in the reaction potential energy surface. Thus, the steps that involve them are avoided by concerted mechanisms. In addition, the modifications suggested by our work explain satisfactorily the experiments with isotopic markings previously described. In the conformational study of humulyl cation, it was found that some conformations of FPP produce unstable humulyl cations that react instantly with the double bond located in the proximity of the carbon with electronic deficiencies, as happens with many unstable terpene secondary carbocations. This shows that the initial formation of humulyl cation (eleven carbon ring) is not strictly necessary before the formation of the β-caryophyllene, it all depends on the conformation adopted by FPP. The analysis of mechanisms also shows that the atom and group migrations require stabilization by hyperconjugation through the suitable alignment of the orbital before the migrations, but the energy increases greatly. In addition, the hydrogen-donating and -accepting carbons should be located as close as possible; this condition is very difficult to satisfy if the intermediate is too rigid to significantly change its structure in the TS.

ASSOCIATED CONTENT

Supporting Information. Full optimized geometries of all compounds and full ref 17. This material is available free of charge via the Internet at http://pubs.acs.org.

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