

**Computational Evidence for Tunneling and a *Hidden Intermediate* in the Biosynthesis of
Tetrahydrocannabinol (THC)**

Edyta M. Greer,^{a*} Victor Siev,^a Ayelet Segal,^a Alexander Greer,^{b,c} Charles Doubleday^{d*}

^aDepartment of Natural Sciences, Baruch College of the City University of New York, 17
Lexington Avenue, New York, NY 10010, United States

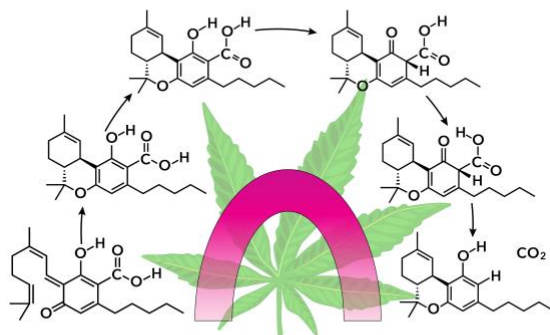
^bDepartment of Chemistry and Graduate Center, Brooklyn College of the City University of New
York, 2900 Bedford Avenue, Brooklyn, NY 11210, United States

^cPhD Program in Chemistry, The Graduate Center of the City University of New York, New York
10016, United States

^dDepartment of Chemistry, Columbia University, 3000 Broadway, MC 3142, New York, NY
10027, United States

*Corresponding authors. Edyta M. Greer (edyta.greer@baruch.cuny.edu) and Charles Doubleday
(ced3@columbia.edu)

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Abstract

Quantum tunneling is computed for a reaction sequence that models conversion of the *ortho*-quinone methide of cannabigerolic acid **1** to the decarboxylated product (-)-*trans*- Δ^9 -tetrahydrocannabinol (THC, **3**). This calculation is the first to evaluate multidimensional tunneling in this sequence. Computations were carried out with POLYRATE and GAUSSRATE using B3LYP/6-31G(d,p) to examine the mechanism of THC **3** formation. The pentyl chain on THC **3** and its precursors was replaced with a methyl group to compute tunneling contributions to the rates of four separate steps: (i) initial Diels-Alder reaction of the quinone methide with the trisubstituted alkene end-group of the geranyl **1Z-CH₃** to give **2Z-CH₃**. (ii) An acid catalyzed keto-enol tautomerization, which converts **2rZ-CH₃** to **4rZ-CH₃**, and (iii) carboxyl rotamerization converting **4rZ-CH₃** to **4E-CH₃**, and (iv) decarboxylation that converts **4E-CH₃** to **3-CH₃**. Tunneling contributions to the rate constants of step (i)-(iv) are between 19% and 76% at 293 K. In step (ii), nonuniform changes in the zero-point vibrational energy along the reaction path created a shallow minimum in the 0 K free energy. It is a *hidden intermediate* because it is not a minimum

on the potential energy surface, and is detectable only when zero-point energy is taken into account along the reaction path. Predicted kinetic isotope effects would be experimentally observable at temperatures that are convenient to use. This is particularly relevant in the decarboxylation stage of the reaction sequence and has important implications because of its role of THC **3** formation.

Introduction

Quantum tunneling has been found to be important in a number of biological and organic reactions.¹⁻²⁴ In a previous examination of heavy-atom tunneling in 13 common reactions at normal operating temperatures, we found that tunneling accounts for 25-95% of the reaction rate in eight of them.¹³ This suggested that a study of tunneling in a biological reaction sequence would be useful. We chose a sequence leading to THC, (-)-*trans*- Δ^9 -tetrahydrocannabinol **3**, a psychoactive component of *Cannabis sativa* shown in Figure 1.²⁵ Starting with the *ortho*-quinone methide QM (**1**), after its formation from cannabigerolic acid (CBGA), we computed the tunneling contribution to the rate of each step from QM **1** to THC **3** (Figure 1), all of which are found to have a substantial tunneling contribution.

Tunneling contributions were calculated by POLYRATE.²⁶ The transition state was located according to a free energy criterion using canonical variational theory (CVT), which gives the non-tunneling rate constant, k_{CVT} .²⁷ The transmission coefficient κ_{SCT} was then computed using the small curvature tunneling (SCT) method to give the tunneling-corrected rate constant, $k_{\text{SCT}} = \kappa_{\text{SCT}} \times k_{\text{CVT}}$.²⁸⁻³⁰ With this definition, $\kappa_{\text{SCT}} = 1$ indicates no tunneling, $\kappa_{\text{SCT}} > 1$ indicates the presence of tunneling, and the fraction of the rate due to tunneling is $(\kappa_{\text{SCT}} - 1)/\kappa_{\text{SCT}}$. Note that κ_{SCT} is a function of all atoms in the reaction. Further discussion of κ_{SCT} and tunneling is given below and in the Supporting Information.

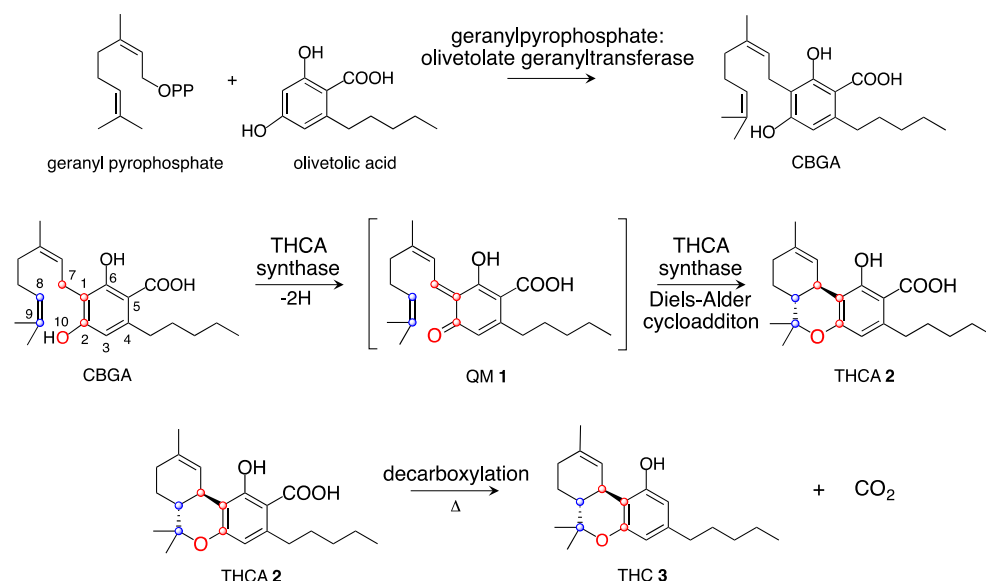


Figure 1. Previously proposed biosynthesis^{31,32} of THCA **2** from geranyl pyrophosphate and olivetolic acid, and the non-enzymatic decarboxylation of THCA **2** to THC **3**.

Our calculations are focused on the sequence of reactions in Figure 1 starting with **1** after its formation from CBGA, and ending with THC **3**. CBGA is formed from the reaction of geranyl pyrophosphate with olivetolic acid via geranylpyrophosphate:olivetolate geranyltransferase.³¹ Tetrahydrocannabinolic acid THCA **2** is then formed by THCA synthase catalyzed transformation of CBGA.³¹ This involves a catalytic hydride transfer from benzylic C(7) of CBGA to flavin adenine dinucleotide and the deprotonation of the phenol O(10) of CBGA by tyrosine oxyanion to produce intermediate QM **1**, which is thought to undergo a hetero-Diels-Alder cyclization to produce THCA **2**.³¹ At this point, in the absence of heat, the plant contains only small amounts of THC **3**.³³ The non-enzymatic decarboxylation of THCA **2** to THC **3** is brought about by smoking, light, or storage.³² As described below, decarboxylation of **2** to **3** is facilitated by tunneling.

Thermodynamic and rate measurements have been reported for the conversion of THCA **2** to THC **3**.³³⁻³⁵ Temperature studies show $E_a = 20.3$ kcal/mol in the solid-state,³⁴ an $E_a = 21.0$ kcal/mol in cannabis extracts,³⁵ and an E_a of 14.1 kcal/mol in the ground-dried hemp plant.³³ In these experimental studies, low pH and increased rates suggested that acids present in the plant serve as catalysts for decarboxylation.³⁴

Computational results for the decarboxylation of THCA **2** to THC **3** have been reported.^{34,36} A formic acid-catalyzed tautomerization was computed with B3LYP/6-31G(d,p), in which the enol form of THCA **2** converted to the keto form and was followed by decarboxylation ($E_a = 19.5$ kcal/mol).³⁴ A methanol catalyzed keto-enol tautomerization of THCA **2** was also computed and found to have a high $\Delta G^\ddagger = 30.4$ kcal/mol.³⁶ In the absence of formic acid or methanol or water, an even higher $\Delta G^\ddagger$ with the value of 50.9 kcal/mol was found.³⁶

In the current work, structure **1Z-CH₃** was used in place of **1** to reduce the number of basis functions as well as high conformational freedom in the pentyl chain, because the tunneling calculations are computationally demanding. They were carried out with POLYRATE²⁶ and the GAUSSRATE³⁷ interface to Gaussian 16³⁸ with the B3LYP/6-31(d,p) functional.^{39,40} Tunneling calculations based on POLYRATE have been applied widely.^{8,11,13,15,18,22,23,41-49} POLYRATE begins by computing the minimum energy path (MEP), a steepest descent path, connecting the saddle point to reactants and products. The transition state is located using canonical variational transition state theory (CVT), at the point of maximum free energy along the MEP, which may deviate from the saddle point. Tunneling and CVT use similar information about the MEP. Both require frequencies of the transverse modes at points along the MEP. The zero-point vibrational energy (ZPE) is then added to the path energy at these points to give the ZPE-corrected MEP, called the V_a^G curve.²⁷ In CVT, the V_a^G curve is the free energy at 0 K. For tunneling, the V_a^G

curve is the barrier through which tunneling occurs. Once computed, the V_a^G curve and related path quantities are used to compute the tunneling transmission coefficient, κ_{SCT} . All of the electronic structure and dynamical information – stationary points on the classical potential energy surface and ZPE and tunneling corrections – are needed to define the mechanism, and in this sense, all are important for THC **3** formation.

Tunneling makes substantial contributions to the four steps shown in Figure 2: the Diels-Alder reaction producing **2Z-CH₃** (quinone methide with trisubstituted alkene end-group of the geranyl), the formic acid catalyzed keto-enol tautomerization of **2rZ-CH₃** to **4rZ-CH₃** (and **2Z-CH₃** to **4Z-CH₃**), the rotamerization of **4rZ-CH₃** to **4E-CH₃**, and decarboxylation converting **4E-CH₃** to **3-CH₃** (Figure 2).

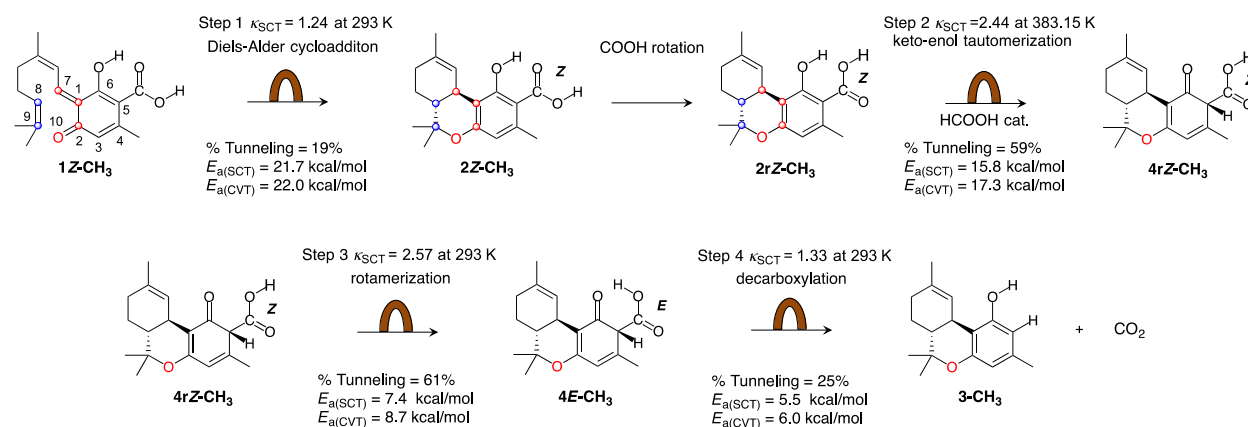


Figure 2. Reactions of model compounds **1Z-CH₃** to **3-CH₃**, in which tunneling contributions are found in four steps.

Results and Discussion

Energies of Stationary Points and Sequence of Reactions. Stationary points related to the parent **1Z** containing the *n*-pentyl chain are shown in Figure 3. To compute tunneling contributions, the pentyl groups were replaced by methyl and the stationary points related to **1Z-CH₃** were reoptimized using initial geometries derived from Figure 3. Upon inclusion of the contribution from tunneling, the predicted E_a is within the range of the reported experimental E_a values.³³⁻³⁵ Replacement of the *n*-pentyl substituent by a methyl group had small effects (0.1 – 0.6 kcal/mol, mean difference 0.3 kcal/mol) on the barrier heights in Figures 3 and 4 (compare Figures S3 and S4 in Supporting Information).

A reviewer suggested that since tunneling is a phenomenon of the reaction as a whole, including all atoms, then changing from pentyl to methyl might exert a mass effect on the transmission coefficient κ_{SCT} . To check this, we computed tunneling for the tautomerization step 2, with a heavy methyl group in which one H atom was given the mass of a butyl group in order to match the mass of pentyl. This allows comparison of κ_{SCT} for step 2 with a methyl vs. heavy methyl. Since both cases involve CH₃, the only difference is the mass. The computed κ_{SCT} values at 383 K are 2.44 for methyl and 2.47 for heavy methyl, not a significant difference. Thus, computational evidence for tunneling in **1Z** and related species in Figure 3 can be inferred from the values of κ_{SCT} computed for **1Z-CH₃** and its related species.

The [4 + 2] cycloaddition of **1Z** to form THCA **2Z** has an activation barrier of $\Delta G^\ddagger=25.3$ kcal/mol, and the reaction is exergonic by 20.0 kcal/mol. In step 2, two rotamers of **2Z**, **2Z-HCOOH** and **2rZ-HCOOH**, interconvert rapidly via a low barrier (Figure 4) before undergoing competitive formic acid catalyzed keto-enol tautomerization to give rotameric ketoacids, **4Z-HCOOH** and **4rZ-HCOOH**. The system operates in the Curtin-Hammett regime at room temperature and above, so the ratio of ketoacid rotamers is governed by the tunneling-corrected

relative energies of the transition states **TS-2Z-HCOOH** and **TS-2rZ-HCOOH**. The 0.8 kcal/mol difference between **TS-2Z-HCOOH** and **TS-2rZ-HCOOH** in Figure 4 implies that **4rZ-HCOOH** is formed about 4 times faster than **4Z-HCOOH** at 298 K. By contrast, in the 1,2-hydrogen shift of trifluoromethyl hydroxycarbene at 10 K, the Curtin-Hammett principle was found not to be operating and the product ratio was dominated by tunneling.⁵⁰

Subsequently, there is a **4rZ** to **4E** rotamerization with an activation barrier of $\Delta G^\ddagger=8.6$ kcal/mol. Finally, decarboxylation of **4E** leads to THC **3** by a low activation barrier of $\Delta G^\ddagger=5.6$ kcal/mol.

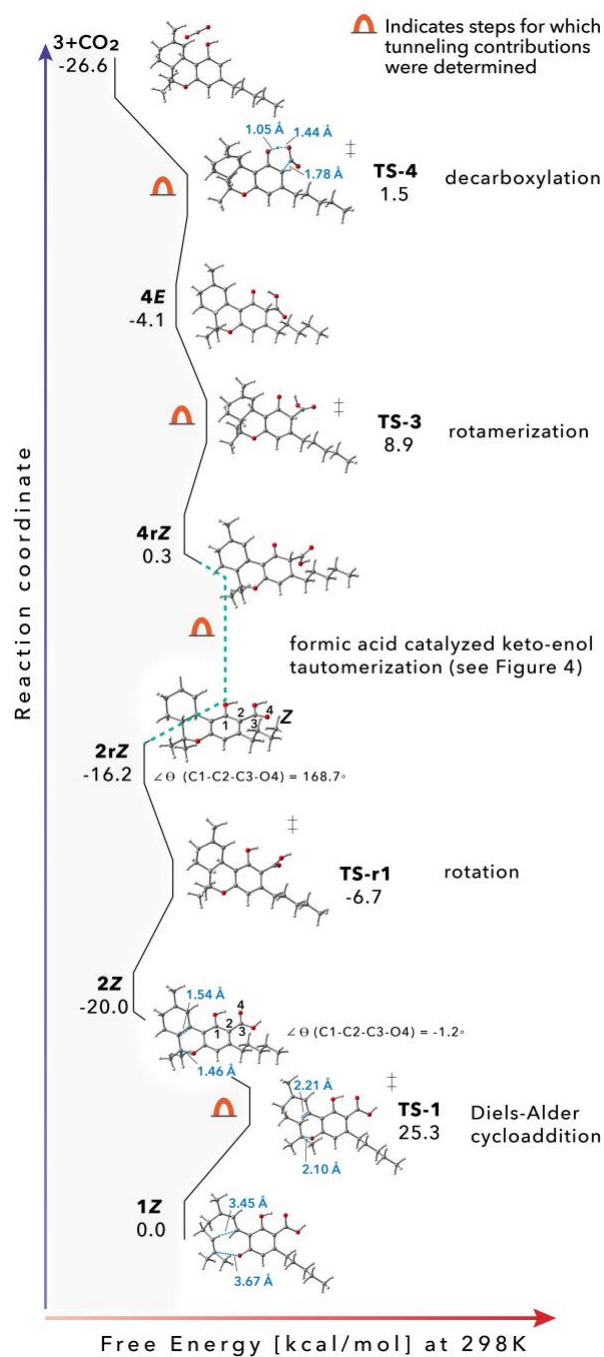


Figure 3. Free energies (kcal/mol) at 298 K at all stationary points in the conversion of QM **1Z** to THC **3** computed with B3LYP/6-31G(d,p). Tunneling was not computed at this stage. **Z** refers to H on COOH *cis* to the C=O, **E** refers to H on COOH *trans* to the C=O.

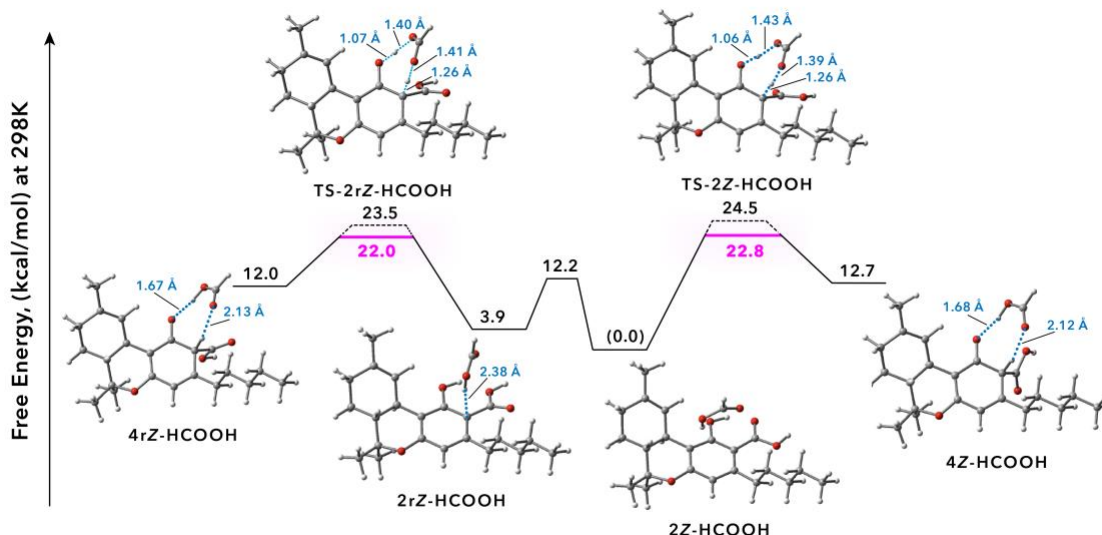


Figure 4. Stationary points for the formic acid-catalyzed tautomerization of **2Z-HCOOH** and **2rZ-HCOOH**. Geometries were optimized at the B3LYP/6-31G(d,p) level of theory. Free energies (ΔG) in black do not include tunneling contributions. Energy values in magenta give the tunnel-corrected E_a values obtained from Arrhenius plots of k_{SCT} vs. $1/T$. (Tunneling was computed after replacing pentyl by methyl as described above.) Since **2Z-HCOOH** and **2rZ-HCOOH** equilibrate rapidly via **TS-r1** (Figure 3), the Curtin-Hammett principle is arising in that tautomerization occurs mainly from the higher energy conformer **2rZ-HCOOH** via the lower energy transition state **TS-2rZ-HCOOH**.

Importance of Tunneling in the 4-Step Sequence in Figure 2. In these calculations, tunneling is computed for all atoms in the reaction, and the fraction of the rate due to tunneling is $(\kappa_{\text{SCT}} - 1)/\kappa_{\text{SCT}}$, where κ_{SCT} is the transmission coefficient. In certain cases, we could gain insight into tunneling with kinetic isotope effect analyses by comparing CVT + SCT Arrhenius plots.

Step 1. Figure 5 and Table 1 show the tunneling contribution for the Diels-Alder reaction of **1Z-CH₃** to **2Z-CH₃** from the transmission coefficients, κ_{SCT} . At 293 K, $\kappa_{\text{SCT}} = 1.24$, indicating

that 19% of the rate is due to tunneling. The pink region of the barrier indicates that 90% of the tunneling occurs within 1.1 kcal/mol of the top. At 293 K, $k_{\text{SCT}} = 9.52 \times 10^{-7} \text{ s}^{-1}$, with a half-life of 202 h (8.4 days), while k_{CVT} (without tunneling) is $7.88 \times 10^{-7} \text{ s}^{-1}$, with a half-life of 244 h (10.2 days). The effective barrier determined from the Arrhenius plot of $\ln k_{\text{SCT}}$ vs. $1/T$ is 21.7 kcal/mol (Figure S6 in Supporting Information), 0.3 kcal/mol lower than the $E_a = 22.0$ kcal/mol obtained from the Arrhenius plot of $\ln k_{\text{CVT}}$ vs. $1/T$ (Figure S5 in Supporting Information). Table 1 shows more details on the temperature dependence on k_{CVT} , k_{SCT} , and half-lives, $t_{1/2}$. An experimentally viable measurement of the KIE for the Diels-Alder reaction could involve Singleton's method with natural abundance ^{13}C ($k^{12}\text{C}/k^{13}\text{C}$) KIEs since CBGA is readily available from the plant.⁵¹

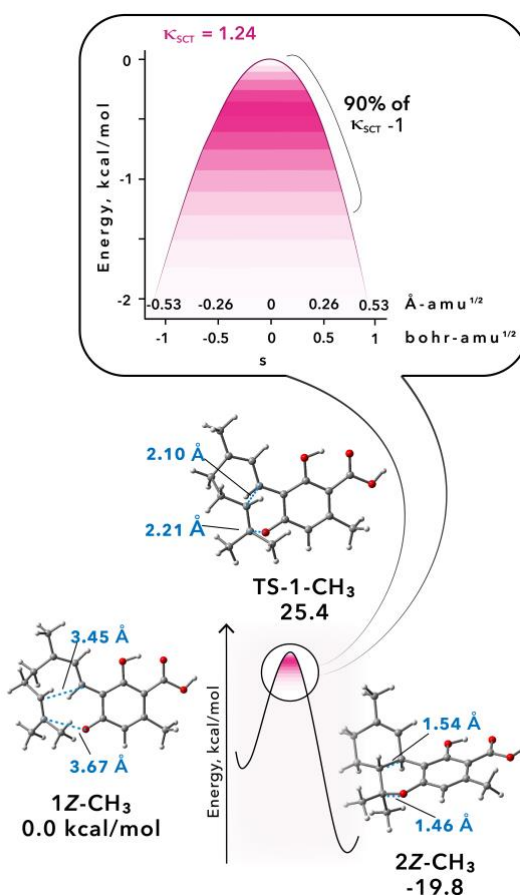


Figure 5. Tunneling contribution in the PES for the Diels-Alder reaction of **1Z-CH₃** to **2Z-CH₃**.

Table 1. Variational transition state theory rate constants (k_{CVT}), rate constants including tunneling (k_{SCT}) and corresponding half-lives ($t_{1/2}$) for selected temperatures for **1Z-CH₃**.

T [K]	k_{CVT} [s ⁻¹]	$t_{1/2}=\ln 2/k_{\text{CVT}}$ [s]	$t_{1/2}=\ln 2/k_{\text{CVT}}$ [hrs]	$k_{\text{CVT/SCT}}$ [s ⁻¹]	$t_{1/2}=\ln 2/k_{\text{SCT}}$ [s]	$t_{1/2}=\ln 2/k_{\text{SCT}}$ [hrs]
250	1.20E-09	5.78E+08	1.61E+05	1.59E-09	4.37E+08	1.21E+05
273.15	5.09E-08	1.36E+07	3.78E+03	6.38E-08	1.09E+07	3.02E+03
293	7.88E-07	8.79E+05	2.44E+02	9.52E-07	7.28E+05	2.02E+02
313	8.74E-06	7.93E+04	2.20E+01	1.03E-05	6.76E+04	1.88E+01
323	2.60E-05	2.66E+04	7.40E+00	3.01E-05	2.30E+04	6.39E+00
333	7.25E-05	9.56E+03	2.66E+00	8.29E-05	8.36E+03	2.32E+00
343	1.90E-04	3.64E+03	1.01E+00	2.15E-04	3.22E+03	8.95E-01
353	4.73E-04	1.47E+03	4.07E-01	5.29E-04	1.31E+03	3.64E-01
373	2.52E-03	2.76E+02	7.65E-02	2.77E-03	2.51E+02	6.96E-02

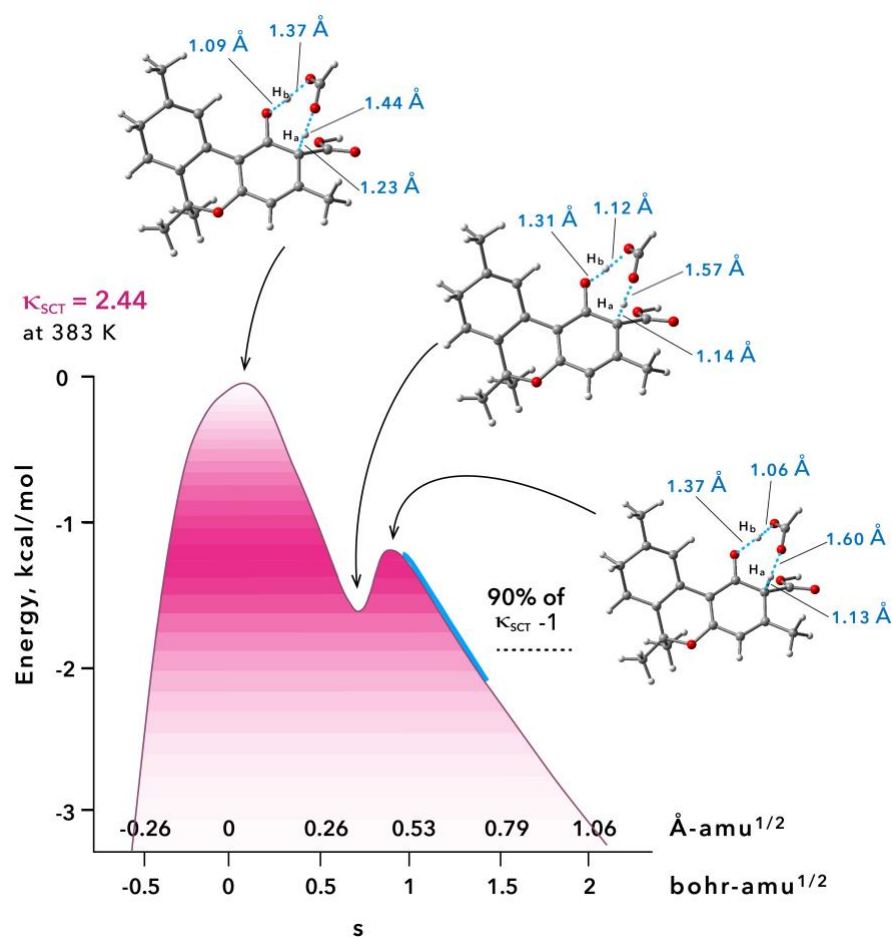
Step 2. Figure 4 shows two competitive processes with barriers within 1 kcal/mol from each other. The Curtin-Hammett principle implies that the higher energy conformer **2rZ-HCOOH**, that goes through the lower energy transition state is dominant. Figure 6 shows the tunneling contribution of the formic-acid-catalyzed keto-enol tautomerization of **2rZ-HCOOH** to **4rZ-HCOOH** based on the transmission coefficients, κ_{SCT} . We consider here results at 383 K, one temperature for which the THCA decarboxylation experimentally yields THC.³⁴ We compute κ_{SCT} to be 2.44, indicating that 59% of the rate is due to tunneling. Based on Figure 6 we note that the formic-acid-catalyzed keto-enol tautomerization is not a single macroscopic step, but instead is made of two microscopic steps. The local minimum on the V_a^G curve provides evidence for a second step via a *hidden intermediate* – one that is not a minimum on the potential energy surface, but shows up only when the quantum correction for zero-point energy of transverse modes is included. The free energy plots at 0 K, 273 K, 373 K, and 473 K in Figure 6B show temperature dependent minima consistent with an intermediate.

The blue layer on the small barrier of Figure 6A marks the interval over which one of the transverse modes has an imaginary frequency. This indicates that the true MEP in this region is

lower than the curve shown. However, it does not affect the small maximum, which was located along the true MEP where all transverse modes have real frequencies.

For the less stable conformer **2rZ-CH₃-HCOOH** the Arrhenius plot of $\ln k_{\text{SCT}}$ vs. $1/T$ gives an effective barrier (including tunneling) of 15.8 kcal/mol (Figure S10 in Supporting Information). This is 1.5 kcal/mol lower than the $E_a = 17.3$ kcal/mol obtained from the Arrhenius plot of $\ln k_{\text{CVT}}$ vs. $1/T$ (Figure S9 in Supporting Information). At 293 K, $k_{\text{SCT}} = 0.025 \text{ s}^{-1}$, with the half-life of 28.3 s, while k_{CVT} (without tunneling) is 0.0058 s^{-1} , with the half-life of 120 s.

A



B

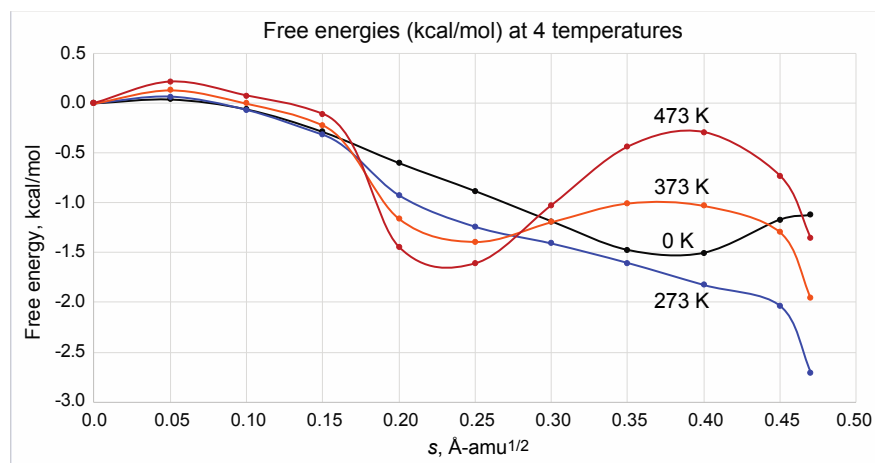


Figure 6. A. Tunneling path (ZPE-corrected MEP, or V_a^G curve) and tunneling contributions to the SCT transmission coefficient κ_{SCT} for formic acid catalyzed tautomerization of **2rZ-CH₃-HCOOH** at 383 K as functions of energy on the V_a^G curve. The blue layer on the small barrier marks the interval over which one of the transverse modes has an imaginary frequency (see text). **B.** Free energy along the MEP for the formic acid catalyzed tautomerization of **2rZ-CH₃-HCOOH** at 4 temperatures: 0 K, 273 K 373 K, and 473 K.

Figure 7 shows $\ln(k_{\text{H}}/k_{\text{D}})$ with inclusion of SCT tunneling (red curve) and without tunneling (blue curve) for tautomerization. Figure 7 reveals a decrease of H/D KIE from 34.0 to 4.86 (and decrease of $\ln(k_{\text{H}}/k_{\text{D}})$ from 3.53 to 1.58) with increasing temperature from 200 K to 500 K. The shape of the red CVT+SCT curve is unusual. The expected signature of tunneling in plots of $\ln(\text{KIE})$ vs. $1/T$ is upward curvature at lower T, as exemplified in Figure 9 below. In such typical plots, the tunneling contribution to the KIE increases at lower T because the decreasing Boltzmann factor for passage over the barrier is offset by tunneling through the barrier at a lower energy (therefore with a higher Boltzmann factor than passage over the top). In Figure 7, however, the red CVT + SCT plot has a negative curvature over the entire temperature range (smaller slope at higher values of $1000/T$), indicating that the tunneling contribution is decreasing at lower T. The reason

is the unusual shape of the barrier in Figure 6. Instead of having one barrier, there are two merged barriers in Figure 6A, which greatly increases the thickness of the combined barrier and lowers the tunneling probability. This makes it unfeasible for tunneling to offset the decreasing Boltzmann factor for passage over the barrier at low T. Because negative curvature is predicted over 200–500 K, a practical test could be carried out on the formic acid catalyzed $2\mathbf{rZ} \rightarrow 4\mathbf{rZ}$ conversion with deuterium (D) substitution as shown in Figure 7, conveniently measured over a temperature range large enough to see the curvature.⁵² Finally, we note that many biological reactions incorporate keto-enol tautomerization, and the unusual features of Figures 6 and 7 may well be present in some of these.

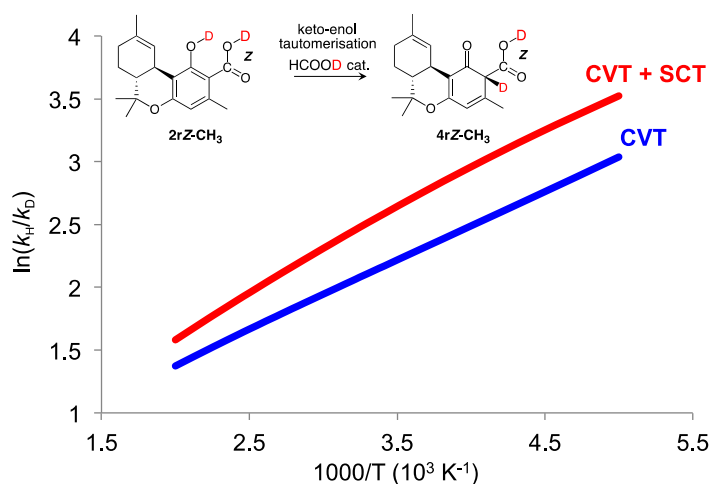


Figure 7. Arrhenius plots of $\ln(k_H/k_D)$ vs $1000/T$ for the formic acid-catalyzed keto-enol tautomerization of $2\mathbf{rZ}\text{-CH}_3\text{-HCOOH}$ to $4\mathbf{rZ}\text{-CH}_3\text{-HCOOH}$ involving the stepwise transfer of two protons (first H_a , second H_b see Figure 6). The calculation involved three deuterium atoms; two in $2\mathbf{rZ}\text{-CH}_3$ and one in formic acid.

Step 3. Figure 8 shows the tunneling contribution for the rotamerization of **4rZ-CH₃** to **4E-CH₃** from the transmission coefficients, κ_{SCT} . At 293 K, κ_{SCT} equals 2.57, indicating that 61% of the rate is due to tunneling. 90% of this tunneling occurs within 2.2 kcal/mol from the top of the barrier (Figure 8). At 293 K, $k_{\text{SCT}} = 5.51 \times 10^6 \text{ s}^{-1}$, with the half-life of 126 ns, while $k_{\text{CVT}} = 2.15 \times 10^6 \text{ s}^{-1}$, with the half-life of 322 ns. Thus, the effective barrier determined from the Arrhenius plot of $\ln k_{\text{SCT}}$ (including tunneling) vs. $1/T$ is 7.4 kcal/mol (Figure S12 in Supporting Information) and is 1.3 kcal/mol lower than the $E_a = 8.7$ kcal/mol barrier obtained from the Arrhenius plot of $\ln k_{\text{CVT}}$ (without tunneling) vs. $1/T$ (Figure S11 in Supporting Information).

Figure 9A shows the $\ln(k_{\text{H}}/k_{\text{D}})$ for reaction in Figure 8 with inclusion of SCT tunneling (red curve) and without tunneling (blue curve). There is a *Z* to *E* rotamerization of the OH hydrogen accompanied by internal rotation around the C–C bond of **4rZ-CH₃** to **4E-CH₃**. Intriguingly, for acetic acid and benzoic acid derivatives the *Z* form is the more stable isomer, whereas in our case due to intramolecular H-bonding in the latter, the *E* form is the more stable.^{53,54} The red curve in Figure 9A is non-linear and curves upward at lower temperature because the tunneling contribution increases at lower temperature. This trend has been observed in other reactions in which tunneling is important.^{11,13,22} The blue line in Figure 9A is linear because tunneling is absent. The rotamerization studied in this section is reminiscent of other work showing tunneling contribution in the rotamerization of oxalic acid.²¹ With oxalic acid rotamerization, the *Z* to *E* conversion has a 9.7 and 10.4 kcal/mol barrier where tunneling occurs at 3K in a Neon matrix.²¹ Such matrix techniques have helped in deducing rotamerization energies, as well detecting reactive intermediates and tunneling.^{16,19,53-56} In our case the *Z* to *E* rotamerization has an 8.6 kcal/mol barrier and tunneling is also potentially trackable, although the temperatures computed here for

4rZ and **4E** are in equilibrium. Table S6 (Supporting Information) shows more details on the temperature dependence on k_{CVT} , $k_{\text{CVT/SCT}}$ and, half-lives $t_{1/2}$.

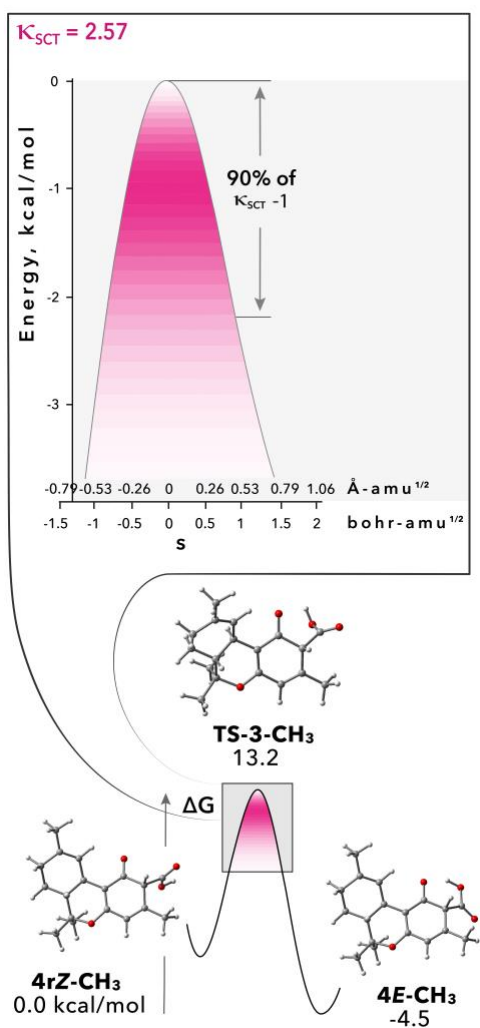


Figure 8. Tunneling contribution in the PES for the isomerization of **4rZ-CH₃** to **4E-CH₃**.

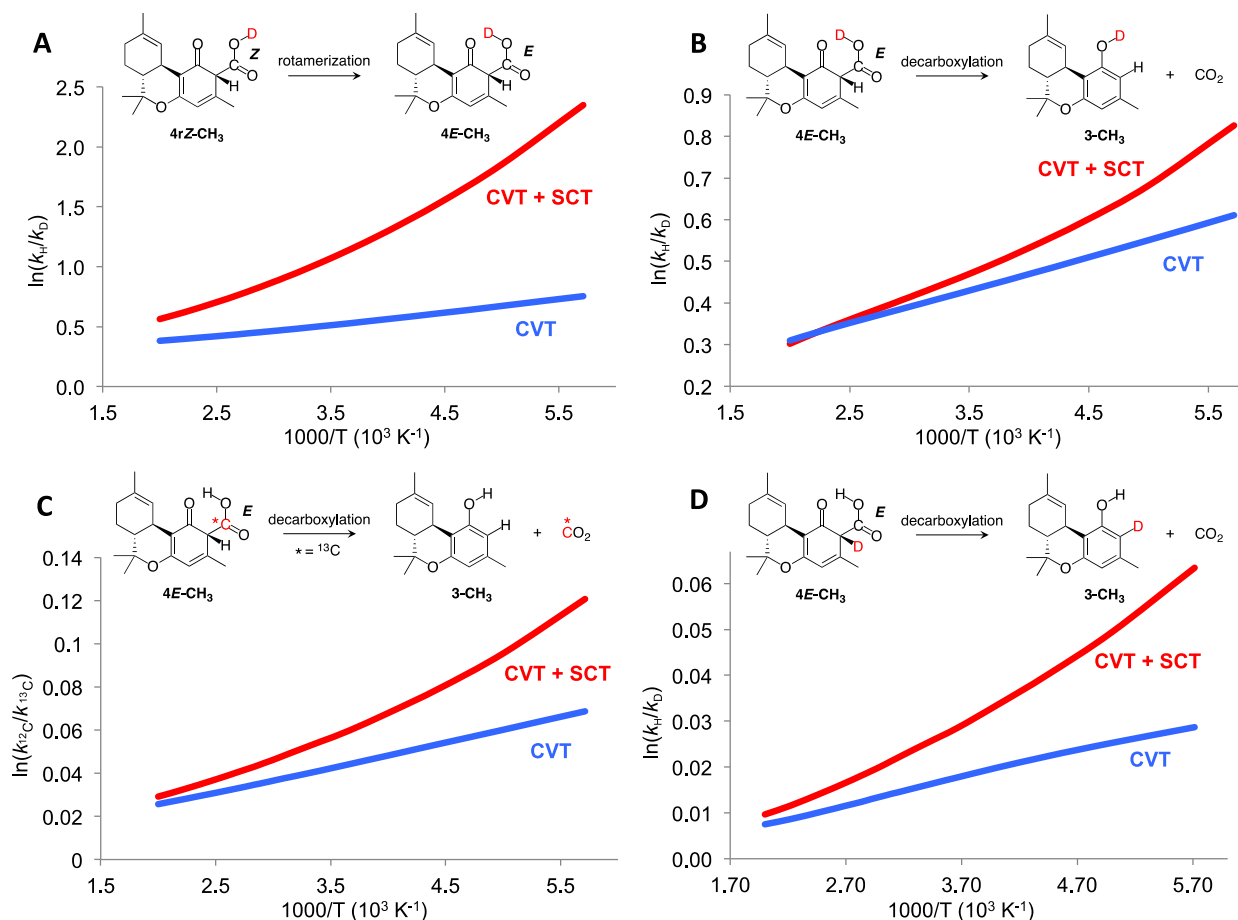


Figure 9. **A.** Arrhenius plots of $\ln(k_H/k_D)$ vs $1000/T$ for the Z/E isomerization step of $4rZ-CH_3$ involving H-rotation **B.** Arrhenius plots of $\ln(k_H/k_D)$ vs $1000/T$ for the hydrogen transfer during decarboxylation of $4E-CH_3$. **C.** Arrhenius plots of $\ln(^{12}C/^{13}C)$ vs $1000/T$ for the loss of CO_2 during decarboxylation of $4E-CH_3$. **D.** Arrhenius plots of $\ln(2^\circ H/D \text{ KIE})$ vs $1000/T$ for the rehybridization during hydrogen transfer during decarboxylation of $4E-CH_3$.

Step 4. Figure 10 shows the tunneling contribution on the decarboxylation of $4Z-CH_3$ to $3-CH_3$ from the transmission coefficients, κ_{SCT} . At 293 K, κ_{SCT} equals 1.33, indicating that 25% of the rate is due to tunneling. Tunneling contributes to κ_{SCT} within 2.0 kcal/mol of the barrier tip and ~90% of the tunneling contribution occurs within 1.1 kcal/mol from the top of the barrier. At

293 K $k_{\text{SCT}} = 3.27 \times 10^8 \text{ s}^{-1}$, while $k_{\text{CVT}} = 2.61 \times 10^8 \text{ s}^{-1}$. The effective barrier determined from the Arrhenius plot of $\ln k_{\text{SCT}}$ vs. $1/T$ is 5.5 kcal/mol (Figure S14 in Supporting Information) and is 0.5 kcal/mol lower than the $E_a = 6.0$ kcal/mol obtained from the Arrhenius plot of $\ln k_{\text{CVT}}$ vs. $1/T$ (Figure S13 in Supporting Information).

Figures 9B-9D show KIEs for decarboxylation including tunneling (red curve) and excluding tunneling (blue curve). There are three major movements of nuclei: (1) Figure 9B shows the Arrhenius plot of $\ln (k_{\text{H}}/k_{\text{D}})$ vs. $1000/T$ for the hydrogen transfer during decarboxylation of **4Z-CH₃**. (2) Figure 9C shows Arrhenius plots of $\ln (k^{12\text{C}}/k^{13\text{C}})$ vs. $1000/T(\text{K})$ for the loss of CO₂ during decarboxylation of **4Z-CH₃**. (3) Figure 9D shows Arrhenius plots of $\ln (2^\circ \text{H/D KIE})$ vs. $1000/T$ for the rehybridization during hydrogen transfer during decarboxylation of **4Z-CH₃**. Figures 9B-9D show significant curvature in the Arrhenius plots with inclusion of tunneling (red curves) even though the H/D KIE values in Figures 9B and 9D are lower than the Kim/Kreevoy criteria of H/D KIE of 7.⁵⁷ Thus Figure 9B is showing the predicted H/D KIE including SCT tunneling range from 1.35 at 500 K to 2.29 at 175 K. In Figure 9C, the ¹²C/¹³C isotope effects range from 1.010 at 500 K to 1.070 at 175 K. In Figure 9D the H/D KIEs range from 1.030 at 500 K to 1.128 at 175 K. The curvature, if observed experimentally, would be consistent with tunneling, in which the loss of the acid C=O stretch $\sim 1700 \text{ cm}^{-1}$ and H–O stretch $\sim 3500 \text{ cm}^{-1}$ in **4E** could be trackable by matrix isolation IR spectroscopy.²¹ Table S8 (Supporting Information) shows more details of the computed temperature dependence on k_{CVT} , $k_{\text{CVT/SCT}}$ and half-lives, $t_{1/2}$.

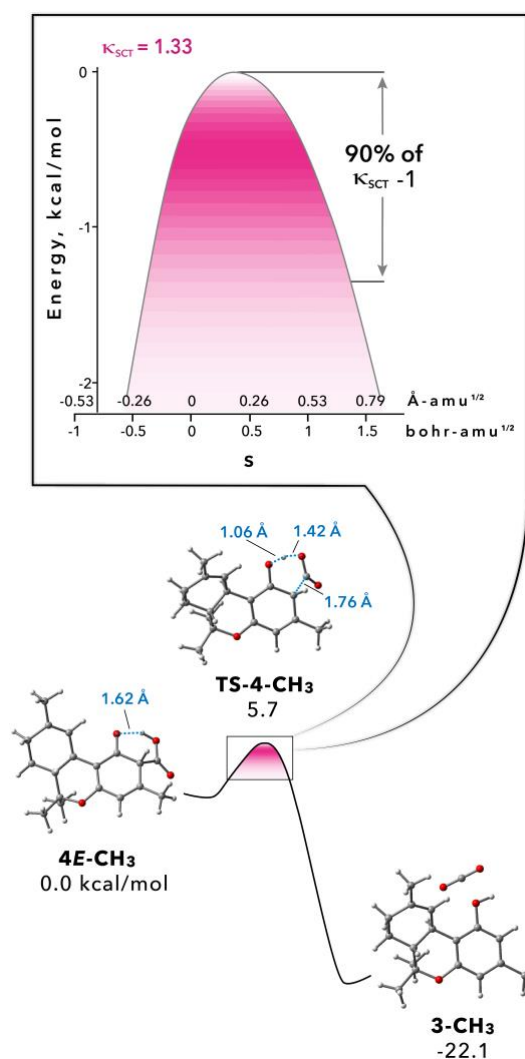


Figure 10. Tunneling contribution in the PES for the decarboxylation of **4E-CH₃** to **3-CH₃**.

Mechanism

Results from our DFT calculations provide evidence for tunneling in the conversion of the quinone methide **1Z-CH₃** to **3-CH₃**. This reaction models the **1Z** formed from CBGA, to reach THC **3**. The initial formation of **1Z-CH₃** is important, as we do not find a computationally viable route to **2Z-CH₃** by a simultaneous deprotonation of C(6)-OH and C(7)-hydride uptake, and subsequent base deprotonation of C(2)-OH and ring closure.⁵⁸ Instead, computations point to a Diels-Alder reaction in **1Z** between the quinone methide moiety and the trisubstituted alkene end-

group of the geranyl substituent. What is key is that the entire reaction sequence to THC **3** contains significant amounts of tunneling (Figures 5-10).

Let us highlight our computed results at 293 K for steps 1, 3, and 4, and at 383 K for step 2 as they are experimentally relevant. Our results show that (i) tunneling for the Diels-Alder reaction of **1Z-CH₃** to **2Z-CH₃** is 19%. (ii) For results at 383.15 K (relevant to burning condition), tunneling in the formic acid-catalyzed keto-enol tautomerization of **2rZ-CH₃-HCOOH** to **4rZ-CH₃-HCOOH** is 59%. (iii) Tunneling contribution to the rotamerization of **4rZ-CH₃** to **4E-CH₃** is 61%. Finally, (iv) tunneling of **4Z-CH₃** to **3-CH₃** with CO₂ expulsion is 25%.

According to Figure 4, the formic acid catalyzed keto-enol tautomerization proceeds largely through the higher energy conformer **2rZ-HCOOH**, which leads to the lower energy transition state. Depending on conditions, experimental results on decarboxylation of THCA **2** show an E_a range of 21.0 to 14.0 kcal/mol.³³⁻³⁵ Our theoretical results show an effective E_a of 15.8 kcal/mol with inclusion of tunneling for the formic acid-catalyzed keto-enol tautomerization of **2rZ-CH₃-HCOOH** to **4rZ-CH₃-HCOOH**. Important facets include, a decrease of H/D KIE with increasing temperature running from 34.0 at 200K to about 4.86 at 500K. The lower temperature region of the Arrhenius plot fulfills the Kim/Kreevoy tunneling criteria of H/D KIE of > 7 .⁵⁷ Notice that Figure 6 features a hidden intermediate, which is noteworthy as a result of the second maximum in the V_a^G . The part of the curve from 0.48 – 0.72 Å-amu^{1/2} has a single imaginary transverse frequency ($3i - 23i$ cm⁻¹). However, since the top of the small barrier occurred with all positive transverse frequencies we know that is not an artifact but a true second barrier. The changes in the transverse frequencies along the path create a temperature dependent free energy minimum shown in Figure 6B. This effect of zero-point energy (ZPE) creating a minimum, was first noted by Manz, Meyer, Pollak, and Römelt in a collinear triatomic case,⁵⁹ and by Singleton

et al. in polyatomic case.⁶⁰ We also applied the method of Kraka and Cremer⁶¹ by plotting the scalar curvature of the reaction path vs. s (reaction progress). Both, the V_a^G energy based method and the Kraka/Cremer reaction path curvature method predict a hidden intermediate in the same region of the path (cf. Figure 6 with Figure S18 in the Supporting Information). Tunneling contributions through a ZPE induced minimum are computed here for the first time. In Figure 7, the downward curvature of the red KIE curve with inclusion of tunneling could be experimentally observable and is specifically tied to the large widening of the V_a^G at low energy due to a ZPE-induced hidden intermediate.

Lastly, interrogating the mechanism of THCA **2** decarboxylation not only pointed to the carbon and hydrogen tunneling, a hidden intermediate, but also the formation of **1** for a Diels-Alder reaction with the geranyl terminus. There is growing appreciation of Diels-Alderase processes⁶² and our computed results also provide information of relevance to such processes. It is conceivable, but was not studied on our part, that THCA synthase also lowers the barrier in a synergistic manner with tunneling in formation of THCA **2**. Differences between uncatalyzed and enzyme-catalyzed decarboxylation of orotic acid mechanisms have been reported.⁶³

Conclusion

Measurements of the rate of the THC **3** formation have been carried out in the solid state,³⁴ cannabis extracts,³⁵ and in the ground-dried hemp plant.³³ Given this heterogeneous set of experimental conditions for rate measurements to date, we sought a realistic prediction of the rates and product ratios under a uniform set of conditions. The calculation includes quantum corrections for all degrees of freedom including the reaction coordinate (i.e., tunneling). In this calculation we are the first to evaluate multidimensional tunneling in the biosynthesis of THC **3**. At 293 K, the

percent of the rate due to tunneling in Steps 1–4 is 19% for the Diels-Alder, 76% for tautomerization, 61% for OH rotamerization, and 25% for decarboxylation. Tunneling is clearly an important facet of the mechanism of these reactions.

The tautomerization step gave two unexpected results. First, nonuniform changes in the ZPE of the transverse modes, brought about by changes in the reaction path direction, led to a small secondary maximum in the V_a^G curve (Figure 6A) with an associated minimum next to it. This minimum is a *hidden intermediate* in the sense that it does not appear as a minimum in the classical potential energy curve (the MEP) but is built from ZPE alone. Second, the presence of an additional maximum in the V_a^G curve created a very broad low-energy region of the barrier for tautomerization. This had a large effect on tunneling and the temperature dependence of KIEs for this reaction, leading to a negatively curved plot of $\ln(\text{KIE})$ vs. $1000/T$ (Figure 7), in which the curved region extends over the entire computed region, 200–500 K. The Arrhenius plot curvature is therefore readily observable, and thus would be detectable experimentally, in contrast to the frequent situation where curvature is observable only at very low temperature, which is a facet relevant for bioorganic chemists in the synthesis of THC **3**. We also note that keto-enol tautomerization occurs in many biological reactions, and the unusual features of Figures 6 and 7 may well be present in some of these and are under active examination by our groups.

Computational Section

Density functional theory (DFT) calculations were performed with the Gaussian 16 program³⁸ using the B3LYP functional^{39,40} with a 6-31G(d,p) basis set.⁶⁴ Tunneling calculations were carried out using POLYRATE²⁶ with a GAUSSRATE³⁷ interface to Gaussian 16. The reason for using B3LYP/6-31G(d,p) was threefold: (i) tunneling calculations are resource intensive for

compounds of the size of **1** and related compounds. B3LYP has been shown to be an efficient functional in tunneling calculations by Vetticatt and Singleton.²³ (ii) B3LYP has been applied successfully in many pericyclic systems, including Diels-Alder reactions to which experimental data is available for comparison.⁶⁵ (iii) There is successful application in cases like nitroso-substituted Bergman system⁶⁶ where experimental data is sparse or lacking altogether, which is the case here due to obvious reasons that THC **3** is a schedule I drug and experimental research on it is severely restricted.

Benchmarking was carried out to go beyond B3LYP/6-31G(d,p) and include dispersion and basis set extension with B3LYP-D3, wB97XD, and M06-2X, and the basis set, 6-311+G(d,p). Benchmarking DFT results indicate that the B3LYP/6-31G(d,p) results are very satisfactory in terms of similar barrier heights and widths in the series (see Figure S17 and Table S12, Supporting Information). We had elected to examine step 2 for the benchmarking since it is a key step and represents the RDS, in which decarboxylation arises from to eventually reach THC.

Stationary points on the potential energy surface were characterized as minima or saddle points by frequency analysis. Transition states (TSs) were located variationally based on a free energy criterion using canonical variational theory (CVT) in POLYRATE.²⁷ This involves computing the minimum energy path (MEP), which is the steepest descent path in mass-weighted coordinates connecting the TS with reactant and product. (The MEP is also known as the intrinsic reaction coordinate or IRC.)⁶⁷ Since an accurate MEP is needed for tunneling, DFT calculations were carried out with the integration grid equal to “SuperFineGrid”.

The barrier through which tunneling occurs is the vibrationally adiabatic ground-state curve, V_a^G , defined as the MEP plus the vibrational zero-point energy (ZPE). To generate the MEP we used the Page–McIver algorithm [RPM = pagem]⁶⁸ with a step size of 0.001 Å-amu^{1/2} and

Hessian calculations every 10 steps. Generalized normal mode analyses were carried out in Cartesian coordinates. Rate constants were computed using canonical variational transition state theory (CVT)²⁷ together with the small curvature tunneling (SCT) method.²⁸⁻³⁰

Figure 3 shows B3LYP/6-31G(d,p) free energies at stationary points for the entire sequence from **1** to **3**. Tunneling is not included. A detailed examination of pentyl conformers in Figure 3 was not carried out. Figures 5, 6, 8 and 10 show the energy regions of the B3LYP V_a^G curves, over which tunneling contributes to κ_{SCT} at 293 K or 383 K. The color intensity of each energy slice is proportional to its contribution to κ_{SCT} . The contribution of tunneling to the rate can be estimated as $(\kappa_{\text{SCT}} - 1)/\kappa_{\text{SCT}}$. This represents the fraction of the rate contributed by quantum correction to motion along the reaction path, which includes transmission and reflection. For a barrier height $\Delta E^\ddagger$, the contribution to κ_{SCT} at energy $\Delta E^\ddagger - \epsilon$ is computed as the difference between the Boltzmann-weighted transmission probability at energy $\Delta E^\ddagger - \epsilon$ and the Boltzmann-weighted reflection probability at energy $\Delta E^\ddagger + \epsilon$. The latter are important only at energies just above $\Delta E^\ddagger$ because of the Boltzmann factors. As a result, transmission and reflection cancel near the top of the barrier as shown by the white space in Figures 5, 6A, 8, and 10. In addition, free energy curves were computed for the formic acid catalyzed step 2 (Figure 6B), based on harmonic partition functions of transverse frequencies along the minimum energy path (MEP) computed by POLYRATE.

The small minimum in Figure 6A can lead to recrossings in the classical dynamics of step 2 that slow the reaction. We checked this with canonical unified statistical theory (CUS),⁶⁹ which accounts for recrossing, and the effect is small. The classical rate constant k_{SCT} is reduced by 1% at 383 K and by 0.3% at 313 K. Tunneling is not affected by classical recrossing.

Acknowledgements

EMG, VS, and AS acknowledge support from the National Science Foundation (NSF) (CHE-1956098) and PSC-CUNY (63367-00 51-A). AG also acknowledges support from the NSF (CHE-1856765). This work used Comet and Expanse, the Extreme Science and pEngineering Discovery Environment (XSEDE) clusters at the San Diego Supercomputer Center, which are supported by the NSF (ACI-1548562) through allocations TG-CHE180060 (EMG) and CHE200050 (AG). We thank Leda Lee for help with the graphic arts work.

Supporting Information

The Supporting Information is available free of charge on the ACS Publication website: Calculated structures and energies of species involved in reported reactions, reaction energetics written by POLYRATE for each step 1-4, transmission coefficients $\kappa_{ZCT}(T)$ and $\kappa_{SCT}(T)$, rate constants, half-lives for each step 1-4, Arrhenius plots for each step 1-4, tunneling paths (vibrationally adiabatic ground-state curve, V_a^G) and tunneling contributions to the SCT transmission coefficient κ_{SCT} for formic acid catalyzed tautomerization of **2Z-CH₃-HCOOH** at 383 K, results of benchmarking DFT calculations, scalar curvature analysis for formic acid catalyzed tautomerization of **2rZ-CH₃-HCOOH**, and sample input files for POLYRATE calculations.

Author Information

Corresponding Author

Edyta M. Greer – Department of Natural Sciences, Baruch College of the City University of

New York, 17 Lexington Avenue, New York, NY 10010, United States; orcid.org/0000-0003-4283-0523

Charles Doubleday:

Department of Chemistry, Columbia University, 3000 Broadway, MC 3142, New York, NY 10027, United States; orcid.org/0000-0003-2702-438X

Author

Victor Siev – Department of Natural Sciences, Baruch College of the City University of New York, 17 Lexington Avenue, New York, NY 10010, United States; orcid.org/0000-0002-3166-9650

Ayelet Segal – Department of Natural Sciences, Baruch College of the City University of New York, 17 Lexington Avenue, New York, NY 10010, United States; orcid.org/0000-0001-8553-0841

Alexander Greer – Department of Chemistry and Graduate Center, Brooklyn College of the City University of New York, 2900 Bedford Avenue, Brooklyn, NY 11210, and PhD Program in Chemistry, The Graduate Center of the City University of New York, New York 10016, United States; orcid.org/0000-0003-4444-9099

References

- [1] Bell, R. P. *The Tunnel Effect in Chemistry*. Chapman and Hall, London, **1980**.

- [2] Greer, E. M.; Kwon, K.; Greer, A.; Doubleday, C. Thermally Activated Tunneling in Organic Reactions. *Tetrahedron* **2016**, *72*, 7357–7373.
- [3] Borden, W. T. Reactions that Involve Tunneling by Carbon and the Role that Calculations have Played in their Study. *WIREs Comput. Mol. Sci.* **2016**, *6*, 20–46.
- [4] Schreiner, P. R. Tunneling Control of Chemical Reactions: The Third Reactivity Paradigm. *J. Am. Chem. Soc.* **2017**, *139*, 15276–15283.
- [5] Schreiner, P. R. Quantum Mechanical Tunneling is Essential to Understanding Chemical Reactivity. *Trends Chem.* **2020**, *2*, 980–989.
- [6] Castro, C.; Karney, W. L. Heavy-Atom Tunneling in Organic Reactions. *Angew. Chem. Int. Edit.* **2020**, *59*, 8355–8366.
- [7] McMahon, R. J. Chemistry: Chemical Reactions Involving Quantum Tunneling. *Science* **2003**, *299*, 833–834.
- [8] Tejero, I.; González-García, N.; González-Lafont, À.; Lluch, J. M. I. Tunneling in Green Tea: Understanding the Antioxidant Activity of Catechol-Containing Compounds. A Variational Transition-State Theory Study. *J. Am. Chem. Soc.* **2007**, *129*, 5846–5854.
- [9] Meyer, M. P.; Klinman, J. P. Investigating Inner-Sphere Reorganization via Secondary Kinetic Isotope Effects in the C–H Cleavage Reaction Catalyzed by Soybean Lipxygenase: Tunneling in the Substrate Backbone as Well as the Transferred Hydrogen. *J. Am. Chem. Soc.* **2011**, *133*, 430–439.
- [10] Roston, D.; Kohen, A. A Critical Test of the “Tunneling and Coupled” Concept in Enzymatic Alcohol Oxidation. *J. Am. Chem. Soc.* **2013**, *135*, 13624–13627.

- [11] Cheng, L.; Doubleday, C.; Breslow, R. Evidence for Tunneling in Base-catalyzed Isomerization of Glyceraldehyde to Dihydroxyacetone by Hydride Shift under Formose Conditions. *PNAS* **2015**, *112*, 4218–4220.
- [12] Ciągła, P.; Fita, P.; Listkowski, A.; Radzewicz, C.; Waluk, J. Evidence for Dominant Role of Tunneling in Condensed Phases and at High Temperatures: Double Hydrogen Transfer in Porphycene. *J. Phys. Chem. Lett.* **2016**, *7*, 283–288.
- [13] Doubleday, C.; Armas, R.; Walker, D.; Cosgriff, C. V.; Greer, E. M. Heavy-Atom Tunneling Calculations in Thirteen Organic Reactions: Tunneling Contributions are Substantial, and Bell's Formula Closely Approximates Multidimensional Tunneling at ≥ 250 K. *Angew. Chem. Int. Ed.* **2017**, *56*, 13099–13102.
- [14] Nunes, C. M.; Eckhardt, A. K.; Reva, I.; Fausto, R.; Schreiner, P. R. Competitive Nitrogen versus Carbon Tunneling. *J. Am. Chem. Soc.* **2019**, *141*, 14340–14348.
- [15] Karmakar, S.; Datta, A. Tunneling Control: Competition between 6π -Electrocyclization and [1,5]H-Sigmatropic Shift Reactions in Tetrahydro-1*H*-cyclobuta[*e*]indene Derivatives. *J. Org. Chem.* **2017**, *82*, 1558–1566.
- [16] Zuev, P. S.; Sheridan, R. S.; Albu, T. V.; Truhlar, D. G.; Hrovat, D. A.; Borden, W. T. Carbon Tunneling from a Single Quantum State. *Science* **2003**, *299*, 867–870.
- [17] Michel, C. S.; Lampkin, P. P.; Shezaf, J. Z.; Moll, J. F.; Castro, C.; Karney, W. L. Tunneling by 16 Carbons: Planar Bond Shifting in [16]Annulene. *J. Am. Chem. Soc.* **2019**, *141*, 5286–5293.
- [18] Greer, E. M.; Cosgriff, C. V.; Doubleday, C. Computational Evidence for Heavy-Atom Tunneling in the Bergman Cyclization of a 10-Membered-Ring Eneidyne. *J. Am. Chem. Soc.* **2013**, *135*, 10194–10197.

- [19] Henkel, S.; Huynh, Y.; Neuhaus, P.; Winkler, M.; Sander, W. Tunneling Rearrangement of 1-Azulenylcarbene. *J. Am. Chem. Soc.* **2012**, *134*, 13204–13207.
- [20] Schreiner, P. R.; Reisenauer, H. P.; Ley, D.; Gerbig, D.; Wu, C.-H.; Allen, W. D. Methylhydroxycarbene: Tunneling Control of a Chemical Reaction. *Science* **2011**, *332*, 1300–1303.
- [21] Schreiner, P. R.; Wagner, J. P.; Reisenauer, H. P.; Gerbig, D.; Ley, D.; Sarka, J.; Császár, A. G.; Vaughn, A.; Allen, W. D. Domino Tunneling. *J. Am. Chem. Soc.* **2015**, *137*, 7828–7834.
- [22] Gonzalez-James, O. M.; Zhang, X.; Datta, A.; Hrovat, D. A.; Borden, W. T.; Singleton, D. A. Experimental Evidence for Heavy-Atom Tunneling in the Ring-Opening of Cyclopropylcarbonyl Radical from Intramolecular $^{12}\text{C}/^{13}\text{C}$ Kinetic Isotope Effects. *J. Am. Chem. Soc.* **2010**, *132*, 12548–12549.
- [23] Vetticatt, M. J.; Singleton, D. A. Isotope Effects and Heavy-Atom Tunneling in the Roush Allylboration of Aldehydes. *Org. Lett.* **2012**, *14*, 2370–2373.
- [24] Nandi, A.; Gerbig, D.; Schreiner, P. R.; Weston, T.; Kozuch, S. Isotope-Controlled Selectivity by Quantum Tunneling: Hydrogen Migration versus Ring Expansion in Cyclopropylmethylcarbenes. *J. Am. Chem. Soc.* **2017**, *139*, 9097–9099.
- [25] Gaoni, Y.; Mechoulam, R. Isolation, Structure, and Partial Synthesis of an Active Constituent of Hashish. *J. Am. Chem. Soc.* **1964**, *86*, 1646–1647.
- [26] Zheng, J.; Zhang, S.; Lynch, B. J.; Corchado, J. C.; Chuang, Y.-Y.; Fast, P. L.; Hu, W.-P.; Liu, Y.-P.; Lynch, G. C.; Nguyen, K. A.; Jackels, C. F.; Ramos, A. F.; Ellingson, B. A.; Melissas, V. S.; Villà, J.; Rossi, I.; Coitiño, E. L.; Pu, J.; Albu, T. V.; Steckler, R.; Garrett, B. C.; Isaacson, A. D.; Truhlar, D. G. POLYRATE, Version 2010-A; University of Minnesota: Minneapolis, MN, 2010.

- [27] Truhlar, D. G.; Garrett, B. C. Variational Transition-State Theory. *Acc. Chem. Res.* **1980**, *13*, 440–448.
- [28] Skodje, R. T.; Truhlar, D. G.; Garrett, B. C. A General Small-Curvature Approximation for Transition-State-Theory Transmission Coefficients. *J. Phys. Chem.* **1981**, *85*, 3019–3023.
- [29] Lu, D.-H.; Truong, T. N.; Melissas, V. S.; Lynch, G. C.; Liu, Y.-P.; Garrett, B. C.; Steckler, R.; Isaacson, A. D.; Rai, S. N.; Hancock, G. C.; Lauderdale, J. G.; Joseph, T.; Truhlar, D. G. Polyrate 4: A New Version of a Computer Program for the Calculation of Chemical Reaction Rates for Polyatomics. *Comput. Phys. Commun.* **1992**, *71*, 235–262.
- [30] Liu, Y.-P.; Lynch, G. C.; Truong, T. N.; Lu, D.-H.; Truhlar, D. G.; Garrett, B. C. Molecular Modeling of the Kinetic Isotope Effect for the [1,5]-Sigmatropic Rearrangement of *cis*-1,3-Pentadiene. *J. Am. Chem. Soc.* **1993**, *115*, 2408–2415.
- [31] Shoyama, Y.; Tamada, T.; Kurihara, K.; Takeuchi, A.; Taura, F.; Arai, S.; Blaber, M.; Shoyama, Y.; Morimoto, S.; Kuroki, R. Structure and Function of Δ^1 -Tetrahydrocannabinolic Acid (THCA) Synthase, the Enzyme Controlling the Psychoactivity of *Cannabis sativa*. *J. Mol. Biol.* **2012**, *423*, 96–105.
- [32] Yamauchi, T.; Shoyama, Y.; Aramaki, H.; Azuma, T.; Nishioka, I. Tetrahydrocannabinolic Acid, a Genuine Substance of Tetrahydrocannabinol. *Chem. Pharm. Bull.* **1967**, *15*, 1075–1076.
- [33] Moreno, T.; Dyer, P.; Tallon, S. Cannabinoid Decarboxylation: A Comparative Kinetic Study. *Ind. Eng. Chem. Res.* **2020**, *59*, 20307–20315.
- [34] Perrotin-Brunel, H.; Buijs, W.; van Spronsen, J.; van Roosmalen, M. J. E.; Peters, C. J.; Verpoorte, R.; Witkamp, G.-J. Decarboxylation of Δ^9 -Tetrahydrocannabinol: Kinetics and Molecular Modeling. *J. Mol. Struct.* **2011**, *987*, 67–73.

- [35] Wang, M.; Wang, Y.-H.; Avula, B.; Radwan, M. M.; Wanas, A. S.; van Antwerp, J.; Parcher, J. F.; ElSohly, M. A.; Khan, I. A. Decarboxylation Study of Acidic Cannabinoids: A Novel Approach Using Ultra-High-Performance Supercritical Fluid Chromatography/Photodiode Array-Mass Spectrometry. *Cannabis Cannabinoid Res.* **2016**, *1*, 262–271.
- [36] He, W.; Foth, P. J.; Roggen, M.; Sammis, G. M.; Kennepohl, P. Why is THCA Decarboxylation Faster than CBDA? An in silico Perspective. Submission Date: September 3, **2020**, *ChemRxiv*. URL: <https://chemrxiv.org/engage/chemrxiv/article-details/60c74f7f469df4b057f446d0> (accessed 2022-04-05)
- [37] Zheng, J.; Zhang, S.; Corchado, J. C.; Chuang, Y.-Y.; Coitiño, E. L.; Ellingson, B. A.; Truhlar, D. G. GAUSSRATE, Version 2009-A; University of Minnesota: Minneapolis, MN, 2009.
- [38] Gaussian 16, Revision C.01, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; Li, X.; Caricato, M.; Marenich, A. V.; Bloino, J.; Janesko, B. G.; Gomperts, R.; Mennucci, B.; Hratchian, H. P.; Ortiz, J. V.; Izmaylov, A. F.; Sonnenberg, J. L.; Williams-Young, D.; Ding, F.; Lipparini, F.; Egidi, F.; Goings, J.; Peng, B.; Petrone, A.; Henderson, T.; Ranasinghe, D.; Zakrzewski, V. G.; Gao, J.; Rega, N.; Zheng, G.; Liang, W.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Throssell, K.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J. J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Keith, T. A.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Millam, J. M.; Klene, M.; Adamo, C.; Cammi, R.; Ochterski, J. W.;

- Martin, R. L.; Morokuma, K.; Farkas, O.; Foresman, J. B.; Fox, D. J. Gaussian, Inc., Wallingford CT, 2016.
- [39] Becke, A. D. Density-Functional Thermochemistry. III. The Role of Exact Exchange. *J. Chem. Phys.* **1993**, *98*, 5648–5652.
- [40] Lee, C.; Yang, W.; Parr, R. G. Development of the Colle-Salvetti Correlation-Energy Formula into a Functional of the Electron Density *Phys. Rev. B* **1988**, *37*, 785–789.
- [41] Albu, T. V.; Lynch, B. J.; Truhlar, D. G.; Goren, A. C.; Hrovat, D. A.; Borden, W. T.; Moss, R. A. Dynamics of 1,2-Hydrogen Migration in Carbenes and Ring Expansion in Cyclopropylcarbenes. *J. Phys. Chem. A* **2002**, *106*, 5323–5338.
- [42] Nandi, A.; Solel, E.; Kozuch, S. Carbon Tunneling in the Automerization of Cyclo[18]carbon. *Chem. Eur. J.* **2020**, *26*, 625–628.
- [43] Arbitman, J. K.; Michel, C. S.; Castro, C.; Karney, W. L. Calculations Predict That Heavy-Atom Tunneling Dominates Möbius Bond Shifting in [12]- and [16]Annulene. *Org. Lett.* **2019**, *21*, 8587–8591.
- [44] Kuan, K.-Y.; Singleton, D. A. Vibrationally Hot and Cold Triplets. Sensitizer-Dependent Dynamics and Localized Vibrational Promotion of a Di- π -methane Rearrangement. *J. Am. Chem. Soc.* **2020**, *142*, 19885–19888.
- [45] Liu, Y.-P.; Lynch, G. C.; Truong, T. N.; Lu, D.-H.; Truhlar, D. G.; Garrett, B. C. Molecular Modeling of the Kinetic Isotope Effect for the [1,5]-Sigmatropic Rearrangement of *cis*-1,3-Pentadiene. *J. Am. Chem. Soc.* **1993**, *115*, 2408–2415.
- [46] Ertelt, M.; Hrovat, D. A.; Borden, W. T.; Sander, W. Heavy-Atom Tunneling in the Ring Opening of a Strained Cyclopropene at Very Low Temperatures. *Chem. Eur. J.* **2014**, *20*, 4713–4720.

- [47] Datta, A.; Hrovat, D. A.; Borden, W. T. Calculations Predict Rapid Tunneling by Carbon from the Vibrational Ground State in the Ring Opening of Cyclopropylcarbinyl Radical at Cryogenic Temperatures. *J. Am. Chem. Soc.* **2008**, *130*, 6684–6685.
- [48] Sedgi, I.; Kozuch, S. Heavy-Atom Tunneling in Cu(II)N₆ Complexes: Theoretical Predictions and Experimental Manifestation. *Chem. Sci.* **2020**, *11*, 2828–2833.
- [49] Solel, E.; Kozuch, S. Tuning the Spin, Aromaticity, and Quantum Tunneling in Computationally Designed Fulvalenes. *J. Org. Chem.* **2018**, *83*, 10826–10834.
- [50] Mardyukov, A.; Quanz, H.; Schreiner, P. R. Conformer-Specific Hydrogen Atom Tunneling in Trifluoromethyl Hydroxycarbene. *Nat. Chem.* **2017**, *9*, 71–76.
- [51] Singleton, D. A.; Thomas, A. A. High-Precision Simultaneous Determination of Multiple Small Kinetic Isotope Effects at Natural Abundance. *J. Am. Chem. Soc.* **1995**, *117*, 9357–9358.
- [52] Aziz, H. R.; Singleton, D. A. Concert along the Edge: Dynamics and the Nature of the Border between General and Specific Acid–Base Catalysis. *J. Am. Chem. Soc.* **2017**, *139*, 5965–5972.
- [53] Maçôas, E. M. S.; Khriachtchev, L.; Pettersson, M.; Fausto, R.; Räsänen, M. Rotational Isomerism in Acetic Acid: The First Experimental Observation of the High-Energy Conformer. *J. Am. Chem. Soc.* **2003**, *125*, 16188–16189.
- [54] Amiri, S.; Reisenauer, H. P.; Schreiner, P. R. Electronic Effects on Atom Tunneling: Conformational Isomerization of Monomeric *Para*-Substituted Benzoic Acid Derivatives. *J. Am. Chem. Soc.* **2010**, *132*, 15902–15904.

- [55] Nunes, C. M.; Knezz, S. N.; Reva, I.; Fausto, R.; McMahon, R. J. Evidence of a Nitrene Tunneling Reactions: Spontaneous Rearrangement of 2-Formyl Phenylnitrene to Imino Ketene in Low-Temperature Matrixes. *J. Am. Chem. Soc.* **2016**, *138*, 15287–15290.
- [56] Eckhardt, A. K.; Gerbig, D.; Schreiner, P. R. Heavy Atom Secondary Kinetic Isotope Effects on H-Tunneling. *J. Phys. Chem. A* **2018**, *122*, 1488–1495.
- [57] Kim, Y.; Kreevoy, M. M. The Experimental Manifestations of Corner-Cutting Tunneling. *J. Am. Chem. Soc.* **1992**, *114*, 7116–7123.
- [58] Tan, M.-C.; Zou, Y.; Watanabe, K.; Walsh, C. T. Tang, Y. Oxidative Cyclization in Natural Product Biosynthesis. *Chem. Rev.* **2017**, *117*, 5226–5333.
- [59] Manz, J.; Meyer, R.; Pollak, E.; Römelt, J. A New Possibility of Chemical Bonding: Vibrational Stabilization of IHI. *Chem. Phys. Lett.* **1982**, *93*, 184–187.
- [60] Gonzalez-James, O. M. Kwan, E. E.; Singleton, D. A. Entropic Intermediates and Hidden Rate-Limiting Steps in Seemingly Concerted Cycloadditions. Observation, Prediction, and Origin of an Isotope Effect on Recrossing. *J. Am. Chem. Soc.* **2012**, *134*, 1914–1917.
- [61] Kraka, E.; Cremer, D. Computational Analysis of the Mechanism of Chemical Reactions in Terms of Reaction Phases: Hidden Intermediates and Hidden Transition States. *Acc. Chem. Res.* **2010**, *43*, 591–601.
- [62] Klas, K.; Tsukamoto, S.; Sherman, D. H.; Williams, R. M. Natural Diels–Alderase: Elusive and Irresistible. *J. Org. Chem.* **2015**, *80*, 11672–11685.
- [63] Singleton, D. A.; Merrigan, S. R.; Kim, B. J.; Beak, P.; Lee, J. K. ¹³C Kinetic Isotope Effects and the Mechanism of the Uncatalyzed Decarboxylation of Orotic Acid. *J. Am. Chem. Soc.* **2000**, *122*, 3296–3300.

- [64] Hariharan, P. C.; Pople, J. A. The Influence of Polarization Functions on Molecular Orbital Hydrogenation Energies. *Theor. Chim. Acta.* **1973**, 28, 213–222.
- [65] Birney, D. M.; Lim, T. K.; Koh, J. H.; Pool, B. R.; White, J. M. Structural Investigations into the *retro*-Diels-Alder Reaction. Experimental and Theoretical Studies. *J. Am. Chem. Soc.* **2002**, 124, 5091–5099.
- [66] Greer, E. M.; Lavinda, O. Theoretical Study of the Bergman Cyclization of 2,3-Diethynyl-1-nitrotropylium Ion: Formation of a Nitroxide Radical Amenable to EPR Detection for Biological Applications. *J. Org. Chem.* **2010**, 75, 8650–8653.
- [67] Fukui, K. The Path of Chemical Reactions - the IRC Approach. *Acc. Chem Res.* **1981**, 14, 363–368.
- [68] Page, M.; McIver, J. W., Jr On Evaluating the Reaction Path Hamiltonian *J. Chem. Phys.* **1988**, 88, 922–935.
- [69] Garrett, B. C.; Truhlar D. G. Canonical Unified Statistical Model. Classical Mechanical Theory and Applications to Collinear Reactions. *J. Chem. Phys.* **1982**, 76, 1853-1858.