

# Computational Study on the Reaction Mechanism of the Key Thermal [4 + 4] Cycloaddition Reaction in the Biosynthesis of Epoxytwinol A

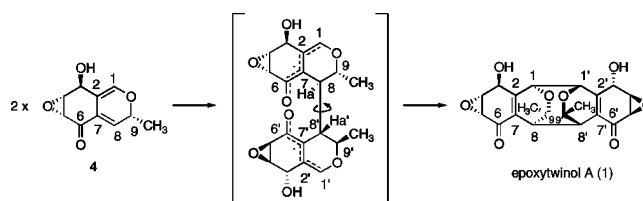
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## ABSTRACT



The key [4 + 4] cycloaddition in the biosynthesis of epoxytwinol A has been established by theoretical calculations to comprise of three processes. The first step is formation of the C8–C8' bond generating a biradical intermediate. Next, rotation about the C8–C8' bond occurs, and finally the C1–C1' bond is formed. Biradicals stabilized by conjugation and two hydrogen bonds are essential for realization of this rare thermal [4 + 4] cycloaddition.

The [4 + 4] cycloaddition reaction<sup>1</sup> generally proceeds under photoirradiation or in the presence of transition-metal catalysts,<sup>2</sup> and genuine thermal [4 + 4] cycloadditions are predicted to be difficult by the Woodward–Hoffmann rules.<sup>3</sup> However, a few exceptional thermal [4 + 4] cycloaddition reactions are known: Highly reactive *o*-quinodimethane and

its derivatives<sup>4</sup> dimerize, while *o*-quinodimethane<sup>5</sup> and corrole<sup>6</sup> react with anthracene or pentacene in a [4 + 4] manner. The dimerization has been proposed to proceed via a biradical mechanism,<sup>4b,e,f</sup> while the mechanism of the latter two reactions has not been investigated in detail.

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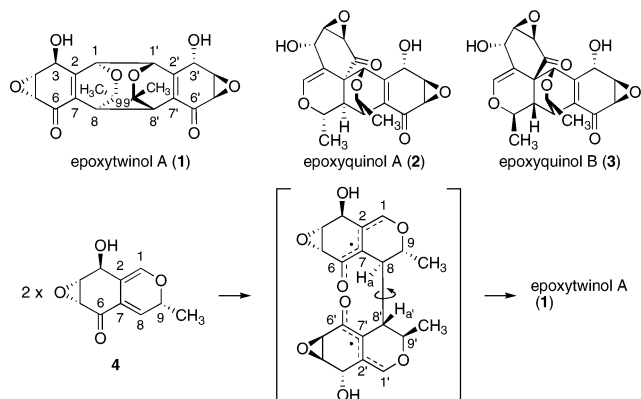
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We have recently isolated a novel pentaketide dimer and angiogenesis inhibitor epoxytwinol A (**1**)<sup>7</sup> from a fungus which also produces structurally different angiogenesis inhibitors such as epoxyquinols A (**2**)<sup>8</sup> and B (**3**) (Figure 1).<sup>9</sup> The latter are thought to be biosynthesized from a 2*H*-



**Figure 1.** Epoxytwinol A (**1**), epoxyquinols A (**2**) and B (**3**), and the reaction path to epoxytwinol A.

pyran monomer by endo and exo Diels–Alder reaction, respectively, while epoxytwinol A (**1**) would be generated by [4 + 4] cycloaddition reaction of the monomer **4**. Epoxytwinol A (**1**) is found to convert gradually into epoxyquinol B (**3**) at room temperature. Recently, we have synthesized all these compounds by biomimetic pathways from **4**,<sup>10</sup> with epoxytwinol A (**1**) being prepared in the dark, indicating that the [4 + 4] cycloaddition proceeds thermally without photoactivation. Li and Porco have also elegantly synthesized epoxytwinol A (**1**) using alkoxy-silanol activation.<sup>10j,11</sup> Epoxytwinol A (**1**) is the first natural product believed to be biosynthesized via the exceptionally rare thermal [4 + 4] cycloaddition, and the mechanism of this reaction is of great interest. In this paper, we disclose the results of our theoretical calculations on this process and on the mechanism of transformation of epoxytwinol A (**1**) to epoxyquinol B (**3**).

In view of the Woodward–Hoffmann rules,<sup>3</sup> a stepwise rather than concerted mechanism is expected for a thermal

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[4 + 4] cycloaddition reaction. Indeed, stepwise biradical mechanisms have been proposed for the [4 + 4] dimerization of *o*-quinodimethane and its derivatives.<sup>4b,e,f</sup> The density functional B3LYP treatment with the unrestricted formalism (UB3LYP)<sup>12</sup> provides relatively reasonable results for the energy of biradical or biradicaloid species, and the UB3LYP method<sup>13</sup> has been successfully employed to investigate reaction paths involving biradical species or to compare the energetics for stepwise, biradical reaction paths with those for concerted, closed-shell paths.<sup>14</sup> We therefore employed this method in conjunction with the 6-31G(d) basis set to locate the stationary points along the reaction coordinate for the [4 + 4] cycloaddition reaction.<sup>15</sup>

Unrestricted B3LYP solutions were obtained from a HOMO–LUMO mixed initial guess. For the reactant monomer **4**, and the reaction products **1** through **3**, as well as for the reactant complexes, the eigenvalues of *S*<sup>2</sup> for UB3LYP calculations were zero, and the total energies were exactly the same as obtained with the corresponding restricted

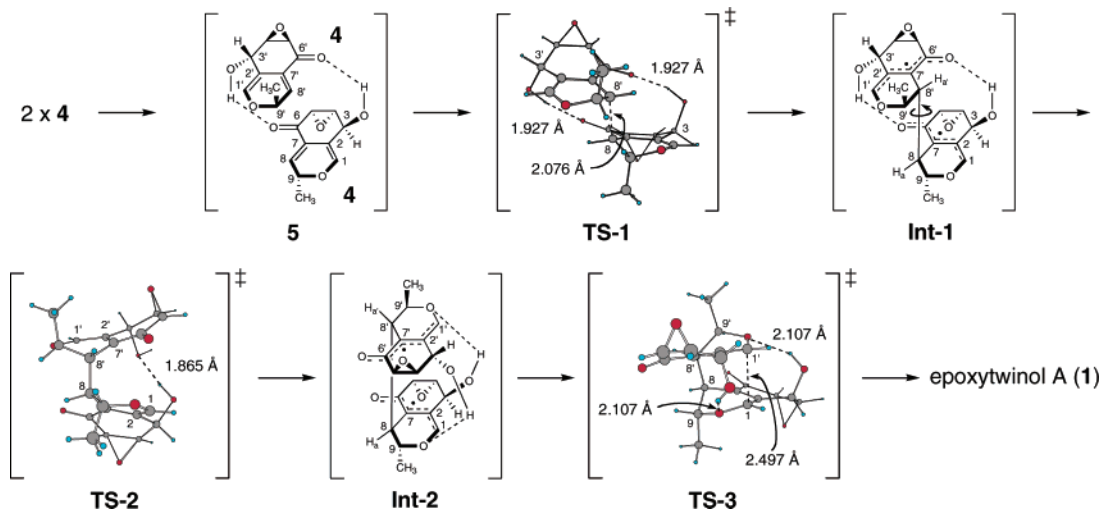
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**Scheme 1.** Reaction Intermediates and Transition States of the Thermal [4 + 4] Cycloaddition of **4** in the Synthesis of Epoxytwinol A (**1**) As Obtained Using B3LYP/6-31G(d) Calculations



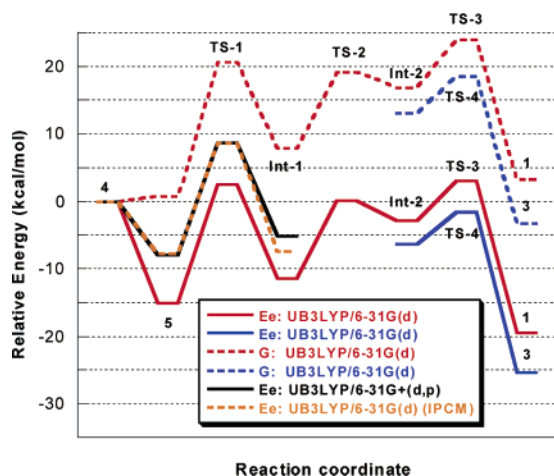
B3LYP (RB3LYP) calculations. A vibrational frequency analysis was carried out on each stationary point.<sup>16</sup> We have confirmed that each stationary point corresponds to a local energy minimum [the number of imaginary frequencies (NImag) = 0], or a transition state [NImag = 1]. In addition, starting from each transition state, an IRC calculation was carried out and the structures of the potential energy minima at both ends of the reaction coordinate were examined. Free energies of the stationary points at 298 K were derived from the B3LYP/6-31G(d) results. These calculations revealed for the first time energy profiles (Figure 2) for the reaction

gas-phase UB3LYP/6-31G(d) potential energy differences when discussing relative stabilities of the stationary points in the following sections.

The first process is formation of the C8–C8' bond. Two monomers **4** preassociate to give complex **5**, which is more stable than two molecules of **4** by 15.1 and 7.9 kcal/mol at the B3LYP/6-31G(d) and B3LYP/6-31+G(d,p) levels, respectively. Counterpoise calculation<sup>17</sup> at these computational levels afforded BSSEs of 7.7 and 2.0 kcal/mol,<sup>18</sup> and hence, the BSSE-corrected stabilization energies for complex **5** are 7.4 and 5.9 kcal/mol.<sup>18</sup> This stabilization can be ascribed to the two hydrogen-bond interactions shown in Scheme 1. From this complex **5**, the C8–C8' bond is formed affording a biradical intermediate **Int-1** via transition state (**TS-1**), in which there are two hydrogen-bond interactions, and the bond lengths of O–H and the forming C8–C8' bond are 1.927 and 2.076 Å, respectively. Each hydroxy group coordinates to a carbonyl oxygen, acting as a Brønsted acid. In **Int-1**, the dihedral angle Ha–C8–C8'–Ha' is –171.3°. The barrier height for the first step is 17.6 kcal/mol,<sup>18</sup> and the radical is stabilized by the hydrogen bond interactions.

The second step is rotation about the C8–C8' bond to afford **Int-2**, in which the dihedral angle Ha–C8–C8'–Ha' is +26.3°. The transition state (**TS-2**) is shown in Scheme 1, and the activation energy for this second step is 11.5 kcal/mol.<sup>18</sup>

In the third step, an intramolecular radical coupling of **Int-2** generates epoxytwinol A (**1**) with formation of the C1–C1' bond, the barrier height for this third step being 6.0 kcal/mol.<sup>18</sup> There are also two hydrogen bond interactions in the transition state (**TS-3**), in which the alcohol protons coordinate with a lone pair of the oxygen of the pyran rings and the bond length O–H is 2.107 Å.



**Figure 2.** Potential (Ee) and free (*G* at 298 K) energy profiles connecting reactant **4**, epoxytwinol A (**1**), and epoxyquinol B (**3**).

pathway of the [4 + 4] cycloaddition reaction. The potential energy profile and the free energy profile both suggest a three-step mechanism involving biradical intermediates (Figure 1). Unless specifically stated, we will refer to the

(16) The “Finegrid” implemented in GAUSSIAN program was employed for the B3LYP calculations and the “Tight” option was applied for geometry optimizations.

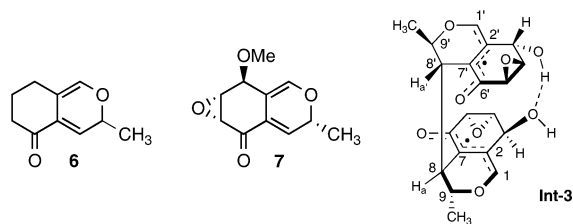
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(18) See the Supporting Information.

Though there is a possible path leading to **Int-2** directly from **4**, its transition-state energy is higher than that of **TS-1** by 9.8 kcal/mol, indicating that its contribution is negligible.<sup>18</sup>

Furthermore, we have carried out additional calculations for the **reactant**, complex **5**, **TS-1**, and **Int-1**; single-point UB3LYP energy evaluations using the larger basis set 6-31+G(d,p), and UB3LYP/6-31G(d) level solution-phase energy calculations using the isodensity polarizable continuum model (IPCM)<sup>19</sup> with  $\epsilon = 2.379$  (toluene is the supposed solvent). It should be noted that using the larger basis set and the IPCM did not alter significantly the above-mentioned gas-phase potential energy profile calculated at the UB3LYP/6-31G(d) level (see Figure 2) and that the calculation is in good agreement with the results of experiments performed in toluene.<sup>10e</sup>

These calculations suggest that the hydroxy group of **4** plays an essential role in this thermal [4 + 4] cycloaddition reaction by forming hydrogen bonds throughout the course of the reaction, from initiation until formation of the final product. The importance of this hydroxy group is supported experimentally by the observations that only Diels–Alder adducts were obtained in the reaction of **6** having no hydroxy or epoxy groups<sup>10d</sup> and in the reaction of **7** containing a methoxy group in place of the hydroxy group<sup>11</sup> (Figure 3).

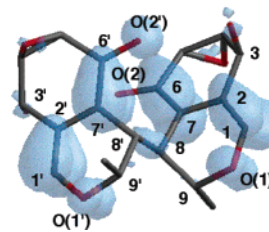


**Figure 3.** Structures of **6**, **7**, and **Int-3**.

$C_2$  symmetry is preserved throughout the reaction except at **TS-2**, where the hydrogen bonds recombine. It should be noted that the intermediate radical is rather stable owing to the delocalization of spin density through O1–C1–C2–C7–C6–O2. The spin density of **Int-1**, for example, is shown in Figure 4, which clearly shows the delocalization of the radical.

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(20) All hydrogens have been omitted for clarity.



**Figure 4.** Spin density surface (0.002 electrons/au<sup>3</sup>) of **Int-1**.<sup>20</sup>

Calculations indicate that the transformation of epoxytwinol A (**1**) to epoxyquinol B (**3**) also involves three steps. Homolytic cleavage of the C1–C1' bond affords biradical intermediate **Int-2**. Rotation around the C8–C8' bond generates **Int-3**, in which the dihedral angle of Ha–C8–C8'–Ha' is +58.7° (Figure 3). Epoxyquinol B (**3**) is generated by a coupling reaction between C1 and C7'. The transition state energy going from epoxytwinol A (**1**) to epoxyquinol B (**3**) is 22.6 kcal/mol, and the latter is thermodynamically more stable than the former by 6.0 kcal/mol, which explains the facile transformation of the former to the latter.

In summary, we have shown that the [4 + 4] cycloaddition of 2*H*-pyran **4** consists of three consecutive steps involving biradical intermediates. The first step is formation of the C8–C8' bond with generation of a biradical intermediate (**Int-1**). The next is rotation about the C8–C8' bond, and the last is radical coupling forming the C1–C1' bond. The biradicals are stabilized by delocalization, while two hydrogen-bonding interactions are essential for realization of this exceptionally rare thermal [4 + 4] cycloaddition reaction. Biradicals are also involved in the transformation of epoxytwinol A (**1**) into epoxyquinol B (**3**).

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**Supporting Information Available:** Cartesian coordinates and absolute energies for all reported structures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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