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Transannular Proton Transfer in the Cyclization of Geranylgeranyl Diphosphate to Fusicoccadiene, a Biosynthetic Intermediate of Fusicoccins

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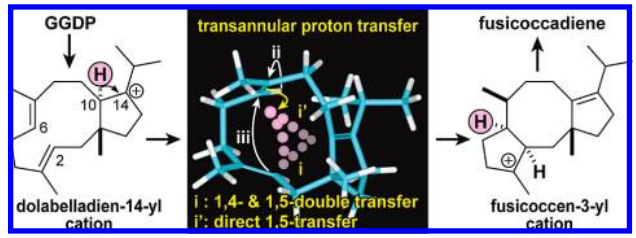
ABSTRACT



Enzymatic cyclization of geranylgeranyl diphosphate to fusicoccadiene involves a transannular proton transfer process. Label distribution in the cyclized products derived from deuterium-labeled GGDPs showed that a proton generated from C-10 migrates to C-6 in the intermediary dolabellane framework prior to the second ring formation. Although a direct 1,5-proton transfer would achieve this process, semiempirical MO calculations suggested an alternative pathway, which involves successive 1,4- and 1,5-proton transfers using C-2 as a springboard.

Fusicoccins (FCs)[1] are a family of diterpenoids isolated from *Phomopsis amygdali* that show strong biological activity as a consequence of interaction with 14-3-3 proteins[2] both in higher plants[3] and amphibian fertilized eggs.[4] We have recently succeeded in isolating a cDNA of *PaFS*, encoding the diterpene cyclase responsible for fusicoccin biosynthesis from mycelia of *P. amygdali*.[5] The recombinant protein of the PaFS, expressed as a fusion protein with glutathione *S*-transferase (GST), converted all-*trans*-geranylgeranyl diphosphate (GGDP) into fusicocca-2,10(14)-diene (**1**),[6] a hydrocarbon intermediate leading to aglycones of FCs. Here, using the recombinant GST-PaFS, we investigated the details of the cyclization mechanism of GGDP to **1** using deuterium-

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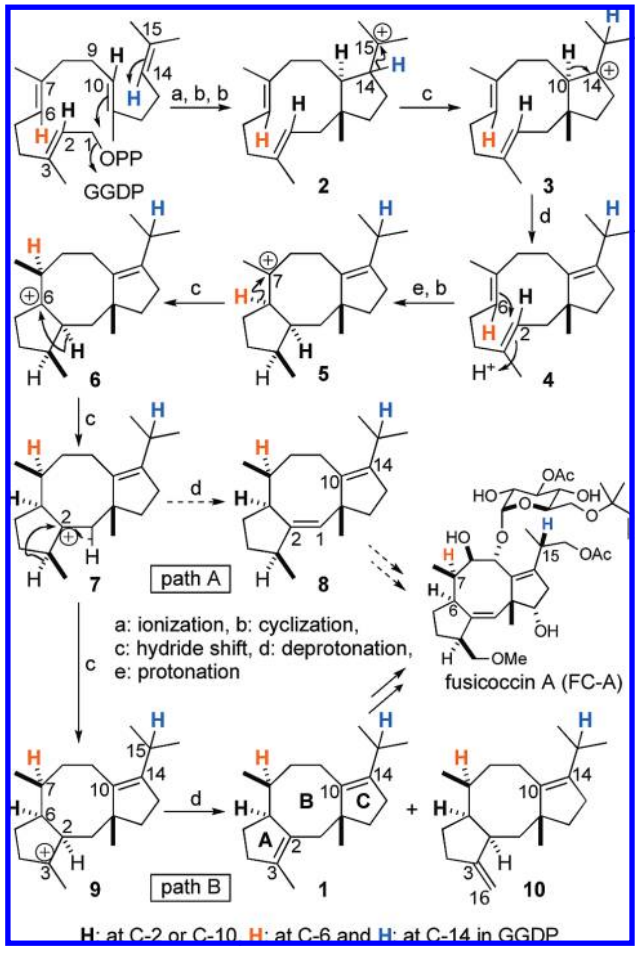
labeled GGDPs. The deuterium distribution patterns of enzymatically derived **1** and other minor congeners led us to propose a new mechanism involving a transannular proton transfer in the cyclization process.

Earlier studies in the 1970s by British and Italian groups, using labeled mevalonates, clarified that three of the four olefinic hydrogen atoms at C-2, C-6, C-10 and C-14 of GGDP are retained at C-6, C-7 and C-15 of FC-A (Scheme 1). It was experimentally evident that the C-6 and C-14 hydrogen atoms of GGDP migrate to C-7 and C-15 of FC-A, respectively. Therefore, the hydrogen at C-6 of FC-A must originate from either C-2 or C-10 of GGDP. Although their experiments could not distinguish these alternative possibilities, two research groups proposed the same cyclization pathway (Scheme 1: path A), in which the C-6 hydrogen of FC-A originates from the C-2 hydrogen of GGDP and the C-10 hydrogen of GGDP is lost as a proton during the formation of δ-araneosene (**4**)[7] from dolbelladien-14-yl cation (**3**).[8] They also proposed that fusicocca-1,10(14)-diene (**8**) is the initially formed hydrocarbon in fusicoccin biosynthesis. However, we have already reported that **1** is the genuine biosynthetic intermediate based on corroborative experimental evidence.[6] **1** was actually isolated from the mycelial extract of the fungus together with (+)-δ-araneosene (**4**) and fusicocca-3(16),10(14)-diene (**10**); this occurrence suggested the intermediacy of fusicoccen-3-yl cation (**9**) (Scheme 1: path B). Therefore, we have temporarily proposed that **9** is formed from **7** by another 1,2-hydride shift.[9] However, all of these hypotheses were based on assumptions regarding the origin of the C-6 hydrogen of FC-A without any experimental evidence.

Scheme 1. Proposed Cyclization Mechanisms in Earlier Studies, Path A[7] and Path B[6]



To verify this assumption, we first carried out the enzymatic cyclization reaction[5] using [1-$^2H_2$,2-^{2}H]GGDP (>95 atom % $^2H_2$ at C-1 and ~50 atom % ^{2}H at C-2)[10] as a substrate of the recombinant GST-PaFS.[6] If the cyclization proceeds along either path A or path B in Scheme 1, all deuterium labels should be retained in **1**. The enzymatically derived **1**, however, showed the parent ion at *m*/*z* 274, only two mass units larger than that of the unlabeled material. Instead, the parent ion of **10**, the minor enzymatic product, appeared at *m*/*z* 275/274 = 1:1, confirming that all three deuterium labels were retained. These results demonstrate that the label at C-2 remained at the original position until formation of **9** and was then eliminated as a proton in the final deprotonation step giving rise to **1**. Therefore, the hydrogen atom at C-6 of **1** and FC-A must originate from C-10 of GGDP, not from C-2 as was proposed previously.[7,9] This conclusion was further confirmed by the fact that both **1** and **10**, derived from [9-$^2H_2$,10-^{2}H]GGDP[10] (>95 atom % $^2H_2$ at C-9 and ~85 atom % ^{2}H at C-10), retained all labels of the precursor. These experiments, together with earlier information, lead to the conclusion that the destinations of the four olefinic protons at C-2, C-6, C-10 and C-14 of GGDP are C-2, C-7, C-6 and C-15 in **9**, respectively, and eliminated the possibility of protonation by an environmental proton in the step from **4** to **5**. In other words, the proton generated in the first cyclization process was trapped intramolecularly and initiated the second cyclization leading to the fusicoccane skeleton. Importantly, δ-araneosene (**4**), another minor product, retained all labels in both experiments. To explain this fact, the hydrogen of C-10 of GGDP must be transferred to C-6 prior to the C-2−C-6 bond formation. Thus, the putative cyclization pathway from GGDP to **1** shown in Scheme 1 needs to be totally revised.

We now propose a new cyclization pathway as shown in Scheme 2. The proton generated from C-10 of **3** is captured transannularly by the β-face of the C-6−C-7 double bond in a Markovnicov manner, a 1,5-proton transfer (blue arrow in Scheme 2), to dolabelladien-7-yl cation (**11**). Subsequent

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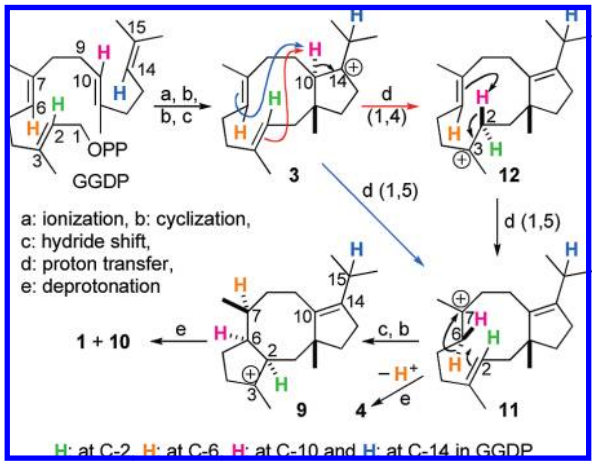
Scheme 2. New Cyclization Mechanisms of Enzymatic Formation of Fusicoccadienes from GGDP



1,2-hydride shift of the original hydrogen at C-6α to C-7 followed by the A/B ring formation leads directly to fusicoccen-3-yl cation (**9**), in which the four olefinic protons of GGDP are correctly located. Elimination of the hydrogen at C-6α of **11**, instead of the 1,2-hydride shift, leads to *δ*-araneosene (**4**), in which labels are also correctly distributed. The new cyclization mechanism is much simpler than path B in Scheme 1 in regards to the number of 1,2-hydride shifts during the process from **3** to **9**. Another discriminating feature is the direction of the A/B ring formation process. The second cyclization from the dolabellane to the fusicoccane skeleton occurs from the C-2 to C-6 cation in Scheme 2, whereas it is from the C-6 to C-2 cation in Scheme 1.

The above-mentioned direct pathway (1,5-proton transfer) from **3** to **11** seems reasonable. However, calculative studies discussed below suggested an alternative pathway. A 1,4-proton transfer from C-10 to C-2 (red arrow in Scheme 2) occurs first to lead to dolabelladien-3-yl cation (**12**). Then, a 1,5-proton transfer from C-2 to C-6 gives rise to **11**.

There are four major conformers in dolabelladien-14-yl cation (**3**) taking into account the orientations of the two double bonds in the 11-membered ring. Among them, only one conformation (C-3-Me up, C-7-Me up) is suitable to provide the correct stereochemistries of the cyclized products. Since the hydrogen atoms at C-10 of **3** (heat of formation by MOPAC2007/PM6[11] = 131.8 kcal/mol), C-6*β* of **11** (126.6 kcal/mol) and C-2*β* of **12** (126.3 kcal/mol) are located in the interior space of dolabellane skeleton, interconversion of these energetically *pseudo*-degenerative cation species seems to take place by small energy and geometry changes along with the proton transfers (Figure 1).

However, no transition states for the direct conversion of **3** to **11** by the 1,5-proton transfer could be found. Instead, the transition states from **3** to **12** (1,4-proton transfer) and **12** to **11** (1,5-proton transfer) were obtained by MOPAC-2007/PM6 calculations (Figure 2). The results of these

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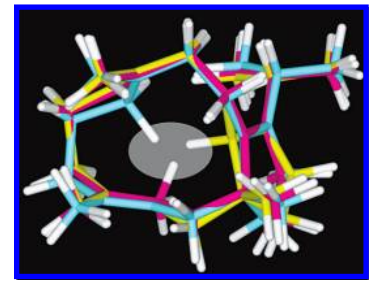

Figure 1. Proximity of the interior hydrogen atoms (in a gray oval) of dolabelladien-14-yl cation (**3**: yellow), 7-yl cation (**11**: cyan) and 3-yl cation (**12**: magenta).

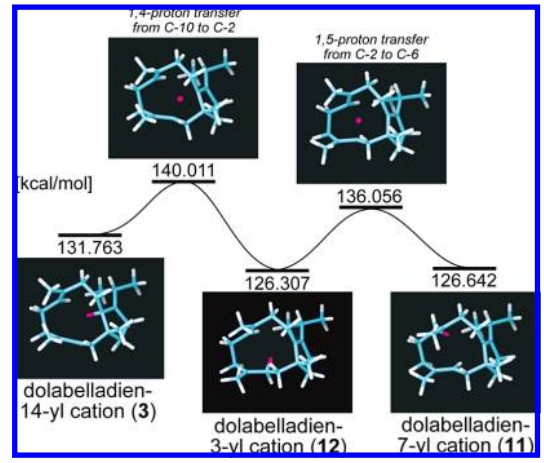

Figure 2. Energy profile of interconversion of **3**, **12** and **11** by MOPAC2007/PM6 calculations. The migrating proton is shown in magenta.

calculations may be rationalized by the fact that, in **3**, (i) the distance from the hydrogen at C-10 to C-2 (2.14 Å) is shorter than to C-6 (2.33 Å), and (ii) the direction of the p-orbital of the C-2−C-3 double bond is more suitable than that of the C-6−C-7 double bond for accepting the proton generated from C-10 as shown in Figure 3.

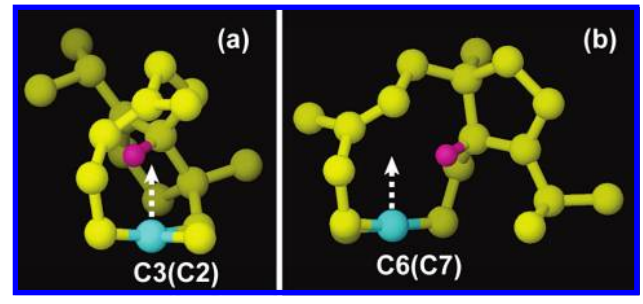

Figure 3. Stereostructure of dolabelladien-14-yl cation (**3**): (a) viewed from C-3 to C-2, (b) viewed from C-6 to C-7. Dotted arrows show the direction of p-orbital of the double bonds. The migrating proton is shown in magenta.

In the calculation (Figure 2), dolabelladien-3-yl cation (**12**) is slightly more stable than 7-yl cation (**11**), which must be the precursor for further transformation. Probably, in an enzymatic environment, some perturbation makes **11** favorable over **3** and **12** to favor the newly proposed pathway. As a simple model, we placed one molecule of water, which stabilizes **11** by hydrogen bonding to the acidic hydrogen at C-6α and a dipole−dipole interaction with the cationic center (Figure 4).

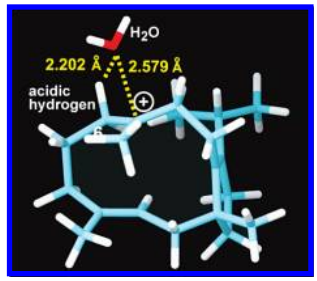

Figure 4. Optimized structure of a H_2O stabilized model of the dolabelladien-7-yl cation (**11**) by MOPAC2007/PM6 calculations.

Even in this simple model, the predominance of **11** (63.1 kcal/mol) over **3** (74.4 kcal/mol) and **12** (69.9 kcal/mol) was rationalizable as shown in Figure 5. To clarify the actual perturbation in the enzymatic reaction, structural studies of PaFS are in progress.

It is well-known that a proton generated in the first cyclization step is captured intramolecularly by the other double bond (1,5-proton transfer) to initiate the second cyclization in taxadiene formation.[10,12] An intramolecular 1,5-proton transfer was also predicted by molecular energy calculations in tricodiene biosynthesis.[13] An intramolecular 1,4-proton transfer was experimentally proven in abietadiene biosynthesis.[14] Our newly proposed pathway is another

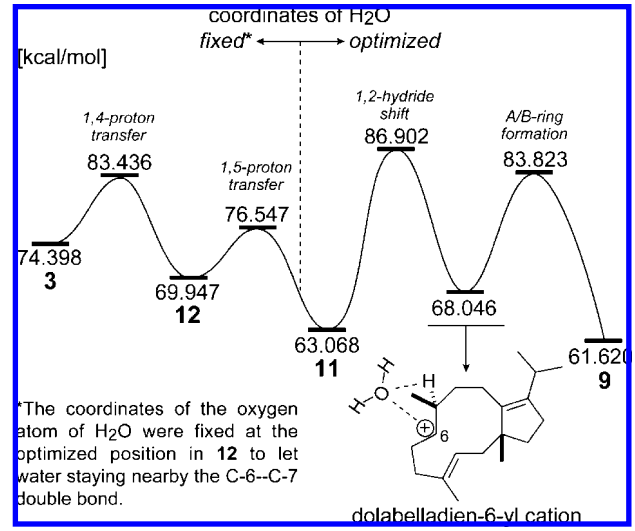

Figure 5. Energy profile from the dolabelladien-14-yl cation (**3**) to the fusicoccen-3-yl cation (**9**) in the presence of H_2O by MOPAC2007/PM6 calculations.

example of such a mechanism. Although it is impossible to distinguish between the two possible pathways in Scheme 2 by experimental evidence, a double proton transfer mechanism is certainly worth considering based upon the results of the calculations. Similarly, the double proton transfer mechanism has been previously proposed based on theoretical calculations in the case of taxadiene biosynthesis.[15]

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Supporting Information Available: GC-MS data of the enzymatic reactions and MOPAC2007/PM6 MO calculation data with coordinates with/without perturbation of H_2O. This material is available free of charge via the Internet at http://pubs.acs.org.

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