WATER ROLE ON DIELS-ALDER REACTION OF PRENYLATED FLAVONOID FORMATION IN *Boesenbergia pandurata*: MECHANISM STUDY

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Abstract

Panduratin A is a prenylated flavonoid derivative from *Boesenbergia pandurata* with many potential biological activities. The biogenesis of this compound and its derivatives is believed to involve a Diels-Alder reaction between monoterpenoid and chalcone derivatives. This study provides insight into modeling biogenesis through the Diels-Alder reaction for Panduratin A and derivatives biosynthesis. We are using M06-2X/6-31G(d)//PM6 level of theory to explore the potential energy surfaces, asynchronicity degree, and global electron density transfer. Explicit water was applied to mimic physiological conditions. Contrary to the fact that water accelerates this reaction through hydrogen bonding catalysis, we found that water could slow this reaction. These results suggest that this reaction proceeds very slowly under physiological conditions, and enzymes catalyze this reaction.

Keywords*: asynchronous, DFT, Diels-Alder, panduratin A, prenylated flavonoids*

Introduction

Boesenbergia pandurata, commonly known as "Temu Kunci" in Indonesia, belongs to the family *Zingiberaceae* whose rhizome has been used by Southeast Asians in folk medicine, such as dysentery, aphthous ulcer, dry mouth, and muscular pain (Ching *et al.*, 2007). In addition, Rhizomes extract from this plant has various biological activities, such as anticancer (Nurrachma *et al.*, 2018), antibacterial (Rahman *et al.*, 2016), aphrodisiac (Ongwisespaiboon and Jiraungkoorskul, 2017), antioxidant (Lee *et al.*, 2020), anti-inflammatory (Saah, 2019), and anti-viral (Kanjanasirirat *et al.*, 2020). The flavonoid derivatives in the rhizome are responsible for those activities mentioned before. One of them is Panduratin A, a primary bioactive compound found in the rhizome of *B. pandurata* (Won *et al.*, 2021). Previous studies showed that Panduratin A has similar activities to the rhizome extract of *B. pandurata*, such as anticancer (Kirana *et al.*, 2007), antibacterial (Park *et al.*, 2005), antioxidant (Shindo *et al.*, 2006), anti-inflammatory (Yun *et al.*, 2003), and anti-viral (Kanjanasirirat *et al.*, 2020). This shows that Panduratin A is a secondary metabolite that carries out the biological activity of *B. pandurata*, which deserves further investigation.

Panduratin A is a prenylated flavonoid derivative isolated from ethanol extract of *B. pandurata* rhizome (Salama *et al.*, 2013). This compound has been reported to be artificially produced by tissue culture technique. Various proposed biosynthetic pathways showed that Panduratin A could be built from chalcone derivative and geranyl via Diels-Alder cycloaddition with the help of prenyltransferase enzymes (Yadnya-Putra

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et al., 2014; Chahyadi *et al.*, 2014). Besides, Panduratin A formation by Diels-Alder cycloaddition was also successfully proved based on the biomimetic synthesis, using *(E)*–ocimene as geranyl substituent and methyl cinnamate as chalcone derivative (Pasfield *et al.*, 2013; Nasir *et al.*, 2017). The utilization of water as a solvent is known to increase the reactivity of the Diels-Alder reaction (Cortes-Clerget *et al.*, 2021). This fact can become an argument that water as a physiological medium can influence Diels-Alder cycloaddition in natural product formation. Yet, further study into the Diels-Alder mechanism of Panduratin A formation in nature is not fully understood.

So, our article focused on exploring the water effect on Diels-Alder cycloaddition on panduratin A formation and its isoforms, namely nicolaidesin B, panduratin H, and panduratin I. This work involved transition state searching of reactions and possible pathways that might occur in their formation by in-silico study. This kind of method is also already conducted and gives deep insight into the Diels-Alder mechanism study since 1997 (Padwa *et al.*, 1997; Jasiński, 2016; Hammoudan *et al.*, 2023; El Ghozlani *et al.*, 2020). Lindler *et al.* reported that Diels-Alder cycloaddition is not always a concerted reaction but can be a stepwise reaction proven by an in-silico approach (Linder and Brinck, 2012). This phenomenon is characterized by the asynchronicity degree in the reaction's transition state. That value could be related to the reactivity of the reaction as it tends to lower barrier energy (Vermeeren *et al.*, 2021). Moreover, the reaction rate of the Diels-Alder could be driven by the flow of electron density from diene into dienophile, called global electron density transfer/GEDT (Domingo, 2014a). It also could determine whether the cycloaddition reaction is non-polar or polar.

Research Methods

(E)-ocimene and chalcone's derivatives were used as reactants in forming Panduratin A, Nicolaidesin B, Panduratin I, and Panduratin H. Potential energy surfaces (PES) diagram of each compound were made to obtain the initial structure of reactant complex and transition state. Then, all reactants, products, and transition state structures were optimized using a PM6 semi-empirical method. This method is considered for the time-cost of electronic structure calculation (Loco *et al.*, 2022). In addition, PM6 was more accurate in predicting geometries involving hydrogen bonds than other semi-empirical methods, such as AM1, PM3, RM1, and even Becke 3-parameters DFT method (Stewart, 2007). In contrast, The Minnesota suite of density functional methods, M06-2X, was used for single-point energy calculation rather than the Becke Lee-Yang-Parr parameter (B3LYP) method regarding data reliability (Zhao and Truhlar, 2008; Walker *et al.*, 2013). Calculation of structure optimization and single-point energy was performed in the gas phase. Zero-point energies (ZPE) were also included for relative energy correction in each structure (Hallooman *et al.*, 2018). Obtained transition state structure after optimization was verified with IRC (Intrinsic Reaction Coordinate) calculation and the presence of one imaginary frequency. It was used to visualize whether the transition structures connect the Diels-Alder product with the reactants (Dykstra *et al.*, 2005). Besides, other structures were characterized as stationary points by the absence of imaginary frequency. The asynchronous degree (Δd) was determined by the length difference of two single bonds forming in the reaction's transition state (Ben El Ayouchia *et al.*, 2018). Global electron density transfer (GEDT) was examined using NBO analysis of transition state structures (Domingo, 2014b).

Results and Discussion

Two different reaction models were used to investigate the role of water molecules as catalysts. In the first model, the reaction proceeded only between two reactants, while in the second model, water was introduced purposely to mediate interaction between reactants. The computational method of M06-2X/6-31G(d)//PM6 was used. The results showed that Nicolaidesin B formation proceeded in different ways when water was absent as a catalyst. As shown in Figure 1, *(E)*-ocimene and pinostrobin chalcone as reactants reacted through the hetero Diels-Alder mechanism which produced unsaturated ether intermediates. The prenyl group in the chalcone acts as the electron-donating group which causes electron repulsion between the α-carbon atom in the chalcone and C1 in *(E)*-ocimene. As a result, the ordinary Diels-Alder reaction is quite difficult to occur in the formation of Nicolaidesin B. Therefore, through the hetero Diels-Alder reaction, the α,β-unsaturated ketone in the chalcone acts as a diene, while *(E)*-ocimene functions as a dienophile. This allows rational charge transfer between the reactants. In contrast, the formation of Panduratin A proceeds via the usual Diels-Alder reaction, in which the β-carbon atom in the chalcone accepts a flow of electrons from C1 in *(E)*-ocimene. This observation also shows the effect of the ortho position on the ease of product formation in the Diels-Alder reaction.(Morell *et al.*, 2008).

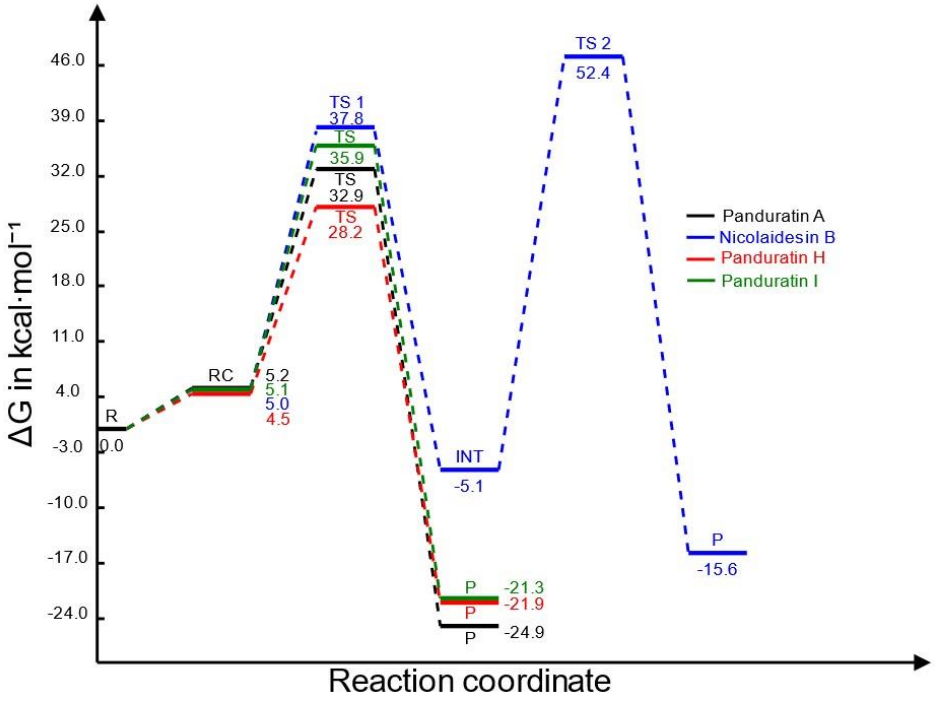


Figure 1. The free energy profile relative to reactants (ΔGrel) is calculated at the theoretical level M06-2X/6-31G(d)//PM6 without water as solvent. (R: Reactant, RC: Reactant Complex, TS: Transition State, P: Product)

From the thermokinetic point of view, Panduratin H is easier to form than the other isomers because it has the lowest energy barrier, which is around 28.22 kcal/mol. Furthermore, Panduratin A is in second place with an activation energy of around 32.90 kcal/mol. This pattern also applies to their stereoisomers, namely Panduratin I and Nicolaidesin B. The energy topology of Nicolaidesin B shows two transition states during the reaction process. The Claisen rearrangement step

of the dihydropyran intermediate appears to be the rate-determining step because of its higher energy barrier. This may be due to steric hindrance between the allylic functional group and other substituents on the dihydropyran ring. In addition, the steric hindrance and ring stiffness also result in a boat-shaped transition state, which is usually less desirable in sigmatropic rearrangements [3,3] (Gül *et al.*, 2010).

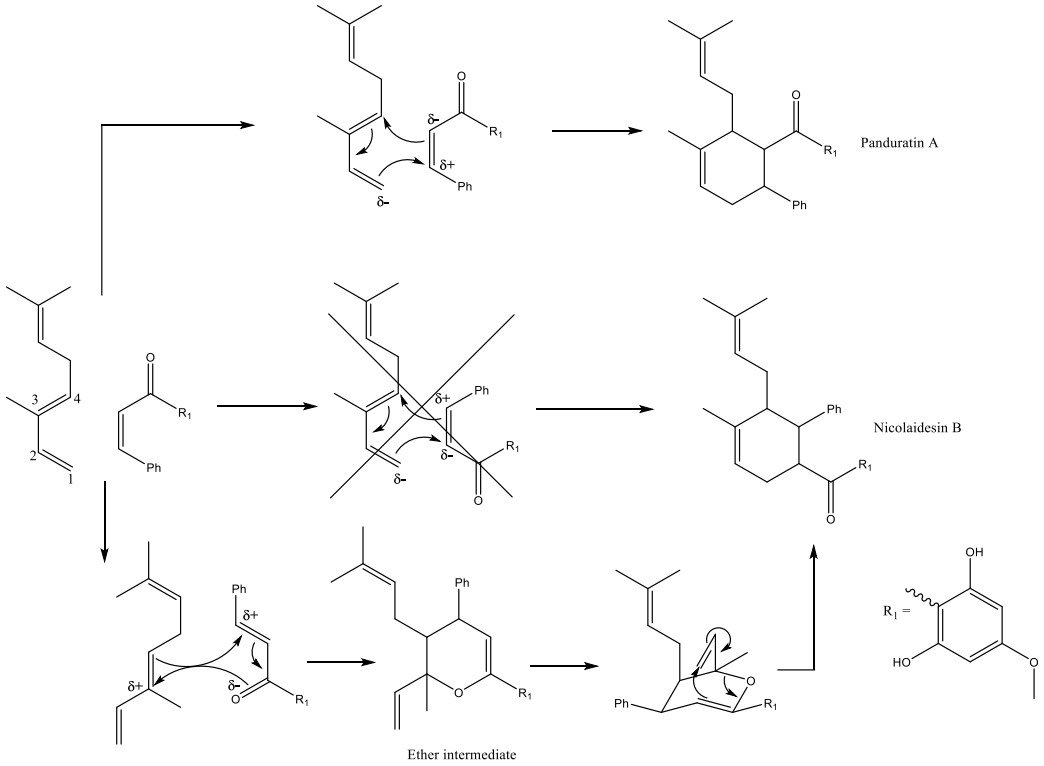
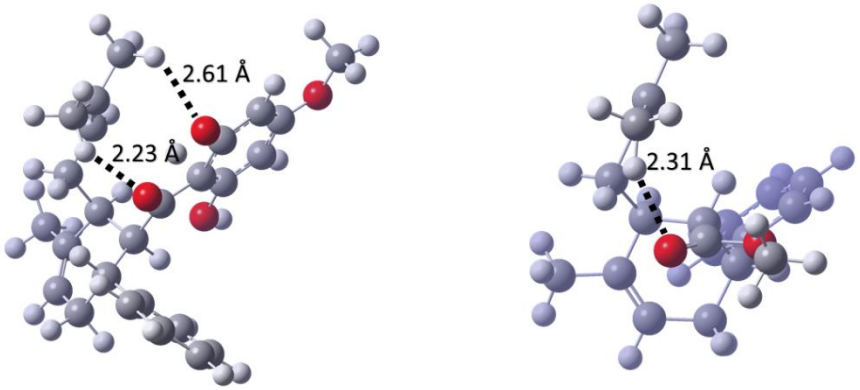


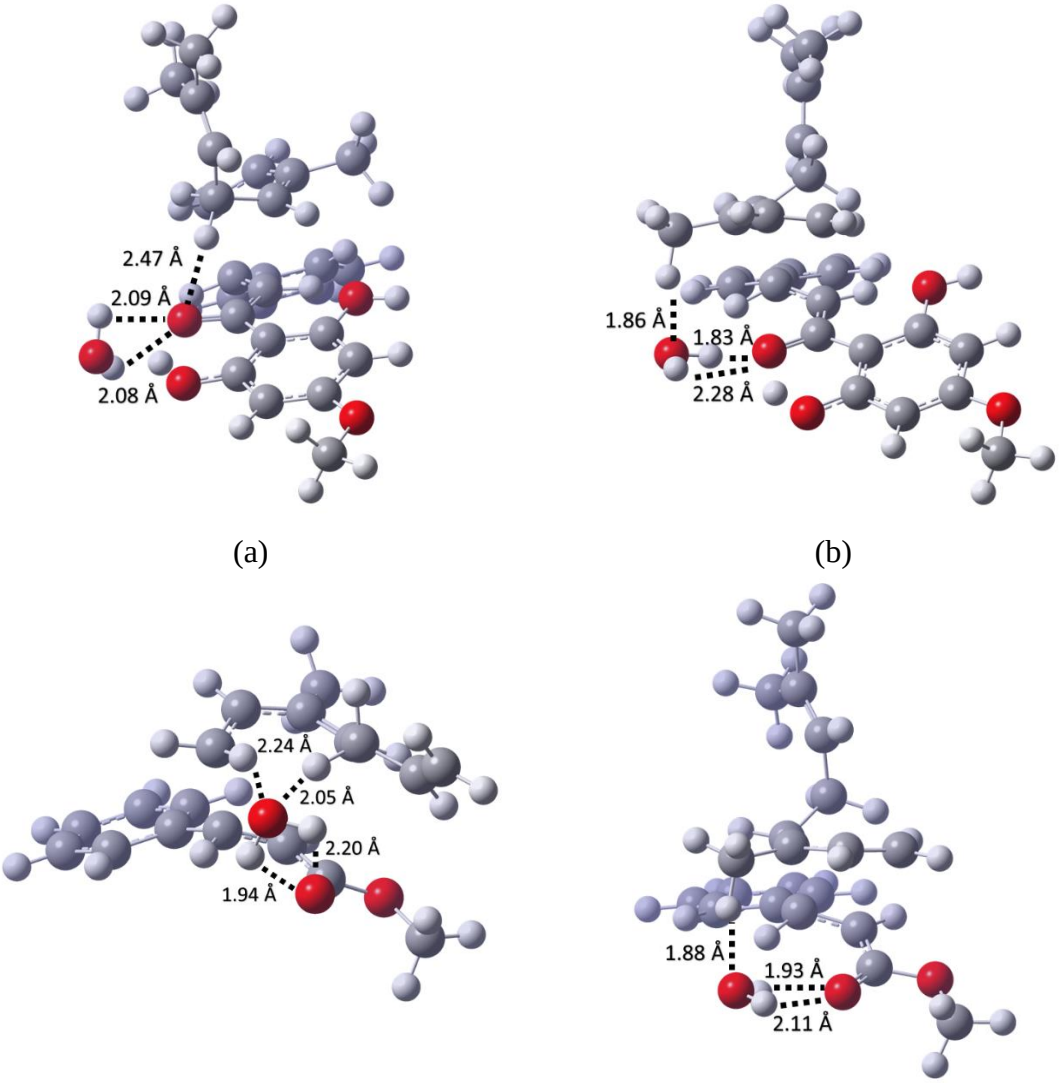
Figure 2. Proposed mechanism pathways of Panduratin A and Nicolaidesin B formation

Although Panduratin H is superior in terms of thermokinetics, Panduratin A has the most stable product compared to the other three isomers. Non-covalent interactions between prenyl and acyl groups play a role in this stability. In Panduratin H, the carbonyl in the ester has an interaction such as a hydrogen bond with one of the methyl groups of the prenyl group. However, in Panduratin A, the oxygen atom in the phenolic ring interacts with the two groups, as shown in Figure 2. This additional interaction makes Panduratin A more difficult to react or decompose. The same pattern holds for Nicolaidesin B and Panduratin I, which lack non-covalent interactions to make them more stable.



(a) (b)

Figure 3. Visualization of non-covalent interaction in (a) Panduratin A. (b) Panduratin H.



(a) (b)

(c) (d)

Figure 4. Visualization of water interaction with reactants in the transition state of (a) Panduratin A. (b) Nicolaidesin B. (c) Panduratin H. (d) Panduratin I.

When a water molecule is involved as a catalyst in the reaction, it is seen that all isomers have a higher activation energy. Their activation energy increases to around 41.59 - 54.97 kcal/mol. Panduratin H still has the lowest barrier energy, followed by Panduratin A, but the trend is slightly different. In this model, Panduratin I has the highest activation energy compared to Nicolaidesin B, as opposed to when water is not present as a catalyst. From Figure 3, the water molecule is positioned near the electron-withdrawing group (EWG) of the dienophile. The molecule interacts with several groups in the range of 1.82 - 2.47 Å. Besides Panduratin A, all water molecules are located between the α, β-unsaturated carbonyls of the chalcone and interact via hydrogen bonds with the carbonyl groups. In addition, there are also non-covalent interactions with proton of *(E)*-ocimene at different distances. In Nicolaidesin B and Panduratin I, water has an interaction with a methyl group with the same affinity, as seen from a distance. Meanwhile, there are two slightly weaker interactions with methylene and C=C-H on *(E)*-ocimene in Panduratin H. Overall, however, Panduratin H has more intermolecular interactions to stabilize its transition state structure, which could explain the lower energy barrier than other compounds. In Panduratin A, water lies between the carbonyl and aromatic rings. It also differs from the others because of the carbonyl part of the chalcone that undergoes hydrogen bonding interactions with methylene and C=C-H in *(E)*-ocimene. Therefore, the presence of water has no significant effect on the stabilization of the *(E)*-ocimene complex with chalcone. However, this suggests that water can mediate the Diels-Alder reaction on Nicolaidesin B, which previously used a two-step mechanism in the absence of water.

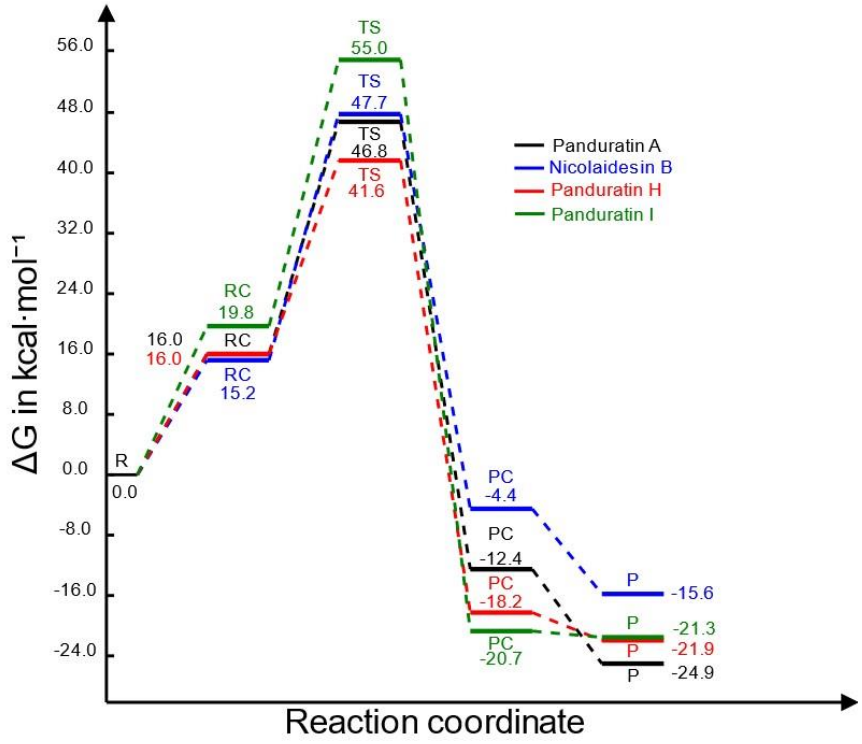


Figure 5. The free energy profile relative to reactants (ΔGrel) is calculated at the theoretical level M06-2X/6-31G(d)//PM6 with water as solvent. (R: Reactant, RC: Reactant Complex, TS: Transition State, PC: Product Complex, P: Product),

According to Vermeeren et al., increasing the degree of asynchronicity (Δd) in the Diels-Alder reaction can lower the activation energy (Vermeeren *et al.*, 2021). However, our results showed that the presence of water exhibited an increase in activation energy although the degree of asynchronicity increased. Based on their degree of asynchronicity, Panduratin A and Panduratin H are categorized as asynchronous Diels-Alder reactions, either with or without water as a catalyst (Isamura and Lobb, 2022). Meanwhile, Panduratin I and Nicolaidesin B have Δd values of more than 0.95 Å in the presence of water molecules, which means they undergo a stepwise Diels-Alder reaction. This stepwise mechanism is also seen in the IRC formation of Nicolaidesin B and Panduratin I. The IRCs have the same feature: broad peaks with the formation of new bonds along these peaks. After reaching the transition state, there is an intermediate structure with one bond from the Diels-Alder product already formed. Then, another bond will be formed along the slope of the reaction coordinate. However, no other transition state could be found in the reaction to prove this claim.

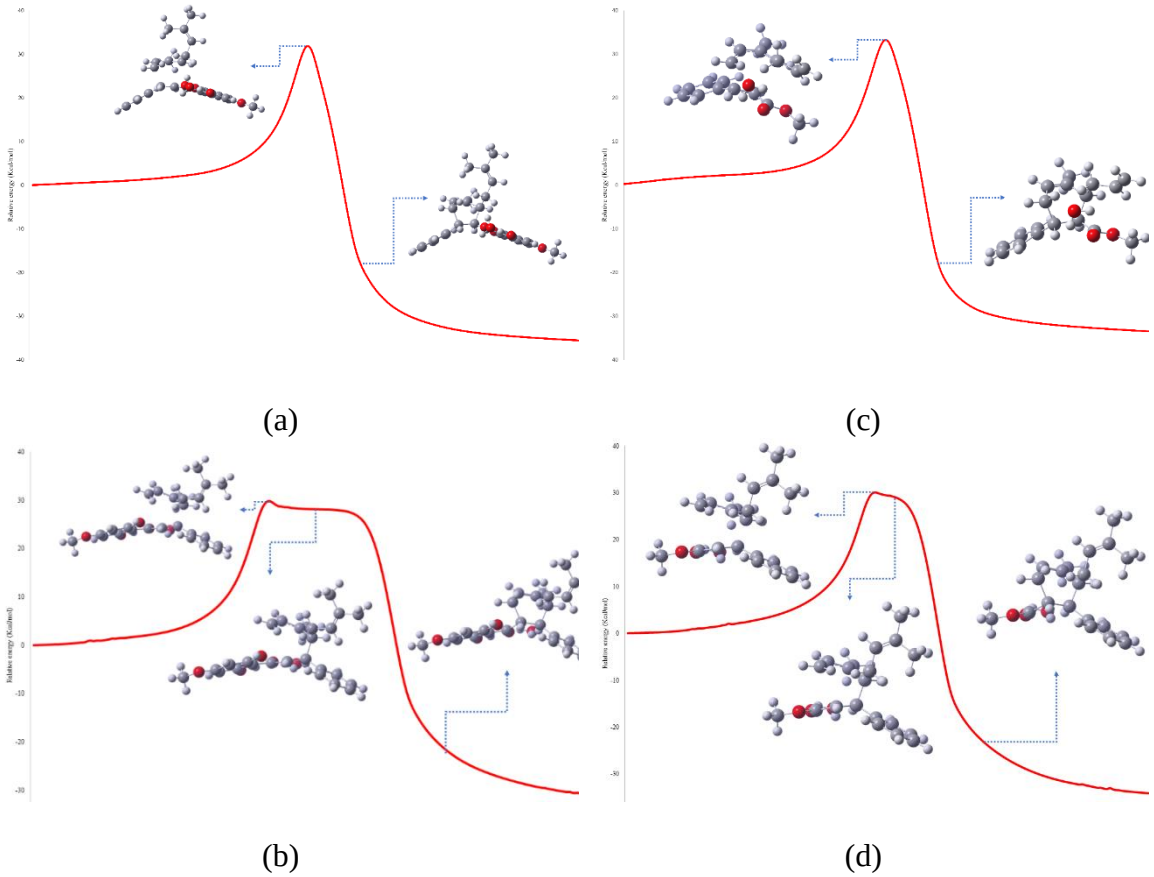


Figure 6. Intrinsic Reaction Coordinate (IRC) of (a) Panduratin A. (b) Nicolaidesin B. (c) Panduratin H. (d) Panduratin I.

Tabel 1. Asynchronous degree of Diels-Alder transition state in Panduratin A, Nicolaidesin B, Panduratin H, and Panduratin I with the presence and absence of water

Compound	Δd –Without Water (Å)	Δd –With Water (Å)
Panduratin A	0.63	0.67
Nicolaioidesin B	-	1.22
Panduratin H	0.55	0.64
Panduratin I	0.23	1.12

The water molecules in Panduratin I tend to make the reaction more asynchronous. A similar thing can also be seen in the use of a Lewis acid catalyst in the Diels-Alder reaction (Sakata and Fujimoto, 2020). Without it, Panduratin I falls between moderate synchronous and asynchronous Diels-Alder reactions. This also shows that the presence of water can increase the polarity of the Diels-Alder reaction (Domingo and Sáez, 2009). This can be verified by increasing the GEDT values of the formation of all isomers when water is involved. But again, there is a contradiction with previous studies because even though the presence of water increases the degree of asynchronous and GEDT values, the activation energy is higher than the reaction without water as a catalyst. Moreover, neither pattern supports the claim that there is a high correlation between GEDT and activation energy in the second reaction model. Although the results provide supporting evidence with previous studies in the first reaction model. Isamura and Lobb suggest that this low correlation could be due to the use of different groups of dienes and dienophiles in this study (Isamura and Lobb, 2022). In addition, it is possible that the water in the reaction does not have a role as a catalyst but rather as a solvent.

Table 2. Global Electron Density Transfer (GEDT) of Diels-Alder transition state in Panduratin A, Nicolaidesin B, Panduratin H, and Panduratin I with the presence and absence of water

Compound	GEDT –Without Water	GEDT –With Water
Panduratin A	0.19	0.20
Nicolaioidesin B	-	0.37
Panduratin H	0.19	0.21
Panduratin I	0.17	0.32

Conclusions

Based on the data interpretation, Panduratin A and its isomers show a polar Diels-Alder reaction with Panduratin A, H, and I categorized as asynchronous mechanisms. Nicolaidesin B is formed by a two-step mechanism consisting of hetero Diels-Alder, continued by Claisen rearrangement. With the existence of water, it could mediate the Diels-Alder reaction for all isomer formations. However, it does not serve as a catalyst despite its increased asynchronous degree and GEDT value of all formations. This existence leads to higher activation energy, which could be concluded that enzymes are necessary to accommodate the biosynthesis of Panduratin A and its isomers in *B. pandurata.*

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