SCAVENGING OF HYDROXYL RADICAL BY KINSENOSIDE ORIGINATED FROM ANOECTOCHILUS ROXBURGHII: A DFT APPROACH

Nguyen Minh Thong[1*], Dinh Tuan[2], Hoang Thi Thu[3], Phan Tu Quy[3], Pham Cam Nam[4]
[1]The University of Danang, Campus in Kon Tum; nmthong@kontum.udn.vn
[2]Hue University's College of Sciences
[3]Tay Nguyen University
[4]The University of Danang, University of Science and Technology; pcnam@dut.udn.vn

Abstract - A density functional theory (DFT) study on the hydroxyl radical scavenging properties of Kinsenoside originated from *Anoectochilus roxburghii* is presented**.** Two mechanisms, single electron transfer (SET) and hydrogen atom transfer (HAT) are considered. The thermochemical results demonstrate that the SET mechanism is not plausible to occur. With respect to the HAT, the interaction of OH• radical with Kinsenoside is also studied in detail by establishing potential energy surface (PES). This result strongly confirms that the C8–H bond decides the radical scavenging activity of Kinsenoside with activation Gibbs free energy ($\Delta G^{\neq}$) and rate constants (*k)* of 1.1 kcal/mol and 5.9×10^{-8} cm^3/molecules, respectively.

Key words - Antioxidant; Free radicals; Kinsenoside; DFT

1. Introduction

Anoectochilus roxburghii belongs to the genus *Anoectochilus (Orchidaceae)*, which is widespread in tropical and subtropical regions such as Vietnam, Laos and south of China [1]. *Anoectochilus roxburghii* has been considered to be one of the valued folk medicinal plant because it has been used to prevent and treat diabetes, hyperliposis, hepatitis, and tumors [1-4]. Since various biological and pharmacological activities of herbs are related to their antioxidant activities, the discovery of a new antioxidant originated from natural compounds has still abstracted much attentions from researchers.

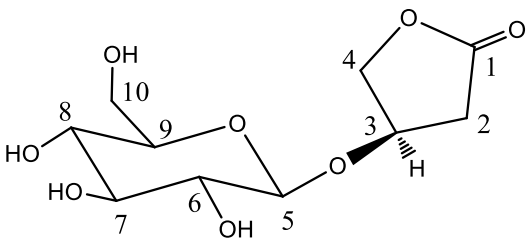


Figure 1. *Structure of Kinsenoside*

Kinsenoside, 3-(R)-3-β-D-glucopyranosyloxybutanolide (Figure 1), is a 3-hydroxybutanolide derivative and a major ingredient of *Anoectochilus roxburghii.* In particular, Kinsenoside is a good potential antioxidant [5-7]. Although experimental results on the antioxidant capacity of Kinsenoside are well reported in literature, there are neither theoretical investigations nor analysis of the free radical scavenger capacity of Kinsenoside. Among the biologically relevant reactive oxygen species (H_2O_2, $O_2^{\bullet-}$, and OH•, etc.), hydroxyl radicals are highly reactive species which can react with various kinds of compounds and causing cell membrane damage in all living organisms. The most well-known mechanisms by which antioxidants exert their action against free radicals involve the so called hydrogen atom transfer (HAT) and single electron transfer (SET). Therefore, the aim of the present work is to carry out a theoretical study on the activity of Kinsenoside in scavenging OH• radical *via* these major mechanisms. In this study, all possible active sites of Kinsenoside that can scavenge OH• radical have been examined, and the thermodynamic and kinetic data of the corresponding channels have been also obtained.

2. Theoretical and computational methods

All computations were performed using the Spartan'14 [8] and the Gaussian 09 suite of programs [9]. The conformational space of Kinsenoside was obtained by the combination of the Merck Molecular Force Field (MMFF) and the Monte-Carlo method in Spartan software. To reveal the most stable conformer of Kinsenoside, Gaussian was used for all further calculations. Each compound and the related species were optimized at the M052X/6-311G(d) level of theory. To build the potential energy surface of the reaction between OH• radical and the studied molecules, the transition states, intermediates and products were optimized and calculated at the same level of theory. All transition states were characterized by only one single imaginary frequency. The intrinsic reaction coordinate (IRC) calculation was also carried out at the same computational level of theory to ensure each transition state connects to an expected reactant and product. The integral equation formalism of the polarizable continuum model (IEF-PCM) was used for this system to estimate the solvent effect on the enthapies [10, 11].

Rate constant (*k*) was computed following the transition state theory (TST) and 1 M standard state in the gas phase according to the equation (1) as follows:

$$k = \sigma\kappa\frac{k_B \mathrm{T}}{h}e^{-(\Delta G^{\#})/\mathrm{RT}} \quad (1)$$

where σ is the reaction symmetry number, k_B is the Boltzmann constant, T is the temperature, *h* is Planck constant, R is the gas constant, $\Delta G^{\#}$ is the Gibbs free energy of activation, and κ is tunneling corrections which are calculated using the Wigner [12] and Eckart [13] methods. The kinetic study was performed using the Eyringpy code [14, 15].

3. Results and discussion

3.1. Optimized molecule geometry

The equilibrium geometry of antioxidant molecule is a very important factor which affects its antioxidant efficiency. In order to get the structure of the stable conformer of molecule, in this work, the series consisting

of 100 low energy conformers were obtained in total 841 possible gas-phase conformations by using the MMFF based Monte-Carlo method. The energies of these conformers are 40 kJ/mol difference from the global maxima which had been excluded. Ten conformers with the lowest total energy were selected for further optimization. The re-optimization and frequency calculations of these structures were carried out at the M052X/6-311G(d) level of theory. In this way, the most stable structure for Kinsenoside is found and shown in Figure 2.

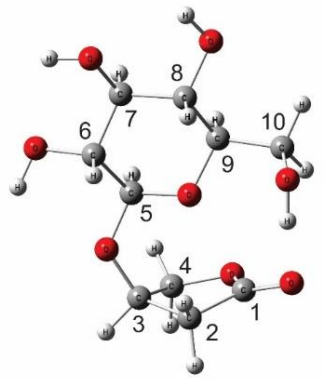


Figure 2. *Optimized structure of Kinsenoside*

3.2. Single Electron Transfer (SET)

Kinsenoside (KIN–H) compound transfers a single electron to OH• radical according to the SET mechanism as follows:

$$\mathrm{KIN-H + OH^{\bullet} \rightarrow KIN-H^{+\bullet} + OH^{-}} \quad (2)$$

As can be seen in Table 1, the electron transfer between OH• and Kinsenoside is not thermodynamically preferred since both the reaction enthalpy (ΔH) and Gibbs free energy (ΔG) in gas and solvent phase are positive values. It is also clear that the ΔH and ΔG values significantly decrease when the calculation is done in aqueous media. The ΔH of SET reaction changes remarkably in gas phase, benzene and water corresponding to 202.6, 135.7 and 85.6 kcal/mol. The corresponding ΔG drops from 202.7 kcal/mol in gas phase, 135.5 kcal/mol in benzene and 84.7 kcal/mol in water. Consequently, the theoretical ΔH, ΔG values in Table 1 shows that SET process is more favorable to occur in solvent phase (water) which can enhance the stabilization of the ionic species solvation.

Table 1. *Reaction enthalpies (ΔH) and Gibbs free energies (ΔG) of SET reaction calculated at the M052X/6-311G(d) level of theory*

Mechanisim	ΔH (kcal/mol)			ΔG (kcal/mol)		
	gas	benzene	water	gas	benzene	water
SET	202.6	135.7	85.6	202.7	135.5	84.7

3.3. Hydrogen Atom Transfer (HAT)

As already known from the literature [16-18], the hydrogen atom transfer (HAT) is a possible mechanism of the hydroxyl radical in which the hydroxyl radical and antioxidant react. The hydroxyl radical takes a hydrogen atom from the C/O–H bond of the Kinsenoside (KIN–H), which generates a Kinsenoside radical (KIN•) and a water molecule as below:

$$KIN - H + OH^{\bullet} \rightarrow KIN^{\bullet} + H_2O \quad (3)$$

The potential energy surfaces (PES) were calculated for the reaction between OH• and Kinsenoside to better understand H donation process. Sixteen pathways of reaction between OH• radical and Kinsenoside are shown in Figure 3A for C–H bond and Figure 3B for O–H bond. Moreover, IRC plots for all transition states related to reactions of OH• radical with Kinsenoside are presented in Figure S1 of Supporting Information (SI).

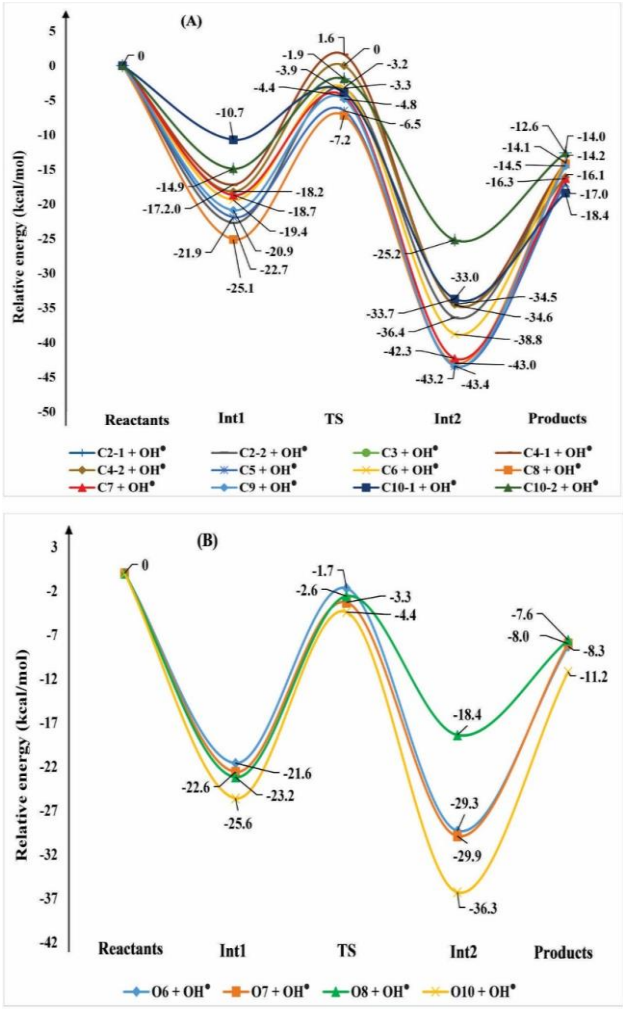


Figure 3. *Potential energy surface of abstraction reaction between OH• radical and Kinsenoside for C–H bonds (A) and for O–H bonds (B)*

As seen in Figure 3, the tendency for all reaction positions of Kinsenoside with OH• is quite similar. The first intermediates (Int1) are formed with their energies −25.6 to −10.7 kcal/mol lower than that of the reactants. Afterwads, the pathways of reactions pass transition states (TS) which are characterized by the relative energy varying from −7.2 to 1.6 kcal/mol. In this state, the H atom of the O/C–H bond is located approximately midway between the O/C and O atom of OH• radical. For example, in case of abstraction at C8 position, the H-OH and C8-H distances are 1.375Å and 1.197Å, respectively (Figure S2 of SI). After passing TSs, the second intermediates (Int2) are formed lying at −43.4 to −18.4 kcal/mol below the

reactants. The final products of the reaction between OH• and KIN–H are KIN• radical and water with the variation of their relative energies from −18.4 to −7.6 kcal/mol.

Moreover, thermodynamic and kinetic parameters of abstraction reaction between OH• radical and Kinsenoside such as: activation ($\Delta G^{\neq}$) and reaction (ΔG) Gibbs free energies and rate constants (*k)* are summarizes in Table 2. Because of the negative ΔG values varying from –19.8 to –8.5 kcal/mol, all H-abstraction reactions are found to be exergonic and spontaneous.

As can be seen in Table 2, the $\Delta G^{\neq}$ of HAT reactions of all reaction sites varies from 1.1 kcal/mol to 8.8 kcal/mol while the rate constants are in the range of 5.9×10^{-8} – 4.6×10^{-13} cm^3/molecule.s. It is clearly observed that the H-abstraction reaction at C8 position has a lower energy barrier and proceeds faster than other positions with $\Delta G^{\neq}$ and *k* of 1.1 kcal/mol and 5.9×10^{-8} cm^3/molecule.s, respectively. This result strongly confirms that the C8–H bond plays an important role in the radical scavenging activity of Kinsenoside.

Table 2. *Activation ($\Delta G^{\neq}$), reaction (ΔG) Gibbs free energies and rate constants (k) at 298K for the abstraction of the hydrogen atom in different positions[(a)] of Kinsenoside calculated at the M052X/6-311G(d) level of theory in the gas phase*

Positions	ΔG (kcal/mol)	$\Delta G^{\neq}$ (kcal/mol)	*k* cm^3/molecule.s
C2-1	-15.2	8.8	3.1×10^{-13}
C2-2	-15.2	5.8	1.0×10^{-10}
C3	-13.9	8.2	4.6×10^{-13}
C4-1	-17.2	7.0	3.4×10^{-11}
C4-2	-17.2	7.3	2.5×10^{-12}
C5	-18.6	2.2	1.1×10^{-9}
C6	-15.6	4.2	2.4×10^{-10}
C7	-17.4	3.6	1.9×10^{-10}
C8	-15.6	1.1	5.9×10^{-8}
C9	-15.3	3.2	7.7×10^{-10}
C10-1	-19.8	4.5	5.1×10^{-11}
C10-2	-13.8	5.7	3.2×10^{-11}
O6	-9.2	6.1	3.1×10^{-10}
O7	-9.0	5.1	1.2×10^{-9}
O8	-8.5	5.3	6.1×10^{-10}
O10	-12.7	4.6	1.7×10^{-8}

[a] *Carbon atoms is numbered in Figure 2. The numbering of oxygen atoms corresponds to the carbon to which they are bonded*

4. Conclusions

Based on the results of the present study, some conclusions can be drawn as follows:

- The Gibbs free energy (ΔG) calculated for all SET and HAT reactions between Kinsenoside and OH• radical reveals that the HAT reactions are exergonic and more preferred than the SET reaction. Thus, SET mechanism does not contribute significantly to the scavenging of hydroxyl radical.

- The PES of abstraction reaction between OH• radical and Kinsenoside for all O/C–H bonds has been studied in detail.

- In the H-abstraction among different positions, the reaction at C8–H position is determined as the most favored with $\Delta G^{\neq}$ and *k* of 1.1 kcal/mol and 5.9×10^{-8} cm^3/molecule.s, respectively. This result may provide the evidence to explain the other biological activities according to computational point of view

Acknowledgements. This research is funded by Funds for Science and Technology Development of the University of Danang under project number B2017-ĐN08-03.

REFERENCES

[1] Shao Q., et al., "Effects of shade treatments on photosynthetic characteristics, chloroplast ultrastructure, and physiology of Anoectochilus roxburghii", *PLoS One*, 9, 2014, e85996.

[2] Cui S. C., et al., "Antihyperglycemic and antioxidant activity of water extract from Anoectochilus roxburghii in experimental diabetes", *Exp Toxicol Pathol*, 65, 2013, 485-8.

[3] Zhang Y., et al., "Antihyperglycemic activity of kinsenoside, a high yielding constituent from Anoectochilus roxburghii in streptozotocin diabetic rats", *J Ethnopharmacol*, 114, 2007, 141-5.

[4] Shao Q., et al., "Essential oils extraction from Anoectochilus roxburghii using supercritical carbon dioxide and their antioxidant activity", *Industrial Crops and Products*, 60, 2014, 104-112.

[5] Liu Q., et al., "The semisynthetic spin-labelled derivatives of 3-hydroxybutanolide as potential oxidative stress inhibitors", *Nat Prod Res*, 28, 2014, 1037-44.

[6] Liu Q., et al., "3-Hydroxybutanolide derivatives and flavonoid glucosides from Anoectochilus roxburghii", *Phytochemistry Letters*, 8, 2014, 109-115.

[7] Liu Z. L., et al., "The vascular protective properties of kinsenoside isolated from Anoectochilus roxburghii under high glucose condition", *Fitoterapia*, 86, 2013, 163-70.

[8] [8].Deppmeier B. J., et al., *Spartan*, 2002: Irvine, CA: Wavefunction.

[9] Frisch M. J., et al., *Gaussian 09*, 2009, Gaussian, Inc.: Wallingford, CT, USA.

[10] Cancès E., et al., "A new integral equation formalism for the polarizable continuum model: Theoretical background and applications to isotropic and anisotropic dielectrics", *The Journal of Chemical Physics*, 107, 1997, 3032-3041.

[11] Tomasi J., et al., "The IEF version of the PCM solvation method: an overview of a new method addressed to study molecular solutes at the QM ab initio level", *Journal of Molecular Structure-theochem*, 464, 1999, 211-226.

[12] Wigner E., "On the Quantum Correction For Thermodynamic Equilibrium", *Physical Review*, 40, 1932, 749-759.

[13] Eckart C., "The Penetration of a Potential Barrier by Electrons", *Physical Review*, 35, 1930, 1303-1309.

[14] Dzib E., et al., "Eyringpy: A program for computing rate constants in the gas phase and in solution", *International Journal of Quantum Chemistry*, 119, 2018, e25686.

[15] Dzib E., et al., *Eyringpy 1.0.2*, 2018: Cinvestav, Mérida, Yucatán.

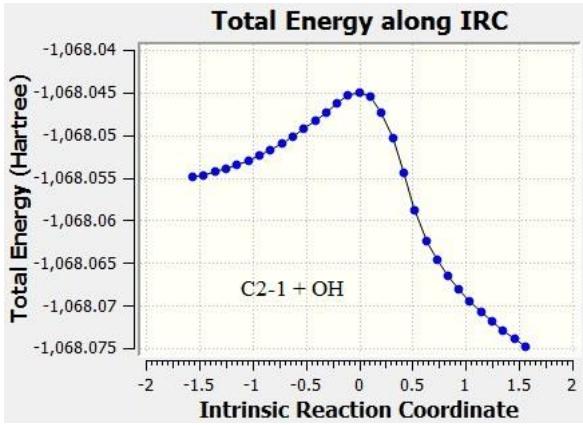
[16] Ngo Thi Chinh, et al., "A DFT analysis on the radical scavenging activity of oxygenated terpenoids present in the extract of the buds of Cleistocalyx operculatus", *RSC Advances*, 7, 2017, 39686-39698.

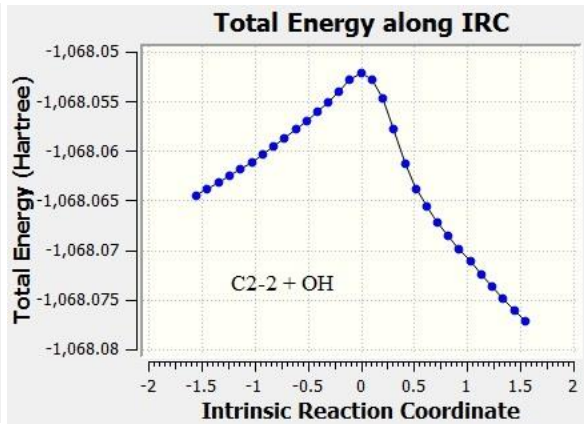
[17] Ngo Thi Chinh, et al., "Insight into the antioxidant properties of non-phenolic terpenoids contained in essential oils extracted from the buds of Cleistocalyx operculatus: a DFT study", *RSC Advances*, 6, 2016, 30824-30834.

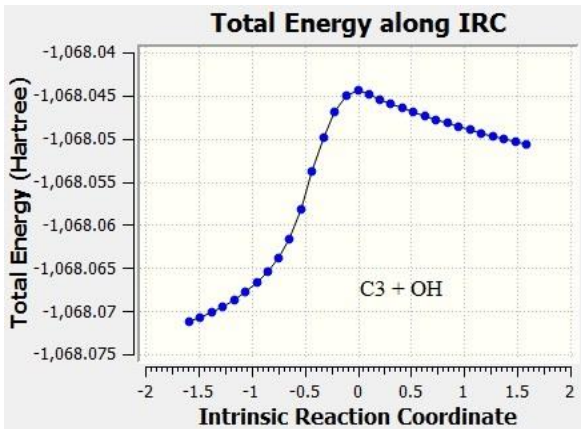
[18] Thong Nguyen Minh, et al., "Theoretical investigation on the bond dissociation enthalpies of phenolic compounds extracted from Artocarpus altilis using ONIOM(ROB3LYP/6-311++G(2df,2p):PM6) method", *Chemical Physics Letters*, 613, 2014, 139-145.

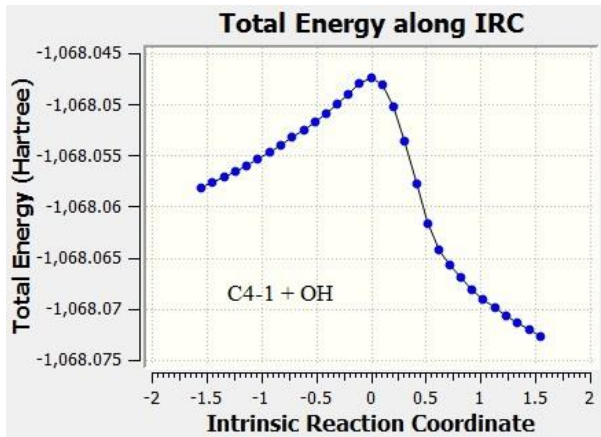
Supporting information

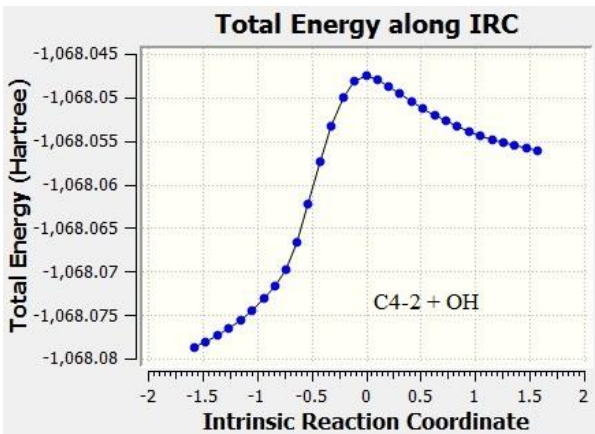
Figure S1. *IRC plots for all transition states related to reaction of OH• radical with Kinsenoside calculated at M052X/6-311G(d) level in the gas phase*

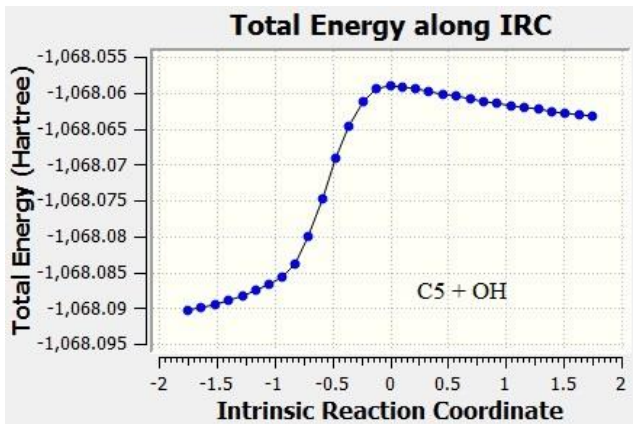


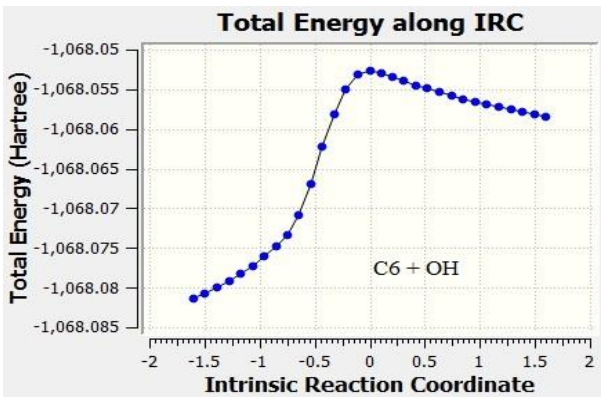


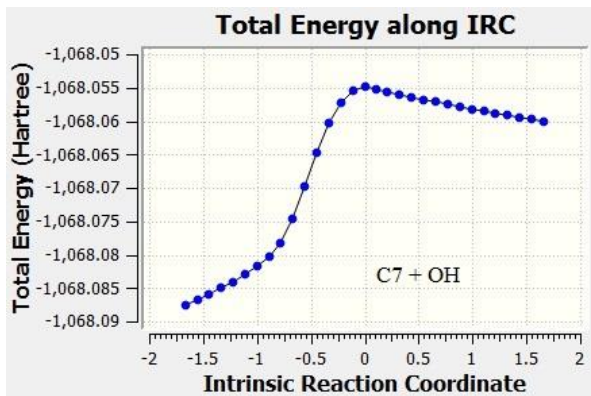












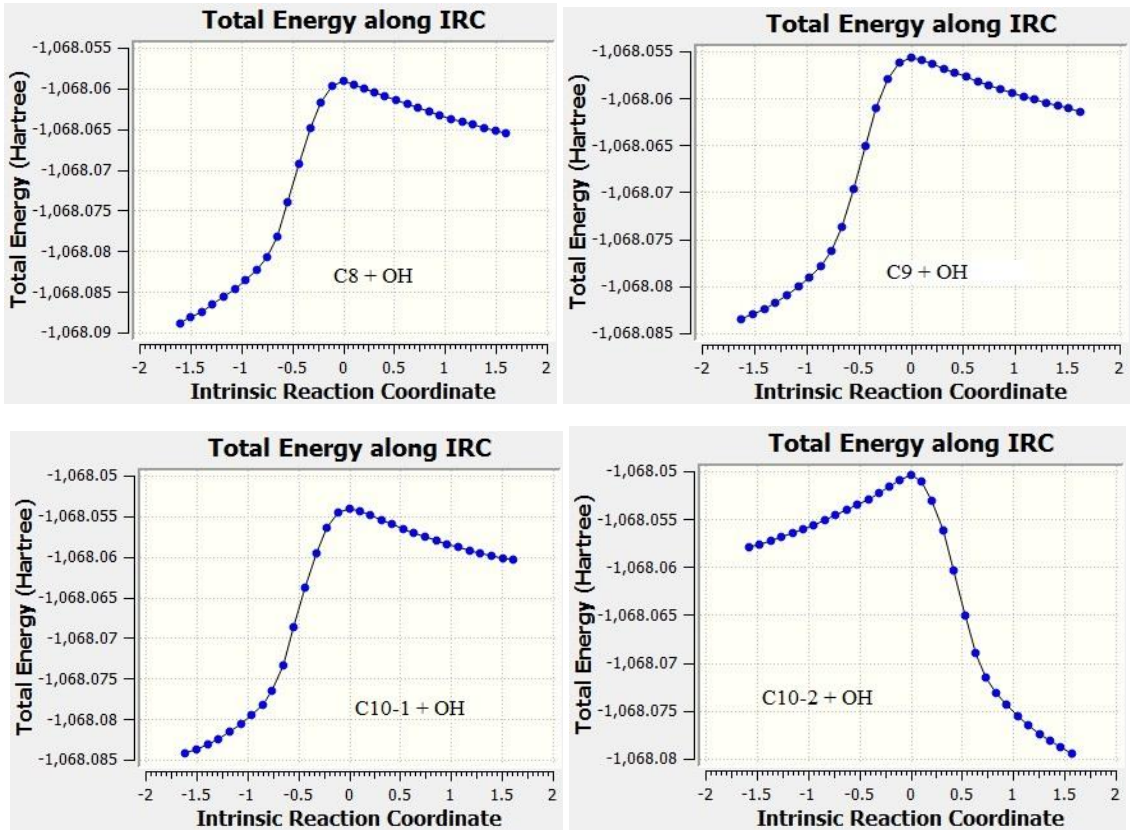
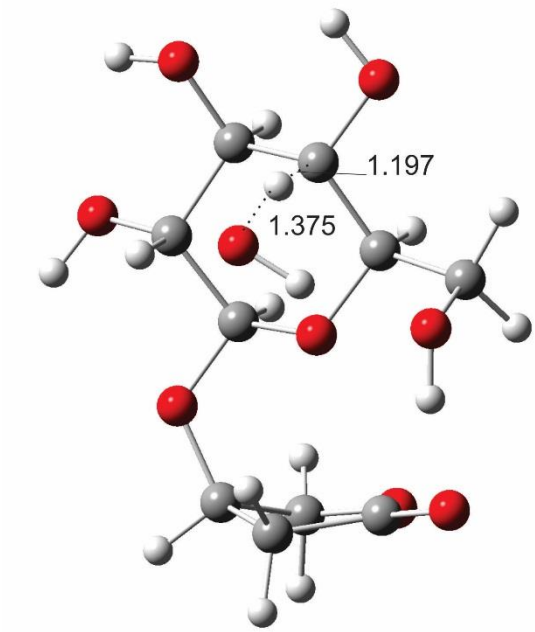


Figure S2. *Optimized geometry of the transition state for the abstraction of the hydrogen atom at C8 position of Kinsenoside calculated at the M052X/6-311G(d) level of theory. Distances are reported in Å*



(The Board of Editors received the paper on 12/8/2019, its review was completed on 25/3/2020)