New Mechanistic Insights: Why Do Plants Produce Isoprene?

Nassim Zeinali, Mohammednoor Altarawneh,* Dan Li, Jomana Al-Nu‘airat, and Bogdan Z. Dlugogorski

School of Engineering and Information Technology, Murdoch University, 90 South Street, Murdoch, Western Australia 6150, Australia

Supporting Information

ABSTRACT: In this article, we argue that the primary role of isoprene is to remove the singlet delta oxygen (O$_2$ $^1\Delta_g$) that forms inside plants by ultraviolet excitation rather than to provide heat protection or scavenge ozone, OH, or other reactive oxygen species (ROS) in the gas phase. By deploying a quantum chemical framework, we address for the first time the exact mode of isoprene reactions with O$_2$ $^1\Delta_g$, the most prominent ROS that causes damage to leaves. Initial reactions of isoprene with O$_2$ $^1\Delta_g$ comprise its addition at the two terminal carbon atoms. The two primary open-shell adducts that appear in these reactions undergo 1,2-cycloaddition to generate methyl vinyl ketone and methacrolein, the sole products detected from in-home (i.e., inside of plants) oxidation of isoprene. Formation of other products, comprising the peroxy O–O bonds, is kinetically insignificant. Furthermore, these adducts are thermodynamically too unstable to diffuse outside of plants. Oxidation of isoprene with O$_2$ $^1\Delta_g$ does not produce new ROS (such as OH or HO$_2$), supporting the well-documented role of isoprene as an effective ROS scavenger. Deploying a solvation model reduces the energy requirements for the primary pathways in the range of 10–56 kJ/mol. The present results indicate that plants attach significant value to the in-home protection against O$_2$ $^1\Delta_g$ by investing carbon and energy into the formation of isoprene, in spite of the appearance of the cytotoxic methyl vinyl ketone as one of the reaction products. (The same chemical species also form in unrelated gas-phase reactions involving isoprene and other ROS.) This finding explains the primary reason for the appearance of the dynamic biosphere–atmosphere exchange of methyl vinyl ketone.

1. INTRODUCTION

Emission of isoprene (2-methyl-1,3-butadiene) from many terrestrial plants accounts for nearly one-third of the annual global budget of volatile organic compounds (VOCs) from all biogenic and anthropogenic sources combined.1 Owing to its high chemical reactivity, once emitted to the atmosphere, isoprene plays a crucial role in many atmospheric phenomena.2 Mounting evidence indicates a plausible thermoslike protection of leaves from certain types of heat shocks.3 However, the most discussed role of isoprene is the mitigation of the effect of reactive oxygen species (ROS) in plants.4 By acting as an ROS scavenger, isoprene can prevent visible damage caused by exposure of leaves to ozone or OH radicals and reduce the loss in their photosynthetic capacity. Nonetheless, isoprene–ROS reactions remain to be elucidated, especially those occurring inside plants.

The lowest excited state of singlet delta oxygen (O$_2$ $^1\Delta_g$) constitutes the most prominent ROS causing damage to leaves6 in such a way that this exotic species accounts for over 80% of lipid oxidation within leaf tissues.7 Photo-induced activation produces O$_2$ $^1\Delta_g$ in plants, mainly mediated by the excited state of the triplet chlorophyll ($^3$Chl*).8 The literature has documented well the O$_2$ $^1\Delta_g$ scavenging properties of isoprene.9 Isoprene acts as an effective quenching agent for many ROS, including the O$_2$ $^1\Delta_g$.10 Through the use of rose bengal as a photo-sensitizer, experiments9b,10 have demonstrated that isoprene protects plants against the damaging effect of O$_2$ $^1\Delta_g$. In fact, ultraviolet (UV) excitation of an isoprene/ground state oxygen mixture results in the formation of O$_2$ $^1\Delta_g$.10 Because the biochemical synthesis of isoprene displays light-dependency, the resistance to O$_2$ $^1\Delta_g$ correlates positively with the light intensity.10 However, the exact mode of isoprene attacks on O$_2$ $^1\Delta_g$ remains largely unclear. Jardine et al.11 have experimentally simulated a “within-plant” oxidation process of isoprene to report methyl vinyl ketone and methacrolein as the sole oxidation products. The authors linked the formation of this exotic species also form in unrelated gas-phase reactions involving isoprene and other ROS.) This finding explains the primary reason for the appearance of the dynamic biosphere–atmosphere exchange of methyl vinyl ketone.
these two compounds with the functionality of isoprene as an ROS scavenger but without pointing to $O_2 \Delta_g$ in particular.

Here, we suggest that the main role of isoprene is to remove $O_2 \Delta_g$ that forms inside of plants by the photo-induced activation process. From this perspective, by deploying quantum chemical calculations, this article provides a detailed mechanistic account of the reactions of $O_2 \Delta_g$ with isoprene. Because of the highly transient nature of $O_2 \Delta_g$ (lifetime of a few microseconds), the reaction chemistry of $O_2 \Delta_g$ constitutes an excellent candidate for a purely theoretical scrutiny. The reaction mechanism and the kinetic parameters developed herein will assist in formulating an atomic-scale understanding of the protective functions of isoprene in plants against ROS, particularly $O_2 \Delta_g$.

2. METHODOLOGY

Normally, treatment of the biradical character exhibited by the adduct of isoprene and $O_2 \Delta_g$ would require the application of multireference wave function methods such as CASSCF and CASPT2. The pioneering work by the Yamaguchi group has demonstrated that the energies of the biradical systems computed by any single-determinant method can be significantly improved by the application of a simple approximate spin-projection (AP) scheme. In this scheme, an approximate spin-projected energy ($E^{AP}$) can be derived from the energies of the broken-symmetry ($E^{BS}$) and pure high-spin ($E^{HS}$) states

$$E^{AP} = f^{AP}E^{BS} - (f - 1)E^{HS}$$

where $f^{AP}$ denotes the spin-projection factor,

$$f^{AP} = \frac{(S^2)^{HS} - s(s + 1)}{(S^2)^{BS} - (S^2)^{HS}}$$

and $(S^2)^{HS}$ and $(S^2)^{BS}$ signify the expectation values of spin contamination pertinent to the pure high-spin and broken-symmetry states, respectively. Application of the AP methodology to various biradical systems yields B3LYP-derived energies to within 4.2–12.6 kJ/mol of the corresponding values obtained with more expensive multireference methods. In a series of contributions, Cremer and co-workers showed that unrestricted density functional theory (UDFT) methods perform surprisingly well in deriving energies and geometries of singlet biradicals.

Accordingly, we deploy the density functional theory (DFT) functional of B3LYP with the extended 6-311+G(d,p) basis set to investigate the biradical system of isoprene and $O_2 \Delta_g$. Final energies are corrected using the AP approach as described above, whenever the reacting species (including reactants, products, and transition states) attain a biradical character. As an initial accuracy benchmark, we calculate the triplet–singlet delta enthalpic gap of the oxygen molecule to be 85.4 kJ/mol, in a relatively good agreement with the corresponding experimental value at 94.1 kJ/mol. Standard UB3LYP calculations result in a significantly larger enthalpy gap of 161.5 kJ/mol, demonstrating the necessity of applying the AP approach. Table S1 enlists the calculated thermal enthalpies and the expected spin contamination values, that is, values required in calculations based on the AP methodology. We carry out all structural optimizations and energy calculations using the Gaussian 09 suite of programs. We have carefully confirmed the identity of each transition structure by performing intrinsic reaction coordinate (IRC) calculations.

The ChemRate code facilitates the calculation of the reaction rate constants, in the temperature range of 300–600 K. We obtain the Fukui indices for electrophilic attack ($f^1$) and the electronic Hirshfeld charges with the aid of DMol3 code. The Fukui $f^1$ indices serve as a molecular descriptor, reflecting the tendency of a certain site in a chemical species to undergo an electrophilic addition.

3. RESULTS AND DISCUSSION

The structure of isoprene features a conjugated $\pi$ system, where the digits 1 and 2 denote two sites for the addition of $O_2 \Delta_g$.

(Figure 1). Figure 2 depicts the mechanism of the reactions of isoprene with $O_2 \Delta_g$, with the geometries of intermediates and transition structures portrayed separately in Figures S1 and S2.

The electrophilic properties of $O_2 \Delta_g$ enable the introduction of oxygen atoms into olefins, dienes, and aromatic structures through three main categories of reactions: 1,2-cycloaddition to double bonds, 1,4-cycloaddition to aromatic rings, and the so-called concerted ene reaction. Generally, the nonconcerted mechanism underlying the reactions of typical dienes with the $O_2 \Delta_g$ passes through diradical intermediates. For instance, the diradical peroxo adduct has been previously observed in the case of cis-1,3-butadiene. Similarly, in this study, the simulations led to two diradical intermediates ($M_1$ and $M_{14}$).

All attempts to locate the transition structures preceding 1,2-, 1,4-cycloadditions or a direct abstraction of a methyl hydrogen atom (i.e., an ene reaction) converge to the peroxo adducts $M_1$ and $M_{14}$, in which the oxygen molecule binds to the terminal carbon atoms at sites 1 and 14, respectively. If it operates in our system, ene reaction should result in the formation of an allylic hydroperoxide upon a shift in the position of the most electron-rich double bond (i.e., species $M_3$ in Figure 2). If the occurrence of an ene is a feasible opening channel, this would violate the role of isoprene as an ROS scavenger because it results in the generation of another potent ROS species, namely the OH radical, through dissociation of the weak O–OH bond.

Although peroxirane and zwitterionic intermediates were detected on the potential energy surface in the reaction between butadiene and $O_2 \Delta_g$, despite our best efforts, we were unable to locate these intermediates. Nonetheless, conversion of the initially formed diradical peroxy-type adduct into peroxoene was shown to be a highly endothermic process by about 100 kJ/mol. Analogously, a related study predicted that a zwitterionic adduct resides nearly 40 kJ/mol above the zwitterionic adduct $M_3$ and $M_{14}$.

Rotation of the peroxy O–O bond in $M_1$ and $M_{14}$ could potentially generate syn and anti conformers. However, performing a partial optimization along the corresponding dihedral angles leads to structures in which the outer oxygen atom either absorbs a hydrogen atom or adds to the carbon skeleton (one of the products in Figure 2); viz., stable syn and anti conformers do not arise in $M_1$ and $M_{14}$.

The $M_1$ and $M_{14}$ moieties, in addition to their transition structures $TS_1$ and $TS_{12}$, possess an open-shell character. On
the basis of AP-corrected enthalpies (298.15 K), the reaction barrier of TS\textsubscript{12} (99 kJ/mol) overshoots that of TS\textsubscript{1} by 51 kJ/mol. To elucidate the different reactivities for the addition of O\textsubscript{2} to sites 1 and 2, Figure 3 displays the calculated Fukui indices and electronic charges on isoprene. The higher electron densities and $f^{-1}$ values at site 2 in comparison with the analogous estimates at site 1 concur with the lower activation barrier of TS\textsubscript{1} with reference to TS\textsubscript{12}.

Figure 2. Reaction routes in the isoprene + O\textsubscript{2} system obtained by the calculation level of B3LYP/6-311+G(d,p). The values in bold and italics signify reaction and activation enthalpies computed at 298.15 K, respectively (in kJ/mol).

Figure 3. Fukui $f^{-1}$ indices (in bold) and Hirshfeld charges (in brackets).

The two initial adducts, M\textsubscript{1} and M\textsubscript{14}, reside at a similar level above the entrance channel. As Figure 2 depicts, the initial adduct M\textsubscript{1} branches into seven exit routes, whereas M\textsubscript{14} launches six channels. In the main channel, a 1,2-cycloaddition occurs via TS\textsubscript{7} overcoming a modest enthalpic barrier of 46 kJ/mol and forms the M\textsubscript{7} adduct. A very exothermic C−C bond fission ($−376$ kJ/mol) in M\textsubscript{7} provides the experimental product for in-house oxidation of isoprene, the methyl vinyl ketone molecule. This process takes place through a barrier of 92 kJ/mol embedded in TS\textsubscript{8}.

The transition states TS\textsubscript{2}, TS\textsubscript{4}, and TS\textsubscript{6} from the main channel, along with TS\textsubscript{13} and TS\textsubscript{14} signify H-migration reactions, in which the outer oxygen atom abstracts a hydrogen atom from the neighboring carbon atoms through sizable reaction barriers amounting to 151, 166, 80, 86, and 143 kJ/mol, respectively. Fission of the relatively weak O−OH bond in the structure of hydroperoxide intermediates (such as M\textsubscript{5} and M\textsubscript{16}) requires modest bond dissociation enthalpies in the range of 141−147 kJ/mol. Nonetheless, significantly higher barriers for the formation of allylic hydroperoxide (80−166 kJ/mol) with reference to the barrier of the main route (46 kJ/mol)
indicate that the formation of the propagating OH radical from the unimolecular rearrangement of M1 is highly unlikely. All our attempts to find transition states connecting M1 directly with M3 and M5 have led to TS2 and TS4, respectively. This interpretation has been guided by detailed IRC calculations and vibration analysis. The formation of hydroperoxide M6 from M1, as well as M15 from M14, occurs via H abstraction from the CH₂ site by the terminal oxygen atom of the peroxy group, with modest reaction barriers of 80 kJ/mol (TS7) and 86 kJ/mol (TS15), respectively. Transition structures TS9 (137 kJ/mol) and TS10 (67 kJ/mol) engender 1,3- and 1,4-cycloadditions commencing from M1. Formation of the six-membered ring, M11 structure, from M1 and M14 proceeds via similar reaction barriers through TS10 and TS18. The peroxy O−O bonds in the molecules are typically very weak, that is, 150 kJ/mol. This explains the absence of M10, M11, and M19 from the products of isoprene oxidations initiated by O₂¹Δg. Both M1 and M14 are capable of forming short-lived dioxirane species M13 and M21 via a two-step pathway, initiated by 1,2-hydrogen transfer characterized by the TS11 (115 kJ/mol) and TS19 (117 kJ/mol) transition states. The corresponding biradical intermediates of M12 and M20 then pass through a barrierless unimolecular cyclization to form the highly stable heterocyclic peroxides M13 and M21. Because the reaction barriers associated with TS11 and TS19 significantly overshoot the barriers of the primary channels (TS7 and TS15), synthesis of dioxiranes from the reaction of isoprene with O₂¹Δg is largely negligible.

On the basis of the reaction and activation barriers displayed in Figure 2, the channel associated with the attack of O₂¹Δg molecule on the double bond at site 2 produces methyl vinyl ketone predominantly. Likewise, addition of oxygen at site 1 preferentially forms the second experimentally detected product of methacrolein. The kinetic parameters listed in Table 1 reveal that the overall isoprene + O₂¹Δg reaction proceeds mainly by the addition of O₂¹Δg at site 2 (M1) and forms methyl vinyl ketone molecule in a three-step mechanism
(M_1 → M_2 → M_8 + M_9). All other channels display negligible importance, including isomerization of M_1 into the six-membered cyclic peroxide M_{11}.

To investigate the solvent effect on the reaction energetics for the two main pathways (i.e., formation of M_8 and M_9 from M_1 and M_{11}, respectively), we deploy a polarizable continuum model (PCM) \(^{27}\) at the B3LYP/6-311g+(d,p) level of theory. Figure 4 contrasts the energies obtained in the gaseous and aqueous media. The PCM approach simulates the solvent effects by generating multiple overlapping spheres inside of a dielectric continuum, around the atoms within the molecule. The PCM predicts a systematic energy reduction in the range of 10 kJ/mol (TS_{13}) to 56 kJ/mol (TS_{15}) for transition states, intermediates, and final products along the two primary channels. Overall, the effect of the solvent is limited to reducing the energy penalties whereas the product distribution (i.e., based on kinetic values in Table 1) remains unchanged with reference to the gas-phase system.

Methyl vinyl ketone also constitutes a major product from the OH-initiated atmospheric oxidation of isoprene.\(^{28}\) However, in accordance with the experimental finding of Jardine et al.,\(^{11}\) we have shown that methyl vinyl ketone also appears from a within-plant oxidation of isoprene with O_2. Jardine et al.\(^{11}\) have indicated, this calls for a reconsideration of the biogenic life cycle of isoprene and its oxidation products. Clearly, plants devote a significant effort into their protection against O_2 + M by producing isoprene in a diurnal cycle\(^{7}\) that reflects the formation of O_2 + M in spite of the appearance of the cytotoxic methyl vinyl ketone as the reaction product.

## ASSOCIATED CONTENT
2 Supporting Information
The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acsomega.6b00025.

Table S1 (thermal enthalpies and spin contamination values for all species), Figures S1 (geometries of stable products and adducts), and Figure S2 (geometries of transition structures) (PDF)

## AUTHOR INFORMATION
Corresponding Author
*E-mail: m.altarawneh@murdoch.edu.au. Phone: (+61) 8 9360 7507.

Notes
The authors declare no competing financial interest.

## ACKNOWLEDGMENTS
This study has been supported by grants of computing time from the National Computational Infrastructure (NCI) and from the Pawsey Supercomputing Centre, Australia, as well as funds from the Australian Research Council (ARC). N.Z. and J.A.-N. thank Murdoch University for the award of postgraduate scholarships.

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