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Synthesis of 1-(2,2-Dimethylpropyl)-Cyclopropene (1-DCP) as an Ethylene Antagonist

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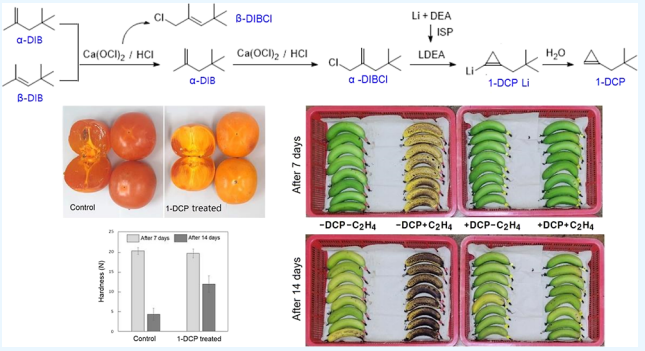
ABSTRACT: Ethylene is a gaseous hydrocarbon molecule known as a plant hormone that promotes fruit ripening and senescence. Efficiently controlling ethylene is a central key to maintaining the quality of agricultural products. The current study uncovered a synthetic method for 1-(2,2-dimethylpropyl)-cyclopropene (1-DCP) as a cyclopropene derivative to inhibit ethylene action in fruit ripening and senescence. We synthesized 1-DCP using α-diisobutylene through a two-step process, including allylic chlorination by hypochlorite and HCl, followed by α-elimination of the allylic chloride using a strong base, lithium diethylamide. GC−MS and NMR analyses demonstrated that 1-DCP was synthesized efficiently with 35% yield and 95% purity. When treated as an aqueous emulsion on plants, including persimmon and banana fruits, 1 mM 1-DCP showed effective inhibition of ethylene action by delaying the flesh softening and peel degreening, which are representative phenomena of fruit ripening and senescence induced by ethylene. Our data demonstrated that 1-DCP could be synthesized and used as a sprayable ethylene antagonist for pre- or post-harvest growth regulation in plants and fruits.



INTRODUCTION

Phytohormones regulate plant growth and development, including auxin, gibberellin, cytokinin, abscisic acid, and ethylene. Ethylene plays a crucial role in various developmental aspects throughout the plant life cycle.[1,2] Microarray analysis of gene expression has revealed that approximately 7% of the 6,000 genes are down- or up-regulated by ethylene.[3] For instance, it is involved in early developmental processes, including seed dormancy and germination, root and shoot growth, flowering, and sex determination, as well as final stages, including flower senescence and fruit ripening.[4] However, the adverse effects of ethylene in promoting plant senescence and fruit ripening may result in reduced quality of horticultural products and consequent economic loss.[5] Hence, the control of ethylene is a significant concern in horticultural production.[2,6,7]

Plants synthesize ethylene from methionine through the Yang cycle.[8,9] Ethylene biosynthesis involves the conversion of methionine to S-adenosyl-L-methionine (SAM) by SAM synthetase, followed by 1-aminocyclopropane-1-carboxylic acid (ACC) synthesis by ACC synthase from SAM. ACC is then oxidized by ACC oxidase in the presence of iron (Fe^{2+}), ascorbate, and oxygen to produce ethylene.[10] The conversion of SAM to ACC is the rate-limiting step in the ethylene biosynthesis pathway. ACC synthase is activated by stress or at specific stages of development, including fruit ripening. One ethylene control strategy involves inhibiting the enzymes involved in ethylene biosynthesis. For instance, aminoethoxyvinylglycine (AVG) inhibits the pyridoxal enzyme ACC synthase to reduce the preharvest drop of apple or peach fruits.[11] Another strategy of ethylene control is to antagonize its action. As a hormone, ethylene must bind to a specific receptor protein to initiate signal transduction, leading to gene expression and physiological responses.[12] In the 1970s, Sisler discovered that cyclic olefins could inhibit ethylene action by irreversibly binding to the putative receptor protein.[13−15] He also found that among the various cycloolefins, cyclopropene has a high binding affinity for ethylene receptors and is the most effective compound for blocking ethylene action at gaseous concentrations as low as nL/L. His invention, 1-methylcyclopropene (1-MCP), is widely used as an agent to maintain the freshness of postharvest horticultural products.[16,17]

However, the practical application of 1-MCP has a limitation due to its gaseous nature at ambient temperature, with a boiling point of 10−12 °C. Accordingly, 1-MCP could be used in a closed system to expose plant material to an optimum concentration for a sufficient duration. We hypothesized that a

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higher carbon number of cyclopropene derivatives would increase the boiling point, potentially facilitating the control of their concentration and inhibiting ethylene reactions in an open space. We synthesized a new ethylene inhibitor, 1-(2,2-dimethylpropyl)-cyclopropene (1-DCP), from commercially available building blocks.

MATERIALS AND METHODS

Materials. Diisobutylene (Daejung Chemicals and Metals, Korea), calcium hypochlorite (CAS 7778-84-3, Samchun Chemicals, Korea), hydrochloric acid (CAS 7647-01-0, Daejung), lithium (1−6 mm granule, Thermo-Fisher Scientific, USA), diethylamine (Daejung), isoprene (Samchun), diethyl ether (Daejung), Celite 545, and a-cyclodextrin (Henrikang Biotech, China) were obtained commercially with reagent grade. All reagents and catalysts were used as received, unless otherwise indicated. Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were obtained by Bruker-500 MHz spectrometry in $CDCl_3$ solutions. NMR chemical shifts were reported relative to residual $CDCl_3$ (7.25 ppm). Proton-decoupled ^{13}C NMR spectra were acquired, with chemical shifts reported relative to $CDCl_3$ (77.0 ppm). Chromatograms were obtained using gas chromatography (Shimadzu GC 2010, Japan) equipped with a flame-ionization detector (FID) and capillary column (HP-5, non-polar, dimension 0.25 mm × 30 m, FT 0.25 μm). The column temperature was held at 60 °C for 2 min, then increased to 200 °C at 25 °C/min. Mass spectra were acquired using gas chromatography (Thermo-Fisher TRACE 1610) connected to a mass spectrometer (ISQ7610) and equipped with a capillary column of TraceGold-5SiMS (non-polar, dimension 0.25 mm × 30 m, film thickness 0.25 μm). The oven temperature program started at 50 °C, then gradually increased to 300 °C at 10 °C/min, and held for 10 min at 300 °C.

Preparation of α-DIB from α/β-DIB Mixture. The building block of 1-DCP synthesis, α-diisobutylene (α-DIB, CAS 107-39-1), was separated from a commercial mixture of α/β-isomers of diisobutylene (DIB, CAS 25167-70-8), as follows. 200 g of calcium hypochlorite (active ingredient content 70%) was dissolved in 1 L of distilled water by ultrasonication, followed by centrifugation to remove the precipitate and obtain a hypochlorite solution. Chilled DIB (328 mL, 2 mol, 3:1 α/β-isomer mixture) was added to the cold hypochlorite solution (511 mL, 1 mol OCl^-) in a 1 L pressure-resistant bottle (max$P$ = 1.5 bar, Duran Pressure plus bottle, code 10130780). Hydrochloric acid (36% HCl, 85.8 mL, 1 mol) was added to a small container, which was carefully transferred into the pressure bottle to prevent the HCl from mixing with the hypochlorite solution before the bottle was capped. The bottle was tightly capped and vigorously shaken for 3 min. During the shaking process, high temperature (60−70 °C) and pressure were formed, and the reaction was completed. After transferring the resulting mixture to a 1 L separatory funnel to remove the lower aqueous phase, the upper phase was collected and refrigerated for 1 h to remove a small amount of water to obtain a mixture of α-DIB and 1-chloro-2,4,4-trimethylpent-2-ene (β-DIBCl, 328 mL, colorless). Repeating the above process six times generated 2 L of crude mixtures. For fractional distillation, a 1 L two-necked round-bottom flask was equipped with a stirring bar, a thermometer, a 30 cm Vigreux column, a Liebig condenser (0 °C coolant), a 500 mL mass cylinder as a distillate receiver, and a heating mantle. 1 liter of the α-DIB/β-DIBCl mixture was distilled to give 500 mL of the first fractional distillation product (colorless, purity 93% by GC). The above process was repeated twice to obtain 1 L of α-DIB. For secondary distillation, 1 L of α-DIB was added into the fractional distillation system to purify at a distillation rate of 7 to 8 mL/min. The distillation was terminated when 90% volume of the reactant was recovered (α-DIB 900 mL, colorless, density = 0.73, final yield 63%, purity 98% by GC). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.83 (dd, J = 2.5, 1.4 Hz, 1H), 4.63−4.61 (m, 1H), 1.93 (s, 2H), 1.77 (s, 3H), 0.92 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 144.02, 113.73, 51.66, 31.35, 30.06, 25.27. GCMS $[C_3H_5]^+$ = 41, $[C_4H_7]^+$ = 55, $[C_4H_9]^+$ = 57 base peak, $[C_5H_9]^+$ = 69, $[C_7H_{13}]^+$ = 97, $[C_8H_{16}]^+$ = 112 molecular ion peak.

Synthesis of α-DIBCl by Allylic Chlorination of α-DIB. Alpha-DIB was converted to 2-(chloromethyl)-4,4-dimethyl-1-pentene (α-DIBCl) through allylic chlorination using hypochlorite and HCl. Hypochlorite was prepared as described previously. Cold α-DIB (157 mL, 1 mol) was added into 613 mL of cold hypochlorite solution (1.2 mol OCl^-) in the 1 L pressure-resistant bottle. Cold hydrochloric acid (114 mL, 1.3 mol) was added into a small container, which was transferred carefully into the bottle to prevent the HCl from mixing with the hypochlorite solution in the bottle before the bottle was capped. The bottle was tightly capped and shaken vigorously for 3 min. During the shaking process, the color of the mixture was changed from green to colorless, and the chlorination was completed. High temperature (60−70 °C) and pressure were formed in the bottle. After transferring the resulting mixture to a 1 L separatory funnel to remove the lower aqueous phase, the collected supernatant was dehydrated under anhydrous sodium sulfate. The above process was repeated five times to obtain 800 mL of crude α-DIBCl. The crude α-DIBCl (800 mL) was purified using a distillation system, including a 1 L two-necked round bottom flask with a magnetic bar, a thermometer, a splash head adapter, a 30 cm Liebig condenser (0 °C coolant), a graduated bottle as a distillate receiver in an ice-water bath, a heating mantle, and a vacuum line. By heating under a 50−200 mBar vacuum, 70% of the reactant volume was recovered as a distillate of α-DIBCl (560 mL, colorless, 75% yield, purity 80% by GC). α-DIBCl ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.28 (d, J = 1.1 Hz, 1H), 4.96 (s, 1H), 4.07 (d, J = 0.9 Hz, 2H), 2.09 (s, 2H), 0.93 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 143.24, 118.11, 49.78, 46.35, 31.43, 29.75.

Synthesis of 1-DCP Lithium Salt by α-Elimination of α-DIBCl Using LDEA. Lithium diethylamide (LDEA) was prepared for the α-elimination of α-DIBCl. A 500 mL three-necked round bottom flask was equipped with a magnetic bar, thermometer, and a 30 cm Dimroth condenser (0 °C coolant) with a gas bubbler. Diethylamine (52.8 mL, 0.5 mol) and diethyl ether (200 mL) were added to the reaction flask and purged with Ar gas. Lithium granules (3.51 g, 0.5 mol, granule size 1−6 mm) were weighed under Ar gas and quickly added to the flask. Isoprene (ISP, 27.8 mL, 0.275 mol) was injected into the flask through the rubber septum using a syringe with vigorous stirring. The mixture's temperature rose and boiled at 38−40 °C within 3 min after ISP injection, and the reaction mixture was refluxed at 35−36 °C constantly. When the lithium disappeared in 30 min, the LDEA reaction was terminated. The flask containing LDEA was cooled to −40 °C using a cold ethanol bath under Ar. Alpha-DIBCl (50 mL, 0.24 mol) was slowly injected into the flask through the septum using a syringe while stirring. The injection speed was controlled to prevent the temperature from exceeding −10

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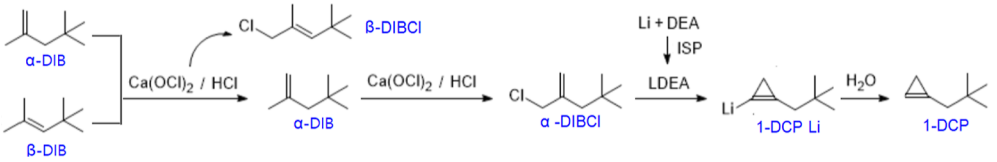

Figure 1. Synthetic scheme of 1-DCP. 1-DCP was synthesized from building block α-diisobutylene. Allylic chlorination and α-elimination generated the lithium salt of the final product, followed by protonation to give 1-DCP.

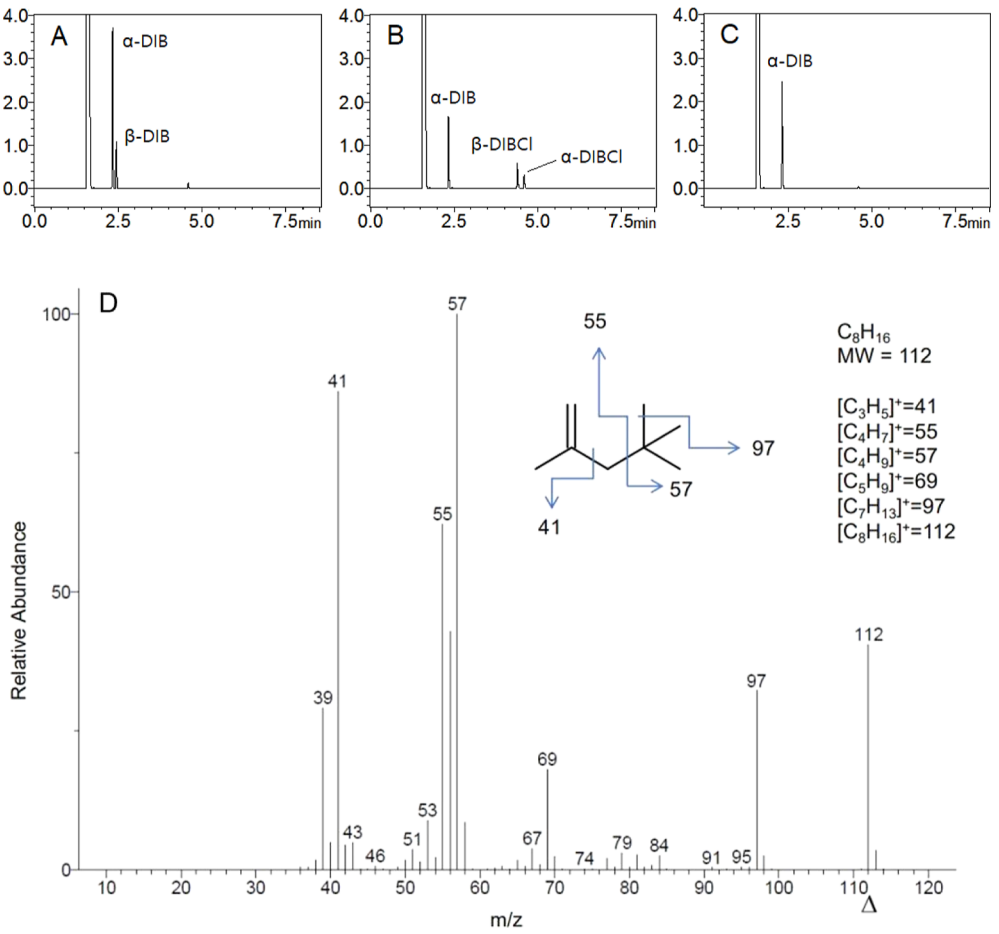

Figure 2. Chromatograms and the mass spectrum of DIB. Commercial α/β-DIB mixture (A). Commercial mixture was chlorinated, producing an α-DIB/β-DIBCl mixture (B). Distillation-purified α-DIB (C). Mass spectrum of α-DIB showed the molecular ion peak ($[C_8H_{16}]^+$ = 112) and the base peak ($[C_4H_9]^+$ = 57 (D).

°C, as α-DIBCl reacts violently with LDEA, causing the temperature to rise steeply and potentially boil over. After completion of the α-DIBCl injection, the bath was exchanged for ice water, and the temperature was allowed to increase gradually. When the flask temperature reached 10−12 °C and the white LiCl precipitate was formed spontaneously, the ice water bath was removed, and the mixture was stirred at room temperature for 15 min. The reaction product was vacuum-filtered using a glass frit filter funnel with Celite 545 to remove the precipitates, resulting in a yellow filtrate containing 1-DCP lithium salt. Before use, Celite was dried at 120 °C overnight to prevent the moisture in Celite from reacting with the Li salt, which can clog the filter pores and impede filtration. The solvent and volatile byproducts were removed from the filtrate using a rotary evaporator at 50 °C to yield 1-DCP lithium salt as a thick brown liquid (35.4 g).

Synthesis of 1-DCP from 1-DCP Lithium Salt and Purification. The 1-DCP lithium salt (35.4 g) was protonated by slow addition to 100 mL of ice water in a 250 mL beaker while stirring. The water temperature was maintained at 0−4 °C, as lithium salt can react too violently with water at higher temperatures. The resulting solution was transferred to a 250 mL separatory funnel to separate the lower aqueous phase from the 1-DCP in the upper phase. After washing with brine to remove water, the obtained 1-DCP (25.4 g, brown oil) was purified through vacuum distillation using a short path distillation apparatus, including a 50 mL two-necked round flask with a magnetic bar, a thermometer, a short path distill head (−10 °C coolant), a 50 mL receiver flask placed in a cold ethanol bath (below −40 °C), a heating mantle, and a vacuum line. By heating under a vacuum of less than 5 mBar, the solution temperature was initially maintained at 5−10 °C, then increased to 35 °C, at which point the distillation process was terminated. Distillation gave the final product 1-DCP (9 g, colorless, density 0.77, boiling point ca. 120 °C) with 95% purity and a 35% overall yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ

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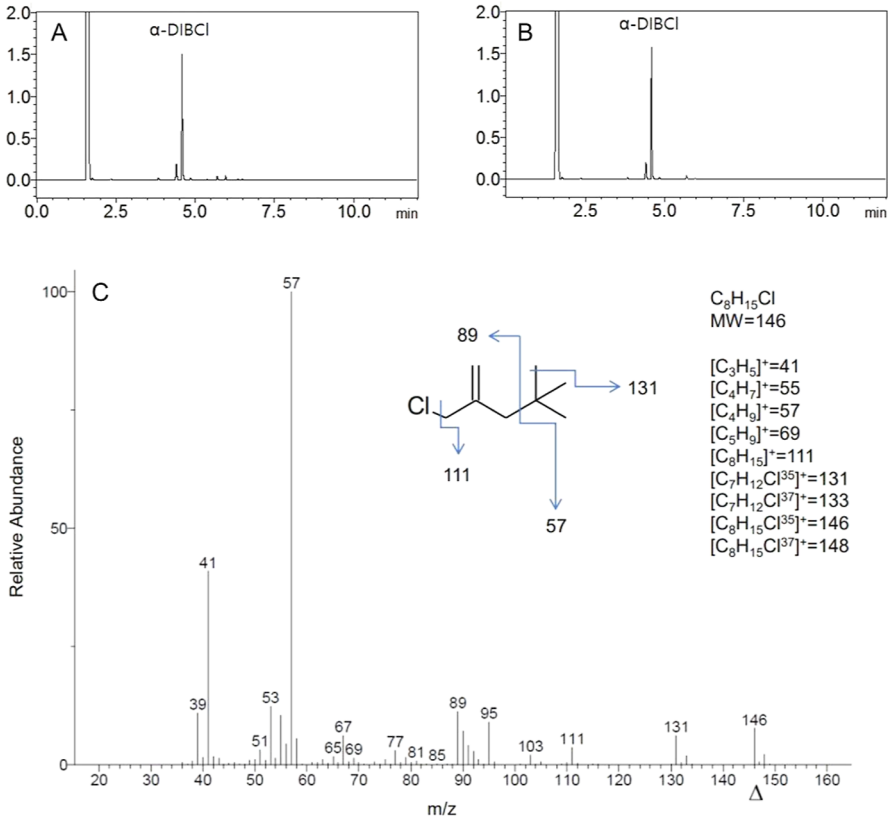

Figure 3. Chromatograms and mass spectrum of α-DIBCl and 1-DCP. Crude α-DIBCl (A). Distill-purified α-DIBCl (B). Mass spectrum of α-DIBCl showed the molecular ion peak ($[C_8H_{15}Cl]^+$ = 146) and the base peak ($[C_4H_9]^+$ = 57). Chlorine isotope fragments showed 3:1 ratio of Cl^{35}/Cl^{37} peaks as shown by $[C_7H_{12}Cl^{35}]^+$ = 131, $[C_7H_{12}Cl^{37}]^+$ = 133, $[C_8H_{15}Cl^{35}]^+$ = 146, and $[C_8H_{15}Cl^{37}]^+$ = 148 (C).

6.52−6.49 (m, 1H), 2.39 (d, J = 0.8 Hz, 2H), 0.99 (s, 9H), 0.90 (d, J = 1.9 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 118.96, 99.06, 40.78, 30.03, 29.55, 5.91.

Preparation of α-CD Inclusion Complex of 1-DCP. In a 1 L vacuum-resistant bottle, 40 g of α-cyclodextrin (α-CD) was dissolved in 400 mL of distilled water by vigorous shaking, and the solution was degassed through repeated vacuum cycles. 5 mL of 1-DCP was added to the bottle. The bottle was capped tightly and shaken vigorously for 15 min, and the inclusion complex of 1-DCP with α-CD was formed as a white precipitate. The suspension containing the white solids was vacuum-filtered using a Buchner funnel with grade 3 Whatman filter paper, and the solid was recovered as a filter cake. The wet filter cake was vacuum-dried in a desiccator at room temperature for 24 h and ground using a mortar and pestle to obtain a solid, powdery inclusion complex of 1-DCP with α-CD (1-DCP/α-CD molar ratio = 0.73, 1-DCP content 92 μL/g). The prepared inclusion complex was stored at room temperature. For plant treatment purposes, the inclusion complex (4 g) was dissolved in dimethyl sulfoxide (DMSO, 10 mL) to obtain a solution of 1-DCP (200 mM) in DMSO. This 1-DCP/DMSO solution was diluted and emulsified in an appropriate volume of water to obtain the optimal concentration for the treatment in the field.

Treatments of 1-DCP on Plants. Four non-astringent persimmon trees of cultivar Taechu (*Diospyros kaki*), grown at the Sweet Persimmon Research Institute in Gimhae, Korea, were selected for treatments on October 7, 2022. The persimmon fruits on two trees were sprayed with a solution containing a spreading agent (0.025%, Kava, Farm Hannong) as the control group. On another two trees, the fruits were sprayed with an aqueous emulsion of 1-DCP (1 mM) and a spreading agent (0.025%) using a manual sprayer as the treated group. The fruits from each treatment group were harvested on October 24, 2022, and stored at room temperature. The flesh hardness of fruits was measured using a texture analyzer (TA-XT2, Stable Micro Systems, UK) after 7 and 14 days of storage.

In addition, immature banana fruits were harvested from a tree cultivated in a facilitated greenhouse in Sancheong-gun, Korea. On the day of harvest, 16 fruits were dipped for 2 s in a solution containing a spreader agent (0.025%, Kava, Farm Hannong) as the control group. Another 16 fruits were immersed in an aqueous emulsion of 1-DCP (1 mM) and a spreader agent (0.025%) as the treated group. The fruits were dried at room temperature. The next day, halves of the fruits in each group were placed in 40 L containers, and ethylene gas was injected into the containers to expose the fruits to ethylene (10 ppm, 24 h). All fruits were stored in an open space at room temperature to observe changes in peel color and deterioration.

Statistical Analysis. The two-tailed t-test was performed for two-group comparisons. $P < 0.05$ is considered statistically significant.

RESULTS

Preparation of α-DIB. 1-DCP synthesis was initiated from a commercially available diisobutylene (DIB) (Figure 1). Commercial DIB exists as a 3:1 mixture of α/β-isomers (Figure 2A). However, using the DIB mixture for 1-DCP synthesis was impractical due to the significantly decreased

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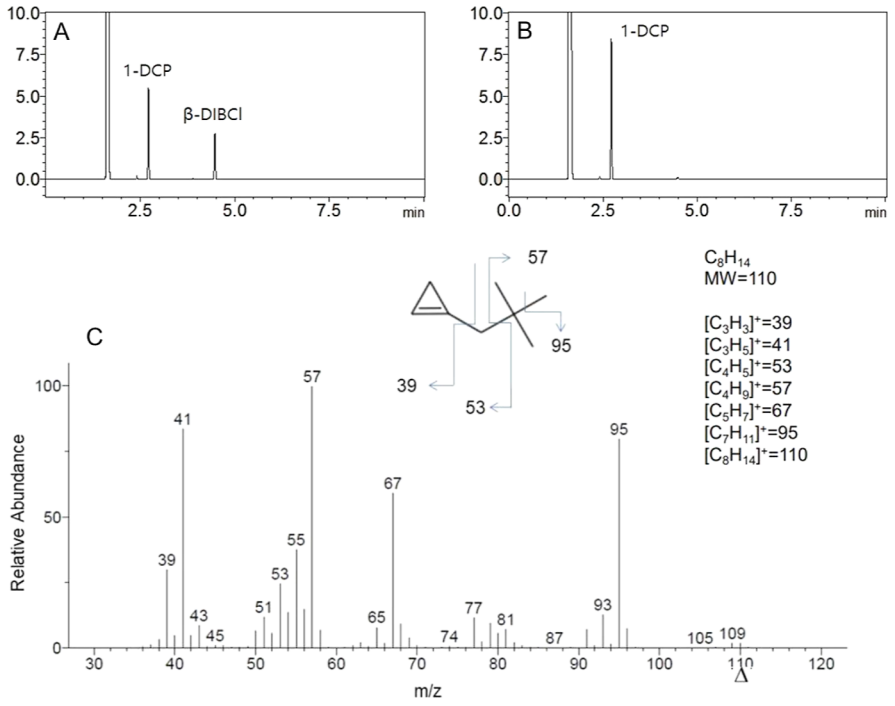

Figure 4. Chromatograms and mass spectrum of 1-DCP. 1-DCP was prepared from α/β-DIB (A) or α-DIB (B). GC−MS analysis showed the molecular ion peak ($[C_8H_{14}]^+$ = 110) and the base peak ($[C_4H_9]^+$ = 57) (C), including major fragments of $[C_3H_3]^+$ = 39, $[C_3H_5]^+$ = 41, $[C_4H_5]^+$ = 53, $[C_5H_7]^+$ = 67, and $[C_7H_{11}]^+$ = 95, confirming the structure of 1-DCP.

yield and contained β-DIBCl impurity, as shown in Figure 4A, which could not be easily removed from the final 1-DCP product. Initially, we planned allylic chlorination of DIB and α-elimination of allylic chloride to produce 1-DCP. However, β-DIB interferes with the original synthetic scheme, so we decided to remove it from DIB. Alpha- and β-DIB were not easily separated through simple distillation due to their similar boiling points. It was discovered that by chlorinating DIB using hypochlorite and HCl under the conditions (molar ratio of DIB/OCl^-/HCl = 2:1:1), β-DIB was selectively chlorinated before α-DIB. This chlorination reaction leads to a mixture of α-DIB and 1-chloro-2,4,4-trimethylpent-2-ene (β-DIBCl), which can be readily separated via fractional distillation due to the higher boiling point of β-DIBCl (Figure 2B). Alpha-DIB was obtained with 63% yield and 98% purity (Figure 2C). GC−MS analysis showed the molecular ion peak ($[C_8H_{16}]^+$ = 112) and the base peak ($[C_4H_9]^+$ = 57), as well as specific fragments, including $[C_3H_5]^+$ = 41, $[C_4H_7]^+$ = 55, $[C_5H_9]^+$ = 69, and $[C_7H_{13}]^+$ = 97, which confirmed the α-DIB (Figure 2D). Further data from ^{1}H and ^{13}C NMR analyses are shown in Supporting Information Figures S3 and S4, respectively.

Allylic Chlorination of α-DIB. Next, α-DIB was allylic-chlorinated using hypochlorite. The hypochlorite (1.95 mol/L, OCl^-) was prepared from calcium hypochlorite ($Ca(OCl)_2$), which contains a higher portion of hypochlorite ions compared to its alternative reagent, sodium hypochlorite (NaOCl). Mixing α-DIB with hypochlorite and HCl (molar ratio of α-DIB/OCl^-/HCl = 1:1.2:1.3) in a pressure-resistant bottle resulted in the rapid completion of the allylic substitution of α-DIB with chlorine within 2−3 min. High temperature and pressure were formed immediately when hypochlorite and HCl were mixed due to the spontaneous formation of chlorine gas; however, the pressure decreased when the reaction was completed as the chlorine was consumed. The synthesized immiscible α-DIBCl could be recovered from the aqueous reaction mixture as a supernatant (Figure 3A). The crude product was purified through fractional distillation to give α-DIBCl in 70% yield and 80% purity (Figure 3B).

The mass spectrum of α-DIBCl showed a molecular ion peak $[C_8H_{15}Cl]^+$ at m/z = 146 and a base peak $[C_4H_9]^+$ at m/z = 57 (Figure 3C). In addition, the molecular ion and a fragment ion of m/z = 131 exhibited a characteristic isotope peak pattern with a ratio of Cl^{35}/Cl^{37} = 3:1, indicating the presence of chlorine. Chlorine isotopes were shown in $[C_7H_{12}Cl^{35}]^+$ = 131, $[C_7H_{12}Cl^{37}]^+$ = 133, $[C_8H_{15}Cl^{35}]^+$ = 146, and $[C_8H_{15}Cl^{37}]^+$ = 148 (Figure 3C). ^{1}H NMR and ^{13}C NMR analysis data were presented in Supporting Information Figures S5 and S6.

LDEA Synthesis and Cyclopropenation. For the cyclopropenation of α-DIBCl through an α-elimination reaction, lithium diethylamide (LDEA) was synthesized in situ. During the synthetic process, lithium reacted smoothly with diethylamine in the presence of isoprene as an electron-donating catalyst and diethyl ether as a solvent. The reaction was exothermic and completed within 30 min; however, it was observed that the deterioration of the lithium metal caused by exposure to the atmosphere reduced the reactivity, resulting in a longer reaction time and lower LDEA yield. After completion of the LDEA synthesis, α-DIBCl was allowed to react with the LDEA in the same pot by adding it slowly at sub-zero temperature. The reaction was very exothermic and resulted in the formation of cyclopropene lithium salt and byproducts, diethylamine, and white precipitates of lithium chloride. The lithium salt was soluble in diethyl ether, whereas the lithium chloride byproduct was not soluble in a solvent; the products could be separated easily by filtration on a glass frit filter. The lithium salt formation can also be advantageous for removing the volatile byproducts and solvent through evaporation since the salt remains unaffected by vacuum conditions, while byproducts (methylbutene and diethylamine) and solvent

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evaporate. Treating the crude product with ice-cold water generated a crude 1-DCP as the immiscible supernatant. The crude 1-DCP was purified further to give 34−36% yield and 95−97% purity after vacuum distillation (Figure 4B).

GC−MS analysis demonstrated the molecular ion peak ($[C_8H_{14}]^+$ = 110) and the base peak ($[C_4H_9]^+$ = 57) of 1-DCP. Major fragments, including $[C_3H_3]^+$ = 39, $[C_3H_5]^+$ = 41, $[C_4H_5]^+$ = 53, $[C_5H_7]^+$ = 67, and $[C_7H_{11}]^+$ = 95, confirmed the final structure of 1-DCP (Figure 4C). ^{1}H NMR and ^{13}C NMR analysis also confirmed the final structure of 1-DCP (Supporting Information Figures S1,S2).

Encapsulation of 1-DCP in Cyclodextrin. Due to its tendency to undergo polymerization, the final product 1-DCP could not be stored at room temperature. We developed a method to encapsulate 1-DCP within cyclodextrin as a host molecule. We tested α-cyclodextrin (α-CD) and β-cyclodextrin (β-CD) for encapsulation and found that α-CD exhibited a higher capacity for accommodating 1-DCP compared to β-CD. The inclusion ratio of 1-DCP in α-CD and β-CD was 92 and 17 μL/g, respectively. The inclusion complex in α-CD was not soluble in water but very soluble in dimethyl sulfoxide (DMSO). In 10 mL of DMSO, we could dissolve at least 4 g of 1-DCP/α-CD complex. The inclusion complex could be stored at room temperature and emulsified in water at an appropriate concentration.

Effects of 1-DCP on Fruit Ripening. Non-astringent Taechu persimmon fruits were treated by spraying with 1 mM of 1-DCP on trees before harvest, and after harvest, the changes in flesh softening were determined during storage at room temperature (Figure 5). After 7 days of storage, the flesh hardness of the fruits was measured as 20.2 N in the untreated group and 19.5 N in the 1-DCP-treated group, showing no significant difference. However, after 14 days of storage, the hardness of the control and treated fruits decreased to 4.3 and 11.9 N, respectively, indicating reduced flesh softening in 1-DCP-treated fruits. In banana fruits, the effect of 1-DCP treatment was determined in combination with ethylene treatment. Ethylene-treated fruits completely lost their green color on the peel within 7 days and turned black after 14 days. However, when treated with 1-DCP before ethylene treatment, the color change induced by ethylene was blocked entirely, resulting in a green peel color similar to non-treated control fruits (Figure 6).

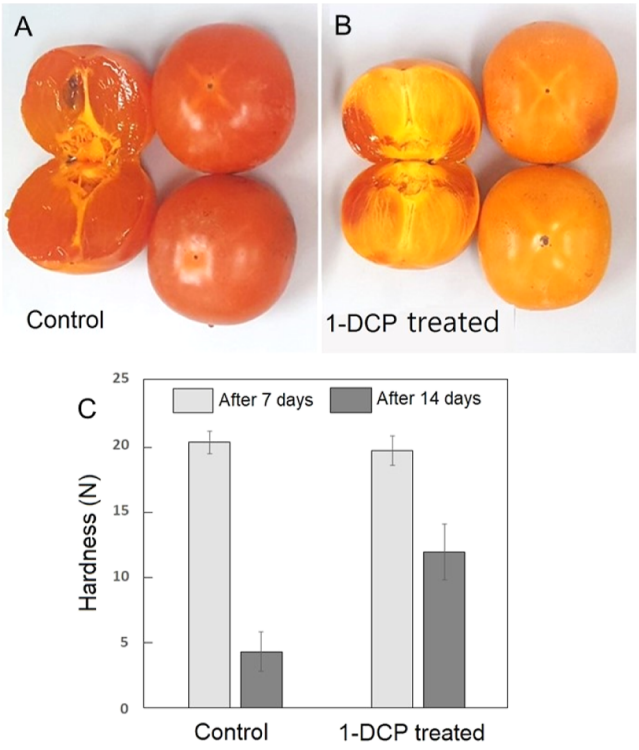

Figure 5. Effect of 1-DCP treatment on the flesh softening of non-astringent Taechu persimmon fruits. The fruits were sprayed with 1 mM 1-DCP emulsion on trees before harvest. Photographs were taken 14 days after storage (A,B), and the flesh hardness was measured at 7 and 14 days at room temperature (C). Vertical bars show SE, and the p-value between treatments after 14 days was 0.03 (n = 8).

Figure 6. Effect of ethylene and 1-DCP treatment on the color changes in banana fruits. The fruits were dipped in 1 mM 1-DCP emulsion after harvest (+DCP), and a part of them was treated with 10 ppm ethylene for 24 h (+C_2H_4). Photographs were taken 7 and 14 days after treatment during storage at room temperature.

DISCUSSION

Synthesis of 1-DCP. Cyclopropene or its 1-methyl derivative can be synthesized through α-elimination of allyl or β-methallyl halide as a building block using a strong base.[18−21] However, larger 1-substituted cyclopropenes are synthesized primarily through the 1,2-elimination of halocyclopropanes, which are synthesized from a suitable long-chained haloalkene using carbene-type reagent.[22−25] The current study demonstrates that 1-(2,2-dimethylpropyl)-cyclopropene (1-DCP) could be synthesized through the α-elimination of 2-(chloromethyl)-4,4-dimethylpent-1-ene (α-DIBCl), which was synthesized by allylic chlorination of 2,4,4-trimethylpent-1-ene (α-DIB) (Figure 1). Alpha-DIB (CAS 107-39-1) is commercially available but costly. However, we could prepare α-DIB from an α/β-isomer mixture of diisobutylene (CAS 25167-70-8), which is also commercially available at a lower cost. To synthesize α-DIBCl as a precursor of 1-DCP, we allylic-chlorinated α-DIB using hypochlorite. Typically, allylic chlorination is achieved using N-chlorosuccinimide (NCS), chlorine gas,[26,27] or hypochlorite with a Lewis acid.[28] However, it was discovered that a combination of an aqueous hypochlorite solution, regardless of sodium or calcium salt, and HCl is an efficient reagent for chlorinating the allylic carbon of allylic alkene. This chlorination method appears to be more convenient than previous reports. After allylic chlorination, α-DIBCl is cyclopropenated through an α-elimination reaction using a strong base to convert to 1-

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Figure 7. In silico molecular docking of 1-DCP with α-CD. AutoDock Vina software (version 1.1.2) was used for docking simulation. The α-CD structure was extracted from PDB ID 3L2M (https://www.rcsb.org/) using PyMOL software.

DCP. Standard strong bases to dehydrohalogenate allylic chlorides are organometallic bases, including phenyllithium,[19] sodium or lithium amides,[18,20,21] or potassium *tert*-butoxide.[23] However, only lithium alkylamides could cyclopropenate α-DIBCl efficiently among these options. Solid lithium amides are pyrophoric, and only lithium diisopropylamide (LDA) is commercially available as a solution containing high boiling point byproducts and solvents that include ethylbenzene and cyclohexane. When LDA is used for the cyclopropenation reaction, it produces diisopropylamine as a byproduct with a high boiling point. Instead, we synthesized lithium diethylamide (LDEA) in situ, which contains methylbutene as a highly volatile byproduct and diethyl ether as a solvent, and could be removed by evaporation. Our experiments showed that LDEA is more advantageous than LDA for 1-DCP purification. Treating α-DIBCl with LDEA leads to the formation of a lithiocyclopropene as a lithium salt product, which was protonated to give the final product 1-DCP in 35% yield. The yield was compatible with previous reports, as the highest reported yield was 39% when using $NaN(TMS)_2$.[21]

Inclusion of 1-DCP in α-CD. Cyclopropenes possess high strain energy and are highly reactive.[17,29] Many cyclopropenes, including cyclopropene or 1-MCP, undergo auto-polymerization at room temperature and even at dry ice temperature and cannot be stored under such conditions. Although minor degradation was observed over a month at −40 °C, 1-DCP cannot be stored at room temperature for more than a day. Encapsulation of 1-MCP with α-cyclodextrin (α-CD) as the guest and host molecules completely prevents polymerization and enables its storage at ambient temperature.[16,30] 1-DCP could be encapsulated in α-CD in the molar ratio of 1-DCP/α-CD = 0.73:1. The molecular docking simulation showed that 1-DCP perfectly fits the cavity of α-CD (Figure 7). The encapsulation of 1-DCP with α-CD appears to prevent its polymerization by isolating each 1-DCP molecule, allowing it to be stored at room temperature without any degradation for several months. However, the problem arose with the solubility of the inclusion complex, as it became insoluble in water. We found that the complex is soluble in dimethyl sulfoxide (DMSO), and the complex in DMSO can be formulated into an emulsion with water as a sprayable or dipping agent for plants.

1-DCP as an Ethylene Antagonist. The softening of persimmon fruits is caused by cell wall degrading enzymes, which are activated by ethylene.[31] However, in our study, this ethylene response was inhibited by spraying the fruits with a 1-DCP emulsion on the tree before harvest. Similarly, in banana fruits, the effect of ethylene treatment, which generally leads to peel degreening by activating the chlorophyll-degrading enzyme, chlorophyllase,[32] was entirely nullified by prior dipping treatment with 1-DCP in our experiment. These results demonstrate the antagonistic effects of 1-DCP against ethylene.

Genetic analysis has shown that in the absence of ethylene, the ethylene signaling pathway is maintained in a suppressed state by ethylene receptors. However, when ethylene binds to a receptor, the suppressing activity is inactivated, leading to the release of ethylene responses,[33,34] but cyclopropene compounds inhibit the effects of ethylene.[35,36] It is believed that cyclopropenes bind to the Cu(I) ion coordinated in the receptor's binding site, similar to ethylene or other ethylene agonists.[36−38] The unsaturated electron-rich π bond in olefinic hydrocarbons appears to play a role in their binding with the metal cation. However, unlike ethylene, which binds reversibly, Pirrung et al. proposed that highly strained cyclopropene can form covalent bonds with amino acid residues of the binding site through the formation of a carbenoid resulting from the opening of the strained ring structure during the copper binding process.[39] This irreversible covalent bonding permanently prevents the existing receptor protein from binding to ethylene. However, if newly synthesized proteins substitute the inactivated receptors, the ethylene response could be restored. Extending the substituted alkyl chain length on the 1-position of the cyclopropene can also inactivate the receptor, similar to small cyclopropene or 1-MCP molecules; the same was observed with 1-DCP in our experiments.[40] In this regard, the unsaturated π bond and strained ring structure of cyclopropene compounds seem to be the crucial functional moiety for inhibiting ethylene binding, with no apparent limitations on the substituent size. However, reports indicate that the ethylene-inhibiting activity of cyclopropene compounds is diminished when an alkyl group is substituted at the 3-position of cyclopropene or when heteroatoms such as oxygen or nitrogen are incorporated into the substituent.[25,41] Unlike the ethylene inhibiting ability, which is not significantly affected by the size of the alkyl substituent, the physical properties of cyclopropene compounds, such as boiling point, stability, and solubility, will be substantially influenced by the substituent. This means that manipulating the substituent can be a promising strategy for developing a cyclopropenyl compound that can be used as a readily available ethylene antagonist for dipping or spraying applications. 1-MCP is a gaseous cyclopropene that cannot be applied in open spaces. However, our study demonstrated that by replacing the substituent at the 1-position with a five-carbon dimethylpropyl group instead of a methyl group, its boiling point could be increased sufficiently to maintain the liquid phase at ambient temperature until it is absorbed by plants when applied through dipping or spraying methods in open spaces.

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CONCLUSIONS

1-DCP, a cyclopropene derivative with a 1-substituent consisting of a five-carbon dimethylpropyl group, could be synthesized practically from α-DIB through allylic chlorination using hypochlorite, followed by cyclopropenation of the resulting allylic chloride, α-DIBCl, using LDEA. 1-DCP could be encapsulated in α-CD and formulated as a sprayable aqueous emulsion. When the emulsion of 1-DCP was treated on plants in an open space, ethylene responses were inhibited effectively. Our data demonstrate that the new molecule 1-DCP showed critical ethylene inhibition, acting as an anti-aging molecule in plants. If its toxicological safety is confirmed, 1-DCP could be considered to be a commercially useful spraying agent as an ethylene antagonist.

ASSOCIATED CONTENT

Data Availability Statement

This published article and its Supporting Information files include all data generated or analyzed during this study.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c04220.

^{1}H and ^{13}C NMR spectra for all compounds and mass spectra for α/β-DIB (PDF)

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Dr. S.-J.C. participated in research design, experimental performance, manuscript drafting, and data analysis. Dr. G.-H.A. participated in research design, experimental implementation, manuscript drafting, and data analysis. Dr. K.H.L. analyzed the data and prepared the manuscript. Dr. W.J.J. participated in data analysis, manuscript drafting, and revision.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Iqbal, N.; Khan, N. A.; Ferrante, A.; Trivellini, A.; Francini, A.; Khan, M. I. R. Ethylene Role in Plant Growth, Development and Senescence: Interaction with Other Phytohormones. *Front. Plant Sci.* **2017**, *8*, 475.

(2) Bleecker, A. B.; Kende, H. Ethylene: a gaseous signal molecule in plants. *Annu. Rev. Cell Dev. Biol.* **2000**, *16*, 1−18.

(3) Van Zhong, G.; Burns, J. K. Profiling ethylene-regulated gene expression in Arabidopsis thaliana by microarray analysis. *Plant Mol. Biol.* **2003**, *53*, 117−131.

(4) Lin, Z.; Zhong, S.; Grierson, D. Recent advances in ethylene research. *J. Exp. Bot.* **2009**, *60*, 3311−3336.

(5) Ahmed, Z. F. R.; Palta, J. P. A Postharvest Dip Treatment with Lysophosphatidylethanolamine, a Natural Phospholipid, May Retard Senescence and Improve the Shelf Life of Banana Fruit. *Hortscience* **2015**, *50*, 1035−1040.

(6) Abeles, F. B.; Morgan, P. W.; Saltveit, M. E., Jr. *Ethylene in Plant Biology*; Academic Press: New York, 2002.

(7) McManus, M. T. *The Plant Hormone Ethylene*; Wiley-Blackwell: Oxford, 2012.

(8) Yang, S. F. Regulation of Ethylene Biosynthesis. *HortScience* **1980**, *15*, 238−243.

(9) Yang, S. F. H.; Hoffman, N. E. Ethylene biosynthesis and its regulation in higher plants. *Annu. Rev. Plant Physiol.* **1984**, *35*, 155−189.

(10) Hamilton, A. J.; Lycett, G. W.; Grierson, D. Antisense gene that inhibits synthesis of the hormone ethylene in transgenic plants. *Nature* **1990**, *346*, 284−287.

(11) Çetinbaş, M. B.; Butar, S.; Onursal, C. E.; Koyuncu, M. A. The effects of pre-harvest ReTain [aminoethoxyvinylglycine (AVG)] application on quality change of "Monroe" peach during normal and controlled atmosphere storage. *Sci. Hortic.* **2012**, *147*, 1−7.

(12) Binder, B. M. The ethylene receptors: Complex perception for a simple gas. *Plant Sci.* **2008**, *175*, 8−17.

(13) Sisler, E. C. Partial Purification of an Ethylene-binding Component from Plant Tissue. *Plant Physiol.* **1980**, *66*, 404−406.

(14) Sisler, E. C. S.; Serek, M. Inhibitors of ethylene responses in plants at the receptor level: Recent developments. *Physiol. Plant.* **1997**, *100*, 577−582.

(15) Sisler, E. C. The discovery and development of compounds counteracting ethylene at the receptor level. *Biotechnol. Adv.* **2006**, *24*, 357−367.

(16) Blankenship, S. M. D.; Dole, J. M. 1-Methylcyclopropene: A review. *Postharvest Biol. Technol.* **2003**, *28*, 1−25.

(17) Rademacher, W. J. Plant growth regulators: Backgrounds and uses in plant production. *Plant Growth Regul.* **2015**, *34*, 845−872.

(18) Fisher, F. A.; Applequist, D. E. Synthesis of 1-methylcyclopropene. *J. Org. Chem.* **1965**, *30*, 2089−2090.

(19) Closs, G. L. K.; Krantz, K. D. A Simple Synthesis of Cyclopropene. *J. Org. Chem.* **1966**, *31*, 638.

(20) Magid, R. M. C.; Clarke, T. C.; Duncan, C. D. Efficient and convenient synthesis of 1-methylcyclopropene. *J. Org. Chem.* **1971**, *36*, 1320−1321.

(21) Binger, P.; Wedemann, P.; Brinker, U. H. Cyclopropene: A new simple synthesis and its Diels-Alder reaction with cyclopentadiene. *Org. Synth.* **2000**, *77*, 254−256.

(22) Al Dulayymi, J. R. B.; Baird, M. S.; Simpson, M. J.; Nyman, S.; Port, G. R. Structure based interference with insect behaviour - Cyclopropene analogues of pheromones containing Z-alkenes. *Tetrahedron* **1996**, *52*, 12509−12520.
(23) Sheshenev, A. E. B.; Baird, M. S.; Croft, A. K.; Bolesov, I. G. Generation and stereoselective transformations of 3-phenylcyclopropene. *Tetrahedron* **2009**, *65*, 10036−10046.
(24) Sisler, E. C. S.; Serek, M.; Dupille, E.; Goren, R. Inhibition of ethylene responses by 1-methylcyclopropene and 3-methylcyclopropene. *Plant Growth Regul.* **1999**, *27*, 105−111.
(25) Rubin, M.; Sherrill, W.; Kim, R. Synthesis of cyclopropenes via 1,2-elimination of bromocyclopropanes catalyzed by crown ether. *Synthesis* **2009**, *2009*, 1477−1484.
(26) Reed, S. F. J. Allylic Halogenation of α-Methylstyrene. Preparation of α-Chloromethylstyrene[1a]. *J. Org. Chem.* **1965**, *30*, 3258.
(27) Easton, C. J.; Edwards, A. J.; McNabb, S. B.; Merrett, M. C.; O'Connell, J. L.; Simpson, G. W.; Simpson, J. S.; Willis, A. C. Allylic halogenation of unsaturated amino acids. *Org. Biomol. Chem.* **2003**, *1*, 2492−2498.
(28) Moreno-Dorado, F. J. G.; Guerra, F. M.; Manzano, F. L.; Aladro, F. J.; Jorge, Z. D.; Massanet, G. M. CeCl3/NaClO: a safe and efficient reagent for the allylic chlorination of terminal olefins. *Tetrahedron Lett.* **2003**, *44*, 6691−6693.
(29) Carter, F. L.; Frampton, V. L. Review of the chemistry of cyclopropene compounds. *Chem. Rev.* **1964**, *64*, 497−525.
(30) Szejtli, J. *Cyclodextrin Technology*; Springer: Netherlands, 2014.
(31) Goulao, L. F. O.; Oliveira, C. Cell wall modifications during fruit ripening: when a fruit is not the fruit. *Trends Food Sci. Technol.* **2008**, *19*, 4−25.
(32) Thomas, P. J.; Janave, M. T. Effect of temperature on chlorophyllase activity, chlorophyll degradation and carotenoids of Cavendish bananas during ripening. *Food Sci. Technol.* **2007**, *27*, 57−63.
(33) Hua, J. M.; Meyerowitz, E. M. Ethylene responses are negatively regulated by a receptor gene family in Arabidopsis thaliana. *Cell* **1998**, *94*, 261−271.
(34) Binder, B. M. Ethylene signaling in plants. *J. Biol. Chem.* **2020**, *295*, 7710−7725.
(35) Sisler, E. C. S.; Serek, M. Compounds controlling the ethylene receptor. *Bot. Bull. Acad. Sinica* **1999**, *40*, 1−7.
(36) Sisler, E. C. G.; Grichko, V. P.; Serek, M. *Interaction of ethylene and other compounds with the ethylene receptor: Agonists and antagonists*; Springer-Verlag, 2006.
(37) Schaller, G. E. B.; Bleecker, A. B. Ethylene-Binding Sites Generated in Yeast Expressing the Arabidopsis ETR1 Gene. *Science* **1995**, *270*, 1809−1811.
(38) Rodríguez, F. I.; Esch, J. J.; Hall, A. E.; Binder, B. M.; Schaller, G. E.; Bleecker, A. B. A copper cofactor for the ethylene receptor ETR1 from Arabidopsis. *Science* **1999**, *283*, 996−998.
(39) Pirrung, M. C.; Bleecker, A. B.; Inoue, Y.; Rodriguez, F. I.; Sugawara, N.; Wada, T.; Zou, Y.; Binder, B. M. Ethylene receptor antagonists: strained alkenes are necessary but not sufficient. *Chem. Biol.* **2008**, *15*, 313−321.
(40) Sisler, E. C. A.; Alwan, T.; Goren, R.; Serek, M.; Apelbaum, A. 1-substituted cyclopropenes: Effective blocking agents for ethylene action in plants. *Plant Growth Regul.* **2003**, *40*, 223−228.
(41) Grichko, V. New volatile and water-soluble ethylene antagonists. *Russ. J. Plant Physiol.* **2006**, *53*, 523−529.

https://doi.org/10.1021/acsomega.3c04220
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