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1-substituted cyclopropenes: Effective Blocking Agents for Ethylene Action in Plants

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Abstract

A series of 1-alkane substituted cyclopropenes has been prepared and tested as ethylene antagonists using banana fruits as an assay system. 1-Methyl-, 1-ethyl-, 1-propyl-, 1-butyl-, 1-pentyl-, 1-hexyl-, 1-heptyl-, 1-octyl-, 1-nonyl-, and 1-decylcyclopropene were all very active compounds. 1-Methylcyclopropene protected bananas from ethylene with a minimum concentration of 0.7 nl.l^{-1} after a 24 h exposure. As the carbon chain length was extended the minimum requirement increased some, but starting with 1-butylcyclopropene, the minimum concentration requirement declined and many cyclopropenes were required in lower concentrations than 1-methylcyclopropene. The time of protection at ambient temperature (22–23 °C) was 12 d for 1-methyl-, 1-ethyl-, 1-propyl-, and 1-butylcyclopropene. 1-Pentylcyclopropene protected bananas for 14 d, 1-hexylcyclopropene for 20 d, 1-heptylcyclopropene for 21 d, 1-octylcyclopropene for 25 d, 1-nonylcyclopropene for 35 d, and 1-decylcyclopropene for 36 d.

Abbreviations: CP – cyclopropene, 1-MCP – 1-methylcyclopropene, 1-ECP – 1-ethylcyclopropene, 1-PCP – 1-propylcyclopropene, 1-BCP – 1-butylcyclopropene, 1-PentCP – 1-pentylcyclopropene, 1-HCP – 1-hexylcyclopropene, 1-HeptCP – 1-heptylcyclopropene, 1-OCP – 1-octylcyclopropene, 1-NCP – 1-nonylcyclopropene, 1-DCP – decylcyclopropene, 3,3-DMCP – 3,3-dimethylcyclopropene

Introduction

Cyclopropenes have been shown to be effective blocking agents for the ethylene receptor in plants (Sisler et al. 1996a, 1996b, 1999, 2001). They compete with ethylene before binding, but do not appear to compete once they are bound (Dupille and Sisler 1995). After an extended period of time the bananas become sensitive to ethylene again, but it is not known if the cyclopropenes are released intact or changed. CP, 1-MCP, and 3,3-DMCP were the first cyclopropenes tested as ethylene antagonists. At 23 °C, CP and 1-MCP protected for 12 d and 3,3-DMCP protected for 7 d. Other cyclopropenes have now been prepared and tested. These include mono, di, and tri, substituted cyclopropenes substituted in the 1,2, and 3 positions (Sisler et al. 2001). Although they showed interesting effects, none were more effective, concentrationwise, than 1-MCP. They protected bananas for differing periods of time from 3 to 12 d. In the previous studies, those cyclopropenes substituted with a methyl group in the 1-position usually protected for longer periods of time than those substituted in other positions. 3,3-Di-substituted cyclopropenes are more

stable than many other cyclopropenes and this property may be valuable in practical applications, however, the present study was undertaken to determine the effect of extending the chain length in the 1-position of cyclopropenes on their ability to protect plants against ethylene.

Materials and methods

Chemicals

All of the compounds were prepared by published procedures. 1-MCP was prepared by the method of Magid et al. (1970). It was kept at room temperature in the gas phase. No loss was noted for several weeks. Other 1-cyclopropenes were prepared from the respective 2-bromoalkenes (Cousseau 1980) and bromoform using 50% NaOH to produce a carbene and form a 1,1,2-cyclopropane. This reacted with methyllithium at dry ice temperature (Al Dulayymi et al. 1996, 1997a, 1997b) to form the cyclopropenes. After distillation and passing a gas chromatographic column only a single iodine reactive compound was present. Identification was confirmed by NMR. This peak was used to monitor the amount of cyclopropene during the course of experiments. Little or no concentration change was noted in ether solutions during 14 d at −20 °C. Long time storage was at −80 °C. At this temperature cyclopropenes are stable indefinitely (Al Dulayymi et al. 1997a).

Gas chromatography

Gas chromatography measurements were made on a GP Carbopack C 80/100 0.2% Carbowax 1500 obtained from Supelco, Supelco Park, Bellefonte PA 16823-0048. Separation was done at 75 °C, 135 °C or 175 °C depending on the compound. A quantization was done by using the equivalent alkene compound as a standard since none of these compounds were available except by synthesis.

Plant material

Green Bananas (*Musa sapientum* L.) were obtained from a commercial vendor and within 2 d used as an assay system to determine minimum concentration and time of insensitivity of bananas to ethylene. All cyclopropene values reported are for the compound in the gas phase. For the low molecular weight compounds, treatment was made by injecting the respective cyclopropene as a gas into the treatment jar with a hypodermic syringe. For 1-PentCP and higher molecular weight compounds, they were pipetted in ether solution on to filter paper in the jar to increase the surface area and facilitate evaporation. The amount of ether used had no effect on bananas. Usually 15 concentrations were used in the experiments. After a 24 h exposure to the respective cyclopropenes in 3 l jars, bananas were treated with 333 $\mu l.l^{-1}$ of ethylene for 18 h. The minimum concentration of cyclopropene that protected the bananas was determined. To determine the time of insensitivity, bananas were exposed to a saturating amount of the cyclopropenes. After 24 h, the bananas were removed and kept at 22–23 °C. Each day a sample was exposed to 333 $\mu l.l^{-1}$ of ethylene for 18 h. The day the bananas became sensitive to ethylene and consequently ripened within 3 d was recorded as the duration of insensitivity to ethylene. The amount of each compound that would completely block the ethylene response in 5 h was completely overcome by high amounts of ethylene (1000 $\mu l.l^{-1}$) by adding both to the banana simultaneous. If the ethylene was added after 5 h, the bananas were protected against ethylene.

Measurements

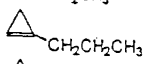
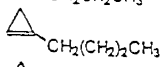
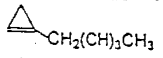
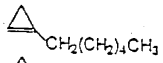
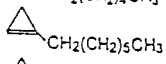
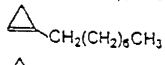
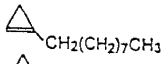
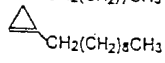
Chlorophyll content was determined with a reflective chlorophyll meter (Field Scout CM1000, Spectrum Technologies Inc. 23839 W. Andrew Rd. Plainfield, Il 60544) calibrated by the method of Arnon (1949).

Results

Concentration of cyclopropenes required to protect bananas

Two basic parameters were used to characterize the effective concentration of cyclopropenes (Sisler et al. 1996a, 1996b). One was the minimum concentration needed to protect against a saturating amount of ethylene after a single 24 h exposure of the banana to the respective cyclopropenes. The second was the time required for the banana to become sensitive to ethylene after being exposed to a saturating amount of the respective cyclopropene. In previous studies 0.7 $nl.l^{-1}$ of the smallest cyclopropenes CP and 1-MCP protected the bananas (Sisler et al. 1996b). The effect of a treatment with various 1-substituted

Table 1. Minimum concentration and time of protection of banana fruits by various cyclopropenes against ethylene.

Compound	Structure	Concentration ($nl.l^{-1}$)[a,b]	Time (days)[c,d]
CP		0.7±0.05	12
1-MCP		0.7±0.05	12
1-ECP		4.0±0.4	12
1-PCP		6.0±0.3	12
1-BCP		3.0±0.1	12
1-PentCP		0.5±0.01	14
1-HCP		0.4±0.01	20
1-HeptCP		0.4±0.01	21
1-OCP		0.3±0.01	25
1-NCP		0.4±0.02	35
1-DCP		0.3±0.01	36

[a]Minimum amount of gas needed to give protection against chlorophyll degradation. [b]Values are means of 3 replicates ± SE. [c]Time bananas remained insensitive to ethylene after exposure to saturating amounts of cyclopropenes in the absence of ethylene. Ethylene (1000 $\mu l.l^{-1}$) completely prevented protection if ethylene and cyclopropene were added simultaneously. [d]Readings were to the nearest day.

cyclopropenes on chlorophyll degradation in banana fruit peel varied according to substitution (Table 1). The addition of a carbon to the 1- substituted carbon chain gave 1-ECP and increased the requirement to 4 $nl.l^{-1}$. Adding a second carbon gave 1-PCP and increased the requirement to 6 $nl.l^{-1}$. As the chain was further extended, the required concentration declined to 3 $nl.l^{-1}$ for 1-BCP. A chain length of 5 gave 1-PentCP and decreased the required concentration to 0.5 $nl.l^{-1}$. This pattern continued to the highest chain lengths. A chain length of 6, gave 1-HCP and lowered the requirement to 0.4 $nl.l^{-1}$ and the 7 carbon 1-HeptCP also required 0.4 $nl.l^{-1}$. With the 8 carbon chain 1-OCP 0.3 $nl.l^{-1}$ are needed. With the 9 carbon 1-NCP and the 10 carbon 1-DCP 0.4 $nl.l^{-1}$ and 0.3 $nl.l^{-1}$ were required to protect the peel. The pulp required a higher amount for protection since at low levels of cyclopropene the banana readily softened while the peel remained green. It is thought that as the molecular weight of the compound increased diffusion into the banana is slower and this becomes evident at the higher molecular weights. There may be other factors involved, but 1-NCP and 1-DCP did protect the pulp for long periods of time if the concentration was increased. The results in Table 1 were unexpected and surprising since it had generally been accepted (Burg and Burg 1967) that small compounds are more active than larger ones, and as substituant size was increased, biological activity with respect to concentration would decline and the larger ones would be inactive.

The concentrations necessary to protect bananas as a function of time are presented in Figures 1 and 2 for some of the cyclopropenes. It is apparent that shorter exposure times required higher amounts of the respective cyclopropene for protection. The individual values are given in the graphs, but it appears that a 4 h exposure required approximately 6–10 times as

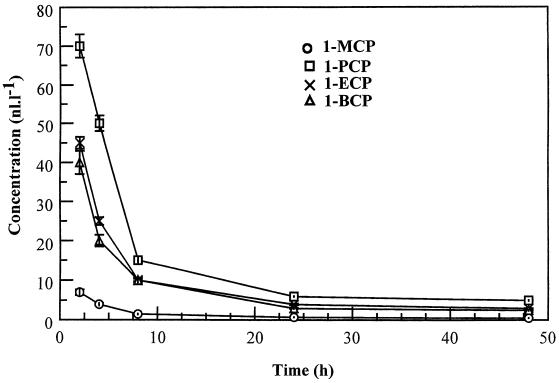


Figure 1. Plot of the minimum concentrations of 1-MCP, 1-ECP, 1-PCP and 1-BCP vs time for the protection of bananas against ethylene at 23 °C. Chlorophyll content after exposure to ethylene was used as a measure of protection. Values are mean of 3 experiments ± SE. All cyclopropenes values are for the compound as a gas.

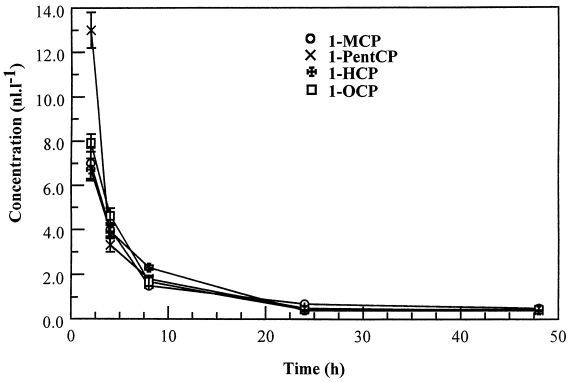


Figure 2. Plot of the minimum concentrations of 1-MCP, 1-PentCP, 1-HCP, and 1-OCP vs time for protection of bananas against ethylene at 23 °C. Values are mean of 3 experiments ± SE. All cyclopropene values are for the compound as a gas.

much as a 24 h exposure, and an 8 h exposure required approximately 2–5 times as much cyclopropene. Most of the higher molecular weight compounds seem to bind about as rapidly as the lower ones suggesting evaporation time into the gas phase was not a large factor.

It has been reported (Sisler and Serek 1997) that a plot of the logarithm of the concentration *vs* the logarithm of the time gave a straight line for 1-MCP in carnations. A similar plot of these compounds also approximated a straight line (Figure 3). Some deviation of the values from 24–48 h may mean the reaction was complete by 24 h in those cases. As these compounds bind, probably the binding site goes to zero concentration, without any reverse reaction, but the reaction may be complex.

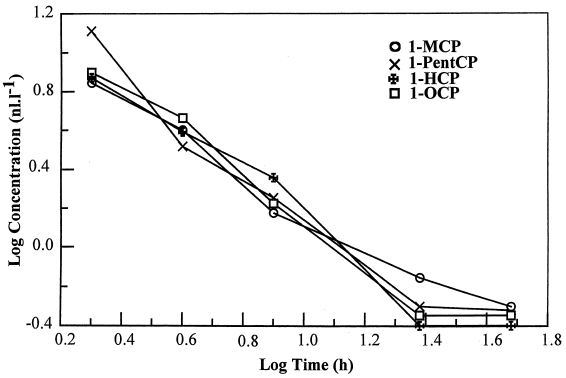


Figure 3. Plot of the logarithm of the minimum concentration of 1-MCP, 1-PentCP, 1-HCP, and 1-OCP *vs* the logarithm of the protection of bananas against ethylene.

Time of insensitivity to ethylene after exposure to cyclopropenes

After exposure to CP and 1-MCP, bananas were insensitive for 12 d at 23 °C (Sisler et al. 2001). Other cyclopropenes were insensitive from 3 to 12 d (Sisler et al. 2001). Some of the cyclopropenes in the present study protected for 12 d (1-ECP, 1-PCP, and 1-BCP), but starting with 1-PentCP (Table 1) protection time increased for a single 24 hr exposure as the number of carbons in the compound increased reaching 36 d for 1-DCP.

Discussion

Compounds that compete with ethylene for binding and action have been known for many years (Sisler and Pian 1973; Sisler 1991), but the use of cyclopropenes for the control of ethylene responses is a recent introduction. A number of cyclopropene ethylene antagonists have now been tested and 1-MCP is commercially available.

Burg and Burg (1967) found the order of activity for alkenes in giving an ethylene response was ethylene 1, propylene 140 and 1-butene 140,000 for a 1/2 maximum response. Longer chain alkenes are not active ethylene agonists. From this information it was concluded that activity was inversely related to molecular size because the unsaturated end of the molecule must attach to a position of limited access. Since cyclopropenes are thought to act by binding to the ethylene receptor (Sisler et al. 1996a), it seems likely that many of the chemical factors relating to binding to the receptor and giving an ethylene re-

sponse would be similar to ethylene agonists. By these rules, only small cyclopropenes would bind to the receptor. This study with 1-substituted cyclopropenes gave results completely different from what was expected. As might be expected, antagonistic activity did decline slightly as the chain in the 1-position on 1-MCP was lengthened; however, starting with 1-PentCP and continuing to 1-DCP the cyclopropenes were more effective than 1-MCP. It has been shown by Scatchard plot analysis that cylopropenes inactivate the receptor (Dupille and Sisler 1995; Sisler et al. 1996a), and it was supposed that they do so by being present on the receptor and prevent ethylene from binding. By analogy to 1-MCP, a reasonable interpretation of the data is that all of the cyclopropenes bind to the receptor in the same way. Large molecules do bind to the receptor. The largest molecule in this study contained 13 carbon atoms. It could be argued that cyclopropenes take the putative metal out of the receptor and inactivate it, or simply capture the metal before it gets to the receptor. That seems unlikely because the time of receptor inactivation by cyclopropenes is from 3–36 d depending on the compound, then activity is restored. If the metal were removed from the receptor, the plant would probably replace it. It seems unlikely that this large range of differences would appear for the restoration of activity if the metal were removed and replaced. The compounds should exhibit about the same time of inactivation. It is suggested that the receptor is inactivated for different periods of time because after binding. the cyclopropenes stay attached to the putative metal and the time differences are probably due to a combination of vapor pressure differences (boiling point) and hydrophobic interaction of the cyclopropene with some plant component(s). These would result in less molecular motion. The major force keeping the inhibitor on the receptor is thought to be ring strain but there do appear to be other forces which hold them longer. Increasing the chain length should not alter ring strain much (Sisler et al. 2001). For whatever the reason, it seems apparent that large molecules can inactivate the receptor. There must be many more active cyclopropenes possible with different chemical groups attached to the cyclopropene molecule or to the side chain(s), and some of them may have interesting physiological properties. Further work is needed to determine this. The cyclopropenes tested and found to be active so far are volatile compounds and are applied to the plant in the gas phase. It is not known if nonvolatile cyclopropenes would inactivate the receptor, but it is a matter that should be investigated.

Acknowledgements

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