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ANTI-ETHYLENE EFFECTS OF *CIS*-2-BUTENE AND CYCLIC OLEFINS*

EDWARD C. SISLER†§ and SHANG FA YANG‡

†Department of Biochemistry, North Carolina State University, Raleigh, NC 27650, U.S.A.; ‡Department of Vegetable Crops, University of California, Davis, CA 95616, U.S.A.

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Abstract—The ability of butenes and cyclic olefins to induce an ethylene response or to counteract ethylene's action in etiolated pea seedlings was investigated. 1-Butene gave an ethylene-like response at 25 000 μl/l. *cis*-Butene exhibited no ethylene-like response but appeared to overcome the action of 0.3 μl/l. ethylene at the same concentration. *trans*-Butene was without effect. 2,5-Norbornadiene competitively inhibited ethylene's action with a K_i of 170 μl/l. (computed as a gas). The K_i for norbornene was 360 μl/l. This value represents about one-half of the activity given by 2,5-norbornadiene. Other K_i values obtained were 488 μl/l. for 1,3-cyclohexadiene, 870 μl/l. for 1,3-cycloheptadiene, 1100 μl/l. for cyclopentene, 4700 μl/l. for 1,4-cyclohexadiene, and 6 ml/l. for cyclohexene. Cyclohexane and benzene were without effect.

INTRODUCTION

Ethylene, a product of plant metabolism, elicits hormonal responses in plants. Various other unsaturated compounds also give a similar response [1]. Burg and Burg [2], in a detailed study with a number of olefins, concluded that a double bond must be adjacent to the terminal carbon atom to elicit a response. Other compounds such as carbon monoxide and acetylene are also active, although the level required to elicit a response is much higher than with ethylene. Sisler [3] reported a number of additional compounds such as isocyanides that gave a response similar to that of ethylene and proposed electron acceptance with its consequential *trans* effect as a model for their action. It should be noted that cyanide induces an increased respiration in tissues with cyanide-insensitive respiration, and ethylene can also induce the same response [4].

Ethylene action is known to be inhibited by two antagonists: CO_2 [2] and Ag^+ [5]. These compounds have been used as a diagnostic test for ethylene action. Although propylene mimics the action of ethylene [2], Dollwet and Seeman [6] observed that propylene counteracted the ethylene action in the pea epicotyl growth test. This raises a new perspective with respect to these compounds. Thus, olefins may have ethylene-like activity, anti-ethylene activity, or both.

Although Sisler and Pian [7] have reported that some cyclic olefins are capable of counteracting the action of ethylene, which increases respiration rate in tobacco leaves, the relative ability of these compounds to counteract ethylene action was not studied. In this investigation, we compared the responses of butenes, the simplest isomeric straight-chain olefins and further characterized the structural requirements of cyclic olefins for anti-ethylene activity.

RESULTS

Effect of butenes on growth and ethylene-binding in pea plants

Burg and Burg [2] reported that 1-butene elicited an ethylene response (inhibition of elongation) in pea segments with a one-half maximum activity at 27 000 μl/l. The results presented in Table 1, for intact pea seedlings, agree with those results. The plants were shorter in the presence of 25 000 μl/l. of 1-butene, and a triple response [1] was observed. As reported by Burg and Burg [2], *trans*-2-butene was inactive, and no significant effect was observed in the present experiments at 25 000 μl/l. *cis*-2-Butene was also reported to be inactive by Burg and Burg [2]; however, in the present experiments, a promotion of elongation by 25 000 μl/l. of *cis*-2-butene was noted either in the absence of added ethylene or in the presence of 0.3 μl/l. of ethylene. *cis*-2-Butene at 25 000 μl/l can apparently overcome much of the effect of 0.3 μl/l. of ethylene, since the plants were nearly as long as the control and the triple response characteristic of ethylene treatment was absent. Using 50 000 μl/l. of *cis*-butene, we obtained a K_i value of 7100 μl/l. Although Burg and Burg [2] reported *cis*-2-butene to be inactive, they probably would not have observed this anti-ethylene effect in their experiments since, in their assay media, Co^{2+} ion, which is known to inhibit ethylene biosynthesis, was included [8]. Thus, in the absence of endogenous ethylene, the anti-ethylene effect of *cis*-butene would not have been readily detected.

1-Butene and *cis*-2-butene inhibited ethylene binding (Table 2) to a greater extent than *trans*-2-butene, again suggesting that the *trans* form does not interact with the

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Table 1. Effect of butenes on pea epicotyl elongation in the presence and absence of added ethylene

Compound	Increase in length (cm ± s.e.)	
	$-C_2H_4$	$+C_2H_4$ (0.3 μl/l.)
None	13.2 ± 0.3	4.9 ± 0.3
1-Butene (25 000 μl/l.)	8.2 ± 0.4	5.2 ± 0.3
cis-2-Butene (25 000 μl/l.)	16.4 ± 0.4	10.6 ± 0.3
trans-2-Butene (25 000 μl/l.)	12.2 ± 0.3	4.9 ± 0.3

When 50 000 μl/l was used, a K_i value of 7100 was obtained for *cis*-butene at the 0.05 confidence level.

Table 2. Effect of butenes on [^{14}C]ethylene binding by pea epicotyls

Compound	[^{14}C]Ethylene bound (dpm ± s.e./g fr. wt)	Inhibition (%)
None	13.8 ± 0.3	0
1-Butene	7.6 ± 0.2	45
cis-2-Butene	8.1 ± 0.3	41
trans-2-Butene	12.6 ± 0.4	9

Exposure was to 25 000 μl/l. of butene.

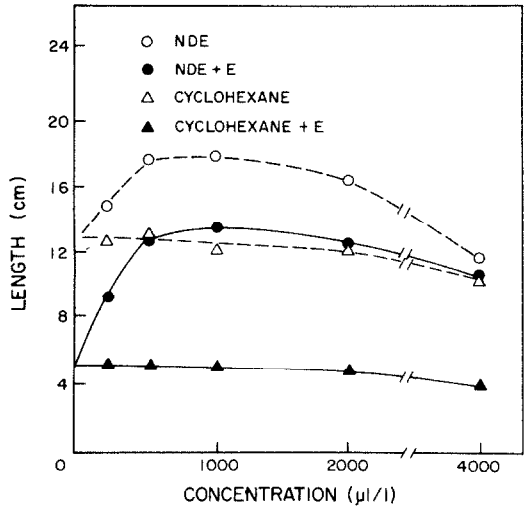


Fig. 1. Effect of 2,5-norbornadiene, a compound capable of overcoming the effect of ethylene, on pea epicotyl elongation in the presence or absence of 0.3 μl/l. of ethylene, and the effect of cyclohexane, a compound not capable of overcoming the effect of ethylene, on pea epicotyl elongation in the presence and absence of 0.3 μl/l. of ethylene. (○---○) 2,5-Norbornadiene; (●——●), 2,5-norbornadiene $+C_2H_4$; (△---△) cyclohexane; (▲——▲) cyclohexane $+C_2H_4$.

binding site to the same extent as 1-butene and *cis*-2-butene. This same effect is observed in the binding of butenes to Ag^+ ion (Table 4) [9].

Effect of 2,5-*norbornadiene on the growth of pea seedlings*

When pea seedlings were grown in the presence of increasing concentrations of 2,5-norbornadiene, the plants increased in length until a concentration of approximately 1000 μl/l. was reached (Fig. 1). As the concentration was further increased, the length of the epicotyl decreased. At a concentration of 25 000 μl/l., death of the tissue occurred. 2,5-Norbornadiene also overcame the effect of 0.3 μl/l. of ethylene; at 600 μl/l. or higher concentrations the length of epicotyl increased to the level of control (cyclohexane) tissues, indicating that the ethylene effect was overcome (Fig. 1). However, at 16 000 μl/l., little or no growth occurred in either the presence or absence of ethylene.

The increase in length due to the low concentration of 2,5-norbornadiene is thought to be due to the ability of 2,5-norbornadiene to counteract the effect of endogenous ethylene. The subsequent decline in growth as the concentration was increased was probably due to its toxic effect rather than the ethylene response it might have elicited. Cyclohexane, a compound which does not stimulate growth either in the presence or in the absence of added ethylene, also caused a decline in length as the concentration was increased (Fig. 1). Benzene also did not stimulate growth in either the presence or absence of added ethylene, but the decline in growth as the concentration was increased was much more marked than with any other compound used. Similarly, all of the cyclic olefins tested inhibited growth at high concentrations. However, the possibility that these olefins, which counteract ethylene action, may elicit a small ethylene response at high concentrations cannot be ruled out.

Compounds which inhibit the action of another can do so in a number of different ways, and kinetic analysis is necessary to determine the type of inhibition. Although the amount of product *in vivo* is unknown, the Lineweaver–Burk plot has been employed to analyse ethylene effects on the pea stem elongation [2]. A Lineweaver–Burk plot of data at a number of different ethylene levels (range 0–1 μl/l.) revealed typical competitive inhibition of ethylene action by 2,5-norbornadiene. It was observed that as the concentration of ethylene was increased, V_{max} (or the maximum inhibition of growth) was the same in the presence and in absence of 2,5-norbornadiene. The K_i obtained at 1080 μl/l. of 2,5-norbornadiene was essentially the same as that obtained at 4340 μl/l. The K_i values were calculated from the equation $(K_m)\text{app} = K_m(1 + [I]/K_i)$ [10], where (K_m)app is the apparent K_m in the presence of inhibitor, [I] is the concentration of inhibitor, and K_i is the dissociation constant of the receptor–inhibitor complex. K_m is obtained from $-1/K_m$, which is the intercept on the x-axis in the absence of inhibitor, whereas the apparent K_m is obtained from $-1/(K_m)$app, which is the intercept on the x-axis in the presence of inhibitor. The derived value for K_m for ethylene was 0.1 μl/l., which is identical to that obtained by Burg and Burg [2] for pea stem elongation. The K_i value for 2,5-norbornadiene at 1085 μl/l. was estimated to be 170 μl/l.

Inhibition by various cyclic olefins

Since *cis*-butene was capable of counteracting ethylene action, we examined a number of cyclic olefins for their anti-ethylene activity. Of those compounds tested, 2,5-

Table 3. The inhibition of ethylene action by various compounds*

Compound structure	Name	K_i (μl/l.)	Ratio
	2,5-Norbornadiene	170	1
	Norbornene	360	2
	1,3-Cyclohexadiene	488	3
	1,3-Cycloheptadiene	870	5
	Cyclopentene	1100	7
	1,4-Cyclohexadiene	4650	27
	Cyclohexene	6060	35
	Cyclohexane	∞	—
	Benzene	∞	—

*The lower the K_i value, the more inhibitory the compound. The value of K_i (μl/l.) is the concentration of inhibitor which will double the apparent K_m for ethylene [10]. The ratio is K_i of compound/K_i of 2,5-norbornadiene.

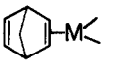
norbornadiene was the most effective inhibitor of ethylene action (Table 3). The K_i value of 360 μl/l. obtained for norbornene means that this compound is approximately one-half as active on a molar basis as 2,5-norbornadiene. Since 2,5-norbornadiene has two double bonds, its effective concentration would be twice its molecular concentration. This then would mean that both 2,5-norbornadiene and norbornene are equally effective with respect to their double bonds. 1,3-Cyclohexadiene is approximately one-third as active as 2,5-norbornadiene while 1,4-cyclohexadiene is only 1/27 as effective. Most of the cyclic olefins tested exhibited some anti-ethylene action. Cyclohexane and benzene were inactive.

DISCUSSION

That ethylene forms complexes with metals has been known since 1827. Burg and Burg [2] conducted a detailed study of the structural requirements of olefins for ethylene action and found that olefins that exert ethylene-like biological activity bind to Ag^+ in the same order as they express biological activity. It is expected that any metal high in the transition series will have binding properties similar to those of silver ion [11]. Ethylene and other olefins are known to bind to metal ions such as Cu^+ and their binding properties can be influenced not only by the metals but also by their coordination ligands.

In the past, most experiments were designed to show which compounds were capable of eliciting an ethylene-like response. However, Sisler and Pian [7] reported that 2,5-norbornadiene seemed to counteract ethylene in a reversible manner. Later, it was shown that this compound interacts with an ethylene-binding component in a Triton X-100 extract of mung bean sprouts [12]. In the present report, the *in vivo* competitive nature of 2,5-norbornadiene with respect to ethylene action is shown with a K_i of 170 μl/l.

Since norbornene, which has only one double bond, is approximately one-half as effective as 2,5-norbornadiene, it would seem likely that binding of the double bond in the five-membered ring to the metal receptor occurs, but such a binding does not elicit an ethylene response. Silver is reported [11] to bind in a linear manner to one side of 2,5-norbornadiene, a mode which may also apply to the ethylene-binding site. It is most probable that it binds as follows:



Although 1,3-cyclohexadiene is not as effective in counteracting ethylene action as is 2,5-norbornadiene, it is much more effective than 1,4-cyclohexadiene. It is of interest (Table 4) that the relative binding constants of Ag^+ to 2,5-norbornadiene, norbornene, cyclopentene and cyclohexene are 1, 1:2, 1:4.5 and 1:32, which are in parallel with the relative activity of these compounds to counteract ethylene action observed in these experiments (Table 3); the extent of binding of cyclic olefins to Ag^+ has been shown to be correlated with ring strain [13].

Experiments reported here and the data obtained from the literature for binding to Ag^+ illustrate that binding alone is not sufficient for ethylene action. Some compounds which bind to Ag^+ (Table 4) and apparently bind in plants elicit an ethylene response, while others exhibit anti-ethylene action.

In the case of butenes, a double bond at the end of the carbon chain (1-butene) is capable of mimicking the action of ethylene, whereas *trans*-butene is totally without effect, perhaps because of its steric hindrance, which interferes with the interaction. Although both 1-butene

Table 4. Literature values of K_0 for olefin binding to silver

Aliphatic olefins	$K_0 \times 10^4$	Ratio	Cyclic olefins	$K_0 \times 10^4$	Ratio
Ethylene	24400	1:	2,5-Norbornadiene	5720	1:
Propylene	4300	1:5.5	Norbornene	2680	1:2
1-Butene	1568	1:15.6	Cyclopentene	1190	1:4.8
cis-2-Butene	808	1:30	Cyclohexene	184	1:32
trans-2-Butene	286	1:85			

K_0 is the equilibrium constant for the Ag^+-complex formation of the olefin dissolved in carbon tetrachloride in equilibrium with Ag^+ ions in 1 M $AgNO_3$ [13].

and *cis*-2-butene are capable of competing with ethylene for binding sites, *cis*-2-butene elicits no biological action and, hence, counteracts ethylene response. On the contrary, once bound, 1-butene elicits biological action, as does ethylene. To elicit ethylene activity, Burg and Burg [2] have concluded that an olefin with its double bond attached to an unsubstituted methylene group (CH_2=C<) is an essential feature. Since *cis*-2-butene, 2,5-norbornadiene, norbornene and other anti-ethylene olefins investigated in this study are all substituted olefins, we may assume that substituted olefins can bind to the receptor site but do not elicit a response. The reason *cis*-butene does not induce an ethylene-like response may be that due to the electron-repelling property of methyl groups at both ends of the double bond, it does not withdraw electrons to such an extent that activity is expressed. *cis*-Olefins are more strained than *trans*-olefins, and this tends to increase bonding to metal-ion complexes while weakening the double bond, as indicated by the bond length [14]. Cyclic olefins are also strained. According to Beverwijk *et al.* [11], increases in electron density at the carbon–carbon double bond enhance a σ-type bond and weaken the π-type bond, and both σ- and π-bonding are important in stability.

The *trans* effect, a model known in inorganic chemistry, first alluded to in 1893 by Werner [15], and developed later [16], has been proposed as a mechanism of action for ethylene in plants [3]. Compounds capable of π-electron acceptance, such as ethylene, carbon monoxide, and cyanide, are high in the *trans* effect series. Although it is not entirely clear how this works, one idea is that as a nucleophilic ligand approaches a metal, the presence of another ligand which can remove excess density will stabilize the complex and enhance the rate of ligand substitution [17]. Ethylene, cyanide and carbon monoxide are all capable of exhibiting this *trans* effect [16]. At present, we would speculate that olefins that possess substituents on both carbons of the double bond may bind, but are not capable of sufficient back-acceptance of electrons to elicit ethylene-like activity. Thus, they serve as competitive inhibitors of ethylene by occupying the binding sites without eliciting ethylene activity. These compounds appear to be useful for overcoming ethylene's action *in vivo*. When 2,5-norbornadiene was applied to green-mature tomato, it was shown to be effective in inhibiting the onset of ripening (Su, L., unpublished results). They may also prove valuable in purifying the ethylene-binding component from plant sources [18], since inhibitors are often used in affinity chromatography to remove enzymes or receptors selectively from solutions of other proteins. In addition, these compounds should also be useful in separating ethylene effects from other effects in the testing of growth regulators or environmental responses, since ethylene binding can readily be blocked.

EXPERIMENTAL

Plant material. Pea seeds (*Pisum sativum* L. cv Alaska) were soaked overnight, with aeration, and then germinated in paper towels. The peas were placed *ca.* 2 cm from the top edge of the towel. The towels were folded and placed in a container with 2 cm distilled water so that the seed was *ca* 6 cm above the water surface. The containers were loosely covered. After 3 days at 25° in the dark, each sample consisting of 20 plants was transferred for treatment under a green safe light to a 20 l. glass jar containing KOH pellets to remove CO_2.

Treatment with liquid compounds was conducted by placing the specified amount of liquid into a vial with cheesecloth to increase the surface area. The jar was quickly enclosed after the addition of the liquid compound. Gas samples were injected through a serum stopper. After 4 days, the jars were opened and the length of the epicotyl was measured [8]. The concns of all compounds are reported as if they were gases. Even though many were liquid at normal temps, all readily vapourized. Norbornene is a solid at room temp., but it was applied on cheesecloth and was observed to vapourize completely within 10 min.

Chemicals. 1-Butene (bp 6°) was obtained from Matheson Chemical Co., Newark, CA; *cis*-2- and *trans*-2-butenes (bp 4° and 1°, respectively) were obtained from Pfaltz and Bauer Inc., Stamford, CT. Other chemicals were obtained from Aldrich Chemical Co., Milwaukee, WI. All chemicals tested for C_2H_4 activity or anti-C_2H_4 activity were subjected to GC analysis [1] and shown to be free of interfering olefins, with the exception that all of the butenes contained trace amounts of propylene which was removed by condensing butenes under a stream of air at −40°. However, this amount of propylene had no measurable effect on the growth of peas or on ethylene binding.

Ethylene binding. Ethylene-binding measurements were made essentially as described by Sisler [18]. $^{14}C_2H_4$ (120 mCi/mmol), obtained from Amersham Corp., was trapped as the $Hg(ClO_4)_2$ complex [9]. The $^{14}C_2H_4$ complex was pipetted into 25 ml Erlenmeyer flasks within a container having 50 g samples of pea epicotyls which had been allowed to stand for 5 hr after harvesting to allow wound C_2H_4 to subside. After sealing and injecting the respective butenes, the $^{14}C_2H_4$ was released by injecting saturated LiCl into the $^{14}C_2H_4$–$Hg(ClO_4)_2$ complex. The final $^{14}C_2H_4$ concn was 0.03 μCi/l. of air. A magnetic stirrer was used to facilitate release of C_2H_4 from soln [9]. Two hr later, the pea epicotyls were removed from the treatment container. After standing in air for 4 min, the epicotyls were put into a second container of *ca* 0.5 l. vol. containing $Hg(ClO_4)_2$ soln in vials to trap the $^{14}C_2H_4$. A piece of fibreglass filter was included in the vial to increase the surface area. After 24 hr, the samples were removed, scintillation fluid was added, and the radioactivities were then determined.

REFERENCES

1. Abeles, F. B. (1973) *Ethylene in Plant Biology*. Academic Press, New York.
2. Burg, S. P. and Burg, E. A. (1967) *Plant Physiol.* **42**, 144.
3. Sisler, E. C. (1977) *Tobacco Sci.* **21**, 43.
4. Solomos, T. and Laties, G. G. (1974) *Plant Physiol.* **54**, 506.
5. Beyer, E. M., Jr. (1976) *Plant Physiol.* **58**, 268.
6. Dollwet, H. A. and Seeman, R. E. (1975) *Plant Physiol.* **56**, 552.
7. Sisler, E. C. and Pian, A. (1973) *Tobacco Sci.* **17**, 68.
8. Lau, O. L. and Yang, S. F. (1976) *Plant Physiol.* **58**, 114.
9. Hepner, F. R., Trueblood, K. N. and Lucas, H. J. (1952) *J. Am. Chem. Soc.* **74**, 1333.
10. Segel, I. H. (1976) *Biochemical Calculations*, 2nd edn. John Wiley, New York.
11. Beverwijk, C. D. M., Van der Kerk, G. J. M., Leusink, A. J., and Noltes, J. G. (1970) *Organomet. Chem. Rev.* **5**, 215.
12. Sisler, E. C. (1982) *J. Plant Growth Regul.* **1**, 211.
13. Traynham, J. G. and Olechowski, J. R. (1959) *J. Am. Chem. Soc.* **81**, 571.
14. Hartley, F. R. (1969) *Chem. Rev.* **69**, 799.
15. Werner, A. (1893) *Z. Anorg. Chem.* **3**, 267.
16. Chatt, J., Duncanson, L. A. and Venanzi, L. M. (1955) *J. Chem. Soc.* 4456.
17. Purcell, K. F. and Kotz, J. C. (1977) *Inorganic Chemistry*, pp. 702–706. W. B. Saunders, Philadelphia.
18. Sisler, E. C. (1980) *Plant Physiol.* **66**, 404.