Ethylene Production of Ethyl Propylphosphonate, Niagara 10637

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ABSTRACT

The response of pea seedlings (Pisum sativum var. Alaska) to ethyl propylphosphonate is similar to the effects of low levels of ethylene. Since ethyl propylphosphonate generates ethylene when exposed to oxygen in combination with a reduced metal ion, it seems probable that its plant growth-retardant properties are due to ethylene.

Ethyl propylphosphonate has been reported to retard the growth of a number of woody species (15). The most apparent change in the growth pattern of treated plants was a reduction in internode length. Apical growth as well as subsequent development of sub-apical and lateral shoot growth was inhibited. In addition to growth retardation, ethyl propylphosphonate caused similar physiological responses such as an increase in cold hardiness, induction of seed germination, changes in root development, and alteration of flowering and fruiting.

Because some of these physiological responses have been attributed to the action of the plant hormone, ethylene (1-10, 12, 13), it is important to know whether ethyl propylphosphonate can be a source of ethylene. A model system for generating ethylene was reported earlier (11) in which various chemicals having a certain basic molecular structure, namely Y-CH2-CH2-X, have been shown to evolve ethylene in a redox-coupled cleavage reaction. The X-substituent can be

-\( \text{O} \), -\( \text{OR} \), -\( \text{O-C-R} \), -\( \text{O-C-OH} \), or -\( \text{O-P-O}^+ \),

while the Y-substituent may be -\( \text{H} \), -\( \text{OH} \), or -\( \text{S-CH}_3 \). It is reasonable to expect that propylphosphonate could function similarly to phosphate as an X-substituent and generate ethylene from the ethyl group. In addition, copper metal was also tested for its ability to function as the reduced metal part of the couple.

MATERIALS AND METHODS

The Niagara 10637 used was from a solution of 88.2% ethyl propylphosphonate and 11.8% wetting agent, Tween 80. One milliliter of the Niagara 10637 was dissolved in distilled water to make 1 liter of solution (5.80 \( \times \) 10\(^{-3} \) M). Ten milliliters of this solution were used in most of the experiments. The Niagara solutions were syringed into 50-ml Erlenmeyer flasks sealed with serum caps, and were thermostated at 25°C on a Dubnoff metabolic shaking incubator. A weight of 1.95 g of FeSO\(_4\) from Mallinckrodt and a weight of 0.50 g of CuO from Baker and Adamson were used in the experiments. In the short time study with CuO the samples were magnetically stirred at ambient temperature and at a constant rate on a Precision Scientific Company Mag-Mix. For each point observed, four individual samples were run.

Three phosphate buffer solutions of pH 2.0, 6.8, and 11.8 were prepared from 0.1 M H\(_2\)PO\(_4\) and 0.1 M NaOH, and were used to investigate the possibility of any pH dependence of the cuprous or ferrous systems. Ten milliliters of each buffer were pipetted into 50-ml flasks, together with samples of CuO or ferrous sulfate. One milliliter of a Niagara solution, containing 1 ml of the 88.2% ethyl propylphosphonate preparation per 100 ml of distilled water was syringed into the serum-capped flasks and was thermostated at 25°C on the incubator.

The copper wire used was from a spool of 20-gauge wire from Anchor. The copper surface was rinsed three times with benzene, three times with ethanol, and three times with distilled water just prior to use. To study the evolution of ethylene mediated by

![Fig. 1. Ethylene and propylene production by ferrous sulfate. The reaction mixture contained 1.95 g of FeSO\(_4\) in 10 ml of a 5.80 \( \times \) 10\(^{-3} \) M ethyl propylphosphonate solution.](image-url)
The reaction mixture contained 0.50 g of Cu$_2$O in 10 ml of a 5.80 × 10$^{-4}$ M ethyl propylphosphonate solution.

First injected into the tube. When a gas sample was removed from the aerobic samples, an equal aliquot of room air was injected into the tube. The oxygen dependence study of the ferrous ion system was done in the buffered solution, pH 2.0.

Gas samples of 0.50 cc were analyzed on a Beckman GC-4 gas chromatograph equipped with a 290-cm-long, 0.318-cm diameter stainless steel column packed with Porapak Q, 50 to 80 mesh, purchased from Waters Associates, Inc., Framingham, Massachusetts. The amount of ethylene and propylene was calibrated by comparison of the unknown with the elution pattern of standard gas mixtures.

An experiment was conducted to test the effect of ethyl propylphosphonate on etiolated pea seedlings. Pea seeds from W. Atlee Burpee Company were leached for 24 hr before being planted in presoaked vermiculite and then placed in a light-tight ventilated growth chamber. When the seedlings had reached a height of 2 to 3 cm, they were sprayed with a solution containing 4000 mg/l of ethyl propylphosphonate. They received two more treatments on consecutive days. Spraying was done outside the chamber in the dark room with a minimal amount of green filtered light. The controls were exposed to the same amount of reduced light. One set of controls was not treated, whereas another set of controls was sprayed with a phosphate buffer of the same pH and molarity as the Niagara spray solution, containing as much Tween 80 as the test solution.

RESULTS

All three of the systems studied generated ethylene, with the cuprous system being the most effective, the ferrous system the least, and the metallic copper system being intermediate. Figure 1 shows the time dependence of ethylene and propylene produced in the ferrous system under aerobic conditions. In Figure 2 the initial phases of ethylene and propylene production in the cuprous system are shown. Figure 3 shows ethylene and propylene production in the copper coil system. The propylene production exceeds that of ethylene by a factor of around 10. In Figure 4 the dependence of ethylene production on substrate level is shown, and Table I gives the results of dependence of the reaction on oxygen. Figure 5 shows the effects of spraying etiolated pea seedlings with copper metal, 1.00-g pieces of copper wire were shaped into coils by winding them around a 6-mm diameter glass tube. These coils, together with the Niagara solution, were thermostated at 25°C as above. All experiments were performed in quadruplicate.

Ethylene production in the absence of oxygen was studied by vacuum-degassing specially constructed high vacuum vessels containing Cu$_2$O or FeSO$_4$ in a side arm and 10 ml of the Niagara solution. The tubes were degassed four times with an oil diffusion pump to 17 μ at liquid nitrogen temperatures, and were then sealed off with a torch. The Cu$_2$O or FeSO$_4$ was decanted from the size arm into the ethyl propylphosphonate solution, and the tubes were kept under vacuum until the time of sampling. The break seal was broken, and the tube was filled with N$_2$. Whenever a gas sample was withdrawn, an equal amount of N$_2$ was first injected into the tube. When a gas sample was removed from the aerobic samples, an equal aliquot of room air was injected into the tube. The oxygen dependence study of the ferrous ion system was done in the buffered solution, pH 2.0.

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a solution containing 4000 mg/l of ethyl propylphosphonate. The thickening and kinks in the stems developed after each spraying. A general reduction in growth, as well as a tighter plumular hook and an inhibition of leaf expansion, can be seen in the treated pea seedlings when compared with the controls.

Table 1. Oxygen Dependence of the Formation of Ethylene from Ethyl Propylphosphonate, Niagara

<table>
<thead>
<tr>
<th>System</th>
<th>Conditions</th>
<th>Moles of Ethylene per Mole of Niagara</th>
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</thead>
<tbody>
<tr>
<td>Cu&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Aerobic</td>
<td>2.8 × 10&lt;sup&gt;-2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cu&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Anaerobic</td>
<td>8.0 × 10&lt;sup&gt;-5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fe&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>Aerobic</td>
<td>4.3 × 10&lt;sup&gt;-4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fe&lt;sup&gt;3+&lt;/sup&gt;</td>
<td>Anaerobic</td>
<td>9.0 × 10&lt;sup&gt;-4&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

DISCUSSION

According to our earlier hypothesis (11), ethyl propylphosphonate would be expected to yield ethylene. Comparison of Figure 1 with Figure 2 shows that the ferrous system is much less effective than the cuprous system. This difference might be expected from the more favorable oxidation-reduction potential in the cuprous-oxygen couple. Although the heterogeneous cuprous oxide system cannot be interpreted from the standpoint of quantitative chemistry, the qualitative observation that the cuprous species participates as an electron donor in the proposed system is substantiated.

The formation of propylene indicates that the carbon-phosphorous bond can also be cleaved and is an example of a new X-constituent.

Treatment of pea seedlings with ethyl propylphosphonate produced physiological responses which are known to be caused by ethylene. The thickening and shortening of pea stem internodes are apparent, and the kinks may be taken as a rudimentary state of the normal transverse geotropic response. All of these effects have been attributed to ethylene by Neljubow (12) and Knight and Crocker (10). Two of the most sensitive physiological responses to ethylene are the thickening of the plumular hook and the inhibition of leaf or terminal bud expansion in pea seedlings (14). These two phenomena are readily seen in Figure 5. From the results reported above, we can propose that ethyl propylphosphonate produces ethylene and propylene in vivo, from a mechanism similar to the model system by using reduced metal ion and
oxygen as described earlier (11), and some of the known responses to ethylene are observed.

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LITERATURE CITED