Hadacidin, a New Plant-growth Inhibitor Produced by Fermentation

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After screening several hundred cultures of fungi, actinomycetes, and bacteria isolated from various soils for plant growth regulating activity, a fungus culture, F7-2578 (identified later as *Penicillium purpureascens*), was found which had a dwarfing effect on pea plants. A foliage spray of the culture filtrate inhibited the growth of the stems, producing shortened internodes, and also inhibited the growth of the roots. The growth of the young expanding leaves of bean plants was greatly inhibited by spraying the fermentation broth of culture F7-2578 on the young plants.

Isolation of the growth inhibitor from the fermentation broth was undertaken and the active component was obtained in crystalline form. This substance was identical to another crystalline product which was isolated at about the same time from another fungus culture, F1-2270 (*Penicillium frequentans*), as the active component exhibiting antitumor activity in embryonated eggs (1,4). The active antitumor agent was characterized as the sodium salt of *N*-formylhydroxyminoacetic acid and was named hadacidin (4).

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\begin{align*}
\text{CH}_2 & \text{ - COONa} \\
N & \text{ - OH} \\
H & \text{ - C = O}
\end{align*}
\]

This report is concerned with the plant growth regulating properties of the fermentation broths of *P. purpureascens* and *P. frequentans* and the crystalline active component, hadacidin.

**Materials and Methods**

*Preparation of the Fermentation Broths.* The inhibitor-producing culture, F7-2578, was isolated from a soil collected from the entrance to an animal burrow east of Ogden, Utah, and identified as *Penicillium purpureascens*. The culture was grown on potato dextrose agar slants. A suspension of spores in 5 ml of distilled water from a slant culture was used to inoculate a 250 ml Erlenmey er flask containing 50 ml of a medium containing 40 g glucose, 10 g of corn steep liquor, and 20 g of Ealmine (enzymic digest of lactalbumin) in 1 liter of distilled water. The flask was incubated on a rotary shaker for 7 days at 28° and then the contents were filtered through 1 layer of cheesecloth and centrifuged for 10 minutes at 3000 rpm. The centrifuged broth of culture F7-2578 at various dilutions was used for treating the plants in most of the greenhouse tests. Crystalline hadacidin was prepared from larger fermentation batches by extracting the dried broths with methanol and crystallizing from a water-ethanol mixture as reported previously (4).

*Plant Bioassays.* The pea plant assays used for screening fermentation broths for plant growth regulating activity were carried out using Alaska peas. The seeds were planted in sand and when the plants were 2.5 cm in height, the plants were transferred to Hoagland's nutrient solution. Seven plants were supported with wads of cotton in V-shaped notches cut along 1 edge of a thin slat of redwood. The slat was placed across a large rectangular polyethylene dishpan containing the nutrient solution. Five more slats were placed over the same pan and when the plants were 7.5 cm tall, 1 slat with 7 plants was removed and the foliage was sprayed with the centrifuged broth. One week after treatment, the lengths of the roots and shoots were measured and compared to untreated controls. The roots and shoots were also separated and weighed.

Using bean plants and measuring the growth of the leaves, another bioassay which was more sensitive, specific and quantitative than the pea-dwarfing test was developed for the determination of hadacidin in fermentation broths. In the greenhouse, pinto bean plants were grown individually in 8-cm paper pots filled with garden soil. Serial dilutions of the centrifuged broths were sprayed to runoff on the foliage of young bean plants at the stage when their first trifoliate leaves were just starting to expand and were 1 to 2 mm in width. Seven days after treatment the widths of the first trifoliate leaves were measured and compared to untreated controls. Three plants were used for each treatment so that 9 leaves were measured for each dilution of the broth. This bioassay was used to compare the activity of different cultures and different media. It was also used to

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guide the chemical isolation by determining the activity of the different fractions. After the active component, hadacidin, was isolated in crystalline form, a curve was prepared by plotting the average growth in width of the leaves against the corresponding concentrations of crystalline hadacidin in the spray. The amount of hadacidin in the various fermentations broths was determined from this standard curve.

Results

Effect on Pea Plants. In the bioassay using Alaska peas, a spray of the culture filtrate of F7-2578 reduced the average height of 14 pea plant stems from 367 mm for the controls to 163 mm for the treated plants. The length of the stem internodes was greatly reduced and the plants appeared dwarfed. Little or no injury to the treated leaves or growing points was noted. Root growth was inhibited slightly by the application to the foliage.

Purified fractions did not cause as much dwarfing of peas as that shown by the crude broth. This was thought to be due to the fact that sprays containing the purified material in water would not adhere to the waxy pea leaves while the crude broths gave good wetting of the leaves. The effect of sprays containing crystalline hadacidin on the growth of pea plants is shown in table I. In all cases the sprays contained 0.1% Tween 20 as a wetting agent. Sprays containing 1000 mg/liter crystalline hadacidin and higher concentrations inhibited both shoot and root growth. Even with the wetting agent added, the spray retention was not as good as when the crude fermentation broth was used. At the highest spray level of 8000 mg/liter, no growth of the stems or roots occurred after treatment. Flowering was delayed in all hadacidin treatments and was completely prevented at the high levels.

Effect on Bean Plants. Fermentation broths of F7-2578 which dwarfed pea shoots, completely stopped the growth of young bean leaves for 1 week after spraying the broth on the foliage. The bioassay using pinto bean plants was more sensitive than the pea-plant bioassay. Broth dilutions as high as 1 to 16 caused over 90% inhibition in growth of the young trifoliate leaves.

The sensitivity of the bean assay is demonstrated in table II which compares the inhibition of leaf growth caused by crystalline hadacidin isolated from culture F7-2578 with the crystalline product isolated from culture F1-2270 as an antitumor agent. Both products were about equal in their leaf growth inhibiting activity. A level of 500 mg per liter stopped leaf expansion almost completely.

A fermentation broth of F7-2578 was also compared in the bean bioassay test with the fermentation broth of F1-2270 after incubation under the same conditions. From the standard curves it was found that the F7-2578 broth contained 5.9 g of the active ingredient, hadacidin, per liter and the F1-2270 broth contained 3.6 g per liter. The freeze-dried broth of F7-2578 which represented a yield of 22 mg of dried solids per ml of broth was found to contain 24% active ingredient by the bean bioassay.

In a test for translocation, a solution containing 1000 mg of crystalline hadacin per liter was painted on the primary leaves of 3 pinto bean plants. The average width of the untreated trifoliate leaves was 5 mm after 1 week compared to 29 mm for the controls. In another experiment 2.5 mg of crystalline hadacidin was applied to 1 primary leaf of each of 12 bean plants. After 4 hours the treated primary leaves of 2 plants were removed and this was repeated after 24 and 48 hours. Growth in width of the young trifoliate leaves was inhibited 26% when the treated primary leaf was removed after 4 hours, showing that some translocation out of the treated leaf had occurred. When the treated leaf was removed after 24 hours 58% inhibition resulted and removal after 48 hours caused 80% reduction in growth of the young leaves. In each case the reduction in growth was based on the growth of the young leaves of untreated plants which had 1 primary leaf removed after the same period. These results indicated that the inhibitor was translocated downward out of the treated

<table>
<thead>
<tr>
<th>Crystalline Hadacidin mg/l</th>
<th>Avg length of shoots mm</th>
<th>Total weight of shoots g</th>
<th>Total weight of roots g</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>352</td>
<td>163</td>
<td>8.5</td>
</tr>
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<td>346</td>
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<td>9.4</td>
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<td>355</td>
<td>13.1</td>
<td>8.1</td>
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<tr>
<td>2000</td>
<td>249</td>
<td>9.3</td>
<td>8.2</td>
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<tr>
<td>4000</td>
<td>184</td>
<td>7.0</td>
<td>5.7</td>
</tr>
<tr>
<td>8000</td>
<td>96</td>
<td>3.7</td>
<td>3.5</td>
</tr>
</tbody>
</table>

* Averages are based on 7 plants for each treatment.

Table II

Comparison of the Effects of Crystalline Hadacidin from Broths of 2 Different Penicillia Species on Growth in Width of Bean Leaves

<table>
<thead>
<tr>
<th>Crystalline material mg/l</th>
<th>% Inhibition of bean leaf expansion*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product from P. purpureuscens</td>
<td>Product from P. frequentans</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>62</td>
<td>24</td>
</tr>
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<td>125</td>
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<td>98</td>
</tr>
<tr>
<td>1000</td>
<td>98</td>
</tr>
</tbody>
</table>

* The % inhibition is based on the growth of the untreated controls which expanded from 1 mm to 30 mm in 7 days. Each figure represents the average of 9 trifoliate leaflets on 3 plants for each treatment.
leaves and up the stem to the young leaves in a short time.

When 4 bean plants were sprayed with each of 4 levels of hadacidin (125, 250, 500, and 1000 mg/liter), the elongation of the stems was inhibited for 6 or 7 days. However, after 12 days an enhancement of stem elongation occurred on those plants treated with 125, 250, and 500 mg/liter (fig 1). No stimulation occurred at the highest rate of 1000 mg/liter.

**Application to Tomato Roots.** Bean plants failed to respond to hadacidin when it was applied to the soil, so studies were initiated to determine the effect when it was applied to roots growing in nutrient solutions. Tomato plants were chosen for the study. At a concentration of 10 mg of hadacidin per liter, the root growth was markedly inhibited for a period of 8 days, then the growth of lateral roots was enhanced. At 50 and 250 mg/liter, the growth of the roots was completely stopped. Stem growth was increased at 10 mg/liter, but it was inhibited 40% at 50 mg/liter and stopped completely at 250 mg/liter. Under these conditions where the hadacidin was applied to the roots, root growth was inhibited more than stem growth.

**Effect on Other Plants.** The undiluted broth of culture F7-2578 was sprayed on the foliage of 14 different plant species growing in the greenhouse. This spray caused a reduction in stem growth of sunflower, cucumber, bean, tomato, rice, zinnia, oat, rye, and crabgrass. A second spray applied to the crabgrass inhibited its growth and prevented flowering. Flowering in oats was delayed more than 2 weeks by 1 application, and flowering was also delayed in sunflower, zinnia, and pinto beans. Leaf size was reduced in several species in addition to beans. Some plants were not affected by the same concentrations which stunted others. These tolerant plants included 2 varieties of peas, perennial ryegrass, and wheat. A culture filtrate of F7-2578 of greater hadacidin concentration than the previous batch completely killed crabgrass after one application.

**Paper Chromatography of Fermentation Broths.** Paper chromatography of F7-2578 broth in n-butanol: acetic acid: water (4: 1: 1; v/v) gave a spot at Rf 0.3 which could be located as an acidic spot with indicator sprays or as a reducing spot when sprayed with potassium permanganate solution. A number of one-dimensional paper chromatograph strips were prepared from the broth and the strips were cut into small sections and placed in the bottom of petri dishes. The paper sections were moistened with water and tomato seeds were placed in the dishes for germination tests. The sections at Rf 0.3 inhibited the growth of the roots, but other sections from the remainder of the chromatograph strips had no effect on the roots of germinating tomato seeds. The results of these tests indicated that no other growth inhibitors were present in the broth except the component at Rf 0.3. When crystalline hadacidin was tested, it was found to give the same color tests and same Rf value as the active component in the broth.

**Reversal Experiments.** Since culture filtrates containing hadacidin had a dwarfing effect on pea and bean plants and gibberellic acid had a growth enhancing effect, these 2 materials were tested as a combination spray in the bean plant bioassay. Ten days after treatment, the average width of the 6 trifoliate leaflets for each treatment was 29 mm for the controls, 37 mm for the 1 mg/liter gibberellic acid spray, 11.5 mm for the combined spray containing 1 mg/liter gibberellic acid and the hadacidin broth diluted 1 to 10 and only 3 mm for the combination of 50 mg/liter gibberellic acid plus hadacidin broth diluted 1 to 2. The average height of the bean plants was 212, 615, 500, and 280 mm, respectively for the same 4 treatments. Thus, hadacidin greatly reduced the enhancement of stem elongation caused by gibberellic acid and counteracted completely the enhancing effect of gibberellic acid on leaf enlargement.

Twenty naturally occurring amino acids including glycine were tested individually at a spray concentration of 2500 mg/liter in combination with 250 mg/liter of crystalline hadacidin in the bean plant bioassay to determine if any of them would reverse the inhibitory effect of hadacidin on leaf growth. A number of other materials including, adenosine, guanine, uracil thymine, cytosine, citric acid, succinic acid, pyruvic acid, sucrose, glucose, reduced glutathione, calcium, pantothenate and riboflavin were tested in the same way. Glycine gave no reversing activity. Aspartic acid, asparagine, glutathione, and calcium pantothenate and riboflavin appeared to cause a partial reversal of the dwarfing effect of hadacidin on bean leaves. Riboflavin completely counteracted the inhibition in leaf growth caused by hadacidin. It was felt that riboflavin inactivated the hadacidin by photoxidation, so another experiment was run in which hadacidin was sprayed on the upper leaf surfaces and riboflavin was applied to the lower leaf surfaces. Under these conditions, riboflavin had no effect. When pantothenate, aspartic acid, and asparagine

![Fig. 1. The effect of concentration of crystalline hadacidin in the spray on stem elongation of bean plants when measured 12 days after treatment. The enhanced stem growth at 12 days at levels of 125, 250, and 500 mg/liter, was preceded by a period of inhibited stem growth the first week after treatment with hadacidin.](image-url)
were retested by spraying on the lower leaf surfaces, no reversing effect was obtained, but glutathione caused slight reversing of the hadacidin activity.

Discussion

The studies reported here indicate that a potential herbicide and dwarfing agent was produced in the fermentation broths of certain fungus cultures. The higher activity of the fermentation broths over the crystalline material was thought to be due to natural wetting agents in the broths which aided the penetration of the active component, since no other growth-inhibiting components could be detected in the broths.

Although hadacidin caused dwarfing of many plants when applied as a foliage spray, it had no effect when added to the soil. Apparently hadacidin was inactivated in the soil, since application to the roots of plants growing in nutrient solutions resulted in dwarfing effects on the roots and stems.

The growth-inhibiting action of hadacidin in the presence of glycine was investigated. In this respect, it is interesting to compare hadacidin with another amino acid analog, O-methylthreonine, which exhibited herbicidal effects (3). The growth-inhibiting and bleaching effect of O-methylthreonine on plants could be reversed by isoleucine (3). However, neither glycine nor any of the common naturally occurring amino acids caused any reversal of the dwarfing effect of hadacidin on plant growth.

Hadacidin counteracted the growth-enhancing effect of gibberelic acid on bean leaf growth (2) and stem elongation, but this was considered to be the resultant effect of 2 plant growth regulators rather than a true antigibberellin effect.

Summary

A new type of dwarfing agent was produced by fungal fermentation and shown to possess interesting plant growth-regulating properties. The fermentation broth of Penicillium purpurescens produced dwarfing effects on certain crop plants and killed crabgrass when the broth was applied as a foliage spray. The active dwarfing component was isolated in crystalline form and identified as N-formylhydroxyaminoacetic acid (hadacidin). A foliage spray of hadacidin caused a dwarfing effect on Alaska peas grown in nutrient solution. Stem internodes were shortened, root growth was inhibited, and flowering was delayed. Sprays containing 125 to 500 mg/liter of hadacidin inhibited the expansion of young leaves of bean plants and stopped elongation of the stems for 1 week, but this was followed by an enhancement of stem growth the following week. Several tests indicated that hadacidin was translocated from the primary leaves of bean plants to the young trifoliolate leaves where it inhibited their growth. Hadacidin had no effect on plants when applied to the soil, but when applied to the roots of tomato plants growing in nutrient solution, it caused severe stunting of both roots and shoots.

Literature Cited


