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# PHYSIOLOGICAL RESPONSES OF LIGHT-GROWN CUCUMBER HYPOCOTYLS TO HELMINTHOSPOROL AND ITS DERIVATIVES

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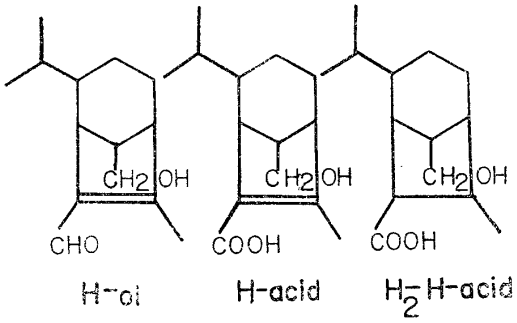
Helminthosporol, helminthosporic acid and dihydrohelminthosporic acid stimulated hypocotyl elongation of light-grown cucumber seedlings. The relative activities of the three compounds were in the order $H_2$-H-acid> H-acid> H-ol. The higher the dosage, the longer the promotion of the hypocotyl elongation lasted. The response of the hypocotyl to H-acid depended on the presence of cotyledons. As the amount of the cotyledon attached to the hypocotyl was reduced, the response decreased.

When IAA-oxidase was estimated as IAA metabolized per dry weight of the hypocotyl, there was an inverse relationship between IAA-oxidase activity and H-ol-induced elongation of the hypocotyl. However, there was no difference between control and H-ol treated materials when IAA metabolized per plant was compared. It is unlikely that the mechanism of H-ol action is closely related to IAA-oxidase activity of the plant.

TAMURA et al. isolated a new growth promoting substance, H-ol, from the fungus, *Helminthosporium sativum*. The rice seedling test was used as a bioassay (*1*). This compound has the following structure (H-ol).




Helminthosporol has been reported to promote leaf sheath elongation in many cultures of rice (*2, 3*), hypocotyl elongation in light-grown cucumber (*3*)

Abbreviations: H-ol, helminthosporol; H-acid, helminthosporic acid; $H_2$-H-acid, dihydrohelminthosporic acid: IAA, indole-3-acetic acid; $GA_3$, gibberellin $A_3$.

and dark-grown lettuce seedlings (*4*). It promotes $\alpha$-amylase activity in embryoless barley endosperm (*5*, *6*) and rice endosperm (*7*). Breaking of dormancy of artichoke tuber by H-ol has been reported (*8*). Helminthosporic acid and $H_2$-H-acid, derivatives of H-ol, are also biologically active in these assays (*4*, *5*, *8*, *9*). The authors have shown that H-ol, H-acid and $H_2$-H-acid are active in leaf sheath elongation of *dwarf*-5 mutants of maize (*10*). Helminthosporol and H-acid have recently been reported to be active in several other biological systems (*11–13*). On the other hand, H-ol is inactive in the dwarf pea assay, the *dwarf*-1 and *dwarf*-2 maize assays and the lettuce seed germination test. It is also inactive in the pea straight growth test for auxin (*3*). The pattern of growth induced by H-ol and its derivatives is very similar to that induced by gibberellins. In this paper the authors will present data on the growth and IAA-oxidase activity of light-grown cucumber seedlings in response to H-ol, H-acid and $H_2$-H-acid.

## MATERIALS AND METHODS

In the preparation and treatment of light-grown cucumber hypocotyls the method described by KATSUMI et al. (*14*) was followed. Cucumber seeds (*Cucumis sativus* "National Pickling") were soaked in tap water for 2–3 hr and planted in moist vermiculite (Zonolite #2) to a depth of 1 cm. Seedlings were raised on a growth bench in the laboratory or in an incubator at approximately 28° under fluorescent light (5,000–8,000 lux). When the hypocotyls reached a length of about 3 cm, they were marked 20 mm below the cotyledonary node with India ink. This 20 mm portion was called the *hypocotyl unit*. Compounds were dissolved in 90% ethanol and 10 $\mu$l of these solutions dropped to the apical bud of the intact seedling. Ethanol treatment was made in the control. The length of the hypocotyl unit of each seedling was measured after 1 to 6 days of growth. Elongation over the initial 20 mm length served as measure of response. The size of samples was ten (n=10) unless otherwise indicated. The mean values with or without standard errors are shown in the text.

For the determination of IAA-oxidase activity, seedlings were harvested and the hypocotyls were excised from them 3 days after treatment. Half of the hypocotyl batch was used for determining length and dry and fresh weights of the hypocotyls. The remaining material was stored at −15° for IAA-oxidase extractions. This frozen material was ground together with dry ice in a porcelain mortar. Phosphate buffer (0.077 M, pH 5.9) was then added, and the macerate homogenized. The homogenate was centrifuged at 9,000 × *g* for 30 min at 0°. The supernatant served as the enzyme solution. The concentration of the enzyme solution was adjusted so that 1 ml represented the extract from one hypocotyl. The reaction mixture was prepared according to the method of GOLDACRE et al. (*15*), and contained 1 ml each of $2 \times 10^{-3}$ M IAA, $10^{-3}$ M 2, 4-dichlorophenol and enzyme solution, and 6 ml of phosphate buffer solution pH 5.9, 0.033 M.

The reaction mixture was incubated at 25° for 30 min, the residual IAA was assayed by the SALKOWSKI reaction using GORDON and WEBER's reagent (*16*). The optical density read at 530 mμ was converted into μg of IAA using a calibration curve prepared with known amounts of IAA.

## RESULTS

### *Biological activity*

Helminthosporol, H-acid and $H_2$-H-acid were applied at the dosages, 1, 10, 100, and 200 μg/plant. The biological activity for cucumber hypocotyl elongation was determined 3 days after treatment. The results are shown in Fig. 1. All three compounds were active in promoting hypocotyl elongation, the responses being proportional to the applied dosages. The order of activity is $H_2$-H-acid>H-acid>H-ol. Relative activities of these three compounds differed from each other approximately by factors of ten. Fig. 2 shows the elongation of cucumber hypocotyl units induced by H-ol, H-acid, $H_2$-H-acid and $GA_3$.

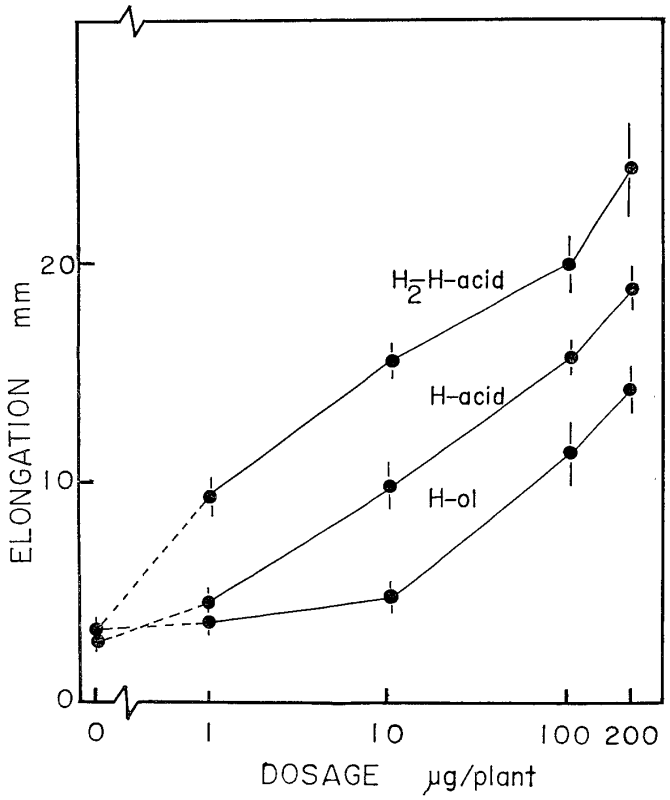



Fig. 1. Responses of hypocotyl units to H-ol, H-acid and $H_2$-H-acid at varying dosages. Measurements made 3 days after treatment. Each curve was obtained from separate experiments. Each point represents the mean of the responses of 10 different plants. Standard errors indicated with vertical lines.

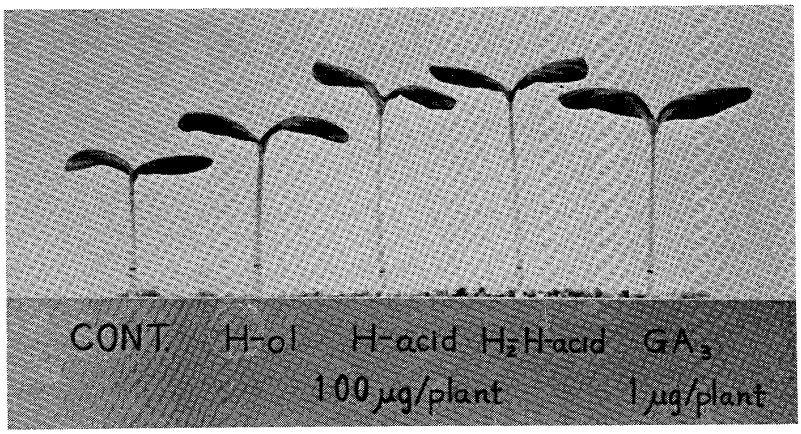



Fig. 2. Elongation of cucumber hypocotyl units induced by H-ol (100 $\mu$g), H-acid (100 $\mu$g), $H_2$-H-acid (100 $\mu$g) and $GA_3$ (1 $\mu$g). Photograph taken 3 days after treatment.

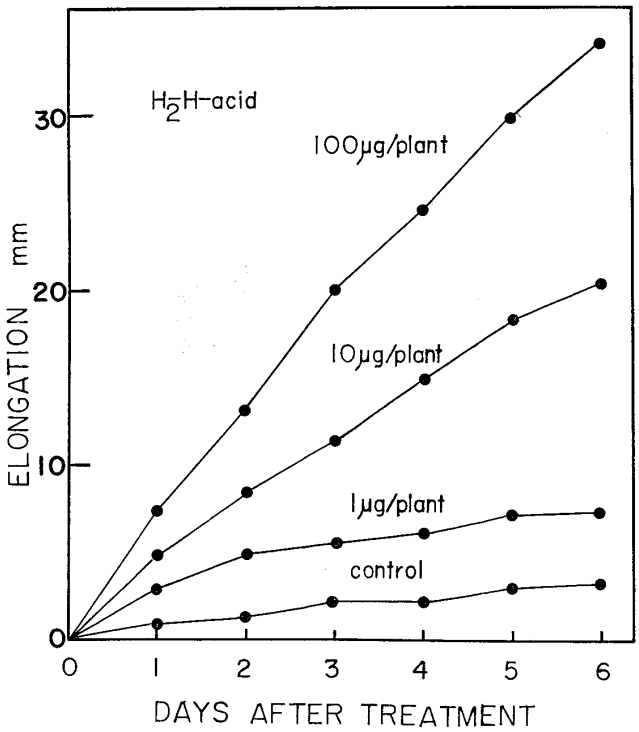



Fig. 3. Time course of response of hypocotyl units to different dosages of $H_2$-acid. The length measured at one day intervals for six days. Each point represents the mean of the responses of 10 different plants.

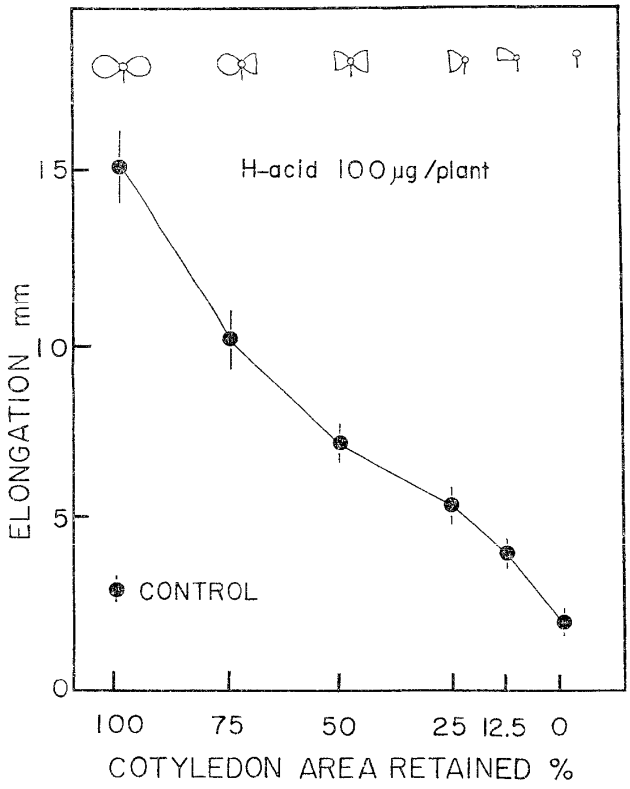



Fig. 4. Responses of hypocotyl units to H-acid in the presence of varying amounts of cotyledon attached. The length measured three days after treatment. Each point represents the mean of the responses of 10 different plants. Standard errors of the means are indicated with vertical lines.

The morphological appearances of the hypocotyls were similar to each other for the four compounds.

*Time course curves*

Dihydrohelminthosporic acid was added to cucumber seedlings and measurements made of the hypocotyls at one day intervals over a period of six days. The results are given in Fig. 3. Increased and stable growth rates were obtained with higher dosages. Experiments with H-acid and H-ol gave similar results, which are not detailed here.

*Cotyledon effect on H-acid-induced elongation*

Prior to treatment portions of one or both cotyledons were removed from the seedlings leaving from 0 to 100% cotyledons attached to the hypocotyls. Helminthosporic acid was applied to these seedlings at a single dosage of 100 µg/plant. Measurements were made three days after treatment. The results are shown in Fig. 4. The response of the plants to H-acid decreased

TABLE I

*Effect of helminthosporol on the growth and IAA-oxidase activity of the hypocotyl of light-grown cucumber seedlings* [a]

| | Control | Helminthosporol, μg/plant | | |
|---|---|---|---|---|
| | | 10 | 100 | 200 |
| Fresh weight (mg) | 82 | 111 | 153 | 159 |
| Dry weight (mg) | 2.4 | 3.0 | 4.8 | 4.1 |
| IAA-oxidase activity per dry weight | 11.7 | 9.7 | 5.9 | 6.9 |
| IAA-oxidase activity per plant | 28.5 | 29 | 28.5 | 28.3 |

[a] Measurements made 3 days after treatment. For fresh and dry weights and length, each value in the columns of control, 10 and 100 represents the mean of 15 plants, and in the column of 200 that of 6 plants. Enzyme activity expressed as μg IAA metabolized/30 min indicated.

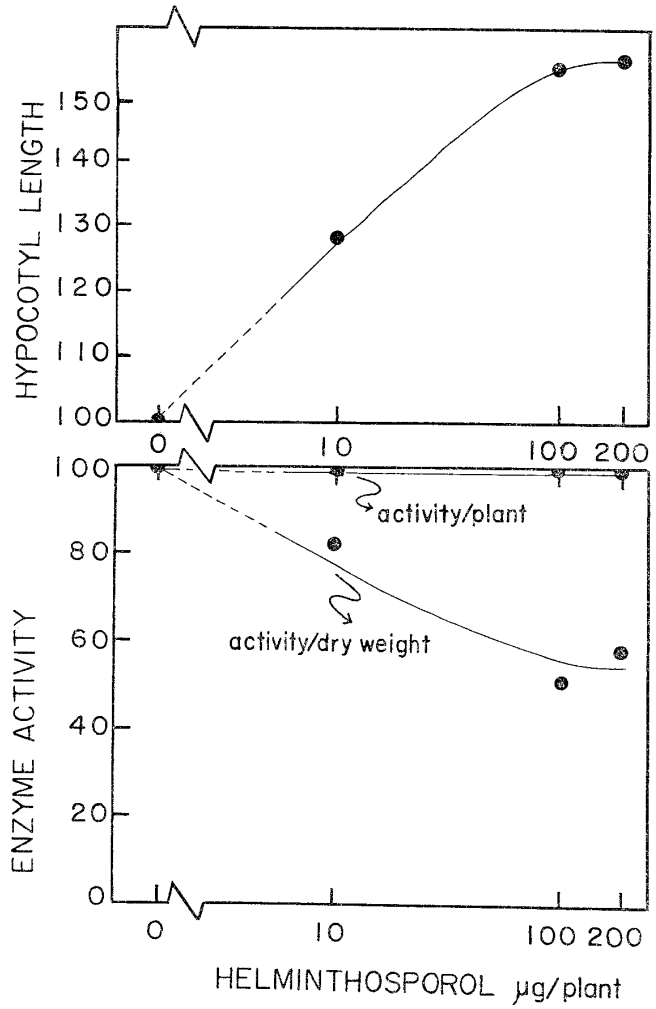



Fig. 5. Inverse relationship between IAA-oxidase activity and H-ol-induced elongation of light-grown cucumber hypocotyls. Data are presented in Table I. Elongation and enzyme activity were expressed as percentage of control.

remarkably as the amount of cotyledons attached was reduced. Thus, the amount of H-acid-induced growth was approximately proportional to the amount of the remaining cotyledons.

*IAA oxidase activity*

IAA-oxidase activity was determined as both IAA metabolized per dry weight of the hypocotyl and IAA metabolized per hypocotyl. The data are presented in Table I. IAA-oxidase activity determined as IAA metabolized per dry weight was less in the H-ol-treated materials. The greater the H-ol dose, the smaller was the enzyme activity. The treatment with 100 $\mu$g H-ol reduced the activity by half that of control. As clearly illustrated in Fig. 5, there was an inverse relationship between IAA-oxidase activity and H-ol-induced elongation of the hypocotyl. On the other hand, if IAA oxidase activity was estimated as IAA metabolized per hypocotyl, there was no difference between control and the H-ol-treated materials.

## DISCUSSION

Light-grown intact cucumber hypocotyls have been shown to respond to IAA, synthetic auxins and gibberellins (*14*). The fact that these hypocotyls also respond to H-ol and its derivatives makes them still more useful for interaction studies and also for screening several classes of growth regulators.

The relative activities of the compounds reported here ($H_2$-H-acid>H-acid>H-ol) would suggest that carboxylation and hydrogenation of these compounds are factors that control their biological activity. While the relative biological activities could be attributed to solubility properties of the molecules, it is also possible that the activity of H-ol may be a result of its conversion to H-acid since this occurs spontaneously at room temperature (*4*). Nothing is known about the metabolic interconversion of these three compounds.

Since the morphological appearance of the hypocotyls responding to these three compounds and $GA_3$ are very similar to each other and the time course curves (Fig. 3) are also very similar to that of $GA_3$ (*14*), the nature of the growth response induced by these three compounds may be the same as that of the growth response induced by gibberellin.

It has been previously reported (*17*) that gibberellin-induced elongation of the cucumber hypocotyl is quantitatively related to the amount of cotyledons present. The suggestion has been made that this cotyledon-gibberellin interaction is due to the presence of a diffusible factor supplied by the cotyledons. However, no such factor has been isolated from cucumber cotyledons. Indole-3-ethanol has recently been shown to be a naturally occurring growth regulator in cucumber seedlings, being produced in the cotyledons and active in the hypocotyl; however, it does not appear to have the properties predicted for the "cotyledon factor" (*18*). Our results show that the presence of cotyledons is also required for H-acid-induced elongation of the cucumber hypocotyl. Whether or not this cotyledon-H-acid interaction is due to the

same hypothetical cotyledon factor(s) is unknown. The presence of the cotyledons is also required in order that cucumber hypocotyl segments respond to cobaltous ions and to secondary alcohols (*19*), although the segments respond to indole-3-ethanol in the absence of the cotyledons (*19*).

It is controversial that the mechanism of gibberellin action is directly or indirectly related to IAA-oxidase activity. Several authors have indicated that IAA-oxidase activity is lowered by gibberellin treatment (*20–22*). HALEVY (*22*) had demonstrated that there was an inverse relationship between IAA-oxidase activity per dry weight and gibberellin-induced elongation of the cucumber hypocotyl. In the present study with H-ol a similar result was obtained as far as the enzyme activity per dry weight was assayed. However, the mechanism of H-ol action via the IAA-oxidase system is unlikely, since no difference in the activity between the control and H-ol treated materials was observed when IAA-oxidase activity was determined as IAA metabolized per plant. This suggests that the total activity of IAA-oxidase per seedling was not changed in the presence of H-ol.

It is concluded that H-ol, H-acid and $H_2$-H-acid are very similar to gibberellins at least in their physiological effects on cucumber hypocotyl growth.

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