Plant Physiol. (1992) 100, 1059–1061
0032-0889/92/100/1059/03/$01.00/0

Received for publication May 4, 1992
Accepted July 21, 1992

***Communication***

# Isolation and Identification of Lepidimoide, a New Allelopathic Substance from Mucilage of Germinated Cress Seeds

**Koji Hasegawa*, Junya Mizutani, Seiji Kosemura, and Shosuke Yamamura**

*Mizutani Plant Ecochemicals Project, Eniwa RBP Center Building, Megumino Kita 3–1–1, Eniwa, Hokkaido 061–13, Japan (K.H., J.M.); and Department of Chemistry, Faculty of Science and Technology, Keio University, Hiyoshi, Yokohama 223, Japan (S.K., S.Y.)*

## ABSTRACT

**A new allelopathic substance that promoted the shoot growth of different plant species but inhibited the root growth was isolated as an amorphous powder from mucilage of germinated cress (*Lepidium sativum* L.) seeds. This substance was identified as sodium 2-*O*-rhamnopyranosyl-4-deoxy-*threo*-hex-4-enopyranosiduronate (designated lepidimoide) from the mass and the nuclear magnetic resonance and infrared spectra coupled with some chemical evidence. Lepidimoide promoted the hypocotyl growth of etiolated *Amaranthus caudatus* L. at concentrations higher than 3 μM and inhibited the root growth at concentrations higher than 100 μM. The growth-promoting activity in hypocotyls was 20 or 30 times as much as that of gibberellic acid.**

The term "allelopathy" was coined by Molisch (4) to refer to both detrimental and beneficial biochemical interactions among all classes of plants, including microorganisms. When seeds of several different species of plants or etiolated seedlings were cultured together with cress seeds in a Petri dish, shoot growth of the plants was significantly promoted by cress seeds, but root growth was inhibited. The growth of cress seedlings was not influenced by cress seeds (2; Fig. 1). The allelopathy was not caused by contact with cress seeds, but was due to the mucilage of the germinated cress seeds, since seeds or seedlings of different species placed apart from the cress seeds on an agar plate also showed the effect (2). Longman and Callow (3) and Ray et al. (5) reported that the mucilage of germinated cress seeds contained the polysaccharides that reduced binding of *Pythium aphanidermatum* to cress roots. However, our recent studies demonstrated that the allelopathic substance(s), the $M_r$ of which was below $5 \times 10^3$, was different from the mucilage polysaccharides (1).

In this article, we report isolation and identification of an allelopathic substance from mucilage of germinated cress seeds and also report on some preliminary aspects of its biological activity.

## MATERIALS AND METHODS

### Plant Material

Three thousand cress seeds (*Lepidium sativum* L.) were allowed to imbibe in distilled-deionized $H_2O$ for 1 h and put on a stainless steel net (3 mm mesh) in a stainless steel tray ($40 \times 40 \times 3$ cm$^3$) containing 1.6 L of $H_2O$. The seeds on the net, in contact with the $H_2O$, were cultured at 25°C in the dark for 2 d. The culture solution was aerated with an air pump during the culture period.

### Purification

The culture solution was filtered through Toyo No. 1 filter paper and evaporated to dryness in vacuo at 35°C. The concentrate was divided into acetone-soluble and -insoluble fractions. The biological activity of growth promotion of *Amaranthus caudatus* L. hypocotyls and growth inhibition of the roots was detected in the acetone-insoluble fraction. This fraction was dissolved in 10 mL of $H_2O$ and was then fractionated into three parts, of $M_r$ above $10^5$, from $10^5$ to $5 \times 10^3$, and below $5 \times 10^3$ by molecular exclusion chromatography (Mol cut, Millipore Corp.). The biological activity was detected in the fraction of $M_r$ below $5 \times 10^3$, and this was evaporated to dryness in vacuo at 35°C.

### HPLC

The concentrate (approximately 150 mg) dissolved in $H_2O$ was purified by reverse phase HPLC (Waters, μBondasphere 5 μm $C_{18}$-100Å; 19 mm × 15 cm; 100% $H_2O$, flow rate 5 mL/min; 214 nm detector). The biological activity was found in fractions with retention times of 5 to 8 min. The eluate was concentrated in vacuo at 35°C and further purified by a second HPLC step (YMC Packed Column AQ-324 S-5 120A ODS; YMC Co. Ltd., Kyoto, Japan; 100% $H_2O$, 1 mL/min, 214 nm detector). The eluate at retention time 17.0 to 17.8 min showed biological activity and was evaporated to dryness in vacuo at 35°C. It gave 6.5 mg of an amorphous powder. We checked whether its occurrence could be ascribed to contamination by microorganisms, but this was not found to be the case. The activity could also be detected in mucilage

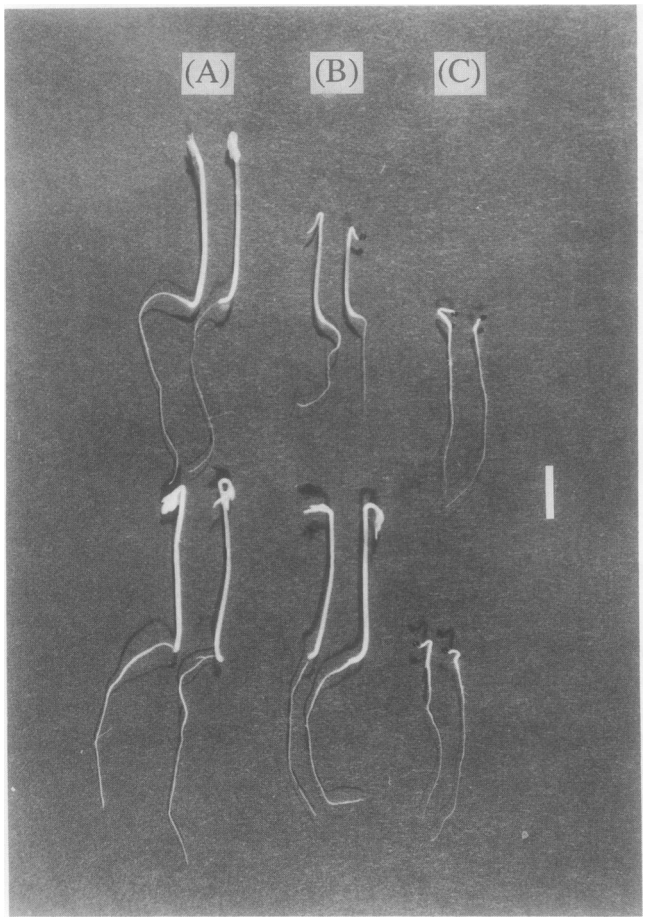


**Figure 1.** Interaction of cress and *A. caudatus* L. seedlings. A, Cress seeds were cultured together with other cress seeds. B, *Amaranthus* seeds (upper) were cultured with cress seeds (below). C, *Amaranthus* seeds were cultured with *Amaranthus* seeds. Ten seeds of the same or different species were cultured in a Petri dish (3 cm) in the dark at 25°C for 5 d. Scale bar, 1 cm.

of germinated cress seeds when the cress seeds were surface sterilized in 0.5% sodium hypochlorite for 5 min, washed in sterile water, and cultured in sterile water.

### Spectrometric Analyses

Optical rotation was determined with a JASCO DIP-360 polarimeter. The IR spectrum was obtained in glycerol with a JASCO A-202 spectrophotometer. The UV spectrum in $D_2O$ was obtained with a JASCO UVIDEC-610A spectrophotometer. The $^1H$ NMR spectrum was taken on a JEOL JNM-GX400 NMR spectrometer, and the fast atom bombardment mass spectrum was recorded in glycerol matrix.

### Bioassay

Ten seeds of *A. caudatus* L. were placed on filter paper moistened with 0.8 mL of test solutions in a 3-cm Petri dish. Petri dishes were kept in the dark at 25°C for 5 d, and then hypocotyl length was measured.

The 5-mm coleoptile sections of 3-d-old etiolated oat (*Avena sativa* L. cv Victory) seedlings were incubated in 1% sucrose solution (pH 5.4) containing various concentrations of lepidimoide for 6 h at 25°C in the dark. After incubation, the length of each section was measured.

## RESULTS AND DISCUSSION

### Determination of Isolated Compound

Lepidimoide (Fig. 2) was isolated as an amorphous powder from mucilage of germinated cress seeds. The high resolution FAB mass spectrum showed an $M^+ + Na$ peak at $m/z$ 367.0591, indicating a molecular formula of $C_{12}H_{17}O_{10}Na_2$ ($\Delta - 2.6$ molecular mass units). Its optical rotation $[\alpha]_D^{19}$ was +87.8 (c 0.032, $D_2O$). Its IR spectrum revealed the presence of a carboxylate group (1590 $cm^{-1}$) and a hydroxyl group (3300 $cm^{-1}$). $^1H$ NMR ($D_2O$) spectral data were as follows: δ5.72 (1H, d, $J$ = 3.2 Hz, j), 5.17 (1H, d, $J$ = 1.6 Hz, a), 5.07 (1H, d, $J$ = 2.3 Hz, g), 4.26 (1H, dd, $J$ = 6.9, 3.2 Hz, i), 4.08 (1H, dd, $J$ = 3.4, 1.6 Hz, b), 3.79 (1H, dq, $J$ = 9.7, 6.8 Hz, e), 3.76 (1H, dd, $J$ = 9.7, 3.4 Hz, c), 3.72 (1H, dd, $J$ = 6.9, 2.3 Hz, h), 3.31 (1H, dd, $J$ = 9.7, 9.7 Hz, d) and 1.80 (3H, d, $J$ = 6.8 Hz, f), where lower case letters refer to corresponding groups in Figure 2. The UV spectrum was $\lambda_{max}$ 225 nm (ε approximately 2100).

When treated with acetic anhydride-pyridine-methanol at room temperature overnight, lepidimoide was converted into the corresponding methyl ester with five acetoxy groups, the mass spectrum of which indicated that the molecular formula was $C_{23}H_{30}O_{15}$ ($m/z$ 546.1564 [$M^+$]). The IR spectrum indicated no hydroxyl absorption and, in the $^1H$ NMR ($CDCl_3$) spectrum, exhibited signals for five acetoxyl methyl protons (δ 2.00 [3H, s], 2.05 [3H, s], 2.10 [3H, s], and 2.14 [3H × 2, s]) and a signal for the methoxyl proton (δ 3.88 [3H, s]). The nuclear Overhauser effect experiments with the methyl ester gave valuable data. Irradiation at δ 5.17 (H of a) increased 7.3 and 8.3% of the intensity of H of b and H of g, respectively. Irradiation at δ 5.07 (H of g) increased 8.3, 6.9, and 13.6% of the intensity of H of a, b, and h, respectively. These data indicated the structure of lepidimoide to be sodium 2-*O*-rhamnopyranosyl-4-deoxy-*threo*-hex-4-enopyranosiduronate (Fig. 2).

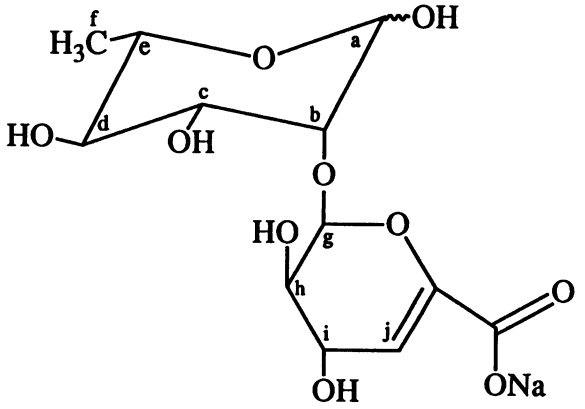


**Figure 2.** Chemical structure of lepidimoide.

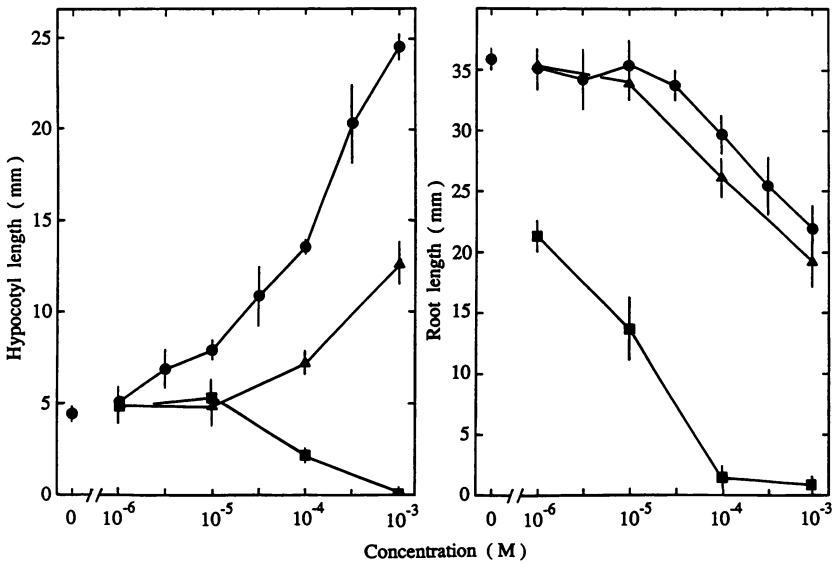


**Figure 3.**. Effects of lepidimoide (●), $GA_3$ (▲), and IAA (■) on the hypocotyl (left) and root (right) growth of etiolated *A. caudatus* L. seedlings.

## Biological Activity of Lepidimoide

The effects of lepidimoide, $GA_3$, and IAA on the growth of etiolated *A. caudatus* L. seedlings were demonstrated (Fig. 3). Lepidimoide promoted hypocotyl growth at concentrations higher than 3 μM, but inhibited root growth at concentrations higher than 100 μM. It is suggested that lepidimoide may render many different plant species susceptible to growth retardation. The promotion of hypocotyl growth by lepidimoide was 20 to 30 times as much as that produced by $GA_3$ treatment, suggesting that lepidimoide was a potent growth substance. The growth inhibition of roots by lepidimoide was not significantly different from that shown by $GA_3$. IAA inhibited hypocotyl and root growth of *Amaranthus* seedlings. On the other hand, lepidimoide showed no auxin activity in the *Avena* coleoptile section test (data not shown).

These results suggest the possibility that cress plants may protect themselves by exuding from roots an allelopathic substance (lepidimoide) and polysaccharides (1, 3, 5), which affect the growth or differentiation of different plant species and microorganisms.

## ACKNOWLEDGMENT

The authors thank Midori Amano for expert technical support.

## LITERATURE CITED

1. **Hasegawa K, Amano M, Mizutani J** (1992) Allelopathic substances produced from cress seedlings. Weed Res **37:** 71–73
2. **Hasegawa K, Amano M, Urashima M, Li H, Mizutani J** (1992) Allelopathy of cress germinated seeds and tomato seedlings. Weed Res **37:** 68–71
3. **Longman D, Callow JA** (1987) Specific saccharide residues are involved in the recognition of plant root surfaces by zoospores of *Pythium aphanidermatum*. Physiol Mol Plant Pathol **30:** 139–150
4. **Molisch H** (1937) Der Einfluss einer Pflanze auf die andere-Allelopathie. Gustav Fischer Verlag, Jena.
5. **Ray TC, Callow JA, Kennedy JF** (1988) Composition of root mucilage polysaccharides from *Lepidium sativum*. J Exp Bot **39:** 1249–1261