Pergamon

0031-9422(95)00159-X

Phytochemistry, Vol. 39, No. 5, pp. 1031–1032, 1995

0020-7462/95 $9.50 + 0.00

LEPIDIMOIDE, AN ALLELOPATHIC SUBSTANCE IN THE EXUDATES FROM GERMINATED SEEDS

KOSUMI YAMADA, TOYOAKI ANAI* and KOJI HASEGAWA*†‡

Graduate School of Science and Technology, Kobe University, Nada-ku, Kobe 657, Japan; *Institute of Applied Biochemistry, University of Tsukuba, Tsukuba, Ibaraki 305, Japan; †Plant Ecochemicals Research Center, Eniwa RBP, Eniwa, Hokkaido 061–13, Japan

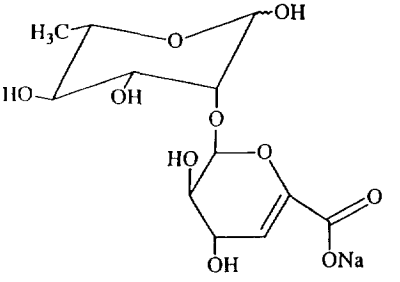
(*Received in revised form* 2 *February* 1995)

Key Word Index—*Lepidium sativum*; Cruciferae; allelopathy; growth promotion; lepidimoide.

Abstract—The occurrence in the plant kingdom of the allelopathic substance, lepidimoide, was studied. Lepidimoide is widespread in the exudates from all plant species studied. Lepidimoide occurred in especially large amounts in the exudates of germinated seeds of sunflower and buckwheat, but it was also detected in those of rice, lettuce, slender smaranth, leek and persian speedwell. Lepidimoide amounts did not differ greatly among genera or families.

INTRODUCTION

The term 'allelopathy' was coined by Molisch [1] to refer to both detrimental and beneficial biochemical interactions among all classes of plants, including microorganisms. It has recently been reported that when seeds or seedlings of different species of plants were cultured together with cress seeds (*Lepidium sativum* L.) in a Petri dish, the shoot growth of the plants was significantly promoted [2, 3]. These results suggested that the germinated cress seeds secrete growth-promoting allelopathic factor(s) to their environment. A new growth-promoting substance as one of the allelopathic factor(s) was isolated from the mucilage of germinated cress seeds and identified as an uronic acid derivative bearing an α,β-unsaturated carboxylate bonded to rhamnose via an α-glucoside linkage (designated lepidimoide) by spectral analysis and some chemical evidence [4]. Lepidimoide was synthesized from D-glucose and α-L-rhamnose and its absolute configuration was unambiguously determined to be Na 2-*O*-L-rhamnopyranosyl-4-deoxy-α-L-threo-hex-4-enopyranosiduronate (**1**) [5]. Lepidimoide also significantly promoted the hypocotyl growth of *Amaranthus caudatus* L. The growth-promoting activity was greater than that of gibberellic acid [4]. Moreover, lepidimoide-like activity was also found in the exudates from seeds of various weed and crop plants [6]. However, the occurrence in the plant kingdom of lepidimoide has not been previously reported. We studied the occurrence of lepidimoide in a number of plant species by physicochemical determination.



RESULTS AND DISCUSSION

The occurrence of lepidimoide in the exudates from seeds of various plant species was studied on the basis of HPLC retention time and biological activity of lepidimoide (Table 1). Twenty-four plant species (12 genera, 22 families) were used. All plant species used showed lepidimoide-like activity which promoted the hypocotyl growth of *Amaranthus caudatus* L. [6]. Lepidimoide was widely detected in the exudates from seeds of all plant species studied, although its amount was different among the species: the largest amount of lepidimoide occurred in the exudates of sunflower and buckwheat. The exudates of corn, oat, mitsuba, parsley, carrot, cress, cabbage, pea, okra, spinach and tomato also contained large quantities. On the other hand, less was detected in rice, lettuce, slender amaranth, leek and persian speedwell. However, lepidimoide amounts did not differ greatly among genera, families or Dicotyledoneae compared to Monocotyledoneae.

‡Author to whom correspondence should be addressed.

Table 1. The occurrence of lepidimoide in the exudates from seeds of various weed and crop plants

Genus	Species	Lepidimoide
Gramineae	corn (*Zea mays* L.)	***
	oat (*Avena sativa* L.)	***
	timothy (*Phleum pratense* L.)	**
	barnyard grass (*Echinochloa crus-galli* L.)	**
	rice (*Oryza sativa* L.)	*
Compositae	sunflower (*Helianthus annuus* L.)	****
	burdock (*Arctium lappa* L.)	**
	lettuce (*Lactuca sativa* L.)	*
Umbelliferae	mitsuba (*Cryptotaenia japonica* Hassk.)	***
	parsley (*Petroselinum crispum* Nym.)	***
	carrot (*Daucus carota* L. var. *sativa* DC.)	***
Amaranthaceae	livid amaranth (*Amaranthus lividus* L.)	**
	cockcomb (*Amaranthus caudatus* L.)	**
	slender amaranth (*Amaranthus viridis* L.)	*
Cruciferae	cress (*Lepidium sativum* L.)	***
	cabbage (*Brassica oleracea* L. var. *capitata* L.)	***
Polygonaceae	buckwheat (*Fagopyrum esculentum* Moench.)	****
Mimosaceae	pea (*Pisum sativum* L.)	***
Malvaceae	okra (*Abelomoschus esculentum* Moench.)	***
Chenopodiaceae	spinach (*Spinacia oleracea* L.)	***
Solanaceae	tomato (*Lycopersicon esculentum* Mill.)	***
Liliaceae	asparagus (*Asparagus officinalis* L.)	**
	leek (*Allium tuberosum* Rottl.)	*
Scrophulariaceae	persian speedwell (*Veronica persica* Poir.)	*

**** $\geqslant$ 10 μg seed^{-1}; *** 10 $\sim$ 1 μg seed^{-1}; ** 1 $\sim$ 0.1 μg seed^{-1}; * below 0.1 μg seed^{-1}.

EXPERIMENTAL

Determination of lepidimoide. Plant seeds were sterilized with 1% NaOCl for 30 min and rinsed with H_2O. The wet seeds were incubated in a Petri dish containing H_2O in the dark at 25° for 2 days. Each dish had 100 seeds. Three sizes of Petri dishes (ϕ 4.5, 7 and 9 cm) were used in proportion to the seed size. The culture soln was filtered through Toyo No. 1 filter paper and evapd to dryness *in vacuo* at 40°. The concentrate dissolved in H_2O was applied to a cation exchange cartridge column (Sep-Pak CM, Waters). The unadsorbed fraction was applied to an anion exchange cartridge column (Sep-Pak QMA, Waters) and then eluted with 10 ml of 0.1% CF_3CO_2H. The effluent was evapd to dryness *in vacuo* at 40°. The concentrate dissolved in H_2O was purified by HPLC [Tosoh, SCX(H^+); ϕ 27 × 150 mm; 0.2 mM CF_3CO_2H; flow rate 2.5 ml min^{-1}; 214 nm detector]. R_t of authentic lepidimoide was 10.0 min. Eluate containing lepidimoide was collected and evapd to dryness *in vacuo* at 40°. The residue dissolved in H_2O was finally purified by HPLC (Tosoh, Amide-80; ϕ 7.8 × 250 mm; MeCN–0.01 mM CF_3CO_2H = 3 : 1; 2.0 ml min^{-1}; 214 nm detector). Lepidimoide was eluted at R_t 12.0 min. Overall recovery of lepidimoide during the purification procedures was *ca* 60%. Whether sample and lepidimoide was equivalent, was confirmed with co-chromatography and analysis of biological activity of sample and authentic lepidimoide. After the area of the sample peak was determined, the amount of lepidimoide was calculated from a standard curve. Lepidimoide amounts calculated from the physicochemical assay almost coincided with those from the bioassay (data not shown).

Bioassay. The biological analysis was performed as followed; six seeds of cockcomb (*Amaranthus caudatus* L.) were placed on filter paper moistened with 0.5 ml of test soln in a 3-cm Petri dish. Petri dishes were kept in the dark at 25° for 4 days, and hypocotyl length measured.

Acknowledgements—This work was supported in part by grants from the Ministry of Education, Science and Culture, Japan [general (B) No. 06454649 to K.H.] and a Naito Foundation Natural Science Scholarship (to K.H.)

REFERENCES

1. Molisch, H. (1937) *Der Einfluss einer Pflanze auf die andere Allelopathie.* Gustav Fischer, Jena.
2. Hasegawa, K., Amano, M., Urashima, M., Li, H. and Mizutani, J. (1992) *Weed Res. Jpn* **37**, 68.
3. Hasegawa, K., Amano, M. and Mizutani, J. (1992) *Weed Res. Jpn* **37**, 71.
4. Hasegawa, K., Mizutani, J., Kosemura, S. and Yamamura, S. (1992) *Plant Physiol.* **100**, 1059.
5. Kosemura, S., Yamamura, S., Kakuta, H., Mizutani, J. and Hasegawa, K. (1993) *Tetrahedron Letters*, **34**, 2653.
6. Hasegawa, K., Amano, M., Asakawa, C., Kakuta, H. and Mizutani, J. (1993) *Weed Res. Jpn* **38**, 109.