Pergamon

Phytochemistry, Vol. 47, No. 1, pp. 1–2, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

PII: S0031-9422(97)00532-3

GROWTH-PROMOTING ALLELOPATHIC SUBSTANCE EXUDED FROM GERMINATING *ARABIDOPSIS THALIANA* SEEDS

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(*Received in revised form* 27 *May* 1997)

Key Word Index—*Arabidopsis thaliana*; Cruciferae; seed exudate; allelopathy; growth-promoting substance; lepidimoic acid; lepidimoide.

Abstract—A potent growth-promoting allelopathic substance was located from the exudates of germinating *Arabidopsis thaliana* (line Shokei) seeds and identified by analysis of its spectral data as 2-*O*-L-rahmnopyranosyl-4-deoxy-α-L-*threo*-hex-4-enopyranosiduronoic acid (lepidimoic acid). © 1997 Published by Elsevier Science Ltd

INTRODUCTION

It has recently been reported that growth-promoting allelopathic factors are exuded from germinating seeds of various plant species into their environment [1, 2]. Of the allelopathic factors, a novel potent growth-promoting substance was isolated from exudates of germinating cress (*Lepidium sativum*) seeds and identified as sodium 2-*O*-rhamnopyranosyl-4-deoxy-α-L-*threo*-hex-4-enopyranosiduronate (designated lepidimoide) [1, 3]. Lepidimoide has been reported not only to promote shoot growth in seedlings of various plant species but also to inhibit the loss of total chlorophyll in excised oat leaf segments [4] and abscission in bean petiole explants [5]. On the other hand, *Arabidopsis thaliana*, which has frequently been employed to elucidate the mechanism of development and differentiation in the life cycle of plants in terms of molecular biology, also secretes growth-promoting allelopathic factors during its germination [6]. We have isolated an allelopathic factor from exudates of germinating *A. thaliana* seeds and determined its structure.

RESULTS AND DISCUSSION

Exudates from germinating *A. thaliana* seeds, after repeated molecular exclusion and anion-exchange chromatography, preparative TLC on C_{18} and HPLC, yielded 1.2 mg of a colourless oil, which showed a potent growth-promoting activity in the cockscomb hypocotyl growth test (Fig. 1). The ^{1}H NMR spectra and R_f value on TLC of **1** and the acetate of its methyl ester were in agreement with those of 2-*O*-L-rhamnopyranosyl-4-deoxy-α-L-*threo*-hex-4-enopyranosiduronoic acid (lepidimoic acid) and its acetate [3], which were synthesized from D-glucose and

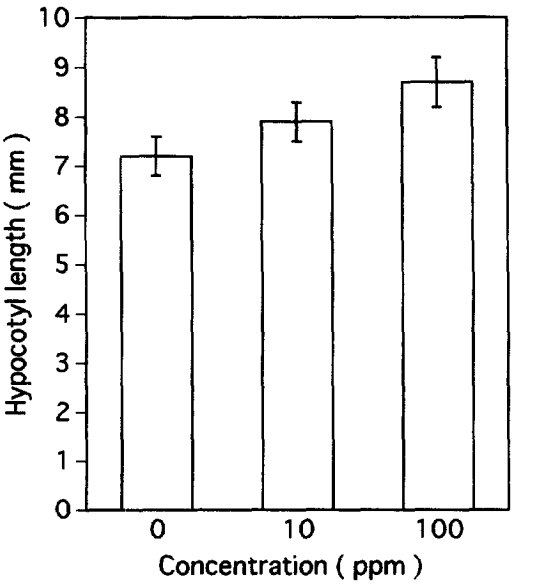

Fig. 1. Effect of lepidimoic acid on hypocotyl growth of cockscomb. Each value is the average of 10 measurements; bars indicate s.e.

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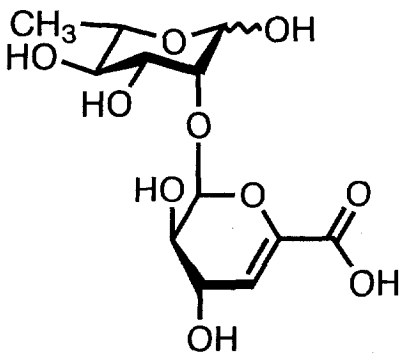

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α-L-rhamnose [3]. Compound **1** has not been isolated from a natural source so far. Lepidimoic acid showed as high growth-promoting activity for the cockscomb hypocotyl growth test as lepidimoide [7]. Furthermore, application of lepidimoide promoted hypocotyl growth, flowering and seed production in *A. thaliana* [8]. Lepidimoic acid may play important roles in the growth and differentiation of *Arabidopsis* itself as well as in allelopathy.

EXPERIMENTAL

Extraction and isolation. Arabidopsis thaliana (L.) Heynh. (line Shokei) seeds (155 g) were cultured in 7.8 l of H_2O in the dark at 5° for 1 day. The culture soln was filtered through Toyo no. 1 filter paper and evapd to dryness *in vacuo* at 35°. The concentrate (*ca* 8.1 g) dissolved in H_2O was fractionated into three parts, of M_r above 10^5, from 10^5 to 10^4, and below 10^4 by molecular exclusion chromatography (Novacell, Filtron Technology Corporation, U.S.A.). Biological activity was determined using the cockscomb hypocotyl elongation test. Promoting activities were detected in the frs of M_r below 10^4 and from 10^4 to 10^5. The former fr. was concd to dryness *in vacuo* at 35°. The concentrate (*ca* 1.4 g) dissolved in H_2O was chromatographed on an anion-exchange column (2.5 × 20 cm) (Q-Sepharose fast flow, Pharmacia) with a H_2O–NH_4HCO_3 solvent system by increasing the NH_4HCO_3 concn in a series of 0.2 M steps (150 ml $step^{-1}$) after washing with 500 ml of H_2O. All frs were applied to a Sephadex G-25 column (2.5 × 20 cm) to remove low M_r substances (below 180). The promotive activities were detected in the 0.2 M and 0.6 M frs. The eluate (0.2 M) showing highest activity was concd to dryness and sepd by TLC (RP-18 F254 S 20 × 20 cm, Merk), H_2O: MeOH (1:1). The eluate was concd and applied to an anion-exchange cartridge column (Sep-Pak QMA, Waters) and then eluted with 10 ml of 0.1% TFA. The eluate was evapd to dryness *in vacuo* at 35°. The concentrate dissolved in H_2O was purified by HPLC (Tosoh, SCX (H^+); ∅21.5 × 150 mm; 2 mM TFA; flow rate 3.5 ml min^{-1}; 214 nm detector). The active eluate (R_t 6.5–8.0 min) was finally purified by HPLC (Tosoh, SCX (H^+); ∅7.8 × 300 mm, H_2O; flow rate 0.8 ml min^{-1}; 214 nm detector). The active fr. was evapd to dryness *in vacuo* at 35°, giving 1.2 mg of a colourless oil.

Bioassay. Ten seeds of cockscomb (*Celosia argentea* var. *cristata* (L.) Kuntze) were placed on filter paper moistened with 0.5 ml of test soln in a 3-cm Petri dish, cultured in the dark for 4 days at 25° and hypocotyl lengths were measured.

Acetylation. The substance was treated with $TMSCHN_2$ in MeOH at room temp for 5 min and then reacted with Ac_2O–pyridine at room temp. overnight.

Lepidimoic acid (**1**). ^1H NMR (400 MHz, D_2O): δ 5.72 (1H, *d*, *J* = 3.2 Hz), 5.17 (1H, *d*, *J* = 1.6 Hz), 5.07 (1H, *d*, *J* = 2.3 Hz), 4.26 (1H, *dd*, *J* = 6.9, 3.2 Hz), 4.08 (1H, *dd*, *J* = 3.4, 1.6 Hz), 3.79 (1H, *dq*, *J* = 9.7, 6.8 Hz), 3.76 (1H, *dd*, *J* = 9.7, 3.4 Hz), 3.72 (1H, *dd*, *J* = 6.9, 2.3 Hz), 3.31 (1H, *dd*, *J* = 9.7, 9.7 Hz), 1.18 (3H, *d*, *J* = 6.8 Hz). Acetate of Me ester of **1**. TLC; R_f 0.55 ($CHCl_3$–MeOH, 100:1). Spectral data identical to lepidimoic acid acetate.

Acknowledgements—This work was supported in part by a Grant-in-Aid for University Research Projects of the Ministry of Education, Science and Culture, Japan to K.H.

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