



ALLELOPATHIC POTENTIAL OF 5-CHLORO-6-METHOXY-2-BENZOXAZOLINONE

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Key Word Index—*Zea mays*; Gramineae; allelopathy; benzoxazolinone; 5-chloro-6-methoxy-2-benzoxazolinone; maize; phytotoxicity.

Abstract—A new benzoxazolinone, 5-chloro-6-methoxy-2-benzoxazolinone (Cl-MBOA) caused growth inhibition of roots and shoots of oat (*Avena sativa*), timothy (*Phleum pratense*), crabgrass (*Digitaria sanguinalis*), ryegrass (*Lolium multiflorum*), cockscomb (*Amaranthus caudatus*), cress (*Lepidium sativum*) and lettuce (*Lactuca sativa*); increasing the dose of Cl-MBOA increased inhibition. The concentrations for 40% inhibition of root growth were 0.12, 0.14, 0.17, 0.19, 0.46, 0.57 and 0.76 mM for timothy, crabgrass, ryegrass, oat, cockscomb, cress and lettuce, respectively, and the concentrations for 40% inhibition of shoot growth were 0.25, 0.28, 0.36, 0.40, 7.9, 12.5 and 19.6 mM for timothy, crabgrass, ryegrass, cockscomb, cress and lettuce, respectively. The contents of Cl-MBOA in shoots and roots of 14-day-old maize (*Zea mays*) seedlings were 37.5 and 8.7 $\mu\text{g g}^{-1}$ fr. wt. The presence of this compound in maize seedlings, coupled with its effect on growth, suggest that it may play an important role in maize allelopathy. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Maize and certain other Gramineae plants contain a group of secondary metabolites known as benzoxazolinones [1–4]. These compounds and their precursor, hydroxamic acid, have been shown to be protective agents in the defence of the Gramineae against insects [5–9] and pathogens [10–12]. In addition, these compounds have been involved in growth regulation of plants [13–17] and allelopathic effects of cereals [18–20].

A new benzoxazolinone was recently isolated from light-grown shoots of maize seedlings (*Zea mays*) and it was identified from spectral data as 5-chloro-6-methoxy-2-benzoxazolinone (Cl-MBOA) [21]. This finding motivated us to focus further attention on its biological activity and endogenous content in the plant.

RESULTS AND DISCUSSION

Cl-MBOA contents in 14-day-old maize seedlings were determined by HPLC analysis and amounted to $37.5 \pm 3.2 \mu\text{g g}^{-1}$ fr. wt in shoots and $8.7 \pm 1.1 \mu\text{g g}^{-1}$ fr. wt in roots. Cl-MBOA was extracted and isolated

from light-grown maize seedlings without chlorine containing reagents [21]. These results suggest that Cl-MBOA may be a naturally occurring new benzoxazolinone. Chlorinated benzoxazolinones have also been isolated from maize seedlings [17, 22] and confirmed to be naturally occurring [22].

The biological activity of Cl-MBOA isolated from maize shoots [21] and synthetic Cl-MBOA were tested using growth of shoots and roots of oat seedlings. Their activities were identical (Fig. 1) and both inhibited the growth of roots and shoots at concentration greater than 0.03 and 0.1 mM, respectively. The concentrations of both Cl-MBOA for 40% growth inhibition of the roots and the shoots were 0.19 and 0.40 mM, respectively. Synthetic Cl-MBOA was therefore substituted for Cl-MBOA isolated from the maize shoots in all remaining bioassays.

Figure 2 shows the effects of Cl-MBOA on the growth of roots of the test plants. When percentage elongation was plotted against logarithm of dose, the dose-response curves of the plants for Cl-MBOA were linear between 20–70% inhibition for timothy, crabgrass and ryegrass, and between 20–50% inhibition for cockscomb, cress and lettuce. The doses required for 40% inhibition, as interpolated from the dose-response curves, were 0.12, 0.14, 0.17, 0.46, 0.57 and 0.76 mM for timothy, crabgrass, ryegrass, cockscomb, cress and lettuce, respectively.

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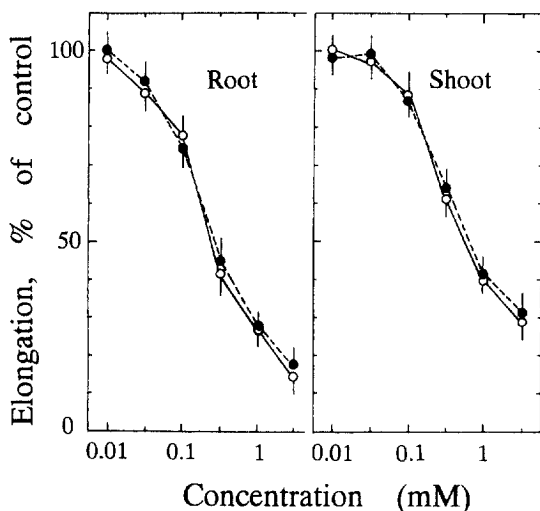


Fig. 1. Effect of isolated (○) and synthetic (●) Cl-MBOA on growth of roots and shoots of oat seedlings. Means $\pm$ s.e. from three replicate experiments with 10 plants each are shown. Elongation of control seedlings was 37.3 ± 1.9 mm and 21.7 ± 1.2 for the roots and the hypocotyls, respectively.

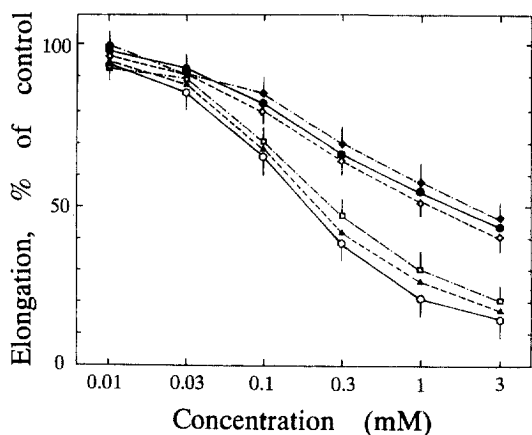


Fig. 2. Effect of Cl-MBOA on growth of roots of timothy (○), crabgrass (▲), ryegrass (□), cockscomb (◇), cress (●) and lettuce (◆). Means $\pm$ s.e. from three replicate experiments with 10–15 plants each are shown. Elongation of control seedlings was 22.8 ± 1.7 , 21.7 ± 1.4 , 33.6 ± 2.1 , 18.7 ± 1.1 , 28.6 ± 1.9 , 21.5 ± 1.6 mm for timothy, crabgrass, ryegrass, cockscomb, cress and lettuce, respectively.

Figure 3 shows the effects of Cl-MBOA on the growth of shoots of the test plants. Its activity against cress, cockscomb and lettuce was less than that on crabgrass, ryegrass and timothy. The dose required for 40% inhibition were 0.25, 0.28, 0.36, 7.9*, 12.5* and 19.6* mM for timothy, crabgrass, ryegrass, cockscomb, cress and lettuce, respectively. Asterisks indicate that values were estimated by extrapolating from the dose-response curves.

Cl-MBOA inhibited the growth of the roots and the shoots of the test plants at concentrations greater than 0.03 and 0.1 mM, respectively, and increasing the dose

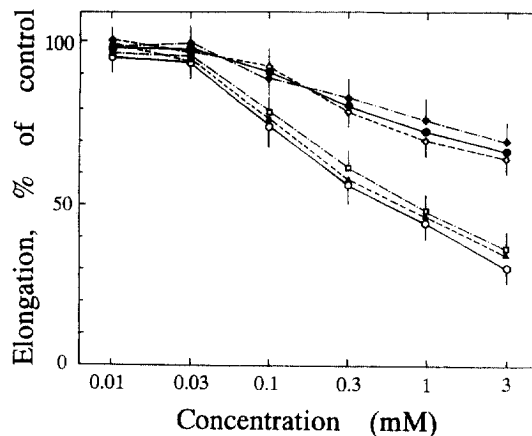


Fig. 3. Effect of Cl-MBOA on growth of shoots of timothy (○), crabgrass (▲), ryegrass (□), cockscomb (◇), cress (●) and lettuce (◆). Means $\pm$ s.e. from three replicate experiments with 10–15 plants each are shown. Elongation of control seedlings was 13.1 ± 0.7 , 11.7 ± 0.8 , 19.2 ± 1.5 , 7.9 ± 0.6 , 15.3 ± 0.9 , 12.5 ± 0.7 mm for timothy, crabgrass, ryegrass, cockscomb, cress and lettuce, respectively.

increased the inhibition (Figs 1–3). Using 40% growth inhibition, the effectiveness of Cl-MBOA on the roots of the test plants was 2- to 28-fold that on the shoots of the same plants. The sensitivity of monocotyledonous seedlings (timothy, cockscomb, oat and ryegrass) to Cl-MBOA were 6- to 24-fold and 19- to 78-fold that of dicotyledonous seedlings (cress, crabgrass and lettuce) for roots and shoots, respectively. Thus, Cl-MBOA was more effective on roots than shoots, and on species of the Monocotyledoneae.

The occurrence of Cl-MBOA in the maize seedlings and its effectiveness on growth (Figs 1–3) suggest that it could act as an allelochemical to other plants after decomposition of the plant in the soil as do other benzoxazolinones [19, 23, 24].

EXPERIMENTAL

Plant material

Seeds of maize (*Zea mays* L. cv. Popcorn; Takii Ltd., Kyoto, Japan) were sown on two sheets of moist filter paper in trays and grown at 25° in daily cycles of 12 h light–12 h dark in a growth chamber. Light was provided from above with two white fluorescent lamps (3.2 W m^{-2} at plant level). After 14 days, shoots and roots of seedlings were harvested, rinsed with H_2O and frozen at -70° until extraction of Cl-MBOA.

Quantification of Cl-MBOA

Frozen plant material was homogenized in Me_2CO , filtrated and concd at 35° *in vacuo* to give an aq. residue [21]. The residue was adjusted to pH 7.5 with 1M K-Pi buffer and partitioned ($3 \times$) against an equal vol. of EtOAc. The EtOAc phase was evapd to dryness

after drying (Na_2SO_4). The crude material was chromatographed on a column (2×60 cm) of silica gel (50 g, silicagel 60, 70–230 mesh; Merck), eluted with benzene–EtOAc containing increasing amounts of EtOAc (10% and 100 ml per step); Cl-MBOA was eluted with 40–60% EtOAc in benzene. After evapn. the residue was dissolved 20% aq. MeOH (2 ml) and loaded on to a reverse-phase C_{18} Sep-Pak cartridge (Waters). The cartridge was eluted first with 20% aq. MeOH (20 ml), to remove impurities, and then with 40% aq. MeOH (20 ml) to release Cl-MBOA. The sample of Cl-MBOA was injected onto a column for HPLC (0.8×30 cm; μ Bondasphere C_{18} ; Waters, 2 ml min^{-1} , H_2O –MeOH (1:4); detection at 250 nm}. The R_f of Cl-MBOA was 36.3 min under these conditions. Quantification was performed by measuring peak areas. The peak frs of the Cl-MBOA did not contain other UV absorbing substances as determined by comparing the UV spectrum of the peak frs with that of the pure sample. The overall recovery of inhibitor through the entire quantification process was ca 80%.

Synthesis of Cl-MBOA

MBOA (80 mg) prepared from 3-methoxyphenol [25] was dissolved in benzene (10 ml) and sulfuryl chloride (0.2 ml) added to the soln. The reaction mixt. was stirred at room temp. for 10 min, poured into ice- H_2O (50 ml) and extracted with EtOAc (3×20 ml). The organic extracts were combined, dried (Na_2SO_4) and filtrated. The solvent was removed *in vacuo* and the residue was separated by TLC (silica gel 70 F_{254}) with *n*-hexane–EtOAc (1:1) to give Cl-MBOA (R_f 0.62).

Bioassays

Seeds of oat (*Avena sativa*), timothy (*Phleum pratense*), crabgrass (*Digitaria sanguinalis*), ryegrass (*Lolium multiflorum*), cockscomb (*Amaranthus caudatus*), cress (*Lepidium sativum*) and lettuce (*Lactuca sativa*) were obtained from Takii Ltb., sown on filter paper and allowed to germinate in the dark at 25° for 1–3 days. Cl-MBOA was dissolved in Me_2CO and added to a sheet of filter paper in a 3 cm Petri dish and dried. The filter paper in the Petri dish was moistened with 1 ml 0.05% (v/v) aq. Tween 20. Germinated Seedlings (10–15) were arranged on the filter paper and allowed to grow in the dark at 25° for 2–3 days. The length of shoots and roots was then measured with a ruler, and the percentage elongation of growth calculated with reference to the elongation of control plants.

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