Repellents against Earthworms, Hydroxamic Acids and Isothiocyanate, from UV-B Damaged Maize and Radish

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Hydroxamic acids (4-hydroxy-1,4-benzoxazin-3-ones) and 4-methylthio-3-butenyl isothiocyanate as repellents against earthworms, have been isolated from UV-B damaged maize (*Zea mays* L.) seedlings and roots of Japanese radish (*Raphanus sativus* L.).

Earthworms, which are very helpful in the formation of vegetable mould, though weak in sunlight, are one of the most important living creatures in relation to agricultural crops, wild plants and ecosystem functions. Increased exposure to UV-B on the surface of the earth due to the depletion of stratospheric ozone is known to cause a variety of physiological and morphological responses in plants.[1] In response to ultraviolet radiation, plants are synthesized and exude volatile and non-volatile compounds from their damaged tissues,[2] some of which act as potential toxins to earthworms. In this communication we wish to report the first isolation of repellents and their biological activity.

In a preliminary study of the occurrence of repellents in seedlings of UV-B damaged- and dark-grown maize,[3] earthworms[4] in the absence of light preferred to burrow into maize seedlings grown in the dark rather than into maize seedlings exposed to UV-B. An active fraction was separated from the seedlings by partitioning between EtOAc and water. The EtOAc fraction of the UV-B damaged seedlings caused considerable repellency, and this activity was higher than that in the fractions from the dark-grown plants. This finding suggests that the repellent(s) against earthworms are released into the rhizosphere soil in root exudates from damaged maize seedlings rather than nondamaged ones. The repellents were isolated from UV-B damaged maize seedlings (1100 g, FW) and identified as 2,4-dihydroxy-7,8-dimethoxy-1,4-benzoxazin-3-one (**1**, DIM$_2$BOA, 215 mg) and 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (**2**, DIMBOA, 430 mg) by spectral analysis.[3] Two benzoxazolinones, 6,7-dimethoxy-benzoxazolin-2-one (**3**, DMBOA) and 6-methoxy-benzoxazlin-2-one (**4**, MBOA), obtained by spontaneous decomposition from the corresponding hydroxamic acids,[5] have also been isolated as repellents against earthworms from UV-B damaged maize seedlings. The repellent from radish was isolated and identified as 4-methylthio-3-butenyl isothiocyanate (**5**, MTB-ITC)[6] by the same manners (Figure 1).

Hydroxamic acids (**1**, **2**) exist predominantly as the corresponding stable β-glucosides (**6**, **7**) in the living young maize plants.[7] These glucosides did not exhibit repelling activity, but they are enzymatically converted to active hydroxamic acids by endo-β-glucosidase upon plant cell disruption. Consequently, under the irradiation of UV-B, hydroxamic acids are nonvolatile compounds that can be released to the soil by root exudation. Since earthworms preferred maize seedlings containing low hydroxamic acids to high hydroxamic acids, the presence of increased hydroxamic acids from the root of maize into the rhizosphere soil has been related to avoidance behavior of the earthworms (Figure 2).

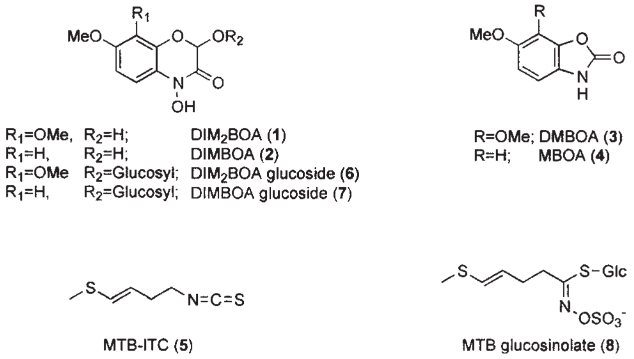

Figure 1. Repellents and their precursors from UV-B damaged maize and radish.

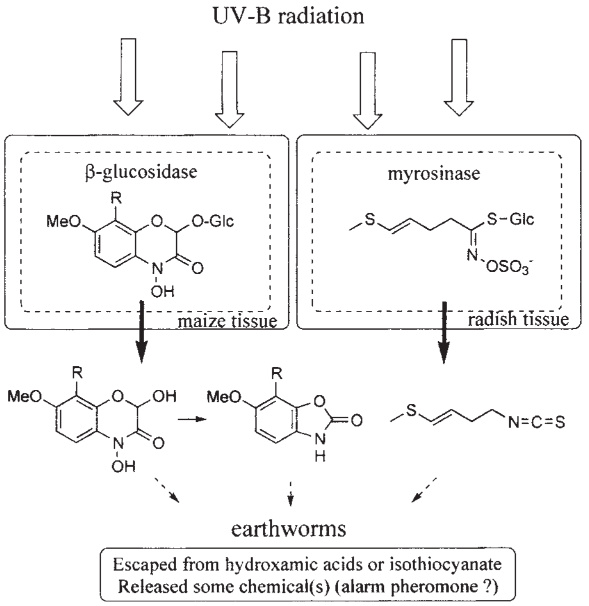

Figure 2. Repellent action of earthworms with UV-B damaged maize and radish.

Most chemical fertilizers are comparatively harmless for earthworms, but an exception is ammonium sulfate. Ammonium sulfate undoubtedly has high toxicity. This fertilizer makes an unpleasant strong acid for the earthworms when it is originally used for neutral soil or soil, which is slightly acid, and the earthworms move away. The hydroxamic acids showed stronger repellent action to earthworm (**1**: 10–50 ppm, **2**: 20–60 ppm) than the ammonium sulfate (50–100 ppm); in addition, benzoxazolinones showed repellent action at the same level as the ammonium sulfate. Surprisingly, it was found during experimentation on the repellent action of the hydroxamic acids on agar that the earthworms when moving away from the repellent would release some yellow chemical(s) such as an alarm pheromone. Moreover, other earthworms which has been left on agar with this yellow chemical(s) writhed in great agony, and escaped from there. Consequently, the performance of earthworms will be lower on UV-B damaged maize accumulating high concentrations of hydroxamic acids in its roots, and as a result, the soil will become impoverished. Such a result is not a phenomenon seen only in maize seedlings. Actually, MTB-ITC (**5**), the pungent principle of the radish,[8] showed stronger repellent action (1–10 ppm) than the hydroxamic acids. This compound occurs in the tissue of radish as 4-methylthio-3-butenyl glucosinolate (**8**, MTB glucosinolate), which is hydrolyzed by myrosinase (thioglucosidase) when the radish is damaged. Considering the formation mechanism of these compounds, an increase in UV-B will exert a similar influence on other plants, resulting in decrease in the activity of earthworms in the earth surface, and cause major adverse effect on the ecosystem functions.

It is well known that hydroxamic acids are secondary metabolites present in major agricultural crops such as wheat, maize, rye and related monocotyledons, and they play a major role in the defense of cereals against insects, fungi and bacteria, in the detoxification of herbicides and in allelopathic effects of the crop.[7] Recently, it has been reported that these properties have considerable, economical effects for agricultural systems. Long-term depletion of the stratospheric ozone layer contributes to an increase in terrestrial solar UV-B radiation. In any event, it is necessary that an increase in the repellents by UV-B damage should be comprehensively investigated to confirm its effects on the ecosystem functions.

References and Notes

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3 Plant material: Seeds of maize (*Zea may* L. cv. Astro Vantam, Sakata Seed Co., Yokohama, Japan) were imbibed for 24 h in a tray in the dark in running tap water, sown on a moist filter paper, and incubated at 25 °C under red light for 1 day. The germinated seeds were then incubated on two trays of 0.6% agar in the dark at 25 °C for 10 days. Uniform dark-grown, ten-day-old seedlings were divided into two groups: one group was left in darkness at 25 °C, and the other group was irradiated with UV-B fluorescent tube [λ_{max} 302 nm, Funakoshi Co., detected with UV-monitor (λ_{max} 313 nm, 0.22 W m^{-2}, 0.40 kJ m^{-2}, Eko Instruments Trading Co.)] at 25 °C. After 30 minutes, earthworms were released into each tray in the dark.

4 *Earthworms, Eisenia fetida* and one of *Pheretima* species, were collected at Hiyoshi campus in Yokohama.

5 Hydroxamic acids and related compounds have been reported as growth inhibitors related to the phototropism. S. Kosemura, S. Yamamura, T. Anai, and K. Hasegawa, *Tetrahedron Lett.*, **35**, 8221 (1994).

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