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LIGHT-ACTIVATED PLANT GROWTH INHIBITORY ACTIVITY OF *cis*-DEHYDROMATRICARIA ESTER, ROSE BENGAL AND FLUOREN-9-ONE ON LETTUCE (*Lactuca sativa* L.)

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ABSTRACT

The polyacetylene allelopathic compound *cis*-dehydromatricaria ester (*cis*-DME) was extracted from the roots of goldenrod, *Solidago altissima* L. *cis*-DME strongly inhibited the growth of lettuce (*Lactuca sativa* L. c. v. sacramento) on its radicles and hypocotyls in the presence of light whereas less or no significantly different effects were observed in the dark. The light-activated plant growth inhibitory (PGI) activity of *cis*-DME was more obvious in lettuce hypocotyls than in radicals. Its PGI activity was compared with the known phototoxic compounds rose bengal and fluoren-9-one. Although the light-activated PGI of *cis*-DME was not as high as that of rose bengal, and the dose-response relationship was slightly different with those of rose bengal and fluoren-9-one, *cis*-DME showed a very typical pattern of light-enhanced PGI activity against lettuce seedlings. The inhibition on the growth of lettuce radicles and hypocotyls highly enhanced by the presence of light. A growth enhancement effect was also observed on the radicles of lettuce when treated with lower concentrations of fluoren-9-one. A photosensitization mechanism is proposed here that the PGI activity of *cis*-DME may involve photosensitization and active oxygen such as singlet oxygen. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Many naturally occurring products from plants possess various biological activities such as herbicidal and plant growth regulatory (PGI) activities. Natural PGIs are used by plants to compete with other plants for nutrition.

However, natural products that have light-activated toxicity (phototoxicity) have only been primarily studied in insects for their possible usage as insecticides (Heitz, J. R. and Downum, K. R., 1987). A light-activated toxicity is also called photo-dependent activity or phototoxicity. It is referred to as the biological activity that is greatly enhanced or only occur in the presence of light. Toxicity of many natural products and dye compounds was found to be induced or enhanced by light (Heitz, J. R. and Downum, K. R., 1987). On the other hand, although many allelopathic interactions between plants have been proven to be chemically mediated, few of such effects have been related to light. Polyacetylene and trithiophene compounds are found predominately in the compositae plants and are the best known group of natural products that have light-activated toxicities toward fungi, bacteria, nematodes, insects and plants (Campbell *et al.*, 1982; Lam, J., 1988).

Goldenrod (*Solidago altissima* L.) is a compositae weed which is said to be brought to Japan after the World War II. This weed expanded very quickly all across the country of Japan, causing many hazardous problems in agricultural fields and along the railroads. *S. altissima* often flock together, and usually there are less or no other weeds thriving around their flocks. Researches have revealed that many long-chain polyacetylene compounds containing conjugated triyne bonds are the allelochemicals related to this phenomenon. *cis*-Dehydromatricaria ester (*cis*-DME) is one of those compounds, and found mostly in the roots (200-400 ppm). It has been reported to have PGI activity (Kobayashi, A. *et al.*, 1974), nematocidal activity, insecticidal activity against house fly, and photo-dependent ovicidal activity (Kawazu, K. *et al.*, 1977, 1980; Kagan, J. *et al.*, 1984). Tsao also found that this allelochemical had synergistic effect on the insecticidal activity of allethrin against house fly, whereas *cis*-DME itself had no direct toxicity against both adult and larval house flies (Tsao, R., Ph. D. Thesis, Kyushu University, 1991). Based on the fact that *cis*-DME is primarily a plant growth inhibitor (PGI), and many compounds of the same category have photosensitization or light-activated effect, we propose a hypothesis here that the PGI effect of *cis*-DME is enhanced by the light, and in the real ecosystem, its isomer *trans*-DME, formed by light radiation may play an identical biological role as does the *cis*-DME. In this study, we studied the photochemistry of *cis*-DME and light-enhanced activity of *cis*-DME, rose bengal and fluoren-9-one to lettuce seedlings, with the emphasis on *cis*-DME.

METHODS AND MATERIALS

1. Extraction, Purification and Identification of *cis*-Dehydromatricaria Ester:

cis-DME was extracted from the goldenrod roots (Fig. 1). The roots of goldenrod (1.0 Kg) were collected locally in Fukuoka, Japan in October, cut into ca. 2-cm long, soaked in 3 L of methanol, and kept in dark at 4 °C for two weeks. The extract was roughly filtered through a glass wool stopped funnel, and then concentrated to ca. 1/5 of its original volume. The concentrated aqueous extract was then extracted with hexane (100 ml × 3) in a 1000-ml separatory funnel. The combined hexane layer was dried over anhydrous Na_2SO_4 and further concentrated with a rotary evaporator below 40 °C to nearly dry. The resulting crude extract was redissolved in a minimum amount of hexane (~15 ml). Yellow crystals appeared from this solution after standing overnight at -20 °C. The crystals were

filtered and recrystallized from CCl_4 followed by identification with the instrumental analyses and melting point measurement.

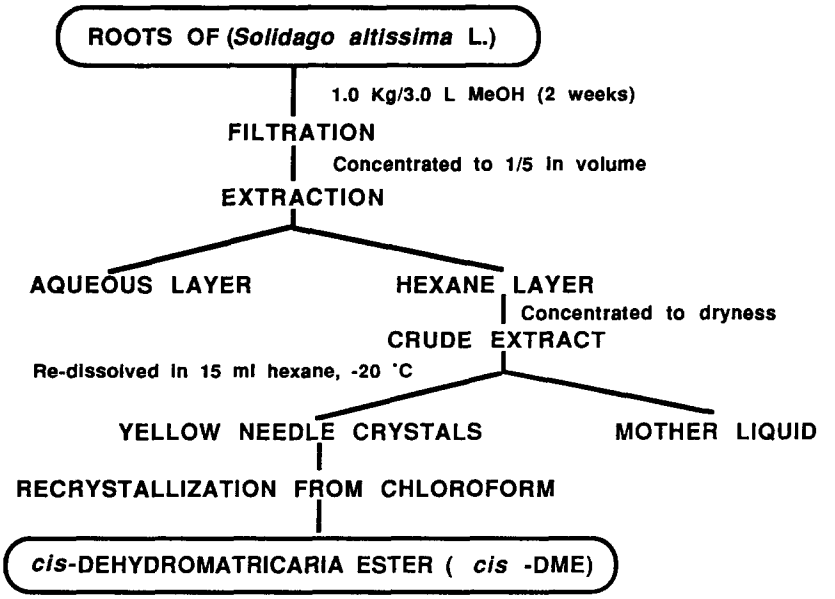

Fig. 1. The flow chart of the procedures used in extraction and purification of *cis*-DME.

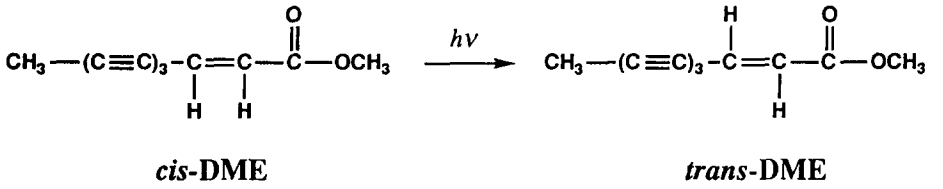

Fig. 2. Photoisomerization of *cis*-DME to *trans*-DME. The light source can be either ambient room light or UV ligh, and the reaction media can be either liquid or solid surfaces.

2. Photoisomerization of *cis*-DME to *trans*-DME.

Only *cis*-DME naturally exists in the plant. However, this natural product is relatively photolabile. It

isomerizes to *trans*-DME rapidly in the presence of light. When *cis*-DME was put in the subdued room light, or irradiated under a UV light, it *trans*formed to its *trans* isomer (Fig. 2).

The structures of *cis*-DME and *trans*-DME were confirmed with instrumental analyses melting point measurement. Gas chromatography was carried out on a Shimadzu GC-7A equipment. A glass column (2.6 m × 3.2 mm) containing 15% DEGS on 60-80 mesh Chromosorb WAW was used. The column temperature was maintained at 220 °C. The retention times for *cis*- and *trans*-DME were 12.42 and 7.86 min, respectively, and the TLC R_f values, 0.31 and 0.42 (hexane: EtOAc = 5:1). The proton NMR (nuclear magnetic resonance) spectra were recorded on a JEOL• JNM FX-100 instrument and the mass spectra on a Shimadzu GC-MS 9020DF. *cis*-DME: ^{1}H-NMR δ_{TMS} ($CDCl_3$) ppm: 2.02 (3H, s, CH_3-C), 3.78 (3H, s, CH_3O), 6.14 (1H, d, J =12 Hz, HC=CH(C=O)-), 6.26 (1H, d, J =12 Hz, HC=CH(C=O)-); MS *m/z* 172 (M^+), 157, 141, 113, 87; mp 116 °C. *trans*-DME: ^{1}H-NMR δ_{TMS} ($CDCl_3$) ppm: 2.02 (3H, s, CH_3-C), 3.78 (3H, s, CH_3O), 6.32 (1H, d, J =16 Hz, HC=CH(C=O)-), 6.69 (1H, d, J =16 Hz, HC=CH(C=O)-); MS *m/z* 172 (M^+), 157, 141, 113, 87.

Rose bengal, fluoren-9-one and other chemicals and solvents used in the experiments were purchased from Nacalai Tesque Inc., Japan.

3. Lettuce Growth Bioassay.

Lettuce petri dish test is a fast bioassay method in evaluating chemicals' effect on plant growth. Acetone solution of *cis*-DME was applied onto a filter paper which was lined in a petri dish (10 cm in diameter) and moistened with 5 ml of deionized water. The treated dishes were then put in a dark fume hood for 10 min to allow the acetone to evaporate. Methanol was used for rose bengal and fluoren-9-one. Ten lettuce (*Lactuca sativa* L. cv. sacramento) seeds were then placed onto the moistened filter paper. And the dishes were incubated in a controlled growth room at 25±1 °C, relative humidity 60±5% with a 12-h dark and light photoperiod. The PPFD (Photosynthetic photon flux density) of the light was 80 $\mu E/m^2/sec$. The dark experiments followed the same procedures except that the treated petri dishes were covered with aluminum foil. Activities were evaluated by measuring the length of the hypocotyl and radicle of the lettuce on the fifth day. Each treatment was tested in triplicate.

RESULTS AND DISCUSSION

cis-DME is a photolytically unstable chemical compound. When an acetone solution (10%) of *cis*-DME was irradiated with UV light of a rotary photochemical reactor (RH400-10W, Riko Chemical Industries, Ltd., Japan) for more than 30 min at 300-370 nm, it reached to an equilibrium, yielding 30-36% of the *trans* isomer under either oxygen or nitrogen atmosphere (Table 1, Fig. 2.). This isomerization reaction showed that oxygen was not a factor affecting the photolytic *trans*formation of *cis*-DME to *trans*-DME (Table 1). Further irradiation of *cis*- or *trans*-DME lead to unknown compound(s) with very high polarity. *trans*-DME (Fig. 2) can also be obtained by exposing *cis*-DME under UV light (λ = 254 nm) for 2 hr on glass or silica gel TLC (thin layer chromatography) surfaces. Separation and purification of *trans*-DME were carried out by using TLC (precoated E. Merck silica gel plate 60 F_{254})

with the developing solvent system hexane: ethyl acetate = 5:1 (v/v).

Table 1. Photoisomerization rates of *cis*-DME in acetone solution under oxygenated and nitrogenated conditions.

	Irradiation time (min)			
	30	60	150	360
	Yield of *trans*-DME (%)			
Oxygen	33.7	36.4	35.8	-
Nitrogen	30.1	31.9	31.7	31.1

Chemical interaction between different plants is a complicated process. It is affected by many environmental factors such as temperature, moisture, soil pH and sunlight. Many naturally occurring polyacetylene compounds have been shown to have photosensitizing activity. This photosensitization makes them more toxic under light to various microorganisms such as bacteria, viruses and fungi, nematodes and insects (Lam, J., 1988). These polyacetylene compounds often require UV-A light (320-400 nm) for their toxicity or other biological activities (Towers, G. H. N. and Champagne, D. E., 1988).

Kobayashi *et al.* reported that *cis*-DME could be extracted not only from the root of goldenrod but also from the surrounding soils. However, its isomer, *trans*-DME could only be found in the soils and no in vivo *trans*-DME has been found (Kobayashi, A. *et al.*, 1980). They also reported that both of the two isomers inhibited similarly on the growth of other plants (Kobayashi, A. *et al.*, 1980). In our study we found that *cis*-DME rapidly photoisomerized to its *trans* isomer when it was exposed to the light (Table 1), and the PGI activity was largely depending on the light. Although separate test with *trans* isomer was not conducted in our test, it is considered that *trans*-DME may play an equal role as its *cis* counterpart in the photo-activated or photo-enhanced bioactivity. When *cis*-DME is released as an exudate from the root of the goldenrod weed, or from the leaves dropped to the surrounding soil, it may be isomerized to *trans*-DME by such environmental factors as sunlight and soil microorganisms. This is supported by the above study of Kobayashi *et al.* that either *cis* and *trans*-DME be extracted from the surrounding soils of the plant, and verified by our study on the photo*trans*formation of *cis*-DME. As is discussed later, we think that the isomerization reaction itself may not be an important factor in the photo-activated PGI activity of *cis*-DME, but the photosensitization process of both isomers may play an important role.

Fig. 3 shows the inhibition of lettuce radicles and hypocotyls by *cis*-DME. The lengths of both parts of the lettuce seedling were markedly reduced even at the 5 ppm level. This growth inhibition of radicles and hypocotyls was observed under both light and dark conditions. However, *cis*-DME showed significantly stronger PGI effect on lettuce radicles and hypocotyls when there is light present. The photo-dependent effect was more obvious in lettuce hypocotyls than in radicles. In the dark experiment (Fig. 3b), *cis*-DME did not have any significant effect on

hypocotyls up to 20 ppm and only about 18% inhibition at 50 ppm. However, when the lettuce were incubated in the light, a 5 ppm *cis*-DME solution had 20% inhibition compared to the control (0%). This PGI effect was further enhanced when lettuce was exposed to higher concentrations of *cis*-DME. Hypocotyls of lettuce treated with 50 ppm *cis*-DME only had half the length of the control. The light effect on radicals was not as remarkable as on hypocotyls, however, the photo-dependent toxicity was still very clear (Fig. 3a). Radicle growth of the *cis*-DME treated and light incubated lettuce was inhibited at least 10% more than those dark incubated at any concentration tested (Fig. 3a). At 5 ppm, *cis*-DME inhibited the growth of radicles by 12% in dark and 29 % in light, and inhibition rates at 50 ppm were 24% in dark and 34% in light, respectively.

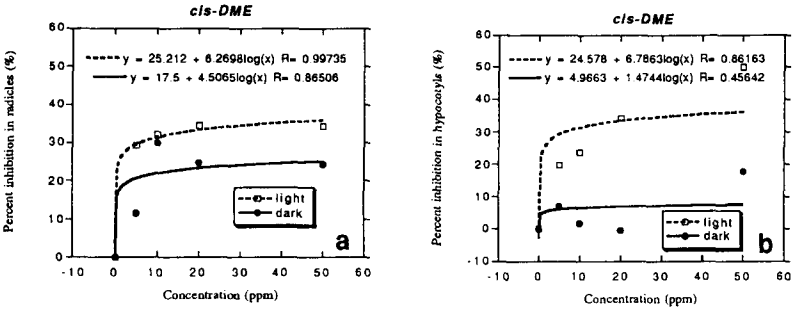

Fig. 3. Growth inhibitory effect of *cis*-DME on lettuce seedlings. The lengths of lettuce hypocotyls and radicles were measured on day 5. The inhibition rate was calculated as follows: % inhibition = [(length of control - length of treatment)/length of control]*100

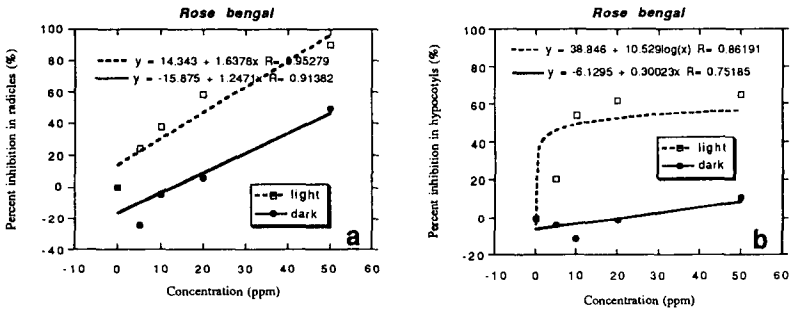

Fig. 4. Growth inhibitory effect of rose bengal on lettuce seedlings. The lengths of lettuce hypocotyls and radicles were measured on day 5. The inhibition rate was calculated as follows: % inhibition = [(length of control - length of treatment)/length of control]*100

Even stronger light-activated PGI effect was observed in rose bengal, i. e. significantly higher PGI effect on both radicles and hypocotyls; no significant effect was observed in dark in hypocotyls (Fig. 4). Being a known photo-dependent compound, rose bengal is a strong plant growth inhibitor (PGI) of lettuce seedlings. When there is light present, rose bengal inhibited almost 90% of the radicles and 65% of the hypocotyls at 50 ppm. Even at lower concentrations, this dye was a strong inhibitor of both radicles and hypocotyls. One interesting observation in rose bengal test is that at lower concentrations, the growth of treated lettuce seedling was actually enhanced in both radicles and hypocotyls (the negative inhibition) when incubated in dark (Fig. 4). At 5 ppm in the dark experiment, lettuce treated with rose bengal had the highest elongation effect (25%) in radicles; and 11% on the hypocotyls at 10 ppm.

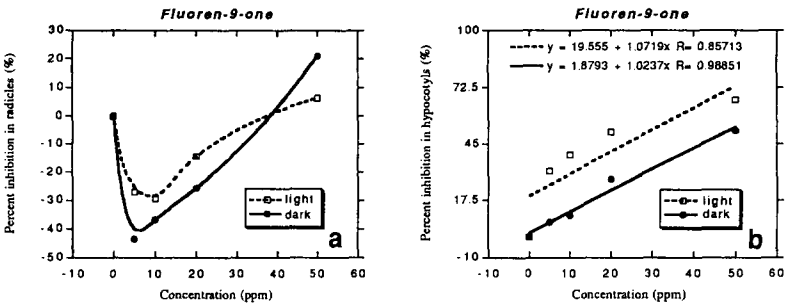

Fig. 5. Growth inhibitory effect of fluoren-9-one on lettuce seedlings. The lengths of lettuce hypocotyls and radicles were measured on day 5. The inhibition rate was calculated as follows: % inhibition = [(length of control - length of treatment)/length of control]*100

Photo-dependent effect on lettuce growth of fluoren-9-one was not as clear as in the *cis*-DME and rose bengal tests. The effect of fluoren-9-one was totally different on different plant parts. In the hypocotyls, the growth was inhibited and the inhibition rate was about 15% higher when light is present (Fig. 5b). However, the growth enhancement effect (the radicle or hypocotyl elongation effect) observed at lower concentrations in rose bengal test was even more significant in fluoren-9-one test. In this test, lettuce treated with 5-20 ppm fluoren-9-one strongly enhanced the growth of the radicles, whether in the presence of light or in the dark experiment. Radicles of lettuce treated with 10 ppm fluoren-9-one was about 30% longer than the blank control in the light, and about 45% longer in the dark when treated with 5 ppm fluoren-9-one (Fig. 5a). It has been reported that the carboxylic acid derivatives of fluoren-9-one have morphological effect to plants (Lejczak, B. *et al.*, 1985). Some morphological changes such as extra side roots and root hairs were also observed on fluoren-9-one treated lettuce seedlings in this experiment.

The reason that we used rose bengal and fluoren-9-one is to compare the PGI activity of *cis*-DME with the known photodynamic compounds. Rose bengal is a well-known compound that has strong photodynamic action on various organisms. Rose bengal is reported to have photo-dependent toxicities on plant tissues (Knox, I. P. and Dodge, A. D., 1984), some insects (Pimprikar, G. D. and Coign, M. J., 1987) and microorganisms (Bezman, S.

A. *et al.*, 1978). Its mode of action has been proven to produce singlet oxygen by light-excited rose bengal molecules (Rodgers, M. A. J., 1987; Pimprikar, G. D. and Coign, M. J., 1987). In this study, rose bengal showed the most typical photo-induced activity on the lettuce hypocotyls and radicles (Fig. 4). Rose bengal under light radiation showed not only the PGI activity but also some morphological effect. Lettuce treated with rose bengal at >50 ppm and incubated in light, stopped growth after germination, and the radicle of some seedlings treated with lower concentrations became rusty and the hypocotyls were bent. At the same time, another interesting effect was observed on these lettuce seedlings that one or two side roots or root hairs appeared from the joint of radicle and hypocotyl or the bottom of the rusty part, whereas no such morphological effect was observed in the control. Lettuce grown under the dark condition did not show any such morphological abnormality, even if treated with rose bengal. The morphological changes may be caused as a result of the reaction between the singlet oxygen and the cytoplasmic components in lettuce.

As stated earlier, photosensitization may be much more important a factor in *cis*-DME's PGI activity. Many polyacetylene compounds show photo-induced activity by producing singlet oxygen. These compounds usually have characteristic conjugated double or triple bonds in their molecules, and show a variety of biological activity to various insects, plants, and fungus (Kobayashi, A. *et al.*, 1974; Downum, K. R. *et al.*, 1982; Wat, C. K. *et al.*, 1980; McLanchlan, D., 1984; Arnason, J. T. *et al.*, 1987; Marchant, Y. Y. and Cooper, G. K., 1987; Towers, G. H. N. and Champagne, D. E., 1987). α-Terthienyl and phenylheptatriyne are probably the best examples of natural polyacetylene with photo-dependent activity. α-Terthienyl shows a wide range of activity against plants, fungus, bacterial and insects (Downum, K. R. *et al.*, 1982). Phenylheptatriyne has also a variety of toxicities. These two compounds as well as many other derivatives of them have been proved to be very efficient singlet-oxygen generators, and many of their activities depend on the presence of light (McLachlan, D. *et al.*, 1984). The conjugated system of *cis*- or *trans*-DME makes these special compounds capable of absorbing the ultraviolet and/or visual component of the light, thus readily be excited by light and consequently show various bioactivities themselves or act as a photosensitizer to "help" other molecules show those functions. Often, they can transfer the acquired energy to the molecules such as oxygen and water, producing very active radicals, singlet oxygen, superoxide anion, hydroperoxy radical, hydrogen peroxide, and hydroxyl radical (Arnason, T. *et al.*, 1981; Garcia F. J. *et al.*, 1984; McLachlan, D. *et al.*, 1984). These are very strong reactant which can react with nucleic acid, protein and other cytoplasmic components of the living organisms, and finally influence and threaten the life of them. Strong photosensitizers such as rose bengal thus cause sever physiological or morphological damages to the plants as shown in this study. Photo-induced toxicity mediated by active oxygen and other highly reactive states is also known as photodynamic action (Campbell, G. *et al.*, 1982; Kagan, J. *et al.*, 1984). *cis*-DME has been shown a possibility to produce singlet oxygen in vitro (Wat, C. -K. *et al.*, 1980, McLachlan, D., 1984), based on this fact and our result, it can be concluded that *cis*-DME may follow the same mechanism as that shown by rose bengal, α-terthienyl and phenylheptatriyne can be drown. That is, the mode of action of *cis*- and/or *trans*-DME is possibly mediated by an excited stage of oxygen, the singlet oxygen.

CONCLUSION

The ecological chemistry of natural products in the real ecosystem is very complicated. There are many ways for one particular plant to use its defense chemicals or allelochemicals to affect other organisms. As our results showed, *cis*-DME was not only a good plant growth inhibitor in the goldenrod, but its bioactivity to some extent depends on the presence of light, that is its biological activity is photo-dependent. Since sun light is an indispensable member of the ecosystem, radiation from sun light must play a very important role in the photo-dependent plant growth inhibitory activity of *cis*-DME.

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