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Original Article

Action Mechanism of DLH-1777, a Novel 4-Pyridone-3-carboxamide Herbicide: Peroxidizing Activity and Accumulation of Porphyrins*

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The mode of action of a 4-pyridone-3-carboxamide derivative, DLH-1777 [5-bromo-*N*-(2, 6-diethylphenyl)-1, 4-dihydro-1, 6-dimethyl-4-oxo-2-propyl-3-pyridinecarboxamide], was studied on cotyledons from etiolated or green seedlings of cucumber (*Cucumis sativus* L. cv Shimoshirazu-jibai) and compared with that of oxyfluorfen (2-chloro-4-trifluoromethylphenyl 3′-ethoxy-4′-nitrophenyl ether). The herbicides both caused the leakage of ninhydrin-positive amino acids in treated etiolated cotyledons exclusively in a light (200 μE $m^{-2} \cdot sec^{-1}$ PAR). Lipid peroxides were detected in the cotyledons treated with 10 μM DLH-1777 after 1-hr exposure to the light. DLH-1777 and oxyfluorfen both induced ethane production in green cotyledon discs. On the other hand, gabaculine (3-amino-2, 3-dihydrobenzoic acid), a porphyrin biosynthesis inhibitor, markedly alleviated the amino acid leakage caused by DLH-1777 or oxyfluorfen. DLH-1777 as well as oxyfluorfen induced the accumulation of protoporphyrin IX, a photosensitizing porphyrin, which is believed to cause light-dependent membrane distruction. These results suggest that DLH-1777 belongs to the same group as photobleaching and peroxidizing herbicides such as oxyfluorfen and oxadiazon as far as the mechanism is concerned, and that it is phytotoxic through inducing abnormal accumulation of protoporphyrin IX in treated tissues by interfering their porphyrin biosynthesis.

INTRODUCTION

DLH-1777 [5-bromo-*N*-(2,6-diethylphenyl)-1,4-dihydro-1,6-dimethyl-4-oxo-2-propyl-3-pyridinecarboxamide], a novel 4-pyridone-3-carboxamide derivative, is a herbicide that is highly active against a wide range of upland weeds and safe to corn by pre- and post-emergence treatment[1] (Table 1). DLH-1777 absolutely requires light for its action and in light it causes rapid bleaching and necrosis in treated plants,[1] which are the common symptoms of photobleaching diphenylether (DPE) type herbicides.[2,3]

Carotenoid pigments have been claimed to be photoreceptors for such light-dependent activity of DPE herbicides.[2,4,5] However, studies of action spectra on various plants have indicated that red light that cannot be absorbed by carotenoid pigments is effective in the action of acifluorfen and the cyclic imide S-23142.[6-9] On the other hand, the action mechanisms of acifluorfen and oxyfluorfen are dependent on the production of singlet oxygen[11,12] that peroxidizes lipids under light.[5,10] According to recent studies,[13-17] a series of these photobleaching and peroxidizing herbicidal actions is due to abnormal accumulation of photosensitizing porphyrins, especially protoporphyrin IX (Proto).

In the present paper, we have tried to confirm the effects of DLH-1777 on 1) membrane disruption by lipid peroxidation, 2) accumulation of porphyrins in cucumber

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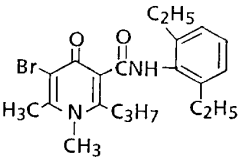
Table 1 Physical properties and toxicity of DLH-1777.

Chemical structure:	
Molecular weight:	419.37
Physical state:	White crystalline solid
Melting point (°C):	184.5–185.5
Water solubility (28°C):	21 ppm
Toxicity:	Acute oral LD_{50} for mouse; >5000 mg/kg, Mutagenicity; negative

cotyledons. The mode of action of DLH-1777 is also compared with that of oxyfluorfen.

MATERIALS AND METHODS

1. *Plant Material*

Cucumber seeds (*Cucumis sativus* L. cv Shimoshirazu-jibai) were obtained from Takii Seed Co., Ltd. (Kyoto). They were seeded in pots with vermiculite, watered with distilled water (DW) and grown at 28°C in the dark for 5 days or with 15-hr daylength at an intensity of 50 μE $m^{-2}\cdot sec^{-1}$ PAR for 7 days. The etiolated and green cotyledons were excised and used for subsequent experiments.

2. *Leakage of Amino Acids*

Twenty etiolated cotyledons were treated with 20 ml of a buffer medium [50 mM HEPES (pH 7.2), 0.5 mM $CaSO_4$] containing 10 μM DLH-1777 or oxyfluorfen with or without 500 μM gabaculine in a glass vial in the dark for 16 hr. Six cotyledons selected from each treatment vial were plunged into 5 ml of DW and incubated with gentle shaking at 28°C in the dark for 6 hr or in a white fluorescent light (200 μE $m^{-2}\cdot sec^{-1}$ PAR) for 1 to 8 hr. A 2-ml portion of the bathing solution was sampled for the determination of amino acids. The amount of amino acids leaked from the cotyledons was determined through a colorimetric reaction with ninhydrin according to the method of Yemm & Cocking.[18] From the beginning of herbicide treatment to the end of sampling of amino acid solution, all procedures were conducted under a dim green light (0.02 μE $m^{-2}\cdot sec^{-1}$ PAR).

3. *Ethane Production*

Ethane production in submerged cucumber leaf discs was determined by the method of Sato *et al.*[19] with slight modifications. Leaf discs (4 mm in diameter) of green cotyledons were prepared with a brass cork borer and submerged in the buffer medium by vacuum infiltration. Forty leaf discs were treated with 5 ml of the buffer medium containing DLH-1777 or oxyfluorfen in a glass vial (10 ml volume). The vials were sealed with rubber stoppers and incubated in a white fluorescent light (267 μE $m^{-2}\cdot sec^{-1}$ PAR) at 28°C for 28 hr. One milliliter of headspace gas was sampled from the vial and injected into a gas chromatograph with a flame ionization detector (Shimadzu, Model GC-R1A). A stainless-steel column (3$\phi\times$2 m) filled with activated alumina (60–80 mesh) was used with a helium carrier gas flow rate of 50 ml/min. The injector temperature was 150°C and the column temperature was 70°C. The relative peak area of ethane gas was calculated from ethane standard (Gaskuro Kogyo Co., Ltd.).

4. *Lipid Peroxides Determination*

The same etiolated cotyledons treated with 10 μM DLH-1777 in the amino acid leakage experiment were excised and placed on filter paper with DW in a petri dish (12 cm in diameter). The petri dishes were placed in a white fluorescent light (200 μE $m^{-2}\cdot sec^{-1}$ PAR) at 25°C for 1 to 6 hr. Eight cotyledons were homogenized with 6 ml of the buffer medium and centrifuged at 2000$\times g$ for 5 min. The supernatant was diluted to one fifth of the original concentration and a 1-ml portion of this solution was used for the determination of the amount of lipid peroxides according to the coloration method of Kanazawa *et al.*[20] with Determiner® LPO (Kyowa Medix Co., Ltd.).

5. *Porphyrin Analysis*

Porphyrin pigments were extracted according the method of Duggan & Gassman.[21] Twenty etiolated cotyledons were put in 15 ml of the buffer medium containing 10 μM DLH-1777 or oxyfluorfen and incubated at 25°C in the dark for 3 to 8 hr. The cotyledons were homogenized in 6 ml of acetone/0.1 M NH_4OH

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(9/1, v/v). From the resulting acetone extract lipoproteins and cell debris were removed by centrifugation at 30,000×*g* at 0°C for 20 min. Carotenoids were removed from the aqueous acetone solution by extraction with hexane. The hexane-washed acetone layer was analyzed with a fluorescense spectrophotometer (Hitachi, Model F-3000). All extractions were conducted under a dim green light (0.02 μE $m^{-2}\cdot sec^{-1}$ PAR).

6. Chemicals

Chemicals were dissolved in acetone and kept as stock solutions. Final concentrations of the solvent were below 0.1% (v/v). In all cases, controls were treated with the same amounts of the solvent as in the assay. DLH-1777 [5-bromo-*N*-(2,6-diethylphenyl)-1,4-dihydro-1, 6-dimethyl-4-oxo-2-propyl-3-pyridinecarboxamide] was synthesized. Oxyfluorfen (2-chloro-4-trifluoromethylphenyl 3′-ethoxy-4′-nitrophenyl ether) was purified from commercial emulsified liquid formulation. Gabaculine hydrochloride and protoporphyrin IX were obtained from Sigma Chemical, St. Louis, Mo., USA.

RESULTS AND DISCUSSION

Since solute leakage is one of the characteristics and quantitatively measurable parameters of phytotoxicity induced by photobleaching herbicides,[5,9] the leakage of ninhydrin-positive amino acids from etiolated cucumber cotyledons was measured. Etiolated cotyledons from cucumber seedlings grown in the dark for 5 days were treated with 10 μM DLH-1777 and oxyfluorfen and incubated for 16 hr in the dark before irradiation so that the herbicides could be incorporated into cotyledons adequately. Preliminary experiments showed that the effects of DLH-1777 and oxyfluorfen on amino acid leakage reached maximum at 10 μM (data not shown). Both DLH-1777 and oxyfluorfen markedly increased amino acid leakage from cotyledons as incubation time elapsed (Fig. 1). In the dark, however, there was no virtual amino acid leakage from the treated cotyledons (Table 2). These results demonstrated that the solute leakage of DLH-1777 as well as the herbicidal property was totally light-dependent.

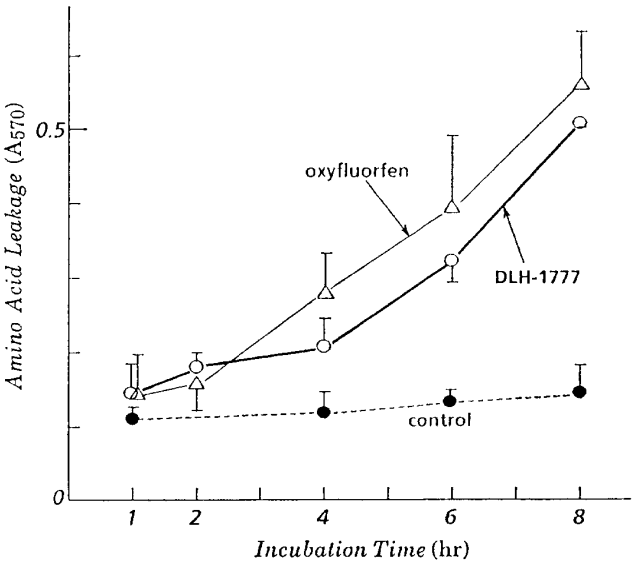

Fig. 1 Time course of amino acid leakage from cotyledons of 5-day-old etiolated cucumber seedlings.

Cotyledons were treated with 10 μM of DLH-1777 (○) and oxyfluorfen (△) for 16 hr in the dark and incubated under a white light (200 μE $m^{-2}\cdot sec^{-1}$ PAR) at 28°C. Closed circle (●) means untreated control. Absorbance of the bathing solution was read at 570 nm (A_{570}). Bars are standard errors.

Table 2 Effect of 10 μM DLH-1777 and oxyfluorfen on amino acid leakage from cotyledons of 5-day-old etiolated cucumber seedlings in the dark.

	Control	DLH-1777	Oxyfluorfen
Amino acid leakage (A_{570})	0.105 (±0.011)	0.100 (±0.023)	0.108 (±0.010)

Cotyledons were treated with the herbicides for 16 hr and incubated for 6 hr in the dark. Absorbance of the bathing solution was read at 570 nm (A_{570}).

Many studies[19,22,23] have shown that ethane, a decomposition product of unsaturated lipid hydroperoxides, is a useful index of *in vivo* lipid peroxidation in the tissues treated with peroxidizing herbicides. We measured the ethane production in submerged green leaf discs to evidence of membrane distruction in green-developed tissues. Sato *et al.*[19] have reported that ethane production in green cucumber discs treated with 0.3 μM S-23142, a

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potent peroxidizing herbicide, reached maximum 20 to 30 hr after treatment under a white light. In our preliminary experiment, however, 10 μM DLH-1777 caused no significant ethane production in green cucumber cotyledon discs after 10-hr of incubation (data not shown), so we increased the incubation time under light to 28 hr. Both DLH-1777 and oxyfluorfen induced ethane production in the discs, but DLH-1777 was less active and required higher concentrations to reach the maximum level of effect than oxyfluorfen (Fig. 2). However, 10 μM DLH-1777 and oxyfluorfen showed no significant difference in activating amino acid leakage from etiolated cotyledons (Fig. 1). These results indicated that yellow, achlorophyllous tissues were more sensitive to DLH-1777 than green-developed tissues. They may explain the higher herbicidal property of DLH-1777 by pre-emergence than post-emergence application.[1]

In the presence of O_2, photobleaching DPE herbicides such as oxyfluorfen and acifluorfen produce lipid peroxides in the treated tissues, which cause membrane distruction leading to solute leakage of amino acids and ethane production.[5,7,23] We quantitatively determined lipid peroxides in etiolated cucumber cotyledons. Lipid peroxides were detected in the DLH-1777-treated cotyledons after 1-hr of exposure to light, but not in the control (Fig. 3). As shown in Fig. 1, leakage of amino acids was significantly enhanced 2 to 4 hr after DLH-1777 treatment. These results suggested that lipid peroxides was produced first, followed membrane distruction and leakage of amino acids.

Matringe & Scalla,[13] and Lydon & Duke[15] have found that porphyrin synthesis inhibitors, 4,6-dioxoheptanoic acid and gabaculine, completely eliminate the solute leakage activity of acifluorfen-methyl in achlorophyllous and green-developed tissues. Gabaculine inhibits porphyrin synthesis by inhibiting the biosynthesis of 5-aminolevulinic acid.[24] Aiming to confirm the protective effect of gabaculine, we examined the leakage of amino acids from the etiolated cotyledons treated with DLH-1777 and oxyfluorfen in the presence of gabaculine. The amino acid leakage caused by DLH-1777 or oxyfluorfen was markedly alleviated by gabaculine (Fig. 4), which indicated the possibility that porphyrin(s) played a role in the light-activated phytotoxicity of DLH-1777 in the same manner as peroxidizing DPE herbicides.

Porphyrin extractions were made after 3 to 8 hr incubation with 10 μM DLH-1777 and oxyfluorfen in the dark and the extracts were

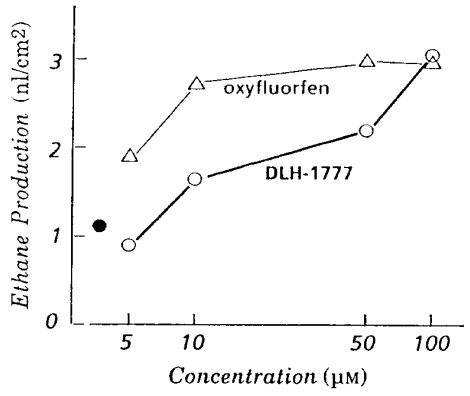

Fig. 2 Effects of DLH-1777 (○) and oxyfluorfen (△) on the ethane production in cotyledon disks of 7-day-old green cucumber seedlings under a white light (267 μE m^{-2}·sec^{-1} PAR) at 28°C for 28 hr.

Closed circle (●) means untreated control.

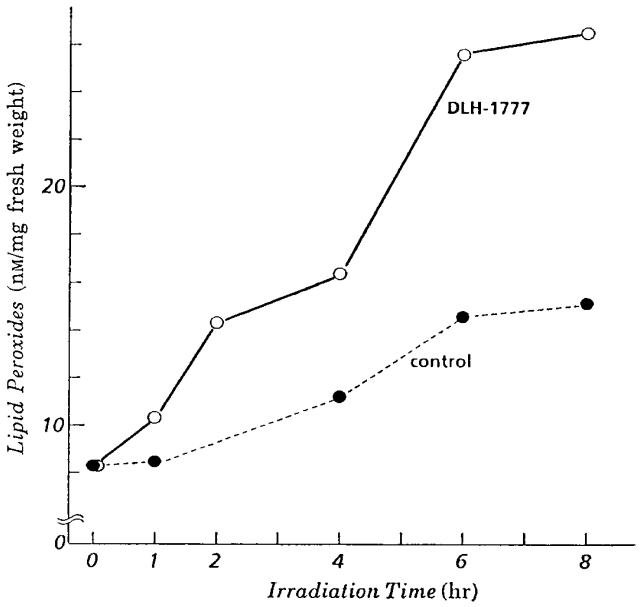

Fig. 3 Effect of DLH-1777 on lipid peroxidation in cotyledons of 5-day-old etiolated cucumber seedlings.

Cotyledons were treated with 10 μM of DLH-1777 for 16 hr in the dark and irradiated with a white light (200 μE m^{-2}·sec^{-1} PAR) at 25°C.

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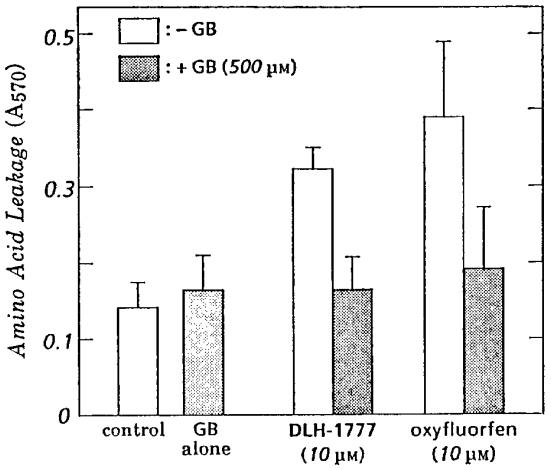

Fig. 4 Effect of 500 μM gabaculine (GB: 3-amino-2,3-dihydrobenzoic acid) on the activity of DLH-1777 and oxyfluorfen as expressed by amino acid leakage from cotyledons of 5-day-old etiolated cucumber seedlings.

Cotyledons were treated with the herbicides for 16 hr in the dark and incubated under a white light (200 μE $m^{-2} \cdot sec^{-1}$ PAR) at 28°C for 6 hr. Absorbance of the bathing solution was read at 570 nm (A_{570}). Bars are standard errors.

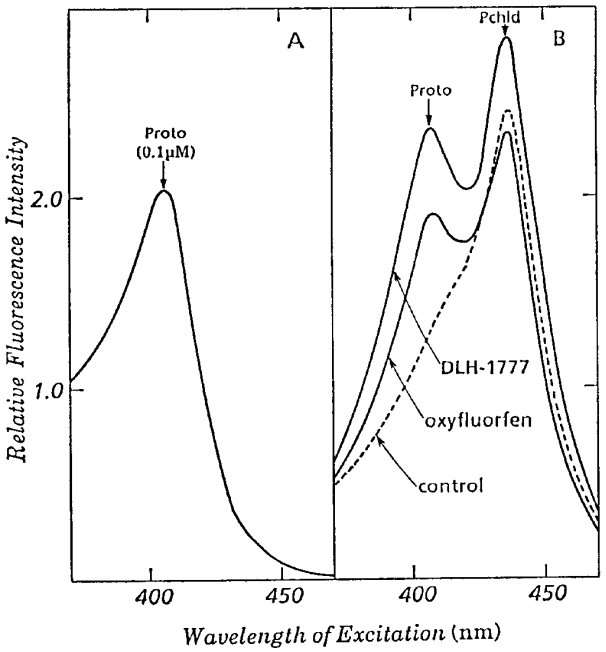

Fig. 5 Fluorescence excitation spectra (emission wavelength: 633 nm) of protoporphyrin IX (Proto) standard (A) and the extracts of etiolated cucumber cotyledons (B).

Cotyledons were treated with 10 μM of DLH-1777 and oxyfluorfen for 8 hr in the dark and extracted with acetone/0.1 M NH_4OH (9/1, v/v).

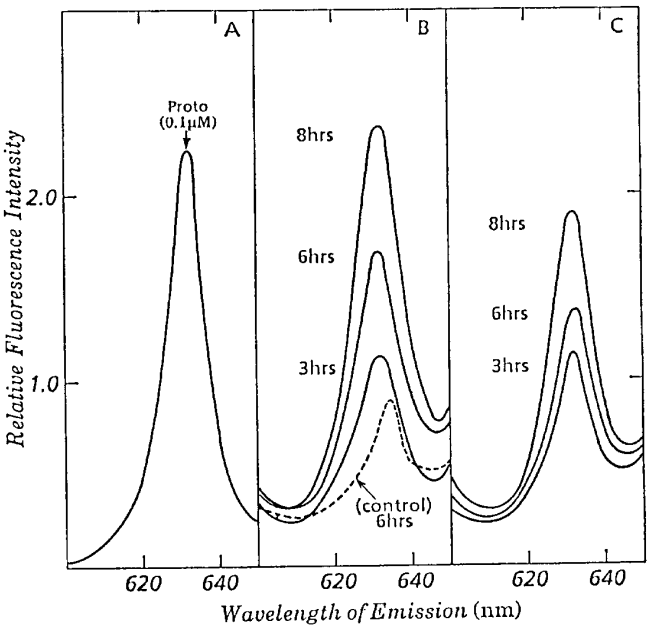

Fig. 6 Fluorescence emission spectra (excitation wavelength: 405 nm) of protoporphyrin IX (Proto) standard (A) and the extracts of etiolated cucumber cotyledons (B) and (C).

Cotyledons were treated with 10 μM of DLH-1777 (B) and oxyfluorfen (C) for 3, 6 and 8 hr in the dark and extracted with acetone/0.1 M NH_4OH (9/1, v/v).

spectrofluorometrically examined. At an emission wavelength of 633 nm, the excitation spectrum of the control acetone extract had a major peak of protochlorophyllide (Pchld) at about 438 nm and a shoulder at about 405 nm (Fig. 5B). By contrast, the samples treated with DLH-1777 and oxyfluorfen for 8 hr had a marked peak at about 405 nm (Fig. 5B), which closely matched the fluorescence spectrum of a Proto standard (Fig. 5A). Pchld was detected at nearly the control level in treatments with both DLH-1777 and oxyfluorfen. In the emission spectra (excitation wavelength: 405 nm), the levels of accumulation of Proto in the cotyledons treated with the herbicides markedly increased with time (Fig. 6). The effect of DLH-1777 on porphyrin synthesis was the same as that observed with oxyfluorfen.

The results of our present study indicate that DLH-1777 has a mode of action consistent with the recent model,[13-17] and that the herbicidal property of light-dependent, bleaching and peroxidizing herbicides comes from their ability to induce the accumulation of Proto, a porphyrin, in treated plants. It is

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believed that Proto, under light, induces the formation of singlet oxygen, which strongly oxidizes membrane lipids[5,10-12] and causes rapid bleaching by triggering oxidative photodestruction of chloroplast membranes and pigments.[25,26] Not only DPE herbicides such as acifluorfen[13-17] and oxyfluorfen[17,27,28] but non-DPE herbicides observed to act in a similar fashion to DPEs (heterocyclic 5-membered ring compounds such as oxadiazon,[29] chlorphthalim,[17,27,28] S-23142,[30] and TNPP-ethyl[31] and the pyridine derivative LS 82-556[13] that relatively resemble DLH-1777 in structure) have been reported to cause high levels of Proto accumulation in the treated plants. However, common structure-activity relationships between these diverse herbicide groups have not been determined.

Nicolaus *et al.*[28] have reported that photosynthesis inhibition by diuron and anaerobic condition by nitrogen atmosphere reduced the production of Proto in *Scenedesmus* caused by peroxidizing herbicides, because photosynthesis or respiration provided NADPH, ATP and carbon skeleton necessary for the buildup of Proto. Masuda *et al.*[32] have said that Proto accumulation caused by acifluorfen-methyl is due to the stimulation of 5-aminolevulinic acid synthesis induced by the decrease of heme content that seems to accelate the accumulation of photosensitizing tetrapyrroles. In any event, Proto is considered responsible for all or major part of the photobleaching activity of acifluorfen and other related photobleaching and peroxidizing herbicides.[33]

Recently Matringe *et al.*[34] have shown that acifluorfen-methyl strongly inhibits protoporphyrinogen IX oxidase that catalyzes the transformation of protoporphyrinogen IX to Proto. They have also suggested that Proto accumulation is caused by the autooxidation of accumulated protoporphyrinogen IX. Matringe *et al.*[35] have reported that this type of herbicidal mode of action was exerted by non-DPE peroxidizing herbicides such as oxadiazon, LS 82-556 and M & B 39279.[36] We think that DLH-1777 shares the major part of such a mechanism.

More detailed studies on conformation and electrostatic analysis of compounds that have the same mechanism seem necessary to define the activities of light-dependent, bleaching and peroxidizing herbicides from the viewpoint of structure.

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REFERENCES

1) Y. Morishima, H. Osabe, Y. Goto, K. Masamoto & H. Yagihara: *Weed Res. Jpn.* **35**, 282 (1990) (in Japanese)
2) S. Matsunaka: *J. Agric. Food Chem.* **17**, 171 (1969)
3) K. Wakabayashi, K. Matsuya, H. Ohta & T. Jikihara: "Advances in Pesticide Science," ed. by H. Geissbuhler, Part 2, Pergamon, Oxford, pp. 256–260, 1979
4) O. Fadayomi & G. F. Warren: *Weed Sci.* **27**, 598 (1976)
5) G. L. Orr & F. D. Hess: *Plant Physiol.* **69**, 502 (1982)
6) D. E. Vanstone & E. H. Stobbe: *Weed Sci.* **27**, 88 (1979)
7) P. Böger: *Z. Naturforsch. C* **39**, 468 (1984)
8) M. P. Ensminger & F. D. Hess: *Plant Physiol.* **77**, 503 (1985)
9) R. Sato, E. Nagano, H. Oshio, K. Kamoshita & M. Furuya: *Plant Physiol.* **85**, 1146 (1987)
10) K. J. Kunert & P. Böger: *Weed Sci.* **29**, 169 (1981)
11) S. O. Duke & W. H. Kenyon: *Z. Naturforsch. C* **42**, 813 (1987)
12) P. Haworth & F. D. Hess: *Plant Physiol.* **86**, 672 (1988)
13) M. Matringe & R. Scalla: *Plant Physiol.* **86**, 619 (1988)
14) D. A. Witkowski & B. P. Halling: *Plant Physiol.* **87**, 632 (1988)
15) J. Lydon & S. O. Duke: *Pestic. Biochem. Physiol.* **31**, 74 (1988)
16) H. Kouji, T. Masuda & S. Matsunaka: *J. Pesticide Sci.* **13**, 495 (1988)
17) G. Sandmann & P. Böger: *Z. Naturforsch. C* **43**, 699 (1988)
18) E. W. Yemm & E. C. Cocking: *Analyst* **80**, 209 (1955)
19) R. Sato, E. Nagano, H. Oshio & K. Kamoshita: *Pestic. Biochem. Physiol.* **28**, 194 (1987)
20) K. Kanazawa, S. Miyamoto, H. Ashida, K. Yamada, G. Danno & M. Natake: *Agric. Biol.*

NII-Electronic Library Service

Pesticide Science Society of Japan

Chem. **49**, 2799 (1985)
21) J. Duggan & M. Gassman: *Plant Physiol.* **53**, 206 (1974)
22) R. Lambert, G. Sandmann & P. Böger: *Pestic. Biochem. Physiol.* **19**, 309 (1983)
23) K. J. Kunert: *Z. Naturforsch. C* **39**, 476 (1984)
24) C. M. Hill, S. A. Pearson, A. J. Smith & L. J. Rogers: *Biosci. Rep.* **5**, 775 (1985)
25) W. H. Kenyon, S. O. Duke & K. C. Vaughn: *Pestic. Biochem. Physiol.* **24**, 240 (1985)
26) G. L. Orr & F. D. Hess: *Am. Chem. Soc. Symp. Ser.* **181**, 131 (1982)
27) K. Wakabayashi, G. Sandmann, H. Ohta & P. Böger: *J. Pesticide Sci.* **13**, 461 (1988)
28) B. Nicolaus, G. Sandmann, H. Watanabe, K. Wakabayashi & P. Böger: *Pestic. Biochem. Physiol.* **35**, 192 (1989)
29) S. O. Duke, J. Lydon & R. N. Paul: *Weed Sci.* **37**, 152 (1989)
30) R. Sato, H. Oshio, H. Koike, Y. Inoue, S. Yoshida & N. Takahashi: Proc. 1988 Conf. The Society for Chemical Regulation of Plants, p. 85, 1988 (in Japanese)
31) D. Yanase & A. Andoh: *Pestic. Biochem. Physiol.* **35**, 70 (1989)
32) T. Masuda, H. Kouji & S. Matsunaka: *Pestic. Biochem. Physiol.* **36**, 106 (1990)
33) J. M. Becerril & S. O. Duke: *Plant Physiol.* **90**, 1175 (1989)
34) M. Martringe, J. M. Camadro, P. Labbe & R. Scalla: *Biochem. J.* **260**, 231 (1989)
35) M. Matringe, J. M. Camadro, P. Labbe & R. Scalla: *FEBS Lett.* **245**, 35 (1989)
36) P. M. Derrick, A. H. Cobb & K. E. Pallett: *Pestic. Biochem. Physiol.* **32**, 153 (1988)

要　　約

新規 4-ピリドン-3-カルボン酸アミド系除草剤，DLH-1777 の作用機構：膜脂質過酸化活性とポルフィリン類の生体内蓄積

森島靖雄，長部広和，後藤幸久

光要求性除草活性を有する DLH-1777 の作用機構を，キュウリ子葉を用いて調べた．DLH-1777 は，oxyfluorfen と同様に 10 μM 処理によって，光の照射下で黄化子葉からのアミノ酸の漏出を顕著に増加させ，過酸化脂質を蓄積した．また緑化子葉への処理によって，リーフ・ディスクからのエタンの発生を誘起した．したがって DLH-1777 は，膜脂質の過酸化によって生体膜を損傷させていることが示唆された．一方，ポルフィリン生合成阻害剤である gabaculine は，DLH-1777 や oxyfluorfen の黄化子葉からのアミノ酸の漏出活性を低下させたが，除草剤のみを処理した子葉中には protoporphyrin IX の異常蓄積が認められ，その量が経時的に増加することが観察された．以上のことから DLH-1777 は，acifluorfen や oxyfluorfen，あるいは oxadiazon などの光要求性除草剤と共通の作用機構を有しており，植物体内に光増感過酸化物質である protoporphyrin IX を異常に蓄積させることによって，一連の膜脂質の過酸化反応を誘起し除草活性を発現するものと推察された．

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