

## Original Article

# The Relationship between the Bleaching Pattern on Radish Cotyledons and Hydrophobicity of Herbicidal 3-Phenoxybenzamides

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When herbicidal 3-phenoxybenzamides were applied to radish seeds, the expanded cotyledons were bleached to different extents. The bleached states of cotyledons were classified into three patterns, A, B and C according to the extent of bleaching. Compounds with lower hydrophobicity ( $\log K_{ow}$  2.57–3.89,  $K_{ow}$ : octanol/water partition coefficient) caused total bleaching of both cotyledons (pattern A), while more hydrophobic compounds bleached only partially: one cotyledon was bleached completely and the other only at the marginal area (pattern B,  $\log K_{ow}$  4.35, 4.43) or both cotyledons were bleached partially (pattern C,  $\log K_{ow}$  4.78, 5.01). It was suggested that the bleaching patterns varied according to the difference in mobility due to hydrophobicity.

## INTRODUCTION

Carotenoids, having poly-conjugated double bonds in the molecule, are one of the major constituents of photosynthetic pigments.<sup>1)</sup> One of the most important roles of carotenoids in chloroplasts is to protect photosynthetic apparatus from destruction by singlet oxygen ( $^1O_2$ ), which is produced through energy transfer from triplet state chlorophyll ( $^3Chl$ ).<sup>2)</sup> Accumulation of  $^1O_2$  damages living cells to an extremely harmful extent, such as destruction of chlorophyll and breaks down cellular membranes.<sup>3)</sup> Carotenoids with poly-conjugated double bonds quench both  $^3Chl$  and  $^1O_2$ .<sup>2)</sup>

A number of herbicides have been developed which interfere with carotenoid biosynthesis, especially at the level of phytoene desaturation.<sup>4)</sup> Plant bodies are bleached when the chlorophyll is destroyed, due to a sharp decrease of carotenoids. 3-Phenoxybenzamides are a series of such herbicidal compounds which inhibit phytoene desaturase.<sup>5,6)</sup>

When radish seeds were treated with 3-phenoxybenzamides, the expanded cotyledons showed three different bleaching patterns. We report in this paper the relationship between the bleaching patterns of cotyledons and the hydrophobicity of compounds.

## MATERIALS AND METHODS

### 1. Chemicals

All melting points (mp) are uncorrected.

3-Phenoxybenzamide derivatives were synthesized by the method of Noguchi *et al.*<sup>7)</sup> For example, compounds **1** and **9** were synthesized as follows. 3-Phenoxybenzoic acid 10.71 g (50.0 mmol) was dissolved in thionyl chloride 29.74 g (250.0 mmol), dry pyridine 0.40 g (5.0 mmol) and benzene 200.0 ml. After this was stirred at room temperature for 10 hr, excess thionyl chloride was removed. Distillation of the resulting residue *in vacuo* yielded acid chloride. 3-Phenoxybenzoyl chloride 6.98 g (30.0 mmol) was dissolved in AcOEt 5.0 ml, and this solution was added dropwise to a stirred mixture of methylamine 7.76 g (100.0

mmol, 40% aq. solution) and AcOEt 30.0 ml under ice-water cooling, which was stirred at room temperature for another 1 hr. Then the reaction mixture was washed with 1.0 N HCl, saturated aq. NaHCO<sub>3</sub> and water, successively, and dried over anhyd. MgSO<sub>4</sub>. Evaporation of the solvent yielded a crude brown solid. Recrystallization from AcOEt-hexane gave 9.78 g of *N*-methylamide **1** as white crystals (86.1%, yield from 3-phenoxybenzoic acid), mp 101°C. <sup>1</sup>H NMR δ<sub>TMS</sub><sup>CDCl<sub>3</sub></sup> ppm: 2.97 (3H, d, *J*=5.0 Hz), 6.00 (1H, brs), 6.86–7.46 (9H, m).

NaH 0.32 g (8.0 mmol, 60% in oil) was added to a dry THF 10.0 ml solution of **1** 1.14 g (5.0 mmol), and stirred at room temperature under an N<sub>2</sub> atmosphere for 2 hr. 1-Iodopentane 1.03 g (5.2 mmol) was added to the mixture and stirred for another 3 hr. The reaction mixture was then poured into water and the organic layer was extracted with AcOEt. This resulting AcOEt layer was dried over anhyd. MgSO<sub>4</sub> and concentrated *in vacuo*. Purification of the resulting crude material by silica gel column chromatography (AcOEt-hexane as eluent) gave 0.54 g (44.8% yield) of *N*-methyl-*N*-pentylamide **9** as a colorless oil. *n*<sub>D</sub><sup>23.5</sup> 1.5534. <sup>1</sup>H NMR δ<sub>TMS</sub><sup>CDCl<sub>3</sub></sup> ppm: 0.85 (3H, brt), 0.98–1.74 (6H, m), 2.96 (3H, s), 3.00–3.60 (2H, m), 6.86–7.46 (9H, m).

Purity of each compound was more than

99% as measured by HPLC analysis. Structures and physical properties of the synthesized compounds are listed in Table 1.

## 2. Bioassay

Each test compound was dissolved in 0.5% aq. DMSO at a concentration of 1600 μM. About 100 radish seeds (*Raphanus sativus* cv. Shirokubi-natsudaikon) were soaked in the test solution for 24 hr, sown in pots filled with soil and grown at 30°C under continuous light (30,000 lux). The cotyledons were observed after 5 days when they had fully expanded.

## 3. Measurement of log *K*<sub>ow</sub>

Log *K*<sub>ow</sub> values were measured by the flask shaking method<sup>9)</sup> except for highly hydrophobic compound **10** (see Table 3).

The log *K*<sub>ow</sub> value of compound **10** was estimated by HPLC method (Sumipax ODS A-217 column, 4.6 mm i.d. × 150 mm, 70% aq. MeOH as eluent, flow rate 1.0 ml/min).<sup>9)</sup> The following equation was obtained:

$$\log K_{ow} = 2.59 \log k' + 1.32 \quad (1)$$

$$k' = (t_R - t_0)/t_0, r = 0.999$$

where *t*<sub>R</sub> was the retention time of compounds **5–9** and *t*<sub>0</sub> was the retention time of KI.

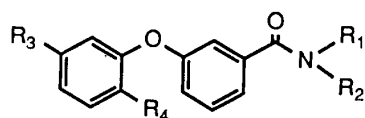
The log *K*<sub>ow</sub> value of **10** was estimated by Eq. (1).

## RESULTS AND DISCUSSION

A radish seed has two cotyledons, large and small, and the small cotyledon is covered by the large one. As shown in Fig. 1, the two cotyledons grew to almost the same size 5 days after germination. There are four leaf surfaces in a young seedling, two adaxial and abaxial surfaces of each of the large and small cotyledons.

When 3-phenoxybenzamide derivatives (**1–10**) were applied to radish seeds, bleaching on each leaf surface was different. We divided the states of cotyledons into three patterns, A, B and C according to whether the whole or marginal area was bleached for each leaf surface. Figure 1 shows untreated leaves and typical bleaching patterns A, B and C. The bleaching states of four leaf surfaces by 3-phenoxybenzamides are summarized in Table 2.

Table 1 Structures and physical properties of 3-phenoxybenzamide derivatives.



Compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	mp (°C)/ <i>n</i> <sub>D</sub> <sup>23.5</sup>
<b>1</b>	CH <sub>3</sub>	H	H	H	101 <sup>a)</sup>
<b>2</b>	CH <sub>3</sub>	H	CH <sub>3</sub>	CH <sub>3</sub>	91
<b>3</b>	iso-C <sub>3</sub> H <sub>7</sub>	H	CH <sub>3</sub>	CH <sub>3</sub>	111
<b>4</b>	tert-C <sub>4</sub> H <sub>9</sub>	H	CH <sub>3</sub>	CH <sub>3</sub>	89
<b>5</b>	CH <sub>3</sub>	CH <sub>3</sub>	H	H	1.5534
<b>6</b>	C <sub>2</sub> H <sub>5</sub>	CH <sub>3</sub>	H	H	1.5739
<b>7</b>	<i>n</i> -C <sub>3</sub> H <sub>7</sub>	CH <sub>3</sub>	H	H	1.5655
<b>8</b>	<i>n</i> -C <sub>4</sub> H <sub>9</sub>	CH <sub>3</sub>	H	H	1.5605
<b>9</b>	<i>n</i> -C <sub>5</sub> H <sub>11</sub>	CH <sub>3</sub>	H	H	1.5534
<b>10</b>	<i>n</i> -C <sub>6</sub> H <sub>13</sub>	CH <sub>3</sub>	H	H	1.5485

<sup>a)</sup> mp were uncorrected.

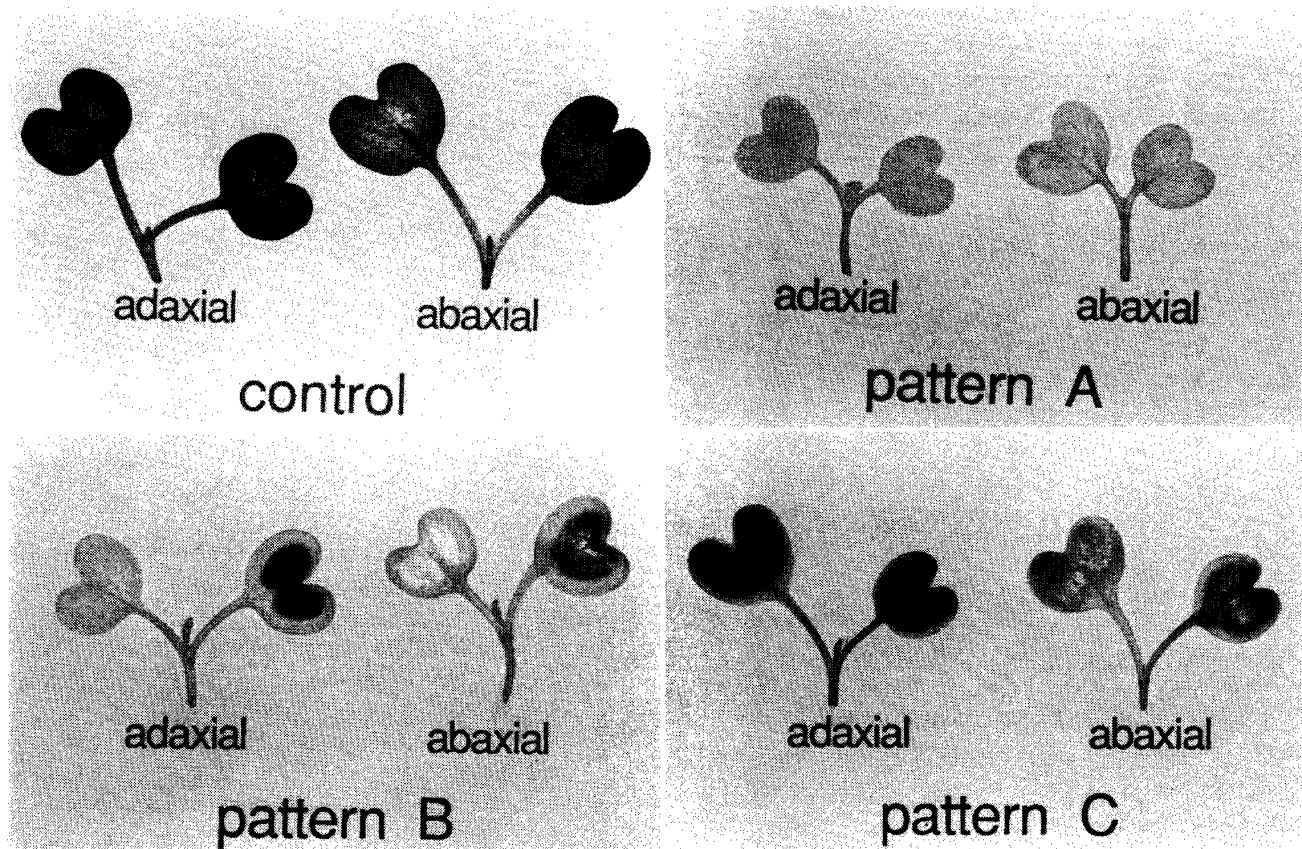


Fig. 1 Typical bleaching patterns of treated and untreated radish cotyledons.

Pattern A: treated with compound **8**, Pattern B: treated with compound **9**, Pattern C: treated with compound **10**.

Left: large cotyledon, Right: small cotyledon.

Table 2 Bleaching patterns of radish (*Raphanus sativus* cv. Shirokubi-natsudaikon) cotyledons.<sup>a)</sup>

Pattern	Large cotyledon		Small cotyledon	
	Ad. <sup>b)</sup>	Ab. <sup>c)</sup>	Ad.	Ab.
A	W <sup>d)</sup>	W	W	W
B	W	W	M <sup>e)</sup>	M
C	M	W	M	M

<sup>a)</sup> Radish seeds that imbibed a test solution. Bleaching pattern of cotyledons was observed 5 days after imbibition.

<sup>b)</sup> Adaxial surface.

<sup>c)</sup> Abaxial surface.

<sup>d)</sup> Whole surface bleached.

<sup>e)</sup> Only marginal area bleached.

Pattern A: Cotyledons were completely bleached.

Pattern B: The large cotyledon was bleached completely but the small cotyledon marginally.

Pattern C: Cotyledon's adaxial surfaces were both bleached marginally, but the abaxial surface of the large cotyledon was bleached completely and the small cotyledon only marginally.

Table 3 shows compounds in order of increasing  $\log K_{ow}$  values and with bleaching patterns of cotyledons. The  $\log K_{ow}$  values of these amides varies from 2.57 to 5.01. Compounds with pattern A (**1**, **2**, **5–8**) had lower  $\log K_{ow}$  values than compounds with pattern B (**3** and **9**) or pattern C (**4** and **10**). There were two turning points for bleaching patterns at  $\log K_{ow}$  values of 3.89–4.35 (between patterns A and B) and 4.43–4.78 (between patterns B and C).

As shown in Table 1, four of the 3-phenoxy-benzamides used in this study were *N*-monoalkylamides and six were *N,N*-dialkylamides. Among the four *N*-monoalkylamides, three amides had two methyl groups on the phenyl

Table 3 Log  $K_{ow}$  values and bleaching patterns of 3-phenoxybenzamide derivatives.

Compound	log $K_{ow}$	Bleaching pattern
<b>5</b>	$2.57 \pm 0.01^{a)}$	A
<b>1</b>	$2.86 \pm 0.00$	A
<b>6</b>	$2.98 \pm 0.01$	A
<b>7</b>	$3.44 \pm 0.00$	A
<b>2</b>	$3.76 \pm 0.01$	A
<b>8</b>	$3.89 \pm 0.03$	A
<b>3</b>	$4.35 \pm 0.03$	B
<b>9</b>	$4.43 \pm 0.06$	B
<b>4</b>	$4.78 \pm 0.03$	C
<b>10</b>	$5.01^{b)}$	C

<sup>a)</sup> Measured by the flask shaking method.  
Mean  $\pm$  S.D. of six replicates.

<sup>b)</sup> Estimated by HPLC method.

ring and one had no substituent on the phenyl ring. Clarke *et al.*<sup>5)</sup> and Sandmann<sup>6)</sup> made structure-activity investigations with 3-phenoxybenzamides against phytoene desaturase from blue-green alga *Aphanocapsa*. In their studies all the compounds listed in Table 1 inhibited phytoene desaturase and the order of inhibiting activity was almost same. Eventually, the bleaching patterns of cotyledons were not dependent on the difference in chemical structure, nor on the degree of inhibiting activity against phytoene desaturase, but on the physical properties such as the hydrophobicity described by log  $K_{ow}$ .

There have been many reported cases in which the hydrophobicity of compounds is correlated with their permeation and translocation in plant systems.<sup>10-12)</sup> In general, translocation of highly hydrophobic compounds in plants is limited. Uchida<sup>13)</sup> examined the translocation of dialkyl dithiolanylidene malonate derivatives in rice plants following uptake by the roots. In his study hydrophilic compounds moved through the whole plant body, while hydrophobic compounds remained in the roots and basal fractions. Briggs *et al.*<sup>14)</sup> examined the distribution of two series of chemicals, *O*-methylcarbamoyloximes and substituted phenylureas, in barley shoots and observed the same as above. Barak *et al.*<sup>15)</sup> studied the translocation of pesticides in the stems of pepper, cotton and bean, and

found that comparatively hydrophobic pesticides were hardly mobile.

Our conclusion is that the bleaching patterns of cotyledons vary according to the difference in translocation of herbicidal 3-phenoxybenzamides and that mobility of the compounds are correlated with their hydrophobicity described by log  $K_{ow}$ . Details of this investigation will be presented in our next paper.

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#### REFERENCES

- 1) M. F. Hipkins: "Advanced Plant Physiology," ed. by M. B. Wilkins, Pitman Publishing Ltd., Massachusetts, pp. 219-248, 1984
- 2) D. Siefertmann-Harms: *Physiol. Plant.* **69**, 561 (1987)
- 3) K. J. Kunert & A. D. Dodge: "Target Sites of Herbicide Action," ed. by P. Böger & G. Sandmann, CRC Press, Boca Raton, pp. 45-63, 1989
- 4) G. Sandmann & P. Böger: "Target Sites of Herbicide Action," ed. by P. Böger & G. Sandmann, CRC Press, Boca Raton, pp. 25-44, 1989
- 5) I. E. Clarke, G. Sandmann, P. M. Bramley & P. Böger: *Pestic. Biochem. Physiol.* **23**, 335 (1985)
- 6) G. Sandmann: "Pesticide Science and Biotechnology," ed. by R. Greenhalph & T. R. Roberts, Blackwell Scientific, Oxford, pp. 43-48, 1986
- 7) H. Noguchi, S. Hashimoto, S. Kitamura, S. Matsuo, A. Mine & K. Kamoshita (Sumitomo Chemical Co., Ltd.): Jpn. Kokai Tokkyo Koho JP 54-66631 (1979)
- 8) T. Fujita, J. Iwasa & C. Hansch: *J. Am. Chem. Soc.* **86**, 5175 (1964)
- 9) C. V. Eadsforth: *Pestic. Sci.* **17**, 311 (1986)
- 10) F. Kerler & J. Schönherr: *Arch. Environ. Toxicol.* **17**, 7 (1988)
- 11) G. G. Briggs, R. H. Bromilow & A. A. Evans: *Pestic. Sci.* **13**, 495 (1982)
- 12) F. C. Hsu, R. L. Marxmiller & A. Y. S. Yang: *Plant Physiol.* **93**, 1573 (1991)
- 13) M. Uchida: *Pestic. Biochem. Physiol.* **14**, 249 (1980)
- 14) G. G. Briggs, R. H. Bromilow & A. A. Evans: *Pestic. Sci.* **14**, 492 (1983)
- 15) E. Barak, B. Jacoby & A. Dinoor: *Pestic. Biochem. Physiol.* **20**, 194 (1983)

## 要 約

除草活性化合物 3-フェノキシベンズアミド類  
の疎水性とダイコン子葉の白化パターン

高橋政弘, 宮門正和, 田中鎮也

3-フェノキシベンズアミド類縁体をダイコンの種子に浸漬処理すると, 展開した子葉は化合物により異なる白化状態を示した. 子葉の白化状態は2枚の子葉の裏表, 計4面での白化の程度に基づいて三つのパターン (A, B, C) に分類することができた. 化合物の疎水性と

白化パターンとの関係を調べると, 疎水性の比較的小さい化合物 ( $\log K_{ow}$  2.57~3.89) では2枚の子葉とも全面が白化した (パターン A). 一方, より疎水性の大きい化合物では部分的に白化しただけであり, 1枚の子葉が完全に白化し, もう1枚は周辺だけが白化したもの (パターン B,  $\log K_{ow}$  4.35, 4.43) と, 2枚の子葉とも周辺のみ白化したもの (パターン C,  $\log K_{ow}$  4.78, 5.01) に分かれた. 子葉の白化パターンの変化は化合物の疎水性の差に基づいた移行性の違いによって起こっていることが示唆された.