Original Article

Peroxidizing Phytotoxicities of 1,2-Alkylene-1,2,4-triazolidines and 3,4-Alkylene-1,3,4-thiadiazolidines

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The influence of 1,2-alkylene-1,2,4-triazolidines and 3,4-alkylene-1,3,4-thiadiazolidines on peroxidizing phytotoxicities was investigated using corn etioplast protoporphyrinogen-IX oxidase (Protox), autotrophic *Scenedesmus acutus* cells and *Echinochloa utilis*. Both types of compounds inhibited Protox, caused ethane formation, decreased chlorophyll content and inhibited growth of *Scenedesmus* and root-growth of *Echinochloa*, like peroxidizing herbicides such as *p*-nitrodiphenyl ethers and cyclic imides. All compounds were identified as peroxidizing herbicides. Among the 1,2-alkylene-1,2,4-triazolidines, tetramethylene-triazolidines exhibited the strongest peroxidizing phytotoxicity, trimethylene-triazolidines and pentamethylene-triazolidines were found to be less active peroxidizers. The tetramethylene moiety of the triazolidine-type compounds appears to be essential for producing active peroxidizing compounds. Phytotoxicities by triazolidin-one-thiones and triazolidine-dithiones were more than 10 times stronger than those of triazolidine-diones. Replacing carbonyl groups in the five-membered moiety by at least one thiocarbonyl group produces stronger peroxidizing phytotoxicities. Although the Protox inhibition of triazolidine-peroxidizers was 10–70 times stronger than that of the corresponding thiadiazolidine-peroxidizers, the level of other phytotoxic activities was quite similar with both types of peroxidizers. This fact may be caused by isomerization of thiadiazolidines to triazolidines.

INTRODUCTION

Chlorophyll decrease, protoporphyrin-IX accumulation, and light-induced ethane formation in *Scenedesmus acutus* cells as well as root-growth inhibition of *Echinochloa utilis* have been determined as phytotoxic parameters when the so-called peroxidizing herbicides were applied.^{1,2)} Recently, the protoporphyrinogen-IX oxidase (Protox) inhibition with corn etioplasts has been combined with the four phytotoxic parameters mentioned above.^{3,4)} A reliable quantitative correlation has been established with those parameters including Protox inhibition.^{2,5)}

On the other hand, herbicidal activity of a series of 1,2-alkylene-1,2,4-triazolidines (I)^{6,7)} and 3,4-alkylene-1,3,4-thiadiazolidines (II)⁸⁾ derivatives (see Fig. 1) has also been reported. Although relationships between the herbicidal activity and their structures have been studied, the structure-activity relationships concerning the peroxidizing parameters have not yet been fully elucidated. In this study, the phytotoxicities are investigated exhibited by the alkylene triazolidines (I) and alkylene thiadi-

azolidines (II) compared with the five peroxidizing parameters in order to explain the relationship between peroxidizing activity and their structures. We will discuss especially the influence of the alkylene moiety A and five-membered moiety B on Protox inhibition of the compounds (Fig. 1, I and II).

MATERIALS AND METHODS

1. Chemicals

1,2-Alkylene-1,2,4-triazolidine (I) and 3,4-alkylene-1,3,4-thiadiazolidine (II) were synthesized according to Wakabayashi *et al.*^{6,9)} and Yamaguchi *et al.*,⁸⁾ respectively. The typical synthetic procedures are outlined by the following examples.

1.1 1-(4-Bromophenylthiocarbamoyl)-2-ethoxycarbonyl-1,2-trimethylenhydrazine

4-Bromophenyl isothiocyanate (6.4 g, 0.03 mol) in 200 ml benzene was added to 1-ethoxycarbonyl-1,2-trimethylenhydrazine (4.3 g, 0.03 mol) which was prepared by decarboxylation of 1,2-bis(ethoxycarbonyl)-1,2-trimethylenhydrazine.¹⁰⁾ After stirring for 3 hr at room temperature, the solvent was evaporated *in vacuo*.

Fig. 1 Structural moieties of 1,2-alkylene-1,2,4-triazolidines and 3,4-alkylene-1,3,4-thiadiazolidines.

The remaining residue was recrystallized from benzene-hexane to yield 8.7 g (81%) of 1-(4-bromophenylthiocarbamoyl)-2-ethoxycarbonyl-1,2-trimethylenehydrazine, mp 135–138°C. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1} : 3300, 3000, 1700, 1580.

1.2 4-(4-Bromophenyl)-1,2-trimethylene-1,2,4-triazolidin-3-one-5-thione (2)

A mixture of 1-(4-bromophenylthiocarbamoyl)-2-ethoxycarbonyl-1,2-trimethylenehydrazine (3.6 g, 0.01 mol) and sodium acetate (0.3 g) in xylene (60 ml) was refluxed for 5 hr. After evaporation *in vacuo*, the residue was washed with water and recrystallized from benzene-ethanol to give colorless crystals (2.6 g, 83%), mp 158–159°C. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1} : 1710, 1490. ^1H NMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$ ppm: 2.75 (2H, m), 3.99 (2H, t), 4.07 (2H, t), 7.45 (2H, d), 7.68 (2H, d).

1.3 4-(4-Bromophenyl)-1,2-trimethylene-1,2,4-triazolidine-3,5-dithione (4)

A mixture of 1-(4-bromophenylthiocarbamoyl)-1,2-trimethylenehydrazine (2.9 g, 0.01 mol) which was prepared *via* decarboxylation of 1-(4-bromophenylthiocarbamoyl)-2-ethoxycarbonyl-1,2-trimethylenehydrazine,¹¹⁾ carbon disulfide (4.3 g) and potassium hydroxide (0.8 g) in ethanol (70 ml) was refluxed for 3 hr. After evaporation *in vacuo*, diluted hydrochloric acid was added to the residue. The resulting solid was collected and recrystallized from ethanol to give colorless crystals (1.3 g, 34%), mp 213–214°C. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1} : 1480, 1420. ^1H NMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$ ppm: 2.84 (2H, m), 4.26 (4H, t), 7.31 (2H, d), 7.67 (2H, d).

1.4 4-(4-Bromophenyl)-1,2-trimethylene-1,2,4-triazolidine-3,5-dione (1)

4-(4-Bromophenyl)-1,2,4-triazolidine-3,5-dione (2.6 g, 0.01 mol) and powdered potassium hydroxide (1.34 g, 0.02 mol) was dissolved in 50 ml of dimethylformamide (DMF). To the resulting greenish solution, trimethylene bromide (2.1 g, 0.01 mol) was added, and then the mixture was heated under reflux for 1 hr with stirring. Precipitated potassium bromide was filtered off and DMF evaporated *in vacuo*. The residue was recrystallized from ethanol to give 4-(4-bromophenyl)-1,2-trimethylene-1,2,4-triazolidine-3,5-dione (2.2 g, 75%), mp 188–189°C (mp 188–189°C in Ref. 9)). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1} : 1770, 1680, 1480, 1410. ^1H NMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$ ppm: 2.40 (2H, m), 3.80 (4H, t), 7.57 (2H, d), 7.60 (2H, d).

1.5 5-(4-Bromophenylimino)-3,4-trimethylene-1,3,4-thiadiazolidin-2-one (3)

1-(4-Bromophenylthiocarbamoyl)-1,2-trimethylenehydrazine (2.9 g, 0.01 mol) and pyridine (2.4 g, 0.03 mol) were dissolved in 50 ml of dichloromethane. After dropwise addition of a solution of trichloromethyl chloroformate (1 g, 0.005 mol) in dichloromethane (30 ml) at 0°C, the solution was stirred for 24 hr at room temperature, and then poured into ice water. The dichloromethane layer was separated, dried over Na_2SO_4 and the solvent evaporated *in vacuo*. The residue was chromatographed over silica gel (150 g, ethyl acetate as eluent) to give the product (1.5 g, 48%), mp 53–55°C. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1} : 1710, 1620, 1580. ^1H NMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$ ppm: 2.57 (2H, m), 3.84 (2H, t), 3.90 (2H, t), 6.83 (2H, d), 7.42 (2H, d).

1.6 5-(4-Bromophenylimino)-3,4-trimethylene-1,3,4-thiadiazolidine-2-thione (5)

1-(4-Bromophenylthiocarbamoyl)-1,2-trimethylenehydrazine (2.0 g, 0.01 mol) and pyridine (2.4 g, 0.03 mol) were dissolved in 50 ml of dichloromethane. After dropwise addition of a solution of thiocarbonyl chloride (1.4 g, 0.012 mol) in dichloromethane (20 ml) at 0°C, the solution was stirred for 5 hr at room temperature and then added to water. The solid obtained was chromatographed over silica gel to give the title compound (21 g, 55%), mp 142–144°C. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1} : 1620, 1480. ^1H NMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$ ppm: 2.75 (2H, m), 4.02 (2H, t), 4.08 (2H, t), 6.82 (2H, d), 7.43 (2H, d).

Other alkylene triazolidines (**6**, **7**, **9**, **11**, **12** and **14**) and alkylene thiadiazolidines (**8**, **10**, **13** and **15**) listed in Tables 1, 2 and 3 were synthesized in a similar manner and their structures confirmed by IR- and NMR-spectroscopy, and elementary analysis for C, H and N.

The reference peroxidizing herbicide, *N*-(4-bromophenyl)-3,4,5,6-tetrahydrophthalimide (**16**), was prepared according to the method of Ohta *et al.*¹²⁾

Analytical-grade chemicals for algal cultivation and other fine chemicals, such as protoporphyrin-IX and buffers, were purchased from Kanto Chemical Co., Inc., Tokyo, Japan or Sigma Chemie, München, Germany.

2. Phytotoxic Assays of Compounds

2.1 Determination of protoporphyrinogen-IX oxidase (Protox) inhibition

Determination of the Protox inhibition was carried out according to the method of Nicolaus *et al.*^{3,4,13)} Corn (*Zea mays* cv. Anjou) was grown for 6 days in the dark at 30°C. The seedlings were harvested after exposure for 2 hr to the light. After homogenizing the seedlings, purified plastids which contained Protox were prepared by three differential centrifugation steps. Protox activity was measured by formation of protoporphyrin-IX. Protox inhibition was determined after adding the compounds to be tested to the 3-ml assay mixture containing

0.1 M tris-(hydroxymethyl)aminomethane (Tris-HCl, pH 7.3), 1 mM EDTA, 5 mM dithiothreitol (DTT), 0.03% Tween 80 (w/v), 0.3–0.6 mg of etioplast protein and 2–5 μ M protoporphyrinogen-IX. The amount of protoporphyrin-IX formed was determined for 5 min by Hitachi F-2000 fluorescence spectrophotometer, using excitation and emission wavelength of 405 and 632 nm, respectively. The I_{50} (Protox) values were determined by reciprocal plots of protoporphyrin-IX formation (in percent of control) vs. inhibitor concentration (Dixon plot). The pI_{50} (Protox) values were calculated from the equation, pI_{50} (Protox) = $-\log I_{50}$.

2.2 Determination of growth inhibition, chlorophyll decrease and ethane formation using *S. acutus*

Autotrophic *S. acutus* was grown according to Watanabe *et al.*¹⁾

Growth inhibition was determined by packed cell volume (pcv) in a graduated microcentrifuge tube.¹⁾ Chlorophyll content was measured spectroscopically in the extraction mixture (methanol:tetrahydrofuran:5 mM aqueous trifluoroacetic acid = 30:16:5, v/v/v).¹⁴⁾ Indices of growth inhibition and chlorophyll decrease are presented as pI_{50} (Sce) and pI_{50} (Chl), negative logarithms of I_{50} (Sce) and I_{50} (Chl). Ethane was measured using a gaschromatograph equipped with a flame-ionization detector. The I_{50} (Eth), the molar concentra-

tion giving half of the hypothetical maximum of light-induced ethane formation produced by *Scenedesmus* in a 20-hr incubation period, was determined through double-reciprocal plots.¹⁴⁾ These figures have also been called "activity values."⁵⁾

2.3 *E. utilis* root-growth inhibition

Using the method reported previously,¹⁵⁾ the inhibition activity against root-growth of *E. utilis* was determined. Root-growth inhibitory indices are represented as pI_{50} (Ech) values, the negative logarithms of the molar concentration at which the compounds produced a 50% root-growth inhibition.

RESULTS

1. Phytotoxic Activity of 1,2-Alkylene-1,2,4-triazolidines (I) and 3,4-Alkylene-1,3,4-thiadiazolidines (II)

The phytotoxicities of 1,2-alkylene-1,2,4-triazolidines (I) and 3,4-alkylene-1,3,4-thiadiazolidines (II) were determined by inhibition of corn Protox, growth inhibition of *S. acutus*, chlorophyll decrease of its cells, light-induced ethane formation by *Scenedesmus* and root-growth inhibition of *E. utilis*. The results are given as pI_{50} values in Tables 1, 2 and 3. Herein, Protox inhibition, ethane formation and chlorophyll decrease indicate inhibition of chlorophyll biosynthesis, peroxidative destruction of thylakoid membranes induced by light-

Table 1 Phytotoxicities of 1,2-trimethylene-1,2,4-triazolidines (I) and 3,4-trimethylene-1,3,4-thiadiazolidines (II).

<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <p>(I)</p> </div> <div style="text-align: center;"> <p>(II)</p> </div> </div>									
No.	Compound type	X, Y	Z	mp (°C)	pI_{50} (Protox)	pI_{50} (Sce)	pI_{50} (Chl)	pI_{50} (Eth)	pI_{50} (Ech)
1	(I)	O, O	—	188–189	6.21	4.85	5.01	5.15	5.11
2	(I)	S, O	—	158–159	7.27	6.69	6.83	6.53	6.21
3	(II)	—	O	53–55	5.44	6.39	6.41	6.01	5.98
4	(I)	S, S	—	213–214	7.54	6.97	6.96	6.84	6.88
5	(II)	—	S	142–144	6.49	6.51	6.59	6.01	6.28

Table 2 Phytotoxicities of 1,2-tetramethylene-1,2,4-triazolidines (I) and 3,4-tetramethylene-1,3,4-thiadiazolidines (II).

<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <p>(I)</p> </div> <div style="text-align: center;"> <p>(II)</p> </div> </div>									
No.	Compound type	X, Y	Z	mp (°C)	pI_{50} (Protox)	pI_{50} (Sce)	pI_{50} (Chl)	pI_{50} (Eth)	pI_{50} (Ech)
6	(I)	O, O	—	183–185	6.47	4.65	4.68	5.55	5.02
7	(I)	S, O	—	152–153	7.89	6.84	6.91	6.90	6.79
8	(II)	—	O	70–72	5.30	6.54	6.57	6.43	6.60
9	(I)	S, S	—	225–227	8.14	7.19	7.43	7.33	7.18
10	(II)	—	S	130–134	6.13	6.79	6.83	6.26	6.61
16	Reference ^{a)}			182–184	7.30	7.16	7.31	6.10	5.68

^{a)} *N*-(4-Bromophenyl)-3,4,5,6-tetrahydrophthalimide.

Table 3 Phytotoxicities of 1,2-pentamethylene-1,2,4-triazolidines (I) and 3,4-pentamethylene-1,3,4-thiadiazolidines (II).

(I)

(II)

No.	Compound type	X, Y	Z	mp (°C)	pI ₅₀ (Protox)	pI ₅₀ (Sce)	pI ₅₀ (Chl)	pI ₅₀ (Eth)	pI ₅₀ (Ech)
11	(I)	O, O	—	136–138	5.60	<4.00	<4.00	<4.00	5.11
12	(I)	S, O	—	150–153	6.89	5.36	5.46	5.40	5.25
13	(II)	—	O	66–70	5.30	5.24	5.40	5.35	5.15
14	(I)	S, S	—	218–221	7.15	5.78	5.84	5.73	5.41
15	(II)	—	S	102–105	5.30	5.47	5.54	5.40	5.28

dependent radical reactions and the bleaching effect through chlorophyll deficiency, respectively. The pI₅₀ (Sce) and pI₅₀ (Ech) express cell-growth inhibition of *Scenedesmus* and root-growth inhibition against *Echinochloa*, respectively, which reflect greenhouse pot tests using different weeds.⁷⁾ Although the strength of phytotoxic activities were different among the compounds tested, all compounds exhibited Protox inhibition, ethane formation, chlorophyll decrease, cell-growth inhibition and root-growth inhibition.

2. Phytotoxicities of Trimethylene-triazolidines (I) and -thiadiazolidines (II)

Protox inhibition by trimethylene-triazolidines (I: compound 1, 2 and 4) in corn etioplast preparation was measured and expressed as pI₅₀ (Protox) (see Table 1). The order of inhibition by the compounds was as follows; 4 (pI₅₀ (Protox) 7.54) ≥ 2 (pI₅₀ (Protox) 7.27) > 1 (pI₅₀ (Protox) 6.21). The triazolidin-one-thione (2) and triazolidine-dithione (4) were more than 10 times stronger Protox inhibitors than triazolidine-dione (1). The same order was observed for both peroxidative ethane formation and bleaching activity; with figures of 6.84 (4), 6.53 (2) and 5.15 (1) for pI₅₀ (Eth) and 6.96 (4), 6.83 (2) and 5.01 (1) for pI₅₀ (Chl), respectively. Although triazolidin-one-thione (2) and triazolidine-dithione (4) inhibited cell growth of *Scenedesmus* more than 100 times stronger than triazolidine-dione (1), only a 10-fold difference of root-growth inhibition against *Echinochloa* between the compounds (2 and 4) and compound 1 was observed.

The Protox inhibition measured by trimethylene-thiadiazolidines (II: 3 and 5) was more than 10 times weaker than that of their isomers trimethylene-triazolidines (2 and 4), while peroxidizing activity, bleaching activity, *Scenedesmus* cell-growth inhibition and *Echinochloa* root-growth inhibition of compounds (3 and 5) were 1.5–6 times less than those exhibited by compounds (2 and 4).

3. Phytotoxicities of Tetramethylene-triazolidines (I) and -thiadiazolidines (II)

The pI₅₀ (Protox) values of tetramethylene-triazolidines (I: 6, 7 and 9) and the reference peroxidizer (16) are shown in Table 2. The order of inhibition by the compounds was as follows; 9 (pI₅₀ (Protox) 8.14) > 7 (pI₅₀ (Protox) 7.89) > 16 (pI₅₀ (Protox) 7.30) > 6 (pI₅₀ (Protox) 6.47). Although the same order of activity for ethane formation by the compounds was found with 7.33 (9), 6.90 (7), 6.10 (16) and 5.55 (6) for pI₅₀ (Eth), algal cell growth inhibition and chlorophyll decrease ranked as 9 > 16 > 7 > 6. Root-growth inhibition (pI₅₀ (Ech)) of reference (16) against *Echinochloa* was extremely weak, compared with the triazolidines (7 and 9).

Protox inhibition by tetramethylene-thiadiazolidines (II: 8 and 10) was more than 100 times weaker than that by the corresponding tetramethylene-triazolidines (7 and 9), while the phytotoxicities of the compounds (8 and 10) by other parameters were 1.5–10 times less than those of compounds (7 and 9).

4. Phytotoxicities of Pentamethylene-triazolidines (I) and -thiadiazolidines (II)

The phytotoxic activities namely Protox inhibition, peroxidative ethane formation, chlorophyll decrease, cell growth inhibition and root-growth inhibition by pentamethylene-triazolidines (I: 11, 12 and 14) are shown in Table 3. The ranking of the phytotoxic activities was as follows; triazolidine-dithione (14) > triazolidin-one-thione (12) > triazolidine-dione (11), indicating 7.15 (14), 6.89 (12) and 5.60 (11) for pI₅₀ (Protox) and 5.73 (14), 5.40 (12) and <4.00 (11) for pI₅₀ (Eth). The orders were same as those of the trimethylene-triazolidines (1, 2 and 4) and tetramethylene-triazolidines (6, 7 and 9).

Phytotoxic activities by pentamethylene-thiadiazolidines (II: 13 and 15) were found 3–70 times weaker than that of the corresponding pentamethylene-triazolidines (12 and 14).

DISCUSSION

1. 1,2-Alkylene-1,2,4-triazolidines (I) and 3,4-Alkylene-1,3,4-thiadiazolidines (II) as Peroxidizing Herbicides

The first effect of peroxidizing herbicides such as oxyfluorfen and chlorophthalim, is inhibition of Protox which catalyzes the oxidation of protoporphyrinogen-IX to protoporphyrin-IX in the chlorophyll-biosynthesis pathway. This inhibition is followed by an abnormal accumulation of protoporphyrin-IX which functions in the light as a photosensitizer and induces radical reactions with subsequent ethane formation indicating destruction of thylakoid membranes, followed by degradation of cellular constituents such as chlorophylls and carotenoids.^{2,5)} Inhibition of corn etioplast Protox, ethane formation and chlorophyll decrease can be used conveniently to assay peroxidative activity of the compounds.^{2,9)} All the 1,2-alkylene-1,2,4-triazolidines (I) and 3,4-alkylene-1,3,4-thiadiazolidines (II) tested inhibited Protox. Furthermore, the effects on ethane formation, chlorophyll decrease and cell-growth inhibition in *S. acutus* and root-growth inhibition of *E. utilis* were quite similar to those caused by the reference peroxidizing cyclic imide herbicide (16), *N*-(4-bromophenyl)-3,4,5,6-tetrahydrophthalimide. According to these facts, all the compounds (1–15) tested are confirmed to have peroxidizing activity.

2. Influence of Alkylene Moiety A on Peroxidizing Phytotoxicities

Three series of 1,2-alkylene-1,2,4-triazolidines, triazolidine-diones (1, 6 and 11), triazolidin-one-thiones (2, 7 and 12) and triazolidine-dithiones (4, 9 and 14), as well as two series of 3,4-alkylene-1,3,4-thiadiazolidines, thiadiazolidin-ones (3, 8 and 13) and thiadiazolidine-thiones (5, 10 and 15), were compared to check the influence of the alkylene moiety on Protox inhibition and peroxidative ethane formation. Among the three series of triazolidines, the corresponding tetramethylene analogues (6, 7 and 9) were the strongest Protox inhibitors, while inhibition activity of trimethylene analogues (1, 2 and 4) was 2–4 times stronger than that of pentamethylene analogues (11, 12 and 14). This finding may indicate that the tetramethylene moiety of the compounds (6, 7 and 9) is essential for binding to Protox, as predicted by receptor mapping¹⁶⁾. The order of peroxidative ethane formation was as follows; tetramethylene-triazolidines (6, 7 and 9) > trimethylene-triazolidines (1, 2 and 4) > pentamethylene-triazolidines (11, 12 and 14), as shown in Tables 1, 2 and 3. The large difference (more than 10-fold) in peroxidative ethane formation between the trimethylene-triazolidines and pentamethylene-triazolidines may be due to different uptake into *Scenedesmus* cells or due to their metabolic instability in

the cells. In the two series of thiadiazolidines, Protox inhibition by trimethylene analogues (3 and 5) was somewhat stronger (*ca.* 2-fold) than that of the corresponding tetramethylene analogues (8 and 10), and pentamethylene analogues (13 and 15) were rather less active. The order of ethane formation was as follows; tetramethylene analogues (8 and 10) > trimethylene analogues (3 and 5) > pentamethylene analogues (13 and 15). The discrepancy between the order of Protox inhibition and the order of peroxidative ethane formation may be explained by isomerization of thiadiazolidines to triazolidines,^{17,18)} which will be discussed below.

3. Influence of the Five-Membered Moiety B on Peroxidative Phytotoxicities

As shown in Tables 1, 2 and 3, Protox inhibition by triazolidin-one-thiones (2, 7 and 12) and triazolidine-dithiones (4, 9 and 14) was more than 10 times stronger than that of triazolidine-diones (1, 6 and 11). The triazolidine-dithiones were somewhat stronger Protox inhibitors than triazolidin-one-thiones. A similar tendency was observed with ethane formation. Both phytotoxic activities by thiadiazolidin-ones (3, 8 and 13) were almost the same as those of thiadiazolidine-thiones (5, 10 and 15), although a thiadiazolidine-thione is a little stronger inhibitor than a thiadiazolidin-one. These facts indicate that phytotoxic activities become stronger when at least one sulfur atom is introduced at the position of X, Y and Z (see Fig. 1) in the five-membered moiety B of the compounds (I) and (II).

The pI_{50} (Ech) of reference 16 was extremely weak, compared with triazolidines (7 and 8), which may be caused by hydrolysis of reference 16 in the 7 days of incubation.¹⁹⁾

It has been reported that tetramethylene-thiadiazolidin-ones isomerize to tetramethylene-triazolidin-one-thiones in the culture medium or plant tissues,¹⁷⁾ while tetramethylene-thiadiazolidine-thiones are not so easily converted into the corresponding tetramethylene-triazolidine-dithiones even in the culture medium or in plant, because the tetramethylene-thiadiazolidine-thiones are chemically very stable compounds.¹⁸⁾ Protox inhibition assay is a short-term experiment by an isolated enzyme, so this inhibitory activity may indicate the intrinsic inhibition by the compounds. Protox inhibition of trimethylene-triazolidines (2 and 4) and pentamethylene-triazolidines (12 and 14) was 10–70 times stronger than that of the corresponding thiadiazolidines (3, 5, 13 and 15). This evidence confirms that triazolidine is the active form rather than the thiadiazolidine. The trimethylene-thiadiazolidin-one (3) and pentamethylene-thiadiazolidin-one (13) and the corresponding triazolidin-one-thiones (2 and 12) exhibited almost the same activities in long-term experiments (ethane formation, chlorophyll decrease, cell-growth inhibi-

tion and root-growth inhibition), but trimethylene-thiadiazolidine-thione (5) and pentamethylene-thiadiazolidine-thione (15) and the corresponding triazolidine-dithiones (4 and 14) exhibited different activity. This tendency is quite similar to the results reconfirmed using tetramethylene-thiadiazolidines (8 and 10) and tetramethylene-triazolidines (7 and 9) which has been originally reported in our previous papers.^{13,14,17,18)} This fact suggests that a trimethylene-thiadiazolidin-one (3) and a pentamethylene-thiadiazolidin-one (13) isomerize to a trimethylene-triazolidin-one-thione (2) and a pentamethylene-triazolidin-one-thione (12). According to Sato *et al.*, tetramethylene-thiadiazolidin-one (8) is isomerized to the corresponding triazolidine (7) in the presence of *Echinochloa* Seedlings, *Scenedesmus* cells, a homogenate of *Spinacia oleracea*, corn glutathione S-transferase (GST) and equine GST, although tetramethylene-thiadiazolidine-thione (10) barely converts into the corresponding triazolidine (9) under the same conditions.²⁰⁾ In fact, in our preliminary conversion experiments²¹⁾ of thiadiazolidin-ones, which were incubated with equine GST in the presence of reduced glutathione (GSH) for 30 min, the thiadiazolidines (3, 8 and 13) were isomerized to the corresponding triazolidin-one-thiones (2, 7 and 12) in the ratios 51.4, 77.0 and 92.0%, respectively. Trimethylene-thiadiazolidine-thione (5), tetramethylene-thiadiazolidine-thione (10) and pentamethylene-thiadiazolidine-thione (15), however, are not so easily converted into the corresponding triazolidine-dithiones (4, 9 and 14) indicating the conversion ratios 1.2, 4.0 and 23.0%, respectively.²¹⁾

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要 約

1,2-アルキレン-1,2,4-トリアゾリジンおよび3,4-アルキレン-1,3,4-チアジアゾリジン系化合物の植物に対する peroxidizing 作用

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1,2-Alkylene-1,2,4-triazolidine と 3,4-alkylene-1,3,4-thiadiazolidine 系化合物を合成し, それらの植物毒性をトウモロコシの protoporphyrinogen-IX oxidase, *Scenedesmus acutus*, *Echinochloa utilis* を用いて検討した。これらの化合物は, protoporphyrinogen-IX oxidase を阻害し, エタンを発生し, クロロフィルを減少させ, 生長を阻害した。以上のことから, 両化合物群は peroxidizing 除草剤であることが確認された。また, 炭素数 3, 4, 5 の alkylene 鎖をもつ

triazolidine 系化合物のなかでは、炭素数 4 の化合物がもっとも高い植物毒性活性を示した。さらに、triazolidin-one-thione, -dithione 系化合物は -dione 系化合物よりも高い植物毒性活性を示したことから、強い peroxidizing 除草剤を得るためには S 原子の導入が重要であることがわかった。

Protoporphyrinogen-IX oxidase 阻害においては、triazolidine 系化合物は thiadiazolidine 系化合物よりも高い阻害活性を示したが、peroxidative 活性においてはほぼ同等であった。この事実には、thiadiazolidine から triazolidine への異性化反応が関与していることが考えられた。