Peroxidizing Phytotoxic Activity of 1,3,4-Thiadiazolidine-2-thiones and 1,2,4-Triazolidine-3,5-dithiones

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Using autotrophic Scenedesmus acutus cells incubated in the light and Echinochloa utilis culture, a series of isomeric compounds, namely 5-arylimino-3,4-tetramethylene-1,3,4-thiadiazolidine-2-thiones (thiadiazolidine-thiones) and 4-aryl-1,2-tetramethylene-1,2,4-triazolidine-3,5-dithiones (triazolidine-dithiones), were assayed with respect to growth inhibition, decrease of chlorophyll contents, protoporphyrin-IX accumulation and light-induced ethane formation level. The both types of phytotoxic compounds decreased chlorophyll contents, caused protoporphyrin-IX accumulation and ethane evolution, and inhibited growth of Scenedesmus cells. They inhibited also protoporphyrinogen-IX oxidase, which led to rapid accumulation of protoporphyrin-IX, an intermediate of chlorophyll biosynthesis, just like peroxidizing herbicides such as p-nitrodiphenyl ethers and cyclic imides. Our comparative data on different sets of the aforementioned parameters suggest that both the thiadiazolidine-thiones and triazolidine-dithiones are grouped as peroxidizing herbicides, affecting a crucial enzyme in the chlorophyll biosynthesis and inducing ethane formation by cell membrane destruction.

INTRODUCTION

5-Arylimino-3,4-tetramethylene-1,3,4-thiadiazolidin-2-ones have been confirmed as the peroxidizing herbicides by Ogino et al. In this study, we have investigated several phytotoxicities exhibited by 5-arylimino-3,4-tetramethylene-1,3,4-thiadiazolidine-2-thiones (thiadiazolidine-thiones) and their isomers, 4-aryl-1,2-tetramethylene-1,2,4-triazolidine-3,5-dithiones (triazolidine-dithiones), using Scenedesmus acutus and Echinochloa utilis, in order to determine whether the thidiazolidine-thiones are also the peroxidizers or not. In this paper, we report the comparative phytotoxicities exhibited by the isomeric herbicidal compounds, thidiazolidine-thiones and triazolidine-dithiones.

MATERIALS AND METHODS

1. Synthesis of Compounds

Thidiazolidine-thiones 1–9 and triazolidine-dithiones 10–18 (see Tables 1 and 2) were synthesized according to the methods described in patents and papers. The typical synthetic procedures are shown in the following examples.

1.1 1-(4-Chlorophenylthiocarbamoyl)-2-ethoxycarbonylhexahydrocarbazoloidine

To N-ethoxycarbonylhexahydrocarbazoloidine (15.7 g, 0.1 mol) in benzene (100 ml) was added p-chlorophenyl isothiocyanate (16.9 g, 0.1 mol). After stirring 3 hr at room temperature, benzene was evaporated in vacuo. The resulting solid was recrystallized from benzene-cyclohexane to yield colorless crystals (28.2 g,
1- (4-Chlorophenylthiocarbamoyl)hexahydropyridazine

1-(4-Chlorophenylthiocarbamoyl)-2-ethoxycarbonylhexahydropyridazine (6.55g, 0.02 mol) in 5% KOH-ethanol (60ml) was refluxed for 4 hr and then the precipitated K₂CO₃ was filtered. The carbonate was washed with hot ethanol on the filter paper. The combined filtrate was concentrated in vacuo, and the resulting solid was washed with water to yield colorless crystals (4.60g, 90%), mp 130-132°C (mp 131-132.5°C in Ref. 6)).

5-(4-Chlorophenylimino)-3,4-tetramethylene-1,3,4-thiadiazolidine-2-thiones, 2

In the mixture of 1-(4-chlorophenylthiocarbamoyl)-2-ethoxycarbonylhexahydropyridazine (6.55g, 0.02 mol) in 5% KOH-ethanol (60ml) was refluxed for 4 hr and then the precipitated K₂CO₃ was filtered. The carbonate was washed with hot ethanol on the filter paper. The combined filtrate was concentrated in vacuo, and the resulting solid was washed with water to yield colorless crystals (4.60g, 90%), mp 130-132°C (mp 131-132.5°C in Ref. 6)).

Table 1 Phytotoxicities of 5-(arylimino)-3,4-tetramethylene-1,3,4-thiadiazolidine-2-thiones.

<table>
<thead>
<tr>
<th>No.</th>
<th>R</th>
<th>mp (°C)</th>
<th>p₅₀ (E)</th>
<th>p₅₀ (S)</th>
<th>p₅₀ (Chl)</th>
<th>p₅₀ (Eth)</th>
<th>p₅₀ (Protox)</th>
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<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>Oil</td>
<td>4.82</td>
<td>4.85</td>
<td>4.86</td>
<td>5.36</td>
<td>10.8</td>
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<tr>
<td>2</td>
<td>4-Cl</td>
<td>105-108</td>
<td>6.39</td>
<td>6.47</td>
<td>6.63</td>
<td>6.26</td>
<td>20.5</td>
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<tr>
<td>3</td>
<td>4-Br</td>
<td>130-134</td>
<td>6.61</td>
<td>6.79</td>
<td>6.83</td>
<td>6.26</td>
<td>25.3</td>
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<tr>
<td>4</td>
<td>4-OCH₃C₆H₅Cl-p</td>
<td>174-176</td>
<td>5.26</td>
<td>7.66</td>
<td>7.70</td>
<td>6.92</td>
<td>30.7</td>
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<tr>
<td>5</td>
<td>4-Cl, 2-CH₃</td>
<td>101-105</td>
<td>5.54</td>
<td>6.34</td>
<td>6.56</td>
<td>6.29</td>
<td>20.3</td>
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<tr>
<td>6</td>
<td>4-Cl, 2-F</td>
<td>83-86</td>
<td>6.80</td>
<td>6.92</td>
<td>7.17</td>
<td>7.04</td>
<td>31.5</td>
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<tr>
<td>7</td>
<td>4-Cl, 2-F, 5-OCH₃C₆H₅</td>
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<td>8.23</td>
<td>8.14</td>
<td>8.26</td>
<td>7.10</td>
<td>63.5</td>
</tr>
<tr>
<td>8</td>
<td>4-Cl, 2-F, 5-OC₆H₅-i</td>
<td>108-111</td>
<td>7.55</td>
<td>7.86</td>
<td>7.95</td>
<td>6.78</td>
<td>30.7</td>
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<tr>
<td>9</td>
<td>4-Cl, 2-F, 5-SCH₂COOCH₃</td>
<td>98-102</td>
<td>6.57</td>
<td>6.61</td>
<td>6.72</td>
<td>6.41</td>
<td>34.1</td>
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<tr>
<td>19</td>
<td>Oxyfluorfen</td>
<td>84-85</td>
<td>5.76</td>
<td>7.51</td>
<td>7.67</td>
<td>7.38</td>
<td>65.6</td>
</tr>
</tbody>
</table>

Table 2 Phytotoxicities of 4-aryl-1,2-tetramethylene-1,2,4-thiazolidine-3,5-dithiones.

<table>
<thead>
<tr>
<th>No.</th>
<th>R</th>
<th>mp (°C)</th>
<th>p₅₀ (E)</th>
<th>p₅₀ (S)</th>
<th>p₅₀ (Chl)</th>
<th>p₅₀ (Eth)</th>
<th>p₅₀ (Protox)</th>
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<td>10</td>
<td>H</td>
<td>248-250</td>
<td>5.45</td>
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<tr>
<td>11</td>
<td>4-Cl</td>
<td>204-206</td>
<td>7.05</td>
<td>7.21</td>
<td>7.36</td>
<td>7.30</td>
<td>50.3</td>
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<td>12</td>
<td>4-Br</td>
<td>225-227</td>
<td>7.18</td>
<td>7.19</td>
<td>7.43</td>
<td>7.33</td>
<td>67.9</td>
</tr>
<tr>
<td>13</td>
<td>4-OCH₃C₆H₅Cl-p</td>
<td>183-186</td>
<td>6.06</td>
<td>7.76</td>
<td>7.81</td>
<td>7.64</td>
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<tr>
<td>14</td>
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<td>156-159</td>
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<td>6.98</td>
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<td>7.07</td>
<td>63.3</td>
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<tr>
<td>15</td>
<td>4-Cl, 2-F</td>
<td>204-206</td>
<td>7.04</td>
<td>7.63</td>
<td>7.74</td>
<td>7.56</td>
<td>70.3</td>
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<td>16</td>
<td>4-Cl, 2-F, 5-OCH₃C₆H₅</td>
<td>211-215</td>
<td>8.77</td>
<td>8.69</td>
<td>8.77</td>
<td>8.14</td>
<td>87.2</td>
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<tr>
<td>17</td>
<td>4-Cl, 2-F, 5-OC₆H₅-i</td>
<td>172-173</td>
<td>7.91</td>
<td>8.12</td>
<td>8.28</td>
<td>7.17</td>
<td>60.2</td>
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<tr>
<td>18</td>
<td>4-Cl, 2-F, 5-SCH₂COOCH₃</td>
<td>Oil</td>
<td>6.71</td>
<td>6.73</td>
<td>6.85</td>
<td>6.80</td>
<td>56.0</td>
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<tr>
<td>19</td>
<td>Oxyfluorfen</td>
<td>84-85</td>
<td>5.76</td>
<td>7.51</td>
<td>7.67</td>
<td>7.38</td>
<td>65.6</td>
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carbamoyl)hexahydropyridazine (2.56 g, 0.01 mol), pyridine (2.0 g, 0.025 mol) and CH₂Cl₂ (50 ml), thiocarbonyl chloride (1.38 g, 0.012 mol) in CH₂Cl₂ (10 ml) was added dropwise at 0°C. Then the mixture was stirred for 3 hr at room temperature, and was poured into ice-water. The CH₂Cl₂ layer was separated from aqueous phase, dried over Na₂SO₄ and the solvent was evaporated in vacuo. The residues were chromatographed over silica gel (200 g, CHCl₃ as elution solvent) to give a brownish crystals of 2 (2.34 g, 79%), mp 105-108°C (mp 96-97°C in Ref. 2)). Anal. Found: C, 48.52; H, 4.18; N, 14.05; Cl, 11.81; S, 21.46. Calcd. for C₁₂H₁₂N₃ClS₂: C, 48.39; H, 4.06; N, 14.11; Cl, 11.91; S, 21.53%. MS m/z: 297 (M⁺). IR ν/cm⁻¹: 1640, 1420, 1240. 1H NMR δ ppm: 1.88 (4H, m), 3.76 (2H, m), 4.18 (2H, m), 6.79 (2H, d), 7.21 (2H, d).

1. 4 4-(4-Chlorophenyl)-1, 2-tetramethylene-1, 2, 4-triazolidine-3, 5-dithiones, 11

1-(4-Chlorophenylthiocarbamoyl)hexahydropyridazine (2.56 g, 0.010 mol), carbon disulfide (0.9 g, 0.012 mol) and potassium hydroxide (0.73 g, 0.013 mol) were dissolved in ethanol (60 ml). The mixture was refluxed for 3 hr. After removing the solvent in vacuo, dilute hydrochloric acid was added and the resulting white solid was collected. Recrystallization from ethanol-ethyl acetate gave colorless crystals of the dithione 11 (2.6 g, 87%), mp 204–206°C (mp 206–208°C in Ref. 3)). Anal. Found: C, 48.57; H, 4.13; N, 14.15; Cl, 11.95; S, 21.40. Calcd. for C₁₂H₁₁N₃ClS₂: C, 48.39; H, 4.06; N, 14.11; Cl, 11.91; S, 21.53%. MS m/z: 297 (M⁺). IR ν/cm⁻¹: 1500, 1310, 1280. 1H NMR δ ppm: 2.09 (4H, m), 4.10 (4H, m), 7.32 (2H, d), 7.52 (2H, d).

Other thiadiazolidine-thiones (1, 3-9) and triazolidine-dithiones (10, 12-18) listed in Tables 1 and 2 were synthesized in a similar manner and their structures were confirmed by IR-, NMR- and Mass-spectroscopy and elementary analysis for C, H and N (also for halogen and sulfur for some compounds). The reference peroxidizing herbicide, oxyfluorfen, was prepared according to the method of Yih & Switchenbank. Analytical-grade chemicals for algal cultivation and other fine chemicals, such like protoporphyrin-IX and buffers, were purchased from Kanto Chemical Co. Inc., Tokyo, or Sigma. Chem. Co., München, Germany.

2. Biological Tests

2.1 Root growth inhibition of compounds

Using the method reported previously, the inhibitory activity against the root growth of E. utilis was determined. Root growth inhibitory indices were represented as pI₅₀(E), logarithm of a reciprocal molar concentration for 50% inhibition.

2.2 Phytotoxic assays using S. acutus

2.2.1 Determination of growth inhibition (pI₅₀(S)), decrease of chlorophyll contents (pI₅₀(Chl)) and protoporphyrin-IX accumulation

Autotrophic culture of the S. acutus, and growth inhibitory tests using Scenedesmus cells in the presence of compounds were performed according to Watanabe et al. Growth inhibition was determined by packed cell volume in a graduated micro-centrifuge tube. Growth inhibition indices were presented as pI₅₀(S), logarithm of a reciprocal molar concentration for 50% inhibition of cell growth. Total chlorophyll contents in 4 ml of algal cell suspension culture were determined according to Watanabe et al. Cells were collected by centrifugation and incubated with 10 ml of the extraction mixture (Methanol: Tetrahydrofuran: 5 mM aqueous trifluoroacetic acid = 30: 16: 5, v/v/v) at 55°C for 10 min. The chlorophylls in the extraction mixture were determined using spectrophotometer. The pI₅₀ (Chl), the negative logarithm of the molar I₅₀, was used to quantify the influence of the compound on the chlorophyll contents. The pI₅₀ (Chl) means the inhibition of chlorophyll biosynthesis and decomposition of chlorophyll already exist in chloroplasts. Protoporphyrin-IX in the extraction mixture above was determined by high-pressure liquid chromatograph. The value was expressed by nanomolar concentration in 1 ml of packed cell volume (nmol/ml pcv).

2.2.2 Determination of ethane formation, pI₅₀ (Eth)

According to Ogino et al., an adequate amount of each test compound in ethanol was added to each 70 ml of 24-hr-old algal cell culture with a density 1 μl packed cell volume.
per milliliter suspension in a 100 ml reacti-flask containing 5 mM of NaHCO₃. After incubating under continuous fluorescent lamps (approx. 16,000 lux) at 22°C for 20 hr, the amount of ethane evolved was determined by Shimadzu GC-6A Gaschromatograph System equipped with a flame-ionization detector. The I₅₀ (Eth), the molar concentration which gives half of the hypothetical maximum of light-induced ethane formation produced by *Scenedesmus* during incubation period, was estimated by the double-reciprocal plots of the rates of ethane evolution vs. compound concentrations.

2.2.3 Determination of protoporphyrinogen-IX oxidase (Protox) inhibition, pI₅₀ (Protox)

Maize seeds (*Zea mays* cv. Anjou) were soaked in water for 6 hr and germinated on vermiculite for 6 days in darkness at 30°C. Before harvesting the seedlings were allowed to green for 2 to 4 hr in the light (approx. 23,000 lux). Maize etioplasts obtained were prepared by differential centrifugation steps and resulted in 1 to 2 ml of purified plastids which contained Prot ox. Protoporphyrinogen-IX was prepared by reduction of protoporphyrin-IX with sodium amalgam under the stream of nitrogen in the dark. Prot ox activity was measured at 30°C by following formation of protoporphyrin-IX. Prot ox inhibition was determined after adding compounds tested to the 3 ml of assay mixture containing 0.1 M Tris-HCl (pH 7.3), 1 mM EDTA, 5 mM dithiothreitol, 0.03% Tween 80 (w/v), 0.3-0.6 mg of etioplast protein and 2-5 μM protoporphyrinogen-IX, according to Nicolaus *et al.*,11 and Sato *et al.*12 The amount of protoporphyrin-IX formed was determined by Hitachi F2000 fluorescence spectrophotometer with a thermostated cell holder during the first 5-10 min. The pI₅₀ (Protox) was given in the double-reciprocal plots of the initial velocity of protoporphyrin-IX formation vs. the substrate concentration.

**RESULTS**

1. Phytotoxic Actions of Thiadiazolidine-thiones and Triazolidine-dithiones

The inhibitory effects of thiadiazolidine-thiones (1-9 in Table 1) and triazolidine-dithiones (10-18 in Table 2) on cell growth (pI₅₀ (S)) and chlorophyll contents (pI₅₀ (Chl)) were assayed using autotrophic *Scenedesmus* grown in the light. The pI₅₀ (S) and pI₅₀ (Chl) well correlated with pI₅₀ (E) which expresses the root growth inhibition against *E. utilis* (see Eqs. (1) and (2) in Table 3). The pI₅₀ (E) has a good correlation with the herbicidal activity exhibited by so-called peroxidizing herbicides in pot tests using a lot of weeds. The term “bleaching” generally refers to decrease in the amount of photosynthetic pigments, in plants in the presence of a compound as compared with the untreated control. This pigments deficiency can be caused either by inhibition of biosynthesis of the pigments or by the destruction of the pigments formed already. The compounds exhibiting such reaction in plant cells are now called the bleachers.8, 13 All the thiadiazolidine-thiones and triazolidine-dithiones assayed (1-18) decreased the chlorophyll contents in cells grown autotrophically in the light. Although there were some differences in pI₅₀ (Chl) values among the compounds (see Tables 1 and 2), they are now confirmed to be the bleachers. Among them, compounds 7, 8, 16 and 17 have stronger bleaching activity than 19 (oxyfluorfen). The activity of 4, 11, 12, 13 and 15 was almost same as that of 19, and 2, 3, 5, 6, 9, 14 and 18 were less active than 19. A little activity was observed with compounds 1 and

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Quantitative correlations among phytotoxic parameters.</th>
</tr>
</thead>
<tbody>
<tr>
<td>pI₅₀(E) = 0.957 pI₅₀(S) + 0.091 (± 0.222)</td>
<td>(1)</td>
</tr>
<tr>
<td>pI₅₀(E) = 0.942 pI₅₀(Chl) + 0.077 (± 0.223)</td>
<td>(2)</td>
</tr>
<tr>
<td>pI₅₀(S) = 0.991 pI₅₀(Chl) - 0.057 (± 0.036)</td>
<td>(3)</td>
</tr>
<tr>
<td>pI₅₀(Chl) = 1.281 pI₅₀(Eth) - 1.587 (± 0.343)</td>
<td>(4)</td>
</tr>
</tbody>
</table>

* a) pI₅₀(E) data of compounds 4 and 13 were deleted in Eqs. (1) and (2), because their formulation in *Echinochloa* test was not so good to obtain accurate inhibition data.

In the equations, n is the number of compounds, s is the standard deviation, r is the correlation coefficient, the figures in parentheses are 95% confidence intervals.
The Eq. (3) in Table 3 quantifies the correlation with a high probability between growth inhibition \( I_{50} (S) \) and decreased chlorophyll contents \( I_{50} (\text{Chl}) \) by all the compounds (1-18) applied to autotrophic Scenedesmus cells.

2. Protoporphyrin-IX Accumulation, Protox Inhibition and Ethane Formation

Protoporphyrin-IX accumulation in autotrophically grown Scenedesmus cells during 1 hr incubation in the presence of 1 \( \mu \text{M} \) of the test compounds was listed in Tables 1 and 2. The 1 hr incubation was the minimum period to determine the protoporphyrin-IX contents with reliability. Active bleachers (2-9, 11-18) caused protoporphyrin-IX accumulation at the level of 20-85 nmol/ml pcw as well as the peroxidizing bleacher, oxyfluorfen, while the less active chlorophyll bleachers (1 and 10) induced a lesser protoporphyrin-IX accumulation (see Tables 1 and 2). Triazolidinedithiones accumulated more protoporphyrin-IX than thiadiazolidine-thiones (exactly, more than 20 nmol/ml pcw). Protox inhibition by the selected compounds (2-9 in Table 1 and 11-17 in Table 2) in maize etioplast preparation also measured and expressed as \( I_{50} (\text{Protox}) \). The inhibition by triazolidine-dithiones was 50 to 150 times stronger than that by the corresponding thiadiazolidine-thiones. Ethane formation caused by the compounds (1-18) in Scenedesmus cells was indicated as \( I_{50} (\text{Eth}) \). The active chlorophyll bleachers (2-9 and 11-18) had bigger \( I_{50} (\text{Eth}) \) values (more than 6.20), while the less active bleachers (1 and 10) indicated smaller ones.

This hydrocarbon is a convenient indicator of peroxidative destruction of thylakoid membranes induced by light-dependent radical reactions. The bleaching activity, \( I_{50} (\text{Chl}) \), by the compounds (1-18) correlated with the peroxidative ethane formation, \( I_{50} (\text{Eth}) \) (see Eq. (4) in Table 3). All the compounds (1-18) exhibited peroxidizing effects which inhibited Protox, accumulated protoporphyrin-IX and induced ethane formation.

**DISCUSSION**

1. Thiadiazolidine-thiones and Triazolidine-dithiones as Peroxidizing Herbicides

The first effect of the \( p \)-nitrodiphenyl ethers and cyclic imides, confirmed already as so-called peroxidizing herbicides, is a halt of chlorophyll biosynthesis in chloroplasts. This inhibition is accompanied by an abnormal accumulation of protoporphyrin-IX which works in the light as a photosensitizer and induces radical reactions with subsequent destruction of thylakoid membranes, followed by degradation of cellular constituents such like photosynthetic pigments. Four phytotoxic parameters, namely Protox inhibition (\( I_{50} (\text{Protox}) \)), protoporphyrin-IX accumulation, ethane formation (\( I_{50} (\text{Eth}) \)) and decrease of chlorophyll contents (\( I_{50} (\text{Chl}) \)), can be used conveniently to assay peroxidative activity of the compounds in autotrophic Scenedesmus cells. The effects of thiadiazolidine-thiones and triazolidine-dithiones (1-18) on protoporphyrin-IX accumulation, ethane formation, decrease of chlorophyll contents and cell growth (\( I_{50} (S) \)) in Scenedesmus cells were quite similar to those caused by the reference peroxidizing herbicide, oxyfluorfen. Furthermore, the selected compounds (2-9 and 11-17) strongly inhibited Protox. Growth inhibition (\( I_{50} (S) \)) by the compounds against Scenedesmus cells well correlated with growth inhibition of Echinochloa seedlings (\( I_{50} (E) \)), reflecting greenhouse pot tests using different weeds. According to this line of findings, all thiadiazolidine-thiones and triazolidine-dithiones tested are now confirmed as the peroxidizing herbicides, although their strength of activity is different among them.

2. Different Phytotoxic Activities between Thiadiazoline-thiones and Triazolidine-dithiones

It has been reported that the isomeric 5-arylimino-3, 4-tetramethylene-1, 3, 4-thiadiazolin-2-ones and 4-aryl-1,2-tetramethylene-1, 2, 4-triazolidin-3-one-5-thiones with the same N-aryl moiety exhibit almost the same quality and quantity of peroxidizing phytotoxic activity. This observation is now explained to be due to isomerization of the formers to the
latters in the culture medium or plant tissues, in our previous papers.\textsuperscript{1,11}\textsuperscript{11} The isomeric thia-

diazolidine-thiones and triazolidine-dithiones with the same N-aryl moiety, for example 3 and 12, exhibited quite different quantity of phytotoxic activities, indicating 6.61 and 7.18 for \textit{pIs}$_{10}(\text{E})$, 6.79 and 7.19 for \textit{pIs}$_{10}(\text{S})$, 6.83 and 7.43 for \textit{pIs}$_{18}(\text{Chl})$, 6.26 and 7.33 for \textit{pIs}$_{18}(\text{Eth})$, 25.3 and 67.9 (nmol/ml pcv) for protoporphyrin-IX accumulation and 6.13 and 8.14 for \textit{pIs}$_{10}(\text{Protox})$, respectively. The analogous consideration is also true between isomers (1 and 10), (2 and 11), (4 and 13), (5 and 14), (6 and 15), (7 and 16), (8 and 17) and (9 and 18).

The phytotoxic activities of triazolidine-dithiones are always higher than those of the corresponding thia-
diazolidine-thiones. Since the triazolidine-dithiones are chemically very stable compounds and the thia-
diazolidine-thiones are not so easily converted into the corresponding triazolidine-dithiones even in the culture medium or in plants,\textsuperscript{16}\textsuperscript{11}\textsuperscript{17} it may be concluded that each thia-
diazolidine-thiones and triazolidine-dithiones indicate each intrinsic peroxidizing phytotoxic activity.

ACKNOWLEDGMENTS

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の化合物は、Echinochloa の根伸長阻害を示し、また光照射下で Scenedesmus 細胞中で、protoporphyrin-IX を蓄積し、ethane を発生し、chlorophyll を減少させ、細胞の生育を阻害した。すなわち、両者は chlorophyll 生合成経路中の protoporphyrinogen-IX oxidase を阻害し、その結果蓄積する物質が関与して生じる酸素ラジカルによって、チラコイド膜を破壊して ethane を発生する作用機構（peroxidizing mechanism）をもつと考えられる。両者を間では、5-アリールイミノ-3,4-テトラメチレン-1,3,4-チアジアゾリシン-2-テオンの植物毒性が、4-アリール-1,2-テトラメチレン-1,2,4-トリアゾリシン-3,5-ジテオンに比べて高かった。