Isomerization of 3,4-Dialkyl-1,3,4-thiadiazolidines and 3,4-Alkylene-1,3,4-thiadiazolidines by Glutathione *S*-Transferase

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Dialkyl-thiadiazolidine, Dialkyl-triazolidine, Isomerization, Glutathione *S*-Transferase

Peroxidizing herbicides, as inhibitors of protoporphyrinogen IX oxidase (protox), cause accumulation of protoporphyrin IX and lead to a rapid phytotoxic degradation of plant cell membranes in the light. Certain 5-arylimino-3,4-tetramethylene-1,3,4-thiadiazolidin-2-ones are peroxidizing compounds. They are isomerized to the corresponding more active triazolidines with glutathione *S*-transferase (GST) in the presence of reduced glutathione (GSH). The rate of isomerization depends on the arylimino moiety and the presence of carbonyl or thiocarbonyl groups at the thiadiazolidine ring. The following compounds and their isomeric triazolidines were synthesized: 5-(4-Bromophenyl)-3,4-dialkyl-1,3,4-thiadiazolidin-2-ones (**I**), 5-(4-bromophenyl)-3,4-dialkyl-1,3,4-thiadiazolidine-2-thiones (**II**), 5-(4-bromophenyl)-3,4-alkylene-1,3,4-thiadiazolidin-2-ones (**III**), 5-(4-bromophenyl)-3,4-alkylene-1,3,4-thiadiazolidine-2-thiones (**IV**). The phytotoxic activities of these compounds were investigated using *Scenedesmus acutus* and protoporphyrinogen IX oxidase isolated from corn (*Zea mays*) etioplasts. These compounds are peroxidizing compounds, and the triazolidines showed higher activity than the thiadiazolidines. Compounds **I** and **III** were isomerized to the corresponding triazolidines, while compounds **II** and **IV** were not or scarcely converted into their isomers with GST in the presence of GSH. Seemingly, the bulkiness of alkylene or dialkyl groups at the heterocycle influences the isomerization of carbonyl-substituted thiadiazolidines.

Introduction

Ethane formation is a convenient marker (Watanabe *et al.*, 1992; Wakabayashi *et al.*, 1993).

During our studies on the mechanism of action of peroxidizing herbicides, it has become apparent that some protox inhibitors are converted into their more active isomers both in the culture medium of *Echinochloa utilis* and with both (equine) glutathione *S*-transferase (GST) reduced glutathione (GSH) present (Hoshi *et al.*, 1993; Sato *et al.*, 1994; Iida *et al.*, 1995). That is, *N*-aryl-3,4,5,6-tetrahydroisophthalimides and 5-arylimino-3,4-tetramethylene-1,3,4-thiadiazolidin-2-ones were converted into *N*-aryl-3,4,5,6-tetrahydrophthalimides and 4-aryl-1,2-tetramethylene-1,2,4-triazolidin-3-one-5-thiones, respectively. However, 5-arylimino-3,4-tetramethylene-1,3,4-thiadiazolidine-2-thiones were not or scarcely converted into their isomeric 4-aryl-1,2-tetra-methylene-1,2,4-triazolidine-3,5-dithiones under the same conditions. Our group has determined a GST II isoform as the functional catalyst of the conversion (Nicolaus *et al.*, 1996).

The previous results give evidence that isomerization is influenced both by the structure of the arylimino moiety and the presence of carbonyl or thiocarbonyl groups at the thiadiazolidine ring. The effect of an alkylene moiety (moiety A in Fig. 1) on the isomerization has not been studied yet. To evaluate this effect, we synthesized 5-(4-bromophenylimino)-3,4-dialkyl-1,3,4-thiadiazolidines (dialkyl-thiadiazolidines in Fig. 1), 5-(4-bromophenylimino)-3,4-alkylene-1,3,4-thiadiazolidines (alkylene-thiadiazolidines), and their isomeric triazolidines (alkylene-triazolidines and dialkyl-triazolidines).

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Material and Methods

Synthesis (for compounds see Table I, II)

5-(4-Bromophenylimino)-3,4-dimethyl-1,3,4-thiadiazolidin-2-one (**1**), 5-(4-bromophenylimino)-3,4-dimethyl-1,3,4-thiadiazolidine-2-thione (**2**), 5-(4-bromophenylimino)-3,4-diethyl-1,3,4-thiadiazolidin-2-one (**3**), 5-(4-bromophenylimino)-3,4-diethyl-1,3,4-thiadiazolidine-2-thione (**4**), 4-(4-bromophenyl)-1,2-dimethyl-1,2,4-triazolidin-3-one-5-thione (**5**), 4-(4-bromophenyl)-1,2-dimethyl-1,2,4-triazolidine-3,5-dithione (**6**), 4-(4-bromophenyl)-1,2-diethyl-1,2,4-triazolidin-3-one-5-thione (**7**), 4-(4-bromophenyl)-1,2-diethyl-1,2,4-triazolidine-3,5-dithione (**8**), 5-(4-bromophenylimino)-3,4-trimethylene-1,3,4-thiadiazolidin-2-one (**9**), 5-(4-bromophenylimino)-3,4-trimethylene-1,3,4-thiadiazolidine-2-thione (**10**), 5-(4-bromophenylimino)-3,4-tetramethylene-1,3,4-thiadiazolidin-2-one (**11**), 5-(4-bromophenylimino)-3,4-tetramethylene-1,3,4-thiadiazolidine-2-thione (**12**), 5-(4-bromophenylimino)-3,4-pentamethylene-1,3,4-thiadiazolidin-2-one (**13**), 5-(4-bromophenylimino)-3,4-pentamethylene-1,3,4-thiadiazolidine-2-thione (**14**), 4-(4-bromophenyl)-1,2-trimethylene-1,2,4-triazolidin-3-one-5-thione (**15**), 4-(4-bromophenyl)-1,2-trimethylene-1,2,4-triazolidine-3,5-dithione (**16**), 4-(4-bromophenyl)-1,2-tetramethylene-1,2,4-triazolidin-3-one-5-thione (**17**), 4-(4-bromophenyl)-1,2-tetramethylene-1,2,4-triazolidine-3,5-dithione (**18**), 4-(4-bromophenyl)-1,2-pentamethylene-1,2,4-triazolidin-3-one-5-thione (**19**) and 4-(4-bromophenyl)-1,2-pentamethylene-1,2,4-triazolidine-3,5-dithione (**20**) were synthesized as described in previous papers (Sato *et al.*, 1994; Iida *et al.*, 1995). Typical procedures for the synthesis of 5-(4-bromophenylimino)-3,4-dialkyl-1,3,4-thiadiazolidines (**1**~**4**) and 4-(4-bromophenyl)-1,2-dialkyl-1,2,4-triazolidines (**5**~**8**) were carried out as follows.

1,2-Bis(ethoxycarbonyl)-1,2-dimethylhydrazine: 1,2-bis(ethoxycarbonyl)hydrazine (50 g, 0.28 mol) and trimethylbenzylammonium chloride (10 g, 0.06 mol) were dissolved in 300 ml dichloromethane (CH_2Cl_2) and mixed with methyl sulfate (126 g, 1.0 mol) and 50% NaOH – water (108 g), then refluxed for 4 hr. After the CH_2Cl_2 layer was separated, the aqueous layer was extracted by CH_2Cl_2. The combined CH_2Cl_2 layers were dried with Na_2SO_4, and evaporated *in vacuo*. The residue was distilled to give 45 g of oil, bp 80–83 °C at 2 mm Hg.

1-(4-Bromophenylthiocarbamoyl)-1,2-dimethylhydrazine (Wakabayashi *et al.*, 1976 a, b): 1-ethoxycarbonyl-1,2-dimethylhydrazine (7.9 g, 0.06 mol) which was prepared by decarboxylation of 1,2-bis(ethoxycarbonyl)-1,2-dimethylhydrazine was added to 4-bromophenylisothiocyanate (13.0 g, 0.06 mol) in 100 ml benzene. After stirring 24 hr at room temperature, the precipitate was separated by filtration to give 11 g of 1-(4-bromophenylthiocarbamoyl)-2-ethoxycarbonyl-1,2-dimethylhydrazine. This compound (10 g, 0.03 mol) and KOH (4 g, 0.07 mol) were dissolved in 50 ml ethanol, then refluxed for 4 hr and K_2CO_3 was separated by filtration. The filtrate was concentrated *in vacuo*. The remaining residue was recrystallized from ethanol to yield 5.7 g (0.02 mol) of colorless crystals, mp 105–108 °C.

4-(4-Bromophenyl)-1,2-dimethyl-1,2,4-triazolidin-3-one-5-thione (Wakabayashi *et al.*, 1978; Ohta *et al.*, 1980): A mixture of 1-(4-bromophenylthiocarbamoyl)-2-ethoxycarbonyl-1,2-dimethylhydrazine (1 g, 3.0 mmol) and sodium acetate (0.5 g, 6.0 mmol) in xylene 40 ml was refluxed for 5 hr, then the solvent evaporated *in vacuo*. The residue was washed with water and recrystallized from ethanol to give colorless crystals (0.3 g, 1.0 mmol), mp 136–138 °C.

4-(4-Bromophenyl)-1,2-dimethyl-1,2,4-triazolidine-3,5-dithione (Wakabayashi *et al.* 1976 c): A mixture of 1-(4-bromophenyl)-1,2-dimethylhydrazine (1.5 g, 5.5 mmol), carbon disulfide (0.4 g, 5.5 mmol) and KOH (0.4 g, 6.0 mmol) in ethanol was refluxed for 3 hr, and the solvent evaporated *in vacuo*. After diluted hydrochloric acid was added to the residue, the resulting solid was collected and recrystallized from ethanol to give colorless crystals (0.3 g, 0.9 mmol), mp 266–268 °C.

5-(4-Bromophenylimino)-3,4-dimethyl-1,3,4-thiadiazolidin-2-one and 5-(4-Bromophenylimino)-3,4-dimethyl-1,3,4-thiadiazolidine-2-thione (Yamaguchi *et al.*, 1992): 1-(4-Bromophenylthiocarbamoyl)-1,2-dimethylhydazine (1.0 g, 4 mmol) was dissolved in a mixture of pyridine (0.3 g, 4 mmol) and 30 ml CH_2Cl_2. After trichloromethyl chloroformate (0.3 g, 2 mmol) had been added dropwise to this mixture at 0 °C, the mixture was stirred for 24 hr at room temperature and then poured into water. The CH_2Cl_2 layer was sepa-

rated, dried with Na_2SO_4, and evaporated *in vacuo*. The residue was chromatographed over silica gel to give 0.2 g of 5-(4-bromophenylimino)-3,4-dimethyl-1,3,4-thiadiazolidin-2-one, mp 122–126 °C.

5-(4-Bromophenylimino)-3,4-dimethyl-1,3,4-thiadiazolidine-2-thione (0.6 g, 2 mmol) was synthesized from 1-(4-bromophenylthiocarbamoyl)-1,2-dimethylhydrazine (1.5 g, 5.5 mmol) and thiocarbonyl chloride (0.6 g, 5.5 mmol) according to the above method.

The chemical structures were confirmed by melting point, IR- and NMR-spectroscopy, and elementary analysis.

Equine GST, GSH, fine chemicals and the chemicals for *S. acutus* cultivation were purchased from Sigma, Munich, Germany, and Tokyo Kasei Kogyo, Tokyo, Japan.

Determination of ethane formation using Scenedesmus acutus

According to the methods of Shouda *et al.*, (1996), ethane was determined by Shimadzu GC-6A gas chromatography system equipped with a flame-ionization detector, using Unipack S glass column (i.d. 32 mm, GL Science). The pI_{50}(Ethane), the molar concentration giving half of the hypothetical maximum of light-induced ethane formation by *Scenedesmus* during a 20-hr incubation period, was determined through double-reciprocal plots by regression analysis, with the *r* value higher than 0.93.

Determination of protoporphyrinogen-IX oxidase inhibition

According to the methods of Nicolaus *et al.* (1993), preparation of protoporphyrinogen IX and determination of protox inhibition were carried out by regression analysis, with the *r* value found higher than 0.93. The protox inhibition activity of the compound is expressed as pI_{50}(Protox), the negative logarithm of the I_{50} value of protox inhibition.

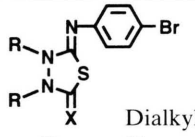
Conversion of thiadiazolidines into triazolidines (Iida et al., 1995)

100 μM of thiadiazolidines (**1–4** and **9–14**), 100 μM of GSH and 0.8–0.9 units of equine GST in potassium phosphate buffer (0.05 M, pH 6.8) was incubated for 30 min at 30 °C in a volume of 0.5 ml. Conversion of thiadiazolidines was determined by a Shimadzu LC-4A HPLC system equipped with a Senshu Pak ODS-1251–120K column (4.5250 mm; Senshu Scientific Co., Tokyo Japan). A solvent mixture of acetonitrile-distilled water (3:2, v/v) was used as the mobile phase (flow rate 1 ml/min) and the eluates were continuously monitored by a UV detector (SSC 3000B, Senshu Scientific Co., Tokyo, Japan) at 210 nm.

Results and Discussion

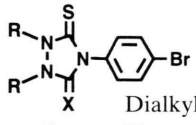
The newly synthesized dialkyl-thiadiazolidines (**1–4**) dialkyl-triazolidines (**5–8**), alkylene-thiadiazolidines (**9–14**), and alkylene-triazolidines (**15–20**) are peroxidizing compounds, because they exhibit both protox inhibition and produce light-induced ethane by *S. acutus* (see Table I and II). The conversion of dialkyl-, and alkylene-thiadiazolidines (**1–4** and **9–14**) into corresponding isomers by equine GST in the presence of GSH were investigated, using HPLC separation. The conversion rate was expressed as triazolidine formed (mol)/thiadiazolidines remained (mol). In a previ-

Table I. Phytotoxic activities of dialkyl-thiadiazolidines and dialkyl-triazolidines.



Dialkyl-thiadiazolidines

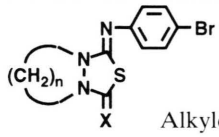
No.	R	X	pI_{50}(Ethane)	pI_{50}(Protox)
1	CH_3	O	5.14	5.43
2		S	4.95	5.51
3	C_2H_5	O	< 4	5.06
4		S	4.15	5.37



Dialkyl-triazolidines

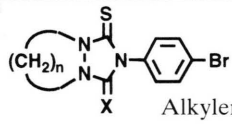
	R	X	pI_{50}(Ethane)	pI_{50}(Protox)
5	CH_3	O	5.43	6.55
6		S	5.12	6.96
7	C_2H_5	O	5.06	6.08
8		S	4.26	5.86

Table II. Phytotoxic activities of alkylene-thiadiazolidines and alkylene-triazolidines.



Alkylene-thiadiazolidines

No.	n*	X	pI_{50}(Ethane)	pI_{50}(Protox)
9	3	O	6.01	5.44
10		S	6.01	6.49
11	4	O	6.43	5.30
12		S	6.26	6.13
13	5	O	5.35	5.30
14		S	5.40	5.30



Alkylene-triazolidines

	n	X		
15	3	O	6.53	7.27
16		S	6.84	7.54
17	4	O	6.90	7.89
18		S	7.33	8.14
19	5	O	5.40	6.89
20		S	5.73	7.17

* n is the number of methylene.

ous paper (Iida *et al.*, 1995), a 2-hr period was used for incubation with GST and GSH. Since it was difficult to compare the conversion rate among 5-(4-bromophenyl)-3,4-alkylene-1,3,4-thiadiazolidin-2-ones under the same condition, a 30-min incubation was applied for the experiments of this study.

The conversion rates of **1**, **3**, **9**, **11** and **13** were 3.4, 0.24, 27.7, 13.6 and 0.53, respectively. Compounds no. **2**, **4**, **10**, **12** and **14** were not converted into their isomers (see Table III). There was also no conversion of dialkyl-triazolidines (**5–8**) and alkylene-triazolidines (**15–20**) into their corresponding dialkyl- and alkylene-thiadiazolidines (data not shown).

In a previous paper, we reported that 5-(4-bromophenylimino)-3,4-tetramethylene-1,3,4-thiadiazolidin-2-one (**11**) having an alkylene group on moiety A isomerized to the corresponding 4-(4-bromophenyl)-1,2-tetramethylene-1,2,4-triazolidin-3-one-5-thione (**17**) but 5-(4-bromophenylimino)-3,4-tetramethylene-1,3,4-thiadiazolidine-2-thione (**12**) was not or scarcely converted into the corresponding 4-(4-bromophenyl)-1,2-tetramethylene-1,2,4-triazolidine-3,5-dithione (**18**) in the presence of GST with GSH (Sato *et al.*, 1994; Iida *et al.*, 1995).

These results indicate that the conversion pattern of the dialkyl-thiadiazolidines having open chain alkyl groups on moiety A (**1–4**) is similar to that of the alkylene-thiadiazolidines (**9–14**) having an alkylene group on moiety A (see Fig. 1). That is, 5-(4-bromophenylimino)-3,4-dialkyl-1,3,4-thiadiazolidin-2-ones were isomerized to the corresponding 4-(4-bromophenyl)-1,2-dialkyl-1,2,4-triazolidin-3-one-5-thiones, but 5-(4-bromophenylimino)-3,4-dialkyl-1,3,4-thiadiazolidine-2-thiones were not or scarcely converted into the corresponding 4-(4-bromophenyl)-1,2-dialkyl-1,2,4-triazolidine-3,5-dithiones.

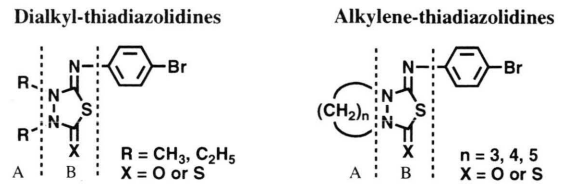


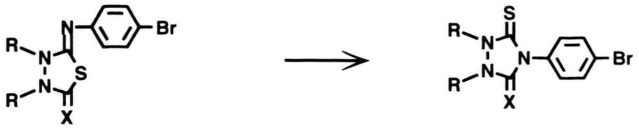
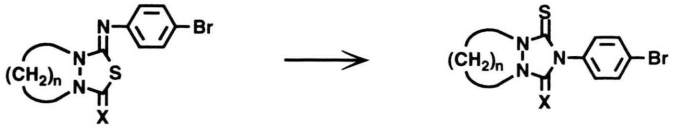
Fig. 1. Structures of dialkyl- and alkylene-thiadiazolidines.

An experiment using equine GST in the absence of GSH did neither convert alkylene-thiadiazolidin-2-ones nor dialkyl-thiadiazolidin-2-ones to the active corresponding triazolidines. Obviously the isomerization mechanism of dialkyl-thiadiazolidines to dialkyl-triazolidines is analogous to that of alkylene-thiadiazolidines to alkylene-triazolidines. Presumably, at first, dialkyl-thiadiazolidines conjugate with GSH in the presence of GST, then possibly the unstable conjugates are rapidly arranged nonenzymatically to the dialkyl-triazolidine via a keto-enol type tautomerism (a possible reaction scheme was published by Sato *et al.*, 1994).

In Table III, the amounts of the non-isomerized thiadiazolidines increased in the order of trimethylene (**9**) < tetra-methylene (**11**) < dimethyl (**1**) < pentamethylene (**13**) < diethyl (**3**). This difference apparently is due to a specific affinity of GST determined by the chemical structure of the compounds assayed.

In case of compound **1**, 71 mmol of **5** was produced from 78.8 mmol of **1** and 14.6 mmol of **7** from 39.0 mmol of **3** in case of **3** (see Table I for nos.). These findings show that the isomerization

Table III. Isomerization of dialkyl-thiadiazolidines and alkylene-thiadiazolidines by equine GST in presence of GSH.

No.	X	Thiadiazolidines remained (μmol)	Triazolidines formed (μmol)	Conversion rate[*1)]
				
1 ($R=CH_3$)	O	21.2	71.0	3.4
2 ($R=CH_3$)	S	85.2	n.d.[*2)]	----[*3)]
3 ($R=C_2H_5$)	O	61.0	14.6	0.24
4 ($R=C_2H_5$)	S	84.5	n.d.	----
				
9 ($n^{*4)}=3$)	O	3.5	86.6	24.7
10 (n=3)	S	88.5	n.d.	----
11 (n=4)	O	5.3	71.9	13.6
12 (n=4)	S	97.5	n.d.	----
13 (n=5)	O	49.6	26.4	0.53
14 (n=5)	S	83.1	n.d.	----

The reaction mixture included 0.1 mM compounds, 0.1 mM GSH and 0.8~0.9 units of GST in potassium phosphate buffer (0.05 M, pH 6.8); incubation for 30 min at 30 °C.
[*1)] Conversion rate = triazolidine formed (μmol)/thiadiazolidine remained (μmol). [*2)] n.d. = not detected. [*3)] ---- = no conversion. [*4)] n = the number of methylene.

of thiadiazolidin-2-ones apparently is influenced by bulkiness of substituents at moiety A together with the carbonyl group of moiety B (see Fig. 1). Specific data will be published elsewhere.

Acknowledgment

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