Phytogrowth-Inhibitory Activity of Sulphur-Containing Compounds. I. Inhibitory Activities of Thiazolidine Derivatives on Plant Growth

Yoshihiko INAMORI,* Chikaaki MURO, Ryoko TANAKA, Atsushi ADACHI, Katsushiro MIYAMOTO and Hiroshi TSUJIBO

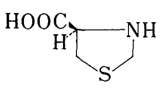
Osaka University of Pharmaceutical Sciences, Kawai, Matsubara-shi, Osaka 580, Japan. *Received March 4, 1992*

(*R*)-(−)-Thiazolidine-4-carboxylic acid (I, *l*-thioproline), (4*R*)-(−)-2-thioxo-4-thiazolidinecarboxylic acid (II), 2-thioxo-4-thiazolidinone (III, rhodanine), 4-oxo-2-thioxothiazolidine-3-acetic acid (IV, rhodanine-3-acetic acid) and thiazolidine (V) showed rather strong inhibitory activity on four kinds of plants examined at the concentration of 3.0×10^{-4} M. In particular, four compounds except for V strongly inhibited the growth of roots of all the plants examined at the concentration of 1.0×10^{-3} M. Furthermore, three compounds except for I and V inhibited the germination of the seeds of some plants at the concentration of 1.0×10^{-3} M. The amount of chlorophyll in the cotyledons of *Brassica rapa* L. treated with 1.0×10^{-3} M of I—V was decreased compared to that of the control group. Of the five compounds, III strongly inhibited the chlorophyll biosynthesis of this plant even at the concentration of 3.0×10^{-4} M.

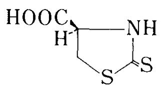
Keywords (*R*)-(−)-thiazolidine-4-carboxylic acid; (4*R*)-(−)-2-thioxo-4-thiazolidinecarboxylic acid; 2-thioxo-4-thiazolidinone; 4-oxo-2-thioxothiazolidine-3-acetic acid; thiazolidine derivative; phytogrowth-inhibitory activity; *Brassica rapa* L.; chlorophyll biosynthesis; inhibition

So far, sulphur-containing herbicides such as thiolcarbamates[1,2] and dithiocarbamate[3] have been synthesized. Asparagusic acid,[4] which contains sulphur atoms in the molecule, has already been isolated from *Asparagus officinalis* L. as a plant growth inhibitor. 1,3-Dithianium[5] and 1,4-dithianium[5-8] derivatives were also found to exhibit phytogrowth-inhibitory activity. Recently, we reported that 2,5-dihydroxy-1,4-dithiane-related compounds[9,10] had rather strong inhibitory activities on plant growth. Compound II (Chart 1, raphanusamic acid), a thiazolidine derivative, has already been isolated from *Raphanus sativus* var. *hortensis* f. *gigantissimus* MAKINO as a plant growth inhibitor.[11] However, there has been no report on the inhibitory activity of II on other plants except for the above plant and *Lactuca sativa* L. var. *longifolia* LAM. Also no work has been done on the phytogrowth-inhibitory activity of other thiazolidine derivatives.

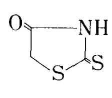
In this work, to expand knowledge of the phytogrowth-inhibitory activity of sulphur-containing compounds, the inhibitory effects of (*R*)-(−)-thiazolidine-4-carboxylic acid (I), (4*R*)-(−)-2-thioxo-4-thiazolidinecarboxylic acid (II), 2-thioxo-4-thiazolidinone (III, rhodanine), 4-oxo-2-thioxothiazolidine-3-acetic acid (IV, rhodanine-3-acetic acid) and thiazolidine (V) were investigated with four kinds of plants. The change of chlorophyll content in cotyledons of *Brassica rapa* L. treated with these compounds was also examined in order to clarify the mechanisms involved.



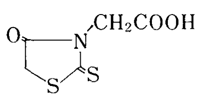
(*R*)-(−)-thiazolidine-4-carboxylic acid (I)



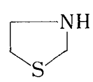
(4*R*)-(−)-2-thioxo-4-thiazolidinecarboxylic acid (II)



2-thioxo-4-thiazolidinone (III)



4-oxo-2-thioxothiazolidine-3-acetic acid (IV)



thiazolidine (V)

Chart 1

Materials and Methods

Chemicals (*R*)-(−)-Thiazolidine-4-carboxylic acid (I, Nacalai Tesque, Inc.), (4*R*)-(−)-2-thioxo-4-thiazolidinecarboxylic acid (II, Aldrich Chemical Co., Ltd.), 2-thioxo-4-thiazolidinone (III, Aldrich Chemical Co., Ltd.), 4-oxo-2-thioxothiazolidine-3-acetic acid (IV, Aldrich Chemical Co., Ltd.) and thiazolidine (V, Nacalai Tesque, Inc.) were used for the phytogrowth-inhibitory activity test. Sodium 2,4-dichlorophenoxyacetate (2,4-D, Tokyo Kasei Industry Co., Ltd.) was employed as a standard.

Plants The seeds of four kinds of plants listed in Table I was used for the inhibitory activity tests.

Phytogrowth-Inhibitory Activity Test[9] Aliquots (1 ml) of I (H_2O), II (dimethylsulfoxide, DMSO), III (DMSO), IV (DMSO), V (DMSO) and 2,4-D (H_2O) were each diluted in 100 ml of sterilized agar (0.8 %, Nacalai Tesque, Inc.) to concentrations of 1.0×10^{-3}—3.0×10^{-5} M. An agar containing chemical or DMSO alone (control) was poured into a 500 ml sterilized biopot. Then, 20 seeds of each plant sterilized with 70% EtOH and 1% NaClO were put on the agar and left for 7d under 9000 lux illumination. The length of the roots was measured and averaged. The phytogrowth-inhibitory activity was expressed as the ratio of the length of roots treated with the chemicals to that of the control (1.00).

Quantitative Analysis of Chlorophyll Contents of *B. rapa* Treated with I—V and 2,4-D The A.O.A.C. method[12] was used to determine the amounts of chlorophyll in the groups treated with I—V and 2,4-D as well as the control group at 7 d after treatment.

Results

Inhibitory Effects of the Five Thiazolidine Derivatives (I—V) on Plant Growth Inhibitory effects of I—V were investigated with four kinds of plants. The results are summarized in Table I. The five compounds inhibited the growth of all of the plants examined at the concentration of 3.0×10^{-4} M. Compounds III and IV showed strong inhibitory activity on the growth of *Daucus carota* L. var. *sativa* DC. even at the low concentration of 3.0×10^{-5} M. In particular, II inhibited the germination of the seeds of *D. carota* var. *sativa*, *B. rapa* and *Brassica rapa* L. var. *chinensis* KITAMURA at the concentration of 1.0×10^{-3} M. Compound IV also exhibited germination inhibition of the seeds of *D. carota* var. *sativa*, *Sesamum indicum* and *B. rapa* var. *chinensis* at the concentration of 1.0×10^{-3} M. Furthermore, III showed the germination inhibition of the seeds of *D. carota* var. *sativa*. and *S. indicum* at the concentration of 1.0×10^{-3} M. Although I did not completely inhibit the germination of the seeds of all of the plants examined at the concentration of 1.0×10^{-3} M, it showed rather strong inhibitory activity at the same concentration. Of the five compounds, V showed the weakest activity. The phytogrowth-inhibitory activity of the five compounds was much lower than that of 2,4-D used as a standard.

Toxicity of III to *B. rapa*. Observation of Growth Process The growth process of *B. rapa* following treatment with III was examined. As shown in Fig. 1, at 2 d after treatment, a strong inhibitory effect was observed in the groups treated with concentrations of 1.0×10^{-3} and 3.0×10^{-4} M. The inhibitory effect increased with the passage of time. The other compounds also showed inhibitory effects (data not shown).

TABLE I. Phytogrowth-Inhibitory Activities of I—V

Plant	Chemical	Growth (ratio)[a] Concentration (M) 1.0×10^{-3}	3.0×10^{-4}	1.0×10^{-4}	3.0×10^{-5}
Daucus carota L. var. *sativa* DC.	I	0.15	0.20	0.49	0.91
	II	—	0.01	0.77	0.58
	III	—	—	0.23	0.23
	IV	—	—	0.05	0.36
	V	0.20	0.69	1.18	1.14
	2,4-D	—	—	—	—
Sesamum indicum L.	I	0.06	0.05	0.09	0.20
	II	0.03	0.12	0.47	1.03
	III	—	0.03	0.11	0.94
	IV	—	0.03	0.56	0.89
	V	0.52	1.08	1.17	1.37
	2,4-D	—	—	—	—
Brassica rapa L.	I	0.03	0.21	0.54	0.74
	II	—	0.53	0.85	0.90
	III	0.05	0.22	0.74	1.06
	IV	0.01	0.22	1.09	0.78
	V	0.64	0.81	1.01	1.20
	2,4-D	0.02	0.02	0.05	0.06
Brassica rapa L. var. *chinensis* KITAMURA	I	0.09	0.13	0.17	0.29
	II	—	0.64	0.91	0.86
	III	0.04	0.16	0.62	0.76
	IV	—	0.10	0.26	0.84
	V	0.59	0.81	1.07	0.88
	2,4-D	—	0.05	0.08	0.09

a) Growth in control experiments after 7 d was taken as 1.00. Quantity of light: 9000 lux. Experimental size: 20 seeds/group, 2 groups. —: no germination.

Figure 2 shows the growth of the groups treated with III and the control at 7 d after treatment. It was found that all the groups showed growth inhibition.

Changes of Amounts of Chlorophyll in Cotyledons As the color of the cotyledons treated with higher concentrations than 3.0×10^{-4} M of I—V changed to yellowish white, the amounts of chlorophyll of the cotyledons were determined. As shown in Table II, the amounts were low in groups treated with 1.0×10^{-3} M of these compounds as compared with that of the control group. In particular, the amounts were markedly low in the group treated with 3.0×10^{-4} M of III; however, the inhibitory effects of I, II and IV at the same concentration were weaker than that of III. The amounts could not be measured in the groups treated with the concentrations of 1.0×10^{-3} M of II and

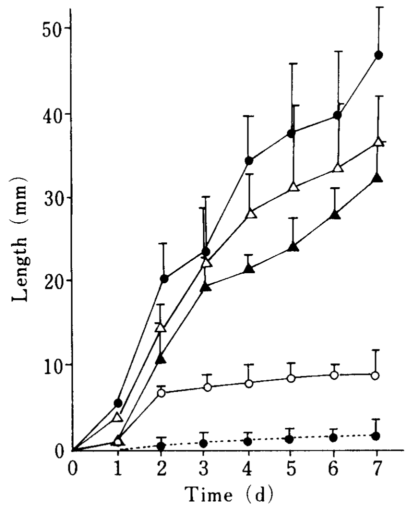


Fig. 1. Inhibitory Effect of III (Rhodanine) on the Growth of *Brassica rapa* L.

---●---, 1.0×10^{-3} M; —○—, 3.0×10^{-4} M; —▲—, 1.0×10^{-4} M; —△—, 3.0×10^{-5} M; —●—, control. Each value represents the mean ± S.D. (n = 20). Experimental size: 20 seeds/group, 2 groups.

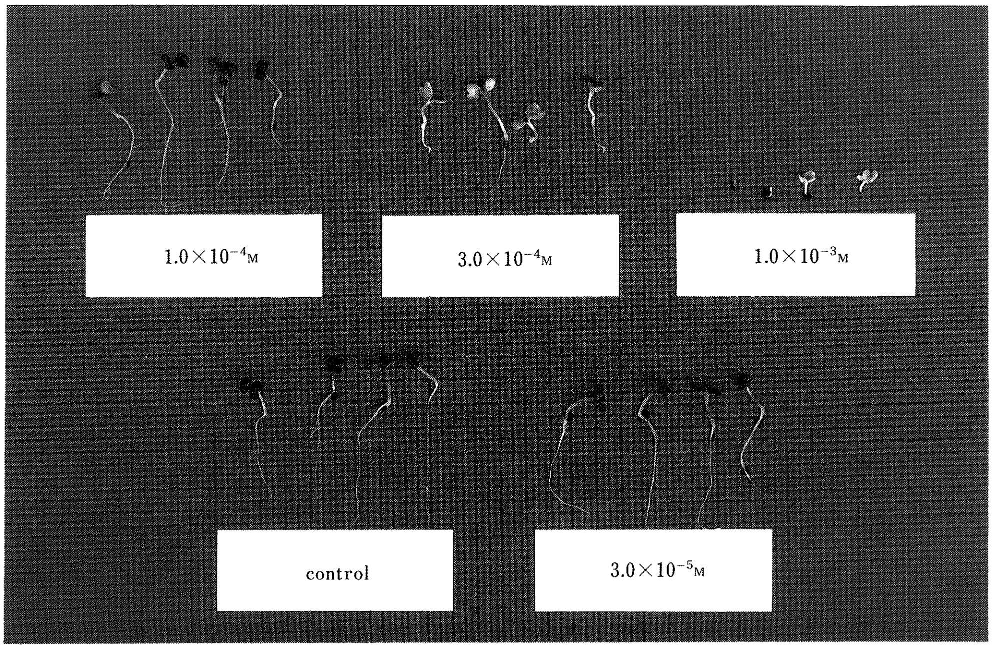


Fig. 2. Inhibitory Effect of III (Rhodanine) on the Growth of *B. rapa* L. at 7 d after Treatment

TABLE II. Chlorophyll Contents of *Brassica rapa* L. at 7 d after Treatment with the Five Thiazolidine Derivatives

Chemical	Concentration (M)	Total chlorophyll (%)[a]	Chlorophyll a (%)	Chlorophyll b (%)
(*R*)-(−)-Thiazolidine-4-carboxylic acid (I)	1.0×10^{-3}	5.575	3.428	2.152
	3.0×10^{-4}	12.596	8.562	4.044
	1.0×10^{-4}	10.579	6.870	3.716
(4*R*)-(−)-2-Thioxo-4-thiazolidine-carboxylic acid (II)[b]	1.0×10^{-3}	—[c]	—	—
	3.0×10^{-4}	13.748	9.478	4.279
	1.0×10^{-4}	25.023	18.009	7.030
2-Thioxo-4-thiazolidinone (III)[b]	1.0×10^{-3}	0.382	0.253	0.129
	3.0×10^{-4}	0.905	0.643	0.262
	1.0×10^{-4}	13.434	6.091	7.356
4-Oxo-2-thioxothiazolidine-3-acetic acid (IV)[b]	1.0×10^{-3}	—	—	—
	3.0×10^{-4}	13.329	9.553	3.784
	1.0×10^{-4}	14.396	13.934	0.468
Thiazolidine (V)[b]	1.0×10^{-3}	12.818	10.713	2.110
	3.0×10^{-4}	17.793	11.836	5.970
	1.0×10^{-4}	13.433	6.974	6.469
Sodium 2,4-dichlorophenoxyacetate (2,4-D)	1.0×10^{-3}	2.049	1.126	0.924
	3.0×10^{-4}	15.075	10.640	4.446
	1.0×10^{-4}	21.168	14.708	6.475
Control				
DMSO		18.570	13.166	5.416
H_2O		18.575	12.688	5.899

a) % (wet wt.). Analytical method: A.O.A.C. method. Observation time: 7 d. *b*) DMSO. *c*) —: not measured.

IV, because of strong germination inhibition. Among these compounds, the inhibitory activity of V was very weak. The inhibitory activities of II—IV at the concentration of 3.0×10^{-4} M were as strong as that of 2,4-D used as a standard, but the inhibitory activity of I at the concentration of 1.0×10^{-3} M was lower than that of 2,4-D.

Discussion

The results indicate that the phytogrowth-inhibitory action might be a common activity of thiazolidine derivatives. Considering that the inhibitory activities of I—IV were much stronger than that of V, the substituent at the C-4 position seems to play an important role in the increase of the phytogrowth-inhibitory activity of thiazolidine-related compounds (Table I). The chlorophyll contents showed a similar tendency (Table II). Compounds II—IV, unlike I, inhibited the germination of the seeds of some plants examined at the concentration of 1.0×10^{-3} M. These results suggest that the thiocarbonyl group of C-2 seems to be useful for increasing germination inhibition of thiazolidine derivatives.

As regards the action of these thiazolidine derivatives on plants, S. Oeriu and I. Oeriu already reported that earlier maturation of plants is caused by treatment with I.[13] Compound II was also isolated as a plant growth inhibitor[11]; however, reports on the activity of II to plant growth were few. In this respect, the broad phytogrowth-inhibitory activity of II in this work is noteworthy. On the other hand, the phytogrowth-inhibitory activities of the other four compounds, except for II, have been reported for the first time in this paper. In addition to thiazolidine derivatives, thiolcarbamates,[1,2] dithiocarbamate,[3] asparagusic acid,[4] 1,3-dithianium and 1,4-dithianium derivatives,[5-8] and 2,5-dihydroxy-1,4-dithiane-related compounds[9,10] have already been reported to have phytogrowth-inhibitory activity. These reports indicate that sulphur atoms in the molecule seem to play an important role in inhibitory activity on plant growth. We are planning to examine the phytogrowth-inhibitory activity of many thiazolidine derivatives.

References and Notes

1) H. Tilles and J. Antognini, U. S. Patent 2913327 (1959) [*Chem. Abstr.*, **54**, 9774*g* (1960)].
2) A. J. Watson, *N. C. Weed Control Conf. Res. Com.*, **14** (1959).
3) A. J. Overman and D. S. Burgis, *Proc. Florida Sta. Hort. Soc.*, **69**, 250 (1956).
4) H. Yanagawa, T. Kato and Y. Kitahara, *Tetrahedron Lett.*, **25**, 2549 (1972).
5) B. Zeeh, K. H. Koenig and J. Jung, *Kem-Kemi*, **1**, 621 (1974).
6) N. Klaus, L. Klaus and S. Klaus, Ger. Offen. 2331184 (1975) [*Chem. Abstr.*, **82**, 156332*p* (1975)].
7) N. Klaus, L. Klaus and S. Klaus. Ger. Offen. 2331185 (1975) [*Chem. Abstr.*, **82**, 170975*g* (1975)].
8) B. Zeeh, J. Jung and C. Rentzea, Ger. Offen. 2217697 (1973) [*Chem. Abstr.*, **80**, 44706*r* (1974)].
9) Y. Inamori, Y. Ohno. S. Nishihata, H. Tsujibo and K. Baba, *Chem. Pharm. Bull.*, **38**, 243 (1990).
10) Y. Inamori, C. Muro, H. Ohishi, K. Miyamoto, H. Tsujibo and K. Baba, *Chem. Pharm. Bull.*, **40**, 536 (1992).
11) T. Hase, *Rev. Latinoamer. Quim.*, **16**, 1 (1985).
12) "Official Methods of Analysis of the Association of Agricultural Chemists," 8th ed., The Association Washington D. C., 1955, p. 122.
13) S. Oeriu and I. Oeriu, Ger. Offen. 1912064 (1968) [*Chem. Abstr.*, **73**, 75984*q* (1970)].