Inhibitive Effects of 1,3-Dithiol Derivatives on the Growth and Sclerotium Formation of *Rhizoctonia solani* Kühn (AG-1)*

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1,3-dithiol derivatives.

Rhizoctonia solani Kühn inciting serious soilborne diseases produces sclerotia, propagules, which are tolerant to various adverse conditions in soil. The inhibition of sclerotium formation with chemicals will be one of the useful control measures to this sclerotium-forming pathogen. Formerly, the authors reported that sulfur-containing amino acids such as cysteine, cystine, homocysteine, taurine and methionine characteristically inhibited the sclerotium formation without any inhibition of mycelial growth¹⁻³⁾. Some of the sulfur-containing compounds such as phenylthiourea, phenylthioethanol, phenylthioacetic acid, dithiothreitol and thioglycolic acid were also inhibitive^{4,5)}. To clarify the relationship between chemical structures of sulfur-containing compounds and the inhibitive activities, the experiments with systematically synthesized 1,3-dithiol derivatives were carried out. The abstracts were reported elsewhere^{6,7)} and the details will appeared in this report.

Isolate R-1 of *Rhizoctonia solani* Kühn was pre-cultured on PSA medium at 28 C for 2 days. The edge of mycelial mat was cut with cork borer of ca. 5 mm in diameter and the mycelial disk was placed on the center of a square cellulose membrane (Visking Co. 4.5×4.5 cm) sheeted on the Hopkins agar medium in 9 cm Petri dish. After incubation at 28 C for 2 days, the mycelial mat was taken with cellulose membrane and transferred on the test medium which contains test chemicals, respectively. Forty derivatives of 1,3-dithiol which were synthesized by one of the authors, Kubota, S., were

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Table 1. Effects of 1,3-dithiol derivatives on sclerotium formation and mycelial growth

Chemicals (No.)	Formula	R ¹	R ²	R ³	R ⁴	Sclerotium formation	Mycelial growth
1		H	H	H	H	149	++
2		CH ₃	H	H	CH ₃	119	+
3		H	OH	H	H	266	++
4		H	-O-	H	H	103	++
5		H	CH ₃	H	H	188	+
6		H	iso-Bu	H	H	2	+++
7		H	Allyl	H	H	94	+
8		H	-(CH ₂) ₃ -	H	H	48	+
9		H	-(CH ₂) ₄ -	H	H	90	++
10		H	-O-	H	H	123	+
11		H	H	H	H	0	-
12		H	-(CH ₂) ₃ -	H	H	92	++
Check						100	++

Concentration: 300 ppm.

donated from the Laboratory of Agricultural Chemicals, Faculty of Agriculture, Kyushu University. Each chemical was added to Hopkins agar medium so as to contain 50, 150 and 300 ppm, respectively. Each plate was incubated at 28 C for 14 days in the dark. Sclerotia formed on the surface of a square cellulose membrane were picked up with forceps, dried at 60 C for 2 days and weighed. Dry weight of sclerotia was represented as an exponential value against the check. Each value in the Tables is mean of 3 replicates. The degree of mycelial growth was recorded as $- \sim +++$, comparing with the growth of the check.

The 1,3-dithiol compounds⁸⁻¹⁰⁾ tested were promotive or inhibitive to sclerotial formation (Table 1, 2). Among the stimulative chemicals, No. 3 and 19 compounds distinctly promoted the formation 2-3 times. While, 24 compounds out of 40 tested were highly inhibitive to the formation. Distinct inhibition was observed on the media containing No. 6, 8, 11, 13-18, 20-23, 25-27, 29, 31-34, 38-40 compounds, respectively, at 300 ppm. Most of the inhibitive compounds had 1,3-dithiane-2-thione structure except for No.6,8,11 compound. Some of them were also inhibitive even at 50 ppm (Table 3). The inhibitive activity of 1,3-dithiane-2-thione derivatives widely changed depending upon substituents. For example, No. 19, the compound substituted at R¹ position by

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Table 2. Effects of 1,3-dithiane-2-thione derivatives on sclerotium formation and mycelial growth

Chemicals (No.)	Formula	R ¹	R ²	R ³	R ⁴	Sclerotium formation	Mycelial growth
13		H	H	H	H	0	—
14		CH ₃	H	H	H	0	—
15		Et	H	H	H	0	—
16		n-Pr	H	H	H	0	—
17		iso-Pr	H	H	H	0	—
18		n-Bu	H	H	H	0	—
19		iso-Bu	H	H	H	343	+
20		n-Pe	H	H	H	0	—
21		Vinyl	H	H	H	0	—
22		Phenyl	H	H	H	0	—
23		H	CH ₃	H	H	0	—
24		H	iso-Pr	H	H	168	+
25		H	n-Bu	H	H	0	—
26		H	iso-Bu	H	H	0	—
27		H	sec-Bu	H	H	0	—
28		H	tert-Bu	H	H	103	+
29		H	Octyl	H	H	45	+
30		H	Phenyl	H	H	160	+
31		H	Benzyl	H	H	0	—
32		H	Allyl	H	H	0	—
33		H	OH	H	H	0	—
34		H	AcO	H	H	0	—
35		H	-(CH ₂) ₃ -		H	140	+
36		H	-(CH ₂) ₄ -		H	128	+
37		CH ₃	H	H	CH ₃	130	+
38		COOEt	H	H	COOEt	0	—
39		CH ₃	CH ₃	H	CH ₃	0	—
40		CH ₃	n-Bu	H	H	0	—
Check						100	≠

Concentration: 300 ppm.

isobutyl group was promotive, whereas others were highly inhibitive. Similar differences were observed among the compounds of which R² and/or R³ were substituted for -(CH₂)₃-, -(CH₂)₄-, isopropyl, *tert*-butyl, phenyl or others (Table 2).

Since 1,3-dithiane-2-thione derivatives were also inhibitive to the mycelial growth at 150–300 ppm, it was simply imagined, at first, that inhibition of the interweaving hyphal growth in the process of the sclerotium formation resulted in the inhibition of the sclerotium morphogenesis. However, some chemicals such as No. 13–17, 23, 25, 27, 29, 38, 39 were inhibitive to the formation without limitation of mycelial growth at 50 ppm. Thus, the mechanism of the inhibition could not be simple. Among the compounds tested, 2-isobutyltrimethylene bis(dimethyldithiocarbamate) (No. 6) was inhibitive to the sclerotium formation and was rather promotive to the mycelial growth. This result resembles with the cases of sulfur-containing amino acids^{1–3)}. Recently, Uno,

Table 3. Effects of 1,3-dithiane-2-thione derivatives on sclerotium formation and mycelial growth at various concentrations

Chemicals (No.)	Sclerotium formation			Mycelial growth (50ppm)
	50	150	300(ppm)	
13	66.8	0	0	++
14	21.4	0	0	++
15	41.7	0	0	++
16	33.4	0	0	++
17	28.9	0	0	++
18	70.4	0	0	+
20	24.5	0	0	+
21	n. t	0	0	n. t
22	21.0	0	0	+
23	8.8	0	0	++
25	20.0	0	0	++
26	158.5	0	0	++
27	53.5	0	0	++
29	75.9	5.6	45	++
31	88.1	0	0	+
32	97.5	0	0	++
33	5.9	0	0	+
34	21.4	0	0	+
38	8.1	3.0	0	++
39	46.5	0	0	++
40	146.5	4.9	0	++
Check	100.0	100.0	100.0	++

n. t: Not tested.

I. *et al.* reported the inhibition of cAMP-dependent sclerotium formation with adenine which inhibits adenylate cyclase activity and postulated the existence of other inhibition mechanisms in sulfur-containing amino acids^{11,12)}. Further investigation with relative compounds of 1,3-dithiols, especially No. 6 compound stated above, will give useful informations on the mechanisms of sclerotium formation and its inhibition.

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和 文 摘 要

松山宣明・矢原三知代・脇本 哲・窪田周平: *Rhizoctonia solani* Kühn (AG-1) の生育と菌核形成におよぼす 1, 3-dithiol 誘導体の抑制作用

含硫アミノ酸および一部の含硫化合物は、*R. solani* の菌核形成を阻害する。化合物の構造と阻害作用の関係を明らかにするため、系統的に合成された 1, 3-dithiol 誘導体40種について検討した(松山ら, 1984, 矢原ら, 1985)。これらの誘導体の作用は複雑で、構造によって促進的に働く場合と抑制的に働く場合がみられた。抑制効果を示した24種の化合物の内、1, 3-dithiane-2-thione 構造を有するものの作用は強く、150—130 ppm の濃度で菌糸の生育および菌核形成をほぼ完全に抑制した。しかし、50 ppm では菌糸生育は抑制せず菌核形成のみを抑制する場合が認められた。

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