Phytogrowth-Inhibitory and Antibacterial Activities of 2,5-Dihydroxy-1,4-dithiane and Its Derivatives

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2,5-Dihydroxy-1,4-dithiane (I) and its derivatives (II—IV) showed rather marked inhibitory activities on the growth of the roots of two plant species. All compounds tested had phytogrowth-inhibitory activities. These compounds markedly inhibited the growth of the two plant species at the concentration of 1.0×10^{-3} M. Seeds of *Brassica rapa* treated with 2,5-dihydroxy-2,5-dimethyl-1,4-dithiane (III) and its diacetate (IV) at the same concentration failed to germinate. Among these compounds, IV showed the most potent inhibitory activity on the two plant species. The radicles of both plant species treated with these compounds at concentrations higher than 1.0×10^{-4} M showed negative geotropism, even though germination occurred. The compounds except for 2,5-diacetoxy-1,4-dithiane (II) also had antibacterial activities. In particular, III had rather marked antibacterial activity and its minimal inhibitory concentration (MIC) for *Staphylococcus aureus* IFO-3060 and *Escherichia coli* IFO-12734 was 4.0 μg/ml.

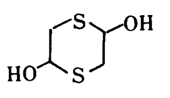
Keywords 2,5-dihydroxy-1,4-dithiane; 2,5-diacetoxy-1,4-dithiane; 2,5-dihydroxy-2,5-dimethyl-1,4-dithiane; 2,5-diacetoxy-2,5-dimethyl-1,4-dithiane; 1,4-dithiane derivative; phytogrowth-inhibitory activity; radicle; negative geotropism; antibacterial activity

Sulfur is an essential element for all organisms, being required for primary metabolites, *i.e.*, amino acids, vitamins, coenzymes and sulfur-containing peptides. Various biologically active substances containing sulfur such as nereistoxin,[1] brugine,[2] gerradine,[3] lenthionine,[4] 1,2,4,6-tetrathiopene,[5] aspargusic acid,[6] brugierol[7] and isobrugierol[7] have been isolated from various organisms. Among these compounds, nereistoxin and brugierol derivatives with insecticidal activity and aspargusic acid with phytogrowth-inhibitory activity have a 1,2-dithiolane skeleton in the molecule. It was also reported that 1,2-dithianum[8] and 1,4-dithianum derivatives[8-11] showed phytogrowth-inhibitory activities. These reports indicate the importance of the biological activities of the dithiane compounds. However, no work has yet been done on the phytogrowth-inhibitory activity of 2,5-dihydroxy-1,4-dithiane derivatives.

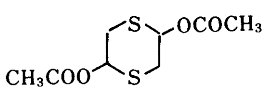
In this work, phytogrowth-inhibitor activities of 2,5-dihydroxy-1,4-dithiane (I, Chart 1) and its derivatives (II—IV, Chart 1) were investigated to extend our knowledge of the biological activity spectrum of dithiane-related compounds. The relationship between the antibacterial[12] and phytogrowth-inhibitory activities and the chemical structures of I and its derivatives (II—IV) is discussed.

Materials and Methods

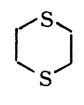
Chemicals 2,5-Dihydroxy-1,4-dithiane (I), 2,5-diacetoxy-1,4-dithiane (II), 2,5-dihydroxy-2,5-dimethyl-1,4-dithiane (III) and 2,5-diacetoxy-2,5-dimethyl-1,4-dithiane (IV) were used for the phytogrowth-inhibitory and antibacterial activity tests. Sodium 2,4-dichlorophenoxyacetate (Tokyo Kasei Industry Co., Ltd.) was employed as a standard. 2,5-Dihydroxy-1,4-dithiane (I, Aldrich Chemical Co., Ltd.): mp 130 °C (dec.). Proton nuclear magnetic resonance (^{1}H-NMR) (DMSO-d_6) δ ppm: 2.80 (2H, dd, J=13.9, 5.8 Hz), 3.71 (2H, dd, J=13.9, 1.5 Hz), 4.93 (2H, dd, J=5.8, 1.5 Hz). 2,5-Diacetoxy-1,4-dithiane (II): A solution of I (1.0 g) in a mixture of Ac_2O (10 ml) and pyridine (1 ml) was heated at 50—60 °C for 40 min and allowed to stand at room temperature overnight. After the reaction mixture had been treated in the usual way, the product was recrystallized from MeOH to give colorless needles, mp 178 °C (dec.). Yield: 690 mg. ^{1}H-NMR ($CDCl_3$) δ ppm: 2.18 (6H, s), 2.81 (2H, dd, J=4.7, 14.6 Hz), 3.69 (2H, dd, J=14.6, 1.8 Hz), 5.83 (2H, dd, J=4.7, 1.8 Hz). 2,5-Dihydroxy-2,5-dimethyl-1,4-dithiane (III, Aldrich Chemical Co., Ltd.): mp 64—65 °C (dec.). ^{1}H-NMR (DMSO-d_6) δ ppm: 1.48 (6H, s), 2.60 (2H, d, J=13.8 Hz), 3.69 (2H, d, J=13.8 Hz), 5.80 (2H, s). 2,5-Diacetoxy-2,5-dimethyl-1,4-dithiane (IV): A solution of III (300 mg) in a mixture of Ac_2O (10 ml) and pyridine (1 ml) was heated at 50—60 °C for 40 min and allowed to stand at room temperature overnight. After the reaction mixture had been treated in the usual way, the product was obtained as a yellow oil. Yield: 250 mg. ^{1}H-NMR (DMSO-d_6) δ ppm: 2.18 (6H, s), 2.35 (6H, s), 3.85 (4H, s).



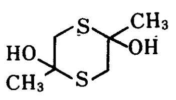
2, 5-dihydroxy-1, 4-dithiane (I)



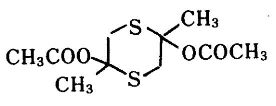
2, 5-diacetoxy-1, 4-dithiane (II)



1, 4-dithiane (V)



2, 5-dihydroxy-2, 5-dimethyl-1, 4-dithiane (III)



2, 5-diacetoxy-2, 5-dimethyl-1, 4-dithiane (IV)

Chart 1

Organisms The plants used were *Brassica rapa* L. and *Medicago sativa* L. The bacteria used were as follows: *Staphylococcus aureus* IFO-3060, *Bacillus subtilis* PCI-219, *Sarcina lutea* IAM-1099, *Escherichia coli* IFO-12734, *Proteus vulgaris* IFO-3851 and *Proteus mirabilis* IFO-3849.

Biological Activity Tests 1) Phytogrowth-Inhibitory Activity Test[13]: Dimethyl sulfoxide (DMSO) solutions of I—IV (each 1 ml) and sodium 2,4-dichlorophenoxyacetate (2,4-D) or DMSO alone (control) were each diluted in 100 ml of sterilized agar (0.8%, Difco Chemical Co., Ltd.) to give a concentration of 1.0×10^{-5}—1.0×10^{-3} M. The agar containing a test chemical or DMSO (control) was poured into a 500 ml sterilized beaker covered with aluminum foil. Then, 20 seeds of two plant species sterilized with 70% EtOH and 1% NaClO were put on the agar and left for 7 d at a light intensity of 9000 lx.[13] The phytogrowth-inhibitory activity was expressed as the ratio of the root length to that of the control (1.00).

2) Antibacterial Activity Test: The antibacterial activity test was carried out by the agar dilution method.[14] The test bacterium was applied to nutrient agar (Eiken Chemical Co., Ltd.) containing various concentrations of I—IV. The plates were incubated at 37 °C for 18 h and the bacterial growth was observed.

Results

Phytogrowth-Inhibitory Activities of 2,5-Dihydroxy-1,4-dithiane (I) and Its Derivatives (II—IV) The inhibitory activities of 2,5-dihydroxy-1,4-dithiane (I) and its derivatives (II—IV) on the two plant species were investigated according to the previous paper.[13] The results are summarized in Table I. All compounds tested markedly in-

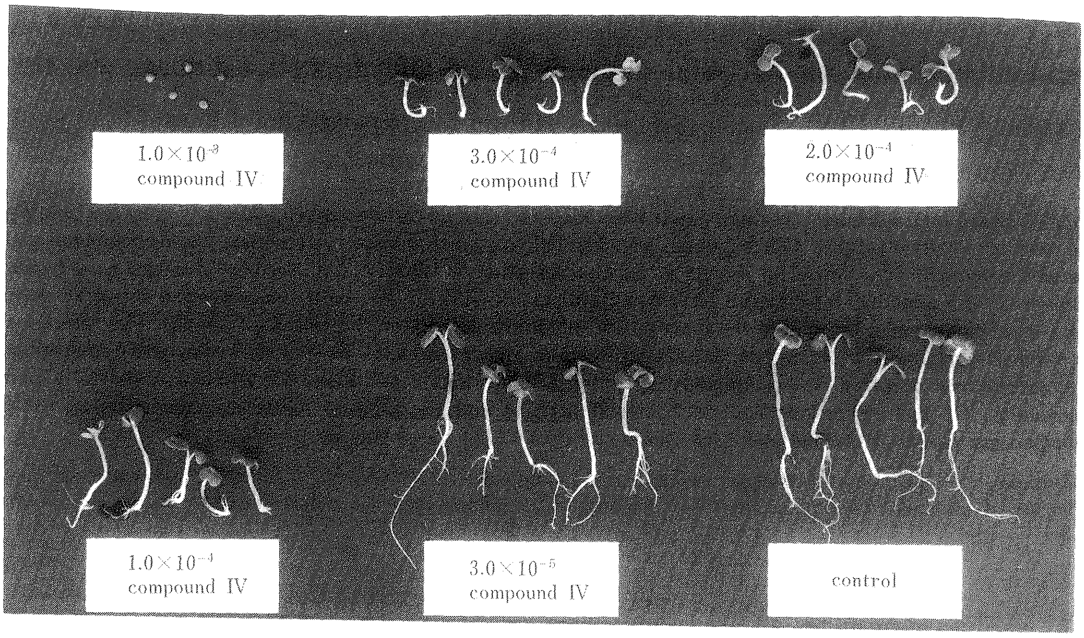


Fig. 1. Inhibitory Effect of 2,5-Diacetoxy-2,5-dimethyl-1,4-dithiane (IV) on the Growth of *Brassica rapa* L. at 7 d after Treatment

Table I. Inhibitory Activities of 2,5-Dihydroxy-1,4-dithiane (I) and Its Derivatives (II—IV) on Plant Growth

Plant	Concentration (M)	Growth (ratio)[a]				
		I	II	III	IV	2,4-D[b]
Brassica rapa L.	1.0×10^{-3}	0.06	0.05	—[c]	—[c]	0.01
	3.0×10^{-4}	0.82	0.06	0.11	0.18	0.01
	2.0×10^{-4}	1.02	0.14	0.52	0.11	0.02
	1.0×10^{-4}	1.36	0.32	0.87	0.28	0.05
	3.0×10^{-5}	1.40	1.01	0.91	0.85	0.10
Medicago sativa L.	1.0×10^{-3}	0.09	0.11	0.06	0.04	0.001
	3.0×10^{-4}	0.69	0.66	0.47	0.36	0.005
	2.0×10^{-4}	0.86	0.86	0.52	0.50	0.01
	1.0×10^{-4}	1.02	1.09	0.87	0.89	0.03
	3.0×10^{-5}	1.04	1.08	0.91	0.89	0.08

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. *b)* Sodium 2,4-dichlorophenoxyacetate. *c)* —: no germination.

hibited the growth of both plant species at the concentration of 1.0×10^{-3} M. In particular, compounds III and IV completely inhibited the germination of *Barssica rapa* L. at the concentration of 1.0×10^{-3} M. The inhibitory activities of the two diacetates II and IV were relatively strong even at the concentration of 1.0×10^{-4} M. Among the compounds, IV had the most potent phytogrowth-inhibitory activity. Figure 1 shows the difference of growth between the group treated with IV and the control group at 7 d after germination. It was found that germination in all the treated groups was inhibited compared with the control group.

Next, the growth process of *B. rapa* L. treated with these compounds was observed with the naked eye. At 1 d after germination, a rather marked inhibitory effect was found in every group treated with a concentration higher than 1.0×10^{-4} M and increased with the passage of time. The radicles in every treated group exhibited negative geotropism.

Table II. Antibacterial Activities of 2,5-Dihydroxy-1,4-dithiane (I) and Its Derivatives (II—IV)

Test bacteria	Antibacterial activity (μg/ml)			
	I	II	III	IV
Gram-positive bacteria				
Staphylococcus aureus IFO-3060	50.0	>500.0	4.0	20.0
Bacillus subtilis PCI-219	50.0	>500.0	8.0	20.0
Sarcina lutea IAM-1099	50.0	>500.0	50.0	90.0
Gram-negative bacteria				
Escherichia coli IFO-12734	20.0	>500.0	4.0	30.0
Proteus vulgaris IFO-3851	50.0	>500.0	20.0	50.0
Proteus mirabilis IFO-3849	50.0	>500.0	100.0	>500.0

Culture conditions: 37 °C, 18 h. Medium: Nutrient agar. Method: Agar dilution method.

Antibacterial Activities of 2,5-Dihydroxy-1,4-dithiane (I) and Its Derivatives (II—IV) The antibacterial activities of 2,5-dihydroxy-1,4-dithiane (I) and its derivatives (II—IV) were examined by the agar dilution method. As shown in Table II, the compounds other than II showed weak antibacterial activities. In particular, III had rather marked antibacterial activity and its minimal inhibitory concentration for both *Staphylococcus aureus* IFO-3060 and *Escherichia coli* IFO-12734 was 4.0 μg/ml. On the other hand, II did not show antibacterial activity against any of the bacteria tested.

Discussion

It was found that 2,5-dihydroxy-1,4-dithiane (I) had phytogrowth and antibacterial activities.

Phytogrowth-Inhibitory Activity Compound I inhibited the growth of the roots of two plant species (Table I). The radicles of both plant species treated with I showed negative geotropism.

Next, the relationship between the phytogrowth-inhibitory activity and the chemical structures of I and its derivatives (II—IV) was examined. The inhibitory activities of II, III and IV were stronger than that of the original compound I. Among them, IV had the strongest inhibitor activity against both plant species (Table I and Fig. 1). The results suggest that the methyl group plays some role in the inhibitory activity. The inhibitory effect was incresed by the introduction of acetyl groups into I and III. However, the reasons for these effects are not clear. At concentrations higher than 1.0×10^{-4} M, all radicles of the two plant species treated with these compounds showed negative seeds Yamaguchi and Street[15] have already reported that negative geotropism of radicles derived from soybean seeds after prolonged storage is due to plant growth regulator imbalance. In fact, we have found that the negative geotropism observed in this work could be normalized by treatment with some plant growth regulators.[16] As regards the phytogrowth-inhibitory activity of dithiane-related compounds, there are some reports on 1,3-dithianum[8] and 1,4-dithianum derivatives.[8-11] However, the phytogrowth-inhibitory activity of 2,5-dihydroxy-1,4-dithiane derivatives is reported for the first time in this paper.

Antibacterial Activity The antibacterial activity of I has already been reported,[12] but the relationship between the antibacterial activity and chemical structures of I and its derivatives (II—IV) has not been investigated as yet. Compound III had rather marked antibacterial activity (Table II). Like the phytogrowth-inhibitory activity, the antibacterial activity of III was stronger than that of I. The findings indicate that the methyl group, as in the case of the phytogrowth-inhibitory activity, plays some role in the antibacterial activity. On the other hand, II showed no antibacterial activity, and the activity of III was stronger than that of IV. The results suggest that free hydroxyl groups at the 2,5-positions of I and III favor the antibacterial activity of 1,4-dithiane-related compounds.

Considering that 1,4-dithiane (V, Chart 1) did not show phytogrowth-inhibitory or antibacterial activity (data not shown), it was concluded that hydroxyl groups or other substituents at the 2,5-positions of the 1,4-dithiane skeleton are necessary for these compounds to show both activities.

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