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Antifungal Activity against Plant-Pathogenic Fungi and Phytogrowth-Inhibitory Activity of 3,3′-Dihydroxy-α,β-diethyldiphenylethane

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3,3′-Dihydroxy-α,β-diethyldiphenylethane (I), an isomer of hexestrol (IV), showed antifungal activity against plant-pathogenic fungi and phytogrowth-inhibitory activity. First, compound I had antifungal activity against all plant-pathogenic fungi examined. The antifungal spectrum of I was similar to those of IV, diethylstilbestrol (III) and 3,3′-dihydroxy-α,β-diethylstilbene (II). However, compound I, unlike II, III and IV, showed antifungal activity against *Ceratocystis fimbriata* IFO-4864. Next, compound I strongly inhibited the growth of roots of two plant species even at the low concentration of 50 ppm. The inhibitory effect of I was as strong as that of sodium 2,4-dichlorophenoxyacetate used as a standard.

It is noteworthy that compound I, in spite of the loss of hormonal side effect, retained the above-mentioned activities.

Keywords——3,3′-dihydroxy-α,β-diethyldiphenylethane; 3,3′-dihydroxy-α,β-diethylstilbene; diethylstilbestrol; hexestrol; oxystilbene-related compound; phytogrowth-inhibitory activity; antifungal activity; hormonal side effect; sodium 2,4-dichlorophenoxyacetate

Nonsteroidal estrogens, diethylstilbestrol (III, Chart 1)[1-3] and hexestrol (IV, Chart 1)[2,4] have been reported to have coronary vasodilator action, phytogrowth-inhibitory activity, ichthyotoxicity, antifungal activity and hypotensive effect by the authors. It was also found that in spite of the loss of hormonal side effect,[5] the above-mentioned activities of 3,3′-dihydroxy-α,β-diethylstilbene (II, Chart 1),[3,6] an isomer of III, were fully retained. Recently, we reported that 3,3′-dihydroxy-α,β-diethyldiphenylethane (I, Chart 1),[7] the dihydrocompound of II, had rather strong coronary vasodilator action on the isolated guinea-pig heart. Compound I like II, showed no hormonal side effect. In this respect, the above-mentioned activities of I and II are noteworthy. However, no work has been done on other biological activities of I except for coronary vasodilator action.

In this work, the antifungal activity against plant-pathogenic fungi and the phytogrowth-inhibitory activity of I were investigated. The results are presented here.

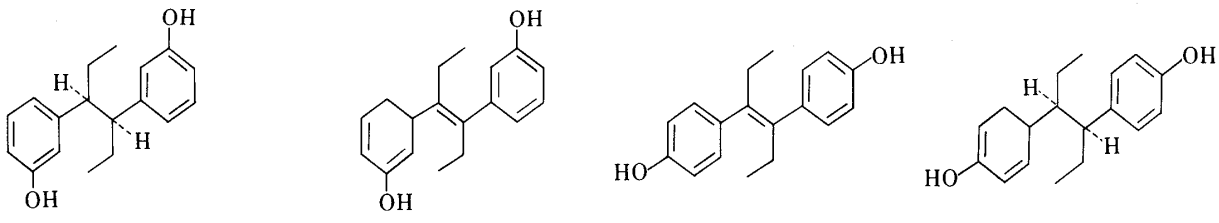

Chart 1

Materials and Methods

Chemicals——3,3′-Dihydroxy-α,β-diethyldiphenylethane (I) was used for the biological activity tests. I was synthesized by the reduction of II [I: mp 100—101 °C (dec.)]. Sodium 2,4-dichlorophenoxyacetate was used as a standard for the phytogrowth-inhibitory activity test.

Organisms——The plant-pathogenic fungi used were: *Boryotinia fuckeliana* IFO-9760, *Pyrenophora graminea* IFO-6633, *Rhizoctonia solani* IFO-30464, *Cochliobolus miyabeanus* IFO-4870, *Ceratocystis fimbriata* IFO-4864, *Fusarium oxysporum* f. sp. *lycopersici* IFO-6531 and *Aureobasidium pullulans* IFO-4464. The plants used were *Brassica rapa* L. and *Raphanus sativus* L. var. *raphanistroides* MAKINO.

Biological Activity Tests——1) Antifungal activity test: Antifungal activity was tested by the agar dilution method. The media used were potato sucrose agar in all cases except for *Fusarium oxysporum* f. sp. *lycopersici* IFO-6531 (potato dextrose agar: Eiken Chemical Co., Ltd.). The test fungi were applied to media containing various concentrations of I. The plates were incubated at 27 °C for 5 d and the growth was observed with the naked eye. 2) Phytogrowth-inhibitory activity test[8]: Aliquots (1 ml) of acetone solution of I and sodium 2,4-dichlorophenoxyacetate were each diluted in 100 ml of sterilized agar (0.8%, Difco Chemical Co., Ltd.) to the concentration of 50 ppm. The agar containing chemicals or acetone alone (control) was poured into a 500-ml sterilized beaker covered with aluminum foil. Then, 20 seeds of each plant sterilized with 70% EtOH and 1% NaClO were put on the agar and left for 7 d under 9000 lux illumination. The lengths of the roots were measured and averaged. The phytogrowth-inhibitory activity was expressed as the ratio of the length of roots to that of the control (1.00).

Results

Antifungal Activity of 3,3′-Dihydroxy-α,β-diethyldiphenylethane (I) against Plant-Pathogenic Fungi

The antifungal activity of I against plant-pathogenic fungi was examined by the agar dilution method. The results are summarized in Table I. Compound I showed antifungal activity against all the plant-pathogenic fungi examined. The antifungal spectrum of I was similar to those of compounds II—IV, but compound I, unlike II—IV, had antifungal activity

TABLE I. Antifungal Activity of 3,3′-Dihydroxy-α,β-diethyldiphenylethane (I) against Plant-Pathogenic Fungi

Fungi	Antifungal activity (μg/ml)			
	I	II[3]	III[3]	IV[4]
Fusarium oxysporum f. sp. *lycopersici* IFO-6531	10.0	4.0	7.0	5.0
Botryotinia fuckeliana IFO-9760	10.0	4.0	50.0	10.0
Pyrenophora graminea IFO-6633	10.0	4.0	10.0	10.0
Aureobasidium pullulans IFO-4464	30.0	25.0	25.0	20.0
Cochliobolus miyabeanus IFO-4870	10.0	25.0	50.0	25.0
Rhizoctonia solani IFO-30464	20.0	25.0	50.0	1000.0
Ceratocystis fimbriata IFO-4864	40.0	1000.0	>1000.0	1000.0

Culture conditions: 27 °C, 5 d. Media: potato sucrose agar (*Fusarium oxysporum* f. sp. *lycopersici* IFO-6531, potato dextrose agar). Method: agar dilution method.

TABLE II. Phytogrowth-Inhibitory Activity of 3,3′-Dihydroxy-α,β-diethyldiphenylethane (I)

Plant	Growth (ratio)[a]				
	I	II[3]	III[3]	IV[4]	2,4-D[b]
Brassica rapa L.	0.03	0.46	0.43	0.49	0.06
Raphanus satvus L. var. *raphanistroides* MAKINO	0.16	0.83	0.65	0.41	0.10

a) Growth in control experiments after 7 d was taken as 1.00. Concentration: 50 ppm. Quantity of light: 9000 lux. Experimental size: 20 seeds/group, 2 groups. *b*) Sodium 2,4-dichlorophenoxyacetate.

against *Ceratocystis fimbriata* IFO-4864.

Phytogrowth-Inhibitory Activity of I

The inhibitory effect of I on two plant species was investigated according to the previous paper.[8] As shown in Table II, compound I showed strong phytogrowth-inhibitory activity even at the low concentration of 50 ppm. The effect of I was much stronger than those of compounds II—IV. The inhibitory activity of I on both plants was as strong as that of sodium 2,4-dichlorophenoxyacetate used as a standard.

Discussion

Like other oxystilbene-related compounds already tested,[1-4,6,7,9,10] 3,3′-dihydroxy-α,β-diethyldiphenylethane (I) was found to have antifungal activity against plant-pathogenic fungi and phytogrowth-inhibitory activity.

Antifungal Activity on Plant-Pathogenic Fungi

Like compounds II—IV, I showed antifungal activity against all the plant-pathogenic fungi tested (Table I). In addition to I—IV, 3,4-*O*-isopropylidene-3,3′,4,5′-tetrahydroxystilbene,[10] having phenolic hydroxyl groups on the stilbene skeleton, shows antifungal activity against plant-pathogenic fungi. Several papers have also appeared on phytolexin which is an oxystilbene compound, in common with I—IV. The findings suggest that antifugal activity against plant-pathogenic fungi might be a common biological activity of oxystilbene-related compounds. Further studies on the antifungal activity of other oxystilbene-related compounds against fungi seem to be desirable.

Phytogrowth-Inhibitory Activity

The inhibitory activity of I was as strong as that of sodium 2,4-dichlorophenoxyacetate used as a standard (Table II). In this respect, the inhibitory activity of I of plant growth is of considerable interest. Growth inhibition of both plants was also found in 3,3′,4,5′-tetrahydroxystilbene[9] and 3,3′,4,5′-tetrahydroxybibenzyl.[9] The results indicate that oxystilbene-related compounds generally have phytogrowth-inhibitory activities. This consideration is supported by the fact that plant growth inhibitors, batatasin III[11] and lunularin,[12] having the same basic skeleton as I were isolated from higher plants. Further studies on the phytogrowth-inhibitory activity of many oxystilbene-related compounds are in progress.

Like II,[5] compound I had no hormonal side effect. In this respect, the above-mentioned biological activities of I are noteworthy.

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