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# Biological Activities of Hexesterol

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·Hexestrol showed antifungal activity on plant-pathogenic fungi, phytogrowth-inhibitory activity and a hypotensive effect on rats. Firstly, hexestrol exhibited antifungal activity on all plant-pathogenic fungi tested except for *Rhizoctonia solani* IFO-30464 and *Ceratoystis fimbriata* IFO-4864. In particular, hexestrol strongly inhibited the growth of *Fusarium oxysporum* f. sp. *lycopersici* IFO-6531 (minimal inhibitory concentration: 5 $\mu$g/ml). Secondly, hexestrol had inhibitory activity on the growth of the root of *Raphanus sativus* L. var. *raphanistroides* MAKINO even at the low concentration of 50 ppm. Thirdly, hexestrol showed a transient hypotensive effect on rats ($-40.00 \pm 2.60$ mmHg, 10 mg/kg, i.v.).

**Keywords**——hexestrol; oxybibenzyl compound; oxystilbene compound; antifungal activity; plant-pathogenic fungus; hypotensive effect; *Fusarium oxysporum* f. sp. *lycopersici* IFO-6531; *Raphanus sativus* L. var. *raphanistroides* MAKINO; phytogrowth-inhibitory activity

As already reported, oxystilbene compounds such as 3,3′,4,5′-tetrahydroxystilbene,[1,2] 3,4-*O*-isopropylidene-3,3′,4,5′-tetrahydroxystilbene,[3,4] diethylstilbestrol[5,6] and 3,3′-dihydroxy-$\alpha,\beta$-diethylstilbene[7,8] have various biological activities, *i.e.*, antifungal activity, ichthyotoxicity, coronary vasodilator action on the isolated guinea-pig heart, phytogrowth-inhibitory activity and a hypotensive effect on rats. It was found that oxybibenzyl compounds, hexestrol (Chart l)[6] and 3,3,′4,5′-tetrahydroxybibenzyl,[2] showed (1) coronary vasodilator action, (2) ichthyotoxicity, and (3) antifungal activity. However, the antifungal activity on plant-pathogenic fungi, the phytogrowth-inhibitory activity and the hypotensive effect on rats have not been investigated in the case of hexestrol as yet. Furthermore, phytoalexins[9,10] which have the basic skeleton in common with hexestrol have been reported. Plant growth inhibitors, batatasin III[11] and lunularin,[12] with the same basic skeleton as hexestrol were also isolated from higher plants. Recently, we reported that diethylstilbestrol,[8] a dehydroderivative of hexestrol, had antifungal activity on plant-pathogenic fungi, phytogrowth-inhibitory activity and a hypotensive effect on rats. Therefore, in this work, the antifungal activity on plant-pathogenic fungi, phytogrowth-inhibitory activity and hypotensive effect on rats were examined in the case of hexestrol to extend our knowledge of the biological activities of oxybibenzyl compounds.

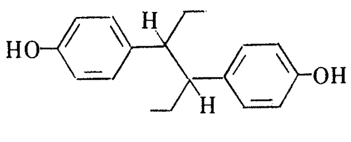

Chart 1. Chemical Structure of Hexestrol

### Materials and Methods

**Chemicals**——Hexestrol (Aldrich Chemical Co., Ltd., mp 186—189 °C) was used. Sodium 2,4-dichlorophenoxyacetate (Nakarai Chemical Co., Ltd.) was used as a standard for the phytogrowth-inhibitory activity test.

**Organisms**——Plant-pathogenic fungi used were as follows: *Aureobasidium pullulans* IFO-4464, *Botryotinia* *fuckeliana* IFO-9760, *Ceratosystis fimbriata* IFO-4864, *Cochliobolus miyabeanus* IFO-4870, *Pyrenophora graminea* IFO—6633, *Rhizoctonia solani* IFO-30464 and *Fusarium oxysporum* f.sp. *lycopersici* IFO-6531. The plants used were *Brassica rapa* L. and *Raphanus sativus* L. var. *raphanistroides* MAKINO. The animals used for the experiments on blood pressure were Wistar strain rats weighing 300—350 g (3 rats/group). The blood pressure prior to drug administration was $130.4 \pm 22.4$ mmHg ($n=9$).

**Biological Activity Tests**——1) Antifungal Activity Test: The antifungal activity test was carried out by the agar dilution method. The media used were as follows: 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 hexestrol. The plates were incubated at 27 °C for 5 d and the growth was observed with the naked eye.

2) Phytogrowth-Inhibitory Activity Test: The inhibitory activity test was carried out according to the method of Hirai *et al.*[13] Namely, acetone solutions of hexestrol and sodium 2,4-dichlorophenoxyacetate were diluted in 100 ml of sterilized agar (0.8%, Difco) to the concentration of 50 ppm. The agar containing hexestrol, sodium 2,4-dichlorophenoxyacetate and 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 a light intensity of 600 lux.[14] The length of the root of each plant was measured and averaged. The phytogrowth-inhibitory activity was expressed as the ratio of the root length to that of the control (1.00).

3) Measurement of Blood Pressure[4]: Systemic blood pressure was measured with a pressure transducer (Nihon Kohden Kogyo Co., Ltd. P-23ID, AP-601 G) following cannulation of the carotid artery in rats under anesthesia with sodium pentobarbital (40 mg/kg, i.p.). Hexestrol was suspended in 5% acacia and administered *via* the femoral vein. It was shown that 5% acacia had no effect on blood pressure.

## Results

### Antifungal Activity of Hexestrol on Plant-Pathogenic Fungi

As shown in Table I, hexestrol showed antifungal activity on all plant-pathogenic fungi tested except for *Rhizoctonia solani* IFO-30464 and *Ceratoystis fimbriata* IFO-4864. In particular, hexestrol strongly inhibited the growth of *Fusarium oxysporum* f. sp. *lycopersici* IFO-6531.

### Phytogrowth-Inhibitory Activity of Hexestrol

As shown in Table II, hexestrol showed inhibitory activity on the root of two kinds of plants even at the low concentration of 50 ppm. The inhibitory activity of hexestrol on *Raphanus sativus* L. var. *raphanistroides* MAKINO was relatively strong. However, the phytogrowth-inhibitory activity of hexestrol was much weaker than that of sodium 2,4-dichlorophenoxyacetate used as a standard.

TABLE I. Antifungal Activity of Hexestrol on Plant-Pathogenic Fungi

| Fungi | Antifungal activity MIC (μg/ml) |
|---|---|
| *Fusarium oxysporum* f. sp. *lycopersici* IFO-6531 | 5.0 |
| *Botrytinia fuckeliana* IFO-9760 | 10.0 |
| *Pyrenophora graminea* IFO-6633 | 10.0 |
| *Aureobasidium pullulans* IFO-4464 | 20.0 |
| *Cochliobolus miyabeanus* IFO-4870 | 25.0 |
| *Rhizoctonia solani* IFO-30464 | 1000.0 |
| *Ceratoystis fimbriata* IFO-4864 | 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. MIC: minimal inhibitory concentration.

TABLE II. Inhibitory Effect of Hexestrol on Plant Growth

| Plant | Growth (ratio)[a] | |
|---|---|---|
| | Hexestrol | 2,4-D[b] |
| *Brassica rapa* L. | 0.49 | 0.06 |
| *Raphanus sativus* L. var. *raphanistroides* MAKINO | 0.41 | 0.10 |

*a*) Growth in control experiments after 7 d was taken as 1.00. Concentration, 50 ppm; quantity of light, 600 lux; experimental size, 20 seeds/group, 2 groups. *b*) Sodium 2,4-dichlorophenoxyacetate.

Table III. Effect of Hexestrol on Blood Pressure in Anesthetized Rats

| Dose (mg/kg) | Mean arterial blood pressure (mmHg) |
|---|---|
| 10 | $-40.00 \pm 2.60$ |
| 20 | $-49.00 \pm 12.0$ |
| 30 | $-55.00 \pm 0.64$ |

Each value represents the mean±S.D. of 3 rats. Route, intravenous injection; body weight, 300—350 g. The blood pressure prior to drug administration was 130.4±22.4 mmHg ($n=9$). Anesthetic: pentobarbital-Na (40 mg/kg, i.p.).

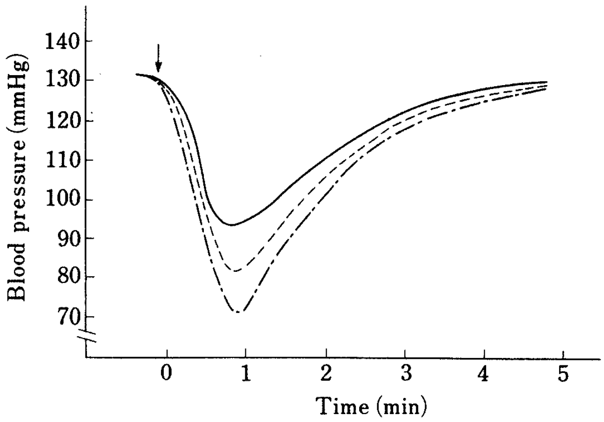


Fig. 1. Effect of Hexestrol on the Blood Pressure of Anesthetized Rats

The value represents the mean of 3 rats. Body weight: 300—350 g. Arrow: injection of 10, 20 or 30 mg/kg of hexestrol.
——, 10 mg/kg; ------, 20 mg/kg; – – – –, 30 mg/kg.

**Effect of Hexestrol on Blood Pressure in Rats**

As shown in Table III, hexestrol showed hypotensive activity (−40 mmHg, 10 mg/kg, i.v.), and the activity increased strongly with increase of the dose of this compound. As shown in Fig. 1, the hypotensive action of hexestrol was transient and the blood pressure recovered to the original level within 5 min. The pattern of the time course of hexestrol was similar to those of other oxybibenzyl and oxystilbene compounds *i.e.*, 3,3′,4,5′-tetrahydroxybibenzyl,[2] diethylstilbestrol,[8] 3,3′,4,5′-tetrahydroxystilbene[2] diethylstilbestrol,[8] 3,3,′4,5′-tetrahydroxystilbene[2]·and 3,4-*O*-isopropylidene-3,3′,4,5′-tetrahydroxystilbene.[4]

## Discussion

In addition to estrogenic effect, coronary vasodilator action and ichthyotoxicity, hexestrol was found to have antifungal activity on plant-pathogenic fungi, phytogrowth-inhibitory activity and a hypotensive effect on rats.

**Antifungal Activity on Plant-Pathogenic Fungi**

Hexestrol showed antifungal activity on plant-pathogenic fungi except for *Rhizoctonia solani* IFO-30464 and *Ceratoystis fimbriata* IFO-4864 (Table I). In particular, hexestrol showed strong antifungal activity on *Fusarium oxysporum* f. sp. *lycopersici* IFO-6531. The antifungal activities of oxybibenzyl and oxystilbene compounds such as hexestrol [MIC ($\mu$g/ml): 5.0],diethylstilbestrol [MIC ($\mu$g/ml): 7.0],[6] 3,4-*O*-isopropylidene-3,3′,4,5′-tetrahydroxystilbene [MIC ($\mu$g/ml): 1.0][4] and 3,3′-dihydroxy-$\alpha,\beta$-diethylstilbene [MIC ($\mu$g/ml): 4.0][8] are all characterized by rather strong growth-inhibitory activity against *F. oxysporum* f. sp *lycopersici* IFO-6531. Further studies on the antifungal activities of many oxybibenzyl and oxystilbene compounds against plant-pathogenic fungi seem to be desirable.

**Phytogrowth-Inhibitory Activity**

Hexestrol, at the concentration of 50 ppm, inhibited the root growth of two kinds of plants (Table II). In particular, hexestrol strongly inhibited the growth of *Raphanus sativus* L. var. *raphnistroides* Makino, though the reason for this is not still clear. The inhibitory activity seems to be intrinsic to oxybibenzyl and oxystilbene compounds; hexestrol, 3,3′,4,5′-tetrahydroxybibenzyl,[1] 3,3′-dihydroxy-$\alpha,\beta$-diethyldiphenylethane,[15] 3,3′,4,5′-tetrahydroxystilbene[1] diethylstilbestrol[8] and 3,3′-dihydroxy-$\alpha,\beta$-diethylstilbene[8] all showed phytogrowth-inhibitory activities.

**Hypotensive Effect on Rats**

Hexestrol showed a transient hypotensive effect on rats (Table III and Fig. 1). The hypotensive effect of hexestrol on rats is considered to be a common pharmacological activity of oxybibenzyl and oxystilbene compounds, because 3,3′,4,5′-tetrahydroxybibenzyl,[2] 3,3′-dihydroxy-$\alpha$,$\beta$-diethyldiphenylethane,[15] 3,3′,4,5′-tetrahydroxystilbene[2] 3,4-*O*-isopropylidene-3,3′,4,5′-tetrahydroxystilbene,[4] diethylstilbestrol[8] and 3,3′-dihydroxy-$\alpha$,$\beta$-diethylstilbene[8] all show similar effects. The hypotensive effects of all oxybibenzyl and oxystilbene compounds tested were transient and the pattern of their time courses was similar to that of hexestrol (Fig. 1). We are now investigating the hypotensive effects of many oxybibenzyl and oxystilbene compounds.

As mentioned above, oxybibenzyl compounds show a broad spectrum of biological activities. However, coralgil with the same basic skeleton as hexestrol was already reported to have the severe side effects of phospholipidosis of the liver[16] and lung cells,[17] and foam cell syndrome.[18] The findings indicate that hexestrol may have the same side effects as coralgil. Therefore, studies on the side effects of hexestrol and other oxybibenzyl compounds in rats are in progress.

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