Hypotensive Effects on Spontaneously Hypertensive Rats and Antifungal Activity on Various Species of *Fusarium oxysporum* of Diethylstilbestrol-Related Compounds

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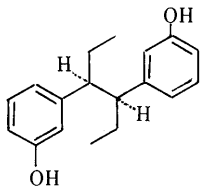
The diethylstilbestrol-related compounds 3,3′-dihydroxy-α,β-diethyldiphenylethane (I), diethylstilbestrol (II) and hexestrol (III) showed hypotensive effects on spontaneously hypertensive rats (SHR) and antifungal activities against all *Fusarium oxysporum* sp. tested. As previously reported, I had strong hypotensive action on normotensive rats at the dose of 10 mg/kg, while II and III showed weak hypotensive effects on these rats at the same dose. In this work, all three compounds also had hypotensive actions on SHR at the same dose. I showed the strongest hypotensive effect (−80.0±5.0 mmHg, 10 mg/kg, i.v.) on both SHR and normotensive rats. The three compounds also had antifungal activities against five kinds of *Fusarium oxysporum* sp. tested. Especially, II strongly inhibited the growth of *Fusarium oxysporum* f. sp. *raphani* IFO-9972 (minimum inhibitory concentration (MIC): 1.0 μg/ml).

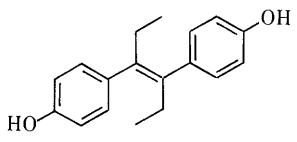
Keywords diethylstilbestrol-related compound; 3,3′-dihydroxy-α,β-diethyldiphenylethane; diethylstilbestrol; hexestrol; hypotensive effect; hypertensive rat; antifungal activity; *Fusarium*; oxystilbene-related compound

Oxystilbene-related compounds such as 3,3′-dihydroxy-α,β-diethyldiphenylethane (I),[1,2,3] diethylstilbestrol (II),[4] hexestrol (III)[5,6] and 3,3′-dihydroxy-α,β-diethylstilbene (IV)[4] (Chart 1) have already been reported to show various biological activities, *i.e.*, antifungal activity, coronary vasodilator action on the isolated guinea pig heart, phytogrowth-inhibitory activity and hypotensive effect on normotensive rats. Among these compounds, I had the strongest action on normotensive rats at the dose of 10 mg/kg. Compound I—IV also exhibited antifungal activities against plant-pathogenic fungi, in particular strongly inhibiting the growth of *Fusarium oxysporum* f. sp. *lycopersici* IFO-6531.[1,4,5]

As a preliminary step in the investigation of the mechanism of hypotensive action of diethylstilbestrol-related compounds, the effects of compounds I, II and III on blood pressure in SHR were examined here. Attention was also focused on their antifungal activity on various species of *Fusarium oxysporum* in an effort to extend the antifungal spectrum of diethylstilbestrol-related compounds.



3,3′-dihydroxy-α, β-diethyldiphenylethane (I)



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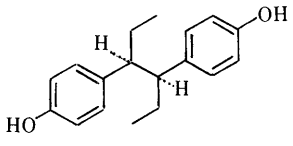
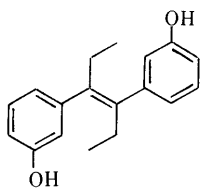


hexestrol (III)



3,3′-dihydroxy-α, β-diethylstilbene (IV)

Chart 1

Materials and Methods

Chemicals 3,3′-Dihydroxy-α,β-diethyldiphenylethane (I),[1] diethylstilbestrol (II)[4] and hexestrol (III)[5,6] were used.

Animals Male spontaneously hypertensive rats (SHR) weighing 324—383 g from the colony raised by the Department of Pharmacology, Jichi Medical School, were used for the experiments on blood pressure. The blood pressure prior to drug administration was 155.0±5.8 mmHg ($n=9$).

Fungi *Fusarium oxysporum* sp. used for the antifungal activity test were as follows: *Fusarium oxysporum* f. sp. *raphani* IFO-9972, *F. oxysporum* f. sp. *conglutians* IFO-6383, *F. oxysporum* f. sp. *cucumernum* IFO-6384, *F. oxysporum* f. sp. *melonis* IFO-6385 and *F. oxysporum* f. sp. *niveum* IFO-4471.

Measurement of Blood Pressure Systemic blood pressure was measured with a pressure transducer (Nihon Kohden Kogyo Co., Ltd., TP-2000T, RM-6000) following cannulation of the carotid artery in rats under anesthesia with sodium pentobarbital (40 mg/kg, i.p.). Compounds I, II and III were suspended in 5% acacia and administered *via* the femoral vein.

Antifungal Activity Antifungal activity test was carried out by the agar dilution method. The medium used was potato dextrose agar (Eiken Chemical Co., Ltd.,). The test fungi were applied to media containing various concentrations of I, II and III. The plates were incubated at 27 °C for 5 d and growth was observed with the naked eye.

Results and Discussion

Effects of 3,3′-Dihydroxy-α,β-diethyldiphenylethane (I), Diethylstilbestrol (II) and Hexestrol (III) on SHR As shown in Fig. 1, three compounds I, II and III had hypotensive actions in SHR, with that of compound I rather strong: the group given 10 mg/kg of I showed a drop in blood pressure of 80.0±5.0 mmHg. This drop was found even at doses of 2.5 mg/kg (−48.0±13.7 mmHg) and 5.0 mg/kg (−76.0±0.6 mmHg). The strong hypotensive effect of I is also supported by our recent paper[3] that this compound demonstrated a relaxing action on the contraction of guinea pig mesenteric artery due to KCl and norepinephrine at low concentrations of 10^{-6}—10^{-5} M. On the other hand, the hypotensive actions of II and III (II: −35.3±7.3 mmHg, 10 mg/kg, i.v., III: −45.7±17.9 mmHg, 20 mg/kg, i.v.) were much weaker. The pattern of the time course of III was different from to those of I and II, and it showed a greater delay in decreasing blood pressure. Following administration of I and II, blood pressure level was not restored to the original level within 30 min, while it recovered to the original level within 25 min

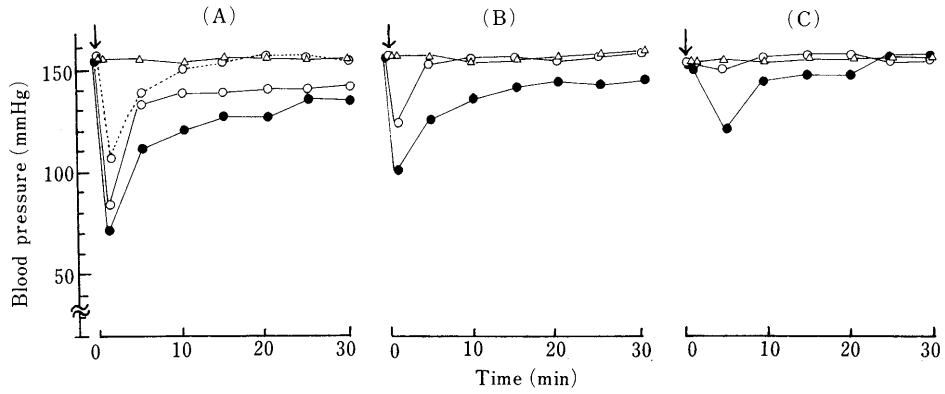


Fig. 1. Effects of (A) 3,3′-Dihydroxy-α,β-diethyldiphenylethane (I), (B) Diethylstilbestrol (II) and (C) Hexestrol (III) on Blood Pressure in SHR

(A) Compound I: ○---○, 2.5 mg/kg; ○—○, 5.0 mg/kg; ●—●, 10.0 mg/kg. (B) Compound II: ○—○, 10.0 mg/kg; ●—●, 20 mg/kg. (C) Compound III: ○—○, 10.0 mg/kg; ●—●, 20 mg/kg. △—△, 5% acacia. Each value represents the mean of 3 rats. Body weight: 324—383 g. Arrow: injection (i.v.). Compounds I, II and III were suspended in 5% acacia and administered *via* the femoral vein. 5% acacia had no effect on blood pressure.

TABLE I. Antifungal Activities of 3,3′-Dihydroxy-α,β-diethyldiphenylethane (I), Diethylstilbestrol (II) and Hexestrol (III) on Various Species of *Fusarium oxysporum*

Fusarium oxysporum species	Antifungal activity (MIC: μg/ml)		
	I	II	III
Fusarium oxysporum f. sp. *raphani* IFO-9972	7.0	1.0	2.0
Fusarium oxysporum f. sp. *conglutians* IFO-6383	5.0	3.0	5.0
Fusarium oxysporum f. sp. *cucumernum* IFO-6384	5.0	4.0	5.0
Fusarium oxysporum f. sp. *melonis* IFO-6385	5.0	6.0	5.0
Fusarium oxysporum f. sp. *niveum* IFO-4471	10.0	5.0	10.0
Fusarium oxysporum f. sp. *lycopersici* IFO-6531	10.0[1)]	7.0[4)]	5.0[5)]

Culture conditions: 27 °C, 5 d. Medium: potato dextrose agar. Assay method: agar dilution method. MIC: minimal inhibitory concentration.

following administration of III. In addition to I,[2)] II[4)] and III,[5)] IV,[7)] 3,3′,4,5′-tetrahydroxystilbene,[8)] 3,3′,4,5′-tetrahydroxybibenzyl[8)] and 3,4-*O*-isopropylidene-3,3′,4,5′-tetrahydroxystilbene,[9)] which are also oxystilbene-related compounds showed hypotensive effects on normotensive rats. The hypotensive action of diethylstilbestrol-related compounds on SHR, however, is reported for the first time in this paper. Considering that SHR are animal models of human hypertension, the strong drop in blood pressure caused by I is noteworthy. Studies on the hypotensive effect of many oxystilbene-related compounds in SHR are in progress.

Antifungal Activities of 3,3′-Dihydroxy-α,β-diethyldiphenylethane (I), Diethylstilbestrol (II) and Hexestrol (III) on Various Species of *Fusarium oxysporum* *Fusarium oxysporum* sp. are well known not only as plant pathogenic fungi, but also causative organisms of keratomycosis; however, no low-toxicity antifungal substances on these fungi have been discovered as yet. As I, II and III have already been found to show antifungal activity against *Fusarium oxysporum* f. sp. *lycopersici* IFO-6531,[1,4,5)] the antifungal activities of these three compounds against five kinds of *Fusarium oxysporum* sp. were further examined by the agar dilution method. The results are summarized in Table I. Compounds I, II and III showed antifungal activities against all *Fusarium oxysporum* sp. tested. In particular, II had the strongest antifungal activity against *F. oxysporum* f. sp. *raphani* IFO-9972 (MIC: 1.0 μg/ml). The results are supported by our previous paper that 3,4-*O*-isopropylidene-3,3′,4,5′-tetrahydroxystilbene (MIC: 1.0 μg/ml)[9)] and 3,3′-dihydroxy-α,β-diethylstilbene (IV, MIC: 4.0 μg/ml)[4)] also showed antifungal activities against *F. oxysporum* f. sp. *lycopersici* IFO-6531. In addition to I, II and III, the oxystilbene-related compounds of 3,3′,4,5′-tetrahydroxystilbene, 3,3′,4,5′-tetrahydroxybibenzyl and 3,4-*O*-isopropylidene-3,3′,4,5′-tetrahydroxystilbene were confirmed to show antifungal activity against these five kinds of *Fusarium oxysporum* sp.[10)] The antifungal activity of oxystilbene-related compounds against various species of *Fusarium oxysporum* is thus noteworthy. Therefore, further studies on the antifungal activity of many oxystilbene derivatives against these fungi are desirable.

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References and Notes

1) Y. Inamori, H. Tsujibo, M. Ogawa, Y. Miki and S. Takemura, *Chem. Pharm. Bull.*, **36**, 3173 (1988).
2) Y. Inamori, M. Ogawa, Y. Ohno, S. Nishihata, H. Tsujibo, Y. Miki and S. Takemura, *Chem. Pharm. Bull.*, **37**, 3137 (1989).
3) Y. Inamori, H. Tsujibo, H. Hachiken, Y. Miki and S. Takemura, *Chem. Pharm. Bull.*, **36**, 1252 (1988).
4) Y. Inamori, M. Kubo, M. Ogawa, H. Tsujibo, Y. Miki and S. Takemura, *Chem. Pharm. Bull.*, **35**, 3502 (1987).
5) Y. Inamori, T. Kobayashi, M. Ogawa and H. Tsujibo, *Chem. Pharm. Bull.*, **36**, 815 (1988).
6) Y. Inamori, M. Kubo, M. Ogawa, M. Moriwaki, H. Tsujibo, K. Baba and M. Kozawa, *Chem. Pharm. Bull.*, **33**, 4478 (1985).
7) Y. Inamori, M. Kubo, H. Tsujibo, M. Ogawa, Y. Saito, Y. Miki and S. Takemura, *Chem. Pharm. Bull.*, **35**, 887 (1987).
8) Y. Inamori, M. Kubo, Y. Kato, M. Yasuda, K. Baba and M. Kozawa, *Yakugaku Zasshi*, **104**, 819 (1984).
9) Y. Inamori, M. Kubo, Y. Kato, M. Yasuda, H. Tsujibo, K. Baba and M. Kozawa, *Chem. Pharm. Bull.*, **33**, 2904 (1985).
10) The antifungal activities of 3,3′,4,5′-tetrahydroxystilbene, 3,3′,4,5′-tetrahydroxybibenzyl and 3,4-*O*-isopropylidene-3,3′,4,5′-tetrahydroxystilbene on five kinds of *Fusarium oxysporum* sp. will be given in the next paper.