Communications to the Editor

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THE ICHTHYOTOXICITY AND CORONARY VASODILATOR ACTION OF DIETHYLSTILBESTROL

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Diethylstilbestrol (I), a nonsteroidal estrogen, showed strong ichthyotoxicity and coronary vasodilator action on isolated guinea-pig hearts. The median ichthyotoxicity tolerance limit (TLm) at 48 h was 3.30 ppm in *Oryzias latipes* TEMMINCK *et* SCHLEGEL and 4.50 ppm in *Carassius auratus* LINNE. The vasodilator activity of I in isolated guinea-pig hearts (ED_{50}: 0.26 μg/heart) was much stronger than that of papaverine (7.0 μg/heart) used as a standard.

KEYWORDS ———— diethylstilbestrol; 3,3',4,5'-tetrahydroxystilbene; 3,4-O-isopropylidene-3,3',4,5'-tetrahydroxystilbene; oxystilbene derivative; ichthyotoxicity; coronary vasodilator action; nonsteroidal estrogen

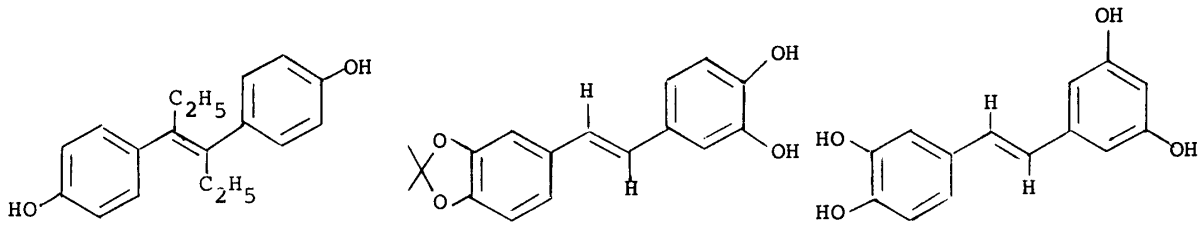
It has been reported that 3,3',4,5'-tetrahydroxystilbene (III, Fig. 1) isolated from the heartwood of *Cassia garrettiana* CRAIB has strong antifungal activity,[1] ichthyotoxicity,[1] phytogrowth-inhibitory activity,[1] coronary vasodilator action on isolated guinea-pig hearts, and hypotensive effects on rats.[2]

Recently, we[3,4] reported that the above-mentioned activities of 3,4-O-isopropylidene-3,3',4,5'-tetrahydroxystilbene (II, Fig. 1), which is chemically derived from III, were much stronger than those of III except for the phytogrowth inhibitory activity.

Subsequently, we examined various biological activities of oxystilbene derivatives to obtain more strongly active substances. As a result, we confirmed that diethylstilbestrol (I, Aldrich Chemical Co., Ltd., mp. 170-172°C, Fig. 1), one of the oxystilbene derivatives, is strongly ichthyotoxic and has coronary vasodilator action on isolated guinea-pig hearts.

In this paper, we wish to report the ichthyotoxicity of I and its coronary vasodilator action on the isolated guinea-pig hearts. It is well known that I has strong estrogenic activity. Also it has antimicrobial activity,[5,6] synergism with antibiotics[7] and hypotensive effects.[8] However, the ichthyotoxicity and coronary vasodilator action of I have not been reported.

The ichthyotoxic activity of I on *Oryzias latipes* and *Carassius auratus* was examined by the method of Sugawara and Koyama.[9] The median tolerance limit (TLm) was calculated according to the Doudoroff method.[10] The TLm at 48 h was, as shown in Table



I II III

Fig. 1. Chemical Structures of Diethylstilbestrol (I), 3,4-O-Isopropylidene-3,3',4,5'-Tetrahydroxystilbene (II) and 3,3',4,5'-Tetrahydroxystilbene (III)

I, 3.30 ppm in *O. latipes* and 4.50 ppm in *C. auratus*, indicating strong toxic effects on both fishes. Recently, we reported that II[4] and III[2] are rather strongly in both fishes. However, the ichthyotoxicity of I was stronger than that of II and III (Table I). These findings suggest that the ichthyotoxicity of oxystilbene derivatives is the intrinsically physiological.

Table I. The Ichthyotoxicity of Diethylstilbestrol (I)

Fish	TLm (48 h, ppm)			
	I	II[4]	III[2]	Rotenone
Oryzias latipes TEMMINCK *et* SCHLEGEL	3.30	14.0	26.5	0.030
Carassius auratus L.	4.50	18.4	31.5	0.033

Calculation of TLm: Doudoroff method.
Temperature: 25°C.
Experimental size: 10 fish/group, 2 groups.

The effect of I on the isolated guinea-pig hearts was investigated by the Langendorff method. The results are summarized in Table II. The action of I was much stronger than that of the papaverine standard. On the other hand, unlike papaverine, I did not show a cardiotonic effect. In this respect I resembles II and III. We previously reported that the hydroxyl group attached to the benzene ring and the *trans*-olefin structure in molecule are necessary for oxystilbene derivatives to show the coronary vasodilator action.[2] Compound (I) also fills the necessary requirements. In fact, phloroglucinol,[11] which has the same polyphenol structure in the molecule as I, II and III, relaxed the smooth muscle. The relaxation of the smooth muscle by curcumine,[12] which has the polyphenol and the *trans*-olefin structure in common with I, II and III, has also been reported. The coronary vasodilator action of oxystilbene derivatives is considered to be an intrinsic phar-

macological activity for two reasons: 1) I, II[4] and III[2] show rather strong coronary vasodilator action and 2) this activity by other stilbene derivatives, i.e., piceid,[13] and rhapontin[13] has also been confirmed.

Table II. Cardiac Effect of Diethylstilbestrol (I) on Isolated Guinea-Pig Heart

Compound	Coronary vasodilation (ED_{50} μg/heart)	Cardiotonic effect
I	0.26	n.e.
II[4]	4.5	n.e.
III[2]	13.0	n.e.
Papaverine	7.0	p.i.

Animals: male Hartley strain guinea-pigs (body weight: 400-500 g). The guinea-pig hearts were rapidly isolated and perfused with Krebs-Hensleit solution according to the Langendorff method. Drugs (0.1 ml in 10% DMSO) were administered directly into the perfusion solution through a connecting rubber tube. DMSO (10%) has no effect on coronary vasodilation. Transducer: Force Diplacement Transducer 45196 (SAN-EI Instrument Co., Ltd.) and MPU-0.5-290-0-3 (Nihon Kohden Kogyo Co., Ltd.)
The relative potency of the test compounds was determined as that perfusion pressure which induced vasodilation by 50% of the maximum response produced by papaverine at 33 μg/heart (ED_{50}).
n.e.: no effect, p.i.: positive inotropic effect.

From the these results it is apparent that diethylstilbestrol (I) has strong ichthyotoxicity and coronary vasodilator action on isolated guinea-pig hearts. The fact that all oxystilbene derivatives examined had the ichthyotoxic and coronary vasodilator action is of considerable interest.
Further studies of I in relation to various biological activities are in progress, together with chemical modification of the mechanisms.

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