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The Biological Activities of Podophyllotoxin Compounds[1)]

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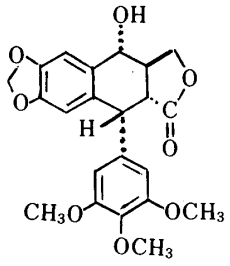
Podophyllotoxin (I), like deoxypodophyllotoxin (II), showed various biological activities. First, I had ichthyotoxic activity: the median tolerance limit at 48 h was 0.73 ppm in *Oryzias latipes* and 1.20 ppm in *Carassius auratus*. Second, I showed inhibitory activities on the growth of two plant species at a concentration of 50 ppm. Third, I showed insecticidal activities against all insects examined except for *Spodoptera litura*. A rather strong insecticidal activity toward *Epilachna sparsa orientalis* larvae was found, with mortality of 95% at 20 ppm. Furthermore, I and II had marked antitumor activities against L 5178 Y mouse leukemic cells *in vitro* [ID_{50} (μg/ml): II, 0.0047 and I, 0.0054].

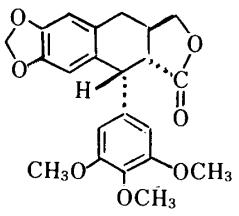
Keywords——podophyllotoxin; deoxypodophyllotoxin; lignan; ichthyotoxic activity; phytogrowth-inhibitory activity; insecticidal activity; antitumor activity; L 5178 Y mouse leukemic cell

Podophyllotoxin (I, Chart 1)[2)] is a lignan with a phenyltetralin skeleton. It has already been reported that I shows mitotic spindle inhibition[3)] and significant antitumor activities.[4,5)] Recently, we reported that deoxypodophyllotoxin (II, Chart 1), having the same basic skeleton as I, exhibits ichthyotoxic,[6)] phytogrowth-inhibitory[6)] and insecticidal activities.[7-9)]

In the present work, we further investigated these activities of I, in order to compare them with those of II. The inhibitory effects of I and II on L 5178 Y mouse leukemic cells *in vitro* were also examined.



podophyllotoxin (I)



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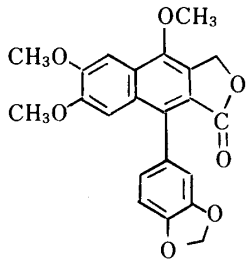
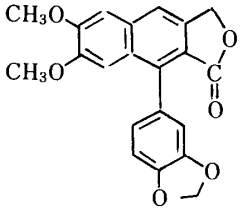


justicidin A (III)



justicidin B (IV)

Chart 1

Materials and Methods

Chemicals——Podophyllotoxin (I, Sigma Chemical Co.) and deoxypodophyllotoxin (II),[10)] which was isolated from the root of *Anthriscus sylvestris* HOFFM by the authors, were used. Rotenone (Kanto Kasei Co., Ltd.) was used as a standard for the ichthyotoxicity test, and sodium 2,4-dichlorophenoxyacetate (Nakarai Chemical Co., Ltd.) for the phytogrowth-inhibitory activity test.

Organisms——The fishes used were *Oryzias latipes* TEMMINCK et SCHLEGEL and *Carassius auratus* L. The plants used were *Brassica rapa* L. and *Medicago sativa* L. The insects used were as follows: adults of *Blattella germanica* L; larvae of *Culex pipiens molestus* FARSKAL; larvae of *Epilachna sparsa orientalis* DIEKE; larvae of *Plutella xylostella*

CURTIS; larvae of *Spodoptera litura* FABRICIUS. The tumor used was L 5178 Y mouse leukemic cell line.

Biological Activity Tests——1) Ichthyotoxicity Test: A method described by Sugawara and Koyama[11] was employed. The median tolerance limit (TLm) at 48 h was calculated according to the Doudoroff method.[12]

2) Phytogrowth-Inhibitory Activity Test: Acetone solutions 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 solution (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 at a light intensity of 600 lux.[13] The length of the root of each plant was measured and averaged. The phytogrowth-inhibitory activity was expressed as the ratio of the length of roots to that of the control (1.00).

3) Insecticidal Activity Test: Bait method[7]: A slice of bread was dipped into the acetone solution of I, then dried in air, and fed to adults of *B. germanica* together with water. The mortality was examined after 13 d. Topical application method[7]: The acetone solution of I was applied to the ventral surface of the abdomen of adults of *B. germanica* which had been anesthetized with CO_2. Subsequently, both bait and water were fed. The mortality was examined after 13 d. Leaf dipping method[7]: i) The leaves of soybean plants were dipped into an aqueous drug solution (containing 0.03% of a spreader, Dine,® Takeda Chemical Industries, Ltd.), then dried in air, and fed to the larvae of *S. litura*. The mortality was examined after 24 h. ii) *P. xylostella* was released on cabbage leaves which had been treated in the same way as described above. The mortality was examined after 24 h. Potato dipping method[7]: Round slices of potato were dipped into an aqueous drug solution (containing 0.03% of Dine®), and fed to the larvae of *E. sparsa orientalis*. The mortality was examined after 48 h. Immersion method[7]: The larvae of *C. pipiens molestus* were released into an aqueous solution of drug. The mortality was examined after 48 h.

4) Assay of Growth-Inhibitory Activity against L 5178 Y Cells: The inhibitory action on the growth of L 5178 Y mouse leukemic cells was determined as described previously.[14] Briefly, the leukemic cells were grown in RPMI 1640 medium (Nissui Co., Ltd.) supplemented with 10% bovine serum at 37 °C. An appropriate amount of the sample in serial half-$\log_{10}$ dilutions was added to 9 volumes of a suspension of cells in the logarithmic phase (1.6×10^5 cells per ml). After 2 d of incubation at 37 °C, the number of cells per tube was counted with a microcell counter, Sysmex CC-110, and the 50% inhibitory dose (ID_{50}) was obtained graphically.

Results

Ichthyotoxic Activity of Podophyllotoxin (I)

The ichthyotoxic activity of I was examined with *Oryzias latipes* and *Carassius auratus*. The results are summarized in Table I. Like II, I showed ichthyotoxic activity: the TLm at 48 h was 0.73 ppm in *O. latipes* and 1.20 ppm in *C. auratus*. Its activity, however, was lower than that of rotenone used as a standard.

Phytogrowth-Inhibitory Activities of Podophyllotoxin (I) and Deoxypodophyllotoxin (II)

The phytogrowth-inhibitory activities of I and II were examined according to the method of Hirai *et al.*[13] and compared. As shown in Table II, both chemicals inhibited the growth of roots of both kinds of plants, but their activity was much lower than that of sodium 2,4-dichlorophenoxyacetate used as a standard. Like the ichthyotoxic activity, the phytogrowth-inhibitory activity of I was weaker than that of II.

Insecticidal Activity of Podophyllotoxin (I)

The insecticidal activity of I on five kinds of insects was examined using various assay

TABLE I. Ichthyotoxicity of Podophyllotoxin (I)

Fish	TLm (ppm, 48 h)		
	I	II[a]	Rotenone
Oryzias latipes TEMMINCK *et* SCHLEGEL	0.73	0.06	0.03
Carassius auratus L.	1.20	0.70	0.03

Calculation of TLm: Doudoroff method. Temperature: 27 °C. Experimental size: 10 fish/group, 2 groups. *a*) Ref. 6.

TABLE II. Inhibitory Effects of Podophyllotoxin (I) and Deoxypodophyllotoxin (II) on the Growth of the Roots of Test Plants

Plant	Growth (ratio)[a]		
	I[b]	II[b]	2,4-D[b,c]
Brassica rapa L.	0.65	0.22	0.06
Medicago sativa L.	0.78	0.24	0.17

a) Growth in control experiments after 7 d was taken as 1.00. *b*) Concentration: 50 ppm. Temperature: 25 °C. Experimental size: 20 seeds/group, 2 groups. Quantity of light: 600 Lux. *c*) Sodium 2,4-dichlorophenoxyacetate.

TABLE III. Insecticidal Activities of Podophyllotoxin (I)[a]

Insect	Method	Concentration (ppm)	Mortality (%)	
			I	II[b]
Blattella germanica[c] (adult)	Bait	1000	40.0	100.0
		500	30.0	100.0
	Topical application	170[f]	25.0	80.0
Epilachna sparsa orientalis[d] (larvae)	Tuber dip	20	95.0	N.T.[g]
		100	100.0	55.0
		500	100.0	85.0
Plutella xylostella[e] (larvae)	Leaf dip	500	0	30.0
		1000	40.9	55.0
Culex pipiens molestus[d] (larvae)	Immersion	20	50.0	90.0
Spodoptera litura[e] (larvae)	Leaf dip	500	0	0

a) Observation time: *c*) 13 d, *d*) 48 h, *e*) 24 h. Temperature: 26 °C. Experimental size: 10 insects/group, 2 groups. *b*) Ref. 7. *f*) μg/g. *g*) Not tested.

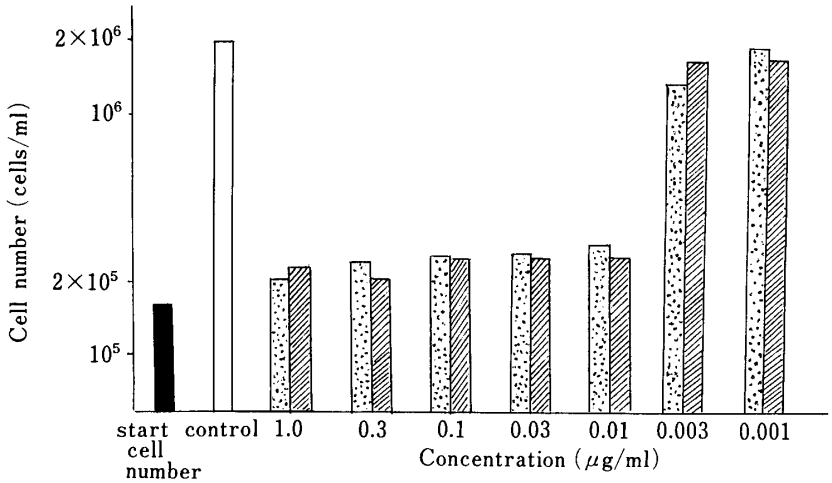


Fig. 1. Effects of Podophyllotoxin (I) and Deoxypodophyllotoxin (II) on the Growth of L 5178 Y Cells *in Vitro* during Incubation for 48 h

□, control; ▨, I; ▩, II; ■, starting cell number: 1.6×10^5, control at 48 h: 2.6×10^6.

methods. As shown in Table III, the insecticidal activity of I was weaker than that of II except in the case of *Epilachna sparsa orientalis* larvae, where it was much stronger than that of II.

Antitumor Activities of Podophyllotoxin (I) and Deoxypodophyllotoxin (II)

The inhibitory effects of I and II on L 5178 Y mouse leukemic cells *in vitro* were investigated. As shown in Fig. 1, both chemicals markedly inhibited the growth of L 5178 Y cells. Complete inhibition by both chemicals at 48 h was observed at 0.01 μg/ml; the 50% inhibition dose (ID_{50}) of II was 0.0047 μg/ml and that of I was 0.0054 μg/ml.

Discussion

It was found that podophyllotoxin (I), like deoxypodophyllotoxin (II), exhibited ichthyotoxic, phytogrowth-inhibitory and insecticidal activities. Both chemicals also showed marked inhibitory activities against L 5178 Y mouse leukemic cells *in vitro*.

(1) Ichthyotoxic Activity

Ichthyotoxic activity of I was examined, taking rotenone, which is typical of the ichthyotoxic components isolated from plants, as a standard. Compound I had a significant ichthyotoxic activity against *O. latipes*; TLm at 48 h was 0.73 ppm (Table I). However, analogous compounds, justicidin A (III) (TLm_{48}: 0.02 ppm),[15] justicidin B (IV) (TLm_{48}:0.04 ppm),[15] and II (TLm_{48}: 0.06 ppm),[6] were more potent (Chart 1).

(2) Phytogrowth-Inhibitory Activity

Although the inhibitory activity of II has been examined,[6] it was tested again by the present method,[13] which is more sensitive. Sodium 2,4-dichlorophenoxyacetate, a herbicide, was used as a standard. Compound I inhibited the growth of the roots of both kinds of plants (Table II), but it was weaker than II. The inhibitory effect seems to be intrinsic to podophyllotoxin related compounds; I, II and podophyllic acid 2-ethylhydrazide[16] all showed phytogrowth-inhibitory activity. Further studies of the effect of I and II on various kinds of plants are in progress.

(3) Insecticidal Activity

Like II[7,8] and other lignans,[17,18] I exhibited insecticidal activity. In particular, it caused the death of 95% of the larvae of *Epilachna sparsa orientalis* at the concentration of 20 ppm (Table III). The insecticidal activity of I was weaker than that of II[7] except for this action on *Epilachna sparsa orientalis*. However, the insecticidal effect of podophyllotoxin derivatives is noteworthy in the following respects: 1) II showed strong delayed insecticidal activities against adults of *Blattella germanica* (LD_{50}: 8.43 μg/insect) and *Culex pipiens pallens* (LD_{50}: 0.34 μg/insect),[19] 2) the insecticidal activity of II is much stronger than that of the conventional insecticide, boric acid-bait agent, and 3) I and II inhibited the pupation of housefly larvae and adult emergence.[20]

(4) Antitumor Activity

The inhibitory effects of I and II on L 5178 Y mouse leukemic cells *in vitro* were investigated for the first time. Both chemicals showed a marked inhibitory activity towards this carcinoma (Fig. 1). It has been reported that the maximum observed *T*/*C* value (%) of I on P-388 mouse leukemia *in vivo* was 171 at 3 mg/kg/d.[21] Unfortunately, these compounds can not be used clinically, because of their high toxicity. Thus, development of less toxic derivatives is required, and studies on the chemical modification of II are in progress. The activities of I were weaker than those of II, but it is not clear whether the difference is due to (1) the decrease in lipid solubility caused by the hydroxyl group of I or (2) the decrease in organotropy of metabolites produced by the hydroxyl group of I. As regards the mechanism

of insecticidal activity, we suggested by the use of the 5th instar larvae of silkworm, *Bombyx mori*, that it was due to damage to the epidermal cells with coagulation of chromatin.[9] The phytogrowth-inhibitory action is thought to be caused by mitotic spindle inhibition.[16] The antitumor activity has been reported to be due to microtubulin inhibition.[3]

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References and Notes

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