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The Biological Actions of Deoxypodophyllotoxin (Anthricin). I. Physiological Activities and Conformational Analysis of Deoxypodophyllotoxin

YOSHIHIKO INAMORI,*,[a] YOSHIAKI KATO,[a] MAYURI KUBO,[a] KIMIYE BABA,[a] TOSHIMASA ISHIDA,[a] KYOSUKE NOMOTO[b] and MITSUGI KOZAWA[a]

Osaka College of Pharmacy,[a] Kawai, Matsubara-shi, Osaka 580, Japan and Suntory Institute for Bioorganic Research,[b] Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618, Japan

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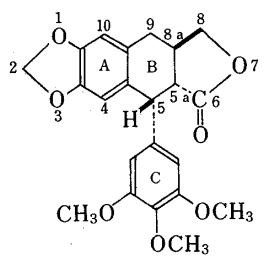
Deoxypodophyllotoxin (I), which was isolated from *Anthriscus sylvestris* HOFFM., has strong toxic activity towards killifish (*Oryzias latipes*) (TLm_{48}: 0.058 ppm) and shrimp (*Altemia salina*) (TLm_{48}: 0.15 ppm). Furthermore, I exhibited the phytogrowth-inhibitory activity against two kinds of plant roots. On the other hand, deoxypicropodophyllin (II), a stereoisomer of I, showed none of these activities.

From these results it can be assumed that the difference of activity between I and II is due to a difference in their conformations. Through analysis of the proton nuclear magnetic resonance spectra it was clarified that 1) the conformation at the 5a position of I is quasi-equatorial, whereas that of II is quasi-axial, and 2) the B-ring of I takes a half-chair form, whereas that of II takes a half-boat form.

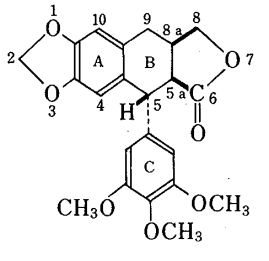
Keywords——deoxypodophyllotoxin; deoxypicropodophyllin; lignan; conformational analysis; toxicity; ichthyotoxicity; phytogrowth-inhibitory activity

Deoxypodophyllotoxin (I) and deoxypicropodophyllin (II) (Fig. 1) are lignans which are widely distributed in higher plants. It has already been reported that I shows antitumor activity[1,2] and a remedial effect on an experimental hepatic lesion in mouse.[3] Recently we reported that I had a broad insecticidal activity spectrum[4,5] and we showed that its action on 5th instar larvae of the silkworm, *Bombyx mori*, involved severe damage to the epidermal cells accompanied with coagulations of the chromatin.[6] In contrast, II, a stereoisomer of I, had no insecticidal activity.[4]

In order to determine the scope of the physiological activity of I in this work, we investigated the ichthyotoxicity and phytogrowth-inhibitory activity of I in comparison with



deoxypodophyllotoxin (I)



deoxypicropodophyllin (II)

Fig. 1. Chemical Structures of Deoxypodophyllotoxin (I) and Deoxypicropodophyllin (II)

those of II. We also studied the conformations of I and II through analysis of the proton nuclear magnetic resonance (^{1}H-NMR) spectra, in order to elucidate the reason for the difference in their activities.

Materials and Methods

Chemicals——Deoxypodophyllotoxin (I) and deoxypicropodophyllin (II) were isolated from the roots of *Anthriscus sylvestris* HOFFM. (Umbelliferae).[7] Rotenone (Nakarai Chemical Co., Ltd.) was used as a standard for toxicity tests, and 2,4-dichlorophenoxyacetic acid sodium salt (Tokyo Kasei Co., Ltd.) as a standard for the phytogrowth-inhibitory activity test.

Organisms——The aquatic organisms used were as follows: *Oryzias latipes* TEMMINCK et SCHLEGEL, *Carassius auratus* L., *Limnodrilus hoffmeisteri* L. and *Altemia salina* L. The plants used were as follows: *Medicago sativa* L. and *Brassica rapa* L.

Physiological Activity Test——1) Toxicity Tests: The method of Sugawara and Koyama[8] was used. The test on *Altemia salina* (shrimp) was carried out in artifical sea water (Nihon Dobutsu Yakuhin Co., Ltd.). TLm (median tolerance limit) at 48 h was calculated according to the Doudoroff method.[9] The effect on *Limnodrilus hoffmeisteri* was expressed as LC_{100} (lethal concentration, ppm) at 48 h.

2) Phytogrowth-Inhibitory Activity Tests:[10,11] Cotton wool in tall Petri dishes (9 cm in diameter) was sterilized by dry heat sterilization. Compounds I and II and 2,4-dichlorophenoxyacetic acid sodium salt were each diluted in 5% acetone to the concentration of 50 ppm. The solutions of these substances and the control (5% acetone and H_2O), 2 ml each, were added to the dishes, then 20 seeds of each plant were put on the sterilized cotton and left for 7 d. The length of the roots of each plant was measured and averaged. The phytogrowth-inhibitory activity was expressed as the ratio to the length of the roots of that of the control group (1.00).

Conformational Analysis of Deoxypodophyllotoxin (I) and Deoxypicropodophyllin (II)——The conformations of I and II were analyzed on the basis of ^{1}H-NMR double resonance experiments. Apparatus: Nicolet NT-360 NMR spectrometer. Solvent: I, C_6D_6; II, $CDCl_3$.

Results

Toxicity of Deoxypodophyllotoxin (I) and Deoxypicropodophyllin (II) to Aquatic Organisms

The toxicities of I and II against *Oryzias latipes* and *Carassius auratus* were examined. As shown in Table I, I showed strong toxicity to both fishes. The TLm (48 h) values of I were 0.058 ppm in *O. latipes* and 0.7 ppm in *C. auratus*. On the other hand, II did not show any

TABLE I. Toxicity of Deoxypodophyllotoxin (I) and Deoxypicropodophyllin (II)

	TLm (ppm, 48 h)		
	I	II	Rotenone
Fishes			
Oryzias latipes TEMMINCK *et* SCHLEGEL	0.058	>30	0.030
Carassius auratus L.	0.70	>13	0.033
Other aquatic organisms			
Altemia salina L.	0.15	>1.0	0.032
	LC_{100} (ppm, 48 h)		
	I	II	Rotenone
Limnodrilus hoffmeistri	0.50	>20	0.1

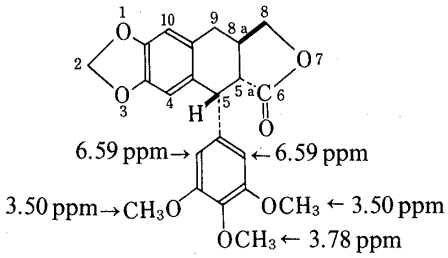
Calculation of TLm: Doudoroff method. Temperature: 27 °C. Experimental size: 10 fishes/group, 2 groups.

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

Plant	Growth (ratio)[a]		
	I	II	2,4-D[b]
Medicago sativa L.	0.35	1.07	0.08
Brassica rapa L.	0.54	1.14	0.09

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

TABLE III. Proton Magnetic Resonance Data for Deoxypodophyllotoxin (I) (δ, ppm, C_6D_6)



Proton	Chemical shift (ppm)	Coupling constant (Hz)	
$H_{2\alpha,2\beta}$	5.30	$J_{H_{2\alpha}H_{2\beta}}$	8.2
H_4	6.48 (s)		
$H_{5\beta}$	4.41	$J_{H_{5\beta}H_{5a}}$	5.0
H_{5a}	1.94	$J_{H_{5a}H_{8a}}$	13.0
		$J_{H_{5a}H_{5\beta}}$	5.0
$H_{8\beta}$	3.03	$J_{H_{8\beta}H_{8a}}$	11.0
		$J_{H_{9\beta}H_{8\alpha}}$	8.5
$H_{8\alpha}$	3.62	$J_{H_{8\alpha}H_{8a}}$	7.0
		$J_{H_{8\alpha}H_{8\beta}}$	8.5
H_{8a}	2.35	$J_{H_{8a}H_{9\beta}}$	12.0
		$J_{H_{8a}H_{9\alpha}}$	5.5
		$J_{H_{8a}H_{8\beta}}$	11.0
		$J_{H_{8a}H_{8\alpha}}$	7.0
		$J_{H_{8a}H_{5a}}$	13.0
$H_{9\beta}$	1.85	$J_{H_{9\beta}H_{9\alpha}}$	16.5
		$J_{H_{9\beta}H_{8a}}$	12.0
$H_{9\alpha}$	2.18	$J_{H_{9\alpha}H_{9\beta}}$	16.5
		$J_{H_{9\alpha}H_{8a}}$	12.5
H_{10}	6.42 (s)		

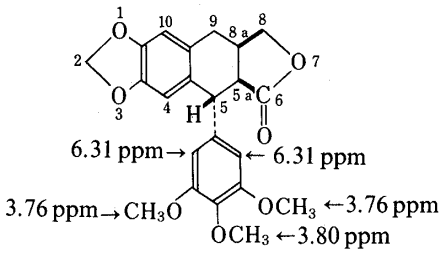
toxicity even at higher concentration.

The toxicity of I and II was also examined with *Altemia salina* and *Limnodrilus hoffmeisteri*. As shown in Table I, I showed strong toxicity against both of them. On the other hand, II did not exhibit any toxicity even at higher concentration.

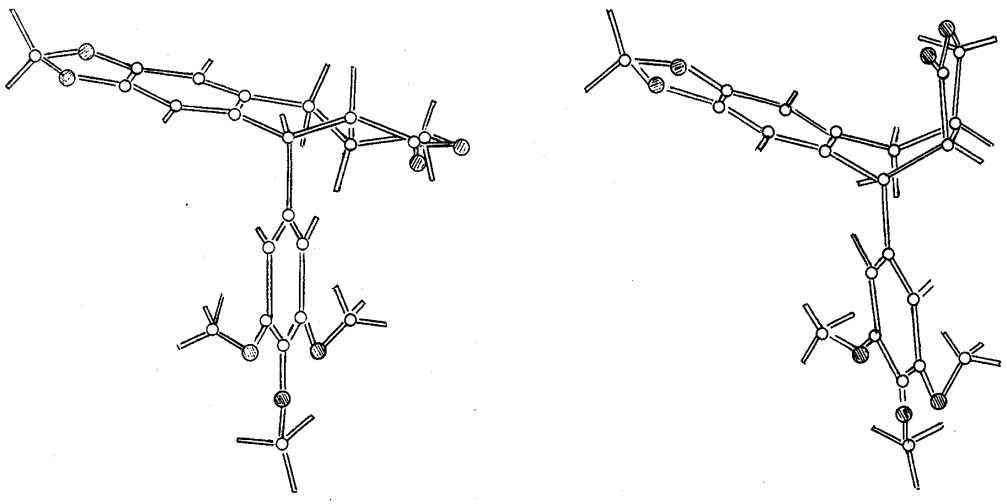
Phytogrowth-Inhibitory Activities of Deoxypodophyllotoxin (I) and Deoxypicropodophyllin (II)

The phytogrowth-inhibitory activities of I and II were examined using two kinds of

TABLE IV. Proton Magnetic Resonance Data for Deoxypicropodophyllin (II) (δ, ppm, $CDCl_3$)



Proton	Chemical shift (ppm)	Coupling constant (Hz)	
$H_{2\alpha,2\beta}$	5.91	$J_{H_{2\alpha}H_{2\beta}}$	9.0
H_4	6.56 (s)		
$H_{5\beta}$	4.35	$J_{H_{5\beta}H_{5a}}$	3.2
H_{5a}	3.32	$J_{H_{5a}H_{8a}}$	9.5
		$J_{H_{5a}H_{5\beta}}$	3.2
$H_{8\beta}$	3.95	$J_{H_{8\beta}H_{8a}}$	3.5
		$J_{H_{8\beta}H_{8\alpha}}$	9.5
$H_{8\alpha}$	4.43	$J_{H_{8\alpha}H_{8a}}$	7.5
		$J_{H_{8\alpha}H_{8\beta}}$	9.5
H_{8a}	3.06	$J_{H_{8a}H_{9\beta}}$	5.5
		$J_{H_{8a}H_{9\alpha}}$	6.5
		$J_{H_{8a}H_{8\beta}}$	3.5
		$J_{H_{8a}H_{8\alpha}}$	7.5
		$J_{H_{8a}H_{5a}}$	9.5
$H_{9\beta}$	2.46	$J_{H_{9\beta}H_{9\alpha}}$	16.0
		$J_{H_{9\beta}H_{8a}}$	5.5
$H_{9\alpha}$	2.84	$J_{H_{9\alpha}H_{9\beta}}$	16.0
		$J_{H_{9\alpha}H_{8a}}$	6.5
H_{10}	6.64 (s)		



deoxypodophyllotoxin (Ia) deoxypicropodophyllin (IIa)

Fig. 2. Possible Spatial Conformations of Deoxypodophyllotoxin (I) and Deoxypicropodophyllin (II)

○, carbon atom; ●, oxygen atom.

plants. As shown in Table II, I inhibited the growth of the roots of both plants at a concentration of 50 ppm. On the other hand, II did not have inhibitory activity at the same concentration.

Conformational Analysis of Deoxypodophyllotoxin (I) and Deoxypicropodophyllin (II)

The conformations of I and II were analyzed through double resonance ^{1}H-NMR spectral measurements. The proton signals of I are summarized in Table III. The coupling constants[12-14] suggested the most probable conformation of I to be Ia (Fig. 2). The conformation is similar to that of hernandin (5-methoxydeoxypodophyllotoxin; isolated from the seeds of *Hernandia ovigera* L.) determined by X-ray analysis.[15]

The proton signals of II are summarized in Table IV. The coupling constants suggested the preferred solution conformation to be IIa (Fig. 2).

Discussion

Deoxypodophyllotoxin (I) is a lignan with a phenyltetraline skeleton and shows antitumor[1,2] and insecticidal[4,5] activities as well as an effect on hepatic lesion in the mouse.[3] In the present study, it was shown that I exhibits ichthyotoxicity as well as phytogrowth-inhibitory activities.

(1) Toxicity to Aquatic Organisms: It is well known that lignans with the phenylnaphthalide skeleton show strong ichthyotoxic activity. Thus, the toxicity of I to aquatic organisms was investigated. Compound I has a significant ichthyotoxic activity against *Oryzias latipes*, and its TLm at 48 h is 0.058 ppm (Table I). The strength of the activity was slightly less than those of justicidins A and B,[16] which are lignans with the phenylnaphthalide skeleton (TLm_{48}: A, 0.02 ppm and B, 0.04 ppm in *O. latipes*). Compound I also showed strong toxicity against *Altemia salina* (TLm_{48}: 0.15 ppm) and *Limnodrilus hoffmeistri* (LC_{100}: 0.5 ppm, 48 h). The strong toxicities of I are consistent with the fact that I exhibits strong insecticidal activity[4] against larvae of *Culex pipiens molestus*.

(2) Phytogrowth-Inhibitory Activities: Since podophyllic acid 2-ethylhydrazide,[17] a podophyllotoxin-related compound is known to have a strong phytogrowth-inhibitory activity, such activity of I was investigated. Compound I, at the concentration of 50 ppm, inhibited the growth of the roots of two kinds of plants, especially the root of *Medicago sativa* (Table II). Although there are many reports[18-20] on constituents of plants which inhibit the growth of the roots of other plants, the only lignans known to have such activity are I and podophyllic acid 2-ethylhydrazide.

(3) Conformational Difference of I and II: As mentioned above, the physiological activities of I and II are quite different from each other. As a first step in elucidating the reason for this, the conformations of I and II were analyzed by means of ^{1}H-NMR measurements. The most significant differences between their conformations are as follows: 1) the bond at the 5a position of I is quasi-equatorial, whereas that of II is quasi-axial, and 2) the B-ring (Fig. 1) of I takes a half-chair form, whereas that of II takes a half-boat one (Tables III and IV, and Fig. 2). Thus the spatial conformations of I and II were concluded to be as shown in Fig. 2. However, it is not clear whether the difference of activities between I and II is due to (1) the difference of their molecular conformations or (2) the difference of their physico-chemical characteristics caused by the difference of their molecular forms. We are now investigating (1) the nature of the receptor of I, and (2) the distribution of II in the tissues of the 5th instar larvae of silkworm, *B. mori*, in comparison with that of I.[5,6]

(4) Mutagenicity: The mutagenicities of I and II were investigated by means of the spot test using the Ames system[21] and the metabolic activation test with S-9 mix. Some activity was expected for the following reasons: 1) I shows antitumor activities,[1,2] 2) the cause of the insecticidal action of I is damage to the epidermal cells of silkworm larvae, accompanied with coagulation of the chromatin,[6] and 3) podophyllotoxin shows chemosterilant activities.[22] However, neither I nor II showed mutagenic activity (data not shown).

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