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## Mechanism of Insecticidal Action of Deoxypodophyllotoxin (Anthricin). III. The Mode of Delayed Insecticidal Action of Deoxypodophyllotoxin

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Deoxypodophyllotoxin (DPT) showed delayed insecticidal activities against female adults of *Culex pipiens pallens*, *Blattella germanica*, *Periplaneta fuliginosa* and *Musca domestica* when tested by the topical application and bait methods. The LD<sub>50</sub> values of DPT were 0.38 µg/insect in *C. pipiens pallens* (7 d) and 8.43 µg/insect in *B. germanica* (9 d). The LD<sub>50</sub> value could not be calculated in *M. domestica*, because the mortality of the control at 4 d was over 10%. DPT also exhibited insecticidal activities against the adults of *B. germanica* and *P. fuliginosa* when tested by the bait method. DPT may be useful as a bait agent, since 1) the strength of insecticidal activity of DPT against *B. germanica* is almost equal to that of boric acid-bait agent and 2) DPT did not show any repellent action against the adults of *B. germanica* even at the high concentration of 0.4%.

**Keywords**—deoxypodophyllotoxin; insecticidal effect; bait method; topical application method; delayed toxicant; *Culex pipiens pallens*; *Blattella germanica*; *Musca domestica*; *Periplaneta fuliginosa*

Deoxypodophyllotoxin (DPT)<sup>1)</sup> is a phenyltetralin-type lignan which was isolated from the root of *Anthriscus sylvestris* HOFFM. It has already been reported that DPT shows antitumor activity,<sup>2,3)</sup> and a remedial effect on an experimental hepatic lesion in mouse.<sup>4)</sup> Recently, we reported that DPT has insecticidal,<sup>5-7)</sup> phytogrowth-inhibitory<sup>8)</sup> and ichthyotoxic activities.<sup>8)</sup> As regards the insecticidal activity, it was confirmed that the symptoms caused by DPT developed slowly; namely, DPT is a delayed toxicant.<sup>5)</sup> This conclusion was supported by the finding that the action of DPT on the 5th instar larvae of silkworm, *Bombyx mori*, involved severe damage to the epidermal cells accompanied with coagulation of the chromatin.<sup>7)</sup> However, no detailed work has been done on the mechanism of delayed insecticidal activity of DPT.

In this work, as a preliminary step to an investigation of the mechanism of delayed insecticidal action, the mode of the insecticidal effect of DPT on three kinds of insects was examined by using two assay methods.

### Materials and Methods

**Chemicals**—DPT was isolated from the root of *Anthriscus sylvestris* HOFFM (Umbelliferae) according to the method of Kozawa *et al.*<sup>1)</sup> Arutandondon (Fumakilla Limited; a bait agent containing 15% boric acid, 2.5 g/tablet) was used as a standard for the bait method.

**Insects**—Female adults of *Culex pipiens pallens*, *Musca domestica*, *Blattella germanica* and *Periplaneta fuliginosa* were used.

**Insecticidal Activity Tests**—1) Topical Application Method: An acetone solution of DPT was applied to the

ventral surface of the abdomen of female adults of *C. pipiens pallens* (0.77  $\mu$ l), *M. domestica* (0.77  $\mu$ l) and *B. germanica* (1.54  $\mu$ l) under ether anesthesia. Both bait and water were available to the insects. The number of deaths was observed at intervals of 24 h for 7–9 d and the LD<sub>50</sub> value was calculated from the dose–mortality relationship.

2) Bait Method: A 2 g tablet of DPT was prepared at a concentration of 0.4% in a mixture of 6 parts of sugar with 4 parts of peanut powder. Female adults of *B. germanica* and *P. fuliginosa* were allowed to feed on the tablet with water in an acryl box (30 × 30 × 6 cm). The number of deaths was observed at intervals of 24 h for 10 d. The dietary intake was calculated as the difference in the weight of sample before and after the examination.

**Temperature**—Every experiment was carried out at 25 ± 2 °C.

## Results

### Insecticidal Activities of DPT by the Topical Application Method

The insecticidal effect of DPT against female adults of *C. pipiens pallens*, *B. germanica* and *M. domestica* was examined by the topical application method. As shown in Tables I, II and III, DPT showed strong delayed insecticidal activities against all three kinds of insects. The insecticidal effects increased with the passage of time and the LD<sub>50</sub> values of DPT were 0.38  $\mu$ g/insect in *C. pipiens pallens* (7 d) and 8.43  $\mu$ g/insect in *B. germanica* (9 d). However, the LD<sub>50</sub> value in *M. domestica* could not be calculated, because the mortality of the control

TABLE I. Insecticidal Effect of DPT against Female Adults of *Culex pipiens pallens*

Concentration of sample (%)	Dose ( $\mu$ g/insect)	Mortality (%)						
		1	2	3	4	5	6	7 (d)
2.0	15.4	6.7	20.0	83.3	96.7	96.7	96.7	96.7
1.0	7.7	3.3	10.0	70.0	90.0	100.0	100.0	100.0
0.5	3.85	0	0	33.3	70.0	73.3	83.3	90.0
0.25	1.925	0	0	10.0	30.0	53.3	76.7	83.3
0.125	0.963	0	3.3	6.7	30.0	46.7	63.6	70.0
0.063	0.481	0	0	13.3	33.3	40.0	66.7	70.0
0.032	0.241	0	0	0	0	13.3	23.3	30.0
0.016	0.120	0	0	0	0	16.7	16.7	16.7
0.008	0.060	0	0	0	0	13.3	13.3	13.3
Control	0.0	0	0	0	0	3.3	3.3	10.0
LD <sub>50</sub> value	( $\mu$ g/insect)	—	—	5.55	1.97	1.10	0.49	0.38

Assay method: Topical application method. Experimental size: 15 insects/group, 2 groups. Temperature: 25 ± 2 °C.

TABLE II. Insecticidal Effect of DPT against Female Adults of *Blattella germanica*

Concentration of sample (%)	Dose ( $\mu$ g/insect)	Mortality (%)								
		1	2	3	4	5	6	7	8	9 (d)
4.0	61.6	3.3	10.0	13.3	40.0	83.3	90.0	93.3	96.7	100.0
2.0	30.8	3.3	10.0	10.0	30.0	46.7	66.7	80.0	83.3	83.3
1.0	15.4	0	0	0	23.3	46.7	53.3	60.0	63.3	66.7
0.5	7.7	6.7	6.7	10.0	16.7	23.3	33.3	46.7	46.7	50.0
0.25	3.85	0	0	3.3	6.7	6.7	13.3	16.7	26.7	26.7
0.125	1.925	0	3.3	3.3	6.7	6.7	6.7	6.7	6.7	6.7
0.063	0.963	0	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3
Control	0.0	3.3	3.3	3.3	3.3	3.3	3.3	3.3	6.7	6.7
LD <sub>50</sub> value	( $\mu$ g/insect)	—	—	—	—	22.21	14.08	10.66	8.98	8.43

Assay method: Topical application method. Experimental size: 15 insects/group, 2 groups. Temperature: 25 ± 2 °C.

TABLE III. Insecticidal Effect of DPT against Female Adults of *Musca domestica*

Concentration of sample (%)	Dose ( $\mu\text{g}/\text{insect}$ )	Mortality (%)						
		1	2	3	4	5	6	7 (d)
2.0	15.4	0	6.7	26.7	40.0	90.0	100.0	—
1.0	7.7	0	0	20.0	66.7	93.3	100.0	—
0.5	3.85	0	6.7	20.0	53.3	100.0	—	—
0.25	1.925	0	0	20.0	56.7	96.7	100.0	—
0.125	0.963	0	0	16.7	43.3	93.3	100.0	—
0.063	0.482	0	0	6.7	6.7	90.0	100.0	—
0.031	0.241	0	3.3	13.3	26.7	70.0	96.7	100.0
0.016	0.120	0	0	3.3	6.7	60.0	100.0	—
Control	0.0	0	0	0	23.3	66.7	83.3	100.0
LD <sub>50</sub> value	( $\mu\text{g}/\text{insect}$ )	—	—	—	—	—	—	—

Assay method: Topical application method. Experimental size: 15 insects/group, 2 groups. Temperature:  $25 \pm 2^\circ\text{C}$ .

TABLE IV. Insecticidal Effect of DPT against Female Adults of *Blattella germanica* and *Periplaneta fuliginosa*

Insect	Test sample	Concentration (%)	Mortality (%)										Amount ingested (g) at 10 d
			1	2	3	4	5	6	7	8	9	10 (d)	
<i>B. germanica</i>	DPT	0.4	2.5	2.5	2.5	22.5	37.5	47.5	57.5	70.0	70.0	75.0	0.19
	Arutandondon	15.0	0.0	12.5	30.0	42.5	60.0	70.0	80.0	82.5	85.0	87.5	0.07
	Control	—	0.0	5.0	5.0	5.0	5.0	5.0	7.5	7.5	7.5	7.5	—
<i>P. fuliginosa</i>	DPT	0.4	0.0	0.0	0.0	0.0	5.0	5.0	5.0	10.0	10.0	15.0	1.87
	Arutandondon	15.0	5.0	5.0	15.0	30.0	50.0	50.0	65.0	70.0	70.0	70.0	0.37
	Control	—	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	—

Assay method: Bait method. Experimental size: 40 insects/group, 2 groups. Temperature:  $25 \pm 2^\circ\text{C}$ .

group at 4 d was over 10%.

After the DPT treatment, spontaneous movement in the insects was little different from that of control insects until 24 h after DPT treatment. However, at 48 h all of the treated groups were paralyzed and most of them were becoming immobilized. Immobilization increased with the passage of time.

#### Insecticidal Activities of DPT by the Bait Method

The insecticidal effect of DPT against female adults of *B. germanica* and *P. fuliginosa* was examined by the bait method. As shown in Table IV, DPT showed delayed insecticidal activity against female adults of *B. germanica*. At 2 d after DPT treatment, the spontaneous movement decreased gradually. After 4 d, mortality (%) increased gradually. The insecticidal effect of DPT was almost equal to that of the conventional insecticide, boric acid-bait agent (Arutandondon, Fumakilla Limited). On the other hand, as shown in Table IV, the insecticidal effect of DPT on female adults of *P. fuliginosa* was very weak.

#### Discussion

The mode of delayed insecticidal action of DPT on three kinds of insects was clarified.

[I] **Topical Application Method**—The toxicity to mosquito adults has not previously been studied. Thus, the mode of insecticidal effect of DPT against female adults of *C. pipiens*

*pallens* was examined. DPT showed delayed insecticidal activity against female adults of *C. pipiens pallens* (Table I). The insecticidal effect of DPT was one-twentieth of that of allethrin ( $LD_{50}$ : 0.019  $\mu\text{g}/\text{insect}$ ). The insecticidal effect of DPT on the mosquito is noteworthy from the following two points of view: 1) as mentioned above, DPT showed delayed insecticidal activity ( $LD_{50}$ : 0.38  $\mu\text{g}/\text{insect}$ ) against female adults of *C. pipiens pallens* and 2) DPT at 5 ppm caused mortality of 80% against the larvae of *C. pipiens molestus*.<sup>5)</sup> Further, DPT showed strong delayed insecticidal activity against female adults of *B. germanica* (Table II). The insecticidal effect of DPT was two-thirds of that of allethrin ( $LD_{50}$ : 5.30  $\mu\text{g}/\text{insect}$ ). As regards the action of DPT on the housefly, it was reported that DPT inhibited the pupation of housefly larvae and adult emergence.<sup>9)</sup> However, the insecticidal effect of DPT against female adults of *M. domestica* has not been reported as yet. In this study, DPT showed delayed insecticidal activity against female adults of *M. domestica* (Table III). However, the insecticidal effect of DPT could not be compared with that of allethrin ( $LD_{50}$ : 0.64  $\mu\text{g}/\text{insect}$ ), because the mortality of the control group at 4 d was over 10%. From these results, it was concluded that DPT showed rather strong delayed insecticidal activities against the above three kinds of insects. The spontaneous movement of the insects treated with DPT was different from that of the groups treated with most conventional insecticides. That is, DPT showed strong toxicity on all three kinds of insects. However, the nature of the difference in mechanism of action between DPT and other insecticides is not clear at present.

**[II] Bait Method**—The insecticidal effect of DPT on female adults of *B. germanica* and *P. fuliginosa* was examined in comparison with the conventional insecticide, Arutandondon (Fumakilla Limited). DPT also showed delayed insecticidal activity against female adults of *B. germanica* (Table IV), but the activity against female adults of *P. fuliginosa* was weaker than that against *B. germanica* (Table IV). However, DPT seems to be potentially useful as a bait agent from the following two points of view: 1) the strength of insecticidal activity of DPT is almost equal to that of the conventional insecticide, Arutandondon (Fumakilla Limited) and 2) DPT did not show any repellent action against adults of *B. germanica* even at the high concentration over 0.4%.

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