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Components of the Root of *Anthriscus sylvestris* Hoffm. II.[1] Insecticidal Activity

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Deoxypodophyllotoxin (anthricin) (I), anthriscinol methyl ether (IV) and (*Z*)-2-angeloyloxymethyl-2-butenoic acid (VI), isolated from the root of *Anthriscus sylvestris* Hoffm., exerted insecticidal activity on the adults of *Blattella germanica*, and on the larvae of *Culex pipiens molestus*, *Plutella xylostella* and *Epilachna sparsa orientalis*. In paticular, the activity of I, *i.e.*, the main lignan component of *A. sylvestris* root, was so strong as to cause the death of 80% of the larvae of *C. pipiens molestus* at a concentration of 5 ppm. The epimer at the 2-position of I, *i.e.*, deoxypicropodophyllin(isoanthricin)(II), however, was not insecticidal.

Keywords——deoxypodophyllotoxin; anthricin; deoxypicropodophyllin; isoanthricin; anthriscinol methyl ether; (*Z*)-2-angeloyloxymethyl-2-butenoic acid; *Anthriscus sylvestris*; insecticidal activity; Umbelliferae

The root of *Anthriscus sylvestris* Hoffm. (Japanese name "Shaku", Umbelliferae) was formerly employed as a kind of the crude drugs "Zengo" in Japan. Even now, it is employed as a hematinic or tonic under the name of "E Shen" in Sichuan Sheng (Shisen Sho) in China. Presently in Japan, the root is soaked in water, and then crushed and pulverized in a dry atmosphere for use as a food. In addition, the young aerial part of this plant is sometimes used for food. Kozawa *et al.*[1] have already made a study on the components of the dried root, isolating the compounds shown in Chart 1, such as lignans, phenylpropanoids and acyloxycarboxylic acid.

In the present study, attention has been focused on the small extent of insect damage in this root in comparison with the damage in other plants of Umbelliferae. This paper describes the physiological activity of the root components on insects.

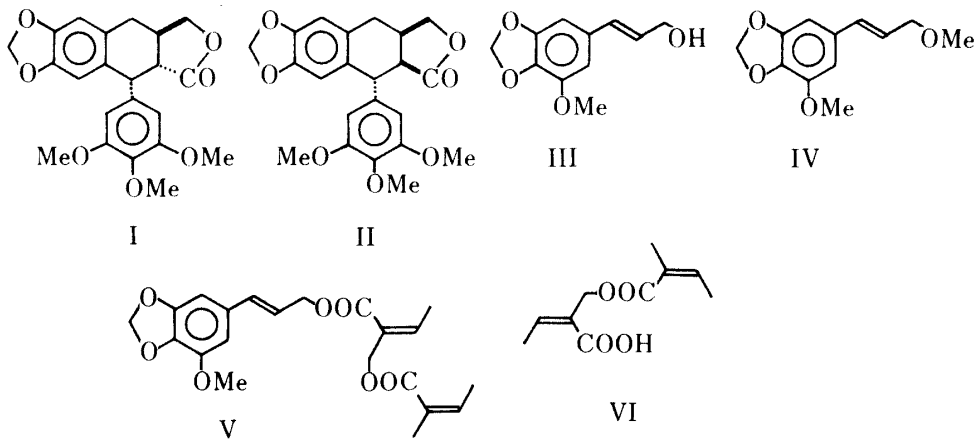

Chart 1

Materials and Notes

Test Samples——The following compounds and extracts were used as test samples: hexane extract of the root; deoxypodophyllotoxin(anthricin)(I); deoxypicropodophyllin(isoanthricin)(II); anthriscinol(III); anthriscinol methyl ether (IV); anthriscusin (V); (*Z*)-2-angeloyloxymethyl-2-butenoic acid (VI).

Test Insects——The following insects were used: adults of *Blattella germanica* L. (cockroach); larvae of *Culex pipiens molestus* Farskal (mosquito); larvae of *Epilachna sparsa orientalis* Dieke (lady beetle); larvae of *Plutella xylostella* Curtis (moth); larvae of *Spodoptera litura* Fabricius (moth).

Insecticidal Activity Test——1) Bait Method: A slice of bread was dipped into the acetone solution of a test drug, then dried in air, and fed to the adults of *B. germanica* together with water. The mortality was examined after 15 d.

2) Topical Application Method: The acetone solution of a test drug was applied to the ventral surface of the abdomen of adults of *B. germanica* which were anesthetized with ether. Both the bait and water were fed to the adults of *B. germanica*. The mortality was examined after 15 d.

3) Leaf Dipping Method: The leaves of soybean were dipped into aqueous drug solution (containing 0.033% 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. *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.

4) Potato Dipping Method: Round slices of potato were dipped in aqueous drug solution (containing 0.033% of Dine®), and fed to the larvae of *E. sparsa orientalis*. The mortality was examined after 48 h.

5) Immersion Method: The larvae of *C. pipiens molestus* were released into an aqueous solution of a drug. The mortality was examined after 24 and 48 h.

Temperature——All the experiments were carried out at 25—27°C.

Results and Discussion

An examination was made on the insecticidal activity of components of the root of *Anthriscus sylvestris* Hoffm. The following compounds had activities: desoxypodophyllotoxin (I) as a lignan, anthriscinol methyl ether (IV) as a phenylpropanoid ether, and (*Z*)-2-angeloyloxymethyl-2-butenoic acid (VI) as an acyloxycarboxylic acid. Among these components, I had a high insecticidal activity on all the test insects except for *S. litura*. Among the insecticidal lignans isolated from plants, diarylhexahydrofuranofuran-type compounds of the phrymarol group[3] isolated from *Phryma leptostachya* L. are well known to have high insecticidal activity. Further, sesamin and sesamolin (furanofuran type), and butyrolactone-type compounds of the hinokinin group are well known to be synergistic with certain insecticidals.[4] In addition to podophyllotoxin, a number of analogous phenyltetraline-type lignans have been reported. However, little is known about the insecticidal activity of those compounds. As regards deoxypodophyllotoxin (I), Russell *et al.*[5] studied the insecticidal activity of the leaves of Libocedrus bidwillii, and found that a concentration of 100 ppm of I gave 33% mortality among larvae of *Musca domestica*. Simultaneously, they reported that the isomer at the 2-position (picro-epimer) was not insecticidal. In the present study, we confirmed that deoxypodophyllotoxin, *i.e.*, the main lignan component of *A. sylvestris*, is more insecticidal than any other component in the plant (Tables I—III).

The insecticidal activity of I was strong enough to give 80% mortality among larvae of *C. pipiens molestus* at a concentration of 5 ppm (Table III). On the other hand, deoxypicropodophyllin (II) was not insecticidal, in accord with the report of Russell *et al.*[5] The difference in activity between I and II suggests a strict stereospecificity in the action mechanism, but further investigation is necessary. In the case of larvae of *C. pipiens molestus*, the mortality caused by I was far higher after 48 h than after 24 h. Delayed insecticidal activity of I was also noted in the adults of *B. germanica*. The mode of activity is being investigated by using larvae of *Bombyx mori*, the silkworm.

In this study, anthriscinol methyl ether (IV) exerted insecticidal activity on the adults of *B. germanica* and the larvae of *P. xylostella*, though the activity was weak. Myristicin, a constituent of *Pastinaca sativa* L., has a substitution pattern similar to that of IV and V. It was reported that myristicin has insecticidal activity on *Epilachna varivestis* Mulsant

TABLE I. Insecticidal Activity of Hexane Extract and Components of *Anthriscus sylvestris* HOFFM. applied into *Blattella germanica* L. (Adults)

Test agent	Bait method		Topical application method	
	Concentration (ppm)	Mortality (%)	Concentration (μg/g)	Mortality (%)
Hexane extract	5000	70	500	100
Deoxypodophyllotoxin(I)	1000	100	80	100
Deoxypicropodophyllin(II)	5000	0	500	10
Anthriscinol(III)	5000	0	500	0
Anthriscinol methyl ether(IV)	5000	80	500	40
Anthriscusin(V)	5000	0	500	10
(*Z*)-2-Angeloyloxymethyl-2-butenoic acid(VI)	5000	40	500	25
Parathion	500	100	50	100

Observation time: 15 d. Temperature: 26°C.
Experimental size: 10 insects/group, 2 groups.

TABLE II. Insecticidal Activity of Topically Applied Deoxypodophyllotoxin (Anthricin)(I) on *Blattella germanica* (Adult)

Concentration (μg/g)	Mortality (%)
170	100
80	100
50	85
25	65
20	45
10	35

Observation time: 15 d. Temperature: 26°C.
Experimental size: 10 insects/group, 2 groups.

TABLE III. Insecticidal Activity of Deoxypodophyllotoxin(I), Anthriscinol Methyl Ether(IV) and (*Z*)-2-Angeloyloxymethyl-2-butenoic Acid(VI)

Insect	Method	Concentration(ppm)	Mortality(%)						
Epilachna sparsa orientalis (larvae)[b]	Tuber dip		I		IV		VI		Parathion
		100	55		0		5		100
		500	85		20		0		100
Plutella xyrostella (larvae)[a]	Lead dip		I		IV		VI		Parathion
		500	55		0		0		100
		1000	30		36.7		6.7		100
Culex pipiens molestus (larvae)[a,b]	Immersion		I		IV		VI		Parathion
			a)	b)	a)	b)	a)	b)	a)
		1	0	35	0	0	0	0	100
		5	5	80	0	0	0	0	100
		20	40	90	5	5	50	50	
Spodoptera litura[a]	Leaf dip		I		IV		VI		Parathion
		500	0		0		0		100
		1000	0		0		0		100

Observation time: *a*) 24 h, *b*) 48 h. Temperature: 26°C.
Experimental size: 10 insects/group, 2 groups.

(Mexican bean beetle) and *Musca domestica* L. (housefly).[6]

(*Z*)-2-Angeloyloxymethyl-2-butenoic acid (VI) is a new type of acyloxycarboxylic acid

contained in some quantity in the root of *A. sylvestris*. It is of interest that VI exerted insecticidal activity on the adults of *B. germanica* and the larvae of *C. pipiens molestus*.

In terms of the structure–activity relationship, it is of interest that IV is a methyl ether of III and that V is an ester derived from VI and III.

The toxic activity of VI on the larvae of *C. pipiens molestus* was different in that the larvae were intoxicated after 4 h, but those larvae which did not die recovered later. This may be a characteristic feature of the action of VI. On the other hand, VI is relatively liable to hydrolysis on account of its acyloxycarboxylic acid structure, and thus, the recovery from intoxication might be due to decomposition of the chemical in the aqueous solution.

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