Chemical Studies on Pyrethroids

Part I. Syntheses of Allethrin Metabolites and their Toxicities

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Allethrin-(E)-ol (IV), allethrin-(E)-al (V) and allethrin-(E)-acid (VI), the metabolites of allethrin (III) in the insect body, were synthesized. Their low toxicities to houseflies seem to support the hypothesis that they are products of the detoxication process of allethrin.

Natural and synthetic pyrethroids are widely used as a domestic insecticide because of their high toxicities to houseflies and mosquitos in comparison with their harmfulness to mammals. In spite of many chemical and biochemical studies on pyrethroids,¹⁾ their metabolic fate in insects and a possible difference in the detoxication mechanism between insects and mammals have not been cleared.

Recently, Yamamoto and Casida²⁾ have applied ¹⁴C-labelled allethrin, a typical synthetic pyrethroid, to houseflies *in vivo* and *in vitro*. They proposed that allethrin is metabolized, in the initial stage, through the oxidation of the methyl group in the isobutenyl side chain of the chrysanthemic acid moiety, the acidic part of allethrin molecule.

After hydrolysis of the metabolite, they separated six compounds in an acid fraction and identified their structures; *trans*-3-(2'-hydroxymethylprop-(Z) and (E)-1'-enyl)-2,2-dimethylcyclopropane carboxylic acids (*trans*-(Z) and (E)-hydroxychrysanthemic acids),³⁾ (Ia) and (IIa) in Fig. 1, *trans*-3-(2'-formylprop-(Z) and (E)-1'-enyl)-2,2-dimethylcyclopropane carboxylic acids (*trans*-(Z) and (E)-oxochrysanthemic acids), (Ib) and (IIb), and *trans*-3-(2'-carboxyprop-(Z) and (E)-1'-enyl)-2,2-dimethyl cyclopropane carboxylic acids (*trans*-chrysanthemum dicarboxylic acid and its (Z)-isomer), (Ic) and (IIc). The main metabolic products were the ones of (E)-series. Therefore, the oxidation of the *trans*-methyl is predominant, and the step-wise oxidation from allethrin (III) to allethrin-

1) M. Matsui and I. Yamamoto, "Naturally Occurring Insecticide," ed. by M. Jacobsen and D. G. Crosby, Academic Press Inc., New York, N. Y., 1971, p. 3.

2) a) I. Yamamoto and J. E. Casida, *J. Econ. Entmol.*, **59**, 1542 (1966).

b) I. Yamamoto, E. C. Kimmel and J. E. Casida, *Agr. Food Chem.*, **17**, 1227 (1969).

3) Throughout this paper, the prefix *trans* refers to the geometry about the cyclopropane rings: The configuration of the trisubstituted double bond is indicated by the (Z, E) nomenclature.

FIG. 1. Derivatives of *trans*-Chrysanthemic Acid.

FIG. 2. Proposed Metabolic Route of Allethrin and the Synthetic Route of the Metabolites.
($\cdots$ metabolic, $\longrightarrow$ synthetic)

(E)-acid (Fig. 2, III $\rightarrow$ IV $\rightarrow$ V $\rightarrow$ VI) may be proposed. It seems also interesting that the last oxidation product, allethrin-(E)-acid (VI) is a demethylated derivative of allethrin-II (VII) which has a strong knockdown or paralytic effect to insects.

This paper describes syntheses of the metabolites of (E)-series; allethrin-(E)-ol (IV), allethrin-(E)-al (V) and allethrin-(E)-acid (VI). Their biological activities are also discussed from the view point of allethrin detoxication process.

Among three oxygenated allethrins, allethrin-(E)-ol (IV) is first synthesized, because the others are supposed to be easily prepared from IV by selective oxidation process. ($\pm$) *trans*-(E)-Hydroxy-chrysanthemic acid (IIa) was already obtained by one of the authors^{2~b)} by pyrolysis or hydrolysis of its *t*-butyl ester. The former method produced fairly large amounts of a high-boiling viscous oil which was identified as a polyester of the hydroxyacid

after the monomer had been recovered by alkaline hydrolysis. Therefore, alkaline hydrolysis of methyl ($\pm$)-*trans*-(E)-hydroxy-chrysanthemate is advisable to prepare much amounts of the starting material.

As the esterification of ($\pm$)-allethrolone with the acid chloride or anhydride is impossible, allethrolone was changed to allethronyl bromide (VIII), which was then heated with silver salt of the acid (II d) in acetone. Because both of the acid and the alcohol were racemate, the product obtained in fairly good yield was a mixture of diastereomers. The product showed a single spot on thin-layer chromatogram. These two isomers could not be distinguished or separated each other with any other chemical or physical methods. Considering that general biochemical studies about the toxicity of allethrin have been performed with an equimolecular mixture of the diastereomers (so called allethrin), this study was performed without separating them.

TABLE I. TOXICITIES OF ALLETHRIN DERIVATIVES TO HOUSEFLY (*Musca domestica* L.) BY THE TOPICAL APPLICATION METHOD

	Allethrin I	Allethrin-(E)-ol	Allethrin-(E)-al	Allethrin-(E)-acid
LD ₅₀ (μ g/body)	1.3	770	460	($\frac{320}{60\%$ killed)

Allethrin-(E)-ol (IV) was oxidized to the corresponding aldehyde (V) with manganese dioxide in chloroform, in high yield.

In the synthesis of pyrethric acid from chrysanthemic acid, Matsui and Yamada^{4,a)} applied atmospheric oxidation to convert *o*-*tert*-butyl ester of IIb to mono-*tert*-butyl ester of IIc. These mild conditions were supposed to be suitable for the conversion of allethrin-(E)-al (V) to allethrin-(E)-acid. In the presence of a catalytic amount of cupric ions (as acetoacetate salt) the aldehyde was shaken in chloroform solution under oxygen for a long time. The yield was low. However, the unreacted material could be recovered and used repeatedly.

To confirm the structure of the oxidation product, allethrin-(E)-acid obtained was methylesterified with diazomethane. The ester was identical in all aspects with an authentic allethrin-II (VII) synthesized through the known method.^{4,5)}

All reaction products were purified by column chromatography, and their purity were checked by thin-layer chromatography (TLC).

The toxicity of the synthesized allethrin derivatives were first tested by topical application to houseflies, and the results are summarized in Table I.

From these results it was indicated that oxygenated allethrins have all very low toxicities to houseflies. However, since these substances are metabolites in insect bodies,

TABLE II. TOXICITIES OF ALLETHRIN DERIVATIVES TO HOUSEFLY (*Musca domestica* L.) BY THE INJECTION METHOD

Numbers in parentheses show the percentages of dead houseflies at those concentrations.

	LD ₅₀ or Kill effect (μ g/body)	
	Exp. 1	Exp. 2
Allethrin I	0.13	0.20
Allethrin II	2.0	1.6
Allethrin-(E)-ol	15 (24%)	15 (12%)
Allethrin-(E)-al	13 (16%)	13 (20%)

the topical application method employed would not be adequate to measure their toxicities. Especially, in the case of such a high LD₅₀ value, penetration of the dosage through body surface should be an important factor for the real toxicity. Therefore, allethrin-(E)-ol and -(E)-al in acetone solution were injected to houseflies at the concentrations from 1.0 to 15 μ g/body, and the calculation of their LD₅₀ values were attempted.

As shown in Table II, toxicities of allethrin-(E)-ol and -(E)-al are too low to calculate their LD₅₀ values under the experimental conditions.

From the above two biological tests, it is obvious that the oxidation of the terminal methyl in the isobutenyl side chain of allethrin molecule leads to a great depression of its toxicity to houseflies. These results also support the idea that the methylene dioxy group in synergist of pyrethroids serves as alternate substrate for hydroxylation of the insecticide.⁶⁾

4) a) M. Matsui and Y. Yamada, *Agr. Biol. Chem.*, **27**, 373 (1963).

b) M. Matsui and H. Meguro, *ibid.*, **28**, 27 (1964).

5) M. Elliot and N. F. Jones, *Chem & Ind.*, **1969**, 270,

6) J. E. Casida, J. L. Engel, E. G. Essac, F. N. Kaniensky and S. Kuwatsuka, *Science*, **153**, 1130 (1966).

The biological test of allethrin-(E)-acid could not be performed from the shortage of the sample, and its toxicity will be re-examined in connection with allethrin II.

EXPERIMENTAL

($\pm$)-*Allethronyl bromide* (VIII). In a 200-ml three-necked flask, 15.2 g (0.1 mole) of allethrolone was dissolved in 50 ml of dry ether and cooled below 0°C. Phosphorous tribromide (9.0 g, 0.034 mole) dissolved in a small amount of ether was added dropwise with cooling to maintain the reaction temperature at 0°C. After the addition, the content was stirred for 2 hr at the same temperature and then poured into ice-water. The ether extract was washed with water and then dried. As the bromide was decomposed above 120°C, the product was purified by distillation at 101~2°C/3 mmHg. Yield; 10.2 g (47%). IR $\nu_{\text{max}}^{\text{film}}$ cm⁻¹: 1720, 1630, 990, 920. NMR (10% in CDCl₃)⁷⁾: 2.1 (3 H, -CH₃), 2.9 (2 H, d, $J=5.0$ cps, β -CH₂-C=), 4.6~5.1 (2 H, multi. =CH₂), 5.3~6.0 (1 H, multi. -CH=C). Anal. Found: C, 50.35; H, 5.58. Calcd. for C₉H₁₁OBr: C, 50.25; H, 5.16.

Silver salt of ($\pm$)-trans-(E)-hydroxychrysanthemic acid (IId). (E)-Hydroxy acid (4.6 g) was dissolved in the equimolecular amount of 5% sodium hydroxide. With stirring at room temperature, 4.2 g of silver nitrate dissolved in 20 ml of water was added, and the reaction mixture was warmed at 60~70°C for a half hour. The precipitated salt was collected by filtration and washed with water and acetone. After drying in a desiccator, the product weighed 7.3 g. Anal. Found: C, 41.29; H, 5.06. Calcd. for C₁₀H₁₅O₃Ag: C, 41.24; H, 5.15.

Allethrin-(E)-ol (IV). To the suspension of anhydrous Ag-salt (IId) (7.7 g) in 50 ml of dry acetone, 6.0 g of allethronyl bromide was added in one portion. Refluxing and stirring were continued for 12 hr. After separation of the precipitated AgBr followed by evaporation of the acetone *in vacuo*, the residual viscous oil weighed 10.0 g, which was then purified by column chromatography (90 g silica gel). After elution with 300 ml of benzene and 300 ml of benzene-

ethyl acetate (9:1), allethrin-(E)-ol was obtained in the fraction eluted 600 ml of benzene-ethyl acetate (4:1). Yield; 4.8 g (60%). The product showed a single spot on TLC (0.2 mm silica gel H, developed with benzene-ethyl acetate (2:1), $R_f=0.48$) IR $\nu_{\text{max}}^{\text{film}}$ cm⁻¹: 3450, 1735~1710 (broad), 1158, 992, 915. UV $\lambda_{\text{max}}^{\text{EtOH}}$ m μ (ϵ): 225 (17500). Anal. Found: C, 71.59; H, 8.08. Calcd. for C₁₉H₂₆O₄: C, 71.67; H, 8.23.

Allethrin-(E)-al (V). A mixture of 1.7 g of allethrin-(E)-ol (IV) and 17 g of manganese dioxide in 50 ml of chloroform was shaken for 4 hr at room temperature. After filtration and distillation of the solvent, the crude oil obtained was separated by column chromatography (20 g silica gel). The expected product was collected as α fraction eluted with 150 ml of benzene-ethyl acetate (9:1) after elution of 150 ml of benzene. Yield: 1.2 g (71%, 0.5 g of (IV) was recovered from benzene-ethyl acetate (4:1) effluent.) TLC: α single spot (the conditions were the same as that of (IV), $R_f=0.91$). IR $\nu_{\text{max}}^{\text{film}}$ cm⁻¹: 1725, 1690, 1155, 992, 915. UV $\lambda_{\text{max}}^{\text{EtOH}}$ m μ (ϵ): 240 (29100). Anal. Found: C, 70.94; H, 7.64. Calcd. for C₁₉H₂₄O₄: C, 72.12; H, 7.65.

Allethrin-(E)-acid (VI). With a catalytic amount of Cu²⁺-acetoacetate, 1.4 g of (E)-al (V) was dissolved in 30 ml of chloroform and was shaken under oxygen for 38 hr.

An acidic fraction was obtained by extraction with 5% sodium bicarbonate and was extracted with ether after acidification. The condensed crude acidic compounds weight 0.25 g, and the amount of the unreacted material recovered from a neutral fraction was 1.2 g. The former was purified by column chromatography (3 g silica gel). The expected product was in the fraction eluted with 25 ml of *n*-hexane-ether (1:1) after elution of 10 ml of *n*-hexane. Yield; 0.20 g. TLC (0.2 mm silica gel H developed with benzene-methanol-acetic acid (45:8:4), $R_f=0.70$). IR $\nu_{\text{max}}^{\text{CS}_2}$ cm⁻¹: 2800~3200 (broad), 1730, 1690, 1150, 992, 915. UV $\lambda_{\text{max}}^{\text{EtOH}}$ (ϵ): 231 (20700). Semicarbazone: mp 218°C (dec.). Anal. Found: C, 60.96; H, 6.80; N, 10.76. Calcd. for C₂₀H₂₇O₅N₃: C, 61.68; H, 6.99; N, 10.79.

Allethrin-II (VII) from VI. The acid (VI) (100 mg) was methylesterified with diazomethane in ether. After the reaction mixture had been washed with 5% sodium bicarbonate and water, the residual neutral oil was chromatographed on a column (silica gel, 10 g). Yield; 80 mg. TLC (0.2 mm silica gel H

7) A. F. Bramwell, L. Crombie, P. Hemesley, G. Pattenden, M. Elliot and N. F. Jones, *Tetrahedron*, **25**, 1727 (1969).

developed with benzene-ethyl acetate (4:1), $R_f=0.70$).
Cochromatography with an authentic allethrin II
showed a single spot. IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 1715~1730,
1652, 1638, 1150, 992, 915. UV $\lambda_{\text{max}}^{\text{EtOH}}$ (ϵ): 233
(21400).^{4~a)}

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