Studies on Piericidin. II. Insecticidal Effects and Respiratory Inhibition of Piericidin A-Related Compounds. Takashi Mitsui*, Takao Sagawa**, Jun-ichi Fukami*, Kazuo Fukunaga*, Nobutaka Takahashi*** and Saburo Tamura***. (* The Institute of Physical and Chemical Research, Saitama, Japan. ** Research Laboratories of Chugai Pharmaceutical Co., Ltd. Tokyo. *** Department of Agricultural Chemistry, The University of Tokyo, Tokyo.) Received June 23, 1969. *Botyu-Kagaku*, 34, 135, 1969.

19. ピエリシジンに関する研究 II. ピエリシジンA関連化合物の殺虫作用およびミトコンドリアの電子伝達系に及ぼす影響

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ピエリシジンA関連化合物の化学構造とイエバエ, ゴキブリ, アブラムシ, ハダニに対する効力の関係を検討した. ピエリシジンAの側鎖およびピリジン核の水酸基をアセチル化すると殺虫力は減じ, 特に側鎖の水酸基が効力に及ぼす影響が大きい. 側鎖の二重結合に水素添加するとやはり効力は減少する.

ワモンゴキブリ筋肉ミトコンドリアの呼吸および酸化的リン酸化に及ぼす影響についても比較した結果, NADH 酸化酵素系阻害においても, 上とほぼ同様の傾向がみられ, 呼吸阻害—殺虫力の間に相関関係が認められる.

Piericidin A and B have been isolated as insecticides from *Streptomyces mobaraensis* and their chemical structures were elucidated by Tamura, Takahashi *et al.*[1~4]

These compounds are powerful inhibitors of mitochondrial electron transport in beef heart mitochondria (Hall 1966,[5] Miji 1968[6]) and in american cockroach muscle mitochondria (Mitsui 1969)[7], and especially they strongly inhibit NADH oxidation system as sensitively as rotenone. It is considered that their insecticidal activities might be based upon their powerful inhibitory effects on respiration.

The insecticidal activities of Piericidin A and B to aphids and mites are, as reported previously[8], as excellent as rotenone and the other miticides.

The aim of this investigation is not only to discover Piericidin A-related compounds that would surpass the parent compound with regard to biological activity and other qualities required of a good insecticide, but also to know the relationship between the insecticidal activity and the inhibitory activity of respiration.

Materials and Methods

The test insects and mites were house fly female adults, *Musca domestica vicina* Macq., 3 to 5 days old, german cockroach male adults, *Blattella germanica* L., green peach aphids, *Myzus persicae* Sulzer, and carmine mites, *Tetranychus telarius*.

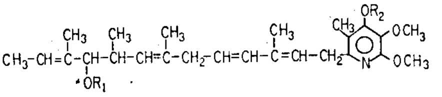
The larvae of house flies were reared in a 50 : 50 mixture of wheat bran and yeast (diet for experimental animals prepared by Oriental Yeast Manufacturing Co.) and the cockroaches with the yeast in a room maintained at 27°C. The green peach aphids were fed on the leaves of egg plants and the carmine mites on the leaves of kidney bean plants in a green house.

The chemicals to be tested, Piericidin A and its related compounds, were summerized in Table 1. Several concentrations of the chemicals were prepared as acetone solution for topical applications and emulsified solution for spray method.

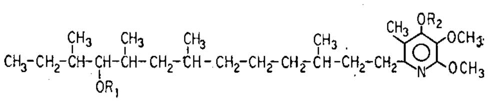
An acetone solution was applied to the dorsal thoracic surface of the house fly and the ventral abdominal surface of the cockroach at the rate of 0.5 and 1.0 μl, respectively. The aphids and mites were directly sprayed with the emulsified solution of the chemicals on the leaves of an egg plant and kidney bean plant, respectively.

After treatment, the house flies and the cockroaches were placed in clean containers and kept at 27°C, and the aphids and mites were kept in a green house. The toxicities of the chemicals were determined by the number of dead-plus-

Table 1. The structure of Piericidin A and its related compounds.



Abbreviation	R_1	R_2
PA (Piericidin A)	H	H
PB (Piericidin B)	CH_3	H
PA-DA	$COCH_3$	$COCH_3$
PA-MA-I	H	$COCH_3$
PA-MA-II	$COCH_3$	H



Abbreviation	R_1	R_2
HPA	H	H
HPA-DA	$COCH_3$	$COCH_3$
HPA-MA-I	H	$COCH_3$
HPA-MA-II	$COCH_3$	H

moribund insects observed at 48 hours after treatment for house flies and cockroaches, and 24 hours after treatment for mites under binocular dissecting microscope. For the aphids, the reduction percentage was calculated by counting the number of aphids/leaf before and 24 hours after treatment. Analysis of the dosage-mortality data was calculated using the probit method of analysis.[9]

The mitochondria from muscle of american cockroach, *Periplaneta americana* L., and rat liver were prepared by following the method of Fukami (1961)[10] and a modified method of Ernster and Löw (1955),[15] respectively. Respiration and oxidative phosphorylation were measured by "Warburg method" with air as gas phase and 0.2 cc of 20% KOH solution in the center well. The composition of the medium for respiration and phosphorylation was as follows; 2.5 mM ATP, 15 mM phosphate buffer (pH 7.4), 7.5 mM $MgCl_2$, 5 mM α-ketoglutarate or 15 mM succinate, 30 mM KCl, 25 mM glucose, hexokinase in excess, and 0.5 cc of mitochondrial suspension containing 6.8 to 7.2 mg of protein, final volume of 2 cc.

The inhibitors were added as 1% ethanol solution at the rate of 0.2 cc per vessel (final concentration of ethanol in medium was 0.1%). Protein determination was made by the biuret method.[12]

Results and Discussion

Insecticidal and miticidal activity

The insecticidal and miticidal activities of the compounds were shown in Table 2 as LD_{50} and LC_{50}. Piericidin A was most active to every insects and mites tested. The other compounds except for PA-MA-I have little efficacy on cockroaches. PA, PA-MA-I and PA-MA-II were effective for carmine mites and PA and PB for green peach aphids, and the other compounds were less effective for both insect and mite.

In general, substitutions of-OH in side chain and in pyridine ring by acetyl radical (R_1, R_2: $COCH_3$) resulted in considerable lessening of the biological activity. Furthermore, acetylation in side chain was inferior in activity to acetylation in pyridine ring. The compound which both -OH were substituted by acetyl radical, PA-DA, had least activity in this series. Hydrogenation of parent structure (HPA series) made the insecticidal activity lessen. Acetylation effect

Table 2. Insecticidal and miticidal effects of Piericidin A and its related compounds.

Compound	Insecticidal and miticidal effect on			
	Carmine mite (LC_{50}: ppm)	Green peach aphid (LC_{50}: ppm)	House fly (LD_{50}: μg/fly)	German cockroach (LD_{50}: μg/roach)
PA	9.3	5.4	0.98	2.45
PB	—	7.6	1.38	—
PA-DA	58.5	945.2	4.27	19.72
PA-MA-I	13.1	47.8	0.99	3.76
PA-MA-II	21.2	144.5	1.05	17.18
HPA	60.9	488.4	10.21	>16
HPA-DA	285.4	536.0	>50	>16
HPA-MA-I	249.8	>1000	15.81	>16
HPA-MA-II	311.8	311.6	32.36	>16

was also observed in this series, and HPA-DA is almost without effect against insects and mites.

Effects on NADH oxidation

The effects of the compounds on mitochondrial respiration of cockroach muscle with α-ketoglutarate as substrate were shown in Fig. 1. I_{50} value (concentration of compound at 50% inhibition) of each compound was also summerized in Table 3. PB and PA-MA-II, which -OH in side chain of parent compound was substituted by $-CH_3$ and $-COCH_3$, respectively, were as sensitive to NADH oxidation system as Piericidin A in american cockroach muscle mitochondria. PAMA-I, which occured acetylation of -OH in pyridine ring of parent compound, was less active to this system.

Hydrogenation of parent structure (HPA series) made the inhibitory effect lessen, and "acetylation effect" was also observed in this series.

The inhibitory effects on NADH oxidation system, as described above, have approximately same tendency as insecticidal and miticidal effects. In contrast with insecticidal and miticidal activity, however, acetylation of -OH in pyridine ring was much more affective to inhibitory effect than acetylation in side chain. Substitution of both -OH in parent structure resulted outstanding decrease in the inhibitory effect; with about 1/20 in activity on PA-DA and with only 24.8% inhibition at the concentration of 10^{-3}Mol of HPA-DA.

I_{50} values on rat liver mitochondria were also

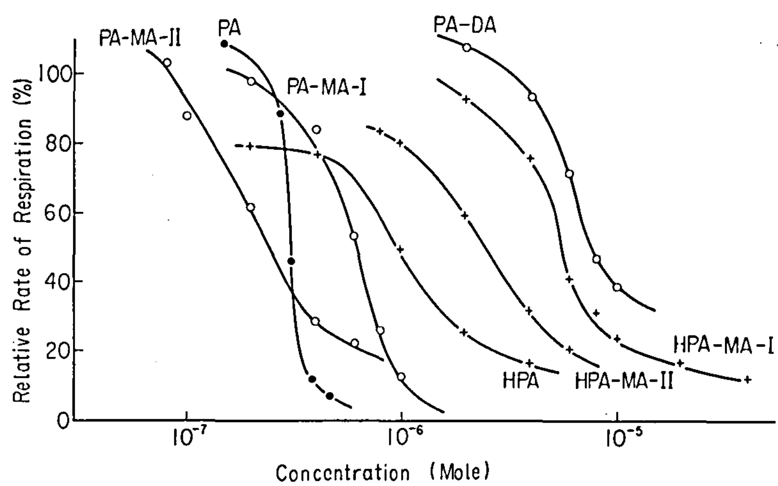


Fig. 1. Effects of Piericidin A-related compounds on mitochondrial respiration of cockroach muscle mitochondria with α-ketoglutarate as substrate.

Table 3. Effects of Piericidin A and its related compounds on mitochondrial respiration and phosphorylation.

Compound	NADH oxidation system I_{50} (Mol) Roach muscle	Rat liver	Succinoxidation system Inhibition (%) at 10^{-3}M of compound	Phosphorylation with succinate Inhibition (%) at 10^{-6}M of compound
PA	3.2×10^{-7}	1.2×10^{-7}	43.4	37.8
PB	2.8×10^{-7}	1.1×10^{-7}	25.9	62.5
PA-DA	7.5×10^{-6}	1.6×10^{-6}	26.6	0
PA-MA-I	6.2×10^{-7}	1.0×10^{-7}	31.8	12.8
PA-MA-II	2.5×10^{-7}	5.8×10^{-7}	28.2	80.0
HPA	9.6×10^{-7}	4.3×10^{-6}	48.9	40.2
HPA-DA	$>10^{-3}$	$>10^{-4}$	9.8	0
HPA-MA-I	5.5×10^{-6}	1.8×10^{-6}	6.6	0
HPA-MA-II	2.5×10^{-6}	2.8×10^{-5}	45.2	0

summerized in Table 3. In this case, they were, in general, parallel to those of cockroach muscle mitochondria in PA series, but HPA series compounds have generally less active to rat liver mitochondria than to cockroach muscle mitochondria.

Effects on succinoxidation system

Effects of the compounds on succinate oxidation were also discussed under the same conditions and summerized as percent in hibition in Table 3. As previously reported[8], Piericidin A inhibited succinate oxidation with only about 40% inhibition at the concentration of 10^{-3}M. None of the compounds tested were more active to this system than Piericidin A. It is considered that inhibition of the succinoxidation system is not important in its insecticidal action.

P:O ratio with succinate

It has generally been observed that P:O ratio with succinate was decreased with Amytal and not with rotenone.[13~15] Piericidin A induced a progressive decrease in the P:O ratio, when added in an amount sufficient to inhibit NADH oxidation system as shown in Fig. 2.

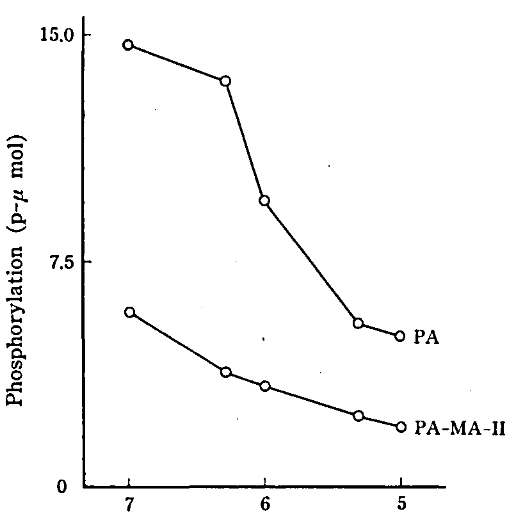


Fig. 2. Effects of PA and PA-MA-II on oxidative phosphorylation with succinate.

PA-MA-II was the most potent inhibitor of phosphorylation (Table 3). HPA was only one in HPA series that has inhibitory effect, and the other compounds in HPA series have little activity at the concentration of 10^{-6}M.

Correlation between insecticidal activity and respiratory inhibition with α-ketoglutarate

Correlation exists between respiratory inhibition (NADH oxidation) of cockroach muscle mitochondria and insecticidal (miticidal) activity as shown in Fig. 3, where -log I_{50} values for respiratory inhibition are plotted against the

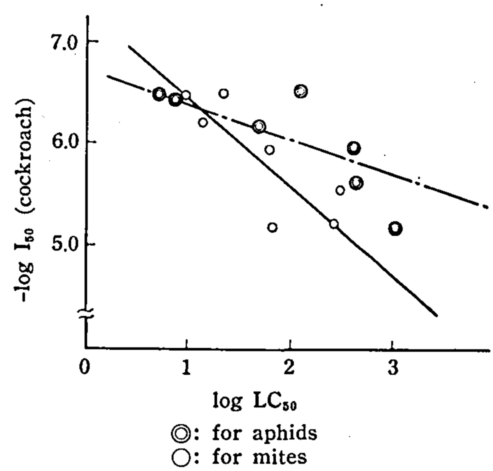


Fig. 3. Relationship of -log I_{50} for inhibition of mitochondrial respiration to LC_{50} for aphids and mites.

insecticidal (miticidal) activity to the aphids and the mites (LC_{50}). Namely, insecticidal and miticidal effects generally increased as respiratory inhibition increased.

From the data reported above, it is probable that the inhibition of NADH oxidation was a prime factor in the mode of insecticidal action of Piericidin A and its related compounds.

Summary

A homologous series of Piericidin A-related compounds was tested against house flies, *Musca domestica vicina* Macq., german cockroaches, *Blattella germanica* L., green peach aphids, *Myzus percicae* Sulzer, and carmine mites, *Tetranychus telarius.*

Acetylation and hydrogenation of parent structure tended to decrease toxicity, and all of them were inferior in toxicity to Piericidin A.

Inhibitory effects on respiration and phosphorylation were also tested against mitochon-

dria of american cockroach, *Periplaneta americana* L., and of rat liver. Correlation existed between the inhibition of mitochondrial respiration and toxicity to green peach apids and carmine mites.

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抄　　録

土壌 TLC を用いた農薬の移動性

Pesticide Mobility: Determination by Soil Thin-Layer Chromatography. Helling, C. S., Turner, B. C. *Science* 162, 562 (1968)

未処理土壌を吸着剤として薄層クロマトグラフィーを行ない，農薬の移動性を R_F 値で表わした．

Lakeland sandy loam (LSL と略), Chillum silt loam (CSL と略), Hagerstown silty clay loam (HSCL と略) を用い通常の方法で薄層を作った．水で展開し，16種の農薬について，その移動性を調べた．どの薬剤についても R_F 値が CSL<HSCL<LSL と増加するのは，土壌中の有機物がこの順に減って行くためと思われる．もし，国際的な規格土壌を用いることができれば，R_F 値による分類ができる．そのような分類にかわるものとして本報告では，HSCL を用いた TLC における R_F 値で，

1) 0～0.09　2) 0.10～0.34　3) 0.35～0.64
4) 0.65～0.89　5) 0.90～1.00

のように便宜上5つにわけ，21種の農薬を分類している．予想されるように酸性の除草剤は，最も移動性がよく，塩素化炭化水素を含む殺虫剤が最も移動性が悪い．　(北村実彬)