Degradations of Vamidothion and Dimethoate in Plants, Insects and Mammals. Osamu Morikawa* and Tetsuo Saito (Laboratory of Applied Entomology, Faculty of Agriculture, Nagoya University, Nagoya) Received June 29, 1966. *Botyu-Kagaku* 31, 130, 1966.

18. バミドチオンおよびジメトエートの解毒分解について

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P^{32}-バミドチオンおよびジメトエートの *in vitro* および *in vivo* における代謝を数種昆虫，植物および哺乳動物をもちいてしらべた．

ワモンゴキブリおよびツマグロヨコバイ成虫によるバミドチオンの *in vivo* における分解をしらべた結果，ジメチル燐酸，脱メチル燐酸，燐酸および未知化合物の4種の化合物が検出された．マウスの尿からも上記4種の代謝物が検出された．一方，稲葉およびリンゴ葉では脱メチル燐酸が検出されなかったが，脱メチルバミドチオンが検出された．

バミドチオンおよびジメトエートの分解におよぼすpHの影響をしらべた結果，ラット肝臓まさい液によるバミドチオンおよびジメトエート分解の最適pHは8.0付近にあり，昆虫まさい液の場合は7.0～7.4であることが明らかとなった．

各種昆虫によるバミドチオンおよびジメトエートの分解量をしらべた結果，ジメトエートの場合はイエバエ成虫，ニカメイガ幼虫，およびモモアカアブラムシ雌虫のまさい液よりもワモンゴキブリ雄成虫のまさい液により強く分解され，バミドチオンの場合にはイエバエ成虫とワモンゴキブリ雄成虫との間に差がなかった．

ワモンゴキブリ雄成虫より摘出した組織のまさい液によるバミドチオンおよびジメトエートの分解量をしらべた結果，両薬剤とも脂肪体まさい液により強く分解され，全消化管および筋肉まさい液でもわずかながら分解されることが明らかになった．

in vitro におけるバミドチオンおよびジメトエートの分解物の同定結果から，ラット肝臓まさい液による S-C 結合の加水分解がみられ，この点が昆虫および植物のまさい液とは異なっているものと考えられる．

The metabolism of dimethoate, *O, O*-dimethyl *S*-(*N*-methyl carbamoylmethyl) phosphorodithioate, which is a relatively low toxic insecticide against mammals, has been studied in detail in insects and plants *in vivo* (Santi and Giacomelli, 1962; Bull *et al.*, 1963; Hacskaylo and Bull, 1963) and in mammals either *in vivo* or *in vitro* (Dauterman *et al.*, 1959; Chamberlain *et al.*, 1961; Uchida *et al.*, 1964).

Vamidothion, *O, O*-dimethy *S*-(*N*-methylcarbamoylethyl thioethyl) phosphorothioate, is closely related with dimethoate in chemical structure. It is interesting to study the metabolism of vamidothion in plants, insects and mammals, just as did in dimethoate.

Materials and Methods

Chemicals. P^{32}-Dimethoate, having specific activity of 3μc/mg, and *O, O*-dimethyl *S*-carboxymethyl phosphorodithioate, designated as carboxy derivative of dimethoate, were provided by Sumitomo Chemical Co. Ltd.

P^{32}-Vamidothion was obtained from Rhône-Poulenc Co. through the courtesy of Shionogi & Co. Ltd. The radioactivity of P^{32}-vamidothion was 0.6μc/mg.

O, O-Dimethyl phosphorodithioic acid was prepared from phosphorous pentasulfide & methanol (Hoegberg and Cassaday, 1951). *O, O*-Dimethyl phosphorothioic acid was prepared by the chlorination of the phosphorodithioic acid and then the hydrolysis of *O, O*-dimethyl thiophosphoryl chloride (Fletcher *et al.*, 1950). *O,O*-Dimethyl phosphate was prepared by the chlorination of dimethyl phosphite and then the hydrolysis of the dimethyl phosphoryl chloride (Kosolapoff, 1950).

O-Methyl *S*-(*N*-methyl carbamoylmethyl) phosphorodithioate, designated as the des-methyl derivative of dimethoate, and *O*-methyl *S*-(*N*-methyl carbamoylethyl thioethyl) phosphorothio

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ate, designated as the des-methyl derivative of vamidothion, were prepared by the refluxing potassium dimethyl dithiophosphate and dimethoate or vamidothion in acetone for two hours (Dauterman *et al.*, 1959). Vamidothion carboxyl derivative, *O,O*-dimethyl *S*-carboxy ethyl thioethyl phosphorothioate, was prepared by the following method ; 0. 1mol of $NaSCH_2CH_2SNa$ was suspended in chloroform and equimolar of α-chloropropionic acid was added dropwisely at 40°C. After stirring for two hours equimolar of dimethyl phosphoryl chloride was added and kept for two hours at 40°C. After filtration, the filtrate was neutralized by the dropwise addition of dilute potassium hydroxide solution and the water layer was concentrated to dryness.

O-Methyl phosphate was prepared by refluxing potassium dimethyl phosphate with equimolar potassium iodide in acetone (Spencer *et al.*, 1958). Other chemicals were commercially obtained.

***In vivo* metabolism of vamidothion.** Adult male of the American cockroach, *Periplaneta americana* L., and adult female of the green rice leafhopper, *Nephotettix cincticeps* Uhler, were topically applied with the propylene glycol solution of P^{32}-vamidothion of dosage of 500 and 2μg per insect, respectively, and they were kept at 25°C. After definite periods, the insects were homogenized with 1 m*l* of chloroform and 1 m*l* of water, chloroform layer was separated by centrifugation, and the water layer were extracted three times with chloroform. Chloroform layers thus obtained were combined. Two m*l* of ethanol was added to aliquote and the mixture was kept overnight in a refrigerator at 5°C. Then, the precipitate was removed by centrifugation and the clear supernatant was concentrated at 45°C by means of a vaccum rotary evaporator. An aliquot of water soluble material was paper chromatographed.

An Adult male of mouse, strain NC-5, was orally treated with 30mg of vamidothion and kept in a metabolic cage. Excreted urine was collected for 24 hours. The collected urine was extracted with chloroform and water as mentioned above.

Leaf chops of apple and rice were placed on 0. 05% water solution of P^{32}-vamidothion at 25°C. After 24 hours, they were homogenized, and then water extracts were prepared as mentioned above.

***In vitro* metabolism of vamidothion and dimethoate.** Adults of male American cockroaches, mixed sexes of adult houseflyies, 4th to 5th insters larvae of rice stem borer, *Chilo suppressalis* Walk., adults of apterous form of green peach aphid, *Myzus persicae* Sulzer, and leaves of rice and cabbage were homogenized with the ice-cold buffer and centrifuged at 3, 000r. p. m. for 10min. The supernatant was used as the enzyme solution. The liver of female rat, Wistar strain, was used for the enzyme preparation as mentioned above.

P^{32}-Vamidothion and P^{32}-dimethoate were used as water solution. The typical reaction medium was as follows:

1/15M Phosphate buffer	2. 0m*l*
1.8×10^{-2}M P^{32}-Vamidothion or -dimethoate	0. 5m*l*
10% Homogenate	0. 5m*l*

The reaction mixture was incubated for two hours at 37°C, then 3m*l* of chloroform was added. Both chloroform and water layers separated were chromatographed and then chromatostrips were submitted for radioassay.

Chromatography. The metabolites were identified or determined by the paper or ion exchange chromatography. The water extracts were applied to Toyo No. 51A filter paper and developed by the following two systems of solvents. The first was the mixture of isopropanol-ammonium hydroxide (75:25) (Plapp and Casida, 1958), the second was the mixture of acetonitrile-water-ammonium hydroxide (40 : 9 : 1) (Hacskaylo and Bull, 1963). After drying, the radioactivity on the paper chromatogram was determined by the Aloka 4π low back ground gas flow paper chromatogram scanner.

The metabolites of dimethoate were also separated by ion exchange chromatography (Dauterman *et al.*, 1959). The radioactivities of eluents were measured by the liquid type glass GM counter.

Results and Discussion

***In vivo* metabolism of vamidothion.** Rf values of related compounds of vamidothion are shown in Table 1. The metabolic products of P^{32}-vamidothion found in insects, animal and plants were given in Table 2. Des-methyl vamidothion

Table 1. Rf values of vamidothion related compounds in paper chromatography using isopropyl alcohol-ammonium hydroxide system.

Compound	Rf
$CH_3O, KO > P(=O)-SCH_2CH_2S-CH(CH_3)CONHCH_3$	0.84
Unknown	0.60
$(CH_3O)_2P(=O)-SCH_2CH_2S-CH(CH_3)COOH$	0.52
$(CH_3O)_2P(=O)-OH$	0.35
$CH_3O, KO > P(=O)-OH$	0.02
H_3PO_4	0.00

was detected only in plants, and no spot corresponding to the carboxyl derivative of vamidothion was detected in all materials. But it is still difficult to conclude that desmethyl vamidothion is not produced within the insects and animal and the carboxyl derivative of vamidothion within all test organisms. P^{32}-Vamidothion used in this experiment was rathr weak in its radioactivity.

Effect of pH on the *in vitro* metabolism of vamidothion and dimethoate. Effects of pH on the degradation of vamidothion and dimethoate in the reaction medium are shown in Fig. 1. The optimum pH for the degradation of the insecticides was about 8.0 for the rat liver homogenates and near 7.0 to 7.4 for the insect homogenates. These difference of optium pH for

Table 2. Metabolic degradation products of P^{32}-vamidothion in insects, mouse and plants identified by paper chromatography.

Material	Percent of radioactivity				
	H_2PO_4	$CH_3O, HO > P(=O)OH$	$(CH_3O)_2P(=O)OH$	unknown	$CH_3O, HO > P(=O)SC_2H_4SCH(CH_3)C(=O)NHCH_3$
American cockroach					
3 hours (normal)	10.2	23.0	35.4	31.4	0
18 hours (paralysis)	16.4	19.1	45.8	18.7	0
Green rice leafhopper					
3 hours (paralysis)	25.5	22.5	29.5	22.6	0
18 hours (paralysis)	28.2	15.7	29.5	26.6	0
Mouse urine					
24 hours (normal)	7.7	16.2	44.7	31.4	0
Rice leaf					
24 hours	10.9	0	5.7	31.0	52.4
Apple leaf					
24 hours	27.3	0	11.0	45.7	16.0

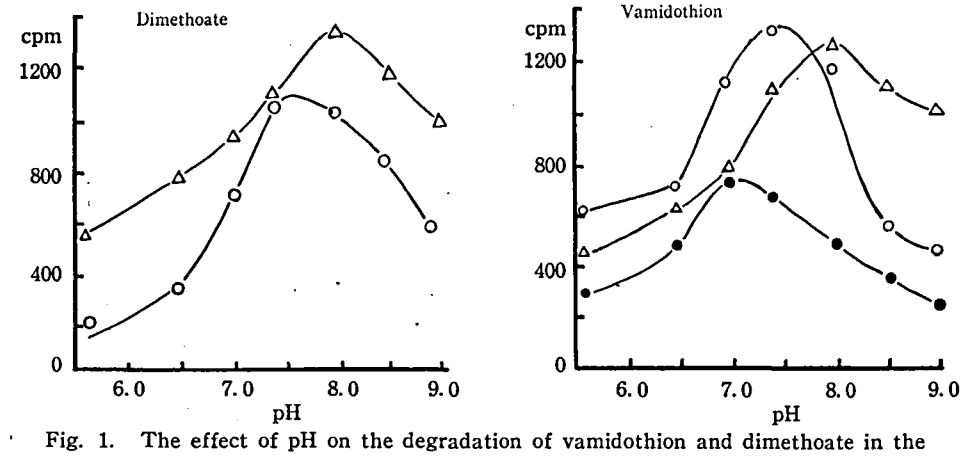

Fig. 1. The effect of pH on the degradation of vamidothion and dimethoate in the homogenate of insect and rat liver.
—○—; House fly, —●—; American cockroach, —△—; Rat liver

the degradation of insecticide may be caused by the difference of the primal degradation site between rat liver homogenate and insect preparation.

Species specificity of the degradation of vamidothion and dimethoate. Total amounts of degradation products of vamidothion and dimethoate were determined at pH 7.4 in the various insect homogenates. Results obtained are shown in Table 3. The cockroach homogenate degradated large amount of dimethoate compared with other insect homogenates did. There was little difference in the degradation amounts of vamidothion between cockroach and house fly homogenates.

Table 3. Amounts of dimethoate and vamidothion degradated by various insect homogenates.

Material	Amount of dimethoate degradated (μg/g/hr.)	Amount of vamidothion degradated (μg/g/hr.)
American cockroach	79.6	13.8
Housefly	9.0	15.5
Rice stem borer	12.4	—
Green peach aphid	10.4	—

Degradations of vamidothion and dimethoate in various tissues. The male adult of the American cockroach was dissected and each tissue was collected in the phosphate buffer, pH 7.4. Total amounts of degradation products of vamidothion and dimethoate by different tissue homogenates were determined (Table 4).

Table 4. Amounts of vamidothion and dimethoate degradated in various tissue homogenates of the American cockroach.

Insecticide	Tissue	Amount of insecticide degradated (μg/g/hr.)
Vamidothion	Total gut	10.1
	Fat body	36.8
	Muscle	9.0
	Total body	16.8
Dimethoate	Total gut	15.7
	Fat body	53.5
	Muscle	19.2
	Total body	25.3

Table 4 shows that vamidothion and dimethoate were hydrolysed greatly by the fat body homogenate, and slightly by the total gut and muscle homogenates.

Nature of metabolic products of vamidothion and dimethoate. After incubation with insects, plants and rat liver homogenates for two hours, several metabolic products of the insecticides were separated by the ion exchange or paper chromatography, and were identified with authentic samples by cochromatography (Tables 5 and 6). Dimethyl phosphorodithioate derived from the hydrolysis of dimethoate at S-C bond was detected only in the rat liver homogenate. Uchida *et al.* (1964) described that the rat liver homo-

Table 5. Metabolites of dimethoate found in insects, plants and rat liver homogenates.

	Material	House fly	American cockroach	Rice stem borer	Rice leaf	Cabbage leaf	Rat liver
Metabolite	$(CH_3O)_2\overset{O}{\overset{\|}{P}}OH$	0%	0%	0%	0%	0%	0%
	$(CH_3O)_2\overset{S}{\overset{\|}{P}}SCH_2COOH$	27.3	17.7	24.0	0	20.0	2.5
	$(CH_3O)_2\overset{S}{\overset{\|}{P}}OH$	6.9	69.5	10.8	0	17.6	58.6
	$CH_3O, HO>\overset{S}{\overset{\|}{P}}SCH_2\overset{O}{\overset{\|}{C}}NHCH_3$	63.1	12.8	65.2	100	40.8	11.6
	$(CH_3O)_2\overset{S}{\overset{\|}{P}}SH$	0	0	0	0	0	25.8
	Unknown	2.7	0	0	0	21.7	1.5

Table 6. Metabolites of vamidothion found in house fly and rat liver homogenates.

Material	Metabolite				
	Unknown	$(CH_3O)_2\overset{O}{\overset{\|}{P}}OH$	$(HO)_2\overset{O}{\overset{\|}{P}}OH$	$CH_3O(HO)\overset{O}{\overset{\|}{P}}SC_2H_4S\overset{CH_3}{\overset{\|}{C}}H\text{-}\overset{O}{\overset{\|}{C}}NHCH_3$	$(CH_3O)_2\overset{O}{\overset{\|}{P}}SC_2H_4S\overset{CH_3}{\overset{\|}{C}}HCOOH$
House fly	0%	65.4	34.6	0	0
Rat liver	55.4%	13.9	30.7	0	0

genate gave two metabolic products of dimethyl phosphorodithioate and carboxy derivative of dimethoate. But in this study, five metabolic products were detected in the rat liver homogenate, and dimethyl phosphorothioate was a major product.

In the hydrolysis products of vamidothion, one spot showing positive reaction with ammonium silver nitrate and Hanes and Isherwood reagents was obtained. However, it has not been identified. Therefore, it seems to be dimethyl phosphorothionate, which was a major product in the rat liver homogenate.

The proposed sites of hydrolysis of dimethoate and vamidothion are shown in Fig. 2. The hydrolysis of S-C bond was specific to the rat liver homogenate.

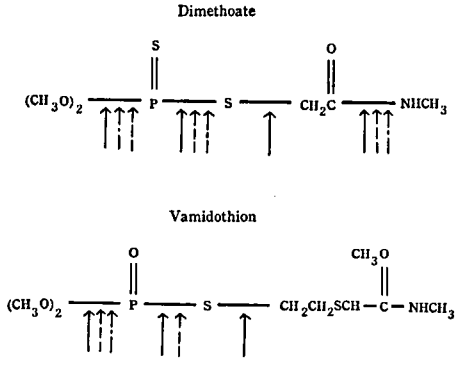

Fig. 2. The sites of hydrolysis of dimethoate and vamidothion. in rat liver, insect and plant homogenates.
⟶ ; Rat liver ⟶ ; Insect ⟶ ; Plant

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Summary

The metabolism of dimethoate and vamidothion in insects, plants and mammals was studied *in vivo* and *in vitro*.

Several metabolic products of P^{32}-vamidothion were detected within insects, plants and mouse urine by paper chromatography. Des-methyl vamidothion was detected only in plants.

The optimum pH for the degradation of dimethoate and vamidothion was about 8.0 for the rat liver homogenate, and 7.0 to 7.4 for the insect homogenates.

The American cockroach homogenate degradates larger amounts of dimethoate than the house fly, rice stem borer and green peach aphid homogenates, but there was a little difference in amount of degradation of vamidothion between the American cockroach and house fly homogenates. Amounts of the insecticides degradated were determined in the several tissues of the American cockroach, and the order of degradation activity was fat body>total gut=muscle.

The hydrolysis of S-C bond of dimethoate and vamidothion was specific to the rat liver homogenate.

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Inhibition of Development of the House Fly by Synergists. Akifumi HAYASHI (Laboratory of Applied Entomology, Taisho Pharmaceutical Co., Ltd. Toshima-ku, Tokyo) Received July 18, 1966. *Botyu-Kagaku* 31, 135, 1966. (with English Summary, 136)

19. 協力剤によるイエバエの発育抑制作用について

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41. 7. 18 受理

数種協力剤のイエバエに対する発育抑制効果を検定した結果，sulfoxide と safroxan は，0.03% 及び0.06%の濃度で，高い抑制効果のある事が認められた．また，allethrin に対して優れた協力効果を示す S-421 が，methylendioxyphenyl 基をもたないにもかかわらず，0.25% の濃度で高い効果を示す事は興味深い，しかし，p. butoxide は，0.25% 以下では発育抑制効果が認められなかった．また局所施用法の結果，safroxan 0.5%，0.25%や salfoxide 0.5%，0.25% 区で，羽化阻害効果のある事を知った．

Mitlin *et al.* (1955)[2] (1956)[3]によって，pyrethroid の協力剤である piperonyl butoxide や，他の methylenedioxyphenyl 化合物が，イエバエの発育を強く抑制する事が報告された．又，酒井 (1960)[4] も，slow down chemical としての作用で将来の研究課題である事を指摘している．著者は，協力剤の作用機作を知る一つの試みとして，本実験を行ない，知見を得たので報告する．

本文に入るに際し，常々御指導をいただいている名古屋大学農学部弥富喜三教授，並びに斎藤哲夫助教授に厚く御礼申し上げる．又，発表を御快諾くだされた当社の上原昭二副社長，研究部長田中一郎博士，及び実験に協力された甘日出正美研究員に謝意を表する．

実験材料及び方法

(1) 供試薬剤　実験に用いた協力剤は，sulfoxide, safroxan, S-421, *n*-propyl isom 及び piperonyl butoxide の5種類で，いずれもテクニカルグレードの

Table 1. Effect of several synergists on the development of the house fly.

Material and conc. (%)		No. larvae tested	Larval period (Mean days)	Per cent pupation	Per cent emergence
Piperonyl butoxide**	1.00	120	8.0	25.0	11.7
	0.50	120	8.0	78.0	51.7
	0.25	120	8.0	98.0	80.0
	0.125	120	8.0	93.0	86.6
Sulfoxide*	0.25	180	9.0	2.8	0.6
	0.125	180	8.3	5.5	0
	0.06	180	8.3	12.8	1.1
	0.03	180	8.3	48.3	5.6
n-propyl isome*	0.25	180	8.4	43.7	31.1
	0.125	180	8.0	83.5	58.9
	0.06	180	7.8	86.1	69.4
Safroxan*	0.125	180	8.5	12.2	0.6
	0.06	180	8.1	52.8	5.0
S-421*	0.25	180	10.3	6.1	4.4
	0.125	180	8.8	51.6	41.1
Untreated*		180	8.1	91.1	70.0

* Nine replications　** Six replications