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Metabolism of Some Carbamate Insecticides in the Green Rice Leafhopper and the Smaller Brown Planthopper*

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When carbonyl-^{14}C-labeled 3,5-xylyl methylcarbamate (XMC) and 3-*tert*-butylphenyl methylcarbamate (terbam) were topically applied on the adults of the green rice leafhopper, *Nephotettix cincticeps* Uhler, and the smaller brown planthopper, *Laodelphax striatellus* Fallén, penetration of radioactivity into the insect body was rapid. When the radioactivity taken up by insects was divided into three fractions, that is, water soluble, organosoluble, and unextracted, most of the radioactivity was found in the organosoluble fraction. Water soluble radioactivity gradually increased in course of time. Although parent compounds occupied 67–98% of organosoluble radioactivity, the ratio of metabolites gradually increased in course of time. Several metabolites were detected by *tlc*-radioautography. The distribution patterns of metabolites were different between the green rice leafhopper and the smaller brown planthopper. *In vitro* metabolism of XMC in microsome fraction of the green rice leafhopper was accelerated by the addition of $NADPH_2$ and was inhibited by piperonyl butoxide. Among several metabolites, *N*-hydroxymethyl derivatives of the insecticides were identified.

INTRODUCTION

Carbamate insecticides have still assumed an important role in rice cultivation for the control of leafhoppers and planthoppers, although resistance development to these insecticides has recently been reported among some strains of the green rice leafhopper, *Nephotettix cincticeps* Uhler.

Metabolism of carbamate insecticides, especially that of carbaryl, has been studied in a number of biological systems, and the metabolic pathways in animals,[1] plants and insects[2] have been reviewed. However the metabolism of carbamate insecticides developed in Japan has not yet been extensively investigated with only a few examples.[3–6] Concerning with the metabolic fate of these chemicals in plant- and leafhoppers, Moriya and Maeda[7] examined *in vivo* metabolism of 2-*sec*-butylphenyl methylcarbamate and *o*-cumenyl methylcarbamate on and in two strains of the green rice leafhopper, and suggested metabolic differences between these two strains. The present study examined *in vivo* and *in vitro* metabolism of 3,5-xylyl methylcarbamate (XMC) and 3-*tert*-butylphenyl methylcarbamate (terbam) by the green rice leafhopper and the smaller brown planthopper, *Laodelphax striatellus* Fallén.

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MATERIALS AND METHODS

1. *Chemicals*

Carbonyl-^{14}C-labeled XMC and terbam were synthesized by the reaction of acetyl-1-^{14}C with 3,5-xylenol or 3-*tert*-butylphenol, respectively, according to the method described by Krishna *et al.*[8] The labeled products were purified by *tlc* (solvent system; ethyl ether: *n*-hexane, 4 : 1). Specific activities were 980 μCi/mM (carbonyl-^{14}C-XMC) and 803 μCi/mM (carbonyl-^{14}C-terbam).

Table 1 Susceptibility of insects to XMC and terbam.[10]

Insecticides	LD_{50} by topical application (ng/insect, female)*		
	Green rice leafhopper		Smaller brown planthopper
	Warabi	Nakagawara	Nishigahara
XMC	9.2	296	4.1
Terbam	5.2	468	1.7

* Average body weight was as follows:
Green rice leafhopper 4 mg/insect
Smaller brown planthopper 1 mg/insect

Unlabeled XMC and terbam were furnished by Hodogaya Chemical Co. Ltd. 3,5–Xylyl *N*–hydroxymethylcarbamate and 3–*tert*–butylphenyl *N*–hydroxymethylcarbamate were synthesized according to the method described by Durden *et al.*[9]

2. Insects

Two strains (Warabi and Nakagawara) of the green rice leafhopper and one strain (Nishigahara) of the smaller brown planthopper were used for this investigation. Insects were reared in the laboratory using rice seedlings. In case of *in vivo* experiment, female adults of two to seven days after emergence were used. For *in vitro* study, male and female adults of two to seven days after emergence were used as enzyme source. Susceptibility of these insects to XMC and terbam was shown in Table 1.

3. In vivo Metabolism

Female adults of two to seven days after emergence were anesthetized in the stream of CO_2 and were topically applied with 0.1 μl/insect of acetone solution containing appropriate amount of labeled chemicals. One half value of LD_{50} of XMC to Warabi strain and its 5-fold value were adopted for the dosal amount. The amount of chemicals actually treated on insects was calculated from the radioactivity applied on a piece of filter paper in a similar way as for insects using a micrometer-driven syringe. Treated insects were kept in a paper cup containing rice seedlings at 27°C. One, 3, 6, and 24 hr after application, each group of 60 to 80 insects was rinsed with acetone to remove radioactivity remaining on the cuticle. Rinsed insects were homogenized in a mortar with 66.7% aqueous acetonitrile. Supernatant solution of the homogenates after centrifuge was extracted with chloroform. Aliquots of acetone, and chloroform and aqueous layers of the supernatant were taken to measure radioactivity. Chloroform extracts were reduced to a suitable volume by evaporation *in vacuo*, and thin layer chromatography was performed on plates 20×20 cm coated with silica gel G 0.5 mm thick. Chromatograms were developed with ethyl ether : *n*–hexane (4 : 1, v/v), and were exposed to X–ray film for appropriate periods and then developed.

4. In vitro Metabolism

Microsome fraction of the green rice leafhopper was obtained according to the method described by Tsukamoto and Casida.[11] Whole bodies of 1,600 adult insects, weighed 4.6 *g*, were homogenized in 30 ml of 0.15 M phosphate buffer (pH 7.4) added with 0.25 M sucrose using Potter-Elvehjem homogenizer in ice-cold bath. Incubation was performed using the microsomal fraction which was sedimented at 100,000 *g* for 60 min from the supernatant fraction of sedimentation at 10,000 *g* for 30 min. Microsome fraction was resuspended in 12 ml of buffer solution, and each of 2 ml, which contained 1.3 mg of total nitrogen, was used for incubation. The substrate, 1 μmole of radiolabeled carbamates in acetone solution was initially added to a 30 ml glass vial, the solvent was evaporated, and then, if necessary, other reaction constituents such as coenzyme (5 μmole of $NADPH_2$) and inhibitor (2 μmole of

piperonyl butoxide) in above-mentioned buffer solution were added making the whole volume 5 ml. After 3 hr incubation at 37°C, the contents were added with 10% trichloroacetic acid, extracted with three portions of 10 ml ethyl ether, and thin layer chromatographic separation was performed.

All determinations of radioactivity were made by a Packard Tricarb Liquid Scintillation Spectrometer Model 3003. Dioxane based cocktail (PPO 3.5 g, POPOP 0.15 g, naphthalene 40 g in 1 *l* dioxane) and toluene based cocktail (PPO 2.5 g, POPOP 0.15 g in 1 *l* toluene) were used for aqueous and organosoluble fractions respectively. In case of insoluble materials, powdered samples were suspended in toluene based scintillator containing 3% CAB-O-SIL® gel powder.

RESULTS AND DISCUSSION

1. *In vivo Metabolism*

Distribution of radioactivity found on and in insect bodies 1, 3, 6, and 24 hours after the treatment was shown in Tables 2 and 3. Disappearance of radioactivity from the surface of insect body was rapid. A greater part of disappearance was resulted from the penetration into the body, because a large amount of radioactivity was found in aqueous and chloroform layers, and insoluble residues of the homogenate, although some amount of radioactivity might be lost by volatilization from the surface as discussed by Moriya and Maeda.[7]

Penetration of chemicals into the smaller brown planthopper body was more rapid than in case of the green rice leafhopper. This phenomenon coincided with the smaller KT_{50}

Table 2 Distribution of radioactive metabolites found on each fraction after topical application of ^{14}C-XMC and terbam on the green rice leafhopper.

Insecticides and dose	Fractions	ng Equivalent per insect			
		Time after application (hr)			
		1	3	6	24
XMC 23.3 ng/insect	Outside	3.37	1.05	0.36	0.18
	Aqueous	0.08	0.23	0.36	0.71
	Organosoluble	9.02	5.60	4.84	5.07
	Unextracted	0.21	0.21	0.20	0.15
	Total*	12.68 (54.4)	7.09 (30.4)	5.76 (24.7)	6.11 (26.2)
XMC 4.7 ng/insect	Outside	0.65	0.44	0.44	0.24
	Aqueous	0.43	0.34	0.50	0.49
	Organosoluble	2.00	1.00	1.72	1.42
	Unextracted	0.45	0.48	0.44	0.31
	Total*	3.53 (75.0)	2.26 (48.1)	3.10 (65.8)	2.46 (52.0)
Terbam 22.2 ng/insect	Outside	3.70	0.76	0.45	0.15
	Aqueous	0.14	0.19	0.35	0.45
	Organosoluble	7.88	11.77	6.71	5.12
	Unextracted	0.19	0.22	0.31	0.10
	Total*	11.91 (53.6)	12.94 (58.3)	7.82 (35.2)	5.82 (26.2)
Terbam 4.6 ng/insect	Outside	0.71	0.43	0.36	0.33
	Aqueous	0.27	0.49	0.46	0.48
	Organosoluble	1.60	2.18	2.03	1.44
	Unextracted	0.53	0.62	0.47	0.39
	Total*	3.11 (67.6)	3.72 (80.8)	3.32 (72.3)	2.64 (57.5)

* Values in parentheses are percents on the basis of the initial dose.

Table 3 Distribution of radioactive metabolites found on each fraction after topical application of ^{14}C–XMC and terbam on the smaller brown planthopper.

Insecticides and dose	Fraction	ng Equivalent per insect			
		Time after application (hr)			
		1	3	6	24
XMC	Outside	0.43	0.38	0.39	0.15
6.7 ng/insect	Aqueous	1.04	1.65	2.17	1.88
	Organosoluble	4.29	3.34	2.72	3.25
	Unextracted	0.11	0.06	0.10	0.08
	Total*	5.65 (84.6)	5.43 (81.4)	5.38 (80.6)	5.36 (80.2)
Terbam	Outside	0.49	0.44	0.62	0.31
8.2 ng/insect	Aqueous	1.57	1.98	2.08	2.20
	Organosoluble	6.30	5.73	3.98	3.63
	Unextracted	0.13	0.13	0.12	0.19
	Total*	8.48 (103.4)	8.28 (100.8)	6.79 (82.7)	6.33 (77.1)

* Values in parentheses are percents on the basis of the initial dose.

values (50% knock down time) of these two insecticides for the smaller brown planthopper.[10] Further experiment in regard to the penetration of carbamates with short intervals after the treatment is needed because more than half a dose disappeared from the surface within one hour after the application.

The amount of radioactivity in solvent fractions of insect homogenates decreased in course of time with simultaneous increase of radioactivity in aqueous layers. It is likely that solvent-soluble insecticides were detoxified to become aqueous metabolites. The formation of water soluble metabolites was larger in lower doses of chemicals in case of the green rice leafhopper, and was larger in the smaller brown planthopper than in the green rice leafhopper.

Three reasons might be considered for low recovery of radioactivity especially in case of the green rice leafhopper. First reason is the excretion of radioactivity with honey dews. The larger recovery ratio in case of the smaller brown planthopper might be caused by smaller excretion of chemicals as a result of early paralysis. Second reason is $^{14}CO_2$ formation by the hydrolytic degradation of carbamates. Zayed *et al.*[12] proved that $^{14}CO_2$ was trapped in the metabolism study of carbonyl–^{14}C–carbaryl by cotton leaf worm, *Prodenia litura*, and Cocks[13] reported the American cockroach, *Periplaneta americana*, metabolized *N*–methyl–^{14}C–carbaryl to form $^{14}CO_2$. Speculative third reason for low recovery is volatilization of insecticides from the body surface. Ohkawa *et al.*[4] mentioned that 3-tolyl methylcarbamate was lost from pea leaves by volatilization. Disappearance of XMC and terbam from the surface of glass plate and filter paper at 27°C was measured as shown in Table 4. Direct volatilization might be overlooked as the cause of low recovery, although the surface structures of glass, paper, and insect body are so different that volatilization from the body surface of insect should be distinguished as Moriya and Maeda[7] discussed. Sugiyama *et al.*[14] also noticed decreasing recovery of radioactivity originated from ^{14}C–carbaryl treated on the black tipped leafhopper, *Bothrogonia iaponica*. Experiments in the metabolic cage of closed system should be performed to clarify the overall fate of carbamate insecticides in plant- and leafhoppers.

Thin layer chromatograms of solvent soluble metabolites were shown in Fig. 1. Although the distribution patterns of metabolites on chromatograms were different between the green rice leafhopper and the smaller brown planthopper, most of radioactivity recovered from the chromatograms was those of parent compounds as shown in Table 5. Parent compounds decreased in course of time with increase

Table 4 Disappearance of ^{14}C–XMC and terbam from the surfaces of glass plate and filter paper at 27°C.*

Time after application (hr)	Percent found	
	XMC	Terbam
Glass plate		
0	100	100
1	13.9	53.6
3	13.9	27.5
6	5.6	18.8
24	5.6	7.2
Filter paper		
0	100	100
1	96.7	97.7
3	104.7	94.1
6	88.2	97.0
24	74.4	91.8

* Initial amounts of chemicals were as follows:
Glass plate: XMC 7.2 ng, terbam 20.7 ng
Filter peper: XMC 485 ng, terbam 573 ng

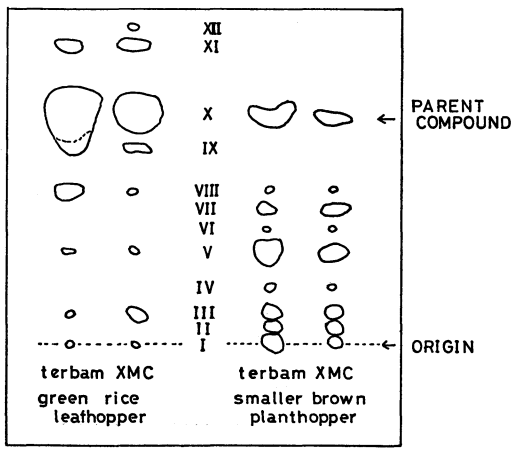

Fig. 1 Distribution of radioactive metabolites on *tlc*-radioautogram of *in vivo* metabolism study.

Table 5 The amount of parent and its degradative metabolites found in organosoluble fraction of the homogenate after *tlc*-radioautography.

Time after application (hr)	ng Equivalent*							
	Green rice leafhopper				Smaller brown planthopper			
	XMC		Terbam		XMC		Terbam	
	P**	M***	P**	M***	P**	M***	P**	M***
1	1.46	0.54	1.35	0.25	3.30	0.99	5.86	0.44
3	0.99	0.01	2.15	0.03	2.14	1.20	5.07	0.67
6	1.47	0.25	1.37	0.66	1.60	1.12	3.52	0.46
24	0.96	0.46	0.95	0.25	2.24	1.01	2.45	1.18

* Initial doses were as follows:

Green rice leafhopper	XMC	4.7 ng/insect
	terbam	4.6 ng/insect
Smaller brown planthopper	XMC	6.7 ng/insect
	terbam	8.2 ng/insect

** Parent compound
*** Metabolites other than parent compound.

of metabolites other than the parent. These metabolites found on the radioautograms are supposed to have intact carbamoyl group, and are expected to be oxidative products of the insecticides. According to Oonnithan and Casida,[15] few metabolites other than corresponding phenols were located on the chromatograms where *Rf* values were larger than those of the parent compounds in this solvent system. Existence of metabolites XI and XII is interesting. These two spots were not detected in case of the planthopper.

2. In vitro Metabolism

Distribution of radioactive spots on *tlc* of ether extracts of incubation mixture was

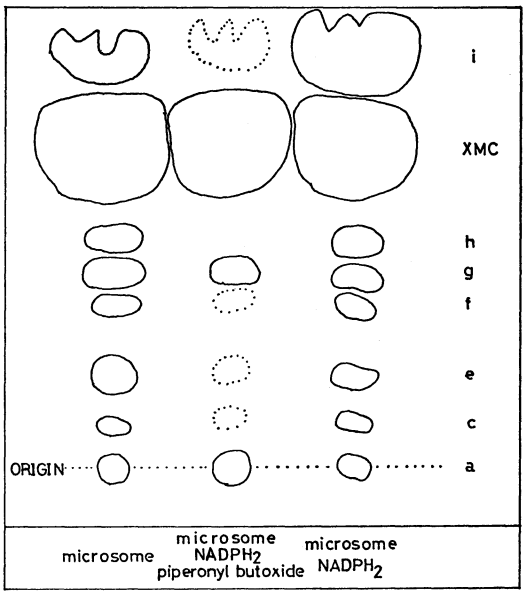

Fig. 2 Distribution of radioactive metabolites on *tlc*-radioautogram of *in vitro* metabolism study.

shown in Fig. 2. Ratio of radioactivity between parent and metabolite "i" was 80 : 20, 60 : 40, and 96 : 4 in the incubation mixtures of microsome only, microsome–$NADPH_2$, and microsome–$NADPH_2$–piperonyl butoxide, respectively. Formation of metabolite "i" (corresponding to XI and/or XII in Fig. 1) was strongly inhibited by the addition of piperonyl butoxide. This fact proves that metabolite "i" was produced through the oxidative metabolism from XMC. Identification of this metabolite has not yet been succeeded because the amount was too small to be analyzed by instrumental analytical procedures except co-chromatography. Metabolism of carbamate insecticides shown by plant- and leafhoppers is not vigorous that is difficult to obtain sufficient amount of metabolic products for identification. Therefore, utilization of ascorbic acid oxidation system which was developed by Brodie *et al.*[16] and applied on Matacil,® 4–dimethylamino–3–tolyl methylcarbamate, by Balba and Saha[17] might be advantageous because XMC was degraded in this system more efficiently than in insects *in vivo* and *in vitro*.

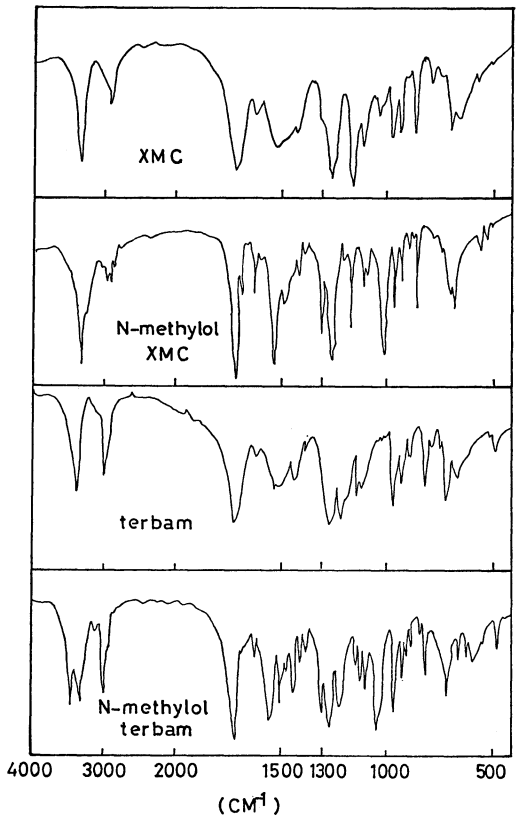

Fig. 3 Infrared spectra of XMC and terbam, and their *N*–hydroxymethyl derivatives.

Among metabolites formed by plant- and leafhoppers, metabolite "e" (corresponding to V in Fig. 1) was tentatively identified to be *N*–hydroxymethyl derivatives of insecticides by co-chromatography. Infrared spectra of synthetic products together with parent compounds were shown in Fig. 3. Strong absorption at $1{,}300^{-CM}$ showed $-CH_2OH$ moiety. Metabolite "e" and synthesized *N*–hydroxymethyl derivatives gave same *Rf* values on *tlc*. Furthermore, hydrolytic products of metabolite "e" and synthesized compounds gave the same *Rf* values with those of phenols which were confirmed by co-chromatography.

Though some aspects of metabolic fate of carbamate insecticides on plant- and leafhoppers have been elucidated in this study, more extensive research should be performed on the metabolism including identification of each metabolite, especially from the standpoint

of resistance problem in the green rice leafhopper.

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要　　約

カーバメート系殺虫剤のツマグロヨコバイおよびヒメトビウンカにおける代謝

風野　光，浅川　勝，富澤長次郎

局所施用したカルボニル-^{14}C-標識3,5-xylyl methylcarbamate(XMC)および3-*tert*-butylphenylmehylcarbamate（ターバム）のツマグロヨコバイおよびヒメトビウンカ体内への浸透は迅速であった．昆虫体からの放射能の回収率は時間の経過とともに低下したが，その原因としてとりこまれた放射能がhoney dewとともに排泄されることおよび呼気として失われることなどが考えられた．とりこまれた放射能を溶媒可溶性，水溶性および抽出残渣に分別したとき，大部分の放射能は溶媒可溶性分画に存在したが，時間とともに水溶性分画の放射能が増加した．溶媒可溶性分画の放射能を*tlc*で分離した結果ではその67～98％は親化合物であったが，代謝物の相対値は時間とともに増加した．ツマグロヨコバイとヒメビウンカでクロマトグラムに相違が認められた．ツマグロヨコバイのミクロゾーム分画による*in vitro*代謝で，XMCは酸化的に代謝されることを確認した．*in vivo*, *in vitro*の代謝物の一つとして*N*-メチル基の酸化された*N*-hydroxymethyl XMCおよび*N*-hydroxymethylターバムを確認した．