without exposure to any insecticide in laboratory. Strains resistant to carbofuran was obtained by treating S strain for 30 generations with carbofuran 10% wettable powder at 30 ~70% mortality. Hereafter, the strain is referred to as Rc-30. The selection was performed on the 3rd and 4th instar nymphs by spray method using the Potter's spay tower (Burkard, UK). Planthoppers which survived 24 hours after treatment were reared on 'Chucheong' rice plant (*Oryza sativa* L.) seedlings (7~10 d after germination) in acrylic cages (26×30×20 cm) at 26 ± 1℃, 65 ± 5% RH, and a photoperiod of 16 : 8 (L : D) hours to produce the offspring. LD_{50} of the resistant strain, RC-30, was 20.3 μg/g and that of the susceptible one, S, was 0.3μg/g, respectively.

Chemicals

^{14}C-Labelled carbofuran (2,3-Dihydro-2,2-dimethyl-[Ring -3-^{14}C]benzofuran-7-yl methyl carbamate) was used. The specific radioactivity of ^{14}C-carbofuran with >99% purity was 18.58 mCi/mmol. The solvents used in this studies were purchased from Sigma.

Enzyme preparation

The BPH microsomes were prepared as follows (Wheelock and Scott, 1992). Abdomens of 1,000 female brown planthoppers were collected in ice powder after freezing at -70℃ and homogenized in 0.1 M sodium phosphate buffer (pH 7.5), containing 10% glycerol (v/v), 1 mM ethylenediaminetetraaceticacid, 0.1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride (PMFS) and 1 mM 1-Phenyl 1-2-thiourea (PTU). After filtration through cheeze cloth, the homogenate was centrifuged at 10,000g for 20 minutes. The supernatant was centrifuged again at 100,000g for one hour. The pellet was resuspended in the same buffer solution without PTU. The final concentration was 100 abdomens/ml buffer. Supernatant and microsomal fractions were used as enzyme solutions in *in vitro* metabolism.

In vitro metabolism

One mililiter of each subcellular enzyme fraction was transferred to 6 ml vial and radio-labelled carbofuran (approximately 300,000dpm) was applied to each fraction. nicotineamideadeninenucleotide reduced form (NADPH) and glutathione (GSH) as cofactors of monooxygenase and gluthathione S-transferase, and piperonyl butoxide(PBO), diethylmalate (DEM) and iprobenfos (IBP) as inhibitors of monooxygenase, glutathione S-transferase and esterase, respectively were also added to each enzyme mixture. Final concentrations of NADPH, PBO, GSH and IBP were 1.1 mM, 0.5 mM, 0.5 mM and 0.5 mM, respectively.

The vials of each subcellular fraction with or without co-factors were placed into the deep freezer (-70 ℃) to stop the reaction after incubating for one hour in a 37℃ water bath. Frozen enzyme mixtures in the vials were thawed and loaded on TLC plates.

Separation and identification of metabolite

For separation and identification of metabolites of carbofuran, two dimensional thin layer chromatography was performed with precoated silica gel 60 F_{254} thin layer chromatoplates (20 x 20 cm, 0.2 mm thickness, Merck, F.R.G.) using hexane-chloroform-ethylacetate = 3:6:1 and bezene-diethylethe- ethanol = 9:3:0.1 as a solvent system. Identification of metabolites was carried out by co-chromatography with authentic compounds. Formulas of carbofuran and its metabolites are presented in Table 1. Non labelled authentic compounds were detected under the UV light, whereas, labelled metabolites were detected with RI image analyzer (Fuji, BAS 2500). The detected spots by RI image analyzer were scraped into 6 ml scintillation bottles, respectively and 5 ml of EP solution was added each bottle. The radioactivities were measured using liquid scintillation counter (Hewlet Pakard).

Results

In vitro metabolites of carbofuran in the microsomal fraction of abdomen of the Rc-30 and susceptible planthopper are presented in Tables 2 and 3, respectively.

Table 1. Structural formulas of carbofuran and its metabolites

Chemicals	Structural formula	Chemical name
Carbofuran		2,3 - dihydro - 2,2 - dimethyl benzofuran - 7 - yl - methyl - carbofuran
3 - Hydroxy carbofuran		2,3 - dihydro - 2,2 - dimethyl 3 - hydroxy benzofuran - 7 - yl - methyl carbmate
3 - Keto carbofuran		2,3 - dihydro - 2,2 - dimethyl 3 - keto benzofuran - 7 - yl - methyl carbamate
Benzofuranol		2,3 - dihydro - 2,2 - dimethyl -7-benzofuranol
3 - Hydroxy benzofuranol		2,3 -dihydro-2,2-dimethyl-3-hydroxy-7-benzofuran
3 - Keto benzofuranol		2,3 - dihydro - 2,2 - dimethyl - 3 - keto - 7 - benzofuran

The amount of carbofuran unmetabolized was over 80% in both strains, whereas, the amount metabolized carbofuran reached less than 3-5% of the total metabolites. There was no significant difference in metabolism of carbofuran with or without NADPH. Similar results were obtained in IBP, GSH, PBO and DEM.

Tables 4 and 5 show *in vitro* metabolism of carbofuran in the supernatant fraction of abdomens of the resistant Rc-30 and susceptible plant hoppers. Similarly, no significant differences in metabolism of carbofuran with or without co-factors or specific inhibitors of detoxifying enzymes was produced.

Table 2. *In vitro* metabolism of carbofuran in the microsomal fraction of abdomens of resistant Rc-30 female adults of brown planthopper

Metabolites	Metabolites[a] (%)					
	None	NADPH	GSH	IBP	NADPH+PB	DEM+GSH
Benzofuranol	2.6±0.2	2.9±0.3	3.1±0.2	2.9±0.2	3.2±0.3	2.7±0.2
3-Ketofuranol	2.8±0.1	2.6±0.2	3.0±0.3	2.8±0.2	3.0±0.0	2.4±0.1
Carbofuran	84.9±0.2	85.2±0.8	84.1±0.9	84.1±0.5	84.3±0.6	84.5±0.6
3-Ketocarbofuran	1.2±0.1	1.4±0.2	2.0±0.2	1.8±0.2	1.1±0.3	2.2±0.2
3-OH benzofuranol	0.3±0.1	0.5±0.0	0.4±0.1	0.5±0.1	0.5±0.1	0.4±0.1
3-OH carbofuran	3.8±0.2	3.0±0.2	3.3±0.4	3.1±0.4	3.6±0.1	3.4±0.2
Origin	4.4±0.2	4.4±0.3	4.2±0.3	4.8±0.4	4.3±0.5	4.4±0.1
Total	100.0	100.0	100.0	100.0	100.0	100.0

[a]Means±standard error.

Table 3. *In vitro* metabolism of carbofuran in the microsomal fraction of abdomens of susceptible(S) female brown planthoppers

Metabolites	Metabolites[a] (%)					
	None	NADPH	GSH	IBP	NADPH+PB	DEM+GSH
Benzofuranol	3.6±0.3	3.1±0.0	3.1±0.3	3.0±0.1	3.4±0.3	3.0±0.1
3-Ketofuranol	3.8±0.1	2.6±0.2	2.9±0.4	3.3±0.3	2.9±0.1	2.8±0.2
Carbofuran	82.3±0.4	84.3±0.3	84.0±0.4	85.0±0.3	83.5±0.4	84.9±0.4
3-Keto carbofuran	1.4±0.2	1.2±0.1	1.4±0.0	1.3±0.1	1.8±0.1	1.3±0.1
3-OH benzofuranol	0.6±0.2	0.4±0.0	0.4±0.1	0.3±0.1	0.6±0.2	0.4±0.2
3-OH carbofuran	3.7±0.3	3.3±0.3	3.5±0.3	2.8±0.3	3.4±0.2	3.1±0.3
Origin	4.6±0.3	5.1±0.4	4.7±0.2	4.3±0.3	4.4±0.1	4.5±0.2
Total	100.0	100.0	100.0	100.0	100.0	100.0

[a]Means±standard error.

Table 4. *In vitro* metabolism of carbofuran in the supernatant fraction of abdomens of resistant(Rc-30) female brown planthoppers

Metabolites	Metabolites[a] (%)					
	None	NADPH	GSH	IBP	NADPH+PB	DEM+GSH
Benzofuranol	2.4±0.1	2.8±0.1	2.1±0.3	2.6±0.1	1.8±0.1	2.7±0.1
3-Ketofuranol	2.4±0.1	2.2±0.3	2.6±0.2	2.6±0.0	2.5±0.1	2.3±0.2
Carbofuran	85.7±0.5	85.6±0.4	85.2±0.7	85.7±0.3	85.4±0.9	85.1±0.5
3-Keto carbofuran	1.5±0.4	1.1±0.2	1.6±0.4	1.1±0.1	1.7±0.3	1.6±0.1
3-OH benzofuranol	0.6±0.0	0.6±0.1	0.5±0.2	0.2±0.0	0.5±0.0	0.4±0.2
3-OH carbofuran	3.2±0.2	3.5±0.0	3.4±0.1	3.4±0.1	3.6±0.0	3.3±0.1
Origin	4.3±0,5	4.3±0.2	4.6±0.4	4.4±0.3	4.6±0.3	4.7±0.3
Total	100.0	100.0	100.0	100.0	100.0	100.0

[a]Means±standard error.

Table 5. *In vitro* metabolism of carbofuran in the supernatant fraction of abdomens of susceptible female brown planthoppers

Metabolites	Cofactors and inhibitors[a]					
	None	NADPH	GSH	IBP	NADPH+PB	DEM+GSH
Benzofuranol	2.4±0.3	2.3±0.1	2.6±0.2	2.4±0.2	2.8±0.5	2.5±0.1
3-Ketofuranol	2.6±0.1	2.8±0.1	2.5±0.2	2.5±0.1	2.1±0.1	2.3±0.0
Carbofuran	86.1±0.3	85.4±0.2	86.2±0.4	85.7±0.2	86.8±0.3	85.8±0.3
3-Keto carbofuran	1.3±0.1	1.2±0.2	1.1±0.2	1.1±0.3	0.9±0.1	1.2±0.2
3-OH benzofuranol	0.5±0.1	0.4±0.1	0.4±0.0	0.4±0.2	0.3±0.0	0.2±0.0
3-OH carbofuran	3.3±0.1	3.3±0.2	3.1±0.2	3.3±0.2	3.5±0.2	3.3±0.1
Origin	3.8±0.1	4.6±0.4	4.1±0.2	4.7±0.2	3.8±0.3	4.8±0.0
Total	100.0	100.0	100.0	100.0	100.0	100.0

[a]Means±standard error.

Discussion

Metabolic detoxification is well known as a major factor in the resistance mechanism of many insect pests (Khan *et al*, 1973; Plapp and Casida, 1969).

Metabolic rate is a very useful indicator in comparing of resistance of the groups of insecticide among insect species. In general, detoxification make xenobiotics more polar by oxidizing or cleaving them. The major characteristic of the carbamates is that oxidative degradation activities through the mixed-function oxidase systems are responsible for their metabolism (Ahn *et al.*, 1993). These results are difficult to accept, because based the chemical nature of these insecticidal carbamic esters, hydrolytic products, such as naphthols and phenols, are often less polar than the parent compounds (Matsumura, 1976). This view is in agreement with that xenobiotic metabolism in animals is geared up to making them more polar so that they can be excreted (Willams, 1959). Structurally poor electrophilic moieties (e.g. substituted phenols and naphthols) are coupled with N-methyl or dimethyl carbamic acid in carbamates, whereas in OP(organophosphorus) insecticides strongly electrophilic substituents are used and thus OP insecticides are naturally susceptible to hydrolytic attack (O'Brien, 1967). Based upon these facts, it is expected that cleavage of esteric bond in carbofuran metabolism will be more or less difficult. These postulates seem to be dependent on both insect species and chemicals.

In my *in vitro* studies, I can not find the significant enzyme in charge of metabolism of carbofuran. This seems to be true at least in *in vitro* study. The opposite postulates have also been reported. Chao *et al.* (1995) reported the importance of microsomal monooxygenase in the metabolism of bendiocarb in flower thrips. However, hydrolysis plays a major role in the metabolism of many carbamates in rats. Schlagbauer and Schlagbauer (1972) showed an importance of hydrolysis responsible for metabolism of carbamates in the rat and have documented the table of percent hydrolysis figures for various compounds.

The portion of hydrolysis in total metabolism varied with compounds. It has been reported that 3-ketocarbofuran phenol and 3-hydroxycarbofuran were the major metabolites of carbofuran in the housefly (Metcalf *et al.*, 1968; Dorough, 1968) indicating that esterase and oxidase systems are responsible for carbofuran metabolism in the insect. Park and Choi (1991) already reported that esterase and mixed function oxidase are responsible for carbofuran metabolism in BPH; about 90% of carbofuran and 80% of fenobucarb were in the water soluble fraction in nine hours. This suggests that carbofuran are metabolized to more polar compounds, which may be 3-hydroxy carbofuran. This is not in agreement with my results. In the metabolic studies, oxidative enzyme did not affect the metabolism of carbofuran. In the present study, these results were in

disagreement with earlier findings with the exception of carbamic ester of carbofuran, which is relatively difficult to be hydrolyzed (O'Brien, 1967).

In *in vivo* metabolism study, the more amount of carbofuran was detected in the resistant strain than susceptible strain when homogenized directly (Yoo *et al.*, 1988) and suggests the conjugation of carbofuran with enzymatic protein as a resistance mechanism in resistant BPH strain. Dual roles of esterase as resistance mechanism and metabolic binding in aphids (Devonshire and Moores, 1982) and in green leafhoppers (Motoyama, 1984) have been reported. From above results, enzymatic or biochemical metabolism is not the main resistance mechanism in the resistant Rc-30 BPH.

This result partially supports the proposal of Yoo *et al.*(1998).

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저항성 및 감수성 벼멸구 체외에서의 카보후란 대사

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요약 : 벼멸구의 카보후란에 대한 저항성 기작을 구명하기 위해 실내에서 카보후란으로 30세대 도태하여 얻은 저항성 계통(LD_{50}; 20.3 μg/g)과 약제를 12년 동안 처리하지 않은 벼멸구 감수성 계통(LD_{50}; 0.3 μg/g)을 완충용액과 마쇄하여, 105,000g에서 2시간 원심분리하여 얻은 상등액(에스테라제층)과 침전물(P450-산화효소층)을 효소액으로 하여 ^{14}C-카보후란을 반응시켜 계통 간 대사물 량의 차이를 조사한 바 저해제(piperonyl butoxide; 산화효소저해제, diethylmalate; 글루타치온 전이효소 저해제, iprobenfos; 에스테라제 저해제)와 보조인자 (NADPH; P-450 산화효소, 글루타치온;글루타치온전이효소)에 상관없이 카보후란의 대사물과 그 양이 계통간 차이가 없었다. 이상의 결과로부터 저항성 벼멸구에서 일반적으로 곤충에서 생화학적 저항성 기구로 잘 알려진 가수 분해 효소의 일종인 에스테라제와 p-450 산화효소, 글루타치온 전이효소의 활성 증가가 저항성 발달에 관여하지 않음을 알 수 있었다.

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* 연락저자