Studies on the Mode of Action of Fenvalerate Enantiomers
II. Cuticular Penetration, Internal Distribution, Penetration
and Absorption into Nerve Tissue and Metabolism
of Fenvalerate Enantiomers in the American
Cockroach, *Periplaneta americana* (L.)¹

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(Received October 13, 1989; Accepted January 22, 1991)

The insecticidal activity, cuticular penetration, internal distribution, penetration and absorption into nerve tissue and metabolism of the pyrethroid insecticide, the *R,S-S* isomer, (*R,S*)- α -cyano-3-phenoxybenzyl (*S*)-2-(4-chlorophenyl)-isovalerate and the *R,S-R* isomer, (*R,S*)- α -cyano-3-phenoxybenzyl (*R*)-2-(4-chlorophenyl)-isovalerate were studied in the American cockroach, *Periplaneta americana* (L.). Results of toxicity tests showed that the relative potency of the *R,S-S* isomer is more than 36 times higher than the *R,S-R* isomer to this insect. The cuticular penetration experiment showed that the *R,S-S* isomer penetrated more rapidly than the *R,S-R* isomer. The internal distribution experiment indicated some difference in the time course of the two compounds in the head, nerve cord and hemolymph. Experiments concerning the penetration and absorption into nerve tissue showed that the *R,S-S* isomer penetrated into nerve tissue more rapidly than the *R,S-R* isomer. The patterns of the *in vivo* metabolism of these two optical isomers were similar and there was little quantitative difference between them. From these results, it was apparent that there was some difference in the pharmacodynamics between the *R,S-S* isomer and the *R,S-R* isomer in the American cockroach. However, this difference seemed to be a minor factor considering the large difference between the bioactivities of these two optical isomers.

INTRODUCTION

It is well-known that the insecticidal activity of pyrethroids highly depends on optical activity of the asymmetric carbon atom (RUGHT, 1985). It is important to explore the mechanism of this difference for a real understanding of the mechanisms of the mode of action of pyrethroids. CLEMENTS and MAY (1977) reported that the reason for the differences in toxicity was based on the ability to disturb nervous conduction. At the target site level, LUND and NARAHASHI (1983) reported that drastic differences exist among optical isomers of pyrethroids in their ability to modify sodium

¹ This research was supported in part by a Grant-in-Aid for Scientific Research Work No. 60440011 from the Ministry of Education, Science and Culture of Japan.

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channel kinetics. Although a number of experiments on the target site has been performed to clarify the difference in biochemical potency of optical isomers, pharmacodynamic studies such as cuticular penetration, internal distribution and penetration into nerve tissue of optical isomers of pyrethroids have been limited.

In the present study, we investigated insecticidal activity, cuticular penetration and internal distribution using two optical isomers of fenvalerate, *R,S-S* and *R,S-R* isomer, in the American cockroach. The *in vivo* metabolism of fenvalerate was also studied to clarify the importance of the mode of action of fenvalerate.

MATERIALS

Insects. American cockroach, *Periplaneta americana* (L.) were reared on water and artificial diet of rat (CE-2®, CLEA Japan Inc., Tokyo, Japan) under 16L-8D, 25°C condition. Two- to 4-weeks old adult males were used throughout the experiments.

Insecticides. *R,S-S* fenvalerate, (*R,S*)- α -cyano-3-phenoxybenzyl(*S*)-2-(4-chlorophenyl)-isovalerate and *R,S-R* fenvalerate, (*R,S*)- α -cyano-3-phenoxybenzyl(*R*)-2-(4-chlorophenyl)-isovalerate, and ¹⁴C-labeled compounds which were labeled at benzyl radical of alcohol moiety (943.5 and 999.0 MBq/mM, respectively) were obtained from Sumitomo Chemical Co. (Osaka, Japan). Stock solution of these compounds were prepared in acetone at the concentration of 1×10^{-3} M.

Physiological saline solution. Physiological saline solution containing (in mM), NaCl 200, KCl 3.1, CaCl₂ · 2H₂O 5.0, was buffered at pH 7.2 with 1/15 M phosphate buffer (NaH₂PO₄ and Na₂HPO₄).

METHODS

Bioassay. Pyrethroids were applied using a microapplicator (Kiya Seisakusyo Co. Ltd., Tokyo, Japan) on the metasterna between ventral abdominal sclerites of CO₂-anesthetized insects. Twelve insects were used at each dose level. Treated insects were placed in plastic cups (10 cm dia.) and kept at 25°C for 24 hr. The control insects were treated with acetone only. Insects which did not respond to pencil tip prodding were considered as dead. The data were analyzed by the method of FINNEY (1971) to determine LD₅₀ and LD₉₅ values using a micro computer (PC-9801VM, Nippon Electric Co., Ltd., Tokyo, Japan).

Cuticular penetration. Insects were treated topically with 2.52 μ g/male of ¹⁴C-labeled fenvalerate (equidose of LD₁₀ of the *R,S-S* isomer) as described above. After evaporation of the solvent, insects were placed individually in scintillation vials. The vials were maintained at 25°C. At 0.75, 1.5, 3, 6, 12 and 24 hr after, the insects were anesthetized with CO₂ and removed from the vials. The insects were washed with 1 ml of ACS-2® scintillator (Aloka Co. Ltd., Tokyo, Japan) 5 times. The radioactivity in the rinsed scintillator was estimated as the surface material (unpenetrated quantities). The rinsed insects were then transferred to petri dishes and allowed to dry. Excreted radiocarbon remaining in the holding vials was also measured by adding 5 ml of ACS-2® to the vials (excreted quantities). The amount of penetrated insecticides was estimated by combusting dried insects in an Aloka ACS-113® sample oxidizer (Aloka Co. Ltd., Tokyo, Japan), and the radioactivity was measured (internal quantities). The radioactivity measurements were performed by an Aloka LSC-700® liquid scintillation spec-

trophotometer (Aloka Co. Ltd., Tokyo, Japan). Experiments were run in triplicate.

Internal distribution. Insects were treated topically with 2.52 $\mu\text{g}/\text{male}$ of ^{14}C -labeled fenvalerate (equidose of LD_{10} of the *R,S*-isomer) as described above. After evaporation of the acetone, insects were placed individually in scintillation vials. The vials were maintained at 25°C for 0.75, 1.5, 3, 6, 12 and 24 hr, respectively. After this incubation, the insects were anesthetized with CO_2 and removed from the vials. At first, 1 μl of hemolymph of the insect was collected with a small glass capillary tube through the metathoracic cuticle between the 2 wings and dispensed into 5 ml of ACS-2® scintillator. Insects were then dissected in physiological saline solution. The head, ventral nerve cord (including three thoracic and six abdominal ganglia), fat body of testis and gut were removed. Each tissue was rinsed in 5 ml of physiological saline solution, and homogenized in a 0.5 ml ACS-2® scintillator using a motor driven POTTER-ELVEHIJEN glass homogenizer. These homogenates were put into vials, then the homogenizer was rinsed with 1.5 ml of ACS-2® scintillator three times. Rinsed solutions were added to homogenates in vials. Radioactivity was measured to determine the concentration of the compounds in each tissue. All experiments were replicated three times.

Penetration and absorption into nerve tissue. The ventral nerve cord was dissected carefully and placed into a vial containing 5 ml of physiological saline solution and kept at 4°C. Test solutions were prepared just before the experiment by adding a small aliquot of the stock solution of ^{14}C -labeled fenvalerate to 1 ml physiological saline solution to make 1×10^{-5} M solution of fenvalerate. Four to five nerve cords were dipped into the test solution at 25°C for an appropriate time. After dipping, nerve cords were rinsed in 5 ml physiological saline solution, homogenized and measured for radioactivity. All experiments were replicated five times.

Metabolism. Adult male American cockroaches were treated topically with two ^{14}C -labeled optical isomers of fenvalerate at LD_{10} doses. Treated insects were placed individually in the glass homogenizers and maintained at 25°C for 24 hr. After this period, the insects were anesthetized with CO_2 and washed three times with 2 ml benzene. Ten percent of aliquot of benzene (unpenetrated quantities) was taken and the radioactivity was determined by an Aloka LSC-700® after addition of 5 ml of ACS-2®. The remainder of the benzene solution was concentrated by a rotary vacuum evaporator to near dryness at 35–40°C. After dropping a small amount of methanol into the residue, a small amount of methanol solution was applied on a silica gel thin layer chromatography (TLC) plate (0.25 mm, F₂₅₄, Merck, Darmstadt, F.R.G.). The insects were homogenized with 3 ml methanol in a homogenizer in which they were kept. The methanol fraction was collected from the homogenizer after centrifuging at 2,500 rpm for 10 min. The sediment was extracted by methanol twice. Ten percent of aliquot of methanol extract was taken from the pooled methanol extract and the radioactivity was measured after addition of 5 ml of ACS-2®. The remainder was evaporated and applied on a TLC plate as mentioned before. The unextractable radiolabeled residue in the insects and excreta was combusted by a sample oxidizer and the radioactivity was measured to determine the amount of unextractable materials. The TLC plate was developed with a solvent system, *n*-hexane: toluene: acetic acid (3: 15: 2, v/v/v) (OHKAWA et al., 1978; MIKAMI et al., 1985). Location of metabolites on the TLC plates was detected by autoradiography with X-ray film (Fuji X-ray film, Fuji Photo Film Co., Ltd., Tokyo, Japan). Unlabeled authentic standards were co-

chromatographed with extracts and *R_f* values of these were determined by absorption of light at 254 nm. Radioactive spots of TLC were scraped into scintillation vials and the amount of radioactivity of each spot was measured. The experiments were carried out four times.

RESULTS

Bioassay

Results of the toxicity tests are shown in Table 1. There was a large difference in the toxicity between the two optical isomers. The LD₅₀ of the *R,S-S* isomer was

Table 1. Susceptibility of male adult American cockroach, *Periplaneta americana* (L.) against *R,S-S* and *R,S-R* isomers at 24 hr after topical application

Compounds	LD ₅₀ (μg/g)	LD ₅₀ (μg/g)
<i>R,S-S</i> isomer	5.42 (4.44–6.53) ^a	11.6 (7.13–14.6) ^a
<i>R,S-R</i> isomer	177 <	—

^a 95% confidence interval.

Fig. 1. The penetration, internal accumulation and excretion of ¹⁴C-fenvalerate at various times after topical application to male adult *Periplaneta americana* (L.). Top: *R,S-S* isomer. Bottom: *R,S-R* isomer. Each point is calculated from five replications. Total recovery: ▲, unpenetrated quantities: ○, internal quantities: ■, excreted quantities: ●.

5.42 $\mu\text{g/g}$ and that of the *R,S-R* isomer was more than 177 $\mu\text{g/g}$, indicating a relative potency of the *R,S-S* isomer to the *R,S-R* of greater than 36:1 against this insect.

Cuticular penetration

Figure 1 shows the penetration, internal accumulation, and excretion of the [^{14}C]-*R,S-S* and the [^{14}C]-*R,S-R* isomers in the American cockroach after treatment with the equidose (LD_{10} of the *R,S-S* isomer). The detected internal amounts of radioactive materials were different between two compounds. The penetrated amounts of the [^{14}C]-*R,S-S* isomer were larger than those of the [^{14}C]-*R,S-R* isomer at 1.5, 6, and 12 hr after treatment. The amount of excreted quantities of the [^{14}C]-*R,S-R* isomer was higher than that of the [^{14}C]-*R,S-S* isomer at 12 hr after treatment. These results indicated that the cuticular penetration rate of the [^{14}C]-*R,S-S* isomer was more rapid than that of the [^{14}C]-*R,S-R* isomer.

Internal distribution

In the case of the [^{14}C]-*R,S-S* isomer, intoxication symptoms were observed at 6 hr after treatment, but no mortality was recorded at 24 hr. The amount of hemolymph collected decreased to less than 1 μl /insect at 24 hr after treatment, so that data at 24 hr were lacking. No intoxication symptoms were observed in insects treated with the [^{14}C]-*R,S-R* isomer during the experiments.

*[^{14}C]-*R,S-S* isomer*

The concentration of the [^{14}C]-*R,S-S* isomer in the insect head, nerve, and hemolymph showed a remarkable fluctuation with time. The concentration in the head increased sharply at 1.5 hr after treatment and then dropped at 3 hr. It increased again at 6 hr and then decreased at 12 hr after treatment (Fig. 2a). A similar pattern was also observed in the nerve cord (Fig. 2b). The concentration in the nerve cord reached a peak at 1.5 hr after treatment. The concentration in the hemolymph increased sharply after treatment and reached a peak at 6 hr after treatment and then decreased (Fig. 2c). The patterns of the gut and the fat body were similar (Fig. 2d, e). Peaks were observed until 6 hr after treatment in the gut and the fat body (but not in the gut). This concentration was maintained for 24 hr. In the fat body, the level of the [^{14}C]-*R,S-S* isomer maintained high for 12 hr and then dropped.

*[^{14}C]-*R,S-R* isomer*

Temporal patterns of the concentration of the [^{14}C]-*R,S-R* isomer were not similar to those of the [^{14}C]-*R,S-S* isomer in all tissues tested. In the head, the peak concentration was reached at 6 hr after treatment and maintained its level for 24 hr (Fig. 2a). Levels of the [^{14}C]-*R,S-S* isomer were higher than those of the [^{14}C]-*R,S-R* isomer at 1.5 hr and 6 hr after treatment. At 12 hr after treatment, levels of the [^{14}C]-*R,S-S* isomer were much higher than those of the [^{14}C]-*R,S-R* isomer. A similar pattern was observed in the nerve cord (Fig. 2b), where the peak of the concentration of the [^{14}C]-*R,S-R* isomer was observed at 1.5 hr, lasting for 24 hr. Levels of the [^{14}C]-*R,S-S* isomer were higher than those of the [^{14}C]-*R,S-R* isomer at 1.5 and 6 hr. At 12 hr after treatment, however levels of the [^{14}C]-*R,S-R* isomer were maintained at much higher concentration than those of the [^{14}C]-*R,S-S* isomer. In the hemolymph, there was a rapid uptake of the [^{14}C]-*R,S-R* isomer. The peak was observed at 1.5 hr and then the concentration

Fig. 2. Time course of the internal distribution of the $R,S-S$ (solid circle) and $R,S-R$ isomers (open circle) in head (a), nerve cord (b), hemolymph (c), gut (d) and fatbody (e) of the American cockroach, *Periplaneta americana* (L.). Each point is calculated from three replications.

decreased to a half of the peak at 6 hr after treatment and was maintained at the same level until 24 hr (Fig. 2c). At 6 and 12 hr after treatment, levels of the $[^{14}\text{C}]-R,S-S$ isomer were much higher than those of the $[^{14}\text{C}]-R,S-R$ isomer. The concentrations of the $[^{14}\text{C}]-R,S-S$ and $[^{14}\text{C}]-R,S-R$ isomers in the fat body were equal for 12 hr. The levels of the concentration of $[^{14}\text{C}]-R,S-R$ isomer increased gradually, while those of the $[^{14}\text{C}]-R,S-S$ isomer concentration decreased sharply (Fig. 2d). In the gut, the concentration of the $[^{14}\text{C}]-R,S-R$ isomer reached a peak at 12 hr, maintaining this concentration until 24 hr after treatment (Fig. 2e).

Penetration into the nerve tissue

The uptake of both the $[^{14}\text{C}]-R,S-S$ and $[^{14}\text{C}]-R,S-R$ isomers into nerve cord *in vitro* reached the highest level by 80 min after treatment (Fig. 3). Level of the $[^{14}\text{C}]-R,S-S$ isomer in the nerve cord was maintained higher than that of the $[^{14}\text{C}]-R,S-R$ isomer. In order to compare the penetration rate of two compounds into the nerve tissue, time constants were estimated. Penetration curves of the $[^{14}\text{C}]-R,S-S$ and the $[^{14}\text{C}]-R,S-R$ isomers were observed to follow a single exponential time course with values of 0.031 and 0.013, respectively. These values were significantly different ($p < 0.05$).

Fig. 3. Time course of the penetration of the *R,S-S* (solid circle) and *R,S-R* isomers (open circle) into the ventral nerve cord of American cockroach, *Periplaneta americana* (L.). Each point is calculated from three replications.

Table 2. Distribution of radioactivity at 24 hr after topical application to male adult American cockroach, *Periplaneta americana* (L.) with ^{14}C -labeled *R,S-S* and *R,S-R* isomers

Compound	Percentage of recovered radioactivity (Mean $\pm$ SD)			
	Unpenetrated ^a	Extracted	Unextracted	Total recovery
<i>R,S-S</i> isomer	13.9 $\pm$ 6.0	36.2 $\pm$ 4.0	52.4 $\pm$ 7.2	66.8 $\pm$ 11.8
<i>R,S-R</i> isomer	28.6 $\pm$ 6.6	32.0 $\pm$ 6.6	39.4 $\pm$ 12.2	79.0 $\pm$ 5.9

^a Means were significantly different at 5% level.

Table 3. Metabolites recovered from American cockroach, *Periplaneta americana* (L.) at 24 hr after topical application with the *R,S-S* and *R,S-R* isomers at equidose (LD_{10} of the *R,S-S* isomer)

Metabolite	Percentage of recovered radioactivity (Mean $\pm$ SD)			
	<i>R,S-S</i> isomer		<i>R,S-R</i> isomer	
	Unpenetrated	Extracted	Unpenetrated	Extracted
fenvalerate	86.6 $\pm$ 5.4	27.6 $\pm$ 3.5	83.4 $\pm$ 6.0	26.1 $\pm$ 5.3
α -CN3PbAlc.		31.0 $\pm$ 5.1		38.1 $\pm$ 4.4
3PbAlc.		6.3 $\pm$ 1.1		5.0 $\pm$ 0.9
3PbAc.		6.3 $\pm$ 1.1		4.7 $\pm$ 1.2
3PbAld.		8.5 $\pm$ 1.8		5.1 $\pm$ 1.1
unknown I ^a		6.2 $\pm$ 2.9		4.7 $\pm$ 1.0
unknown II		3.3 $\pm$ 0.8		5.7 $\pm$ 1.9
unknown III		5.3 $\pm$ 1.0		3.8 $\pm$ 1.0
unknown IV		5.0 $\pm$ 1.0		4.0 $\pm$ 1.2
total metabolites	13.4 $\pm$ 5.4	72.5 $\pm$ 3.5	16.7 $\pm$ 5.9	73.9 $\pm$ 5.3

^a Means were significantly different at 5% level.

Cuticular penetration and metabolism

Unpenetrated, internal and excreted quantities of the [^{14}C]-*R,S-S* and [^{14}C]-*R,S-R* isomers at 24 hr after topical application are shown in Table 2. The total recoveries of radioactivities in the [^{14}C]-*R,S-S* and [^{14}C]-*R,S-R* isomers were 66.1% and 79.0%, respectively. These values were relatively low. From preliminary experiments, amount of ^{14}C -radioactivity in expired CO_2 was less than 1% of the applied dose, thus expired CO_2 gas was not trapped to detect ^{14}C -radioactivity. Results of the metabolism studies are shown in Table 3. The main metabolites of the [^{14}C]-labeled compounds were tentatively identified as 3-phenoxybenzyl aldehyde (3 PBAlde, $R_f=0.58$), 3-phenoxybenzyl (3PBAlc, $R_f=0.56$), 3-phenoxybenzyl alcohol (3PBAlc, $R_f=0.44$), and α -CN 3-phenoxybenzyl alcohol (α -CN 3PBAlc, $R_f=0.28$). The remaining metabolites were not identified and are referred to as unknowns; unknown I ($R_f=0.71$), unknown II ($R_f=0.40$), unknown III ($R_f=0.23$), and unknown IV ($R_f=0.14$). R_f value of the *R,S-S* and *R,S-R* isomers was 0.65.

DISCUSSION

The *R,S-S* isomer showed higher insecticidal activity than the *R,S-R* isomer in various insects (NAKAYAMA et al., 1979; HONMI et al., 1987; NOPPUN et al., 1989; NAGATA et al., unpublished data). This study also showed the potency of the *R,S-S* isomer to be more than 36 times more potent than the *R,S-R* isomer against the American cockroach.

It has been shown that there was no difference in penetration, internal distribution and metabolism between optical isomers of NRDC 157 and NRDC 163 in the American cockroach (SODERLUND, 1979; SODERLUND et al., 1983). However, in this study, there were some differences in cuticular penetration, internal distribution, and penetration into nervous tissue between two optical isomers of fenvalerate. In the cuticular penetration experiment, the *R,S-S* isomer penetrated more rapidly than the *R,S-R* isomer. Similar observations were reported in the diamondback moth, *Plutella xylostella* (L.) (NOPPUN et al. unpublished data) and the rice brown planthopper, *Nilaparvata lugens* STÅL (TESHIMA et al. unpublished data).

In the internal distribution experiments, there was a difference in the temporal patterns of the concentrations in the head and nerve cord between the [^{14}C]-*R,S-S* and *R,S-R* isomers. This phenomenon might be caused by the difference in toxic symptoms between the two compounds. But the difference was observed before the appearance of toxic symptoms, and there was no mortality until 24 hr after application (dose was LD_{10} of the *R,S-S* isomer) under this experimental condition. This may be due to the difference in the behaviour between two compounds.

CLEMENTS and MAY (1977) reported that in the desert locust, *Schistocerca gregaria*, the preparation of the peripheral nervous system was affected more by a low concentration of pyrethroids than the preparation of the central nervous system. They considered that the site of action of pyrethroids was the peripheral nervous system. The difference observed in the concentration of the two optical isomers in the hemolymph may cause the difference of their transportation rate by the hemolymph at the peripheral level. In this study, we examined the concentration of compounds only in the central nervous system, so further study will be needed to examine the concentration of these isomers in the peripheral nervous system.

The concentration of ^{14}C -fenvalerate in an isolated nerve cord *in vitro* increased immediately after dipping into the test solution (5 min after dipping), and then increased slowly and reached a steady state at 80 min after treatment. We observed that more than two phases existed in the penetration of fenvalerate into the nerve tissue. The time constant and amount of the ^{14}C -fenvalerate penetration studies showed that the ^{14}C -*R,S-S* isomer penetrated more rapidly into the nerve tissue than the ^{14}C -*R,S-R* isomer and the amount of the ^{14}C -*R,S-S* isomer was higher than that of the ^{14}C -*R,S-R* isomer. A similar phenomenon was observed in the German cockroach (NAGATA et al., 1989).

Cleavage of fenvalerate (^{14}C -labeled at benzyl position) at the ester bond gives radiolabeled α -cyano 3-phenoxybenzyl alcohol (α -CN 3PBAlc) and unlabeled isovaleric acid. α -CN 3PBAlc is further oxidized to aldehyde (3PBAlc), alcohol (3PBAlc), and acid (3PBAlc) in mammals and plants (OHKAWA et al., 1978; KANEKO et al., 1981, 1984; MIKAMI et al., 1985; TAKAMATSU et al., 1987). The patterns of metabolism of the two optical isomers were similar. There were no quantitative differences in parent compounds (27.6 and 26.1% for the *R,S-S* and *R,S-R* isomers, respectively) and metabolites except unknown I at 24 hr after treatment (Table 3). The amount of unknown I was very low among all of the metabolites. This difference did not affect the total metabolites between the two compounds. Therefore, we can conclude that the metabolism of optical isomers of fenvalerate is not responsible for the differences in their insecticidal potencies.

These results suggested that there was a difference in the pharmacodynamics between the *R,S-S* and *R,S-R* isomers in the American cockroach. Since these differences are not so large, it is not clear how much they are responsible for the differences in insecticidal activities of optical isomers.

ACKNOWLEDGEMENTS

We wish to express our thanks to Dr. V. NOPPUN, Mr. H. TESHIMA, Dr. E. HAYAMIZU, Mr. ADEL RAMZY FARMY, Dr. Y. TSUBAKI and Dr. Y. ITÔ of our laboratory for their helpful comments and continued encouragement.

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