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New Phenomenon

Structure modification of fenvalerate metabolized in *Trichoplusia ni* cells

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The failure of insecticides to control pests is becoming more serious around the world. One of the consequences of this failure is that more insecticides are retained in soil, run-off water, and foods as persistent organic pollutants, resulting in severe environmental pollution. So elucidating the pesticide metabolic process of insects has a huge significance. Fenvalerate, a widely used synthetic pyrethroid pesticide that is effective in controlling insect populations, has encountered the rapid development of resistance [1–3]. The cabbage looper (*Trichoplusia ni*, *Tn*) is a common crucifer pest that has exhibited resistance to the pyrethroid insecticides. In our previous study [4], three P450 monooxygenase isozymes that were functionally connected with the hydroxylation reaction in *Tn* cells were identified in the fenvalerate challenge process. This finding suggested that the P450 might increase the hydrophilicity of fenvalerate by modifying its chemical groups. In the present study, we attempted to examine the chemical structure modification of fenvalerate after it has been metabolized in *Tn* cells, which will help us identify the molecular metabolic basis of the insecticide's action inside the living cells.

Firstly, we extracted fenvalerate metabolic byproducts by diethyl ether. *Tn* cells (*Tn* cell-strain) were cultured in TNM-FH insect medium (BD Biosciences, Franklin Lakes, USA) and treated with fenvalerate at 12.5 μM for 12 h. The cell culture media and cells were then collected. In the control group, fenvalerate was loaded into the medium without *Tn* cells under the same conditions. Because mass impurities in the cell culture media would greatly interfere with the extraction and purification, we collected abundant material from the cell culture system *via* repeated extraction to obtain the fenvalerate metabolic derivative compounds. Silica gel thin layer chromatography (TLC) was tested to determine whether silica gel could separate fenvalerate and its derivative. When the TLC test was proved to be useful, the same conditions were applied to a silica gel column, and a sufficiently pure metabolite was obtained **(Supplementary Fig. S1)**.

Secondly, the extracted metabolic byproducts were analyzed by nuclear magnetic resonance (NMR), mass spectrometry (MS), and elemental analysis. The data of ^{1}H- and ^{13}C-NMR analyses were shown in **Supplementary Material**. The result of NMR measurement (**Fig. 1**) indicated that the α-cyano group and the phenyl group of the 3-phenoxybenzyl were eliminated. Then the molecular weight (MW) was estimated by MS, as shown in **Fig. 1**(C). The molecular formula of the metabolite is believed to be $C_{18}H_{19}ClO_3$; therefore, the theoretical MW should be 318.11. According to the charged molecules and molecule fragments, the MS analysis returned a value of 319.12 *m*/*z* $[M + 1]^+$ for the metabolite, with an MW of 318.12. So, MS results supported the NMR results. The elemental analysis of the fenvalerate yielded (%): C, 71.51; H, 5.28; O, 11.43; and N, 3.33. The calculated theoretical element percentages of the metabolite $C_{18}H_{19}ClO_3$ were C, 67.82; H, 6.09; O, 15.06; and N, 0; the actual assay values were C, 67.57; H, 6.28; O, 14.84; and N, 0. Based on the concordance of two measurements (error $<0.05\%$), we thought that the molecular formula of the metabolite was $C_{18}H_{19}ClO_3$. Thus, the elemental analysis also confirmed the NMR findings.

Pyrethroids are structural derivatives of naturally occurring pyrethrins that primarily contain cyclopropane carboxylic acid moieties (or equivalent groups) linked to aromatic alcohols through a central ester (or ether) bond. One modification to this basic pyrethroid structure, the addition of an α-cyano group to the alcohol moiety, plays a critical role in providing superior insecticidal activity [5]. In the present study, during the *Tn* cell metabolic process, the α-cyano group might suffer a nucleophilic attack, followed by a decarboxylic reaction. The cyano elimination suggested that this modification could reduce the pesticide toxicity to some extent, though the mechanism might not be strong or fast enough to detoxify the fenvalerate before it reached the target. In addition, we also found the benzyl group elimination. Investigations indicated, albeit indirectly, that enhanced metabolic detoxification of fenvalerate was mediated by monooxygenases, not by esterases, and this was the principal mechanism of resistance in the resistant strains [4,6,7]. The elimination of the benzyl group from fenvalerate with a 3-phenoxybenzyl group increased the hydrophilicity of the fenvalerate, which facilitated its excretion.

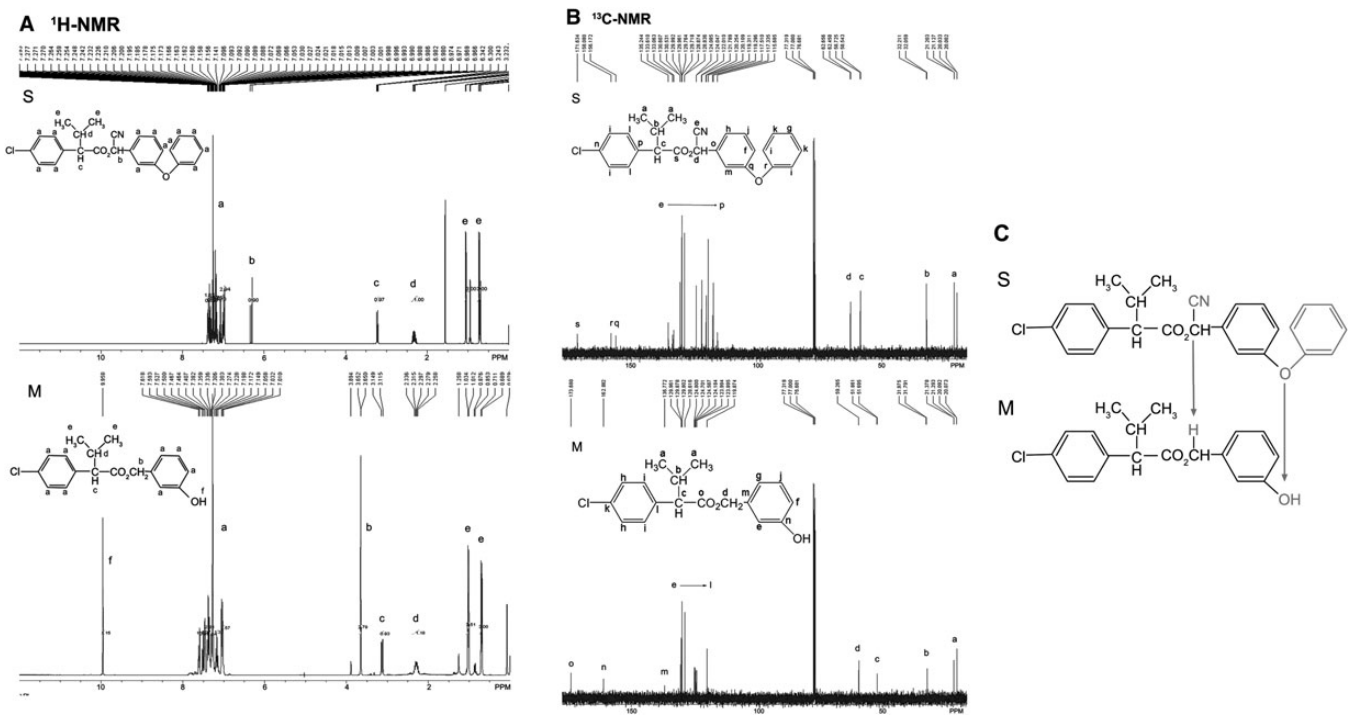

Figure 1 NMR measurement of the fenvalerate metabolite (A) ^{1}H-NMR spectra of the fenvalerate standard (S) and the metabolite (M). The different signals are labeled from 'a' to 'e' (S) and 'a' to 'f' (M) in both the spectra and the structure formulas. The ^{1}H NMR spectra of the metabolite indicate that the phenyl group of 3-phenoxyphenyl in fenvalerate was eliminated. (B) ^{13}C NMR spectra of the fenvalerate standard (S) and the metabolite (M). The different signals are labeled from 'a' to 's' (S) and 'a' to 'o' (M) in both the spectra and the structure formulas. The ^{13}C NMR spectra of the metabolite indicate that the α-cyano group was directly eliminated from the molecule. (C) The molecular structure of the fenvalerate metabolite. S, the molecular structure of fenvalerate standard—$C_{25}H_{22}ClNO_3$; M, the proposed molecular structure of the metabolite—$C_{18}H_{19}ClO_3$. The α-cyano group and the phenyl group of 3-phenoxybenzyl group were eliminated during the metabolic reaction in the *Tn* cells.

Table 1 The toxicity of fenvalerate and the metabolic derivative to *Drosophila melanogaster*

Items	Groups							
	1	2	3	4	5	6	7	8
Fenvalerate								
Concentration (μg/ml)	6.20	7.74	9.68	12.10	15.13	18.91	23.63	Acetone
Mortality (%)	20	25	30	35	50	60	85	0
Derivative								
Concentration (mg/ml)	0.094	0.47	0.94	4.72	9.43	47.15	94.30	Acetone
Mortality (%)	0	0	0	0	0	0	0	0

Finally, the toxicity of the fenvalerate metabolite was investigated, that is, a median lethal concentration (LC_{50}) assay in *Drosophila melanogaster* was carried out. According to the LC_{50} assay, fenvalerate exhibited a dose-dependent mortality with an LC_{50} of 14.062 ± 0.6 μg/ml (**Table 1**). The derivative compound showed 0% mortality, indicating that the fenvalerate metabolic derivative was an inactive metabolite. Therefore, the metabolized fenvalerate lost the configuration necessary to reach and interact with the insect axonal membrane receptor to trigger its neurotoxicity [8].

This study implied that the *Tn* cells were detoxified fenvalerate by modifying the cyano and 3-phenoxybenzyl groups. To date, the metabolite derived from fenvalerate has not been identified. The results elucidate fenvalerate detoxification, and contribute to our understanding of the molecular metabolic basis of its insecticidal properties.

Supplementary Data

Supplementary data are available at *ABBS* online.

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