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Enantiomerization and Enantioselective Bioaccumulation of Benalaxyl in *Tenebrio molitor* Larvae from Wheat Bran

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ABSTRACT: The enantiomerization and enatioselecive bioaccumulation of benalaxyl by dietary exposure to *Tenebrio molitor* larvae under laboratory conditions were studied by HPLC-MS/MS. Exposure of enantiopure *R*-benalaxyl and *S*-benalaxyl in *T. molitor* larvae revealed significant enantiomerization with formation of the *R* enantiomers from the *S* enantiomers, and vice versa. Enantiomerization was not observed in wheat bran during the period of 21 days. For the bioaccumulation experiment, the enantiomer fraction in *T. molitor* larvae was maintained approximately at 0.6, whereas the enantiomer fraction in wheat bran was maintained at 0.5; in other words, the bioaccumulation of benalaxyl was enantioselective in *T. molitor* larvae. Mathematical models for a process of uptake, degradation, and enantiomerization were developed, and the rates of uptake, degradation, and enantiomerization of *R*-benealaxyl and *S*-benealaxyl were estimated, respectively. The results were that the rate of uptake of *R*-benalaxyl (k_{Ra} = 0.052 h^{-1}) was slightly lower than that of *S*-benalaxyl (k_{Sa} = 0.061 h^{-1}) from wheat bran; the rate of degradation of *R*-benalaxyl (k_{Rd} = 0.285 h^{-1}) was higher than that of *S*-benalaxyl (k_{Sd} = 0.114 h^{-1}); and the rate of enantiomerization of *R*-benalaxyl (k_{RS} = 0.126 h^{-1}) was higher than that of *S*-benalaxyl (k_{SR} = 0.116 h^{-1}). It was suggested that enantioselectivtiy was caused not only by actual degradation and metabolism but also by enantiomerization, which was an important process in the environmental fate and behavior of chiral pesticides.

KEYWORDS: *benalaxyl, enantioselectivity, Tenebrio molitor larvae, enantiomerization, bioaccumulation*

INTRODUCTION

It has been estimated that approximately 40% of pesticides are chiral molecules, most of which are commercialized as racemic mixtures due to the limitation of chiral separation and synthesis.[1] Chiral compounds exist as two or more non-superimposable mirror images called enantiomers, often designated *R* and *S*. The enantiomers of a chiral molecule possess the same physicochemical properties except for their optical behavior. However, enantiomers usually exhibit different properties in a chiral environment, which may lead to variations in bioactivity, toxicity, biological uptake, metabolic pathways, degradation, and so on.[2,3] This means that one enantiomer might be safer in the environment than the other. Hence, using an enantiopure enantiomer that is more active than the other instead of the racemate is likely to reduce environmental risk.[4−6]

In some case, some chiral pesticides were configurationally unstable under certain conditions and might undergo enantiomerization, which was observed for mecoprop, dichlorprop, triadimefon, phenthoate, malathion, fenpropathrin, fenvalerate, cypermethrin, and so on.[7−12] Enantiomerization might generate positive or negative influences on environments. On the one hand, an active enantiomer could be converted to an inactive one with more toxicity and persistency; on the other hand, an inactive enantiomer might partly be converted into the active one. Therefore, the possibility of enantiomerizaion of chiral pesticides in environments should be considered to exactly assess the safety of chiral pesticides.

Benalaxyl, methyl-*N*-phenylacetyl-*N*-2,6-xylyl alaninate, is an important broad-spectrum acetanilide fungicide that inhibits mycelial growth of fungi and germination of zoospores. It can be absorbed by the roots, stems, and leaves, and apoplastic movement occurs acropetally to all parts of the plant. Benalaxyl has a chiral carbon consisting of two enantiomers (Figure 1).

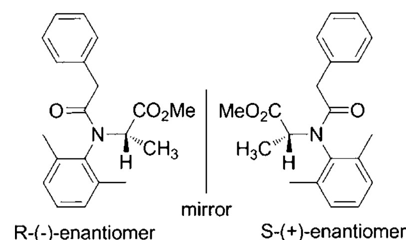

Figure 1. Structures of benalaxyl enantiomers.

The activity of benalaxyl is mainly attributed to the *R*-enantiomer.[13] Differences in degradation between the two enantiomers of benalaxyl were observed in tomato, tobacco, sugar beet, capsicum, cucumber, soil, rabbit plasma, liver microsomes from rat and rabbit, and freshwater alga *Scenedesmus obliquus*.[13−19] It was discussed that the bioaccumulation factor values of *R*-benalaxyl and *S*-benalaxyl from soil to earthworms were 0.53 and 0.46, respectively, demonstrating the differences in the bioaccumulation of the two benalaxyl enantiomers against this nontarget organism.[20] Therefore, it is

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quite significant to further understand the biological behaviors of benalaxyl enantiomers in ecosystems.

Tenebrio molitor, affiliated with coleoptera in insect taxonomy, is a serious stored-product insect pest with a worldwide distribution.[21] The life of *T. molitor* (referring to a growth cycle) is divided into four stages of egg, larva, pupa, and adult, wherein the larva is the most sensitive to environmental contaminants. *T. molitor* larvae are essential food sources for many birds, fishes, reptiles, and amphibians, and they also constitute a significant part of the human diet in some parts of the world,[26] so they are a significant link in transporting environmental contaminants to other organisms; the effects of some chemicals on *T. molitor* larvae have been reported in several studies.[22−25] However, information related to behaviors of chiral pesticides on *T. molitor* larvae in environments is limited. So far, no research has yet been reported for enantiomerization and enantioselective bioaccumulation of benalaxyl enantiomers in *T. molitor* larvae.

In this paper, the enantiomerization and enantioselective bioaccumulation of enantiomers of benalaxyl by dietary exposure to *T. molitor* larvae were studied under laboratory conditions by HPLC-MS/MS. Mathematical models for a process of uptake, degradation, and enantiomerization were developed and explained that enantioselectivtiy was not only caused by actual degradation and metabolism but also by enantiomerization, which played an important role in the environmental fate and behaviors of chiral pesticides.

■ MATERIALS AND METHODS

Chemicals and Reagents. The fungicide *rac*-benalaxyl (>98.0% purity) was provided by the China Ministry of Agriculture Institute for Control of Agrochemicals. The two enantiomers of benalaxyl were prepared via chiral synthesis methods, and the optical purities of (*R*)-(−)-enantiomer and (*S*)-(+)-enantiomer were 99.0 and 98.4%, respectively. Water was purified by a Milli-Q system. Methanol (HPLC grade), *n*-hexane (HPLC grade), and acetonitrile (HPLC grade) were obtained from Dikma Co. (USA). Pyridine, trifluoromethanesulfonic anhydride, methyl (*S*)-(−)-lactate, methyl (*R*)-(+)-lactate, 2,6-dimethylaniline, phenylacetic acid chloride, sodium bicarbonate, toluene, dichloromethane (CH_2Cl_2), ether, ethyl acetate, acetone, acetonitrile (analytical grade), and anhydrous sodium sulfate (Na_2SO_4) were purchased from Beijing Chemical Reagent Co. Ltd. (Beijing, China).

HPLC Conditions. An Agilent 1100 series HPLC (Agilent Technology) was used to determine the optical purity of two enantiopure enantiomers of benalaxyl prepared via chiral synthesis methods. A commercial HPLC cartridge ChiralPAK IC [cellulose tris(3,5-dichlorophenyl-carbamate)] was employed to separate benalaxyl enantiomers in this study. The cartridge purchased from Daicel Chemical Industries (Tokyo, Japan) was 250 × 4.6 mm i.d. with the CSPs immobilized on a 5 μm silica gel substrate. The mobile phase was a mixture of 80% acetonitrile and 20% water with a flow rate of 1.0 mL/min. Chromatographic separation was conducted at 30 °C, the injection volume was 20 μL, and the UV detection wavelength was 210 nm.

HPLC-MS/MS Conditions. HPLC was performed using a Thermo ACCELA series (Thermo Electron Corp., Hopkinson, MA, USA) equipped with an ACCELA autosampler, an ACCELA 600 pump, and 20 and 2 μL flow cells. Enantiomers were separated on a ChiralPAK IC [cellulose tris(3,5-dichlorophenyl-carbamate)]. The cartridge purchased from Daicel Chemical Industries was 250 × 4.6 mm i.d. with the CSPs immobilized on a 5 μm silica gel substrate. The mobile phase was a mixture of 75% acetonitrile and 25% water with a flow rate of 0.5 mL/min. Chromatographic separation was conducted at 20 °C, and the injection volume was 1 μL.

TSQ QUANTUM ACCESS MAX was used for LC-MS/MS analysis (Thermo Electron Corp.). Quantification was achieved in positive-ion mode (ESI+). The signals were received and processed with Thermo Xcalibur 2.2 SP1.48 software. The optimized major working parameters were as follows: spray voltage, 3200 V; vaporizer temperature, 250 °C; sheath gas pressure, 30 psi; auxiliary gas pressure, 10 arbitrary units; capillary temperature, 350 °C; capillary offset, 35 V; and Q2 gas pressure, 1.5 psi. For benalaxyl, transition m/z 326 → 148 was used for quantification, m/z 326 → 292 was used for confirmation, and collision energies were 28 and 15 eV, respectively.

Synthesis of Enantiopure Enantiomers of Benalaxyl. One of the biggest challenges in understanding the chiral selectivity in the biological actions of chiral pesticides is to obtain enough enantiopure enantiomer standards. In the study, two enantiopure enantiomers of benalaxyl were prepared via chiral synthesis methods.

General Procedure.[27,28] (a) Synthesis of *N*-Xylyl-D-methyl Alaninate. Pyridine (1.42 mL, 17.6 mmol, 1.1 equiv) in 30 mL of CH_2Cl_2 was cooled to 0 °C, trifluoromethanesulfonic anhydride (2.8 mL, 16.2 mmol, 1.05 equiv) was added, and the resulting mixture was stirred for 10 min. Methyl (*S*)-(−)-lactate (1.53 mL, 16.0 mmol, 1 equiv) was added dropwise. The reaction mixture was stirred at 0 °C for 30 min and then warmed to room temperature. The mixture was filtered, the filter cake was washed with ether, the filtrate was evaporated in vacuum (<25 °C), and the residue was triturated with ether and filtered. After evaporation of the solvent, the residue was dissolved in 20 mL of dichloromethane, and 2,6-dimethylaniline (1.97 mL, 16.0 mmol, 1 equiv) was added. The reaction solution was stirred at room temperature overnight, and removal of the solvent under vacuum followed by flash chromatography gave a light yellow oil (2.76 g).

(b) Synthesis of *R*-(−)-Benalaxyl. Sodium bicarbonate (0.5 g, 6 mmol, 1.1 equiv) was added to a solution of *N*-xylyl-D-methyl alaninate (1.12 g, 5.5 mmol, 1 equiv) in 30 mL of toluene and cooled to a temperature ranging from 5 to 10 °C; subsequently, phenylacetic acid chloride (0.93 g, 6 mmol, 1.1 equiv) was slowly added dropwise. After 4 h at room temperature, the above solution was washed with saturated sodium chloride, and the organic phase was evaporated under vacuum. The obtained crude product was crystallized with hexane to give 1.2 g of white crystalline solid alaninate with an enatiomeric *R*/*S* ratio = 98 (yield 71%).

(c) Synthesis of *S*-(+)-benalaxyl with methyl (*R*)-(+)-lactate as a starting material followed the above experimental procedure.

***T. molitor* Larvae.** *T. molitor* larvae were purchased from Beijing Jie Matt Biological Technology Co., Ltd., and were reared in ventilated terrariums (25 × 15 × 20 cm). A layer of wheat bran covered the terrarium floor. The population was kept at 25 °C, and the light cycle adopted was 16 h light and 8 h dark.

Wheat Bran Formulation and *T. molitor* Larvae Exposure. To disperse the *rac*-benalaxyl uniformly in 100 g of wheat bran by dry weight, the procedure in the following steps (dilution spike) was adopted. First, racemic benalaxyl was dissolved in acetonitrile to make a stock solution at a concentration of 1000 mg/L, then 2 mL of acetonitrile solution (1000 mg/L) was dissolved into 50 mL of acetone, and, meanwhile, 100 g of wheat bran was soaked in the 50 mL acetone solution of *rac*-benalaxyl, followed by drying in a fuming cupboard overnight. The wheat bran contaminated with enantiopure *R*-benalaxyl and *S*-benalaxyl followed the above experimental procedure.

In this experiment, *T. molitor* in the larva stage (0.1 g mean body weight) was used. Molting or pupating larvae were excluded from the experiment. Before the worms were introduced, they were fed uncontaminated wheat bran for 1 week to acclimate. A piece of paper resting on top of the wheat bran provided a cover for the worms. The paper was gently soaked with water daily to prevent the larvae from becoming dehydrated. Thirty grams of *T. molitor* larvae were exposed to the chemical in each ventilated terrarium containing 7 g of contaminated wheat bran. Food was renewed once a week. All of the terrariums were placed in the dark in an environmental chamber.

For the bioaccumulation experiment, 1 g of *T. molitor* larvae and 0.5 g of wheat bran were collected after an exposure period (1, 2, 3, 9, 24,

72, 168, 336, and 504 h). Wheat bran on the surface of worms was filtered by a sieve, and the worms were frozen at −20 °C in a 50 mL of polypropylene centrifuge tube. Wheat bran samples from each terrarium were also stored at −20 °C. All experiments were performed at 25 ± 0.1 °C and were run in triplicate at each sample point.

Analysis of Benalaxyl Residue. For the sample pretreatment of wheat bran, 15 mL of acetonitrile was added to a 50 mL polypropylene centrifuge tube containing 0.5 g of wheat bran sample. Next, the sample was stirred for 3 min on a vortex mixer, exposed to ultrasonic vibration for 10 min, and then centrifuged at 3000 rpm for 5 min. The extract was transferred to a new tube, the remaining part was extracted again following the same extraction step, and the extracts were combined. The combined extract was filtered through a funnel with about 5 g of anhydrous sodium sulfate to a pear-shaped flask and evaporated to dryness by a vacuum rotary evaporator at 35 °C. The Alumina-N-solid-phase extraction (SPE) (1.0 g) on a cartridge (6 mL) was used to clean up other interfering substances. The cartridge was preconditioned by rinsing with 5 mL of ethyl acetate followed by 5 mL of *n*-hexane and equilibrated with 5 mL of 1:4 ethyl acetate/*n*-hexane. The sample of dry extract was dissolved in 2 mL of 25% ethyl acetate in *n*-hexane, and then the solution was loaded to the SPE cartridge. The cartridge was eluted with an additional 8 mL of 1:4 ethyl acetate/*n*-hexane. The eluates were combined and collected in a pear-shaped flask and evaporated to dryness in vacuum at 35 °C, and the dry extract was diluted to 1.0 mL with acetonitrile (HPLC grade) and filtered through a filter membrane (pore size, 0.22 μm).

For analysis of the *T. molitor* larvae, the samples were thawed for about 15 min at room temperature. Fifteen milliliters of acetonitrile was added to each tube containing 1 g of *T. molitor* larvae sample. Next, the sample was stirred for 3 min on a vortex mixer, exposed to ultrasonic vibration for 10 min, and then centrifuged at 3000 rpm for 5 min. The sample was re-extracted in the same way, and the supernatants were combined. The combined extract was filtered through a funnel with about 5 g of anhydrous sodium sulfate to a pear-shaped flask and evaporated to dryness at 35 °C. For cleanup (fat destruction), 3 × 10 mL of *n*-hexane was added for liquid−liquid partition to extract most of the lipid. The upper layer of *n*-hexane was discarded, and the layer of acetonitrile was evaporated to dryness by a vacuum rotary evaporator. The Alumina-N-SPE (1.0 g) on a cartridge (6 mL) was used to clean up other interfering substances. The cartridge was preconditioned by rinsing with 5 mL of ethyl acetate followed by 5 mL of *n*-hexane and equilibrated with 10 mL of 1:5 ethyl acetate/*n*-hexane. The sample of dry extract was dissolved in 2 mL of 20% ethyl acetate in *n*-hexane, and then the solution was loaded to the SPE cartridge. The cartridge was eluted with an additional 8 mL of 1:5 ethyl acetate/*n*-hexane. The eluates were combined and collected in a pear-shaped flask and evaporated to dryness under vacuum at 35 °C, and the dry extract was diluted to 1.0 mL with acetonitrile (HPLC grade) and filtered through a filter membrane (pore size, 0.22 μm).

Method and Validation. Stock solutions (100 mg/L) of *rac*-benalaxyl and two enantiomers were prepared in acetonitrile, respectively. A series of standard working solutions at 10, 50, 100, 500, and 1000 μg/L concentrations were prepared from the stock solution by serial dilution in acetonitrile. According to the procedure described above, matrix-matched standard solutions were obtained at the same concentrations by adding blank *T. molitor* larvae and wheat bran sample extracts to each serially diluted standard solution. Calibration curves were generated by plotting the peak area of quantification ion transition against the concentrations of the enantiomers with regression analysis.

Blank sample (*T. molitor* larvae and wheat bran) analysis was performed to check interference from the matrix. The slope ratios of the linear calibration functions were calculated to differentiate between the extraction efficiency and the matrix-induced signal suppression/enhancement (SSE). The SSE caused by matrix effects was determined.

Recovery of benalaxyl enantiomers was measured in a blank sample that was fortified at different levels (0.1, 0.5, and 1 mg/L based on five replicates). The samples were left for 1 h to ensure that the spiked samples were evenly distributed. The fortified samples were analyzed, and the recoveries were calculated by comparing the measured concentration to the fortified concentrations. The precision in the condition for repeatability, expressed as the relative standard deviation (RSD), was determined over the entire calibration range.

The stability of the benalaxyl enantiomers was evaluated in the stock solutions and matrix extracts. The stock solutions of the enantiomers, which were prepared 2 months previously, were tested by comparison of injections of a newly prepared working solution. The stability of the spiked *T. molitor* larvae and wheat bran samples for the enantiomers was determined, and all of the samples used in the stability test were stored at −20 °C.

■ RESULTS AND DISCUSSION

Assay Validation. The chiral LC-MS/MS method described above was validated for the analysis of benalaxyl in *T. molitor* larvae and wheat bran. The typical chromatogram is shown in Figure 7; *R*-benalaxyl and *S*-benalaxyl were baseline separated, and no endogenous interference peaks were eluted at retention times of 10.41 min (*R*-benalaxyl) and 11.49 min (*S*-benalaxyl). Residue analysis showed good linearity over the concentration range of 0.01−5 mg/kg, and the mean correlation coefficients (r^2) of *R*-benalaxyl and *S*-benalaxyl were 0.9995 and 0.9997 and the equations of the linear regression were $y = 2.50 \times 10^{-7}x + 0.06502$ and $y = 2.50 \times 10^{-7}x + 0.06183$, respectively. If the concentrations of the analytical extract exceed the linear range of the analysis, the analytical extract should be diluted or concentrated. The matrix

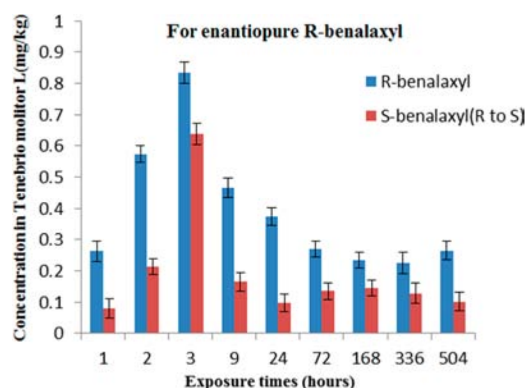


Figure 2. For enantiopure *R*-benalaxyl, concentrations of *R*-benalaxyl and *S*-benalaxyl for a process of enantiomerization in *Tenebrio molitor* larvae.

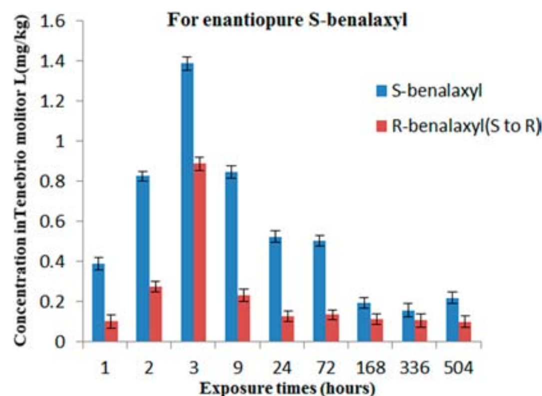


Figure 3. For enantiopure *S*-benalaxyl, concentrations of *R*-benalaxyl and *S*-benalaxyl for a process of enatiomerization in *Tenebrio molitor* larvae.

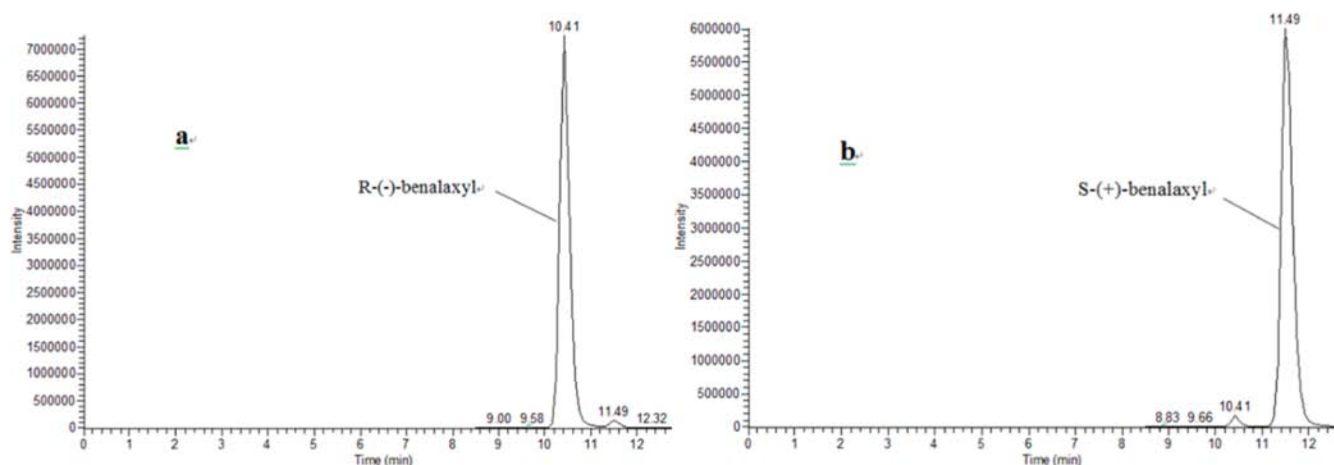
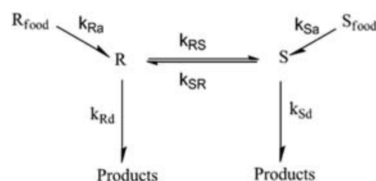


Figure 4. Representative HPLC-MS chromatogram of the expections of (a) enantiopure *R*-benalaxyl in wheat bran and (*b*) enantiopure *S*-benalaxyl in wheat bran after 21 days of exposure. Flow rate = 0.5 mL/min, ultrapure water/acetonitrile = 25:75 (v/v).

Chart 1. Generalized Reaction Scheme with Rates of Uptake, k_{Ra} and k_{Sa}, Rates of Degradation, k_{Rd} and k_{Sd}, and Rates of Enantiomerization, k_{RS} and k_{SR}



effect was calculated by comparing the slope of matrix-matched standard curve with the slope of the standard calibration curve, and the slope ratios were in the range of 0.87−1.13, so there was no significant matrix effect for benalaxyl determination with HPLC-MS/MS based on the proposed method. The mean average recoveries for both enantiomers ranged between 86 and 94% in *T. molitor* larvae and between 90 and 95% in wheat bran, and the relative standard deviations (RSDs) ranged from 2.5 to 3.1% in *T. molitor* larvae and from 2.1 to 2.8% in wheat bran. On the basis of the peak-to-peak signal-to-noise ratio of 3, the limit of detection of the method was 0.002 mg/kg in both *T. molitor* larvae and wheat bran.

Enantiomerization in *T. molitor* Larvae. To assess enantiomerization of benalaxyl enantiomers in *T. molitor* larvae, the wheat bran contaminated with enantiopure *R*-benalaxyl and *S*-benalaxyl was fed to *T. molitor* larvae, respectively. The results are shown in Figures 2 and 3. For enantiomerization of enantiopure *R*-benalaxyl, at the time of 3 h of exposure, the concentration of *R*-benalaxyl rapidly increased to 0.83 mg/kg. Meanwhile, obvious enantiomerization from *R* to *S* was observed and the concentration of *S*-benalaxyl reached 0.63 mg/kg. The concentrations of *R*-benalaxyl and *S*-benalaxyl decreased after 3 h of exposure. The steady-state level was

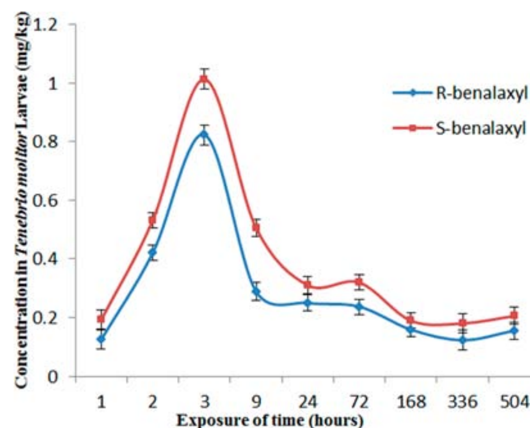


Figure 5. Accumulation curves for benalaxyl enantiomers in *Tenebrio molitor* larvae.

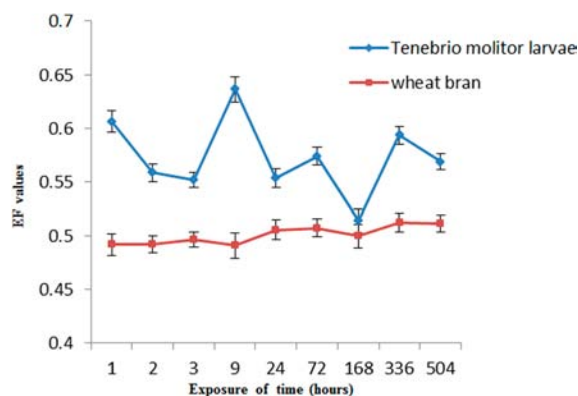


Figure 6. Enantiomeric fraction (EF) of racemic benalaxyl residue in *Tenebrio molitor* larvae and wheat bran.

Table 1. Functions of Benalaxyl Enantiomers in *Tenebrio molitor* Larvae

test material	enantiomer	function	R^2
enantiopure *R*-benalaxyl	*R*	$[R] = 80.953\ e^{-0.287t} - 81.219\ e^{-0.293t} + 0.266$	0.845
	S	$[S] = -160.359\ e^{-0.287t} + 471.648\ e^{-0.293t} - 311.412\ e^{-0.297t} + 0.123$	0.517
enantiopure *S*-benalaxyl	*R*	$[R] = 0.682\ e^{-0.117t} + 2220\ e^{-0.411t} - 2220\ e^{-0.411t} - 0.682$	0.544
	S	$[S] = 2.179\ e^{-0.117t} - 2.462\ e^{-0.411t} + 0.283$	0.813

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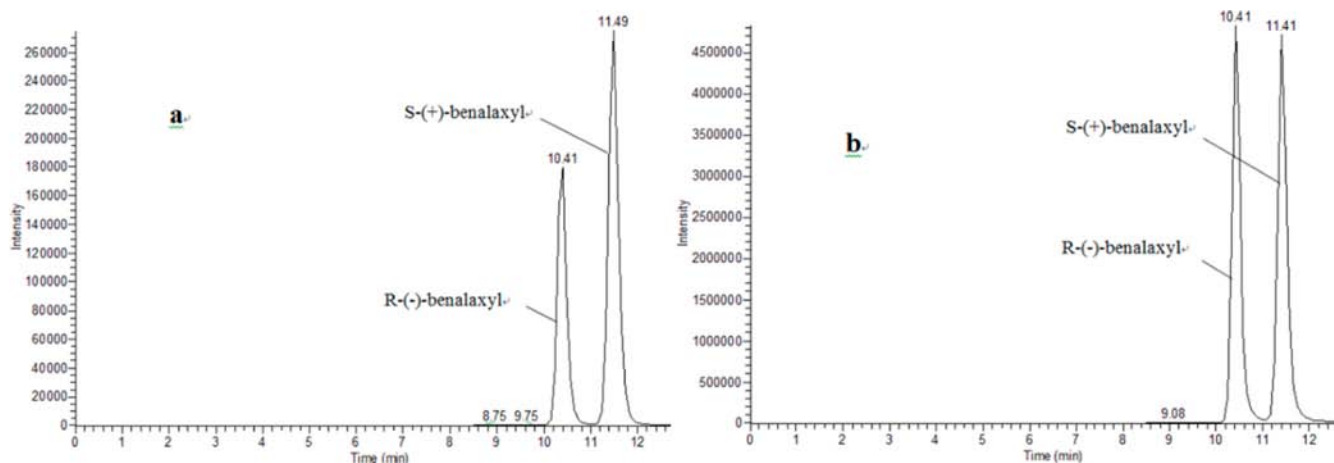

Figure 7. Representative HPLC-MS chromatogram of (a) enantiopure R-benalaxyl in wheat bran and (b) enantiopure S-benalaxyl in wheat bran after 21 days of exposure. Flow rate = 0.5 mL/min, ultrapure water/acetonitrile = 25:75 (v/v).

reached in 3−21 days, and the concentrations of *R*-benalaxyl and *S*-benalaxyl were 0.27 and 0.10 mg/kg, respectively.

Enantiomerization of enantiopure benalaxyl-*S* from *S* to *R* in *T. molitor* larvae was also observed, and the trend of the concentration change was similar to that of *R*-benalaxyl. At the time of 3 h, the concentration of *S*-benalaxyl reached the highest level. At the same time, obvious enantiomerization from *S* to *R* was observed and the concentration of *R*-benalaxyl reached 0.89 mg/kg. After 3 h, the concentrations of *R*-benalaxyl and *S*-benalaxyl decreased until a steady-state level.

It was suggested that benalaxyl enantiomers underwent an obvious enantiomerization with formation of the *R* enantiomers from the *S* enantiomers, and vice versa, in *T. molitor* larvae. We considered that certain enzymes in *T. molitor* larvae might bring about the enantiomerization, and *T. molitor* larvae exhibited a strong excitability for the rapid enantiomerization in a short time. However, the enantiomerization of benalaxyl enantiomers was not found in wheat bran during 21 days, as shown in Figure 4.

One hypothetical pathway of enantiomerization has been reported via deprotonation of an active proton at the chiral center in the view of molecular structure.[29] As seen in Figure 1, the chiral carbon in benalaxyl holds an electron-withdrawing carbonyl group and a conjugated acetanilide functional group. Therefore, the proton on the chiral center is relatively acidic and is thus easily lost to create an intermediate carbanion. Under protic environment, reprotonation regenerates the parent compound from either the top or the bottom face of the anion, thus interconverting enantiomers.

In fact, there should be more chiral pesticides that may undergo such enantiomerization in environmentsn and it is worthy of further investigation.

Kinetic Considerations. For accurately describing the details of enantiomerization of *R*-benalaxyl and *S*-benalaxyl in *T. molitor* larvae, the rate equations for a process involving uptake, enantiomerization, and degradation according to the generalized reaction scheme in Chart 1 were established, and first-order or pseudo-first-order kinetics were assumed.

For the process (uptake, enantiomerization, and degradation) of enantiopure *R*-benalaxyl

$$\frac{d[R]}{dt} = k_{Ra}[R_{wb}] - k_{Rd}[R] + k_{SR}[S] - k_{RS}[R] \quad (1)$$

$$\frac{d[S]}{dt} = k_{RS}[R] - (k_{Sd} + k_{SR})[S] \quad (2)$$

Equations 1 and 2 are first-order linear nonhomogeneous ordinary differential equations, the solution of which is given by the general solution of the homogeneous equation and a particular solution of the nonhomogeneous equation. After Laplace transformation and a method of undetermined coefficients, the general solution of the corresponding homogeneous equation is

$$[R] = A_1 e^{-at} + B_1 e^{-bt} + C_1 \quad (3)$$

$$[S] = A_2 e^{-at} + B_2 e^{-bt} + C_2 e^{-ct} + D \quad (4)$$

For the process (uptake, enantiomerization, and degradation) of enantiopure *S*-benalaxyl

$$\frac{d[S]}{dt} = k_{Sa}[S_{wb}] - k_{Sd}[S] + k_{RS}[R] - k_{SR}[S] \quad (5)$$

$$\frac{d[R]}{dt} = k_{SR}[S] - (k_{Rd} + k_{RS})[R] \quad (6)$$

Similarly, for eqs 5 and 6, the general solution of the corresponding homogeneous equation is

$$[S] = A_3 e^{-a't} + B_3 e^{-b't} + C_3 \quad (7)$$

$$[R] = A_4 e^{-a't} + B_4 e^{-b't} + C_4 e^{-c't} + D' \quad (8)$$

wherein a, b, c, a', b', c', A_1−A_4, B_1−B_4, C_1−C_4, D, and D' are special constants. $[R_{wb}]$ and $[S_{wb}]$ are the concentrations of *R*-benalaxyl and *S*-benalaxyl in wheat bran (mg/kg), respectively. $[R]$ and $[S]$ are the concentrations of *R*-benalaxyl and *S*-benalaxyl in *T. molitor* larvae (mg/kg), respectively. k_{Ra} and k_{Sa} are the uptake rate constants from wheat bran (h^{-1}), k_{Rd} and k_{Sd} are the rates of degradation, and k_{RS} (*R* to *S*) and k_{SR} (*S* to *R*) are the rates of enantiomerization of the *R* and *S* enantiomers, respectively.

By fitting the average concentrations of triplicate in *T. molitor* larvae and time into eqs 3, [4], [7], and [8], a nonlinear dynamic fitting technique provided by SPSS 16.0 gave the estimated values corresponding to homogeneous equations (Table 1), and the rates that led to reasonable approximations of the experimental data were 0.052 h^{-1} (k_{Ra}), 0.061 h^{-1} (k_{Sa}), 0.285 h^{-1} (k_{Rd}), 0.114 h^{-1} (k_{Sd}), 0.126 h^{-1} (k_{RS}), and 0.116 h^{-1} (k_{SR}).

The results indicated that *R*-benalaxyl and *S*-benalaxyl had almost the same uptake rate from wheat bran, and the rate of degradation of *R*-benalaxyl was higher than that of *S*-benalaxyl. In addition, the rate of enantiomerization of *R*-benalaxyl (*R* to *S*) was higher than that of *S*-benalaxyl (*S* to *R*).

In chemically mediated reactions, both enantiomers of a chiral compound interconvert, and the rates of enantiomerization k_{RS} (*R* to *S*) and k_{SR} (*S* to *R*) are equal ($k_{RS}/k_{SR} = 1$).[7] In enzyme-catalyzed reactions, these rates generally differ ($k_{RS}/k_{SR} \neq 1$), and the formation of one enantiomer over the other is favored.[30] Therefore, to some extent, the results of the experiment ($k_{RS}/k_{SR} \neq 1$) illustrating enantiomerization could be attributed to enzyme-catalyzed reactions with enantiomers of benalalxyl in *T. molitor* larvae.

Enantioselective Bioaccumulation in *T. molitor* Larvae. The concentrations of the two benalaxyl enantiomers in *T. molitor* larvae and wheat bran were determined. The result of bioaccumulation in *T. molitor* larvae is shown in Figure 5. The rapid uptake of two benalaxyl enantiomers was observed in *T. molitor* larvae. Concentrations of the two enantiomers both reached the highest level in 3 h, but a rapid decrease of concentrations was observed between 3 and 12 h of exposure. Finally, the concentrations tended to stabilize after 7 days. A paired *t* test for the same time points between *R*-benalaxyl and *S*-benalaxyl yielded a *p* value of 0.001. The paired *t* test exhibited a significant difference between the concentrations of two enantiomers in *T. molitor* larvae at the same sample point, with concentrations of *S*-benealaxyl higher than that of *R*-benealaxyl. Therefore, enantioselectivity occurred when the two benalaxyl enantiomers were accumulated by *T. molitor* larvae. However, the concentrations of the two benalaxyl enantiomers were almost the same in wheat bran. Another paper has reported that the concentration of *R*-benealaxyl was higher than that of *S*-benealaxyl from soil to earthworms,[20] the result of which was just the opposite with that of the experiments, demonstrating the different enantioselectivity in the bioaccumulation of the two benalaxyl enantiomers against different organisms.

The enantiomeric fraction (EF) was employed to measure the enantioselectivity of the bioaccumulation of benalaxyl enantiomers in *T. molitor* larvae. The EF values defined ranged from 0 to 1, with EF = 0.5 representing the racemic mixture. EF was expressed as follows:

$$\mathrm{EF} = [S]/[R] + [S] \qquad (9)$$

The EF values were calculated, and the data are shown in Figure 6. The EF values in *T. molitor* larvae were observed to deviate from 0.5 in the bioaccumulation experiment, and they were maintained approximately at 0.6, so the bioaccumulation of benalaxyl enantiomers in *T. molitor* larvae for this treatment was enantioselective. The EF values of two enantiomers in wheat bran were not obviously observed to deviate from 0.5. There was no significant biodegradation and transformation for both enantiomers by comparing the concentrations in wheat bran in 21 days.

From the results of the experiments, benalaxyl did not accumulate in *T. molitor* larvae significantly and the observed characteristics were that benalaxyl was taken up rapidly and the concentration of benalaxyl reached the highest level in a short time and then decreased quickly until an equilibration. It could be concluded that the low ability of bioaccumulation of benalaxyl in *T. molitor* larvae was attributed to metabolism and degradation at a high rate.

As the rates of degradation and enantiomerization of two enantiomers were calculated before, enantiomerization of benalaxyl in *T. molitor* larvae could be considered as one of the principal causes of enantioselective bioaccumulation of benalaxyl in *T. molitor* larvae. This showed that enantioselectivtiy was caused not only by actual degradation and metabolism but also by enantiomerization. Although enantiomerization could not be observed directly with the racemic compounds, it was easily observed when the enantiopure enantiomers were used.

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Notes

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