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Stereo and Enantioselective Degradation of *β*-Cypermethrin and *β*-Cyfluthrin in Soil

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Abstract *β*-Cypermethrin (*β*-CP) and *β*-Cyfluthrin (*β*-CF) are two important pyrethroid insecticides and both consist of two enantiomeric pairs (diastereomers), i.e. four enantiomers. In this study, the stereo and enantioselective degradation of *β*-CP and *β*-CF in a Tianjin alkaline soil was studied in details by a combination of achiral and chiral HPLC. The results showed that for the two pyrethroids, the *trans*diastereomer was degraded faster than the corresponding *cis*-diastereomer. *β*-CP and *β*-CF were found to be configurationally unstable in this soil, since isomerization between diastereomers was observed during the degradation process. Further enantioselective analysis showed that significant enantioselectivity occurred during the 30 days incubation time. The enantiomeric ratio (ER) values of *cis*- and *trans*diastereomers changed from initial ≈ 1.00 to final 0.79 and 0.55 for *β*-CP, and to 0.64 and 0.48 for *β*-CF, respectively. At last, it was found that *β*-CF was degraded at relatively faster degradation rate and higher enantioselectivity than *β*-CP. Findings from this study may be used to better understand the chiral profiles of *β*-CP and *β*-CF as well as relevant pyrethroid analogues in environment.

Keywords *β*-Cypermethrin · *β*-Cyfluthrin · Stereo and enantioselective degradation · Isomerization · Enantiomeric ratio

Many pesticides are chiral compounds and consist of mixtures of enantiomers having different biological activity. Similarly, studies have shown that different pesticide enantiomers may also exhibit different environmental behavior (Lewis et al. 1999; Müller and Kohler 2004). Knowledge on environmental enantioselectivity is of great importance, since it provides valuable insight about the chiral profiles and risk assessment of chiral pesticides. However, such enantioselectivity was so far mainly investigated for some simple compounds with only two enantiomers, such as organochlorine insecticides, phenoxyalkanoic acid herbicides and acetamide fungicides (Buerge et al. 2003; Buser and Müller 1998; Garrison et al. 2000; Wang et al. 2005). As for many other complex pesticides, such as synthetic pyrethroids consisting of 4–8 enantiomers, information on their isomer or enantiomer selectivity in environmental process was rather scarce or inadequate (Liu and Gan 2004; Qin et al. 2006). This may be attributed to analytical difficulty in separation and identity of multiple enantiomers of these pesticides (Liu and Gan 2004).

Cypermethrin (CP) [(*RS*)-α-cyano-3-phenoxybenzyl-(*RS*)-*cis,trans*-3-(2,2-dichlorovinyl)-1,1-dimethycyclopropanecarboxylate] and Cyfluthrin (CF) (*RS*)-α-cyano-4-fluoro-3-phenoxybenzyl-(*RS*)-*cis,trans*-3-(2,2-dichlorovinyl)-1,1-dimethycyclopropanecarboxylate] are two widely used synthetic pyrethroid insecticides. The two compounds are similar in chemical structure, except that there is a fluorine atom in the phenoxybenzyl group in CF. Because of the substitution of the F atom, CF has higher acaricidal activity than CP. CP and CF are both chiral with three asymmetrical centers (two chiral carbons in the cyclopropyl ring and one chiral carbon at the α-cyano position, see Fig. 1), and therefore have eight optical isomers. Pairs of mirror-image enantiomers constitute diastereomers, and with respect to

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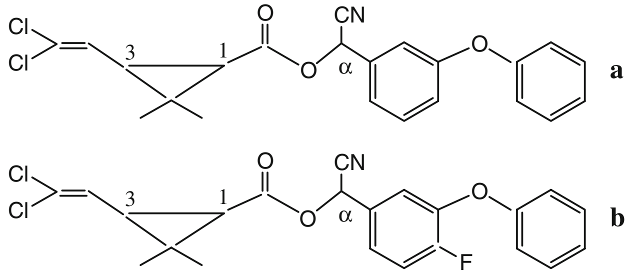

Fig. 1 Chemical structures of CP and CF (**a**: CP, **b**: CF; α, 1 and 3 indicate chiral positions)

the plane of the cyclopropyl ring, the eight enantiomers could therefore be grouped into two *cis* diastereomers of *1R,cis,αS* + *1S,cis,αR* and *1R,cis,αR* + 1*S,cis,αS*, and two *trans* diastereomers of *1R,trans,αS* + *1S,trans,αR* and *1R,trans,αR* + *1S,trans,αS* (Fig. 1 and Table 1). Studies showed that the biological activity or toxicity of CP and CF was mainly derived from only two enantiomers of *1R,cis,αS* and *1R,trans,αS* (Leicht et al. 1996; Liu et al. 2005a). These enantiomers are also intentionally enriched in other commercial formulations to achieve improved insecticidal activity. As seen in Table 1, *β*-cypermethrin (*β*-CP) and *β*-cyfluthrin (*β*-CF) consist of one *cis* diastereomer (i.e. *cis*-*β*-CP or *cis*-*β*-CF) and one *trans* diastereomer (i.e. *trans*-*β*-CP or *trans*-*β*-CF), and α-CP contains only the *cis* diastereomer of *β*-CP (i.e. *cis*-*β*-CP). Since each diastereomer also contains two enantiomers, *β*-CP and *β*-CF both consist of four enantiomers. Currently, *β*-CP and *β*-CF are used to replace racemic CP and CF in many places. In a previous study (Li et al. 2003), we developed a simple and precise method, which combined Matrix Solid-phase Dispersion (MSPD) extraction with achiral and chiral HPLC, for enantiomer determination of several pesticides, including *β*-CP and *β*-CF, in soil. In this study, we applied this method to characterize the stereo and enantioselective degradation of *β*-CP and *β*-CF in soil. Moreover, the purpose of this investigation was also to unveil the difference in enantioselectivity in the degradation of the two pyrethroids and to evaluate the impact of the subtle structural difference on enantioselectivity.

Materials and Methods

CP, CF, *β*-CP (with a *cis:trans* ratio of 1:1), α-CP and *β*-CF (with a *cis*:*trans* ratio of 27:73) standards with purities >97% were all donated by the chemical synthetic group in State Key Laboratory of Elemento-Organic Chemistry, Tianjin, China. Stock solutions were made up at a concentration of 1 mg/mL in hexane and preserved at 4°C. n-Hexane, ethyl acetate and isopropanol were all of analytical grade, redistilled and filtered through a 0.45-μm filter before use. Other solvents and chemicals were of analytical or pesticide residue grade.

A garden soil from Tianjin City in North China was used in this study. The soil was a typical alkaline loam in China with pH value of 8.2. Other properties of this soil were: organic matter 0.72%, sand 34%, silt 40% and clay 26%. Blank determination of the soil prior to fortification revealed no *β*-CP and *β*-CF present (detection limit <0.03 μg/g).

The HPLC system consisted of a Varian model 2010 pump (Varian, Northeast Florham Park, NJ, USA), a Rheodyne model 7125 injector with a 20-μL loop, a Varian model 2050 UV detector set at 230 nm and an Anastar chemical analytical work station (version 5.2). The HPLC columns used were an achiral silica-gel column (Dalian Elite Co. Ltd., China, 250 × 4.6 mm i.d., 5-μm particle size) and an enantioselective Chiralcel OD column (Daicel Chemical Industries Ltd. Japan, 250 × 4.6 mm i.d., 10-μm particle size) both protected with a guard cartridge of the same solid phase (30 × 4.6 mm i.d.).

Two separate experiments were carried out with *β*-CP and *β*-CF, respectively. Portions of 100 g soil (dry weight equivalent) were placed into two 250 mL conical flasks and mixed with 15 mL water. After preincubation in the dark at 25°C for a week, *β*-CP (experiment S1) and *β*-CF (experiment S2) were spiked separately into the soil at a concentration of 5 μg/g of dry soil. After thorough mixing, 15 mL more water was added to give final 23% moisture content. The flasks were then sealed with cotton-wool plugs and stored at 25°C in the dark. During preincubation and incubation processes, distilled water was added at 3–4 days interval to maintain the initial moisture. Soil samples equivalent to 6 g dry soil weight were removed for

Table 1 Enantiomer/diastereomer compositions of CP and CF formulations

	cis-diastereomer		*trans*-diasteromer		*n*
	1R,cis,αS +*1S,cis,αR*	*1S,cis,αS* +*1R,cis,αR*	*1R,trans,αS* +*1S,trans,αR*	*1S,trans,αS* +*1R,trans,αR*	
CP	√	√	√	√	8
β-CP	√ (*cis*-*β*-CP)		√ (*trans*-*β*-CP)		4
α-CP	√ (*cis*-*β*-CP)				2
CF	√	√	√	√	4
β-CF	√ (*cis*-*β*-CF)		√ (*trans*-*β*-CF)		4

n: Number of enantiomers

analysis at different time intervals and frozen at −20°C until they could be analyzed.

The extraction of β-CP and β-CF from soil was carried out by MSPD method (Li et al. 2003). The soil samples collected were placed into a mortar (50 mL capacity). 9 g Florisil adsorbent and 2 mL distilled water were added, followed with gently blending for 10 min to obtain a homogeneous mixture. This mixture was then introduced into a 30 × 1.5 cm i.d. glass chromatographic column containing a coarse frit and 2 cm of anhydrous sodium sulfate. Finally a 1.0 cm layer of anhydrous sodium sulfate was placed on the top of the column and a mixture of n-hexane/ethyl acetate (7:1/v:v) was added to the column and allowed to elute dropwise by gravity. The initial 15 mL eluent was collected into a graduate conical tube and then carefully blown to dryness with a mild nitrogen stream in a water bath of 50°C. The final residue was dissolved in achiral HPLC mobile phase (n-hexane/isopropanol, 100:0.1/v:v), and after filtration through a 0.45-μm filter the sample solution was subjected to achiral HPLC column and quantified using external standard method. The recoveries of β-CP and β-CF at 2.0 and 0.2 μg/g levels were >72% with relative standard deviations <10%.

The enantiomer-specific analysis of β-CP and β-CF was achieved by a combination of achiral and chiral HPLC. The concentration of each diastereomer was determined on the achiral silica-gel column with 1.0 ml/min n-hexane/isopropanol (100:0.1/v:v) as the mobile phase. As for chiral separation, another clean-up procedure was necessary. The remainder samples after diastereomer quantitation were concentrated and injected into the silica-gel column. Mobile phase fractions containing β-CP and β-CF diastereomers were individually collected by observing corresponding UV signals. After gently blown to dryness and dissolved in chiral HPLC mobile phase, the samples were analyzed on the Chiralcel OD column for Enantiomer Ratio (ER) determination. The mobile phase was made of n-hexane fortified with isopropanol and the flow rate was fixed at 1.0 mL/min. One replicate sample per two or three samples was analyzed, and the precision of the enantiomer concentrations as well as ER values was satisfactory with percent difference lower than 10%.

Results and Discussion

On the achiral silica-gel column, the four diastereomers of racemic CP or CF could be baseline separated into four peaks with 100/0.1 n-hexane/isopropanol (v:v) as the mobile phase. The four diastereomers were then labeled as CP-1, CP-2, CP-3 and CP-4 for CP or CF-1, CF-2, CF-3 and CF-4 for CF according to the elution time (Fig. 2). By using the same conditions, β-CP standard gave two peaks with identical retention times of CP-2 and CP-4. Further analysis of α-CP, which contains only the *cis*-diastereomer of β-CP, gave only one peak corresponding to CP-2. Therefore, CP-2 was assigned to be *cis*-β-CP, leaving CP-4 as *trans*-β-CP. In case of β-CF, the injection produced one small peak and one larger peak corresponding to CF-2 and CF-4, respectively. According to the *cis*:*trans* composition of 27:73 of β-CF standard, the first smaller peak was then assigned to the *cis* form (*cis*-β-CF) and the latter larger peak to the *trans* form (*trans*-β-CF). Therefore, for both β-CP and β-CF, the *cis* diastereomer eluted faster than the corresponding *trans* diastereomer. Due to the absence of authentic standards, the first (CP-1 or CF-1) and third (CP-3 or CF-3) eluted diastereomers were not identified in this study. However, assuming a similar rule was followed, it may be postulated that CP-1 or CF-1 was the other *cis* diastereomer and CP-3 or CF-3 the other *trans* diastereomer.

The above achiral HPLC system was also used for quantitative determination of individual diastereomers of β-CP and β-CF in the two soil incubation experiments. In Fig. 3, we gave the chromatograms of soil samples analyzed on the silica-gel column. The concentration of each diastereomer of CP and CF was also summarized in Table 2. The data showed that, for the two pyrethroids, the *trans*-diastereomer (*trans*-β-CP or *trans*-β-CF) was degraded much faster than the *cis*-diastereomer (*cis*-β-CP or *cis*-β-CF). This observation was very consistent with previous studies (Kaufman et al. 1981; Qin et al. 2006; Sakata et al. 1992). In Fig. 3a, it was also noted that a small peak appeared with a retention time equivalent to that of CP-1 during the incubation of β-CP (experiment S1). To certify its identity, this compound was further isolated at HPLC outlet and subjected to GC-MS analysis. As a result, its mass spectrum was very identical with that of CP standard, suggesting it was definitely CP-1. In the case of β-CF (Fig. 3b, experiment S2), a very similar pattern was also observed and a certain amount of CF-1, which was also identified with GC-MS, was yielded during β-CF degradation. Since almost no CP-1 or CF-1 was contained in the fortified β-CP or β-CF (Fig. 3), it is deduced that CP-1 or CF-1 must be formed via the isomerization of β-CP or β-CF diastereomers. Previous studies (Leicht et al. 1996; Liu et al. 2005b) showed that some pyrethroids, including CP and CF, could undergo epimerization at chiral α-carbon position in water media. The isomerization found in this study seemed to proceed via a similar process, since the soil environment also contained a certain amount of water.

It is obvious that isomerization would more or less reduce the efficacy of biologically active enantiomer. However, as seen in Fig. 3, the yield of CP-1 or CF-1 was low, suggesting the isomerization was subtle in the studied soil and would not significantly affect the insecticidal activity of β-CP or β-CF. As for the other diastereomer of

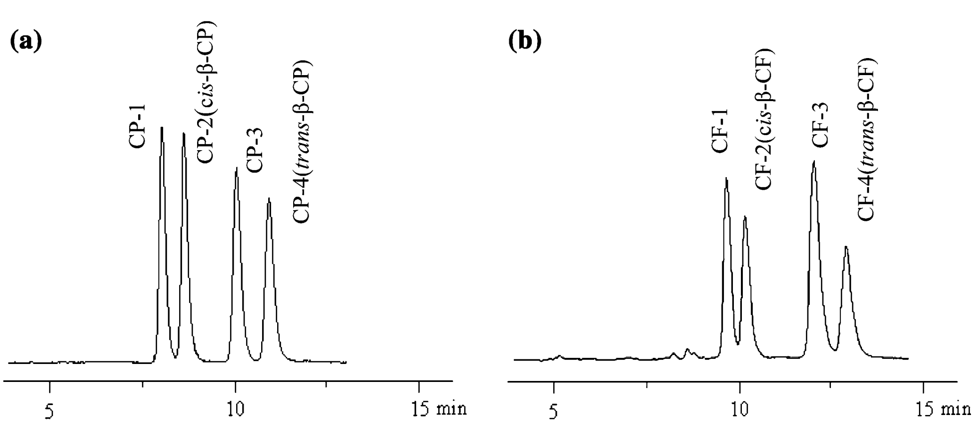

Fig. 2 Separation of racemic CP (**a**) and CF (**b**) on the silica-gel column

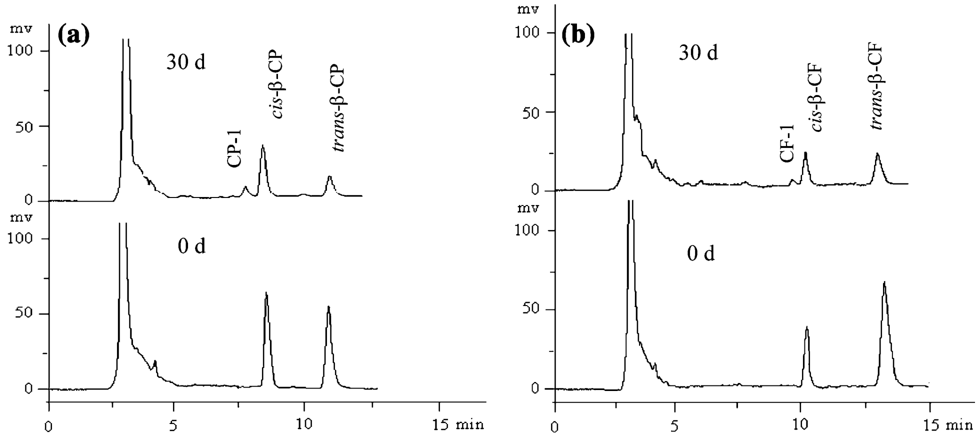

Fig. 3 Achiral HPLC chromatograms of MSPD extracts of soil examples after 0 and 30 days incubation with β-CP (a, experiment S1) and β-CF (b, experiment S2), respectively

Table 2 Concentration (Conc. μg/g) and ER values for the degradation of β-CP and β-CF in soil

Experiment	Diastereomer		Time (days)				
			0	3	7	14	30
S1	*cis*-β-CP	Conc.	2.54	2.42	2.01	1.67	1.19
		ER	0.99	1.01	0.97	0.92	0.79
	trans-β-CP	Conc.	2.67	2.13	1.48	0.92	0.43
		ER	1.00	0.85	0.77	0.57	0.55
S2	*cis*-β-CF	Conc.	1.22	1.10	1.03	0.88	0.47
		ER	0.99	0.94	0.85	0.66	0.64
	trans-β-CF	Conc.	4.22	3.52	2.39	1.11	0.64
		ER	1.03	0.88	0.75	0.52	0.48

CP-3 or CF-3, there was almost no peak of this compound detected during the incubation process, suggesting the yield of CP-3 or CF-3 was nearly negligible.

Because *cis*-β-CP, *trans*-β-CP, *cis*-β-CF and *trans*-β-CF all contain two enantiomers, further enantiomer separation was then carried out on an enantioselective Chiralcel OD column. In this procedure, each diastereomer was individually isolated at HPLC outlet, and after concentration an aliquot was analyzed on the chiral column. The optimized mobile phase consisted of hexane and isopropanol at a ratio of 100/6 (v/v) for *cis*-β-CP and *trans*-β-CP, or 100/2 (v/v) for *cis*-β-CF and *trans*-β-CF. Under these conditions, the two enantiomers from the same diastereomer could be baseline separated rapidly and precisely. Since there is no enantiomer standard available, no effort was made to determine absolute configuration of each enantiomer. For expression convenience, the first eluted enantiomer was all assigned to be enantiomer-1 and the second enantiomer-2, i.e. *cis*-1 and *cis*-2 for the two enantiomers of *cis*-β-CP or *cis*-β-CF, and *trans*-1 and *trans*-2 for the two enantiomers of *trans*-β-CP or *trans*-β-CF. As a measure of enantioselectivity, enantiomer ratio (ER) was defined as the peak area ratio of enantiomer-1 over enantiomer-2. In Table 2, we gave the variation in the ER values of each diastereomer of β-CP and β-CF in the experiments. The chiral HPLC chromatograms of β-CP and β-CF diastereomers after 0 and 30 days incubation were also given in Fig. 4.

On the basis of overall diastereomer concentration determined on the silica-gel column together with the corresponding ER value determined on the chiral column (Table 2), the concentration of each enantiomer could then be measured and calculated. For all the enantiomers, the data fit well to the first-order decay model, with $r^2 > 0.92$ in most cases. In Table 3, we listed the kinetic data (half-life of $T_{1/2}$ and rate constant k) of each enantiomer. All

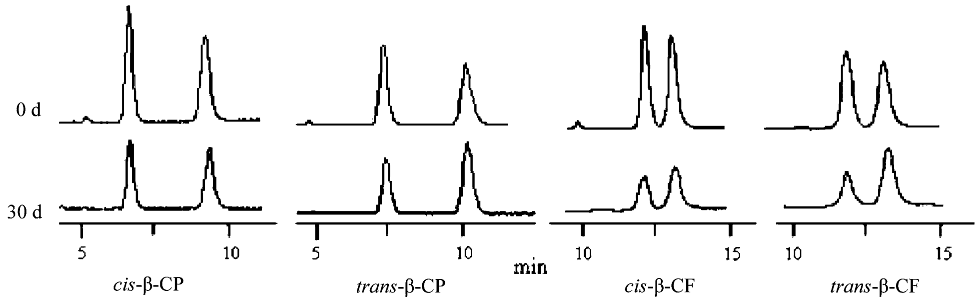

Fig. 4 Chiral HPLC chromatograms of β-CP and β-CF diastereomers in soil after 0 and 30 days incubation

Table 3 First rate constant (k), half-life ($T_{1/2}$) and correlation coefficient (r^2) for the degradation of enantiomers of β-CP and β-CF in soil

Experiment	Compound	Enantiomer	k × 10^2 (d^{-1})	$T_{1/2}$ (d)	r^2
S1	*cis*-β-CP	*cis*-1	2.96	23.42	0.9874
		cis-2	2.21	31.36	0.976
	trans-β-CP	*trans*-1	7.23	9.59	0.9657
		trans-2	5.21	13.3	0.9927
S2	*cis*-β-CF	*cis*-1	4.04	17.16	0.9995
		cis-2	2.44	28.41	0.9106
	trans-β-CF	*trans*-1	7.93	8.74	0.9223
		trans-2	5.47	12.67	0.955

pyrethroid enantiomers exhibited relatively short half-life, with $T_{1/2}$ ranging from 8 to 32 days. At the same time, significant enantioselectivity occurred for each diastereomer (Fig. 4). For all the diastereomers, the first eluted enantiomer-1 was all degraded fairly faster than the later eluted enantiomer-2. Moreover, significant higher enantioselectivity was observed with the *trans*-diastereomer than the *cis*-diastereomer. For *trans*-β-CF as an example, the ER decreased from initial 1.0 to 0.48 after 30 days incubation, whereas the ER of *cis*-β-CF only decreased to 0.68. For both β-CP and β-CF, the degradation rates of four enantiomers decreased in the order of *trans*-1 > *trans*-2 > *cis*-1 > *cis*-2. Therefore, the enantioselectivity would finally result in relative enrichment of *cis*-2 enantiomer for both β-CP and β-CF.

At last, by comparing the degradation of the two pyrethroids, it was found that β-CP and β-CF had similar enantioselective behavior in the studied soil. However, β-CF was degraded at relatively faster degradation rate and higher enantioselectivity than β-CP (Tables 2–3 and Fig. 4). The shortest $T_{1/2}$ was found with *trans*-1 enantiomer of β-CF (8.74 d, Table 3) and the lowest ER value with *trans*-β-CF diastereomer (0.48 at 30 days incubation time, Table 2). Since the incubation conditions were the same for the two pyrethroids, it was likely that the F-atom in CF may play some key role in the metabolism process. Isomer/enantiomer selectivity may be compound and environment-specific and should be determined precisely for better understanding the chiral profiles and fate of chiral contaminants in the environment.

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