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Influence of Soil Properties on the Enantioselective Dissipation of the Herbicide Lactofen in Soils

Jinling Diao,[†] Chunguang Lv,[†] Xinquan Wang,[‡] Ziheng Dang,[†] Wentao Zhu,[†] and Zhiqiang Zhou*,[†]

[†]Department of Applied Chemistry, China Agricultural University, Beijing 100193, China, and [‡]Institute of Quality and Standard for Agro-products, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China

A scheme was developed to elucidate the dissipation behaviors of the two enantiomers of the herbicide lactofen in soils using a normal-phase high-performance liquid chromatograph (HPLC) with UV detector and a column with a cellulose-tri-(3,5-dimethylphenylcarbamate)-based chiral stationary phase (CDMPC-CSP). Eight soils with a wide range of soil properties were studied. Racemic and the enantiopure (*S*)-(+)- and (*R*)-(−)-lactofen were incubated under aerobic and anaerobic conditions. The data from sterilized controls indicated that the dissipation of lactofen was biological. The dissipation was shown to be enantioselective with (*S*)-(+)-enantiomer being degraded faster than the (*R*)-(−)-enantiomer, resulting in residues enriched with (*R*)-(−)-lactofen when the racemic compound was incubated. Lactofen was configurationally stable in soil, showing no interconversion of (*S*)-(+)- to (*R*)-(−)- enantiomer and vice versa. Significant correlations of the enantioselectivity, expressed as ES = $(k_{(S)} - k_{(R)})/(k_{(S)} + k_{(R)})$ of lactofen with soil pH were observed under aerobic and anaerobic conditions. In addition, we found that the enantioselectivity correlated with the soil texture rather than the organic carbon.

KEYWORDS: Enantioselective dissipation; enantiomer; lactofen; soil pH; soil texture

INTRODUCTION

Many pesticides in use are chiral compounds containing stereoisomers. The biological transformation of chiral compounds is often stereoselective, and the uptake, metabolism, and excretion of enantiomers by organisms may also be selective (*1*, *2*). Such enantioselectivity has been shown to result in different distribution patterns (*3*−*5*) and bioaccumulation potentials between enantiomers in the environment (*6*, *7*). The role of enantioselectivity in environmental safety is poorly understood for pesticides, and the knowledge gap is reflected in that the great majority of chiral pesticides are used and regulated as if they were achiral, that is, single compounds. Achiral analysis gives only partial information, so enantioselective analysis is required for a full understanding of the biological behavior of such compounds. Information on the stereoselective dissipation kinetics and bioaccumulation of chiral pesticides will help improve our understanding of the pesticide safety to humans, animals, and the environment.

Lactofen, 2-ethoxy-1-methyl-2-oxoethyl-5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate, has an asymmetrically substituted C atom and consists of a pair of enantiomers; the herbicidal activity mostly originates from the *S*-(+)-enantiomer (*8*) (**Figure 1**). The absolute configuration of the lactofen enantiomers was established by combined use of chemical correlation methods and chiral high-performance liquid chromatography (HPLC) (*9*). Lactofen is a member of the diphenyl ether chemical family (*10*) and was developed by PPG Industries. Lactofen is a restricted-use pesticide and is in the Environmental Protection Agency (EPA) toxicity class I. It is applied as a foliar spray on target weeds (*11*) and is commonly used to control broadleaf weeds in soybeans, cereal crops, potatoes, and peanuts. The metabolism and environmental fate of racemic lactofen in soil have been studied extensively and reviewed (*11*−*15*). Lactofen is of low persistence in most soil types (*11*, *12*); field half-lives range from 1 to 7 days (*11*−*13*). It is rapidly degraded, mainly by microbial activity rather than hydrolysis (*11*), although hydrolysis is more likely at pH 9 and above, conditions that are unlikely in soil environments (*11*, *13*). Aerobic conditions speed the rate of microbial breakdown of lactofen (*11*, *13*, *14*). Regardless of all of these data, the enantioselective dissipation of lactofen in environmental matrices has not been reported.

Enantioselective dissipation in soils has been observed for various chiral pesticides, and some studies suggested that soil properties that have a major impact on the activity of the soil microbial community, such as soil pH, organic carbon, moisture, redox condition, and soil texture, could influence chiral signatures in soils (*16*−*22*). For example, incubation of the chiral pesticides metalaxyl and *cis*-epoxiconazole in soils suggested that enantioselectivity (ES) correlated with soil pH (*18*, *20*). Furthermore, a re-evaluation of published kinetic data from dichlorprop and mecoprop studies indicated similar correlations (*18*). In the

*To whom correspondence should be addressed. Fax: 861062733547. E-mail: zqzhou@cau.edu.cn.

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present study, the enantioselective dissipation of herbicide lactofen was, therefore, investigated in different soils. Eight soils were selected primarily to cover a wide range of soil properties. Furthermore, we evaluated the potential for the enantioselective dissipation of lactofen in aerobic as well as anaerobic soils.

Under certain conditions, some chiral compounds are configurationally unstable and may undergo enantiomerization. For example, two studies reported the enantiomerization of chiral phenoxypropionic acids mecoprop and dichlorprop (*23*, *24*). Therefore, we also investigated the dissipation of lactofen in soil with respect to its stereochemistry using racemic and single pure enantiomers.

EXPERIMENTAL SECTION

Chemicals and Reagents. The analytical standard of *rac*-lactofen (>99% purity) was provided by the China Ministry of Agriculture's Institute for Control of Agrochemicals. The two enantiomers of lactofen were prepared by HPLC (see below). Purified water by a Milli-Q system, ethyl acetate, methanol, *n*-hexane, and 2-propanol (HPLC grade) were from Fisher Scientific (Fair Lawn, NJ). All other chemicals and solvents were analytical grade and purchased from commercial sources.

Soil Sample. Soil samples were collected between June and August, 2006, at 0−10 cm from different agriculture regions of China to represent physicochemically diverse characteristics. None of the soils had been treated with lactofen in the last 5 years. Soil site and some soil characteristics (particle size, texture, pH, and organic carbon) are listed in **Table 1**. Standard equipment was used for sampling. The soils were sieved (2 mm) and air-dried at room temperature and kept in the dark until used within a few days.

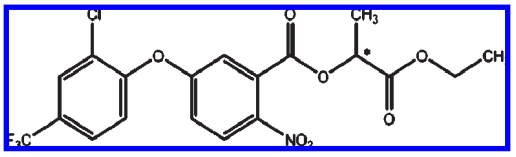

Figure 1. Structure of lactofen.

Table 1. Sampling Sites and Characterization of the Soils Studied

soil site [a]	particle-size sand (%)	silt (%)	clay (%)	soil texture	pH (water)[b]	C_{org} (%)
1 Henan ZhengZhou	55.9	40.9	3.2	sandy loam	8.3	1.1
2 Neimenggu Chifeng	74.4	21.9	3.7	sandy loam	8.1	1.9
3 Heilongjiang Haerbin	44.3	50.8	4.9	silt loam	7.6	5.2
4 Liaoning Dalian	83.2	15.0	1.7	sandy loam	7.2	1.3
5 Shandong Yanzhou	41.9	52.6	5.5	silt loam	6.9	1.1
6 Guangxi Nanning	35.7	47.8	16.4	silt loam	6.7	2.2
7 Zhejiang Cixi	26.0	71.0	3.0	silt loam	5.1	2.5
8 JiangSu Wuxi	36.8	61.5	1.7	silt loam	5.0	1.7

[a] The cities in China. [b] Suspension of soil in water, 1:2.5 (w/w).

Incubation of Lactofen in Soils under Aerobic Conditions. Approximately 100 g of air-dried soils intended for incubation of lactofen under aerobic conditions (soils 1−8, **Table 1**) were placed into 250 mL Erlenmeyer flasks covered with sterile cotton plugs. A portion of the soil (10 g) was first spiked with 0.1 mL of acetone containing 1000 μg of *rac*-lactofen and stirred for 5 min. The spiked soil was then allowed to air dry for 10 min before the remaining soil (90 g) was added and mixed thoroughly for another 10 min (spike level, 10 μg/g dry soil). The soil samples were incubated with a water content of 20−36 g per 100 g dry soil, corresponding to about 60% of field holding capacity (w/w). The soils were incubated at 25 °C in the dark for 5 days while they were covered with sterile cotton plugs. The flasks were weighed regularly, and the loss of water by evaporation was compensated by the addition of distilled water. At appropriate time intervals, aliquots of 5 g of soil (base on dry weight) were removed from each treatment and transferred into a 50 mL plastic centrifuge tube for extraction and analysis. Three replicate samples were taken immediately after pesticide addition and mixing to determine the recovery and reproducibility of extraction in the respective soils (see below).

Incubation of Lactofen in Soils under Anaerobic Conditions. Four soil samples (soils 2, 4, 5, and 8, **Table 1**) were incubated under anaerobic conditions. These experiments were carried out in Petri dishes with a diameter of 9 cm. Portions of 20 g of air-dried soils were placed into separate dishes (15 dishes for one soil, three replicates for one sampling point), in vacuum desiccators (five vacuum desiccators; each vacuum desiccator for one sampling point contained triplicates of each of the four soils for one sampling point). The soil samples were fortified by adding 20 μL of an acetone solution of *rac*-lactofen with a 50 μL syringe (spike level, 10 μg/g dry soil). Adequate amounts of distilled water, previously purged with N_2 to remove O_2, was added to each dish to form a 1 cm water layer, and then, the dishes were sealed. The vacuum desiccators were pumped to vacuum, then filled with N_2, and this procedure was repeated three times. The vacuum desiccators were put into an incubator at 25 °C in the dark. The samples were extracted and analyzed as for the aerobic samples at time zero and four other appropriate times.

To check the redox conditions, a piece of oxygen indicator was added to each vacuum desiccator. The color of the oxygen indicator was red upon anaerobic conditions; on the contrary, it was blue when there was only a small amount of oxygen in the vacuum desiccator. The color of the oxygen indicator stayed red during these anaerobic incubations.

Semipreparative Separation and Incubation of Lactofen Enantiomers. The two enantiomers of lactofen were required to detect potential enantiomerization of the compounds. Separate incubations were carried out with the two enantiomers of lactofen in soils 2, 5, and 8. The tested soils were fortified with the pure enantiomers, respectively, at a level of 10 μg/g and incubated in the same way as the other aerobic and anaerobic soils. The enantiomers of lactofen were separated on CDMPC-CSP [cellulose-tri-(3,5-dimethylphenylcarbamate)-based chiral stationary phase, provided by the Department of Applied Chemistry, China Agricultural University, Beijing]. The CSP was prepared according to the procedure described in the literature (*25*). CSP was packed into a 250 mm × 4.6 mm (i.d.) stainless steel column. *rac*-Lactofen was dissolved in 2-propanol at a concentration of

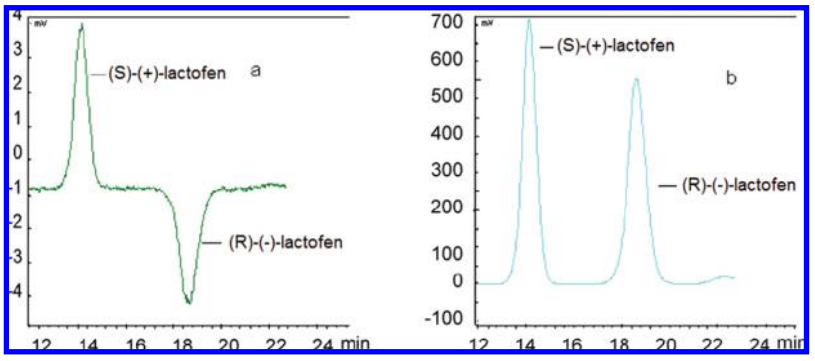

Figure 2. Representative HPLC chromatogram of (**a**) CD of lactofen at 230 nm and (**b**) UV of lactofen at 230 nm (*n*-hexane:2-propanol = 95:5; flow rate, 1.0 mL/min).

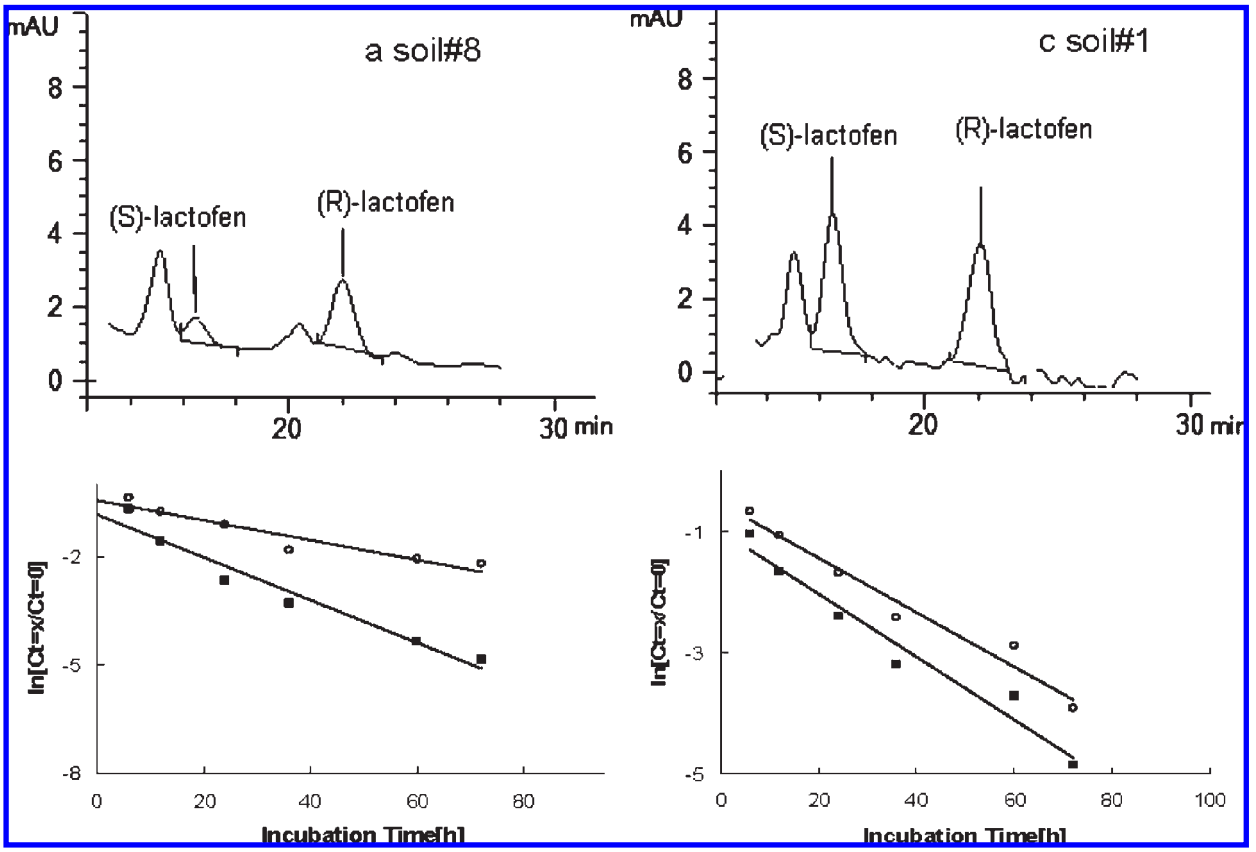

Figure 3. Incubation of *rac*-lactofen in a silt loam soil (soil 8, pH 5) and in a sand loam soil (soil 1, pH 8.3) under aerobic conditions. Chromatograms show elution of (*S*)- and (*R*)-lactofen after incubation times of 36 h (silt loam soil, panel **a**; and sand loam soil, panel **c**). Panels **b** (soil 8) and **d** (soil 1) show the first-order dissipation of (*S*)-lactofen (large squares) and (*R*)-lactofen (large circles) (*n*-hexane:2-propanol = 98:2; flow rate, 1.0 mL/min).

10.0 mg/mL. Chiral separation of the two enantiomers was performed using a mobile phase of hexane/2-propanol (95/5) with a flow rate of 1.0 mL/min at a temperature of 20 °C. The individual enantiomers were collected using a JASCO 2000 HPLC system (JASCO Corp., Japan) equipped with PU-2089 plus pump, UV-2075 plus ultraviolet detector set to 230 nm, CD-2095 plus circular dichroism detector set to 230 nm, and a 7125 Rheodyne injector with a 100 μL sample loop. The enantiomers of lactofen eluted at resolution times of 14.1 and 18.6 min (**Figure 2**). The individual enantiomers were collected manually by observing the UV signal and changing the collection vials accordingly. In this way, the two enantiomers were obtained in high (>99%) enantiomeric purity.

Incubation of Lactofen in Soils under Sterilized Conditions. To determine if enantioselective dissipation was a result of microbially mediated transformations, an additional set of soil samples (soils 2, 5, and 8) was also prepared and subjected to sterilization treatment. Portions of 20 g of air-dried soils were placed into separate 150 mL flasks (21 flasks for one soil, three replicated for each of seven sampling points). The soil samples were fortified by adding 20 μL of an acetone solution of *rac*-lactofen with a 50 μL syringe (spike level, 10 μg/g dry soil). Six milliliters of aseptic water was added to each of the flasks, which were then sealed with film for sterile culture vessels. Sterilization was achieved by autoclaving the samples twice at 121 °C for 60 min, with a 24 h interval between the first and the second autoclaving, to eliminate microbial activity. The sampling times were 6, 12, 24, 48, 84, 120, and 168 h.

Sample Preparation. Three replicate samples were removed from each treatment at different time intervals after pesticide addition and immediately transferred into a freezer (−20 °C) to stop dissipation. For extraction, methanol (20 mL) was added to a 50 mL polypropylene centrifuge tube containing 5 g (dry weight basis) of incubated soil sample. The tube was then stirred for 3 min on a vortex mixer and centrifuged at 4000 rpm for 5 min. The extraction was repeated, and the extracts were combined and evaporated to around 2 mL on a vacuumed rotary evaporator at 45 °C. The residues were extracted three times with a solution of 20 mL of ethyl acetate and 10 mL of aqueous sodium chloride solution (40 g/L) using a 150 mL separatory funnel. The organic phase (ethyl acetate) was collected, concentrated to near dryness, and reconstituted in 1.0 mL of hexane. An aliquot (20 μL) was injected into the HPLC.

Enantioselective HPLC Analysis. Chromatography in this study was performed using an Agilent 1200 Series HPLC (Agilent Technology) equipped with a G1322A Degasser, G1311A Quat Pump, G1329A ALS, and G1314B VWD. The signal was received and processed by Agilent chemstation software. The enantiomers of lactofen were baseline separated on CDMPC-CSP. The detailed HPLC methods and the elution order based on the absolute configuration of lactofen enantiomers are described elsewhere (*9*, *26*). In the present work, the HPLC method was successfully applied to analyze enantiomers of lactofen in soil samples, and the first and second eluted solutes were *S*-(+)-lactofen and *R*-(−)-lactofen, respectively (**Figure 2**). The enantiomeric fraction {EF = area of (*S*)-(+)-enantiomer/[area of (*S*)-(+)-enantiomer + area of (*R*)-(−)-enantiomer]} of the lactofen racemate used in all experimental studies was calculated to be 0.49 (±0.01).

Soil samples were analyzed using a mixture of hexane and isopropanol as the mobile phase. The flow rate was 1.0 mL/min. Chromatographic separation was conducted at room temperature, and UV detection was conducted at 230 nm. No enantiomerization had been observed for lactofen under these analytical conditions. Concentrations were determined by using peak area, assuming the same response factor for the enantiomers and the racemate.

A series of standard solutions (0.3, 3, 30, 60, 90, and 120 mg/L) of racemic lactofen for linearity of the two enantiomers were prepared in isopropanol. The injection volume was 20 μL. Calibration curves were generated by plotting peak area vs the concentration of each enantiomer. The relative standard deviation (RSD) was calculated at the calibration range. The run-to-run precisions of three concentrations (1, 5, and 50 mg/L) for individual enantiomers were determined by injections in six replicates, and the day-to-day precisions were also tested over 6 days by six successive injections each day. The calibration curve for *S*-(+)- and *R*-(−)-enationmer showed an excellent linearity in the range of

0.3−120 mg/L of each enatiomer (n = 6). For *S*-(+)-lactofen, y = 35.368x + 0.491, R^2 = 0.9997; for *R*-(−)-lactofen, y = 34.771x + 3.7546, R^2 = 0.9994. The run-to-run and day-to-day precisions of retention times and peak areas at three concentration levels (1, 5, and 50 mg/L) were also good with RSDs of 0.2−0.8 and 2.6−7.8% for (S)-(+)-lactofen and 0.3−1.1 and 2.7−8.2% for *R*-(−)-lactofen.

Recoveries of lactofen from different soils were determined immediately after fortification. Preliminary experiments showed that the recovery of the above procedure was >90% for the two enantiomers of lactofen in all soils. The limit of quantification (LOQ) for both enantiomers in all samples was found to be 0.3 μg/g based on acceptable RSD of 20%. The limit of detection (LOD) for both enantiomers, defined as the concentration with a signal-to-noise ratio of 3, was 0.1 μg/g of soil samples.

Kinetic Analysis. For all treatments, the data fit well to the first-order decay model, with R^2 ranging from 0.90 to 0.99 (**Figure 3b**,**d** and **Tables 2** and **3**). In this work, we used the rate constant values $k_{(S)}$ and $k_{(R)}$ to measure the enantioselective of the dissipation process, as reported by Buerge and Poiger (*18*). Corresponding rate constants $k_{(S)}$ were determined from the linear range of plots ln[(*S*)] or ln[(*R*)] vs time t (no lag phases were observed in this work):

$$\ln[(S)] = \ln[(S)]_{t=0} - k_{(S)} \times t \quad (1)$$

$$\ln[(R)] = \ln[(R)]_{t=0} - k_{(R)} \times t \quad (2)$$

where [(*S*)] is the concentration for (*S*)-(+)-enantiomer at time t and [(*R*)] is the concentration for (*R*)-(−)-enantiomer at time t.

The ES of dissipation was defined as

$$\mathrm{ES} = \frac{k_{(S)} - k_{(R)}}{k_{(S)} + k_{(R)}} \quad (3)$$

ES is a measure of ES. It reflects the overall trend in enantioselective dissipation. Positive values (0 < ES ≤ 1) indicate a more rapid dissipation of (*S*)-enantiomer, while negative values (−1 < ES ≤ 0) indicate a more rapid dissipation of (*R*)-enantiomer. At an ES value of 0, dissipation is not enantioselective, and at an ES value of 1, dissipation is fully enantioselective.

Table 2. First-Order Rate Constants *k*, Half-Life ($t_{1/2}$), Correlation Coefficient (R^2), and the ES Values of Lactofen in Soils under Aerobic Conditions

soil	enantiomer	k (h^{-1})	$t_{1/2}$ (h)	R^2	ES
1	(*S*)-(+)-lactofen	0.0587	11.8 ± 0.6	0.94	0.093
	(*R*)-(−)-lactofen	0.0487	14.2 ± 0.8	0.96	
2	(*S*)-(+)-lactofen	0.0433	16.0 ± 0.9	0.91	0.162
	(*R*)-(−)-lactofen	0.0312	22.0 ± 0.5	0.91	
3	(*S*)-(+)-lactofen	0.0606	11.4 ± 0.4	0.95	0.192
	(*R*)-(−)-lactofen	0.0411	16.7 ± 0.2	0.97	
4	(*S*)-(+)-lactofen	0.0450	15.4 ± 0.7	0.94	0.186
	(*R*)-(−)-lactofen	0.0309	22.4 ± 0.5	0.96	
5	(*S*)-(+)-lactofen	0.0823	8.4 ± 0.1	0.95	0.217
	(*R*)-(−)-lactofen	0.0530	13.1 ± 0.7	0.96	
6	(*S*)-(+)-lactofen	0.0364	19.0 ± 0.1	0.97	0.230
	(*R*)-(−)-lactofen	0.0228	30.4 ± 0.7	0.98	
7	(*S*)-(+)-lactofen	0.0349	20.0 ± 1.4	0.98	0.432
	(*R*)-(−)-lactofen	0.0138	50.2 ± 0.8	0.93	
8	(*S*)-(+)-lactofen	0.0648	10.7 ± 0.9	0.95	0.363
	(*R*)-(−)-lactofen	0.0303	22.9 ± 0.9	0.91	

RESULTS AND DISCUSSION

Enantioselective Dissipation under Aerobic Conditions. Lactofen transformation in environmental samples was investigated using soils collected from eight geographically distinct locations representing different physicochemical characteristics (**Table 1**). Differences in soil pH, percentage of organic carbon, and soil texture were apparent.

In general, the residues of both enantiomers of lactofen in the eight soils decreased with the time elapsed (**Table 2**). A typical chromatogram and kinetic data from incubation of lactofen in soil 8 and soil 1 show the concentration of (*S*)- and (*R*)-lactofen decreased according to apparent first-order kinetics (**Figure 3a**−**d**). In these soils, the (*S*)-enantiomer was preferentially degraded over the (*R*)-enantiomer, thus showing positive ESs (ES = 0.093 and 0.363, **Table 2**) and enrichment of the (*R*)-enantiomer. In silt loam soil (soils 3 and 5−8), the selective dissipation resulted in relative enrichment of the (*R*)-enantiomer, as shown in **Figure 3a** for soil 8. In sandy loam soil (soils 1, 2, and 4), the ES value was smaller than that in silt loam soil. Especially in soil 1, the two enantiomers degraded at similar rates, and residues were close to racemic (**Figure 3c**). From these results, it can be concluded that the rate of ES in the dissipation of lactofen was closely dependent on the soil type.

Microbial decomposition plays a critical role in stereoselective metabolism of many chiral chemicals in soils (*27*−*30*). Thus, additional chromatographic analyses were performed for an autoclaved aerobic soil sample (soil 2) to determine if the dissipation of lactofen was microbial. In these microbially inhibited microcosms (autoclaved), lactofen loss was minimal (<5%) during 7 days of incubation. On the contrary, in the nonautoclaved sample, lactofen was rapidly degraded about 50% in 3 days (**Figure 4b**). Apparently, any abiotic hydrolysis was much slower than microbial hydrolysis in this soil. Our conclusion is consistent with previous studies, in which lactofen was rapidly degraded, mainly by microbial activity rather than hydrolysis (*31*). For the autoclaved samples, the initial EF was 0.49, the same as for the lactofen standard, and the same autoclaved sample had an EF of

Table 3. First-Order Rate Constants *k*, Half-Life ($t_{1/2}$), Correlation Coefficient (R^2), and the ES Values of Lactofen in Soils under Anaerobic Conditions

soil	enantiomer	k (h^{-1})	$t_{1/2}$ (h)	R^2	ES
2	(*S*)-(+)-lactofen	0.0392	17.8 ± 0.8	0.98	0.156
	(*R*)-(−)-lactofen	0.0286	24.2 ± 0.3	0.95	
4	(*S*)-(+)-lactofen	0.0321	21.6 ± 0.6	0.99	0.191
	(*R*)-(−)-lactofen	0.0218	31.8 ± 1.0	0.97	
5	(*S*)-(+)-lactofen	0.0399	17.4 ± 0.9	0.99	0.184
	(*R*)-(−)-lactofen	0.0275	25.2 ± 0.9	0.96	
8	(*S*)-(+)-lactofen	0.0469	14.8 ± 0.1	0.93	0.419
	(*R*)-(−)-lactofen	0.0192	36.1 ± 1.0	0.93	

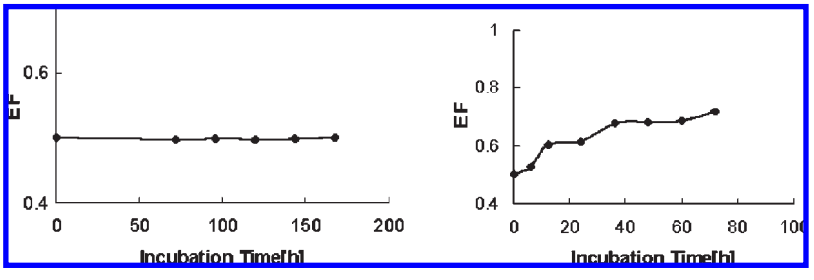

Figure 4. Enantiomeric fraction (EF) of lactofen residues (**a**) in the autoclaved aerobic soil 2 and (**b**) in the nonautoclaved aerobic soil 2.

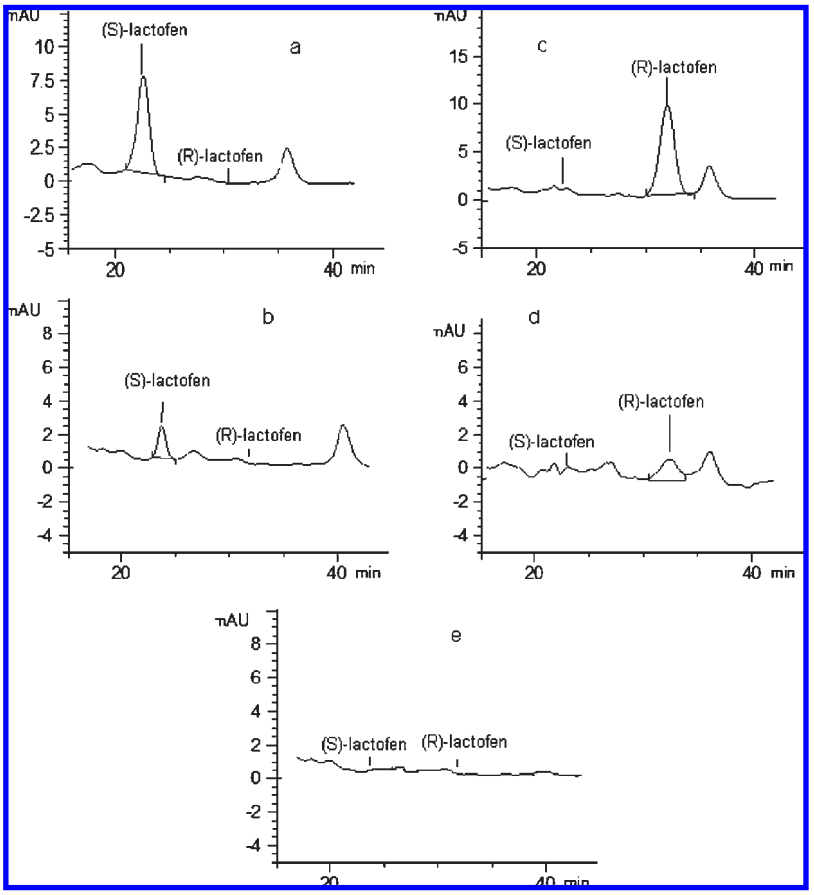

Figure 5. Chromatograms showing elution of lactofen from the incubation of (*S*)-lactofen in aerobic soil 2 after (**a**) 0 and (**b**) 72 h and from the incubation of (*R*)-lactofen in aerobic soil 2 after (**c**) 0 and (**d**) 72 h. (**e**) Chromatogram of blank soil 2. Note the absence of enantiomerization with respect to (*S*)- and (*R*)-lactofen (*n*-hexane:2-propanol = 99:1; flow rate, 1.0 mL/min).

0.49 when analyzed at day 7 (**Figure 4a**). In contrast, for the nonautoclaved aerobic sample, the EF values for lactofen decreased with time elapsed (**Figure 4b**). These results show that the transformation of lactofen was enantioselective, and the dissipation of lactofen was biologically mediated.

Enantioselective Dissipation under Anaerobic Conditions. The dissipation of lactofen was investigated in four soils under anaerobic conditions (**Table 3**). Dissipation of lactofen was enantioselective in the anaerobic soils, the same as that in the aerobic soils. **Table 3** shows the half-lives ($t_{1/2}$), the rate constant values, and ES value of each pair of enantiomers in those four soils under anaerobic conditions. The ES values were positive, as they were in the aerobic soils, and ranged from ES = 0.156 to 0.419 (**Table 3**).

In some other works, the direction of the ES varied with incubation conditions. For example, metalaxyl was usually found to be enriched in the (*S*)-enantiomer; however, the ES reversed in anaerobic dissipation in sewage sludge, resulting in residues enriched in the (*R*)-enantiomer (*32*). In another example, *cis*-permethrin showed significant ES in San Diego Creek sediment under anaerobic conditions, but the direction of selectivity was reversed in aerobic soils, with (*S*)-*cis*-permethrin degrading more slowly than (*R*)-*cis*-permethrin (*33*). The two examples indicate that redox conditions affect the biotransformation of some chiral compounds, as different bacterial consortia are active under aerobic and anaerobic conditions (*34*). However, the data from our study showed that under both aerobic and anaerobic conditions, enantioselective dissipation occurred for lactofen and the direction of ES did not shift; in other words, anaerobic conditions did not significantly alter the enantiomeric preference of the microbial community.

Chiral Stability of Lactofen in Soil. Enantiomerization, that is, the transformation of (*S*)-enantiomer to (*R*)-enantiomer or vice versa, was studied by separate incubations of the two enantiomers under aerobic conditions. No indication was found for enantiomerization (**Figure 5a**−**d**). The chromatograms in panels **a** and **c** show the high enantiomeric purity of (*S*)- and (*R*)-lactofen in soil 2 prior to incubation; the chromatograms in panels **b** and **d** indicate dissipation of (*S*)-(+)- and (*R*)-(−)-lactofen after 72 h of incubation with no appearance of the other enantiomer. We also incubated anaerobic soils with the single pure enantiomers of lactofen. During such incubations, we still could not detect the (*S*)-(+)-enantiomer when (*R*)-(−)-enantiomer was the substrate or the (*R*)-(−)-enantiomer when (*S*)-(+)-enamtiomer was the substrate. On the basis of these results, it can be concluded that enantiomerization did not occur in the enantioselective dissipation of lactofen under either aerobic or anaerobic conditions.

ES of Lactofen Dissipation Correlates with Soil Properties. As described above, a preferential dissipation of (*S*)-(+)-enantiomer occurred in eight aerobic soils. Thus, the ES values of all soils are positive (**Table 2**). The highest ES was observed in soils 8 and 7, the two soils with the lowest pH (pH 5.0 and 5.1, ES = 0.363 and 0.432), and the lowest ES was found in soil 1 with the highest pH (pH 8.3, ES = 0.093). This revealed a clear correlation between aerobic soil pH and ES of dissipation. Interestingly, this phenomenon has also appeared in the anaerobic controls. In four anaerobic soils, the highest ES was detected in soil 8 with the lowest pH (pH 5.0, ES = 0.419), and the lowest ES was

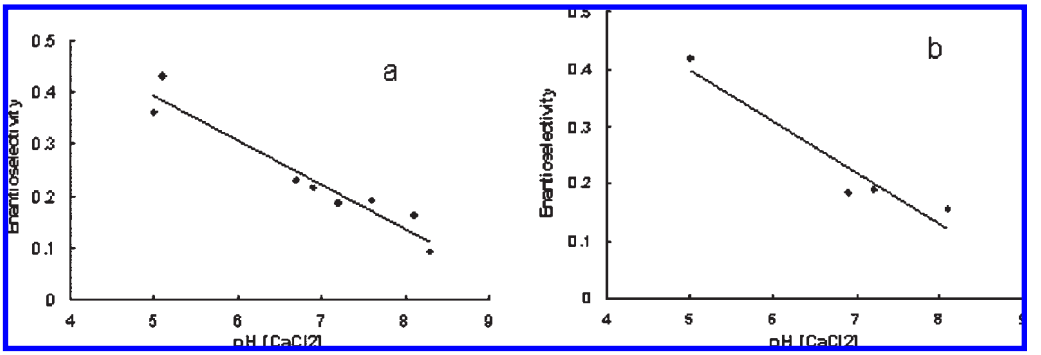

Figure 6. pH-dependent enantioselective degradation of lactofen in (**a**) eight aerobic soils (soils 1−8) and (**b**) four anaerobic soils (soils 2, 4, 5, and 8).

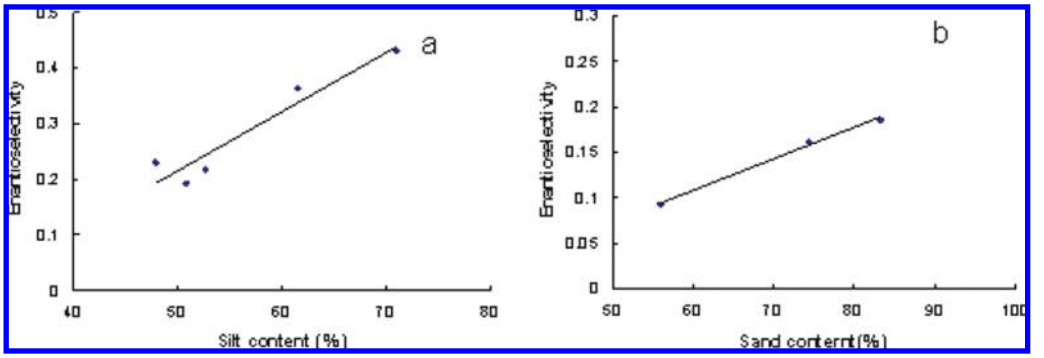

Figure 7. Linear correlation between ES and (**a**) silt content for silt loam aerobic soils (soils 3, 5, and 8) and (**b**) sand content for sandy loam aerobic soils (soils 1, 2, and 4).

discovered in soil 2 with the highest pH (pH 8.3, ES = 0.156, **Table 3**).

The increase of ES values with decreasing pH was fitted with a linear correlation, both in aerobic soils and in anaerobic soils ($r^2 = 0.93$, $p = 0.0011$ and $r^2 = 0.92$, $p = 0.043$, respectively, **Figure 6a**,**b**). Buerge et al. reported that the ES for *cis*-epoxiconazole correlated with the soil pH (*20*). They found that with decreasing pH, the ES of *cis*-epoxiconazole dissipation changed toward more positive ES values, but cyproconazole did not show a clear correlation between soil pH and ES of dissipation. In this paper, the authors summarized correlations between pH and ES found for different chiral compounds, including the metalaxyl, metalaxyl-acid (primary metabolite of metalaxy), dichlorprop, mecoprop, and ethofumesate besides the *cis*-epoxiconazole and cyproconazole. Finally, they suggested that metalaxyl, *cis*-epoxiconazole, dichloprop, and mecoprop showed a clear correlation between soil pH and ES, whereas for the two diastereomers of cyproconazole, metalaxyl-acid and ethofumesate, no statistically significant correlation was observed between soil pH and ES (*18*, *20*, *35*−*38*). From these results, it can be seen that different microorganisms and enzymes are probably involved in the primary dissipation of these compounds, but the mechanism by which pH exerts an effect on ES remains unclear. ES may be dependent on the level of microbial populations or on the level of enzymes responsible for uptake or actual dissipation, etc.

Soil texture and organic matter appear to impact microbial growth, and the enantioselective dissipation of lactofen may be influenced by these parameters. In our studies, we found that with decreasing silt content and increasing sand, the ES of silt loam aerobic soils changed toward less positive ES values. However, for sandy loam aerobic soils, the ES changed toward more positive ES values with decreasing silt content. This suggested a dependence of the ES on the silt and sand content of the soil. When ES was plotted vs silt content and sand content, respectively, a linear correlation was observed for silt loam soils and sandy loam soils ($r^2 = 0.914$, $p = 0.011$ and $r^2 = 0.995$, $p = 0.046$), respectively (**Figure 7a**,**b**). It can be assumed that dissipation of lactofen was enantioselective and was dependent on the soil texture.

In contrast to the correlation between the ES values and the silt content of soil, the ES of lactofen did not show any correlation with the organic carbon content of the soils. This result was similar to previous observations made for other chiral compounds. For example, the ES of metalaxyl did not correlate with organic carbon (*18*), and the ES of epoxiconazole showed a weak (co)-correlation with the organic carbon content of the soils (*20*).

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