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Soil Microbial Effects on the Stereoselective Mineralization, Extractable Residue, Bound Residue, and Metabolism of a Novel Chiral Cis Neonicotinoid, Paichongding

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ABSTRACT: Fate characteristics of the four stereoisomers of paichongding [IPP, 1-((6-chloropyridin-3-yl)methyl)-7-methyl-8-nitro-5-propoxy-1,2,3,5,6,7-hexahydroimidazo[1,2-*a*]pyridine] in aerobic sterilized and nonsterilized fluvio-marine yellow loamy soil were investigated using a ^{14}C tracer technique combined with HPLC and LC-MS/MS. Results showed that the mineralization and bound residue (nonsterile/sterilized soil, % of applied amount) of four stereoisomers of IPP were 1.76−6.10/0.33−0.82 and 12.01−31.20/6.58−20.81 at 100 days after treatment. Seven and five incomplete intermediates of IPP were detected in nonsterilized and sterilized soil, respectively, and a possible degradation pathway was proposed. Degradation mainly occurred on the tetrahydropyridine ring, including oxidation and elimination of the methyl, propyl, and nitro groups. All of these results suggest that soil microbial activity greatly contributes to the epimeride-selective mineralization, formation of bound residue, and degradation of IPP in loamy soil. The identified transformation intermediates could be used for further study on their toxicity to target and nontarget species.

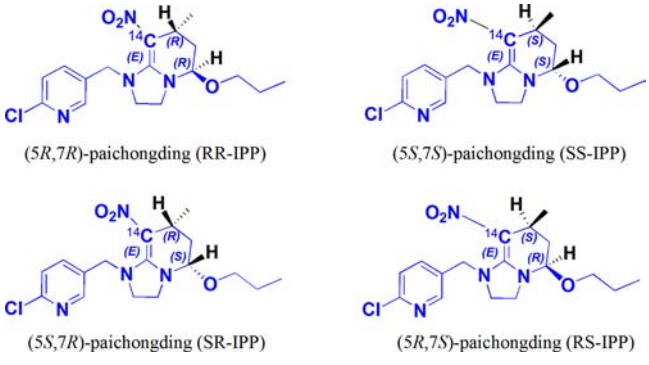
KEYWORDS: *soil microorganism, pesticide, paichongding, stereoselective degradation, mineralization, bound residue*

INTRODUCTION

cis-Nitromethylene neonicotinoids, a new nicotinic family with several chiral members, have been growing rapidly in recent years.[1] However, most of these compounds, as well as approximately 24% of the total chiral pesticides, are sold as racemic products.[2] The introduction of raceme into soil via application of racemic/mixture products may result in the release of inactive isomers into the environment.[3] Furthermore, once in soil, one or more parts of the stereoisomers may be more persistent than other(s) due to the enantioselective transformation influenced by the physicochemical properties of both the pesticides and soil.[3−7] In general, soil microbial effects are considered to be key elements of pesticide enantioselective transformation,[8] which directly affect the selective bioavailability[9] and hence potential for preferable secondary shifts of an isomer from soil, such as leaching to surface water and groundwater and uptake into plants. Moreover, the incomplete selective degradation of chiral neonicotinoids may produce specific intermediates with changed bioactivity and transferability, leading to unexpected adverse environmental effects. However, little is currently known about the enantioselective transformation of cis neonicotinoids.

Paichongding [IPP, 1-((6-chloropyridin-3-yl)methyl)-7-methyl-8-nitro-5-propoxy-1,2,3,5,6,7-hexahydroimidazo[1,2-*a*]-pyridine] is a novel nitromethylene neonicotinoid with a tetrahydropyridine fixed cis configuration and exhibits excellent toxicity toward insects of a broad spectrum.[1,10] In addition, the insecticidal activity of IPP against the imidacloprid-resistant brown planthopper is much higher than that of imidacloprid.[11] Moreover, the yield of IPP will greatly increase to approximately 1000 tones each year (3.3 million hectare equivalent) in China. IPP has two chiral centers, resulting in four stereoisomers (two pairs of enantiomers) known as 5*R*,7*R*-paichongding (RR-IPP), 5*S*,7*S*-paichongding (SS-IPP), 5*S*,7*R*-paichongding (SR-IPP), and 5*R*,7*S*-paichongding (RS-IPP) (Scheme 1). Previous studies of IPP were based on the raceme.[11,12] However, the fate characteristics of IPP, how it is transformed in soils, and whether microbial transformation

Scheme 1. Stereostructures of Four Isomers of Paichongding (IPP)



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leading to enantioselective degradation of incomplete and potential active products occurs are still not known.

In this study, liquid chromatography−tandem mass spectrometry (LC-MS/MS) coupled with ^{14}C labeling was used to track the transformation dynamics and identify the degradation intermediates of single stereoisomer products of IPP in loamy soil. Additionally, the ^{14}C isotope tracer technique was used to investigate the mineralization, bound residue formation, and extractable residue in sterilized and nonsterilized soils under aerobic conditions.

MATERIALS AND METHODS

Pesticide and Chemicals. ^{14}C-Labeled paichongding (^{14}C-IPP), 1-((6-chloropyridin-3-yl)methyl)-7-methyl-[^{14}C]8-nitro-5-propoxy-1,2,3,5,6,7-hexahydroimidazo[1,2-*a*]pyridine, was synthesized and separated by the Institute of Nuclear and Agricultural Sciences, Zhejiang University, China.[13] Four nonlabeled separated stereoisomers of paichongding were obtained from the School of Pharmacy, East China University of Science and Technology, Shanghai, China. Both the chemical purity and radiochemical purity were >98%. Four separated stereoisomers of ^{14}C-IPP were obtained from Daicel Chiral Technologies Co., Ltd., Shanghai, China. 2,5-Diphenyloxazole (PPO, Acros Organics, Geel, Belgium) and 1,4-bis(5-phenyloxazol-2-yl)-benzene (POPOP, Acros Organics) were of scintillation grade and used to prepare scintillation cocktails I and II. All reagents used for HPLC analysis, including acetonitrile and glacial acetic acid, were of chromatography grade. Ultrapure water was prepared using the Milli-Q water purification system (Milli-Q Advantage A10, Molsheim, France). Other organic and chemical solvents were of analytical grade.

Ingredients of scintillation cocktail I were as follows: 0.5 g of POPOP, 7.0 g of PPO, 350 mL of glycol ether, and 650 mL of dimethylbenzene. Cocktail II consists of 0.5 g of POPOP, 7.0 g of PPO, 175 mL of ethanolamine, 275 mL of glycol ether, and 550 mL of dimethylbenzene.

Soil Preparation. The fluvio-marine yellow loamy soil (loamy soil) without previous application of IPP was collected from the surface layer (0−15 cm) of the experimental field in Hangzhou, Zhejiang Province, China. The soils were dried, ground, and passed through a 1 mm sieve. The physicochemical characteristics of the soils are shown in Table 1.

Table 1. Chemical and Physical Properties of Fluvio-Marine Yellow Loamy Soil

property	loamy soil
soil type	fluvio-marine yellow loamy soil
location	Hangzhou, Zhejiang
pH (H_2O)	7.02
OM[a] (g kg^{-1})	30.5
CEC[b] (cmol kg^{-1})	10.83
clay (%)	8.0
silt (%)	71
sand (%)	21

[a]OM, organic matter. [b]CEC, cation exchange capacity.

Sterile fluvio-marine yellow loamy soil (sterile loamy soil) was selected to investigate the effects of soil microorganisms. Soils were sterilized by being subjected to γ rays generated by cobalt-60 at 2 kGy/h for 12 h. After sterilization, 5 mL of 0.01 mol/L NaN_3 was added to maintain sterility. The soils were classified as sterilized and nonsterilized, respectively.

Experimental Setup. The experimental apparatus was modified on the basis of a previous study.[14] Briefly, a 500 mL Erlenmeyer flask was used to incubate the soil samples. Upstream of the soil sample flask, one conical flask filled with distilled water (30 mL) was employed to purge air, whereas two conical flasks filled with 5 M NaOH (30 mL) were used to elute CO_2. In the downstream soil sample flask, one conical flask filled with ethylene glycol (30 mL) and another flask filled with 1.0 M H_2SO_4 (30 mL) were used to trap volatile organic compounds and alkaline volatile products. This was followed by two flasks filled with 0.5 M NaOH (30 mL) to absorb mineralized $^{14}CO_2$ from the soil samples. All incubation flasks were covered with a two-way plug and connected with an airtight tube.

Treatments. All soils were amended with distilled water to adjust the soil moisture content to 30% of the soil water-holding capacity (WHC), after which they were preincubated for about 15 days to revive the microbial activity. First, IPP was dissolved into ddH_2O (0.2% acetonitrile), and then an aliquot of 60 mL of solution (RR-^{14}C-IPP, SS-^{14}C-IPP, SR-^{14}C-IPP, and RS-^{14}C-IPP) was added to approximately 900 g of soil (dry weight equivalent) to give an initial concentration of 8.56 mg kg^{-1} and radioactive concentrations of 1.62×10^6, 1.53×10^6, 1.44×10^6, and 1.63×10^6 Bq/kg, respectively. The ^{14}C-applied soils were mixed thoroughly by subsequently adding distilled water to maintain the soil moisture content at 60% of WHC. After thorough mixing, an aliquot of 1.0 g of soil sample (dry weight equivalent) was combusted using a biological oxidizer (OX-400, R. J. Harvey Instrument, Hillsdale, NJ, USA) at the optimal condition (catalyst zone, 680 °C; combustion zone, 900 °C; combustion time, 4 min), and the radioactivity was used to calculate the data at 0 days. The remaining soil was distributed in triplicate (300 g × 3). Triplicate soil subsamples (1.0 g × 3) were removed from the treated soils, and the radioactivity was measured using a biological oxidizer (OX-400) (recovery rate = 96.0 ± 3.5%, n = 3) to demonstrate the uniform distribution of ^{14}C-IPP. The treated soils were put into a fume cupboard to volatilize the acetonitrile and then immediately moved into 500 mL Erlenmeyer flasks. The experimental system with the treated soils was incubated at 25 ± 1 °C in a cultivation cabinet in the dark. A continuous and low air flow was applied throughout the incubation period via an air pump. To maintain the soil moisture constant at 60% WHC, distilled water was added to compensate for loss by evaporation as required on the basis of the weight of the Erlenmeyer flasks containing soil samples.

Sampling and Measurement of Mineralization, Bound Residue (BR), and Extractable Residue (ER). At 5, 10, 20, 30, 45, 60, 75, and 100 days after treatment (DAT), sampling was performed in triplicate. During sampling, the two NaOH solutions in the downstream flasks were merged and homogenized. A 1 mL aliquot of this homogeneous solution was then dissolved into 10 mL of scintillation cocktail I and kept in the dark for about 24 h to remove chemiluminescence, after which it was subjected to radioassay using an ultralow-level liquid scintillation counter (Quatalus-1220, Perkin-Elmer Co., Turku, Finland) to quantify the amount of mineralized $^{14}CO_2$.

Triplicate soil subsamples (3 × 10.0 g, dry weight equivalent) were collected from the 500 mL Erlenmeyer flasks into 100 mL polypropylene centrifuge tubes, after which the soil subsamples were continuously extracted and shaken for 2 h twice using the method described by Wang et al.[14] Briefly, the samples were extracted using 50 mL of 0.01 M $CaCl_2$, acetonitrile/water (9:1, v/v), methanol, and dichloromethane. At the end of each extraction step, a 1.0 mL aliquot of supernatant was separately dissolved into 10 mL of scintillation cocktail I and the radioactivity (^{14}C-dpm) was measured using a liquid scintillation counter (LSC) (Wallac WinSpectral-1414, PerkinElmer). The ER was defined as the sum of radioactivity from four phases ($CaCl_2$, acetonitrile/water, methanol, and dichloromethane).

After extraction, the soils were air-dried and ground into powder. Next, 1.0 g of soil powder was combusted in a Biological Oxidizer OX-400 (R. J. Harvey Instrument) under optimum conditions (catalyst zone, 680 °C; combustion zone, 900 °C; combustion time, 4 min). The released $^{14}CO_2$ was then absorbed using 15 mL of scintillation cocktail II and radioassayed (^{14}C-dpm) by LSC to determine the amount of BR. The ^{14}C recoveries of combustion were found to be >95%.

Pretreatment, Radioassay, and HPLC-ESI-MS^n. After extraction, the 0.01 M $CaCl_2$ extraction phase was adjusted to pH 3.0 and then extracted with an equivalent volume of acetonitrile/dichloromethane (1:1, v/v) at least three times until no further radioactivity

was observed in the $CaCl_2$ phase. The acetonitrile/dichloromethane (1:1, v/v) phase was merged with the other three extraction steps (acetonitrile/water, methanol, and dichloromethane), after which the sample was filtered through a 0.45 μm filter (Millipore, Ireland) and concentrated to about 5 mL under vacuum using a rotary evaporator (Eyela SB-1000, Eyela Co., Tokyo, Japan) at 40 ± 1 °C. The concentrated mixture of extractions was then dried with nitrogen and further dissolved with an aliquot of 1 mL of chromatography grade acetonitrile, followed by centrifugation at 14000 rpm for 10 min. Finally, the supernatant was passed through a 0.22 μm organic filter for HPLC analysis. A 20 μL aliquot of treated supernatant was then analyzed by a Waters HPLC system connected using a Waters 2998 photodiode array (PDA) detector (Waters Co., USA) and a Diamonsil C_{18} column (5 μm, 250 × 4.6 mm, Dikma Technologies Inc., Lake Forest, CA, USA) with a C_{18} precolumn (5 μm, 30 × 4.6 mm, Dikma Technologies Inc.). The measurement wavelength was set at 268 nm, and the column temperature was kept at 30 ± 1 °C. The mobile phase was composed of A (ddH_2O + 0.1% ethylic acid) and B (acetonitrile + 0.1% ethylic acid). The elution was performed by a gradient procedure (A%/min: 85:0, 85:7, 65:20, 0:40, 0:45, 85:50, 85:65) at a flow rate of 1.0 mL/min. The fractions of the elution were collected into scintillation vials and dissolved in 10 mL of scintillation cocktail I to determine the ^{14}C radioactivity of IPP parent and/or IPP degradations by LSC.

LC-MS/MS analysis was conducted using a Waters 2695 HPLC system coupled with a Bruker Esquire 3000^{plus} ion trap mass spectrometer equipped with an electrospray ionization source (ESI). The separate conditions agreed with those used for HPLC analysis. Esquire 5.0 software was used to control the instrument and acquire the MS data. The ion source temperature was set at 250 °C, and the capillary voltage was −4 kV. During analysis, pure nitrogen was applied for drying and nebulization at a flow of 10 L/min and a gas pressure of 30 psi, and continuous full scanning from m/z 50 to 1000 was performed in positive ion mode. The collision energy for MS^n data was set at 1.0 V, and the collision gas was high-purity helium. Identification of the parent compound IPP and its degradation products was carried out by HPLC-LSC, cochromatography with standards, and LC-MS/MS.

Statistical Analysis. Data are presented as the means and standard errors (means ± SEM) obtained from triplicate analyses. SPSS 19.0 (IBM SPSS Statistics, USA) was used to determine the significance based on one-way ANOVA at $\alpha = 0.05$. Origin 8.0 (Microcal Software, Northampton, MA, USA) was used to plot and edit the figures.

RESULTS AND DISCUSSION

Mineralization and Formation of BR. Mineralization, BR, and ER were summed to calculate the mass recovery. Good mass balance (89.11−105.00% of initial applied radioactivity) was consistently obtained throughout the cultivation experiment.

Significant differences in the mineralization of ^{14}C-IPP between sterilized and nonsterilized loamy soil were found, indicating the involvement of microbial action in the mineralization of IPP in loamy soil. In addition, the apparent differences between epimers of IPP almost completely vanished in sterilized loamy soil when compared with the obvious diastereoselectivity in nonsterilized loamy soil (Figure 1), indicating that microorganisms played a key role in the epimer-selective mineralization of IPP in loamy soil.

For the four isomers of ^{14}C-IPP (RR-IPP, SS-IPP, SR-IPP, and RS-IPP), 1.76, 1.96, 6.10, and 5.82% mineralization rates in the nonsterilized loamy soil were observed from the evolution of $^{14}CO_2$, respectively, whereas only 0.33, 0.35, 0.82, and 0.76%, respectively, were measured in the sterilized soil at 60 days after treatment (Figure 1). These findings implied that the fate of IPP in loamy soil was critically affected by microbial degradation. A similar observation for the herbicide 2,4-D

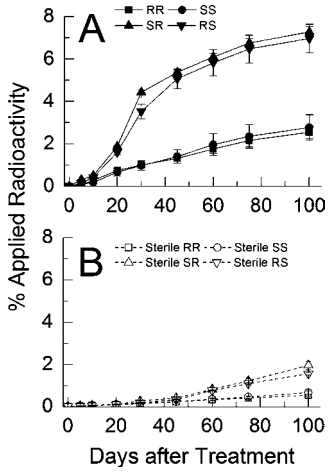

Figure 1. Mineralization of RR-IPP, SS-IPP, SR-IPP, and RS-IPP in (A) nonsterilized loamy soil and (B) sterilized loamy soil. All data represent the average of triplicates ± standard deviation (mean ± SD), $n = 3$.

and the insecticide lindane was reported by several researchers.[15−17] No significant differences ($p > 0.05$) were observed between the enantiomers of IPP (RR-IPP and SS-IPP, SR-IPP and RS-IPP) in both sterilized and nonsterilized soils, indicating that there was no enantioselectivity for the enantiomers of IPP. However, the mineralization of epimers RR-IPP and SR-IPP in nonsterilized loamy soil differed ($p < 0.05$) and reached maximum values of 2.55 and 7.28% at 100 DAT, whereas the corresponding amounts at 100 DAT in sterilized soil were 0.54 and 1.96%, respectively (Figure 2). The

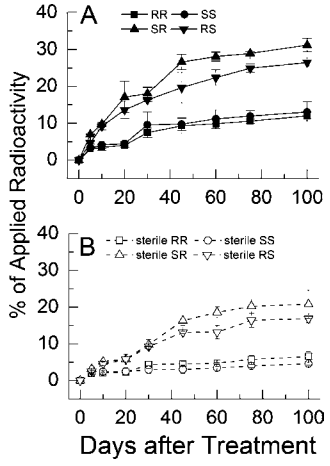

Figure 2. Bound residues of RR-IPP, SS-IPP, SR-IPP, and RS-IPP in (A) nonsterilized loamy soil and (B) sterilized loamy soil. All data represent the average of triplicates ± standard deviation (mean ± SD), $n = 3$.

discrepancy of SR-IPP in sterilized and nonsterilized soil was much higher than that of RR-IPP, indicating that enantiomers SR-IPP and RS-IPP were more available to soil microflora than their epimers RR-IPP and SS-IPP. Moreover, after the soil was sterilized, the gap of mineralization between RR-IPP and SR-IPP narrowed substantially, suggesting that microbes greatly contribute to the epimer-selective mineralization of IPP in loamy soil.

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A lag phase (10 days) was observed during the 100 day incubation of IPP in loamy soil, providing further evidence of the adaptation of soil microbes to the pesticide. These findings are in accordance with the farm field in Hangzhou from which the soil was obtained having no history of IPP treatment.

Similar to mineralization, significant differences ($p < 0.05$) in BR of IPP between the sterilized and nonsterilized loamy soil (Figure 2) were observed, clearly indicating that microbial action greatly affects the formation of BR in loamy soil. Despite gap reduction after sterilization, a small gap was found between the epimers in the sterilized soil, implying that microorganisms were not the only factor involving the selective formation of BR. Original study showed that SR-IPP and RS-IPP were more easily degraded than RR-IPP and SS-IPP. In addition, soil particles and organic materials such as humus and minerals can also selectively bind with IPP. As mineralization and the formation of BR increased, the availability of IPP and/or its degradation intermediates decreased, resulting in a corresponding reduction of ER in both sterilized and nonsterilized loamy soils. Obvious differences ($p < 0.05$) were observed in the ER between the epimers in both sterilized and nonsterilized soils. Moreover, the gap was reduced after the soil was sterilized, clearly indicating that a microbial effect plays a key role in epimer selectivity to IPP and that the differences between epimers may be caused by the generation of specific degradation intermediates.

Parent Degradation. A rapid degradation of SR-IPP and RS-IPP was observed in both sterilized and nonsterilized loamy soils (Figure 3). However, the degradation rate of RR-IPP and SS-IPP in nonsterilized soil was much higher than that in sterilized soil (Figure 3). For example, only 41.85% of the parent compound SS-IPP remained in nonsterilized loamy soil at 20 days after treatment, whereas the corresponding value was 76.64% in sterilized soil, which was much higher than that in nonsterilized soil. These findings indicate that microbial effects make a significant contribution to the degradation of the parent compound, IPP. However, no significant differences in the degradation of enantiomers SR-IPP and RS-IPP were observed between the sterilized and nonsterilized loamy soils, suggesting that parent compounds of enantiomers SR-IPP and RS-IPP were rarely utilized by soil microorganisms. Although the reasons for this are still unclear, the parent residue of enantiomers RR-IPP and SS-IPP was much higher than that of SR-IPP and RS-IPP in both sterilized and nonsterilized soils. The epimer selectivity of IPP may be ascribed to the spatial conformation of IPP stereoisomers, which could lead to the selective chemical degradation involving the organic matter in soil. The combination of RS-IPP and SR-IPP was significantly higher than that of RR-IPP and SS-IPP on the fulvic acid in loamy soil, implying that the combined effect of soil microorganisms and organic matter contributes to the epimer-selective degradation of IPP. These results agreed with previous studies that pesticides could be bound in soil through interaction with soil organic materials such as humic or fulvic acids.[18,19] Microbes were involved in the decrease of parent compounds of RR-IPP and SS-IPP, but not SR-IPP and RS-IPP. This discrepancy was likely due to the selective metabolism of intermediates of IPP. With the 5-day rapid degradation of SR-IPP and RS-IPP, the mineralization increased quickly (Figures 1A and 3A) in nonsterilized soil, whereas it remained at low levels in sterilized soil. These findings confirmed the involvement of microbial action in the mineralization of IPP degradation products.

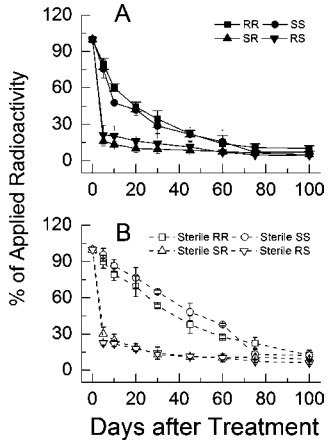
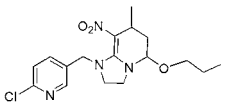
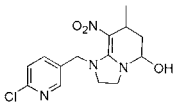
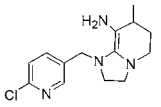
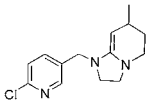
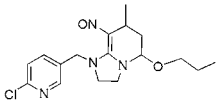
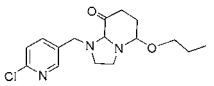
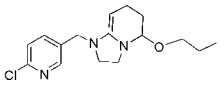
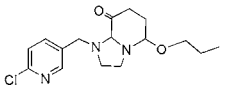


Figure 3. Degradation dynamics of parent compound of RR-IPP, SS-IPP, SR-IPP, and RS-IPP in (A) nonsterilized loamy soil and (B) sterilized loamy soil. All data represent the average of triplicates ± standard deviation (mean ± SD), $n = 3$.

Identification of Degradation Intermediates. After analysis of the radioactivity of the elution separated by HPLC, five and seven radioactive intermediates were identified in both sterilized and nonsterilized loamy soils. LC-MS in ESI positive mode and LC-MS/MS were carried out to further identify the structure of the radioactive intermediates. Tentative structural identification of all intermediates of IPP was conducted by examining the protonated ion $[M + H]^+$ and the fragments pattern and by comparison against authentic standards whenever possible. The elution time, calculated mass, protonated ions, daughter ions, empirical formula, and structure are shown in Table 2. IPP and M1 were confirmed directly by analyzing nonlabeled standards under the same conditions. The retention time of radioactive elution agreed with the standards. Both the calculated mass and fragment pattern were used in the identification. M1 was tentatively identified as 1-((6-chloropyridin-3-yl)methyl)-7-methyl-8-nitro-5-hydroxy-1,2,3,5,6,7-hexahydroimidazo[1,2-*a*]pyridine. Standards were not available for other intermediates; therefore, their structures were deduced by analyzing the fragment patterns. On the basis of this technique, M2 was positively identified as an amino derivative of M1. The protonated molecular ion clearly showed there was an even number of nitrogen atoms of M2 and a mass unit loss of 46. These indicated that the three oxygen atoms were lost from M1 to form amino product M2, 1-(6-chloropyridin-3-ylmethyl)-7-methyl-8-amino-1,2,3,5,6,7-hexahydroimidazo[1,2-*a*]pyridine, which can only accord with the nitrogen rule. The molecular ion of M3 was the daughter ion observed in IPP, M1, and M2 (Table 2), and the mass loss of 15 from M2 likely corresponded to NH, implying that M3, 1-(6-chloropyridin-3-ylmethyl)-7-methyl-1,2,3,5,6,7-hexahydroimidazo[1,2-*a*]pyridine, was the deamination product of M2. The molecular mass of M4 was the daughter ion of IPP, and the loss of 16 mass units was likely a result of nitrosylation of the nitro on IPP. The calculated mass of M4 was also consistent with the photodegradation product nitroso-IPP as reported by Zhao et al.[12] Hence, M4 was tentatively identified as 1-(6-chloropyridin-3-ylmethyl)-7-methyl-8-nitroso-5-propoxy-1,2,3,5,6,7-hexahydroimidazo[1,2-*a*]pyridine. The protonated ion of metabolite M5 suggests that there were odd nitrogen atoms on M5, and the loss of 13 mass units likely

Table 2. Retention Time and Mass Spectrum of Paichongding (IPP) and Its Degradation Intermediates

metabolite	t_R(min)	mass	proposed empirical formulas	proposed structure	daughter ions (relative abundance)
IPP	22.6	366.15	$C_{17}H_{23}ClN_4O_3$		367.2 ([M+H],100), 369.2 (30), 389.1 (M+Na, 25), 350.1(11), 321.1(100), 263.0(93), 281.1(11), 136.9(7)
M1	4.5	324.10	$C_{14}H_{17}ClN_4O_3$		325.4 ([M+H],100), 327.8 (28), 279.1 (100), 195.7 (44), 264.1 (55), 246.1(34), 153.2(25), 295.1 (7), 126.3(10)
M2	6.4	278.13	$C_{14}H_{19}ClN_4$		279.2 ([M+H], 100), 281.2 (29), 264.2 (100), 236.2(13), 208.1 (41), 153.2 (29), 193.1 (29), 126.1 (9)
M3	8.7	263.09	$C_{14}H_{18}ClN_3$		264.1 ([M+H], 100), 266.2 (32), 246.2(100), 236.1(35),196.3(5), 126.1 (7)
M4	18.6	350.15	$C_{17}H_{23}ClN_4O_2$		351.2([M+H], 100), 353.2(28), 333.1(64), 319.2(30), 195.7 (15), 165.7(12), 247.1(100), 126.2(14)
M5	15.6	337.16	$C_{17}H_{24}ClN_3O_2$		338.23 ([M+H], 100), 340.41 (34), 277.8 (100), 236.1 (57), 126.1 (21), 196.2(18), 169.1(27)
M6	17.7	307.14	$C_{16}H_{22}ClN_3O$		308.2 ([M+H], 100), 310.2 (29), 289.9 (100), 264.0 (40), 244.1(40),280.2(37),165.7 (26), 125.7 (24)
M7	14.1	323.14	$C_{16}H_{22}ClN_3O_2$		324.4 ([M+H], 100), 326.8 (28), 306.0 (100), 263.8 (46), 195.7 (59), 136.8 (12), 125.8 (10), 152.7(10)

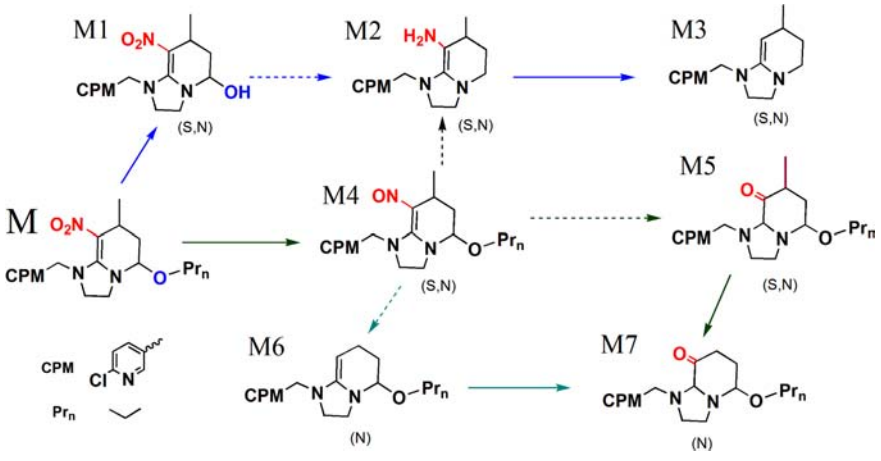


Figure 4. Proposed metabolic pathway of IPP in sterilized (S) and nonsterilized (N) loamy soil.

corresponded to the loss of one nitrogen atom. Moreover, the fragmentation pattern was similar to the keto-product observed in a previous study.[12] On the basis of these characteristics, M5 was preliminarily identified as 1-(6-chloropyridin-3-ylmethyl)-7-methyl-5-propoxy-8-carbonylhexahydroimidazo[1,2-*a*]-pyridine. A similar pattern was observed between M4 and M6 (Table 2), and there were odd nitrogens on M6 according to the even number of M6 ($[M + H]^+$), suggesting the loss of a nitroso group of M4. The difference of total loss units of 43 and nitroso loss of 29 is 14, which likely corresponded to the removal of methyl. Therefore, M6 was tentatively identified as 1-(6-chloropyridin-3-ylmethyl)-5-propoxy-1,2,3,5,6,7-hexahydroimidazo[1,2-*a*]pyridine. M7 was identified as the keto-demethyl derivative, 1-(6-chloropyridin-3-ylmethyl)-5-propoxy-8-carbonylhexahydroimidazo[1,2-*a*]pyridine, because of the similarity in the fragmentation pattern to that of M4 and M6. Finally, the loss of 14 was likely related to CH_2.

Pathways. On the basis of the identified degradation intermediates, a possible degradation pathway in loamy soil under aerobic conditions was proposed (Figure 4). The

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microbial and chemical transformation of IPP revealed three modification patterns of the tetrahydropyridine ring on the IPP parent compound: (a) elimination of propyl or methyl; (b) reduction of nitro to nitroso and amino; and (c) oxidation of tetrahydropyridine.

Initially, the propyl group of the parent compound was removed to form M1, after which the amination reaction occurred on the nitro group of M1 to create amino product M2. M3 was then generated by the deamination of M2 (Figure 4). Next is the nitrosylation of the parent compound. One oxygen was lost to convert nitro to nitroso and form M4, after which the nitroso group of M4 was cleared and oxidation occurred at the tetrahydropyridine to invert C═C to C—C═O and form carbonyl derivative M5, which has also been identified in aqueous solution by Zhao et al.[12] and shown to be similar to the C═N conversion to C═O of imidacloprid.[20−22] All five degradation intermediates described above were detected in both sterilized and nonsterilized loamy soils, whereas M6 and M7 were detected only in nonsterilized soil, which confirms the involvement of microbial degradation in the soil metabolism of IPP. M6 was formed as the demethylation and denitration product of M4. The C═C on the tetrahydropyridine of M6 was then ketonized to form M7 as described in the case above, forming the distinctly different degradation pathways in sterilized and nonsterilized loamy soil (Figure 4).

In this study, the effects of microorganisms were investigated by comparing the behavior characteristics of IPP in nonsterilized and sterilized loamy soils. The results clearly demonstrated that the soil microbial community plays an important role in the epimer-selective degradation of IPP in loamy soil. Specifically, there were significant differences in the mineralization, bound residues, and extractable residues between sterilized and nonsterilized soils, as well as between pairs of epimers. Moreover, soil microbes critically affected the degradation of parent RR-IPP and SS-IPP despite the higher degradation rates of SR-IPP and RS-IPP. Using LC-LSC, LC-ESI-MS, and LC-MS/MS, soil degradation products of IPP were preliminarily identified, and all degradation intermediates observed in nonsterilized soil were also found in sterilized soil except M6 and M7. Possible soil degradation pathways of IPP were proposed. The degradation pathways of IPP were partially consistent with the photodegradation of IPP and imidacloprid, whereas the major metabolic pathways were different. Specifically, the primary soil degradation occurred on the tetrahydropyridine ring, including reduction of the nitro group, demethylation, depropylation, and oxidation. Furthermore, obvious differences in the degradation pathways were found between the sterilized and nonsterilized soil as well as between the epimers in nonsterilized loamy soil, whereas these differences were weakened or vanished in sterilized soil, implying that the microorganisms are the pivotal factor involved in epimeride-selective degradation of IPP in loamy soil.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

IPP, paichongding; SR-IPP, (5*S*,7*R*)-paichongding; RS-IPP, (5*R*,7*S*)-paichongding; RR-IPP, (5*R*,7*R*)-paichongding; SS-IPP, (5*S*,7*S*)-paichongding; BR, bound residue; ER, extractable residue; WHC, water-holding capacity; DAT, days after treatment; LSC, liquid scintillation counter; LC-MS/MS, liquid chromatography−tandem mass spectrometry

REFERENCES

(1) Shao, X.; Zhang, W.; Peng, Y.; Li, Z.; Tian, Z.; Qian, X. *cis*-Nitromethylene neonicotinoids as new nicotinic family: synthesis, structural diversity, and insecticidal evaluation of hexahydroimidazo-[1,2-α]pyridine. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6513−6516.

(2) Williams, A. Opportunities for chiral agrochemicals. *Pestic. Sci.* **1996**, *46*, 3−9.

(3) Garrison, A. W. Probing the enantioselectivity of chiral pesticides. *Environ. Sci. Technol.* **2006**, *40*, 16−23.

(4) Lewis, D. L.; Garrison, A. W.; Wommack, K. E.; Whittemore, A.; Steudler, P.; Melillo, J. Influence of environmental changes on degradation of chiral pollutants in soils. *Nature* **1999**, *401*, 898−901.

(5) Liu, W.; Gan, J.; Schlenk, D.; Jury, W. A. Enantioselectivity in environmental safety of current chiral insecticides. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 701−706.

(6) Buerge, I. J.; Poiger, T.; Müller, M. D.; Buser, H. R. Influence of pH on the stereoselective degradation of the fungicides epoxiconazole and cyproconazole in soils. *Environ. Sci. Technol.* **2006**, *40*, 5443−5450.

(7) Qin, S.; Budd, R.; Bondarenko, S.; Liu, W.; Gan, J. Enantioselective degradation and chiral stability of pyrethroids in soil and sediment. *J. Agric. Food Chem.* **2006**, *54*, 5040−5045.

(8) Harner, T.; Jantunen, L. M. M.; Bidleman, T. F.; Barrie, L. A.; Kylin, H.; Strachan, W. M. J.; Macdonald, R. W. Microbial degradation is a key elimination pathway of hexachlorocyclohexanes from the Arctic Ocean. *Geophys. Res. Lett.* **2000**, *27*, 1155−1158.

(9) Zipper, C.; Nickel, K.; Angst, W.; Kohler, H. P. Complete microbial degradation of both enantiomers of the chiral herbicide mecoprop [(*RS*)-2-(4-chloro-2-methylphenoxy)propionic acid] in an enantioselective manner by *Sphingomonas herbicidovorans* sp. nov. *Appl. Environ. Microbiol.* **1996**, *62*, 4318−4322.

(10) Tian, Z.; Shao, X.; Li, Z.; Qian, X.; Huang, Q. Synthesis, insecticidal activity, and QSAR of novel nitromethylene neonicotinoids with tetrahydropyridine fixed cis configuration and exo-ring ether modification. *J. Agric. Food Chem.* **2007**, *55*, 2288−2292.

(11) Xu, X.; Bao, H.; Shao, X.; Zhang, Y.; Yao, X.; Liu, Z.; Li, Z. Pharmacological characterization of *cis*-nitromethylene neonicotinoids in relation to imidacloprid binding sites in the brown planthopper, *Nilaparvata lugens*. *Insect Mol. Biol.* **2010**, *19*, 1−8.

(12) Zhao, X.; Shao, X.; Zou, Z.; Xu, X. Photodegradation of novel nitromethylene neonicotinoids with tetrahydropyridine-fixed cis configuration in aqueous solution. *J. Agric. Food Chem.* **2010**, *58*, 2746−2754.

(13) Li, C.; Xu, X.; Li, J.; Ye, Q.; Li, Z. Synthesis and chiral purification of ^{14}C-labeled novel neonicotinoids, paichongding. *J. Labelled Compd. Radiopharm.* **2011**, *54*, 775−779.

(14) Wang, H.; Ye, Q.; Yue, L.; Han, A.; Yu, Z.; Wang, W.; Yang, Z.; Lu, L. Fate characterization of a novel herbicide ZJ0273 in aerobic soils using multi-position ^{14}C labeling. *Sci. Total Environ.* **2009**, *407*, 4134−4139.

(15) MacRae, I. C.; Raghu, K.; Castro, T. F. Persistence and biodegradation of four common isomers of benzene hexachloride in submerged soils. *J. Agric. Food Chem.* **1967**, *15*, 911−914.
(16) Boh, C. U.; Ralph, K. C.; Yeang, L. K. Degradation of four commonly used pesticides in Malaysian agricultural soils. *J. Agric. Food Chem.* **1998**, *46*, 1217−1223.
(17) Estrella, M. R.; Brusseau, M. L.; Maier, R. S.; Pepper, I. L.; Wierenga, P. J.; Miller, R. M. Biodegradation, sorption, and transport of 2,4-dichlorophenoxyacetic acid in saturated and unsaturated soils. *Appl. Environ. Microbiol.* **1993**, *59*, 4266−4273.
(18) Bollag, J.-M.; Myers, C. J.; Minard, R. D. Biological and chemical interactions of pesticides with soil organic matter. *Sci. Total Environ.* **1992**, *123*−*124*, 205−217.
(19) Stevenson, F. J. Organic matter reactions involving pesticides in soil. In *Bound and Conjugated Pesticide Residues*; ACS Symposium Series 29; American Chemical Society: Washington, DC, 1976; pp 180−207.
(20) Schippers, N.; Schwack, W. Photochemistry of imidacloprid in model systems. *J. Agric. Food Chem.* **2008**, *56*, 8023−8029.
(21) Mukherjee, I.; Gopal, M. Environmental behaviour and translocation of imidacloprid in eggplant, cabbage and mustard. *Pest Manag. Sci.* **2000**, *56*, 932−936.
(22) Ford, K. A.; Casida, J. E. Comparative metabolism and pharmacokinetics of seven neonicotinoid insecticides in spinach. *J. Agric. Food Chem.* **2008**, *56*, 10168−10175.