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Synthesis of carbon-14 labeled pyraoxystrobin, a novel fungicide

Xun-yue Liu,[a] Qing-fu Ye,[a] Deng-lei Kan,[a] Zheng Zhang,[b]* and Xing-cheng Ding[a]**

SYP-3343, (*E*)-2-(2-((3-(4-chlorophenyl)-1-methyl-1H-pyrazole-5-yloxy)methyl)) phenyl)-3-methoxyacrylate, is a novel fungicide with broad-spectrum and high activity against fungi. In this paper, radioactive pyraoxystrobin, labeled in the pyrazole ring system was achieved from ^{14}C labeled 4-chlorobenzoic acid through substitution, cyclization, and condensation, in an overall chemical and radiochemical yield of 44%. The chemical and radiochemical purity of the title compound was 98.5% and 98.2%, respectively. This labeled SYP-3343 was used as a radiotracer for metabolism, toxicology, mode of action, and other environmental studies.

Keywords: pyraoxystrobin; radiosynthesis; ^{14}C-labeled; SYP-3343

Introduction

SYP-3343, (***E***)-2-(2-((3-(4-chlorophenyl)-1-methyl-1H-pyrazole-5-yloxy) methyl)) phenyl)-3-methoxyacrylate (Figure 1), is a novel pyraoxystrobin fungicide that was independently developed by the ShenYang Research Institute of Chemical Industry in China. It is highly effective and has broad-spectrum activity against *Pseudoperonospora cubensis*, *Blumeria graminis*, *Erysiphe cichoracearum*, *Plasmopara viticola*, and rice blast. It is widely used to control many species of acariens on various crops such as rice, vegetables, and teas.[1–8] SYP-3343 has low toxicity to mammals. Its acute oral lethal dose 50% is 1000 mg/kg in male rats and 1022 mg/kg in female rats. It does not cause skin irritation in rabbits.[9] It obtained temporary registration in China in 2009 for cucumber downy mildew with a use rate of 80–100 g active ingredient per hectare.[10]

Insecticides, fungicides, and some herbicides directly enter into the soils after treatment.[11] Pesticide residues in soils, the dynamics of degradation, and the effects of pesticides on invertebrates, microbiology, and plants are important issues.[12–14] Pesticides degrade differently in different soils under differing conditions. When pesticide degradation is studied under different environmental conditions, a mathematical model can be established combining actual rainfall and temperature, to predict the dynamics of degradation and the effects on the environment.[15–17] At the present time, insufficient data are available on the toxicology of SYP-3343, and therefore, radiolabeled SYP-3343 is necessary to further elucidate the precise mechanism of action and support ongoing studies on the environmental behavior and fate of SYP-3343.[18,19] In this paper, the synthesis of pyrazole ring labeled [^{14}C]-SYP-3343 is described.

Results and discussion

In the design of a procedure for the preparation of a radiolabeled compound for use in environmental fate studies, it is important to locate the C-14 label in a metabolically stable position. In SYP-3343, there are two benzene rings and a pyrazole ring, which may be attractive sites for labeling. We selected the pyrazole ring for labeling, because potential precursors were readily available. Both C-14 labeled 4-chloroacetophenone and 4-chlorobenzoic acid were considered as possible starting materials; however, the yields in the synthesis of **6** from 4-chloroacetophenone were significantly lower than when 4-chlorobenzoic acid was used. The radiolabeled compound, [^{14}C]-pyrazole ring SYP-3343, was synthesized from 4-chlorobenzoic-[carbonyl-^{14}C] acid according to the method shown in Scheme 1. In the preparation of **1**, **3**, and **6**, it was necessary to vigorously eliminate water from the reactions or the yields were significantly decreased. Reaction of carbonyldiimidazole with 4-chlorobenzoic-[carbonyl-^{14}C] acid (**2**) and subsequent reaction with the magnesium salt of mono-ethyl malonate (**1**) yielded ethyl 3-(4-chlorophenyl-3-[^{14}C]-oxopropanoate (**3**) in 98% yield. Reaction of **3** with *N*-methylhydrazine yielded 3-(4-chlorophenyl)-1-methyl-1H-[3-^{14}C]-pyrazol-5-ol (**4**) in 87% yield. Subsequent reaction of **4** and (*E*)-methyl 2-(2-(chloromethyl) phenyl)-3-methoxyacrylate (**5**) yielded **6** in 68% crude yield. The last step of the reaction produced several by-products that required semi-preparative HPLC to remove, resulting in a 51% yield for the last step and 44% overall yield from 4-chlorobenzoic-[carbonyl-^{14}C] acid (**2**). HPLC-mass spectrometry (MS), MS (electron impact [EI]), and ^{1}H-NMR analysis of **6** were consistent with those obtained for unlabeled SYP-3343. The chemical and radiochemical purities were over 98%; the

[a]*Institute of Nuclear Agricultural Science, Zhejiang University, Hangzhou 310029, Zhejiang Province, China*

[b]*Department of Eye Science, Zhejiang University, Hangzhou 310009, Zhejiang Province, China*

**Correspondence to: Zheng Zhang, Eye Center of Affiliated Second Hospital, Zhejiang University 88 Jiefang RoadHangzhou, 310009, Zhejiang, Province, China. E-mail: zhangzhengzju@yahoo.com.cn*

***Correspondence to: Xing-cheng Ding, Institute of Nuclear-Agricultural Science, Zhejiang University, 268 Kaixuan Road, Hangzhou, 310029, Zhejiang, Province, China. E-mail: dingxch@zju.edu.cn*

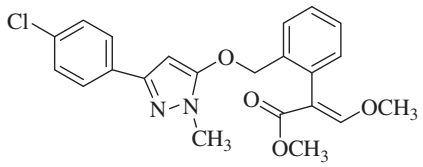


Figure 1. SYP-3343.

specific activity was 5.04 mCi/mmol as determined by HPLC, radio-thin layer chromatography (TLC), and HPLC-LSC (Liquid Scientillation Counting) methods.

Experimental

General

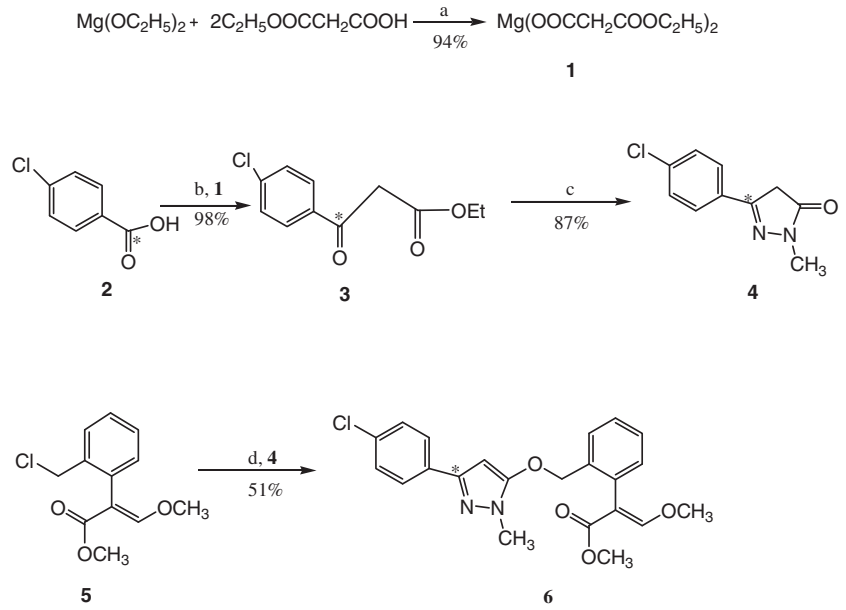
[1-^{14}C]4-Chlorobenzoic acid (15 mCi, 5.1 mCi/mmol) (**2**) was purchased from American Radiolabeled Chemicals Inc., (St. Louis, MO, USA). Paraformaldehyde, 2,5-diphenyloxazole (PPO, scintillation grade, 99%), and 1,4-*bis*(5-phenyloxazol-2-yl) benzene (POPOP, scintillation grade) were obtained from Acros Organics (Geel, Belgium). Methanol for HPLC was of chromatographic grade from SK Chemicals (Seoul, Korea), and ultrapure water was prepared on a Milli-Q academic instrument (Millipore, Alsace, France). The standard of SYP-3343 and the related intermediates (>99%) were provided by the ShenYang Research Institute of Chemical Industry in China. Scintillation cocktail was prepared as follows: PPO (7.0 g) and POPOP (0.5 g) were dissolved in a mixture of xylene (650 mL) and 2-methoxyethanol (350 mL). All other regents were of analytical grade and were commercially available and were used without further purification. ^{1}H-NMR spectra were measured on a Bruker DRX 300 spectrometer (Bruker, Fallanden, Switzerland), and MS was performed on an Agilent 5973 instrument (Agilent Technologies, Santa Clara, CA, USA). HPLC-MS was conducted on an Agilent 1100 LC/MS instrument (Agilent Technologies). HPLC analysis and semi-preparative purifications were completed on a Waters 2695 series system (Waters Co., Milford, MA, USA) consisting of an automated gradient pump, photodiode array detector with Inertsil ODS-3 column (4.6 × 250 mm, GL Science Co., Tokyo, Japan), and Hypersil semi-preparative column (8 mm × 300 mm, Dalian Elite Co., Liaoning, China). TLC was run on GF_{254} plates, and radio-TLC plates were scanned on a Fujifilm BAS-1800II laser-based fluorescence and radioisotope imaging system (Fujifilm Global, Tokyo Japan). Radioactivity was determined with a WinSpectral-1414 liquid scintillation spectrometer (Wallac, Turku, Finland).

*Magnesium ethyl 2-hydroperoxyacrylate (**1**)*

Magnesium ethanolate (3.43 g, 30 mmol) and 2-(ethylperoxy) acrylic acid (7.93 g, 60 mmol) were mixed in anhydrous THF and stirred for 2 h at room temperature. The reaction mixture was concentrated *in vacuo*, to yield (**1**) as a gray solid (8.07 g, 94% yield).

*Ethyl 3-(4-chlorophenyl)-[3-^{14}C]-oxopropanoate (**3**)*

A THF (dried by Na) solution (2 mL) of 4-chlorobenzoic-[carbonyl-^{14}C] acid (3.0 mmol, 5.1 mCi/mmol) was stirred under nitrogen. *N*, *N*-carbonyldiimidazole (535 mg, 3.3 mmol) was added to the reaction mixture, and stirring was continued for 2 h. THF (5 mL) was transferred by using a syringe into the reaction mixture, and stirring was continued for an additional 4 h at room temperature. The reaction mixture was diluted in *t*-butyl methyl ether (30 mL) and washed with water (30 mL, three times). The organic solution was washed with brine, dried over anhydrous Na_2SO_4, and evaporated to afford (**3**) (665 mg, 98%). ^{1}H-NMR (300 MHz, $CDCl_3$), δ(ppm): 7.89 (d, J = 8.7 Hz, 2H, ArH), 7.45 (d, J = 8.7 Hz, 2H, ArH), 4.21 (q, J = 7.1 Hz, 2H, CH_2), 3.97 (s, 2H, CH_2), 1.25 (t, J = 7.1 Hz, 3H, CH_3).



* = ^{14}C

a, Na, THF, 20-25°C; b, *N*,*N*-Carbonyldiimidazole (CDI); c, 40% methylhydrazine in MeOH, HOAc, 90-100°C; d, DMF, anhydrous K_2CO_3, 100-110°C

Scheme 1. Synthesis of [^{14}C] SYP-3343.

*3-(4-Chlorophenyl)-1-methyl-1H-[^{14}C]-pyrazol-5-yloxy)methyl (**4**)*

To a flask was added methanol (10 mL), acetic acid (1.0 mL), and (**3**) (665 mg, 2.9 mmol) in portions together with 40% methyl hydrazine (407 mg, 3.53 mmol). The mixture was refluxed for 8 h at 90–100°C. The solution was cooled and then was extracted with dichloromethane and washed with water. The organic extracts were combined and washed with brine, dried over anhydrous Na_2SO_4, and evaporated to afford (**4**) (532 mg, 87%). ^{1}H-NMR (500 MHz, $CDCl_3$), δ(ppm): 7.60 (d, J = 8.5 Hz, 2H, ArH), 7.40 (d, J = 8.5 Hz, 2H, ArH), 3.58 (s, 2H, CH_2), 3.41 (s, 3H, NCH_3).

(*E*)-2-(2-((3-(4-Chlorophenyl)-1-methyl-1H-^{14}C-pyrazole-5-yloxy) methyl))phenyl)-3-methoxyacrylate (6)

Compound (**4**) (532 mg, 2.6 mmol) was added to a solution of Dimethyl Formamide (DMF) (10 mL), (*E*)-methyl 2-(2-chloromethyl) phenyl)-3-methoxyacrylate (**5**) (675 mg, 2.8 mmol), and anhydrous K_2CO_3 (530 mg, 3.8 mmol) and warmed to 110°C for 4 h. The reaction was checked by using the TLC trace method, and the mixture was diluted in ethyl acetate (30 mL) and washed with water (30 mL, three times) and brine, and dried over anhydrous Na_2SO_4. The solution was filtered, and the mixture was dissolved in a solution of petroleum ether (PE) and Dichloromethane (DCM) (volume ratio 1:1). Silica gel (2.0 g, 100–200 mesh) was added to the solution and mixed for flash chromatography (PE/Ethyl Acetate (EA) 5:1 by volume). Fractions containing the product were combined, and solvent was removed to give impure (**6**). The impure products were subjected to semi-preparative HPLC (column: Diamonsil C_{18}, 10 μm, 250 × 10.0 mm; inject volume: 200 μL; methanol : water 80:20, by volume; flow rate: 4.5 mL/min; detection UV: 254 nm; column temperature: 30°C). Fractions between 7.610 and 8.750 min were collected and concentrated to afford (**6**) (538 mg, 51%). Chemical purity: 98.5%; radiochemical purity: 98.2%; and specific activity: 5.04 are determined by using HPLC,[20] radio-TLC, and HLPC-LSC methods.[21] HPLC-MS (ESI) *m/z*: 413.2($M + H^+$), 414.2($M + H^+ + 1$), 435.1($M + Na^+$). MS(EI, 70 eV) *m/z*(%): 412(M^+, 56), 145.1(100). ^{1}H-NMR (300 MHz, $CDCl_3$), δ(ppm): 7.64 (d, J = 7.0 Hz, 2H, ArH), 7.61 (s, 1H, =CH-), 7.55–7.52 (m, 1H, ArH), 7.39–7.36 (m, 2H, ArH), 7.32 (d, J = 7.0 Hz, 2H, ArH), 7.22–7.19 (m, 1H, ArH), 5.73 (s, 1H, Het-H), 5.03 (s, 2H, CH_2), 3.83 (s, 3H, NCH_3), 3.70 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3). The data were consistent with those in the previous studies.[1–3]

Acknowledgements

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