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Plant Uptake and Metabolism of Nitrofuran Antibiotics in Spring Onion Grown in Nitrofuran-Contaminated Soil

Yinan Wang, K. K. Jason Chan, and Wan Chan*

Department of Chemistry, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong

Supporting Information

ABSTRACT: Environmental pollution caused by the discharge of mutagenic and carcinogenic nitrofurans to the aquatic and soil environment is an emerging public health concern because of the potential in producing drug-resistant microbes and being uptaken by food crops. Using liquid chromatography−tandem mass spectrometry analysis and with spring onion (*Allium wakegi* Araki) as the plant model, we investigated in this study the plant uptake and accumulation of nitrofuran from a contaminated environment. Our study revealed for the first time high uptake and accumulation rates of nitrofuran in the edible parts of the food crop. Furthermore, results indicated highly efficient plant metabolism of the absorbed nitrofuran within the plant, leading to the formation of genotoxic hydrazine-containing metabolites. The results from this study may disclose a previously unidentified human exposure pathway through contaminated food crops.

KEYWORDS: *nitrofuran, root uptake, plant metabolism, green liver, spring onion, LC−MS/MS*

INTRODUCTION

Nitrofuran (Figure 1) is a class of broad-spectrum veterinary drugs for the treatment of microbial infections, with furaltadone

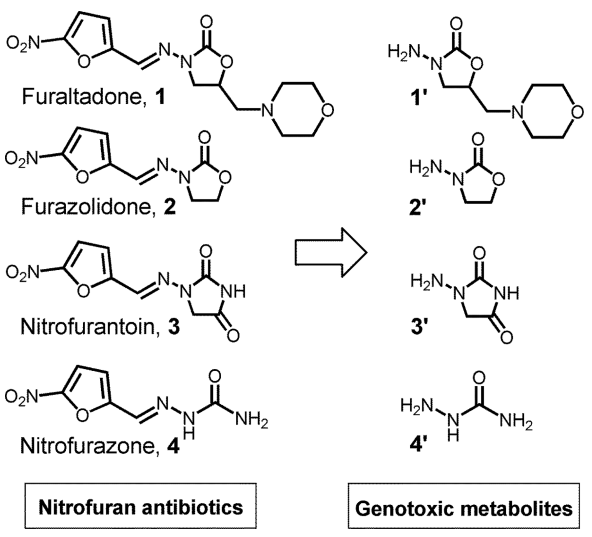

Figure 1. Chemical structures of nitrofurans and their corresponding protein-bound metabolites.

(**1**), furazolidone (**2**), nitrofurantoin (**3**), and nitrofurazone (**4**) being the most widely used nitrofurans.[1−5] They are highly effective in suppressing and killing most kinds of bacteria, fungi, and protozoa, which led them to be used extensively in aquaculture and livestock farming as growth promoters until they were observed to be carcinogenic and mutagenic to humans.[6−10] Currently, the use of nitrofuran in food-producing animals has been banned in many countries, including China, U.S.A., and many European countries.[11,12]

Despite being prohibited from use in food animal production in many countries, the use of nitrofuran in some developing countries is still allowed and illegal use of nitrofuran exists.[5,13] Furthermore, only few studies reported the potential plant uptake of nitrofuran from a contaminated environment, and no information on their plant metabolism is available in the literature.[14,15] Therefore, it is still of high importance to monitor food for nitrofuran contamination as a result of their adverse effect on human health.[16,17] Currently, meat products are routinely sampled by regulatory authorities for nitrofuran residues by detecting their protein-bound metabolites (semicarbazides or carbazates) in the muscles (Figure 1).[18,19]

It is known that most antibiotics, nitrofuran included, are poorly absorbed through the intestines and are largely excreted unchanged.[20] For example, it was reported that over 60% of furazolidone was excreted through urine and feces.[21] These residual nitrofurans in the excrements, together with those used in the aquaculture fisheries, may end up being discharged into the aquatic environment and soil through improper wastewater handling or when applying the animal excreta as fertilizer for food crop production,[14,22,23] leading to concerns that residual antibiotics may be taken up by food crops and enter the human food chain.[24] In this study, we tested the hypothesis that humans may be exposed to nitrofuran through consumption of contaminated food crops grown in polluted soil that have uptaken nitrofuran through the roots and accumulated nitrofuran in their edible parts. This could be an existing "secondary exposure" pathway to nitrofuran, of which we have been unaware.

Using a liquid chromatography−tandem mass spectrometry (LC−MS/MS) method and with spring onion (*Allium wakegi*

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Araki) as the plant model, we investigated in this study the uptake and accumulation of nitrofuran from nitrofuran-contaminated soil. The *in vitro* and *in vivo* metabolisms of nitrofuran in spring onion homogenate and spring onion, respectively, were also studied for the first time. Furthermore, the study also evaluated the plant uptake efficiency and biogeochemical stability of nitrofuran from the soil environment.

MATERIALS AND METHODS

Reagents. Compounds **1**, **2**, **3**, and **4**, 3-amino-5-morpholinomethyl-2-oxazolidinone (**1′**), 3-amino-2-oxazolidinone (**2′**), 1-aminohydantoin hydrochloride (**3′**), semicarbazide hydrochloride (**4′**), [^{13}C,^{15}N$_2$]-semicarbazide ([^{13}C,^{15}N$_2$]-**4′**), 2-nitrobenzaldehyde, and 5-nitro-2-furaldehyde were purchased from Sigma-Aldrich (St. Louis, MO). High-performance liquid chromatography (HPLC)-grade acetonitrile, ethyl acetate, hexane, and methanol were obtained from Tedia (Fairfield, OH). Isotope-labeled compound **4** ([^{13}C,^{15}N$_2$]-**4**) was synthesized by reacting [^{13}C,^{15}N$_2$]-**4′** with 5-nitro-2-furaldehyde, as described previously.[25] Using a similar strategy, the 2-nitrobenzaldehyde derivative of [^{13}C,^{15}N$_2$]-**4′** was also synthesized by reacting [^{13}C,^{15}N$_2$]-**4′** with 2-nitrobenzaldehyde.

***In Vitro* Study.** The *in vitro* experiment was performed by incubating a mixture of compounds **1**, **2**, **3**, and **4** (1 μg/mL) with the homogenate of spring onion leaf/root bulb at 25 °C for an extended period of 12 h (n = 3). Aliquots of the solution were sampled at 0 and 30 min and 1, 2, 4, 8, and 12 h and processed for nitrofuran and associated metabolite (**1′**, **2′**, **3′**, and **4′**) analyses using the methods described below. Using a similar strategy, the control experiment was conducted by incubating a mixture of compounds **1**, **2**, **3**, and **4** (1 μg/mL) with homogenate that was pretreated by heating at 100 °C.

***In Vivo* Study.** The *in vivo* experiment was conducted by cultivating spring onion in nitrofuran-contaminated soil (4 mg/kg). In brief, to the nitrofuran-spiked soil samples that were left in the ambient environment for 2 days, spring onions were cultivated. The plants were left in an ambient environment with a natural day/night cycle and were watered once per week. After growing in the soil for 7, 14, 28, and 56 days, the leaves and root bulbs were harvested separately for analysis (n = 3). The control experiment was performed by analyzing spring onion grown on soil with no nitrofuran contamination and analyzing nitrofuran-contaminated soil with no spring onion cultivated.

Sample Preparation. *For Nitrofuran Analysis.* The leaf and root bulb samples were processed for nitrofuran analysis using a method described previously.[26] In brief, to the homogenized samples (200 mg) was added ethyl acetate (5 mL); the mixture was mixed by vigorous shaking for 5 min and then centrifuged; and the supernatant was collected and dried under a stream of nitrogen. To the dried sample extract, acetonitrile (5 mL) and hexane (3 mL) were added and shaken for 5 min. After centrifugation [4200 relative centrifugal force (rcf)] for 5 min, the acetonitrile fraction was isolated and dried under nitrogen before the internal standard [^{13}C,^{15}N$_2$]-**4** (0.3 μg/mL in methanol, 50 μL) and aqueous 50% methanol (450 μL) were added to the residues for LC−MS/MS analyses.

For Metabolite Analysis. Using a previously reported method,[27] the leaf and root bulb samples were also processed for protein-bound metabolite analysis. In brief, hydrochloric acid (0.125 M, 5 mL) and 2-nitrobenzaldehyde (50 μg/mL in methanol, 200 μL) were added to ground leaf or root bulb samples (200 mg). After vortex mixing, the solutions were incubated at 37 °C overnight to liberate and derivatize the protein-bound metabolites (Figure 2). Sodium hydroxide solution (1 M, 400 μL) was then added to adjust the sample solutions to pH 7.0 (±0.3) before the solutions were washed with *n*-hexane (3 mL) and extracted with ethyl acetate (2 mL). The ethyl acetate extracts were combined and dried under a stream of nitrogen before the derivatized [^{13}C,^{15}N$_2$]-**4′** internal standard (0.6 μg/mL in methanol, 50 μL) and aqueous 50% methanol (450 μL) were added to the residue for LC−MS/MS analyses.

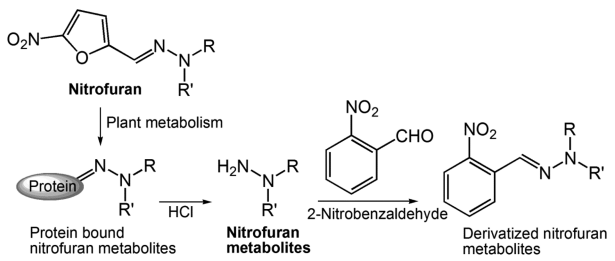

Figure 2. Acid-catalyzed release of the protein-bound metabolites of nitrofuran and simultaneous derivatization of the released metabolites with 2-nitrobenzaldehyde to produce hydrophobic derivatives of enhanced chromatographic performance for LC−MS/MS analysis.

LC−MS/MS Analyses. LC separation of both nitrofurans and their metabolites was performed on a 150 × 2.1 mm inner diameter, 5 μm, GraceSmart RP 18 (Grace, Deerfield, IL) powered by an 1100 HPLC system (Agilent Technologies, Palo Alto, CA). The sample extracts (5 μL) were injected into the column eluted using 20 mM ammonium acetate in water (A) and methanol (B) as the mobile phase at a flow rate of 0.3 mL/min. The gradient elution was programmed with a linear gradient increasing from 10% (20% for nitrofuran metabolites) to 100% B (v/v) in 10 min and holding for 5 min, before being re-equilibrated to initial conditions for 7 min.

The LC eluate was directed to an API 4000 QTRAP LC−MS/MS system (Toronto, Ontario, Canada) operated in positive electrospray ionization (ESI) mode. Quantitative analysis was performed by the multiple reaction monitoring (MRM) mode of the MS/MS system using the optimized instrumental parameters. The m/z values for the qualifying and quantifying MRM transition of the target analytes and internal standard are listed in Table 1, with the dwell time for each transition set to 100 ms.

Table 1. MS/MS Transitions Used To Monitor Nitrofurans and Their Protein-Bound Metabolites, Together with the Log(K_{ow}) Values and Chromatographic Migration Time of the Nitrofuran

	log(K_{ow})	migration time (min)	MRM transitions (m/z)
		Nitrofuran	
1	0.20	8.6	325/252 and 325/281
2	−0.04	8.0	226/122 and 226/139
3	−0.47	7.7	239/95 and 239/139
4	0.23	7.9	199/156 and 199/182
[^{13}C,^{15}N$_2$]-**4**		7.9	202/158
		Metabolites[a]	
1′		8.3	335/262 and 335/291
2′		7.2	236/104 and 236/134
3′		7.0	249/104 and 249/134
4′		7.3	209/166 and 209/192
[^{13}C,^{15}N$_2$]-**4′**		7.3	212/168

[a]After chemical derivatization with 2-nitrobenzaldehyde.

Calibration. Working standard solutions of nitrofuran were prepared by serial dilution of the stock solution mixture of compounds **1**, **2**, **3**, and **4** (2 μg/mL) with blank sample extract. To 450 μL of the working standard solution mixtures with concentrations ranging from 0.01 to 0.5 μg/mL, 50 μL of the internal standard ([^{13}C,^{15}N$_2$]-**4**, 0.3 μg/mL) was added. After vortex mixing, the standard solutions were analyzed by the LC−MS/MS method described above. Calibration curves were established by plotting the peak area ratio of individual nitrofuran to that of the internal standard against the concentration of the nitrofuran in the working standards.

A spring onion sample with no detectable amount of compounds **1′**, **2′**, **3′**, and **4′** was used to prepare the matrix-matched calibration curves of compounds **1′**, **2′**, **3′**, and **4′**. In brief, to the homogenized

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samples (200 mg), 100 μL of the standard mixture of **1′**, **2′**, **3′**, and **4′** (from 0.01 to 1.0 μg/mL; $n = 3$) was added. After vortex mixing, 2-nitrobenzaldehyde (50 μg/mL in methanol, 200 μL) and hydrochloric acid (0.125 M, 5 mL) were added and allowed to react at 37 °C overnight. The derivatized working standard solutions were then extracted with ethyl acetate, added with an internal standard (2-nitrobenzaldehyde derivative of [^{13}C,$^{15}N_2$]-**4′**), and then analyzed by LC−MS/MS, as described above.

Method Validation. The methods were validated for recovery and limit of detection (LOD). From analysis of nitrofurans and their metabolties at the lowest calibration concentration (0.01 μg/mL) and using a previously reported method,[28−30] the LOD was estimated as the amount of analyte that generated a signal 3 times the noise level in analyzing nitrofuran and metabolites.

The recovery of nitrofuran was evaluated by analyzing homogenized samples (200 mg) that were spiked with 450 μL of a nitrofuran mixture of compounds **1**, **2**, **3**, and **4** (0.01, 0.25, and 0.5 μg/mL; $n = 3$). The nitrofuran-fortified samples were vortex-mixed and allowed to stand at room temperature for 5 min before being extracted using ethyl acetate, added with an internal standard, and analyzed using the method described above. The recovery was established by dividing the recovered nitrofuran to that of the spiked amount. Using a similar method, the recovery of the nitrofuran metabolites was also evaluated. In brief, to 200 mg of homogenized samples, 100 μL of a standard mixture of compounds **1′**, **2′**, **3′**, and **4′** (0.1, 0.5, and 1.0 μg/mL; $n = 3$) was added. After vortex mixing, 2-nitrobenzaldehyde (50 μg/mL in methanol, 200 μL) and hydrochloric acid (0.125 M, 5 mL) were added and allowed to react at 37 °C overnight. The sample solutions were then adjusted to pH 7.0 using sodium hydroxide, washed with *n*-hexane (3 mL), and extracted with ethyl acetate before the concentration of the metabolites in the sample extracted was analyzed using the method described above.

Data Analysis. The half-life ($t_{1/2}$) of individual nitrofuran in *in vitro* incubations with plant tissue homogenates were estimated by plotting $\ln([NF_t]/[NF_i])$ versus time,[31] where $[NF_t]$ and $[NF_i]$ represent the concentrations of individual nitrofuran at the time t and the beginning of the experiment, respectively. The concentration factors for leaves and root bulbs were calculated by dividing the concentrations of nitrofuran in plant tissues to that in the soil samples at the harvesting time (day 56).[32]

RESULTS AND DISCUSSION

Root Uptake and Accumulation of Nitrofuran in Spring Onion. Emerging evidence suggests that antibiotics are a new class of environment pollutants.[26,28] The discharged antibiotics in the environment originate from both human medicinal sources and food-producing animal facilities.[24] For example, the contamination with nitrofuran as well as many other types of antibiotics is now widespread in the environment.[23] It is likely that antibiotics may contaminate the human food supply through root uptake into food crops from a contaminated environment,[33−36] in addition to tainted poultry or seafood products.[37]

Among the commonly used antibiotics, the mutagenic and carcinogenic nitrofurans are of particular concern to human health.[5] Previous studies focused on detecting the protein-bound nitrofuran metabolites in meat products,[13,17] with only one study investigating the uptake of compound **1** by algae from contaminated seawater.[26] We hypothesize that food crops grown in nitrofuran-contaminated soil may similarly uptake nitrofuran through the roots and become contaminated.

Using a calibrated and validated LC−MS/MS method (Table 2), we investigated in this study the plant uptake of nitrofuran from a contaminated environment. Choosing spring onion as our plant model and four commonly used nitrofurans **1**, **2**, **3**, and **4**, as model compounds, we revealed that food crops do indeed uptake and accumulate these compounds from

Table 2. Calibration and Validation Parameters of the LC−MS/MS Method for Nitrofuran and Associated Metabolite Analyses

	linear range (μg/mL)	slope	intercept	R^2	LOD (ng/g)	recovery (%)
			Nitrofuran			
1	0.01−0.5	0.0321	0.1506	0.9967	0.2	86.2−87.9
2	0.01−0.5	0.0066	0.0653	0.9981	0.2	86.8−94.4
3	0.01−0.5	0.0023	0.0392	0.9993	0.8	87.2−90.5
4	0.01−0.5	0.0033	0.0588	0.9929	0.7	87.3−92.9
			Metabolites[a]			
1′	0.01−1.0	0.0258	0.3809	0.9978	0.2	90.9−92.0
2′	0.01−1.0	0.0079	0.1693	0.9979	0.4	88.4−91.5
3′	0.01−1.0	0.0023	0.0663	0.9983	0.7	86.5−92.9
4′	0.01−1.0	0.0036	0.1119	0.9963	0.7	89.0−91.8

[a]After chemical derivatization with 2-nitrobenzaldehyde.

contaminated soil into their root bulbs in a time-dependent manner from 0 to 28 days (Figure S1 of the Supporting Information). The presence of nitrofuran was also detected in the leaves in a similar concentration pattern but at lower concentrations, indicating that transport of nitrofuran from the root bulb up to the leaves occurs efficiently by transpiration. Shown in Figure 3 are typical chromatograms obtained from LC−MS/MS of the nitrofuran in leaf samples.

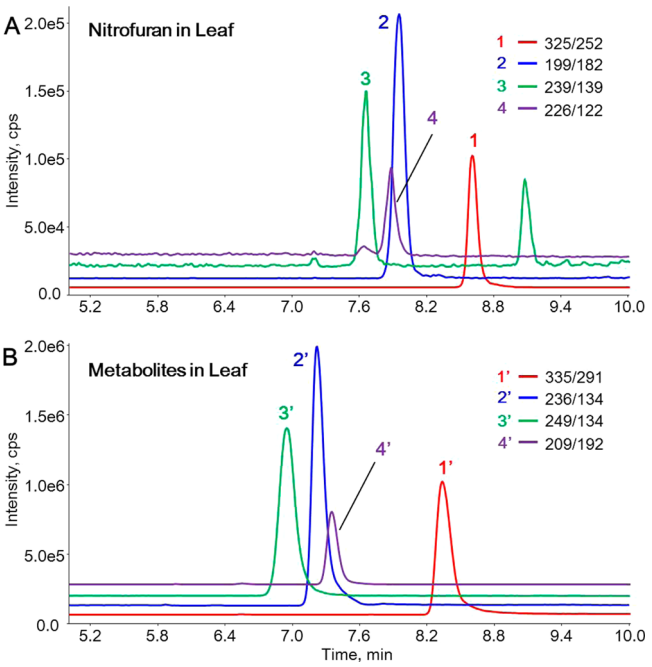

Figure 3. Reconstructred chromatograms from LC−MS/MS analysis of (A) nitrofuran and (B) nitrofuran metabolites (after chemical derivatization with 2-nitrobenzaldehyde) in the leaf of spring onion grown in nitrofuran-contaminated soil.

Data showed a slight decrease in nitrofuran concentrations, in both the root bulbs and leaves from days 28 to 56 (Figure 4); this decrease is parallel to the gradual depletion of nitrofuran in the soil (Figure 4). Nevertheless, the concentrations of the four nitrofurans in both the root bulbs and leaves decreased in the order of **2** > **3** > **1** > **4**, which is in good agreement with their decreasing solubility in water [increasing $\log(K_{ow})$; Table 1]. The close correlation of the $\log(K_{ow})$ values with the

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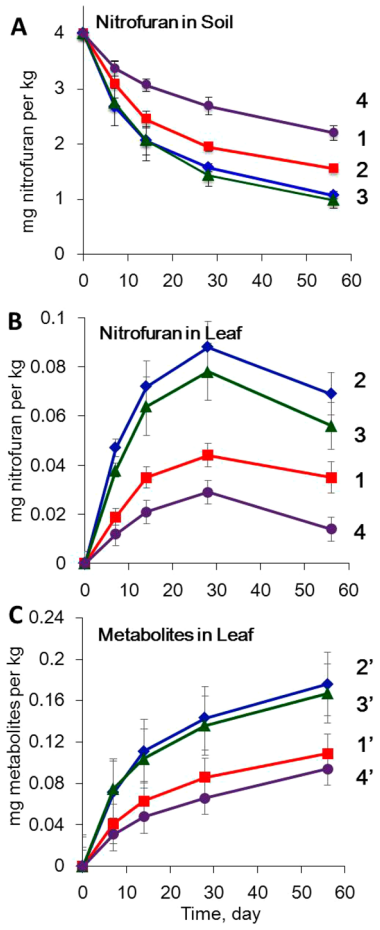

Figure 4. Time-dependent changes in the concentrations of nitrofuran in (A) soil, together with that of (B) nitrofuran and (C) associated metabolites in the leaf of spring onion grown in nitrofuran-contaminated soil.

absorption efficiency strongly suggests that nitrofurans were uptaken into the plants as the roots uptake water from the soil containing dissolved nitrofuran. The absorbed nitrofurans were then transported up the leaves through transpiration. Figure 5 summarized the bioconcentration factors of nitrofuran in the leaves and root bulbs of spring onion, also illustrating a decreasing uptake efficiency in the order of **2** > **3** > **1** > **4** and

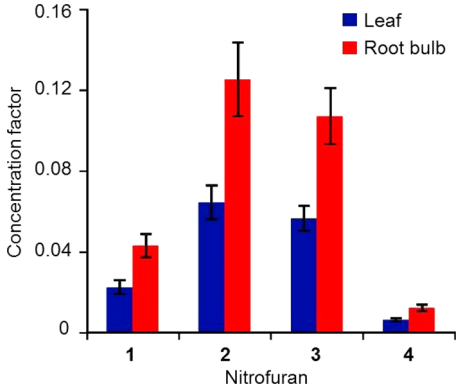

Figure 5. Concentration factors of nitrofurans **1**, **2**, **3**, and **4** in the leaf and root bulb of spring onion grow in nitrofuran-contaminated soil.

higher accumulation levels in the root bulbs compared to the leaves.

Using green algae (*Ulva lactuca*) as their plant model, Leston et al. reported that, when the concentration of compound **1** was monitored in seawater over a 5 day period of aquaculture, roughly 70% of compound **1** in the seawater was degraded by microbes and/or sunlight and 30% was removed by plant uptake.[26] Our study using spring onion demonstrated a comparatively lower uptake and degradation rate of nitrofuran. Specifically, over an extended period of 56 days, we observed that less than 20% of nitrofurans were degraded by microbial/photodegradation (Figure S2 of the Supporting Information), while approximately 50% (except for compound **4** with 34.6% being absorbed) has been absorbed by the plant (Figure 4). This observed difference in uptake and degradation efficiency could be attributed to using different plant species and media for the experiments. Nevertheless, the results from our study could demonstrate that nitrofuran released into the soil environment would likely be more persistent than in the aquatic environment.

Determination of Protein-Bound Metabolites of Nitrofuran in Spring Onion. After demonstration of the ability of spring onion to uptake nitrofuran from a contaminated environment, the study was extended to detect several previously identified protein-bound metabolites from animal and plant tissues.[13,16] These protein-bound, genotoxic metabolites of nitrofuran, carbazates **1′** and **2′** and semicarbazides **3′** and **4′** in particular (Figure 1), are hydrolytic products of the imine bond in nitrofuran. They were used extensively to detect nitrofuran contamination in muscle samples.[17,18] However, the identification of these metabolites in vegetable food crops has not been reported in the literature to this date, to the best of our knowledge. It was proposed that plants are able to perform some xenobiotic metabolism similar to that observed in hepatic metabolism through enzymes present in plant cells.[38,39] Using a validated LC−MS/MS method (Table 2), we tested in this study the hypothesis that the absorbed nitrofuran may be metabolized within the plant cells of spring onion.

In Vitro Study. The goal of this study was to test the feasibility of using the previously identified protein-bound metabolites of nitrofuran as marker molecules for the detection of nitrofuran contamination in food crops.[13,16−19] We carried out *in vitro* incubations of nitrofurans **1**, **2**, **3**, and **4** with the homogenates of leaf and root bulb tissue from spring onion separately (n = 3). The incubated mixtures were sampled at different times, then processed, and analyzed for the presence of nitrofurans and their protein-binding metabolites using the methods described above (Figure 2).

Surprisingly, the metabolite analysis revealed, in both the incubated leaf and root bulb tissue homogenates, a similar and time-dependent increase in the concentrations of nitrofuran metabolites, i.e., compounds **1′**, **2′**, **3′**, and **4′** (Figure 6 and Figures S3 and S4 of the Supporting Information). The parallel nitrofuran analysis also detected a corresponding time-dependent decrease in nitrofuran concentrations during the incubations (Figure 6), with over 60% of nitrofurans converted into their metabolites during a 4 h period of incubation. Table 3 summarizes the half-lives of nitrofuran when incubated with leaf and root bulb homogenates; it showed that the metabolic rates in root bulb homogenate are generally faster compared to the leaf homogenate, indicating that, in the whole plant, the metabolic rate would likely also be faster in the root bulb than

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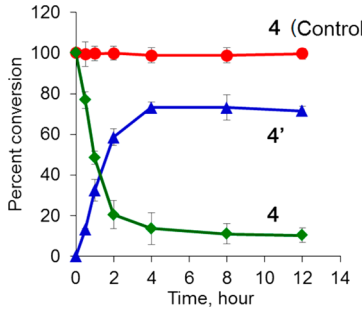

Figure 6. Time-dependent changes in the concentrations of nitrofurazone and its protein-bound metabolites, i.e., semicarbazide, in homogenized fresh leaf samples as well as that of the concentration of nitrofurazone in heat-treated tissue homogenate.

Table 3. Half-Life ($t_{1/2}$) of Nitrofuran in *In Vitro* Incubations with Leaf and Root Bulb Homogenates of Spring Onion

	in leaf homogenate (h)[a]	in root bulb homogenate (h)[a]
furaltadone (**1**)	1.4 ± 0.2	0.9 ± 0.1
furazolidone (**2**)	0.8 ± 0.1	0.7 ± 0.1
nitrofurantoin (**3**)	1.3 ± 0.2	1.1 ± 0.1
nitrofurazone (**4**)	1.1 ± 0.1	0.9 ± 0.1

[a]A total of 200 mg of homogenized plant tissue samples was used in both experiments. Data represent the mean ± 3 times the standard derivation.

the leaves. A half-life of around 1 h was observed for all four nitrofurans, indicating that the plant enzymes can process structurally different nitrofurans efficiently. No noticeable change in the nitrofuran concentration was observed in the control experiments using heat-denatured plant tissue homogenates (Figure 6), which strongly suggests that the degradation of nitrofuran is indeed mediated through enzymes, e.g., nitroreductases, which were previously observed to play a key role in the metabolism of nitrofuran in bacterial and mammalian cells.[40,41]

In Vivo Study. Having successfully detected the protein-bound metabolites of nitrofuran in *in vitro* incubations, we extended the study to the living whole plant and tested for the presence of metabolites **1′**, **2′**, **3′**, and **4′** in the leaf and root bulb samples of spring onion grown in nitrofuran-dosed soil. To our surprise, our study detected all of the targeted nitrofuran metabolites in both the leaf (Figure 4) and root bulb (Figure S1 of the Supporting Information) samples and with the leaf/bulb concentration ratio (~1:2) similar to that observed for the parent drugs. No nitrofuran metabolites were detected in any of the soil samples, indicating that the only source of these metabolites was from plant metabolism and ruling out their formation by soil microorganisms or chemical decomposition. Depicted in Figure 3 are typical chromatograms obtained from LC−MS/MS of the metabolites in leaf samples. To the best of our knowledge, this is the first report of nitrofuran metabolism taking place in a plant.

Interestingly, a similar concentration pattern (**2′** > **3′** > **1′** > **4′**) as that displayed by the parent drugs (**2** > **3** > **1** > **4**) was also observed (Figure 4), indicating a similar rate of metabolism of the four nitrofurans by the enzymes in the plant. Nevertheless, the metabolite concentration showed an increasing trend throughout the entire experiment (from day 0 to day 56), indicating these metabolic products are highly persistent and will accumulate in the plant. A similar high persistence of the metabolites was also observed in the animal tissues from nitrofuran-treated animals in previous studies.[18,42] Because both nitrofurans and their metabolites were identified as secondary species capable of being transferred through contaminated food sources,[5] it is feasible that through bioaccumulation and biomagnification up the food chain, the risk of human exposure to these toxic substances through plants grown in tainted soil would be enhanced.

Using LC−MS/MS methods, we investigated in this study the environmental stability, root uptake, and plant metabolism of nitrofuran, a group of carcinogenic and mutagenic antibiotics, in spring onion. Our results demonstrated for the first time the high persistence of nitrofurans in the soil environment and their highly efficient uptake into food crops. With nitrofuran antibiotics emerging as a new class of environmental pollutants, this study disclosed important but previously unrealized human exposure pathways to nitrofuran. Furthermore, the study revealed an interesting, novel phenomenon of plant metabolism in which nitrofurans were efficiently degraded to their genotoxic metabolites by the living plant cells.[31,43] It is expected that the study will alert food scientists and regulatory authorities to a potential new source of human exposure to nitrofuran.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.7b01050.

Time-dependent changes in the concentration of nitrofuran and associated metabolites in root bulb of spring onion grown in nitrofuran-contaminated soil (Figure S1), time-dependent changes in the concentrations of nitrofuran in soil without spring onion cultivation (Figure S2), time-dependent changes in the concentration of nitrofurans and their protein-bound metabolites in *in vitro* incubation with leaf and root bulb homogenates (Figures S3 and S4) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Telephone: +852-2358-7370. Fax: +852-2358-1594. E-mail: chanwan@ust.hk.

ORCID

Wan Chan: 0000-0001-8479-3172

Author Contributions

Yinan Wang and Wan Chan designed the research; Yinan Wang performed the research; Yinan Wang, K. K. Jason Chan, and Wan Chan analyzed the data; and Wan Chan wrote the paper.

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Notes

The authors declare no competing financial interest.

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