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Identification of metabolites of propyrisulfuron in rats

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ABSTRACT

The metabolites found in the urine, feces and bile of male and female rats administered with ^{14}C-labeled herbicide, propyrisulfuron [1-(2-chloro-6-propylimidazo[1,2-b]pyridazin-3-ylsulfonyl)-3-(4,6-dimethoxypyrimidin-2-yl)urea] were identified by high-performance liquid chromatography (HPLC) with the ultraviolet (UV) and radioisotope (RI) detectors, tandem mass spectrometry and nuclear magnetic resonance (NMR). Administered ^{14}C was excreted into the urine (5.7–29.8%) and feces (64.6–97.4%). Urine and bile samples were concentrated and purified using a solid-phase extraction cartridge, and fecal homogenates were extracted using acetonitrile. Conjugates were hydrolyzed with enzyme or hydrochloric acid solution for identification. The proposed major metabolic reactions of propyrisulfuron are as follows: (1) hydroxylation of the pyrimidine ring, propyl group, and imidazopyridazine ring, (2) O-demethylation, (3) cleavage of the pyrimidine ring, and (4) glucuronic acid and sulfate conjugation. The metabolic patterns found are not different among sulfonylurea herbicides.

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1. Introduction

Sulfonylurea herbicides have been commercially used since 1982 [1]. The strong points of sulfonylurea herbicides are considered as high safety of non-targeted organism, low dosage compared with traditional herbicides, a wide range of weeding spectrum, and selectivity for many crops by structure modification [2–4]. Thus, sulfonylurea herbicides have occupied an important place in selective herbicide. Sulfonylurea herbicides inhibit acetolactate synthase (ALS), which is responsible for synthesizing branched-chain amino acids, leading to the firing [5–7]. However, the problem of sulfonylurea-resistant weeds has emerged because of heavy usage [8]. Mutations in ALS is attributed to the cause of resistance [9,10].

Propyrisulfuron [1-(2-chloro-6-propylimidazo[1,2-b]pyridazin-3-ylsulfonyl)-3-(4,6-dimethoxypyrimidin-2-yl)urea] is a novel sulfonylurea paddy herbicide developed by Sumitomo Chemical Co., Ltd (Fig. 1). Although propyrisulfuron inhibits ALS to eradicate weeds, it is very unique that it can eradicate sulfonylurea-resistant weeds [11,12].

The metabolism of propyrisulfuron in rats (BrlHan:WIST@Jcl (GALAS)) has been investigated in conjunction with toxicological studies for safety evaluation. Results from toxicity studies that have been conducted show low acute toxicity and no observed mutagenicity [12]. The present report deals with the identification of metabolites of propyrisulfuron in rats. Although published reports about mammalian metabolism of sulfonylurea herbicides are extremely limited, some information has been described by Roberts et al. [13]. Cleavage of the sulfonylurea linkage, hydroxylation, O-dealkylation, and glucuronide are shown in this paper, and these reactions are common in other sulfonylurea herbicides, although propyrisulfuron has a unique property of water solubility (0.98 mg/L), which is relatively lower than other sulfonylurea herbicides [from 14.5 mg/L (pyrazosulfuron) to 31.8 g/L (chlorsulfuron)] [13]. For instance, research on the metabolism of azimsulfuron in rats, a sulfonylurea herbicide used for rice weeds, revealed that O-demethylation was the major metabolic pathway, and other minor pathways included the hydroxylation of the pyrimidine ring, cleavage of the sulfonylurea linkage, and glucuronide and sulfate conjugation of the hydroxylated metabolites [13]. The metabolites of propyrisulfuron in animals would be of particular interest because it is extremely important for evaluating the toxicity. In addition, propyrisulfuron has a unique feature of water solubility, which is different from other sulfonylurea herbicides, indicating that there is a possibility of unique metabolites produced in rats. Therefore, we identified the metabolites of propyrisulfuron in rats and discussed the difference of metabolic patterns between propyrisulfuron and other sulfonylurea herbicides in rats.

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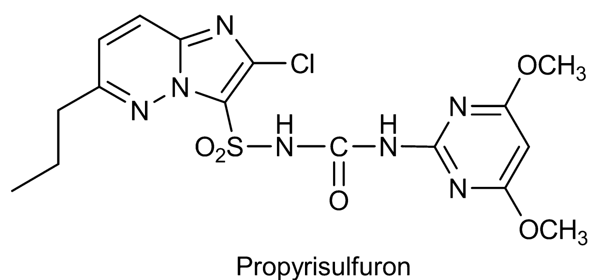

Fig. 1. Chemical structure of propyrisulfuron.

2. Experimental

2.1. Chemicals

[Propyl (pr)-^{14}C]propyrisulfuron and [pyrimidine (pm))-^{14}C]propyrisulfuron were prepared in our laboratory with a specific activity of 2.15 GBq/mmol and 2.04 GBq/mmol, respectively (Fig. 2). The radiochemical purities of the labeled compounds were analyzed by thin-layer chromatography (TLC) prior to use. The results showed that radiochemical purities of these compounds were >99.1% ([pr-^{14}C]propyrisulfuron) and >98.3% ([pm-^{14}C]propyrisulfuron), respectively. Unlabeled propyrisulfuron (purity 100.0%) and seven authentic metabolite standards, unlabeled PISN, PHDU, PIHU, ACPS, ADPM, ADPM-OH and PDMU, were prepared in our laboratory and used for the identification of metabolites (Fig. 3). The structures of synthesized metabolites were confirmed by NMR. Other chemicals were reagent grade unless otherwise noted in the text.

2.2. Thin layer chromatography (TLC) analysis

Pre-coated silica gel 60 F 254 TLC plates (20 cm × 20 cm, 0.25 thickness, Merck, Germany) were employed, with chloroform/acetone/formic acid/water, 40:40:10:5 (v/v) and chloroform/methanol, 9:1 (v/v) as the solvent system. Radioactive compounds on TLC plates were detected by autoradiography using imaging plates (Fuji Photo Film, Tokyo, Japan). These plates were allowed to contact with the TLC plates at room temperature and then were processed with a fluorescent image analyzer (FLA-5000, Fuji Photo Film).

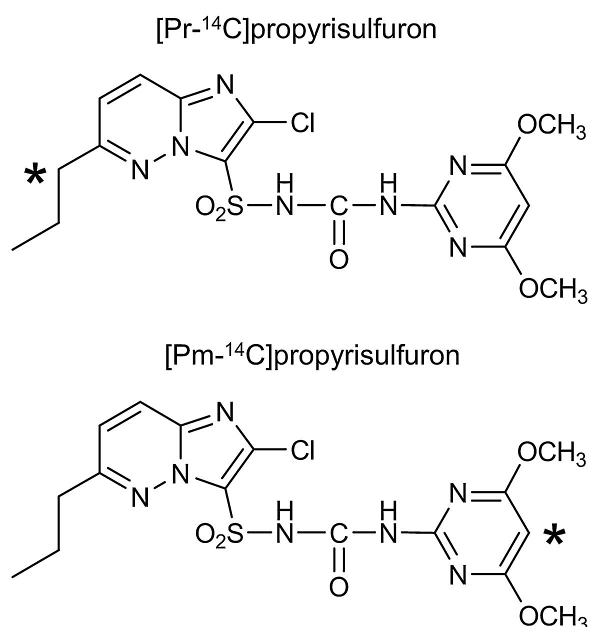

Fig. 2. Chemical structures of [pr-^{14}C]propyrisulfuron and [pm-^{14}C]propyrisulfuron. Asterisks (*) represents the position of ^{14}C.

2.3. High-performance liquid chromatography (HPLC) analysis

HPLC was carried out on a system consisting of an L-7100 or L-6300 HPLC intelligent pump (Hitachi, Ltd., Tokyo, Japan), an L-7400 or L-4200 UV detector (Hitachi, Ltd., Tokyo, Japan), and a Radiomatic 505TR RI detector (PerkinElmer Japan, USA) (RI measurement: 6 s interval) fitted with an Atlantis dC18 column (4.6 mm I.D. × 150 mm, Waters, MA, USA) (column temperature: 20 °C). The injection volume was 5–100 μL depending on the concentration of samples. The mobile phases were acetonitrile: 0.1% (v/v) trifluoroacetic acid in water, (5:95, v/v, 0 min) and (30:70, v/v, 40 min) with a flow rate of 1 mL/min.

2.4. Liquid chromatograph-mass spectrometry (LC–MS) analysis

A liquid chromatograph–mass spectrometer (liquid chromatograph: Finnigan Surveyor, mass spectrometer: LTQ XL, Thermo Fisher Scientific, USA) connected to a Radiomatic 505TR RI detector was used. RI was detected by passing the 1:3 mixture of the mobile phase solution and the scintillation cocktail of Ultimaflow™ AP (PerkinElmer Japan) into the liquid cell of the RI detector. The LC conditions are detailed in Section 2.3. The RI detector was used only for samples with radioactive materials. Electrospray ionization (ESI) was used as the ionization method for mass spectrum analysis, and MS and/or MS^n were measured in the positive or negative ion mode depending on the difference of ionization efficiency of metabolites. Capillary temperature was 200 °C, capillary voltage was 8 V and collision energy was set at 35%.

2.5. Nuclear magnetic resonance (NMR) analysis

Varian UNITY 400 plus type high resolution FT-NMR spectrometer (Agilent, USA) was used to measure the ^{1}H NMR spectrum. Deuterated methanol was used as the solvent.

2.6. Radioanalysis

The radioactivity in urine and bile was measured by a liquid scintillation counter (LSC) (TRI-CARB 2500TR, PerkinElmer Japan). Fecal homogenate was combusted using an oxidizer, and the radioactivity was counted by LSC.

2.7. Animal experiments

Male and female BrlHan:WIST@Jcl (GALAS) rats (Wister Hannover) were obtained from CREA Japan, Inc. Animals were maintained in animal rooms with the following environmental conditions: room temperature between 21 and 25 °C, humidity between 40 and 70%, air ventilation equal to or over 10 times/hour, and 12-h/12-h cycle of light and dark. Before the test substance was administered, animals were housed in econcages (Plastic cage, 290 mm × 340 mm × 170 mm, CREA Japan, Inc.). After the test substance was administered, animals were housed in glass metabolic cages or rat cages. Animals were fed solid feed, CRF-1 (Oriental Yeast Co., Ltd.), and supplied with filtered tap water *ad libitum*. All animal experiments were conducted in accordance with *The Guide for the Care and Use of Laboratory Animals in Environmental Health Science Laboratory of Sumitomo Chemical Co., Ltd.*

Four male and female BrlHan:WIST@Jcl (GALAS) rats seven weeks old were given a single oral dose of [pr-^{14}C]propyrisulfuron at 5 and 1000 mg/5 mL corn oil/kg, and [pm-^{14}C]propyrisulfuron at 5 mg/5 mL corn oil/kg by gavage administration. The low dose

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