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Original Article

Metabolism of Simeconazole in Rats

Koji WAKABAYASHI,* Shingo SADAKANE and Mitsuru ANDO

Agroscience Research Laboratories, Sankyo Agro Co, Ltd., 894 Yasu, Yasu, Shiga 520–2342, Japan

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The excretion, tissue distribution and metabolic fate of simeconazole [(*RS*)-2-(4-fluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-3-trimethylsilyl propane-2-ol] in rats were studied by administering ^{14}C-labeled simeconazole orally to male and female rats at 5 mg/kg b.w. (low dose) and 70 mg/kg b.w. (high dose). The simeconazole was readily absorbed and most of the radioactivity was excreted in the urine and feces within three days. The blood radioactivity level reached a maximum at 4–8 hr and 1–2 hr post-dosing in male and female rats, respectively. The radioactivity in the tissues and organs rapidly decreased with time. Simeconazole was initially metabolized to M1, the hydroxymethyl metabolite. M1 was further metabolized to glucuronide, sulfate, silanols and various dessiloxane compounds.

Keywords: simeconazole, rat, metabolism, sulfate, glucuronide.

INTRODUCTION

Simeconazole [(*RS*)-2-(4-fluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-3-trimethylsilyl propane-2-ol] is an azole antifungal agent used against *Thanatephorus cucumeris*, *Venturia inaequalis*, *Monilinia mali*, *Rhizoctonia solani* AG2-2LP, *Sclerotinia homoeocarpa* and *Ustilago nuda* among others. The mechanism of its antifungal action involves inhibition of the 14-demethylation of lanosterol to form ergosterol, an essential membrane component of fungi. This report describes the excretion, tissue distribution and metabolic fate of simeconazole in rats after the oral administration of a ^{14}C-labeled form of the compound.

MATERIALS AND METHODS

1. Chemical

Triazole-3,5-^{14}C-simeconazole [triazole-3,5-^{14}C-(*RS*)-2-(4-fluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-3-trimethylsilyl propane-2-ol, ^{14}C-simeconazole] was synthesized by Amersham Co. Ltd. The structure and labeled position are shown in Fig. 1. The specific activity was 851 MBq/mmol and the radiochemical purity was higher than 98%, based on a TLC analysis.

2. Treatment of Animals

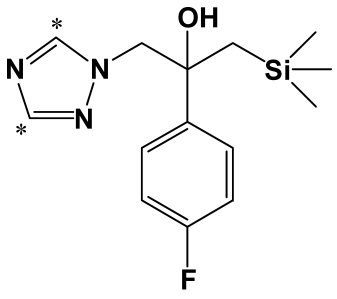
Fischer F-344/DuCrj male and female rats (6 weeks old) were supplied by Charles River Co. Japan Ltd. and were acclimatized for at least one week under the experimental conditions. Food (F-1, Funabashi Nojo Co. Ltd.) and water were provided *ad libitum*.

^{14}C-Simeconazole was diluted as required by the addition of appropriate quantities of non-radiolabeled simeconazole. The solutions for dosing were prepared by dissolving or suspending ^{14}C-simeconazole in a 10% aqueous solution of Tween 80.

Rats were orally administered ^{14}C-simeconazole at a dose of 5 mg/kg b.w. (low dose) and 70 mg/kg b.w. (high dose) using a gastric tube. The high-dose level was one-tenth the acute oral LD_{50} for male and female rats. Rats were fasted overnight pre-dosing and fed approximately 2 hr post-dosing.

3. Excretion

Five male and five female rats were orally administered 5 and 70 mg/kg b.w. of ^{14}C-simeconazole. The treated animals were



^{14}C-simeconazole

Fig. 1. The chemical structure of ^{14}C-labeled simeconazole. (*: ^{14}C-labeled position)

* To whom correspondence should be addressed.
E-mail: kjwaka@sankyo-agro.co.jp

housed individually in metabolic cages to collect urine and feces separately at 24-hr intervals for 7 days. On the 7th day, the rats were sacrificed for the tissue distribution study.

4. Blood Concentration

Six male and six female rats were orally treated with 5 and 70 mg/kg b.w. of ^{14}C-simeconazole. Blood was sampled from three male and three female rats alternately. From the tail vein of the rats at a specified time after the dosing, approximately 50 μl of blood was taken using hematocrit capillary tubes. The blood was weighed and combusted for radioassay. The sampling was conducted at 1, 2, 4, 6, 8, 24, 48, 72, 96, 120, 144 and 168 hr post-dosing.

5. Tissue Distribution

Six male and six female rats were orally administered 5 and 70 mg/kg b.w. of ^{14}C-simeconazole. Three male rats each were sacrificed by decapitation at 6 and 48 hr post-dosing. Three female rats each were sacrificed by decapitation at 2 and 24 hr. The sampling time was decided from the T_{max} and $T_{1/2}$ in the blood concentration study. Rats on day 7 post-dosing in the excretion study were sacrificed for the tissue distribution study. Thereafter, the following organs and tissues were collected for radioassay: blood, plasma, fat, muscle, bone, seminal vesicle, ovaries, uterus, spleen, adrenal gland, kidney, liver, lung, heart, thymus, thyroid, brain and pituitary.

6. Biliary Excretion

Three male and three female rats with bile duct cannulation were administered ^{14}C-simeconazole at a dose of 5 mg/kg b.w. Bile was collected from each rat 0–3, 3–6 and 6–24 hr after the dosing. Urine and feces were collected from each rat at 24 hr post-treatment.

7. Preparation of Samples and Measurement of Radioactivity

7.1. Measurement of radioactivity

Radioactivity was measured using a liquid scintillation counter (LSC-5100, Aloka, abbreviated as LSC). The scintillation cocktail was composed of 8.0 g of 2,5-diphenyloxazole (PPO) and 0.2 g of 1,4-bis(4-methyl-5-phenyl-2-oxazolyl)benzene (dimethyl POPOP) in a mixture of 500 ml each of ethanol and toluene. The counting efficiency was determined by the automatic external standard ratio method. The radioactivity of all samples was measured for 2 min in triplicate with the LSC.

7.2. Preparation of samples for the measurement of radioactivity

The urine and bile were directly mixed with the scintillation cocktail for radioassays using the LSC. The feces were collected and mixed well in a mortar with a pestle. The amount of feces was weighed for combustion. The liver and kidney samples at the C_{max} blood radioactivity level were extracted with acetonitrile/water (8/2, v/v). The fat samples were extracted with hexane. Aliquots of the extracts were mixed with the scintillation cocktail and the residues were combusted for the radioassay. Other organs and tissues were weighed (less than 0.5 g) for combustion.

The samples of feces and tissues were combusted in a sample combustion system (ASC-113, Aloka), in which $^{14}CO_2$ was trapped in monoethanolamine/methanol (2/3, v/v) and mixed with the scintillation cocktail.

7.3. Thin layer chromatography (TLC)

Preparative Silica gel 60 F_{254} plates (Art. 1.05715, MERCK) and RP-18 F_{254S} plates (Art. 15389, MERCK) were used. The developing solvent systems were:

(system 1) TLC plates (60 F_{254}, MERCK) with *n*-butanol/acetic acid/water (8/1/1, v/v).

(system 2) TLC plates (RP-18 F_{254S}, MERCK) with acetonitrile/water (55/45, v/v).

Radioactive areas on the TLC plates were autoradiographically detected with a Bio-imaging analyzer (BAS-1000, Fuji Film, Japan). Unlabeled compounds were detected under a UV lamp at a wavelength of 254 nm.

7.4. High performance liquid chromatography (HPLC)

An HPLC system 600E (Waters) and Symmetry^{TM} C_{18} HPLC column (25 cm×4.6 mm id, Waters) were used for the HPLC analysis. Chromatographic conditions were as follows: mobile phase, acetonitrile/0.2% acetic acid (15/85 or 1/9, v/v); flow rate, 1.0 ml/min; column temperature, 40 °C. Radioactivity in the effluent was monitored using a flow scintillation analyzer (525 TRX, Packard).

8. Isolation and Identification of Major Metabolites

To isolate and identify the urinary and fecal metabolites, simeconazole was administered daily to rats at a dose of 100 mg/kg/day and urine and feces were collected once a day. The feces were extracted with acetonitrile/water=8/2 (v/v), and the extracts were partitioned with dichloromethane under neutral conditions. The aqueous layer was acidified to pH 2 with phosphoric acid and partitioned with ethyl acetate after the addition of 10% sodium chloride. The urine was partitioned in the same manner as the extracts of the feces.

Each metabolite was purified by means of various methods such as by using a cartridge column SEP PAK C_{18} (5 g in weight, Waters), a TLC system, a cartridge column SAX (5 g in weight, International Sorbent Technology) and/or HPLC systems.

The isolated metabolites were subjected to FAB mass spectrometry (JMS-AX500, JEOL) and/or ^{1}H NMR spectrometry (JNM-GX270, JEOL) for identification.

Isolated and identified metabolites were used for the investigation of metabolites in excreta with authentic standards.

9. Metabolites in Excreta

Most of the metabolites were identified by co-chromatography with isolated samples (TLC system 1). M1 and M2 were separated in TLC system 2. M8 stayed at the origin in TLC

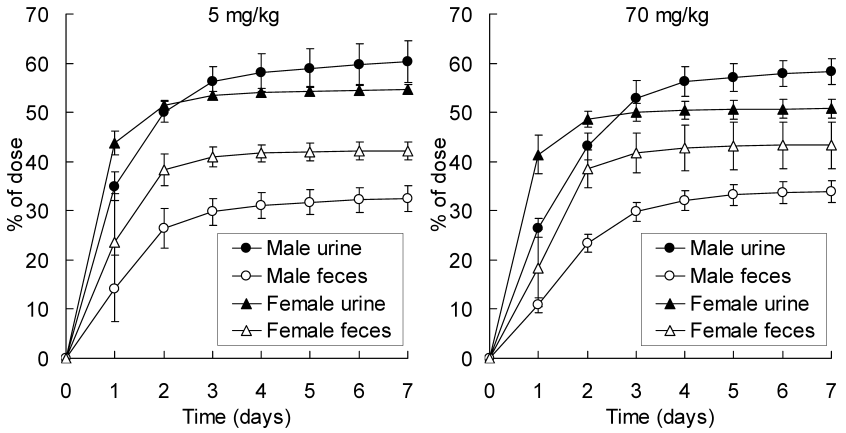


Fig. 2. Cumulative excretion of radioactivity in the urine and feces of rats after an oral administration of ^{14}C-simeconazole.

system 1 but was separated and determined using HPLC system 1.

RESULTS

1. Excretion in Urine and Feces

The excretion of radioactivity in the urine and feces of rats treated with ^{14}C-simeconazole is shown in Fig. 2. At a dose of 5 mg/kg b.w., the total recovery of radioactivity in the urine and feces was 92.7% of the dose in male rats and 96.8% in female rats, within 7 days. The radioactivity in the urinary and fecal excretion was shown to account for 60.3 and 32.4% of the dose in male rats, and 54.6 and 42.2% in female rats, respectively. Most of the urinary and fecal radioactivity was excreted within a 3-day period after the dosing. In a preliminary study, the radioactivity evolved in expiratory air as $^{14}CO_2$ accounted for 0.2% of the dose in both male and female rats. At a dose of 70 mg/kg b.w., the total recovery of radioactivity in the urine and feces was 92.2% of the dose in male rats and 94.2% in female rats, within 7 days. The radioactivity in the urinary and fecal excretion was shown to account for 58.3 and 33.9% of the dose in male rats, and 50.8 and 43.4% in female rats, respectively. No significant difference in the urinary and fecal excretion pattern of radioactivity was observed between dose levels of 5 and 70 mg/kg b.w.

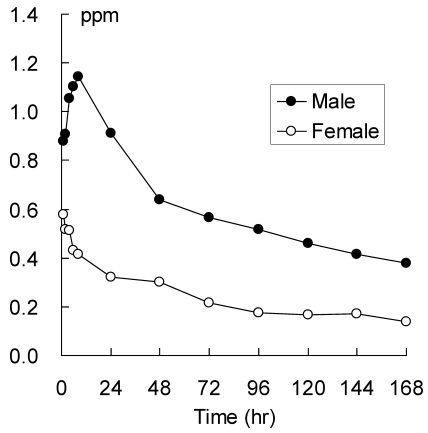


Fig. 3. Time course of change in ^{14}C-levels in blood of rats after an oral administration of ^{14}C-simeconazole at a dose of 5 mg/kg.

2. Blood Concentrations

The concentrations of radioactivity in the blood of rats administered ^{14}C-simeconazole at 5 mg/kg b.w. are shown in Fig. 3. The maximal concentration (C_{max}), time (T_{max}) and half-life of radioactivity in the blood are shown in Table 1. The C_{max} of the radioactivity in the blood of male rats at 5 mg/kg b.w. was 1.14 ppm at 8 hr post-dosing and the half-life was 48 hr. In female rats, the concentration of radioactivity in the blood reached a maximum (C_{max}: 0.58 ppm) at 1 hr after the administration of 5 mg/kg b.w. and the half-life was 26 hr.

3. Tissue Distribution

Table 2 shows the distribution of radioactivity in various tissues and organs.

Based on the data on the concentration of radioactivity in the blood, male rats were sacrificed at 6 and 48 hr and female rats, 2 and 24 hr post-dosing.

In male rats, 6 hr after 5 mg/kg b.w. of ^{14}C-simeconazole was administered, the concentration of radioactivity was highest in the liver (12.62 ppm), followed by the adrenal gland (3.15 ppm), kidney (1.44 ppm), lung (1.37 ppm) and blood

Table 1. T_{max} [a)], C_{max} [b)] and half-life of radioactivity in the blood of rats after an oral administration of ^{14}C-simeconazole

Dose	Sex	T_{max} (hr)	C_{max} (ppm)	Half-life (hr)
5 mg/kg	male	8	1.14	48
	female	1	0.58	26
70 mg/kg	male	4	10.42	86
	female	2	8.08	16

[a)] Time at which the blood radioactivity was maximum. [b)] Concentration of radioactivity in blood at T_{max}.

Table 2. Concentration of radioactivity in tissues of rats after an oral administration of ^{14}C-simeconazole at a dose of 5 mg/kg

Tissue/organ	ppm[a)]					
	Male			Female		
	6 hr	48 hr	168 hr	2 hr	24 hr	168 hr
Whole blood	1.29±0.09	0.77±0.07	0.40±0.02	0.57±0.01	0.33±0.06	0.15±0.01
Plasma	1.30±0.08	—[b)]	—	0.68±0.01	—	—
Fat (subcutaneous)	0.39±0.03	0.10±0.01	0.01±0.00	7.89±0.87	0.11±0.04	<0.01
Fat (peritoneal)	0.35±0.03	0.06±0.01	0.01±0.00	9.83±0.77	0.14±0.09	<0.01
Muscle	0.39±0.02	0.10±0.01	<0.01	0.75±0.00	0.10±0.05	<0.01
Bone	0.30±0.02	0.10±0.01	0.02±0.00	0.61±0.06	0.08±0.03	0.01±0.00
Seminal vesicle	0.46±0.03	0.14±0.01	0.01±0.00	—	—	—
Ovaries	—	—	—	2.21±0.09	0.18±0.07	0.01±0.00
Uterus	—	—	—	1.07±0.22	0.15±0.07	0.02±0.01
Spleen	0.63±0.05	0.31±0.01	0.13±0.01	0.85±0.02	0.17±0.07	0.05±0.00
Adrenal gland	3.15±0.24	0.82±0.16	0.06±0.01	6.28±0.34	1.08±0.25	0.02±0.01
Kidney	1.44±0.12	2.40±0.09	1.47±0.13	2.89±0.33	0.64±0.30	0.78±0.08
Liver	12.62±0.56	6.40±0.58	1.63±0.27	11.44±0.90	1.78±0.77	0.25±0.03
Lung	1.37±0.21	0.77±0.03	0.30±0.02	2.06±0.12	1.40±0.38	0.41±0.01
Heart	0.61±0.05	0.17±0.02	0.03±0.00	1.15±0.04	0.14±0.07	0.01±0.00
Thymus	0.41±0.03	0.12±0.02	0.02±0.00	0.83±0.10	0.13±0.08	<0.01
Thyroid	1.10±0.49	0.31±0.07	—	1.53±0.25	<0.25	—
Brain	0.25±0.01	0.08±0.01	<0.01	1.01±0.04	0.07±0.03	<0.01
Pituitary	0.67±0.15	0.26±0.08	<0.15	1.23±0.31	0.21±0.14	<0.15

[a)] Values are expressed in ppm (μg equivalent of simeconazole/g tissue). [b)] Not collected.

(1.29 ppm). At 48 hr post-dosing, the radioactivity in the tissues had decreased to less than half the initial levels at 6 hr with the exception of the kidney. At 7 days, levels of radioactivity in all the tissues were decreased.

In female rats at 2 hr after the administration of 5 mg/kg b.w., the concentration of radioactivity was highest in the liver (11.44 ppm), followed by the fat (7.89, 9.83 ppm), adrenal gland (6.28 ppm) and kidney (2.89 ppm). The radioactivity in these tissues rapidly decreased at 24 hr post-dosing. The decline in radioactivity was apparently faster than that in male rats. At 7 days post-dosing, the amount of radioactivity in all the tissues was small.

The tissue distribution of radioactivity at 70 mg/kg b.w. was similar to that at 5 mg/kg b.w.

4. *Biliary Excretion*

The bile duct-cannulated rats were orally administered ^{14}C-simeconazole at a dose of 5 mg/kg b.w. The cumulative percentage of radioactivity excreted in the bile of male rats was 52.5 and 70.7% of the dose at 3 and 24 hr, respectively, indicating that the absorption of simeconazole was rapid and the bile was the major route of excretion in rats (see Table 3). The cumulative percentage of radioactivity excreted in the bile of female rats was 18.8 and 57.3% of the dose at 3 and 24 hr, respectively. The biliary excretion of radioactivity was lower in female rats than male rats. Urinary excretion accounted for 4.9 and 13.9% of the dose in bile duct-cannulated male and female rats, respectively, within 24 hr post-dosing. Less than 0.1% of the dose was excreted in the feces of male rats within 24 hr and 0.3% in females. The total recovery of radioactivity from the bile duct-cannulated male and female rats 24 hr after the administration was 75.6 and 71.5% of the dose, respectively. The radioactivity in the carcass was not analyzed in this experiment.

5. *Identification of Major Metabolites*

We isolated one metabolite from the feces and 8 metabolites from the urine of male rats. M1 was isolated from the dichloromethane layer of the feces of male rats. M2, M3 and M4 were isolated from the dicholromethane layer, M5 and M6 were isolated from the ethyl acetate layer, and M7, M8 and M9 were isolated from the aqueous layer of the urine of male rats. The data on ^{1}H NMR and FAB-MS are shown in Table 4. Based on the data, these metabolites were identified as the following, M1: 2-(4-fluorophenyl)-3-hydroxymethyl-dimethylsilyl-1-(1*H*-1,2,4-triazol-1-yl)propan-2-ol, M2: 2-(4-

Table 3. Biliary excretion of radioactivity from bile-duct cannulated rats after an oral administration of ^{14}C-simeconazole at a dose of 5 mg/kg

Time (hr)	Recovery (%)[a)]					
	Male			Female		
	Bile	Urine	Feces	Bile	Urine	Feces
0–3	52.5±9.3	—	—	18.8±6.9	—	—
3–6	9.2±0.8	—	—	17.0±7.2	—	—
6–24	9.0±0.9	4.9±0.8	<0.1	21.5±21.5	13.9±11.1	0.3±0.5
Subtotal	70.7±9.4	4.9±0.8	<0.1	57.3±5.9	13.9±11.1	0.3±0.5
Total	75.6±9.1			71.5±8.5		

[a)] Values are expressed as a percentage of the dose.

fluorophenyl)-3-hydroxydimethylsilyl-1-(1*H*-1,2,4-triazol-1-yl)propan-2-ol, M3: 2-(4-fluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-1,2-diol, M4: 2-(4-fluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propionic acid, M5: 3-(4-fluorophenyl)-3-hydroxy-4-(1*H*-1,2,4-triazol-1-yl)butanoic acid, M6: glucuronide of M1, M7: Sulfate of M1, M8: 2-(4-fluorophenyl)-3-dihydroxymethylsilyl-1-(1*H*-1,2,4-triazol-1-yl)propan-2-ol and M9: glucuronide of M3.

6. Metabolites in Urine and Feces

Radioactive components in the urine and feces excreted within 48 hr post-dosing at 5 mg/kg b.w. are shown in Table 5.

Within 48 hr at a dose of 5 mg/kg b.w., 50.2 and 26.4% of the radioactivity was excreted in the urine and feces of male rats, respectively. The extracts and unextractable residue of the feces accounted for 23.85 and 2.55% of the dose, respectively. The major urinary metabolite identified was M8, accounting for 16.75% of the radioactivity in the urine. The major fecal metabolite identified was M2, accounting for 10.24% of the radioactivity in the feces.

Within 48 hr at 5 mg/kg b.w., 51.4 and 38.3% of the radioactivity was excreted in the urine and feces of female rats, respectively. The extracts and unextractable residue of the feces accounted for 37.13 and 1.17% of the dose, respectively. The major urinary and fecal metabolite identified was a sulfate conjugate of M1 (M7), accounting for 34.88 and 31.59% of the radioactivity in the urine and feces, respectively.

Proportions of radioactive components in the urine and feces of rats at 70 mg/kg b.w. were almost the same as those at 5 mg/kg b.w.

7. Metabolites in the Bile

The proportions of radioactive components in the bile after 5 mg/kg b.w. of ^{14}C-simeconazole was administered are shown in Table 6.

The bile duct-cannulated rats were treated with ^{14}C-simeconazole at 5 mg/kg b.w. In male rats, 70.7% of the radioactivity was excreted in the bile within 24 hr. Proportions of radioactive components in the bile of male rats excreted within 24 hr were analyzed by TLC. One major metabolite was found, which was identified to be a glucuronide of M1 (M6), accounting for 56.54% of the radioactivity (79.97% of biliary radioactivity).

In female rats, 57.3% of the radioactivity was excreted in the bile within 24 hr. The two major metabolites were identified to be M6 and M7 (sulfate of M1), accounting for 35.61 and 16.57% of the radioactivity, respectively (62.15 and 28.92% of the biliary radioactivity, respectively).

DISCUSSION

Almost all the simeconazole was rapidly absorbed and excreted within 72 hr. The excretion of radioactivity seemed to be as fast as for other triazole fungicides in rats.[1–9)]

The major biotransformation reactions of simeconazole in rats are based on the oxidation of the trimethyl silyl group. The initial and most predominant metabolism was hydroxylation of the silyl-methyl group to form M1. This reaction also occurs in other organisms such as plants.[10)] M1 was subsequently conjugated to the glucuronide (M6) and the sulfate (M7) in rats. Further metabolism of M1 occurred at the trimethyl silyl moiety as follows. The methyl group of M1 was oxidized to the silanol metabolite (M2) and silan-diol metabolite (M8). M1, M2 and M8 were further metabolized to a diol metabolite (M3) by oxidative desilylation. M3 was oxidized to a carboxylic acid metabolite (M4) and conjugated to the glucuronide (M9). M5 is a unique metabolite in that its carbon-chain was elongated. The chemical structure of M5 was unequivocally confirmed using co-chromatography with a synthetic compound. The metabolic pathway of M5 is still being examined. The proposed metabolic pathways of simeconazole in rats are shown in Fig. 4.

In this study, two conjugated metabolites of M1, the glucuronide (M6) and sulfate (M7) were identified. The biliary excretion experiment showed that M6 was rapidly excreted in

Table 4. Data on ^{1}H NMR and MS of major metabolites

Metabolite	^{1}H NMR spectrum δppm (CD_3OD)	MS (*m*/*z*)
M1	−0.35 (3H, s, CH_3–Si), −0.14 (3H, s, CH_3–Si), 1.31 (1H, d, *J*=14.5 Hz, C–CH_2–Si), 1.61 (1H, d, *J*=14.5 Hz, C–CH_2–Si), 3.06 (1H, d, *J*=14.0 Hz, Si–CH_2–O), 3.12 (1H, d, *J*=14.0 Hz, Si–CH_2–O), 4.43 (1H, d, *J*=14.2 Hz, N–CH_2–C), 4.49 (1H, d, *J*=14.2 Hz, N–CH_2–C), 6.95–7.04 (2H, m, Ph), 7.37–7.45 (2H, m, Ph), 7.81 (1H, s, triazole), 8.14 (1H, s, triazole)	310 (M+1)
M2[a)]	−0.33 (3H, s, CH_3–Si), 0.06 (3H, s, CH_3–Si), 1.26 (1H, d, *J*=14.5 Hz, C–CH_2–Si), 1.34 (1H, d, *J*=14.5 Hz, C–CH_2–Si), 4.33 (1H, d, *J*=13.9 Hz, N–CH_2–C), 4.43 (1H, d, *J*=13.9 Hz, N–CH_2–C), 6.96–7.02 (2H, m, Ph), 7.28–7.36 (2H, m, Ph), 7.86 (1H, s, triazole), 7.88 (1H, s, triazole)	296 (M+1)
M3	3.67 (1H, d, *J*=11.5 Hz, C$\underline{H}_2$–OH), 3.75 (1H, d, *J*=11.5 Hz, C$\underline{H}_2$–OH), 4.59 (1H, d, *J*=14.3 Hz, N–CH_2–C), 4.65 (1H, d, *J*=14.3 Hz, N–CH_2–C), 6.96–7.05 (2H, m, Ph), 7.41–7.49 (2H, m, Ph), 7.83 (1H, s, triazole), 8.22 (1H, s, triazole)	238 (M+1)
M4	4.47 (1H, d, *J*=14.1 Hz, N–CH_2–C), 4.54 (1H, d, *J*=14.1 Hz, N–CH_2–C), 6.98–7.07 (2H, m, Ph), 7.38–7.45 (2H, m, Ph), 7.84 (1H, s, triazole), 8.21 (1H, s, triazole)	252 (M+1)
M5	2.81 (1H, d, *J*=15.7 Hz, C–CH_2–C), 3.04 (1H, d, *J*=15.7 Hz, C–CH_2–C), 4.53 (1H, d, *J*=14.1 Hz, N–CH_2–C), 4.64 (1H, d, *J*=14.1 Hz, N–CH_2–C), 7.01 (2H, t, *J*=8.9 Hz, Ph), 7.43 (2H, dd, *J*=5.2, 8.9 Hz, Ph), 7.85 (1H, s, triazole), 8.23 (1H, s, triazole)	266 (M+1)
M6	−0.22 (3H, s, CH_3–Si), −0.16 (3H, s, CH_3–Si), 1.60 (1H, d, *J*=7.3 Hz, C–CH_2–Si), 1.66 (1H, d, *J*=7.3 Hz, C–CH_2–Si), 3.00 (1H, d, *J*=12.9 Hz, Si–CH_2–O), 3.18–3.25 (1H, m, sugar), 3.29–3.34 (1H, m, sugar), 3.50–3.56 (1H, m, sugar), 3.54 (1H, d, *J*=12.9 Hz, Si–CH_2–O), 3.75 (1H, d, *J*=9.7 Hz, sugar), 4.08–4.17 (1H, m, sugar), 4.45 (1H, d, *J*=14.3 Hz, N–CH_2–C), 4.51 (1H, d, *J*=14.3 Hz, N–CH_2–C), 6.87–7.01 (2H, m, Ph), 7.32–7.44 (2H, m, Ph), 7.80 (1H, s, triazole), 8.19 (1H, s, triazole)	486 (M+1)
M7	−0.20 (3H, s, CH_3–Si), −0.14 (3H, s, CH_3–Si), 1.41 (1H, d, *J*=14.9 Hz, C–CH_2–Si), 1.62 (1H, d, *J*=14.9 Hz, C–CH_2–Si), 3.55 (1H, d, *J*=12.9 Hz, Si–CH_2–O), 3.62 (1H, d, *J*=12.9 Hz, Si–CH_2–O), 4.45 (1H, d, *J*=13.9 Hz, N–CH_2–C), 4.54 (1H, d, *J*=13.9 Hz, N–CH_2–C), 6.93–7.02 (2H, m, Ph), 7.35–7.43 (2H, m, Ph), 7.77 (1H, s, triazole), 8.10 (1H, s, triazole)	434 (M+2Na−1)
M8	—	298 (M+1)
M9	3.24–3.32 (2H, m, sugar), 3.36–3.45 (1H, m, sugar), 3.49–3.56 (1H, m, sugar), 3.60–3.87 (1H, m, C–CH_2–O), 4.17–4.27 (1H, m, C–CH_2–O), 4.40 (1H, d, *J*=7.7 Hz, sugar), 4.64 (1H, d, *J*=14.1 Hz, N–CH_2–C), 4.74 (1H, d, *J*=14.1 Hz, N–CH_2–C), 6.97–7.04 (2H, m, Ph), 7.43–7.51 (2H, m, Ph), 7.82 (1H, s, triazole), 8.27 (1H, s, triazole)	—

[a)] ^{1}H NMR of M2 was analyzed in $CDCl_3$ solution.

the bile of male rats. In female rats, M6 and M7 were rapidly excreted in the bile. This indicates that female rats have a higher sulfate conjugation rate for M1 and/or a lower glucuronide conjugation rate than male rats. The sulfate conjugation activity of the alcohol group was reported to be greater in the female rat than the male rat.[11–13)] The strong activity for sulfate conjugation of M1 in female rats corresponded with the reported data.

Table 5. Proportions of radioactive components in the urine and feces after a single oral administration of ^{14}C-simeconazole

	Recovery (%)[a]			
Dose	5 mg/kg		70 mg/kg	
Sex	Male[b]	Female[b]	Male[b]	Female[b]
Sampling time	0–48 h	0–48 h	0–72 h	0–48 h
Urine	50.2	51.4	52.8	48.6
M8	16.75	4.81	12.89	5.45
M9 (gluc. of M3)	0.58	n.d.[c]	0.81	n.d.
M6 (gluc. of M1)	2.05	0.83	2.24	0.36
Triazole	7.17	1.84	7.84	1.83
M7 (sulf. of M1)	1.00	34.88	1.09	34.85
M5	8.54	0.97	7.80	1.45
M3 + M4	4.38[d]	1.26[d]	5.07[d]	1.80[d]
M2	7.74	1.79	12.29	n.d.
M1	0.73	4.71	1.73	2.92
Others	1.26	0.31	1.04	n.d.
Feces	26.4	38.3	29.8	38.5
Extracts	23.85	37.13	27.80	37.76
M8	0.49	n.d.	0.44	0.84
M9 (gluc. of M3)	3.55	0.54	1.99	0.16
M6 (gluc. of M1)	0.32	n.d.	n.d.	n.d.
M7 (sulf. of M1)	1.65	31.59	8.23	34.88
M5	1.90	n.d.	1.19	0.09
M3 + M4	1.53[d]	0.38[d]	1.34[d]	0.42[d]
M2	10.24	2.33	5.62	0.20
M1	2.51	1.67	5.01	0.35
Others	1.66	0.62	3.98	0.94
Unextractable residue	2.55	1.17	2.00	0.74
Total	76.6	89.7	82.6	87.1

[a] Values are expressed as a percentage of the dose. [b] These samples were pooled from 5 rats before the analysis. [c] n.d. means not detected. [d] Sum of M3 and M4.

The biliary excretion of radioactivity within 24 hr after the administration of ^{14}C-simeconazole was obviously much greater than the fecal excretion of radioactivity shown in Fig. 2. The glucuronide metabolite M6, the major metabolite in the bile, was not found in the feces of male or female rats. These results indicate that the deconjugation of M6 occurs in both sexes. M6 may have been hydrolyzed to M1 in the intestine and M1 may have been reabsorbed and exposed to the oxidative enzymes.

In the blood concentration analysis, the level of radioactivity was lower in female rats than male rat at every sampling point, and the T_{max} of female rats came earlier than that of male rats. This phenomenon is explained by the rapid excretion of M7 composed in female rats. In contrast to M6, the sulfate metabolite M7 was relatively stable against hydrolyzation in the intestine. The hydrolysis experiment showed that

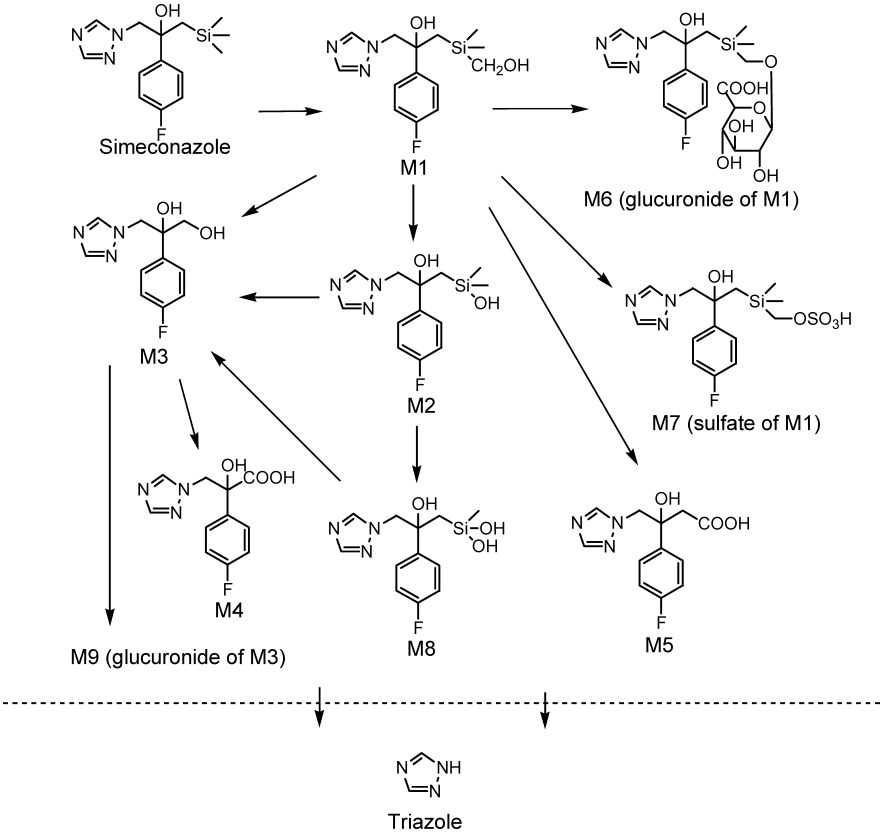


Fig. 4. The proposed metabolic pathways of simeconazole in rats.

Table 6. Proportions (by TLC) of radioactive components in the bile of male and female rats excreted within 24 hr after the administration of ^{14}C-simeconazole at a dose of 5 mg/kg

Metabolites	Recovery (%)[a]	
	Male[b]	Female[b]
M8	0.18	n.d.[c]
M9 (gluc. of M3)	1.34	n.d.
M6 (gluc. of M1)	56.54	35.61
M7 (sulf. of M1)	0.58	16.57
M5	0.38	n.d.
M3) M4	0.05[d]	n.d.
M2) M1	11.52[e]	2.51[e]
Others	0.11	2.61
Total	70.7	57.3

[a] Values are expressed as a percentage of the dose. [b] These samples were pooled from 3 rats before the analysis. [c] n.d. means not detected. [d] Sum of M3 and M4. [e] Sum of M2 and M1.

M7 was stable in the presence of sulfatases from several species. M7 was readily excreted in both feces and urine in rats. As a result of the rapid excretion of M7, the levels of radioactivity in the tissues decreased faster in female than male rats.

M1 has two hydroxyl groups and it is necessary to clarify whether the conjugation occurs on the tertiary or primary hydroxyl group. We predicted the conjugation site based on the change of chemical sifts in the ^{1}H NMR spectra (see Fig. 5). One of the methylene groups is next to the primary hydroxyl group. The conjugation caused a considerable chemical shift, approximately 0.4–0.5 ppm, of the methylene indicating that the conjugation must have occurred on the primary hydroxyl group. Taking into consideration the steric hindrance, conjugation would occur more easily on the primary alcohol group than on the tertiary alcohol group.

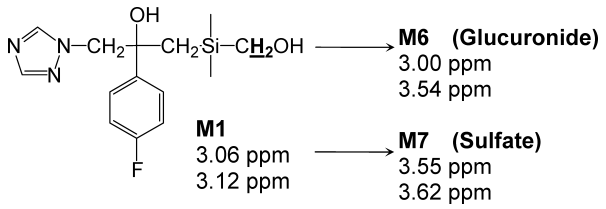


Fig. 5. ^{1}H NMR chemical shift value of the hydroxymethyl group in M1 and that of the conjugation.

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