Metabolism of Pentyl 2-Chloro-4-fluoro-5-(3,4,5,6-tetrahydrophthalimido)phenoxyacetate (Flumiclorac Pentyl, S-23031) in Rats. 2. Absorption, Distribution, Biotransformation, and Excretion

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Male and female Sprague-Dawley rats, 7 weeks old, were given a single oral dose of [*phenyl*-^{14}C]-S-23031 [pentyl 2-chloro-4-fluoro-5-(3,4,5,6-tetrahydrophthalimido)phenoxyacetate] or [*tetrahydrophthaloyl*-1,2-^{14}C]-S-23031 ([*THP*-^{14}C]-S-23031) at 1 or 500 mg/kg. ^{14}C was rapidly excreted into feces and urine, and more than 90% of the dosed ^{14}C was recovered within 48 h post-treatment. ^{14}C levels in tissues and blood were generally low on the seventh day after dosing. However, those with [*THP*-^{14}C]-S-23031 were relatively higher than those with [*phenyl*-^{14}C]-S-23031. S-23031 was rapidly and extensively metabolized. Major fecal metabolites were the sulfonic acid conjugates, and the major urinary metabolite was 5-amino-2-chloro-4-fluorophenoxyacetic acid. The main metabolic reactions were (1) cleavage of the ester linkage, (2) cleavage of the imide linkage, (3) hydroxylation at the cyclohexene ring of the 3,4,5,6-tetrahydrophthalimide moiety or the cyclohexane ring of the cyclohexane-1,2-dicarboxylic acid moiety, and (4) incorporation of a sulfonic acid group into the >C═C< double bond of the 3,4,5,6-tetrahydrophthalimide moiety.

Keywords: *Flumiclorac pentyl; metabolism; rat; absorption; distribution; biotransformation; excretion*

INTRODUCTION

Pentyl 2-chloro-4-fluoro-5-(3,4,5,6-tetrahydrophthalimido)phenoxyacetate (S-23031, flumiclorac pentyl, Resource) is a new selective, postemergence herbicide for control of broad-leaved weeds in soybean fields. The weed control spectrum of S-23031 includes troublesome weeds such as velvetleaf, prickly sida, jimsonweed, and common lambsquarters (Kamoshita *et al*., 1992).

One urinary and four fecal major metabolites of S-23031 were isolated and identified in the previous study (Matsunaga *et al*., 1996). We found unique fecal metabolites having a sulfonic acid group in that study. Sulfonic acid metabolites were also found in metabolic studies of S-23121 (Yoshino *et al*., 1993a) and tetramethrin (Tomigahara *et al*., 1994), which share the same tetrahydrophthalimide (THP) moiety.

The present paper describes the metabolic fate (absorption, distribution, biotransformation, and excretion) of [*phenyl*-^{14}C]- and [*THP*-^{14}C]-S-23031 following oral administration to rats at 1 (low dose) or 500 (high dose) mg/kg.

MATERIALS AND METHODS

Chemicals. Chemicals used and their abbreviations are listed in Table 1. S-23031 labeled uniformly with ^{14}C in the phenyl group ([*phenyl*-^{14}C]-S-23031) with a specific activity of 7.22 GBq/mmol (195 mCi/mmol) or labeled at the alpha-carbons in the THP moiety ([*THP*-^{14}C]-S-23031) with 3.89 GBq/mmol (105 mCi/mmol) was synthesized in our laboratory. The radiochemical purities of the labeled compounds were >99% on thin-layer chromatography (TLC). Unlabeled S-23031 (chemical purity >99%) was also prepared in our laboratory.

AFCA and IMCA were synthesized by Sumika Chemical Analysis Service Ltd. (Osaka, Japan). THPA, 4-OH-THPA, and 1-OH-HPA were synthesized in our laboratory. IMCA-SA, 4-OH-IMCA-SA1, 4-OH-IMCA-SA2, and 5-OH-IMCA-SA were purified from feces, and 4-OH-IMCA was purified from the urine of rat given [*phenyl*-^{14}C]-S-23031, as identified in our previous study (Matsunaga *et al*., 1996) and used as authentic standards for chromatography.

Chromatography. *1. TLC.* TLC analysis was carried out according to the methods described in previous papers (Kaneko *et al*., 1988; Matsunaga *et al*., 1995; Saito *et al*., 1991). Precoated silica gel 60 F_{254} chromatoplates (20 × 20 cm, 0.25 mm layer thickness, Merck, Darmstadt, Germany) and RP-18 F_{254}s chromatoplates (20 × 20 cm, 0.25 mm layer thickness, Merck) were used for analysis and purification. R_f values for the authentic standards and solvent systems used are shown in Table 2. Unlabeled compounds on TLC plates were detected under UV light or by color reaction by spraying Bromocresol Purple (BCP) or 2,6-dichlorophenolindophenol sodium salt. Radioactive spots on the TLC plates were detected by autoradiography using X-ray films (SB-5, Kodak, Rochester, NY), developed with a M6B processor or imaging plates processed with a BAS 2000 image analyzer (Fuji Photo Film Co., Ltd., Minamiashigara, Japan).

2. High-Performance Liquid Chromatography (HPLC). Some metabolites were isolated by preparative TLC, followed by HPLC analysis according to the methods reported previously (Yoshino *et al*., 1993a). HPLC was performed with the following equipment: an L-6200 Intelligent pump (Hitachi Ltd., Hitachinaka, Japan), an L-4200 UV−vis detector (Hitachi), and an LB 507A HPLC radioactivity monitor (Berthold, Wildbad, Germany), using a SUMIPAX ODS A-212 column, 6 mm i.d. × 15 cm, 5 μm particle size (Sumika Chemical Analysis Service). UV detection was carried out at 260 nm except for 1-OH-HPA and IMCA standards, which were detected at 240 nm. Data from UV and radioactivity detectors were collected and analyzed by a C-R7A Chromatopac (Shimadzu Co., Kyoto, Japan). The following gradient conditions were used: solvent a, water (0.1% trifluoroacetic acid); solvent b, acetonitrile; (A) start with 100% a, hold for 10 min, linear gradient to 100% b from 10 to 60 min, and hold at 100% b from 60 to 75 min; (B)

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S0021-8561(96)00312-3 CCC: $14.00

Table 1. Chemical Names of S-23031 and Its Derivatives and Their Abbreviations

abbreviation	chemical name
S-23031	pentyl 2-chloro-4-fluoro-5-(3,4,5,6-tetrahydrophthalimido)phenoxyacetate
IMCA	2-chloro-4-fluoro-5-(3,4,5,6-tetrahydrophthalimido)phenoxyacetic acid
AFCA	5-amino-2-chloro-4-fluorophenoxyacetic acid
4-OH-IMCA	2-chloro-4-fluoro-5-(4-hydroxy-3,4,5,6-tetrahydrophthalimido)phenoxyacetic acid
IMCA-SA	2-chloro-4-fluoro-5-(1-sulfo-1,2-cyclohexanedicarboximido)phenoxyacetic acid
4-OH-IMCA-SA1 and 4-OH-IMCA-SA2[a]	2-chloro-4-fluoro-5-(4-hydroxy-1-sulfo-1,2-cyclohexanedicarboximido)phenoxyacetic acid
5-OH-IMCA-SA	2-chloro-4-fluoro-5-(5-hydroxy-1-sulfo-1,2-cyclohexanedicarboximido)phenoxyacetic acid
THPA	3,4,5,6-tetrahydrophthalic acid
4-OH-THPA	4-hydroxy-1-cyclohexene-1,2-dicarboxylic acid
1-OH-HPA	1-hydroxy-*trans*-1,2-cyclohexanedicarboxylic acid

[a] Presumed to be stereoisomers, though their absolute configurations have not been determined.

Table 2. TLC R_f Values of Authentic Standards

	R_f value in solvent system[a]				
compound	A	B	C	D	E
S-23031	0.76	0.83	0.00	0.47	0.62
IMCA	0.49	0.66	0.36	—[b]	—
AFCA[c]	0.45	0.60	—	—	—
THPA	0.34	0.61	0.75	—	—
4-OH-IMCA	0.34	0.54	0.53	—	—
1-OH-HPA	0.26	0.45	—	—	—
4-OH-THPA	0.11	0.42	0.94	—	—
IMCA-SA	0.06	0.35	0.72	—	—
OH-IMCA-SA (mix)[d]	0.00	0.21	0.82	—	—

[a] Solvent systems: (A) toluene/ethyl formate/formic acid (5:7:1), (B) 1-butanol/acetic acid/water (6:1:1), (C) acetonitrile/water (2:3), (D) chloroform, (E) toluene/diethyl ether (5:1). [b] Not analyzed. [c] Data from the [*phenyl*-^{14}C]-S-23031 study were normalized, and the other data were from the [*THP*-^{14}C]-S-23031 study. [d] Mixture of 4-OH-IMCA-SA1, 4-OH-IMCA-SA2, and 5-OH-IMCA-SA.

Table 3. HPLC Retention Time Values (R_t) for Authentic Standards[a]

chemical	gradient	UV detection (nm)	R_t (min)
IMCA	A	240	38.1
THPA	A	260	22.1
4-OH-THPA	A	260	10.7
1-OH-HPA	B	240	20.7

[a] Gradient conditions are described in the text.

start with 100% a, hold for 10 min, then change to 100% b, and wash the column with 100% b. The flow rate was 2 mL/min. The retention time values of authentic standards by HPLC are shown in Table 3.

Radioanalysis. Quantitation of radioactivity was performed according to the methods described previously (Matsunaga *et al*., 1995; Yoshino *et al*., 1993b). Radioactivity in fecal extracts, urine, and silica gel regions scraped from TLC plates was quantified by liquid scintillation counting (LSC) with a Tri-Carb 460CD spectrometer (Packard, Meriden, CT). Radioactivity in fecal homogenates, unextractable fecal residues, and tissue samples was quantified by combustion analysis using a Tri-Carb 306 sample oxidizer (Packard) prior to LSC. Eight milliliters of Oxisorb-CO_2 and 12 mL of Oxiprep-2 (New England Nuclear, Boston, MA) were used as $^{14}CO_2$ absorbent and scintillator, respectively. ^{14}C recovery was 95% or more for the combustion.

Animal Treatment and Sample Collection. Male and female Sprague-Dawley rats, 6 weeks old, were purchased from Charles River Japan Inc. and maintained in an air-conditioned room at 22−24 °C with an alternate 12-h light and dark cycle for 1 week before use. The rats were allowed free access to pelleted diet (CRF-1, Oriental Yeast Inc., Tokyo, Japan) and water *ad libitum* during all treatments.

For ^{14}C excretion and ^{14}C tissue residue studies, groups of five male and five female rats were given a single oral dose of [*phenyl*-^{14}C]-S-23031 or [*THP*-^{14}C]-S-23031 at 1 or 500 mg/kg. These dose levels were chosen because S-23031 had no observable effect at 1 mg/kg but caused elevated water intake, decrease in spleen weight, and lower activity of alkaline phosphatase in blood at 500 mg/kg dose level in toxicity studies (Yoshida *et al*., unpublished observation). The amounts of radioactivity given were 9.25−9.53 MBq/kg. After administration, all rats were placed individually in Metabolica CO_2 cages (Sugiyamagen Iriki Co., Ltd., Tokyo, Japan) and their feces and urine were collected separately. On the seventh day after dosing, rats were sacrificed by collecting whole blood from their abdominal arteries, and their tissues were dissected out to allow analysis of ^{14}C levels. The following samples were collected: adrenals, blood (aliquots were separated into blood cells and plasma by centrifugation), bone (and bone marrow), brain, fat, heart, kidneys, liver, lungs, muscle, ovaries, pancreas, spleen, testes, thyroid, uterus, and the residual carcass.

For the ^{14}C tissue distribution study, 18 male rats were given a single oral administration of [*phenyl*-^{14}C]-S-23031 at 1 mg/kg (low dose) and 6 groups of rats (3 rats per group) were sacrificed by collecting whole blood from abdominal arteries at 1, 2, 4, 8, 24, and 48 h after dosing, followed by dissection to remove the kidneys and liver.

For the ^{14}C bile excretion study, six male rats underwent a surgical operation on their bile duct. Each bile duct was cannulated *in situ* with a PE-10 Intramedic polyethylene tube (Clay Adams, Becton Dickinson, Franklin Lakes, NJ) as described earlier (Isobe *et al*., 1987). One group of three bile duct-cannulated rats was dosed orally with [*phenyl*-^{14}C]-S-23031 at 1 mg/kg and the other group of three rats at 500 mg/kg. Following the ^{14}C treatment, rats were individually placed in Bollman cages (Natsume Product, Ltd., Tokyo, Japan) until sacrifice. Each rat was sacrificed 48 h postdosing in the same manner as mentioned above.

Quantitation of Metabolites in Feces and Urine. Feces and urine from each rat were collected at 6 h (urine only) and 1, 2, 3, 5, and 7 days after administration. The 0−2 day fecal samples from each rat on each collection day were homogenized in acetonitrile (5 mL/g feces) using a Waring blender (Nihonseiki Co., Tokyo, Japan), and the homogenates were centrifuged at 3000 rpm (*ca*. 1500*g*) for 10 min. The precipitates were then extracted three times with 1:1 acetonitrile/water. The collected fecal extracts were concentrated with a rotary evaporator.

Metabolites in the 0−2 day fecal extracts and urine samples were tentatively identified by TLC and partly by HPLC cochromatography with authentic standards. The quantification of each metabolite was conducted as follows: fecal extracts and urine were subjected to TLC, and then relatively nonpolar metabolites were developed with solvent A (5:7:1 toluene/ethyl formate/formic acid), while polar metabolites were developed with solvent B (6:1:1 1-butanol/water/acetic acid). Two-dimensional TLC with a combination of the above-mentioned solvent systems indicated nonpolar metabolites to be distinguishable from polar metabolites (data not shown).

RESULTS

^{14}C Excretion. Curves for cumulative ^{14}C excretion into feces and urine over 7 days after a single oral administration of [*phenyl*-^{14}C]-S-23031 and [*THP*-^{14}C]-S-23031 are shown in Figure 1. ^{14}C was rapidly and almost completely excreted into urine and feces; more than 93% of the dosed ^{14}C was recovered within 48 h postdosing from all dose groups, and total ^{14}C recoveries within 7 days after dosing were 94−100% of the dosed

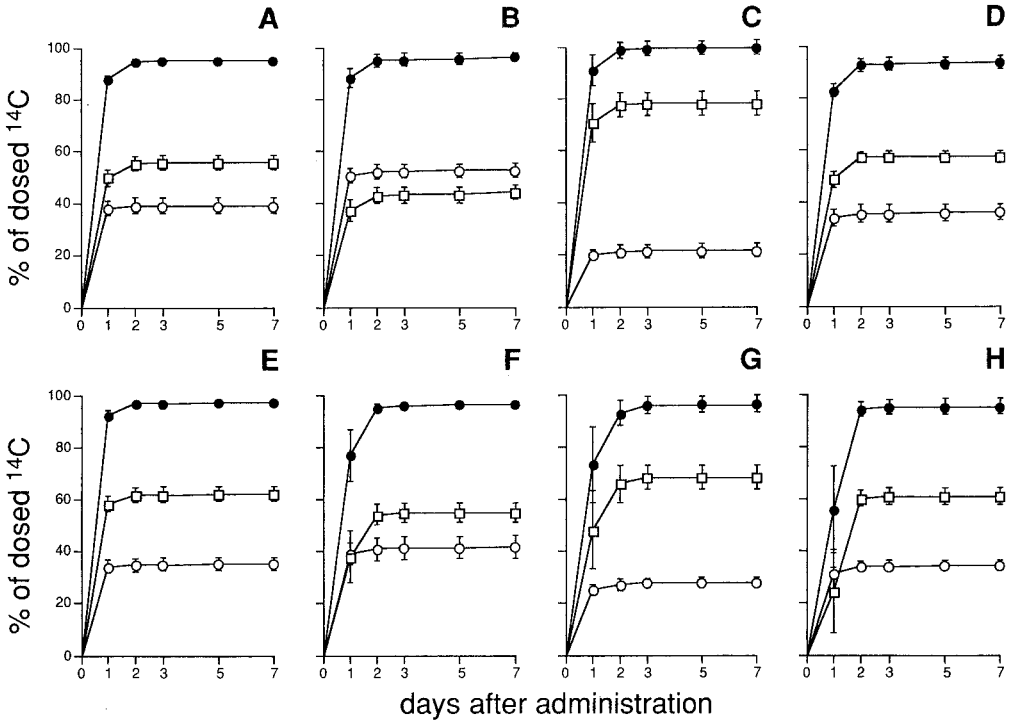

Figure 1. Cumulative ^{14}C excretion into feces and urine of rats after a single oral administration of [*phenyl*-^{14}C]-S-23031 [(A) male, at a dose of 1 mg/kg; (B) female, at a dose of 1 mg/kg; (C) male, at a dose of 500 mg/kg; (D) female, at a dose of 500 mg/kg] or [*THP*-^{14}C]-S-23031 [(E) male, at a dose of 1 mg/kg; (F) female, at a dose of 1 mg/kg; (G) male, at a dose of 500 mg/kg; (H) female, at a dose of 500 mg/kg]. (□) Feces; (○) urine; (●) total. Results are mean ± SD values ($n = 5$).

Table 4. Cumulative ^{14}C Recoveries in Bile, Feces, Urine, and Carcass within 48 h after a Single Oral Administration of [*phenyl*-^{14}C]-S-23031 to Male Rats at 1 (Low Dose) or 500 (High Dose) mg/kg[a]

	% of dosed ^{14}C at		
	6 h after admin	24 h after admin	48 h after admin
	1 mg/kg (Low Dose)		
bile	7.7 ± 6.5	18.0 ± 4.8	18.8 ± 5.5
feces	–[b]	20.3 ± 17.3	33.1 ± 10.6
urine	–	41.3 ± 9.8	55.0 ± 7.0
carcass	–	–	3.9 ± 1.9
total	7.7 ± 6.5	79.5 ± 9.3	110.8 ± 10.2
	500 mg/kg (High Dose)		
bile	8.0 ± 6.1	16.5 ± 10.9	18.5 ± 9.9
feces	–	17.4 ± 16.3	44.0 ± 7.1
urine	–	24.0 ± 7.7	30.9 ± 3.4
carcass	–	–	5.0 ± 0.8
total	8.0 ± 6.1	57.9 ± 31.0	98.5 ± 5.0

[a] Data are means ± SD for three rats. [b] Not applicable.

^{14}C. Fecal ^{14}C excretion predominated except in the females given the low dose of [*phenyl*-^{14}C]-S-23031.

Data for ^{14}C excretion into bile, urine, and feces within 48 h post-treatment are shown in Table 4. Biliary excretion accounted for about 19% of the dosed ^{14}C in both low- and high-dose groups.

^{14}C Tissue Levels. ^{14}C levels in blood, kidney, and liver of male rats after treatment with [*phenyl*-^{14}C]-S-23031 at 1 mg/kg are shown in Figure 2. ^{14}C concentrations reached maxima at 2 h post-treatment, being 0.13, 1.03, and 0.86 μg of S-23031 equiv/g of tissue (ppm) in blood, kidney, and liver, respectively. ^{14}C levels declined thereafter to 24 h postdosing with biological half-lives of 7.7, 4.9, and 4.6 h for blood, kidney, and liver, respectively.

^{14}C tissue residue levels on the seventh day after administration of the ^{14}C-labeled compound are shown in Table 5. ^{14}C tissue residue levels were generally very

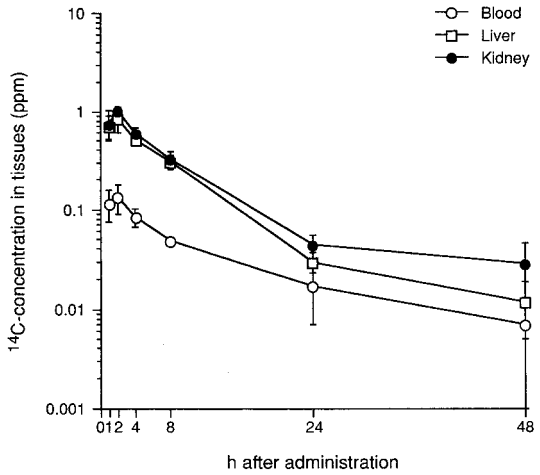

Figure 2. ^{14}C Concentrations in the blood (○), kidney (●), and liver (□) of male rats after a single oral administration of [*phenyl*-^{14}C]-S-23031 at 1 mg /kg. Results are mean ± SD values ($n = 3$).

low, being below the equivalent of 6 ng of S-23031/g of tissue (ppb) and <2.50 ppm in the low- and high-dose groups, respectively. ^{14}C residue levels in blood and kidney were somewhat higher than those in any other of the examined tissues for both sexes of all dosed groups. [*THP*-^{14}C]-S-23031-dosed groups showed relatively higher ^{14}C residues than [*phenyl*-^{14}C]-S-23031 dosed groups. In all groups, tissue and blood contained <0.2% of the dosed ^{14}C.

Metabolites in Bile, Feces, and Urine. Figure 3 shows representative HPLC chromatograms of the metabolite tentatively identified by co-injection with the synthetic standard. Table 6 shows the amounts (expressed as percentages of the dosed ^{14}C) of fecal and urinary metabolites quantified with TLC. The major fecal imide metabolites, IMCA, IMCA-SA, and a mixture of 4-OH-IMCA-SA1, 4-OH-IMCA-SA2, and 5-OH-IMCA-

Table 5. ^{14}C-Labeled Tissue Residues in Male and Female Rats on the Seventh Day after a Single Oral Administration of [*phenyl*-^{14}C]-S-23031 or [*THP*-^{14}C]-S-23031 at 1 (Low Dose) or 500 (High Dose) mg/kg[a]

	μg or ng S-23031 equiv/g of tissue (ppm or ppb)							
	low dose (ppb)				high dose (ppm)			
	[*phenyl*-^{14}C]		[*THP*-^{14}C]		[*phenyl*-^{14}C]		[*THP*-^{14}C]	
tissue	male	female	male	female	male	female	male	female
adrenal	<1.7	<1.2	<3.7	<3.0	<0.64	<0.49	<1.39	<0.99
blood	0.4[d]	0.5[b]	3.1	1.9	<0.14	<0.13	1.08	1.38
bone	<0.3	<0.3	0.9	0.9	<0.13	<0.14	0.37[e]	0.26
brain	<0.3	<0.3	<0.3	<0.3	<0.14	<0.14	<0.13	<0.11
fat	<0.8	<0.6	<0.6	<0.7	<0.29	<0.31	<0.37	<0.24
heart	<0.3	<0.4	0.7	0.4[e]	<0.14	<0.14	0.32	0.18
kidney	0.8	2.4[d]	2.8	5.3	0.21	0.34	1.17	1.16
liver	0.4	0.3[c]	1.0	0.7	0.19[e]	<0.14	0.56	0.30
lung	<0.3	0.5[b]	1.2	0.7	<0.13	<0.14	0.50	0.27
muscle	<0.3	<0.3	0.4[b]	<0.3	<0.13	<0.13	0.18[b]	<0.12
ovary		<0.7		0.7[d]		<0.39		0.30
pancreas	<0.3	<0.3	0.4	0.4[c]	<0.13	<0.13	0.24[e]	0.16[b]
spleen	<0.3	<0.3	0.4	<0.3	<0.13	<0.14	0.20[d]	0.15[b]
testis	<0.3		0.4[e]		<0.14		0.20[d]	
thyroid	<4.6	<3.4	<3.2	<3.5	<1.96	<2.24	<1.59	<1.76
uterus		0.9[b]		0.9		<0.17		0.33
residual carcass	0.7[d]	0.4[c]	1.0	0.8	0.77[e]	0.26[d]	0.53	0.37

[a]Data are the mean values for five rats, but values below the detection limit were excluded from the calculations. [b]Value for one rat. [c]Mean value for two rats. [d] Mean values for three rats. [e] Mean values for four rats.

Table 6. Amounts of Metabolites in Urine and Feces within 2 Days after a Single Oral Administration of [*phenyl*-^{14}C]-S-23031 or [*THP*-^{14}C]-S-23031 to Male and Female Rats at 1 (Low Dose) or 500 (High Dose) mg/kg[a]

	amount (% of dosed ^{14}C)							
	low dose (1 mg/kg)				high dose (500 mg/kg)			
	male		female		male		female	
metabolite	feces	urine	feces	urine	feces	urine	feces	urine
organosoluble fraction								
imide metabolites								
S-23031	0.4−0.7	−[c]	0.6−0.7	−	32.2−35.2	−	27.4−27.7	−
IMCA	9.5−9.6	0.9−3.1	6.6−7.1	1.8−5.5	5.6−14.0	0.5−2.9	5.0−6.7	6.3−6.4
4-OH-IMCA	1.2−2.0	1.8−5.7	0.7−1.2	0.9−4.7	1.2−1.5	0.4−0.8	0.7−1.0	1.3−2.0
IMCA-SA	9.4−12.5	1.2−5.2	7.4−12.7	0.9−3.6	3.1−4.6	0.2−5.3	3.0−6.0	0.3−3.2
OH-IMCA-SA(mix)[b]	5.4−12.9	0.8−1.7	3.4−9.0	0.2−1.7	2.9-5.9	0.3−3.8	0.1−2.8	0.2−0.8
	[*phenyl-ring*-^{14}C] Labeling							
phenyl moiety								
AFCA	4.8	22.5	3.9	29.8	6.3	14.3	5.1	20.8
identified ^{14}C	39.0	27.2	29.3	33.6	66.0	15.7	43.6	29.0
	[*THP*-^{14}C] Labeling							
THP moiety								
THPA	2.7	3.0	2.4	4.0	2.7	1.4	2.4	2.3
4-OH-THPA	1.5	2.9	−	3.2	−	3.8	−	1.6
1-OH-HPA	3.9	−	3.6	−	3.0	−	3.9	−
identified ^{14}C	37.5	21.6	30.0	22.7	52.2	18.0	48.2	16.2
others	10.4−19.2	6.7−13.2	6.0−18.8	18.1−18.4	10.3−12.2	5.6- 9.4	9.1−11.9	6.6−17.9
unextractable ^{14}C	6.0−9.2		7.6−9.1		1.2−4.3		4.0−4.2	

[a] Data show the mean values of five rats. [b] Mixture of 4-OH-IMCA-SA1, 4-OH-IMCA-SA2, and 5-OH-IMCA-SA. [c] Not detected.

SA accounted for 5.0−14.0%, 3.0−12.7%, and 0.1−12.9%, respectively.

The metabolite from the phenyl moiety was AFCA, which was predominant in urine, accounting for 14.3−29.8%.

The metabolites from the THP moiety were THPA, 4-OH-THPA, and 1-OH-HPA. Each of the three metabolites was a minor one, accounting for <4%.

The amount of the parent compound excreted was high in both sexes given the high dose, but very small with the low dose. The values were 0.4−0.7% and 27.4−35.2% in the low- and high-dose groups, respectively.

AFCA was the major metabolite in bile, in which the parent compound could not be detected (data not shown).

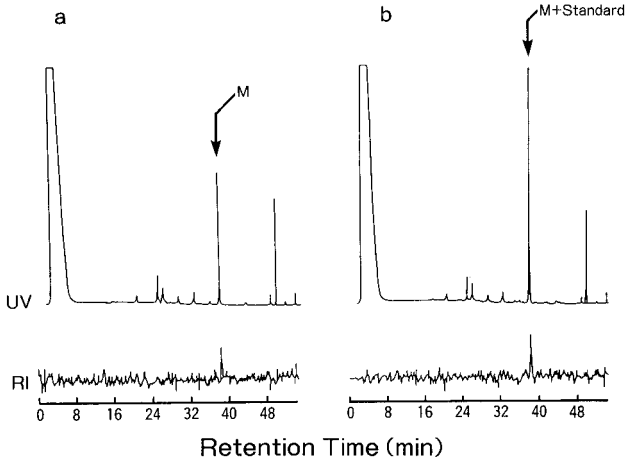

Figure 3. Representative HPLC chromatograms (upper, UV; bottom, radioisotope detection) of (a) the fecal metabolite (M) prepurified by TLC and (b) those of the metabolite (M) co-injected with the IMCA standard.

DISCUSSION

Both types of labeled S-23031 were rapidly and almost completely eliminated from the bodies of both sexes of rats within the examined time period. Urinary ^{14}C-excretion appeared to be greater in females than in males. There are statistically significant sex-related differences determined by Student's *t*-test (provability

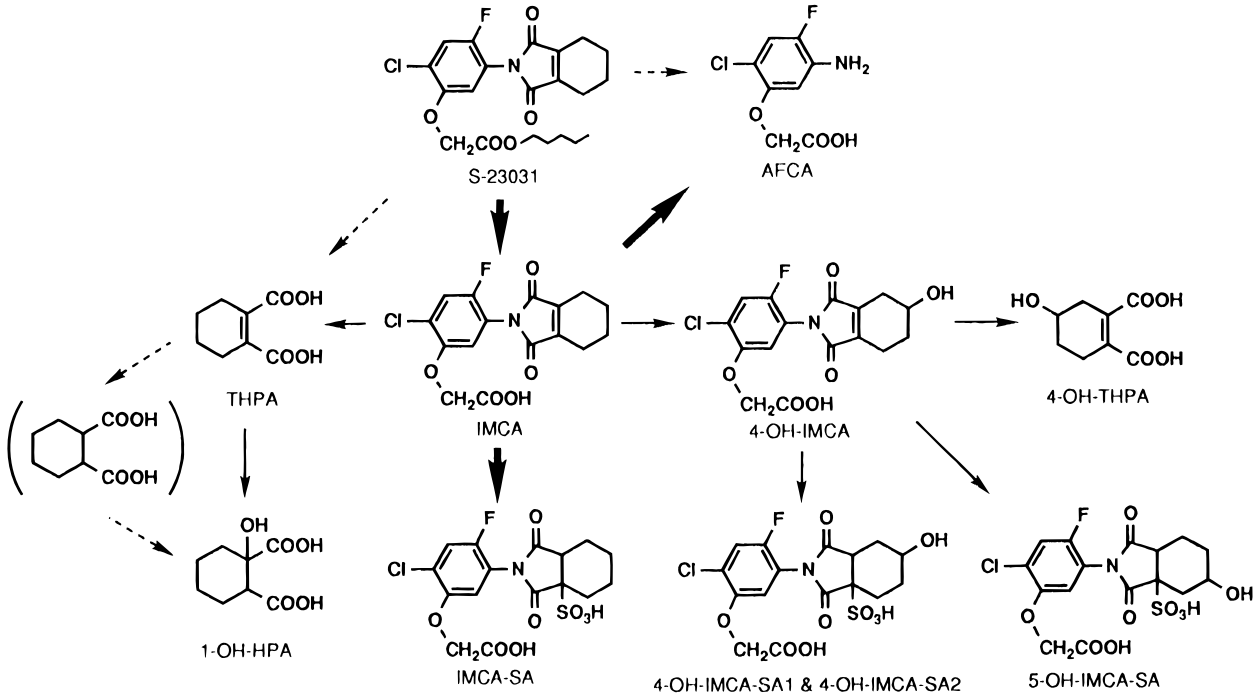

Figure 4. Proposed metabolic pathways of S-23031 in rats.

Table 7. Extent of Each Metabolic Transformation

metabolic transformation	% of dosed ^{14}C							
	[*phenyl*-^{14}C]				[*THP*-^{14}C]			
	low dose		high dose		low dose		high dose	
	male	female	male	female	male	female	male	female
cleavage of ester	65.8	62.2	46.5	44.9	44.4	40.5	27.1	28.4
cleavage of imide moiety	27.3	33.7	20.6	25.9	16.4	13.2	11.0	10.2
hydroxylation	17.5	11.3	8.1	2.6	24.9	18.9	15.5	11.8
incorporation of $-SO_3^-$	24.3	17.5	9.5	3.6	24.7	21.4	16.6	12.7

<0.05) in all dose groups except the low-dose group treated with [*THP*-^{14}C]-S-23031. There is no significance in that group; however, it seems that urinary ^{14}C excretion in females was slightly greater than in males (Figure 1E,F). AFCA was the predominant metablite in urine, being greater in females than in males (Table 6). This diffence may in part account for the above sex-related difference with regard to excretion.

^{14}C tissue residue levels on the seventh day post-treatment were generally low. ^{14}C levels in blood, kidney, and liver reached maxima 2 h post-treatment and declined with $T_{1/2}$ values of 4.6−7.7 h.

On the basis of the metabolites tentatively identified in this study in addition to the metabolites identified in the previous study (Matsunaga *et al.*, 1996), the metabolic pathways shown in Figure 4 are proposed for S-23031 in rats. The major biotransformations of S-23031 in rats were (1) cleavage of the ester bond, (2) cleavage of the imide linkage, (3) hydroxylation of the cyclohexene ring of the THP moiety or the cyclohexane ring of the cyclohexane-1,2-dicarboxylic acid moiety, and (4) incorporation of a sulfonic acid group into the >C═C< double bond of the THP moiety. In addition to the above metabolic pathways, there appears to be a possible pathway leading to the formation of 1-OH-HPA by direct addition of H_2O to the double bond of THPA. The extents (expressed as percentages of the dosed ^{14}C) of each metabolic reaction were calculated and are shown in Table 7. Ester cleavage predominated, which coincides with the fact that S-23031 was rapidly hydrolyzed *in vitro* to IMCA (or further to AFCA) in reaction mixtures containing the liver microsome fraction or plasma (data not shown). Regardless of sex or labeling positions, the high dose led to excretion of large amounts of the parent compound into feces. It is likely that higher fecal excretion of the parent compound with the high dose is due to the dosage exceeding the absorption capacity of the gastrointestinal tract. The absorption rate could be estimated by summing the rates of biliary and urinary excretion, being 74% for the low-dose group and 49% for the high-dose group.

The mechanism of formation of sulfonic acid conjugates was investigated and reported previously (Yoshino *et al.*, 1993a; Matsunaga *et al.*, 1996). It was suggested that sulfite (or bisulfite) converted from sulfate (SO_4^{2-}) by microflora in the body might be nonenzymatically incorporated into the >C═C< double bond. This type of conjugation contributes to detoxication of xenobiotics by transforming them into polar metabolites.

In conclusion, S-23031 orally administered to rats is excreted rapidly and completely from the body in both sexes and without significant persistence in any tissues.

ACKNOWLEDGMENT

We gratefully acknowledge Mrs. Akiko Mitsuzawa (Hashimoto) and Mrs. Tomomi Horimoto (Nishikawa) for skillful technical assistance.

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Received for review April 29, 1996. Accepted November 5, 1996.[⊗]

JF960312E

[⊗] Abstract published in *Advance ACS Abstracts*, December 15, 1996.