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Absorption, Distribution, and Excretion of Suprofen in Mice of Both Sexes

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The disposition and metabolism of DL-2-(4-(2-thienylcarbonyl)phenyl)propionic acid (suprofen, S) were evaluated in male and female mice. The radiometric findings following oral administration of 2 mg/kg of ^{3}H–S to male and female mice showed similar patterns of absorption, distribution, and excretion of the radioactivity. S was rapidly absorbed in mice of both sexes; blood levels after a single oral dose of 2 mg/kg of ^{3}H–S reached maxima within 7.5 min. Blood radioactivity was mostly accounted for by unchanged S. Elimination of ^{3}H–S from the blood was rapid; most of the radioactivity was excreted in the urine and a portion in the feces within 24 h after oral administration of 2 mg/kg of ^{3}H–S or 10 mg/kg of ^{14}C–S. The only tissues with concentrations similar to or higher than that in the plasma were those involved in metabolism and excretion (liver and kidney); other tissue levels, except for the stomach, were all very low and there was no evidence of accumulation of drug-related material in any tissue. Male mouse urine contained S, 2-(4-(2-thienylhydroxymethyl)phenyl)propionic acid, 2-(4-carboxyphenyl)propionic acid, and two unknown metabolites, accounting for about 73% of the urinary radioactivity.

Keywords——suprofen; nonsteroidal anti-inflammatory drug; oral administration to mouse; radiometry; HPLC; synchronized accumulating radiodetector; absorption; distribution; excretion; urinary metabolite

DL-2-(4-(2-Thienylcarbonyl)phenyl)propionic acid (suprofen, S) is a nonsteroidal anti-inflammatory drug (NSAID) that also possesses analgesic and antipyretic activity.[1,2] The effects of S on acute inflammatory reactions and prostaglandin biosynthesis in animal models were comparable to those of ketoprofen and indomethacin (other NSAID's), but S showed a low incidence of ulcerogenic effects on the gastrointestinal tract in rats.[2a] In order to obtain basic information on the pharmacological and toxicological properties, labeled S was prepared, and its disposition and metabolism in animals were studied. This paper describes the absorption, distribution, and excretion of ^{3}H–S in mice of both sexes, and the urinary metabolites of ^{14}C–S in male mice.

Materials and Methods

Chemicals——S, as well as 2-(4-(2-thienylhydroxymethyl)phenyl)propionic acid (S–OH), 2-(4-carboxyphenyl)propionic acid (S–COOH), and ^{3}H– and ^{14}C–S, was synthesized in our laboratories.[3] The specific activities of ^{3}H–S and ^{14}C–S were 3.33 mCi/mg, and 5.4 μCi/mg, respectively, and the radiochemical purity was >98%. Doses with required specific activities were prepared by dilution with appropriate amounts of unlabeled S.

Animals and Dose——Male and female ddy mice (Shizuoka Laboratory Animal Center, Shizuoka) weighing about 25 g were used. All mice were fasted overnight before dosing. Mice were orally given ^{3}H– or ^{14}C–S suspended in 0.5% tragacanth, as a single dose of 2 or 10 mg/kg; all oral doses were administered by gastric intubation. After dosing, the mice were maintained with free access to food and water in individual metabolism cages designed to

permit separate collection of urine and feces.

Radiometry——Urine and feces were collected for 5 d after dosing. Blood samples were obtained from the abdominal aorta at various intervals after dosing (up to 24 h), and plasma was separated promptly thereafter by centrifuging (1500 × *g*, 10 min) the heparinized blood. Tissue samples were dissected out, rinsed in cold saline, blotted with filter paper, and weighed. Portions (0.1—0.2 g) of tissues or whole tissues were processed in an Aloka model ASC-113 sample oxidizer (Aloka Instrument Co., Tokyo). Feces were dried in a desiccator, weighed, and ground to a powder. Aliquots of feces and blood or plasma were processed in the sample oxidizer, and aliquots of urine were directly dissolved in Oxifluor^{R}-H_2O (New England Nuclear Corp., Boston, MA) after dilution with water. Radioactivity was determined in an Aloka model LSC-651 scintillation spectrometer. The radioactivity determined was converted in some cases to equivalents of unchanged suprofen, based on the specific activity of the dosed ^{3}H–S.

Isolation of Plasma or Urinary Metabolites——Plasma obtained by centrifuging the heparinized blood from mice after oral administration of ^{14}C–S was homogenized with 2 × 4 volumes of methanol and centrifuged at 1500 × *g* for 10 min. The resultant supernatants were evaporated to dryness at 40 °C, taken up in 0.5 ml of methanol, and spotted on thin-layer chromatography (TLC) plates. Urine was collected for 24 h after *p.o.* dosing with 10 mg/kg of ^{14}C–S (50 μCi) to male mice. A portion (about 0.04 μCi) of the intact urine was spotted on TLC plates. The origin fraction was incubated with 0.2 ml of 1 N NaOH for 1 h at 37 °C. The resulting hydrolyzed fraction, after neutralization with 1 N HCl, was evaporated to dryness at 40 °C and dissolved in 0.3 ml of methanol; this was rechromatographed with TLC. Plasma and urinary metabolites were determined by high-pressure liquid chromatographic (HPLC) analysis.[4]

Chromatography——Silica gel 60 F_{254} (0.25 nm, thickness) precoated plates (E. Merck, Darmstadt, Germany) were used for TLC. The solvent system was: chloroform–acetone–acetic acid (20 : 3 : 1, v/v/v). The developed plates were scanned with an Aloka model TLC-2B radiochromatogram scanner, and radioactive components were extracted with methanol. Extracts were evaporated to dryness at 40 °C and dissolved in 100 μl of methanol. HPLC was performed using a Shimadzu model LC-2P HPLC (Shimadzu Seisakusho Ltd., Kyoto, Japan) equipped with a synchronized accumulating radiodetector (Aloka model RLC-R17-748).[4] Samples (5 μl) of the methanol extract were injected into the HPLC column directly and measured at 254 nm (0.08 AUFS). Radioactivity was detected at 300 counts full scale (scintillator flow rate = 7.5 ml/min; sampling time = 7 s). Known amounts of ^{14}C–S were injected into the chromatograph and the total counts under the peak were calculated manually. The counting efficiency of this detector was estimated by comparing the total counts with amount of injected ^{14}C.[4] S and its metabolites were separated on a Dupont Zorbax ODS column (4.6-mm i.d. × 25 cm) (E.I. Dupont De NeMours & Co., Wilmington, Delaware 19898). Compounds were eluted with 0.01% acetic acid–acetonitrile (5 : 3, v/v), and the solvent flow rate was 1.5 ml/min. Authentic samples of S, S–OH, and S–COOH were used as standards, and the retention times were 11.0, 6.8, and 3.2 min, respectively.

Results

Blood Levels

Figure 1 shows the concentrations of ^{3}H in the blood of male and female mice given ^{3}H–S

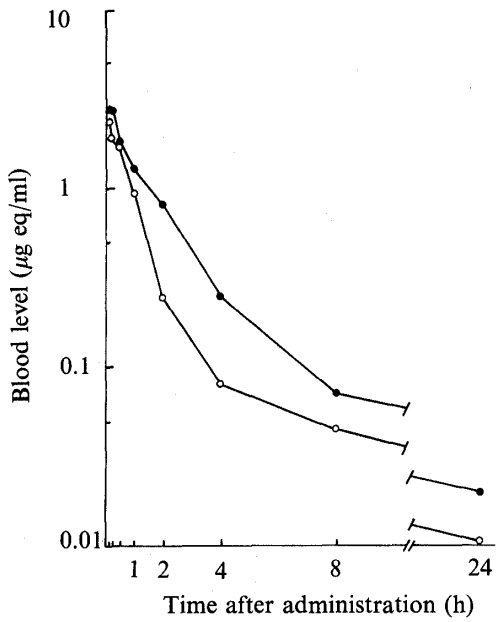

Fig. 1. Blood Levels of ^{3}H in Male (○) and Female (●) Mice after Oral Administration of 2 mg/kg ^{3}H–S

Each point is the mean for 4 mice.

TABLE I. Tissue Levels of Radioactivity in Male and Female Mice after Oral Administration of ^{3}H–S

Tissue	Males					Females				
	Tissue level[a] at					Tissue level[a] at				
	7.5 min	15 min	1 h	4 h	24 h	7.5 min	15 min	1 h	4 h	24 h
Plasma	3.2±0.4	2.7±0.5	0.8±0.1	0.2±0.0	0.1±0.0	4.9±0.2	3.6±0.2	2.1±0.2	0.3±0.1	0.1±0.1
Cerebrum	0.1±0.0	0.1±0.0	0.1±0.0	[b]	[b]	0.2±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
	([b])	(0.1±0.0)	([b])	([b])	([b])	(0.1±0.0)	(0.1±0.0)	(0.1±0.0)	([b])	([b])
Lung	0.7±0.2	1.0±0.2	0.3±0.0	0.1±0.0	0.1±0.0	1.3±0.1	0.9±0.1	0.5±0.1	0.1±0.0	0.1±0.0
	(0.2±0.1)	(0.3±0.1)	(0.1±0.0)	([b])	([b])	(0.4±0.1)	(0.4±0.0)	(0.2±0.0)	([b])	([b])
Heart	0.6±0.1	0.6±0.0	0.2±0.0	0.1±0.0	0.1±0.0	0.9±0.1	0.7±0.1	0.4±0.0	0.1±0.0	0.1±0.0
	(0.1±0.0)	(0.1±0.0)	([b])	([b])	([b])	(0.2±0.0)	(0.2±0.0)	(0.1±0.0)	([b])	([b])
Liver	3.7±0.1	3.8±0.4	1.7±0.2	0.4±0.0	0.2±0.0	4.5±0.2	3.3±0.5	1.7±0.2	0.4±0.1	0.3±0.0
	(6.3±1.6)	(7.2±0.7)	(3.5±0.3)	(0.8±0.0)	(0.4±0.0)	(9.4±0.8)	(6.0±1.2)	(3.4±0.4)	(1.2±0.3)	(0.9±0.4)
Kidney	5.1±1.2	7.3±0.8	3.5±0.8	0.4±0.1	0.1±0.0	6.7±0.4	5.1±0.1	2.6±0.2	0.3±0.1	0.2±0.0
	(3.5±0.8)	(4.9±0.8)	(2.4±0.7)	(0.3±0.0)	(0.1±0.0)	(3.9±0.3)	(3.5±0.6)	(1.5±0.1)	(0.3±0.1)	(0.1±0.0)
Spleen	0.4±0.1	0.5±0.0	0.2±0.0	0.2±0.2	[b]	0.7±0.0	0.5±0.0	0.3±0.0	0.1±0.0	0.1±0.0
	(0.1±0.0)	(0.1±0.0)	([b])	([b])	([b])	(0.1±0.0)	(0.1±0.0)	(0.1±0.0)	([b])	([b])
Adrenal	0.2±0.0	0.3±0.1	[b]	[b]	0.2±0.0	0.9±0.2	0.4±0.1	0.5±0.1	0.1±0.0	[b]
	([b])	([b])	([b])	([b])	([b])	([b])	([b])	([b])	([b])	([b])
Stomach	19.0±2.8	16.2±3.0	4.7±1.2	1.5±0.2	0.2±0.1	5.2±1.4	13.1±1.7	9.3±3.2	3.1±0.7	0.3±0.1
	(8.1±1.0)	(5.2±2.0)	(1.8±0.5)	(0.4±0.2)	(0.1±0.0)	(2.5±0.7)	(5.9±0.8)	(3.7±1.3)	(1.1±0.5)	(0.1±0.0)
Small intestine	0.6±0.2	0.7±0.1	0.8±0.2	0.3±0.1	0.1±0.0	1.3±0.3	0.9±0.1	0.9±0.2	0.1±0.0	0.1±0.0
	(2.0±0.5)	(1.8±0.1)	(1.8±0.3)	(0.8±0.2)	(0.4±0.1)	(4.8±1.9)	(2.9±1.0)	(3.1±0.7)	(0.6±0.1)	(0.3±0.0)
Large intestine	0.4±0.1	0.4±0.0	0.3±0.1	0.3±0.1	0.1±0.0	0.5±0.0	0.4±0.1	0.4±0.0	0.3±0.0	0.2±0.0
	(0.3±0.2)	(0.4±0.0)	(0.3±0.1)	(0.4±0.1)	(0.2±0.0)	(0.5±0.1)	(0.5±0.1)	(0.4±0.0)	(0.3±0.1)	(0.2±0.0)
Fat	0.1±0.0	0.1±0.0	0.1±0.0	[b]	[b]	0.2±0.0	0.2±0.0	0.2±0.0	0.1±0.0	[b]
Muscle	0.1±0.0	0.3±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.3±0.1	0.2±0.0	0.2±0.0	0.1±0.0	0.1±0.0
Testicle (uterus)	[b]	0.1±0.0	0.2±0.0	[b]	[b]	0.8±0.2	0.6±0.3	0.6±0.1	0.2±0.0	[b]
	([b])	(0.1±0.0)	(0.1±0.0)	([b])	([b])	([b])	(0.1±0.0)	(0.1±0.0)	([b])	([b])
Prostate (ovarium)	0.3±0.0	1.4±0.6	1.2±0.4	0.5±0.2	0.1±0.0	0.9±0.1	0.3±0.2	0.5±0.1	[b]	[b]
	([b])	(0.1±0.0)	(0.1±0.0)	([b])	([b])	(0.1±0.0)	([b])	([b])	([b])	([b])

a) Tissue levels are expressed as μg eq/g tissue, and recoveries are given in parentheses as % of dose.
b) <0.05. Values are mean±S.E. for four animals.

as a single *p.o.* dose of 2 mg/kg, the therapeutically effective dose of S.[2b] Blood levels of ^{3}H reached maxima within 7.5 min after dosing, and peak levels of ^{3}H were 2.58 ± 0.34 (mean of four mice, $\pm$S.E.) and 2.96 ± 0.19 μg eq/ml for males and females, respectively. TLC and HPLC analyses of methanol extracts from plasma showed that S accounted for most of the radioactive material circulating in the blood. Oral administration of ^{3}H–S resulted in a biphasic fall-off of the blood concentration with elimination half-lives ($T_{1/2}$) of 0.56 and 3.64 h in males and of 0.55 and 2.56 h in females; ^{3}H in blood was rapidly eliminated and had largely disappeared at 24 h. The area under the curve of blood concentration *vs.* time, estimated from the trapezoidal approximation of total radioactivity data, was significantly ($p < 0.01$) higher in females (5.38 ± 0.37 μg eq·h/ml) than in males (3.08 ± 0.24 μg eq·h/ml).

Tissue Distribution

The tissue distribution was assessed in male and female mice given an oral dose of 2 mg of ^{3}H–S per kg. Groups of four mice were killed at 0.125, 0.25, 1, 4, and 24 h after dosing. Total radioactivity levels in the plasma and selected tissues are shown in Table I. In both sexes, tissue radioactivity levels, except for the intestine, reached a peak within 15 min after dosing, and began to decline thereafter; the small intestine retained the peak level of ^{3}H up to 1 h. Among all the tissues examined, the ^{3}H levels exceeded that in plasma only in the kidney and liver at up to 4 h in males, excluding the stomach, which might have been contaminated by its highly radioactive contents; the tissue/plasma radioactivity ratio ranged from 1.6 to 4.4 for the kidney and from 1.2 to 2.1 for the liver. On the other hand, liver and kidney levels in females were similar to or higher than those in plasma; the tissue/plasma ratio ranged from 1.0 to 1.4 for the kidney and from 0.8 to 1.3 for the liver. All other tissues in mice of both sexes contained less radioactivity, with the brain and fat containing the least at 7.5 min. On the basis of the tissue composition of the mouse,[5] the total radioactivity in the whole blood at 7.5 min accounted for 8.9 and 10.6% of the dose in males and females, respectively. Accordingly, almost 19 and 24% of the dosed amounts of ^{3}H were recovered in liver, kidney, and blood at the time of maximal tissue levels. However, most of the ^{3}H had disappeared from all the tissues 24 h after dosing, as in the case of plasma; all the tissues studied contained less than 1.6% of the dose of ^{3}H. However, very low radioactivity (0.9% or less of the dose) was still detected in the liver.

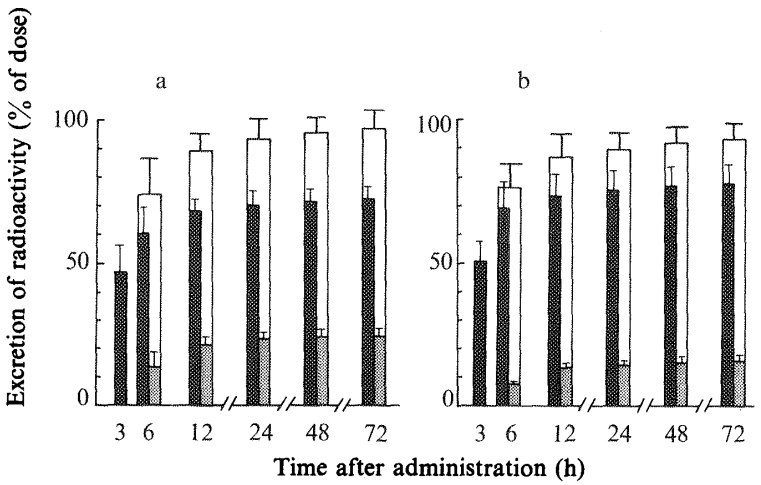

Fig. 2. Cumulative Urinary and Fecal Excretions of ^{3}H after Oral Administration of 2 mg/kg ^{3}H–S to Male (a) and Female (b) Mice

■, urine; ■, feces; □, total.
Data represent the means ± S.E. for 4 animals.

Urinary and Fecal Excretions

Figure 2 shows the excretion of ^{3}H in the urine and feces of mice given ^{3}H–S. Most of the ^{3}H was eliminated *via* the urine. Approximately half of the dose was excreted in the urine within 3 h after dosing in mice of both sexes; 23% or less of ^{3}H was detected in the feces within 24 h. About 95% of the administered ^{3}H was excreted in urine and feces within 72 h after dosing; however, small amounts of ^{3}H (1% of the dose/d) were still being eliminated when the study was terminated after 5 d (data not shown).

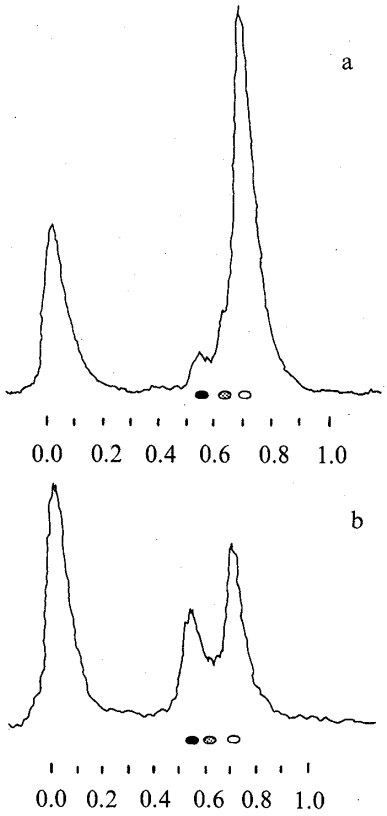

Fig. 3. Radioscannogram of 24-h Urine after Oral Administration of 10 mg/kg ^{14}C–S to Male Mice (a) and a Further Radioscannogram of the Material at the Origin after Alkaline Treatment (b)

S (○), S–OH (◍), and S–COOH (●) are authentic samples.

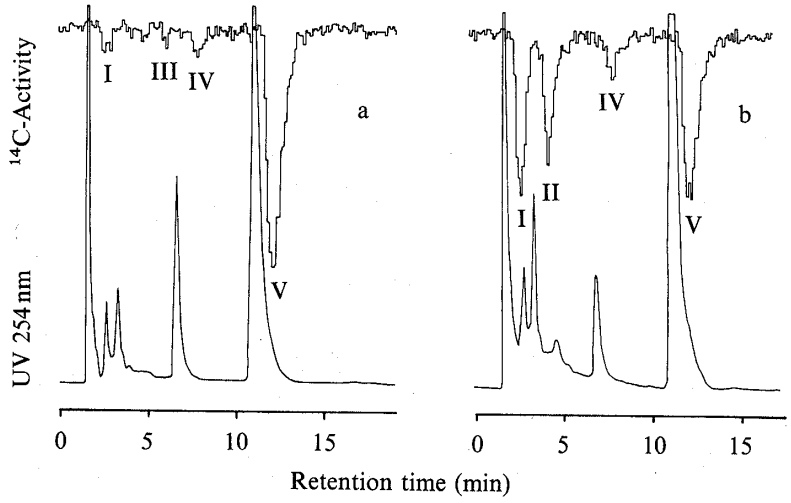

Fig. 4. High-Pressure Liquid Chromatograms of Extracts of Radioactive Components (Rf=0.4—0.9) in TLC

The instrument was equipped with a UV detector and synchronized accumulating radiodetector. The samples (from 24-h urine after oral administration of ^{14}C–S) are the methanol extracts obtained from TLC plates before (a) and after (b) alkaline treatment, as described in the legend to Fig. 3.

Urinary Metabolites

About 70% of the dose was excreted in pooled urine from 3 male mice within 24 h after *p.o.* dosing with 10 mg/kg of ^{14}C–S; 26% of ^{14}C was detected in the feces within 24 h. The result is essentially consistent with that found after administration of 2 mg/kg of ^{3}H–S (Fig. 2). A typical radiochromatogram of urine from mice after ^{14}C–S dosing is shown in Fig. 3. TLC analysis of the intact urine revealed the presence of four radioactive materials; three showed *Rf* values corresponding to those of authentic S, S–OH, and S–COOH, and the fourth was a polar compound(s) at the origin (Fig. 3a). The same results were observed in rechromatography of the material from the origin after alkaline treatment (Fig. 3b). The less polar fractions ($Rf = 0.4$—0.9) of unconjugated (a) and of conjugated (b) metabolites and the origin fraction (b) contained 52.1, 20.7, and 24.0%, respectively, of the excreted activity; the total recovery from the 24-h urine was about 96.8% in these steps. The less polar metabolites were further isolated and determined by HPLC analysis (Fig. 4). HPLC revealed two additional unidentified metabolites (Peaks I and III). S–COOH (Peak II) was found only in conjugated form, while S (Peak V) and S–OH (Peak IV) were excreted mainly as unconjugated forms (5.1 and 2.4 times more than the conjugated forms, respectively). Consequently, the following percentages of the urinary radioactivity of ^{14}C–S were obtained in 24 h; S (about 54%), S–OH (5%), S–COOH (5%), and unknown metabolites (36%).

Discussion

The radiometric findings following oral administration of 2 mg/kg of ^{3}H–S in male and female mice showed similar patterns of absorption, distribution, and excretion of the radioactivity. S was rapidly absorbed after oral administration of a suspension dose in mice of both sexes; blood concentrations of ^{3}H–S reached peak levels within 7.5 min (earliest sampling time). Blood radioactivity was mostly accounted for by unchanged S.

Although ^{3}H was found in all tissues examined, there was no accumulation of drug-related material in any tissue. At times up to 24 h only the gastrointestinal tract, liver, and kidney exhibited concentrations similar to or greater than those in the corresponding plasma samples (Table I). Similar results have been reported for other structurally similar anti-inflammatory agents, *e.g.*, ketoprofen,[6] ibuprofen,[7] and tiaprofen.[8] Since S is extensively bound to rat plasma proteins,[3b] the rather high plasma levels of ^{3}H relative to other tissues might be attributed to this considerable protein binding. Similar results have been reported for other NSAID's.[9–13] The kidney showed high levels of ^{3}H as early as 7.5 min after dosing of the drug, and relatively high concentrations of radioactive material were also observed in the small intestine at 1 h; this might represent contamination by excreted bile. The drug is therefore rapidly excreted through the kidney, and also in the feces, *via* the biliary route, as was observed in previous experiments with rats.[3b] In addition, the persistence of a certain level of ^{3}H in the liver 24 h after dosing might be due to enterohepatic circulation of the drug. The very low levels of ^{3}H in the brain at times up to 24 h indicated that S essentially does not cross the blood-brain barrier. Anti-inflammatory agents have been reported to cause elongation of parturition time and increase in the number of stillborns in rats, possibly due to the suppression of prostaglandin production.[14,15] Suprofen might possibly exert similar effects, inasmuch as suprofen also appears to inhibit prostaglandin synthesis,[2a] and the presence of ^{3}H was also noted in mouse uterus, although the level was much lower than that in plasma. Other tissues showed substantial decreases in concentrations within 24 h.

Both male and female mice rapidly excreted most of the dose in the urine and feces. In male mice given 10 mg/kg of ^{14}C–S, 70% of the dose was excreted in urine 24 h after dosing. S (about 36.9% of the dose), S–OH (3.7%), and S–COOH (3.3%) were detected in the urine. In 24 h urine after *p.o.* dosing of 2 mg/kg of unlabeled S, the metabolite hydroxylated at the 5-

position of the thienyl ring accounted for about 9.7% of the dose; this was determined by mass fragmentography using S-d_4 as an internal standard.[16] This metabolite could not be detected in the present study because of its chemical instability; its decomposition product(s) should be located at the origin (polar metabolites) in TLC (Fig. 3b). These results suggest that more than half of ^{14}C in the polar fraction (about 17% of the dose) might be present as 2-(4-(5-hydroxy-2-thienylcarbonyl)phenyl)propionic acid. Fujimura *et al.* reported that the ulcerogenic effect of S in the rat small intestine was significantly less than those of ketoprofen and indomethacin,[2a] and the decrease in the anti-inflammatory activity of S in the rat 3 d after oral administration was greater than those of the reference compounds.[2b] The excretion of S in urine and feces of mice and rat[3b] was rapid compared to those of ketoprofen[6] and indomethacin.[17] Accordingly, the decrease in the side effects of S and the short duration of S activity in the test animals might be partly due to the rapid excretion of S and/or its metabolites.

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