Metabolism of the Fungicide S-*n*-Butyl S'-*p*-*tert*-Butylbenzyl N-3-Pyridyldithiocarbonimidate(S-1358) in Rats

Hideo OHKAWA, Reiko SHIBAIKE, Toshiko HATANAKA
and Junshi MIYAMOTO

Research Department, Pesticides Division, Sumitomo Chemical Co., Ltd.,
Takarazuka, Hyogo 665, Japan

Received March 25, 1975

S-1358 was rapidly absorbed, metabolized and readily excreted via urine and feces from orally dosed rats. Excretion of radioactivity was almost complete within 4 days. The radioactivity was distributed mainly in stomach, intestines, liver and kidneys. It seems that S-1358 and its metabolites do not persist in organs and tissues following a single oral dosing.

Major urinary metabolites of the benzyl-labeled S-1358 were *p*-(1,1-dimethyl-2-hydroxyethyl)benzyl methyl sulfide [B], *p*-(1,1-dimethyl-2-hydroxyethyl)benzyl methyl sulfone [A], *p*-(1-methyl-1-carboxylethyl)benzyl methyl sulfide [D], *p*-(1-methyl-1-carboxylethyl)benzyl methyl sulfone [C] and their glucuronide conjugates. Fecal metabolites were S-*n*-butyl S'-1,1-dimethyl-2-hydroxyethyl)benzyl N-3-pyridyldithiocarbonimidate [MR], A, B, C and D. These metabolites were also found in the bile. The pyridine-labeled S-1358 gave rise to 2-(3'-pyridylimino)-4-carboxylthiazolidine [HM] and 3-aminopyridine [AP] in the urine, and MR and AP in the feces. Intact S-1358 was a major component of the fecal radioactivity.

Dithiocarbonimidates are a new class of chemicals with fungicidal activity. Among them, S-*n*-butyl S'-*p*-*tert*-butylbenzyl N-3-pyridyldithiocarbonimidate (S-1358) has demonstrated outstanding effects for the control of powdery mildew diseases.¹⁾ This chemical depresses the growth of germ tubes of *Sphaerotheca fuliginea* and *Monilinia fructigena*, causing swelling of their tips.^{1,2)} Although S-1358 does not significantly affect respiration and biosyntheses of proteins, nucleic acids and cell wall, it specifically inhibits the biosynthesis of ergosterol.^{2,3)}

Acute toxicity of S-1358 to mammals is very low. The oral LD₅₀ is 4.9 g/kg for male rats and 2.7 g/kg for female rats.⁴⁾ However, it is necessary to assess further toxicological properties of this chemical for the safe use as an agricultural fungicide.

The present investigation was conducted to elucidate the metabolic fate of S-1358 in the rat, using both the benzyl-labeled and the pyridine-labeled compounds.

MATERIALS AND METHODS

Chemicals. Two radioactive preparations of S-1358 were synthesized in this laboratory⁵⁾: S-1358-¹⁴C labeled at the methylene carbon of the benzyl group (7.0 mCi/mmol) and S-1358-³H labeled at the 5-position of the pyridine ring (65.1 mCi/mmol). The structure of S-1358 and the positions of radiolabeling are shown in Fig. 1. Each of the preparations had a radiochemical purity greater than 99%, as determined by thin-layer chromatography (tlc). The following standard chemicals were also prepared in this laboratory⁶⁾: unlabeled S-1358, bis(*p*-*tert*-butylbenzyl) disulfide [BDS], S-*p*-*tert*-butylbenzyl N-3-pyridyldithiocarbamate (PTB) and 3-pyridylisothiocyanate (NCS). 3-Aminopyridine (AP) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan).

2-Methyl-2-*p*-tolylpropanol was prepared according to the following procedure: To 7 g of potassium cyanide dispersed in 150 ml of dimethyl sulfoxide warmed to 35~40°C, 15 g of α -chloro-*p*-xylene was added over a period of 1 hr. The reaction mixture was stirred for 2 hr, poured into ice-water and then extracted with ether. The ether extract was dried over calcium chloride and concentrated. Distillation of the residue yielded *p*-tolylacetonitrile, bp 68~70°C/1 mmHg. This product (7.5 g) was added dropwise to a stirred mixture of methyl iodide (35 g) and 50% sodium hydride (6 g)

FIG. 1. The Structure of S-1358 [Showing the Positions of ^{14}C - or ^3H -Labeling.

in 50 ml of dimethylformamide-toluene mixture (1/3, v/v) at $50\sim 60^\circ\text{C}$. The reaction mixture was stirred at 80°C for 3 hr, poured into ice-water, extracted with ether, dried over calcium chloride and concentrated. The residue was distilled under reduced pressure to yield 4 g of 1-methyl-1-*p*-tolylpropionitrile, bp $78\sim 80^\circ\text{C}/1\text{ mmHg}$. This compound (4 g) was then hydrolyzed by refluxing in 50% sulfuric acid for 3 hr. The produced carboxylic acid was extracted with ether. After concentration of the ether extract, the residue was recrystallized from *n*-hexane to give a colorless product melting at $83.0\sim 83.5^\circ\text{C}$. The obtained carboxylic acid was quantitatively methylated by reaction with diazomethane in ether to give the corresponding methyl ester. This ester (8 g) was refluxed in 100 ml of dry ether for 30 min with 1 g of lithium aluminum hydride. The reaction mixture was treated with 10 ml of ice-water and then 10 ml of 1 *N* hydrochloric acid, poured into 100 ml of ice-water, and extracted with ether. After concentration, the residue was distilled under reduced pressure to give 7 g of 2-methyl-2-*p*-tolylpropanol, bp $37^\circ\text{C}/1\text{ mmHg}$. Found: C, 80.30; H, 9.79. Calcd. for $\text{C}_{11}\text{H}_{16}\text{O}$: C, 80.44; H, 9.81%.

2-(3'-Pyridylimino)-4-methoxycarbonylthiazolidine was prepared as follows: NCS (1.4 g) was added dropwise to a stirred solution of L-cysteine (1.2 g) in 20 ml of water at $0\sim 1^\circ\text{C}$. The reaction mixture was stirred at room temperature for 2 hr. The resulting precipitate was collected, washed with ether and dried *in vacuo* over phosphorus pentoxide. The obtained solid was added gradually to a stirred solution of diazomethane in ether. The mixture was stirred at room

temperature until the solid dissolves, and then concentrated. The product was purified by passing through a silica gel (70~230 mesh, E. Merck) column. The column (3 $\times$ 50 cm) was developed with 300 ml each of the following sequence of solvents; chloroform-methanol, 10/0, 9/1, 7/3, 5/5 and 3/7, v/v. Each fraction was examined by tlc and fractions of similar composition was combined and concentrated to give a colorless solid, mp $98\sim 100^\circ\text{C}$. Found: C, 50.73; H, 4.40; N, 17.52; S, 13.65. Calcd. for $\text{C}_{10}\text{H}_{11}\text{N}_3\text{O}_2\text{S}$: C, 50.61; H, 4.67; N, 17.70; S, 13.51%.

Spectroscopy. Nuclear magnetic resonance spectra (NMR) were obtained with a model R-20B spectrometer (Hitachi, Japan). Samples were dissolved in appropriate deuterated solvents. Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane.

Infrared spectra (IR) were recorded on a model EPI-G3 spectrometer (Hitachi, Japan). Compounds were dried *in vacuo* over phosphorus pentoxide and analyzed as a KBr pellet or a thin film on a NaCl crystal.

Mass spectrometric analyses (MS) were accomplished on a Shimadzu-LKB 9000 spectrometer (Shimadzu, Japan) at electron energies of 12 and 70 eV. Samples were analyzed by means of a direct insertion probe. Accurate mass measurement was accomplished using a RMU-7M mass spectrometer (Hitachi, Japan) with the $m/e = 242.98564$ peak of perfluorokerosene as reference.

Radioactive analysis. Most samples were directly analyzed for ^{14}C and ^3H contents by liquid scintillation counting (lsc) in a model LSC-230 (Beckmann, U.S.A.) using the following scintillation fluids: A, 2.7 g of 2, 5-diphenyloxazole in 1 liter of toluene; B, 5.0 g of 2, 5-diphenyloxazole and 100 g of naphthalene in 1 liter of dioxane. Selected blood samples and dried feces were combusted in a model 305 sample oxidizer (Packard, U.S.A.) prior to radioanalysis.

Thin-layer chromatography (tlc). Silica gel 60F-

TABLE I. CHROMATOGRAPHIC PROPERTIES OF S-1358 AND ITS METABOLITES

Compound	<i>R_f</i> in solvent system						
	I	II	III	IV	V	VI	VII
S-1358	0.53	0.62	0.76				
MR	0.21	0.20	0.62				
PTB	0.24	0.15	0.60				
BDS	0.73	0.84					
A			0.53	0.68			
B			0.37	0.63			
C			0.30	0.52			
D			0.25	0.44			
HM					0.39	0.18	
AP					0.72		0.28

254 chromatoplates (20×20 cm, 0.25 mm thickness, E. Merck, Germany) were used for both analytical and preparative tlc. The chromatoplates were developed with one of the following solvent systems: I, benzene-ethyl acetate (2/1, v/v); II, chloroform-ether (7/3); III, chloroform-methanol (9/1); IV, chloroform-methanol-formic acid (70/30/2); V, chloroform-methanol-diethylamine (70/30/2); VI, ethyl acetate-methanol (1/1); VII, *n*-hexane-acetone (1/9). *R_f* values of S-1358 and its metabolites are listed in Table I. Radioactive compounds were detected by radioautography using Fuji X-ray film 200 (Fuji Film, Japan) or radioscanning with a model TRM-1B tlc scanner (Aloka, Japan). Unlabeled standard compounds were visualized by u. v. fluorescence quenching and by spraying with 1% palladium chloride solution in 1 N hydrochloric acid (produced brown to yellow spots for several types of sulfur compounds). Percent distribution of radioactive metabolites was determined by scraping gel regions from the chromatoplates and counting them directly with a liquid scintillation spectrometer.

Animal studies. Male Sprague-Dawley rats weighing approximately 200 g were used throughout the present experiments. Each of the radiolabeled preparations was administered by stomach intubation as an aqueous suspension of 10% Tween 80 at a dose of 40 mg/kg, unless otherwise indicated. The treated rats were placed in a metabolism cage, enabling the separate collection of urine, feces and expired carbon dioxide, as described previously.⁷⁾ Urine, feces and air traps were assayed for either ¹⁴C or ³H contents by lsc. For the preparative purpose, sixty rats were orally dosed with either S-1358-¹⁴C or -³H at 300 mg/kg. Urine and feces were collected separately for 48 hr after dosing.

For the estimation of blood levels of radioactivity and unchanged S-1358, rats were dosed with S-1358-¹⁴C by oral intubation. Blood samples were withdrawn from the plexus venosus in orbita at various times after dosing into heparinized tubes and assayed for ¹⁴C content. The pooled blood was hemolyzed by addition of three-time volumes of distilled water and extracted with two-time volumes of ethyl acetate. The ethyl acetate extracts were subjected to tlc in solvent systems I and II. Radioactivity corresponding to unchanged S-1358 on the plate was measured, as described above.

For the whole-body radioautography, S-1358-¹⁴C was administered to rats by oral intubation. At intervals after dosing, the rats were anesthetized with ether and rapidly frozen to -60°C in an acetone-solid CO₂ mixture. The frozen animals were first cut with a jig-saw, embedded in a paste of carboxymethylcellulose and then mounted on the stage of a model JCMT-1A cryomicrotome (JEOL Ltd., Japan) at -20°C. Several saggital sections, 20 μm thick, were

taken through the mid-line and through the kidneys as described by Ullberg.⁸⁾ The sections were freeze-dried overnight and applied to Fuji X-ray film 200.

The bile duct of two rats was cannulated with a nylon cannula under ether anesthesia as described previously.⁹⁾ After recovery, S-1358-¹⁴C was injected into the femoral vein at 40 mg/kg. Bile was collected for up to 21 hr.

Metabolite isolation and purification. The collected urine and bile samples were lyophilized to dryness and the residues were dissolved in methanol. The feces were dried *in vacuo* over phosphorus pentoxide and then extracted with methanol. The methanol extracts from urine, feces and bile were respectively subjected to tlc in solvent systems III and IV for ¹⁴C metabolites and in solvent systems V and VI for ³H metabolites. Amounts of metabolites were determined by lsc of the radioactive gel regions determined by radioautography or tlc radioscanning.

For the preparative purpose, after lyophilization of the pooled urine, the residue was dissolved in methanol and applied to a column (4.5×84 cm) of Amberlite XAD-2 resin (Organo Ltd., Japan). The column was washed with 1 liter of water and then the radioactivity was eluted with methanol (2 liters). The methanol eluate containing more than 95% of the applied radioactivity was concentrated and used for successive purification.

A mixture of ¹⁴C metabolites was applied to the top of a 4.5×60 cm silica gel column (30~70 mesh, E. Merck) and eluted with 800 ml each of chloroform, various proportions of methanol in chloroform mixtures (v/v percent) (5, 10, 20, 30, 40, 50 and 70), followed by methanol. Each fraction (20 ml) was examined for ¹⁴C by lsc and fractions of similar composition were combined.

A mixture of ³H metabolites dissolved in 100 ml of water was applied to a 4.5×30 cm Dowex-1 (Muro-machi Kagaku Kogyo, Japan) column (OH⁻ form) and eluted with 800 ml each of distilled water, 0.1 N formic acid and 0.3 N formic acid. After elution of the void volume, 20 ml fractions were collected and analyzed for ³H by lsc. More than 95% of the applied ³H was eluted in one peak. The fractions in the peak were combined and lyophilized. The residue was dissolved in water and introduced onto a 3.5×45 cm Amberlite CG-50 (Organo Ltd., Japan) column (H⁺ form) which was eluted with 500 ml each of the followings; distilled water, 0.1 N NH₄OH, 0.3 N NH₄OH and 1 N NH₄OH. Each fraction (20 ml) was examined for ³H by lsc and fractions of similar composition were combined. Metabolite HM (*R_f*=0.39 in solvent system V) was successively purified by repeated preparative tlc using solvent system VI.

Enzymic hydrolysis of conjugates. Samples of

urine or bile were dissolved in 10 ml of 0.2 M sodium acetate buffer, pH 5.0, and incubated with excess β -glucuronidase (type B-3, from bovine liver, Sigma Chemical Co., U.S.A.) or aryl sulfatase (type II, from limpets, Sigma) at 37°C for 18 hr. The incubation solutions were lyophilized, extracted with methanol and subjected to tlc in solvent systems I and III.

RESULTS

Excretion, distribution and blood concentration

Figure 2 (A, B and C) shows cumulative excretion of radioactivity in the urine and feces of rats after a single oral administration of each radiolabeled S-1358 at the dose indicated. Excretion of radioactivity was almost complete within 4 days regardless of the positions of radiolabeling and the dose levels. When S-1358- ^{14}C was dosed to rats at 40 mg/kg, the final distribution of ^{14}C 4 days after dosing was 43% in the urine and 54% in the feces with a total accountability of 97%. At 300 mg/kg, 93% of the dosed ^{14}C was eliminated in the urine (36%) and feces (57%) during 6 days. In both cases, no radiocarbon was detected in the expired carbon dioxide. Comparative 4-day excretion from rats receiving S-1358- ^3H at 40 mg/kg was 42% in the urine and 41% in the feces. Recovery of the fecal radioactivity was somewhat lower with S-1358- ^3H than with S-1358- ^{14}C . This resulted in the lower recovery of total radioactivity with the pyri-

dine- ^3H label than with the benzyl- ^{14}C label.

Whole-body radioautography of rats dosed with S-1358- ^{14}C showed that concentrations of ^{14}C were high in stomach, intestines, liver and kidneys 3 hr after dosing (Fig. 3). The radioactivity was hardly detected in the brain or spinal cord. This distribution pattern remained unaltered from 3 hr to 8 hr after dosing.

When S-1358- ^{14}C was orally administered to rats at 40 mg/kg, ^{14}C concentration in blood reached maximal levels of 2.52 and 2.56 μg of S-1358 equivalent relative to g of fresh blood weight at 3 hr and 6 hr, respectively, and decreased thereafter to 0.47 μg at 48 hr after dosing as shown in Fig. 4. The S-1358 level was maximal (1.05 μg) at 1 hr and then rapidly declined to 0.07 μg 24 hr after dosing. The intact S-1358 represented 58.7, 35.7, 16.3 and 6.3% of the total radioactivity at 1, 3, 6 and 24 hr after dosing, respectively. No S-1358 was detected in the blood 48 hr after dosing.

Identification of S-1358- ^{14}C urinary metabolites

Essentially all of the radioactive metabolites were soluble in methanol after lyophilization of 0~48 hr urine from rats dosed with S-1358- ^{14}C . The methanol extract was analyzed by tlc in solvent systems III and IV. Proportion of the metabolites is listed in

FIG. 2. Excretion of Radioactivity in the Urine and Feces from Male Rats Receiving a Single Oral Dose of S-1358.

A, S-1358- ^{14}C at a dose of 40 mg/kg; B, S-1358- ^{14}C at a dose of 300 mg/kg; C, S-1358- ^3H at a dose of 40 mg/kg.

Total radioactivity (●), urinary radioactivity (○), fecal radioactivity (△).

FIG. 3. Radioautograph of Saggital Section of Rat Sacrificed 3 hr after an Oral Dose of S-1358- ^{14}C .

The black areas correspond to high levels of radioactivity. b=brain, sc=spinal cord, l=liver, k=kidney, i=intestines, st=stomach.

FIG. 4. Levels of Total ^{14}C and S-1358 in the Blood after Oral Administration of S-1358- ^{14}C at 40 mg/kg.

Total ^{14}C (○), intact S-1358 (△).

Table II. Four major metabolites, designated as A, B, C and D, accounted for about half of the urinary ^{14}C . The other half was referred to as polar metabolites which were remained at the origin of tlc.

The NMR data of metabolite A, B, C and the methyl ester of D are presented in Table III. These NMR data were similar and indicated the presence of parts of the *tert*-butyl group, the benzyl group and a new methyl group but the absence of the pyridine ring and the *n*-butyl group, suggesting that all

TABLE II. PROPORTIONS OF S-1358- ^{14}C AND ITS METABOLITES EXCRETED BY RATS

% of the administered dose of S-1358- ¹⁴ C				
Compound	Urinary metabolite			Fecal metabolite
	Free	Glucu- ronide	Total	
S-1358				14.2
MR				14.5
A	4.8	9.6	14.4	5.8
B	2.8	6.0	8.8	1.6
C	9.2	0.8	10.0	4.4
D	4.7	1.8	6.5	1.1
Unknowns			3.2	12.2
Total			42.9	53.9

of the metabolites are derived from the *tert*-butylbenzyl moiety of the S-1358 molecule.

The NMR of metabolite A exhibited the loss of three protons from the *tert*-butyl group and the presence of a new methylene proton signal due to the CH_2O group and one proton signal due to the OH group (disappeared on addition of D_2O), indicating hydroxylation of the *tert*-butyl group. A methyl proton signal at 2.80 ppm was assigned to the CH_3SO_2 group because sulfur was detected by elemental analysis (negative to palladium chloride test). By reaction with acetic anhydride in the

TABLE III. NMR DATA OF S-1358 METABOLITES

	Structure	Solvent	Chemical shift (ppm)						others
			a	b	c	d	e	f	
MR		CDCl ₃		4.30 2H,s	7.31 4H,s	1.32 6H,s	3.60 2H,s	2.73 H,br	
A		CD ₃ COCD ₃	2.80 3H,s	4.32 2H,s	7.43 4H,s	1.30 6H,s	3.58 2H,s	4.50 H,br	
B		CD ₃ COCD ₃	2.45 3H,s	3.96 2H,s	7.33 4H,d	1.29 6H,s	3.55 2H,s	3.25 H,br	
C		CD ₃ COCD ₃	2.81 3H,s	4.34 2H,s	7.42 4H,s	1.54 6H,s			6.36 H,br (COOH)
D-ME		CDCl ₃	2.48 3H,s	3.97 2H,s	7.31 4H,s	1.59 6H,s			3.66 3H,s (OCH ₃)

presence of pyridine, metabolite A was converted to the corresponding acetate, which showed a new acetyl proton signal at 1.92 ppm (3H, s) in NMR and a characteristic carbonyl absorption band at 1720 cm⁻¹ in IR. The MS of metabolite A represented a weak molecular ion peak of *m/e* 242 with predominant fragments appeared at *m/e* 211 (M-CH₂OH), 163 (M-CH₃SO₂), 147, 132 and 117. Elemental analysis resulted in the following elemental compositions: Found C, 59.38; H, 7.45. Calcd. for C₁₂H₁₆O₃S; C, 59.48; H, 7.49%. These analytical data established that metabolite A is *p*-(1,1-dimethyl-2-hydroxyethyl)benzyl methyl sulfone.

The NMR of metabolite B was similar to that of metabolite A. Since both methyl and methylene proton signals occurred at somewhat higher field as compared with that of the CH₃SO₂CH₂ moiety of A, this methyl signal was assigned to the CH₃S group (positive to palladium chloride test). In addition, metabolite B reacted with acetic anhydride in the presence of pyridine to yield an acetate whose NMR contained a new methyl proton signal (1.90 ppm, 3H,s) due to the acetyl group and IR showed a carbonyl absorption band

at 1725 cm⁻¹. The MS of the acetate exhibited a molecular ion peak at *m/e* 252 and following fragments; *m/e* 205 (M-CH₃S), 179 (M-CH₂OCOCH₃), 145 and 133. These results confirmed that metabolite B is *p*-(1,1-dimethyl-2-hydroxyethyl)benzyl methyl sulfide.

The NMR of metabolite C exhibited a singlet signal of 6 protons, a methyl proton signal due to the CH₃SO₂, a methylene proton signal due to the benzyl group and aromatic proton signals. The IR showed absorption bands due to the carboxylic acid group (a broad band at 3,000 and a strong band at 1700 cm⁻¹). By reaction with diazomethane, metabolite C was transformed to the corresponding methyl ester whose IR exhibited a carbonyl absorption band at 1725 cm⁻¹ and NMR represented a new methoxy proton signal at 3.67 ppm (3H, s.). The MS of the methyl ester showed the following peaks: *m/e* 270 (M), 211 (M-COOCH₃), 191 (M-CH₃SO₂), 163 and 132. On the basis of these data, metabolite C was identified as *p*-(1-methyl-1-carboxylethyl)benzyl methyl sulfone.

Metabolite D was directly treated with diazomethane to form the methyl ester whose IR showed a carbonyl absorption band at

1725 cm^{-1} The NMR of the ester (D-Me) differed from that of the methyl ester of metabolite C in the chemical shift of both methyl and methylene proton signals which appeared in somewhat higher field. This indicated that the methyl signal corresponds to the CH_3S group because of positive test to the palladium chloride reagent. The MS of the methyl ester showed the following ions: m/e 238 (M), 191 (M- CH_3S), 179 (M- COOCH_3), 163 and 132. On this evidence, metabolite D was identified as *p*-(1-methyl-1-carboxylethyl)benzyl methyl sulfide.

A mixture of polar metabolites was deconjugated by incubation with β -glucuronidase, but not with aryl sulfatase. About 92% of the polar metabolites were hydrolyzed by the action of β -glucuronidase to yield aglycones, which mainly consisted of metabolites A, B, C and D. This indicated that metabolites A, B, C and D are also present as glucuronide conjugates. Proportion of these aglycones is listed in Table II.

Identification of S-1358- ^{14}C fecal metabolites

Fecal metabolites were extracted with methanol and identified by cochromatography with the reference compounds in solvent systems I and III. Proportion of the metabolites is listed in Table II. Although metabolites A, B, C and D were present in the feces, main products were intact S-1358 and MR. They accounted for 28.7% of the dose.

MR was assigned as the hydroxylation product of S-1358 at the *tert*-butyl group, based on the following evidence. The NMR of MR was similar to that of S-1358 and exhibited the presence of the pyridine ring and the *n*-butyl group, the loss of three protons from the *tert*-butyl group and the existence of a new methylene signal due to the CH_2O group, and one proton signal due to the OH group (disappeared on addition of D_2O). This indicated hydroxylation at the *tert*-butyl group. The MS showed the following ion peaks; m/e 388 (M), 358 (M- CH_2O), 332 (M- C_4H_9), 299 (M- SC_4H_9), 255, 193 and 163. Reduction of MR with sodium in liquid ammonia¹⁰⁾

produced 2-methyl-2-*p*-tolylpropanol, which was identified by cochromatography with the synthesized authentic compound ($R_f=0.35$ in *n*-hexane-acetone, 4/1). These results established that MR is S-*n*-butyl S'-(1,1-dimethyl-2-hydroxyethyl)benzyl N-3-pyridyl-dithiocarbonimide.

Identification of S-1358- ^3H metabolites

Radioactive metabolites were extracted with methanol after lyophilization of the pooled urine, and analyzed by tlc in solvent systems V and VI. The main urinary metabolite HM represented more than 90% of the urinary ^3H . AP occurred to a minor extent (Table IV).

TABLE IV. PROPORTIONS OF S-1358- ^3H AND ITS METABOLITES EXCRETED BY RATS

Compound	% of the administered dose of S-1358- ^3H	
	Urinary metabolite	Fecal metabolite
S-1358		21.0
MR		9.5
AP	0.2	1.3
HM	38.8	
Unknowns	2.8	8.7
Total	41.8	40.5

Metabolite HM was purified by ion-exchange column chromatography as described above. The tlc features of HM were not altered during the course of purification. In addition, behavior during purification by ion-exchange column chromatography indicated the presence of both acidic and basic groups in the HM molecule (HM was eluted with 0.1 N formic acid from the Dowex-1 column and with 0.3 N NH_4OH from the Amberlite CG-50 column). Since HM showed broad signals in NMR (CD_3OD), the spin coupling of these signals was not characterized. Therefore, HM was led to derivatives. By reaction with diazomethane, HM gave HM-1 and HM-2 whose R_f values were 0.32 and 0.54 in solvent system III, respectively. HM-1 was transformed to HM-2 by successive methylation with diazomethane. In addition, HM-1 was converted to HM-3 ($R_f=0.63$ in solvent system III) by reaction

TABLE V. NMR DATA OF S-1358-³H METABOLITE HM AND ITS DERIVATIVES

	Structure	Solvent	Chemical shift (ppm)					
			a	b	c	d	e	others
HM		CD ₃ OD	7.80 4H,br	3.60 2H,br	4.50 H,br	8.60 H,br		
HM-1		CDCl ₃	7.78 4H,m	3.55 2H,d	4.70 H,t	6.40 H,br	3.68 3H,s	
HM-2		CDCl ₃	7.75 4H,m	3.41 2H,m	4.39 H,q		3.80 3H,s	3.10 3H,s (NCH ₃)
HM-3		CDCl ₃	7.78 4H,m	3.45 2H,m	5.43 H,q		3.83 3H,s	2.68 3H,s(COCH ₃)

with acetic anhydride in the presence of pyridine. The NMR data of these derivatives are shown in Table V, together with that of HM. The NMR of HM-1 indicated the presence of the pyridine ring, the CH₂CH group (the doublet signal at 3.55 ppm and the triplet signal at 4.70 ppm were decoupled to singlets by irradiation at 4.70 and 3.55 ppm, respectively), the NH group (one proton signal at 6.40 ppm disappeared on addition of D₂O) and the CH₃O group. Ms fragments appeared at *m/e* 237 (M), 178 (M-COOCH₃), 151, 137, 119 and 93. An experimental accurate mass value of 237.0573 for the molecular ion was in good agreement with the theoretical value of 237.0550 calculated for C₁₀H₁₁N₃O₂S. Based on these analytical data, HM-1 was identified as 2-(3'-pyridylimino)-4-methoxycarbonylthiazolidine, which was synthesized by reaction of NCS with L-cysteine followed by methylation with diazomethane. The synthesized compound and HM-1 had identical NMR and tlc features. This evidence confirmed the proposed structure.

The HM-2 exhibited an additional methyl signal due to the NCH₃ group. Ms fragments appeared at *m/e* 251 (M), 198, 192 (M-COOCH₃), 151 and 136. These data indicated that HM-2 is the N-methyl derivative of HM-1.

The NMR of HM-3 showed the presence of a new methyl signal due to the acetyl group. Ms fragments appeared at *m/e* 279 (M), 248 (M-CH₃O), 237 (M-COCH₃), 220 (M-COCH₃), 178, 161 and 151. Accurate mass measurement of HM-3 resulted in the following elemental compositions; C₁₂H₁₃N₃O₂S (Found, 279.0677; calcd., 279.0675). These analytical data established that HM-3 is 2-(3'-pyridylimino)-3-acetyl-4-methoxycarbonylthiazolidine.

Since the three HM derivatives were identified, the structure of HM is assigned as 2-(3'-pyridylimino)-4-carboxylthiazolidine. Also, the reaction processes are shown as follows:

Fecal metabolites were analyzed by tlc and identified by tlc cochromatography with the reference compounds. Proportion of the metabolites is listed in Table IV. The main component was intact S-1358, and MR and AP were also found in the feces.

Bile metabolites

For the assessment of contribution of biliary secretions to the fecal excretion of radiolabeled

FIG. 5. Excretion of Radioactivity in the Bile of Rats after Intravenous Administration of S-1358- ^{14}C at 40 mg/kg.

TABLE VI. PROPORTIONS OF BILE PRODUCTS AFTER INTRAVENOUS ADMINISTRATION OF S-1358- ^{14}C TO THE CANNULATED RATS

Product	% of recovered radiocarbon			
	hr after administration			
	2	4	8	21
Without β -glucuronidase treatment				
Origin	96.7	94.6	95.8	91.3
With β -glucuronidase treatment				
BDS	1.3	0.9	0.8	0.7
S-1358	2.9	2.5	2.2	0.7
MR	16.1	6.7	18.0	4.0
PTB	13.3	4.2	9.1	3.2
A	13.9	43.9	28.6	40.8
B	4.4	7.2	6.5	11.7
C	0.5	0.9	1.3	2.0
D	1.2	2.1	1.9	2.4
Origin	46.5	31.2	31.7	34.5

metabolites, the biliary excretion of ^{14}C metabolites was examined for 21 hr after intravenous administration of S-1358- ^{14}C . As shown in Fig. 5, about 60% of the dose was recovered during 21 hr. After lyophilization of the bile, ^{14}C metabolites were extracted with methanol and then analyzed by tlc in solvent systems I and III. Although intact S-1358, BDS, MR, PTB, metabolites A, B, C and D were present in very small amounts in the bile, about 91 to 97% of the radioactivity were polar metabolites which remained at the origin of the tlc plate. Most of the polar metabolites

were deconjugated by incubation with β -glucuronidase, but not with aryl sulfatase. The relative percentage of the ^{14}C metabolites after β -glucuronidase treatment is listed in Table VI. These results indicated that the major bile products, MR, metabolites A and B, are mostly present as glucuronide conjugates.

DISCUSSION

This study clearly established that S-1358 is extensively metabolized and readily excreted *via* urine and feces from orally dosed rats. On the basis of the results in Figs. 2 and 4, the benzyl-labeled S-1358 and its metabolites appear not to persist in tissues and organs following a single oral dosing. The lower recovery of radioactivity with S-1358- ^3H than with S-1358- ^{14}C may be due in part to exchange under the acidic conditions in stomach or hydroxylation at the labeled position (the 5-position of the ring).

The identified metabolites account for more than 80% of the dose of both the benzyl- and pyridine-labeled S-1358. The proposed metabolic pathways are shown in Fig. 6. Most of the identified metabolites derived from both S-1358- ^3H and ^{14}C except MR has been

FIG. 6. Proposed Metabolic Pathways of S-1358 in the Rats.

cleaved at the dithiocarbonimidate linkage, indicating the great lability of this linkage to metabolic attack. The ease of metabolism also results from the attack at the *tert*-butyl group of the molecule, namely all of the benzyl-labeled metabolites underwent hydroxylation at the *tert*-butyl group or subsequent oxidation to the corresponding carboxylic acids. When S-1358 or its metabolites such as MR are cleaved at the dithiocarbonimidate linkage, substituted benzyl mercaptans could be produced and they are subsequently methylated to give the corresponding methyl sulfides. The sulfides are further oxidized to the corresponding sulfoxes. On the other hand, coincident with the liberation of the mercaptans, the pyridylimino moiety appears to conjugate with either free or peptide-bonded cysteine to give the final product 2-(3'-pyridylimino)-4-carboxylthiazolidine.

It has been demonstrated that Captan is degraded by reaction with thiol compounds to form transient thiophosgene, which subsequently reacted with either cysteine or glutathione to give a thiazolidine derivative.¹¹⁾ In fact, Captan is metabolized in rats to give thiazolidine-2-thione-4-carboxylic acid, which is not further metabolized in the rats.¹²⁾ Cyanide is also detoxicated by a pathway in which it is conjugated with cysteine to form 2-iminothiazolidine-4-carboxylic acid which is excreted in the urine.¹³⁾ In addition, dimethyldithiocarbamate (DDC) fungicides are transformed enzymatically in plants into L-DDC-alanine, which is converted non-enzymatically into thiazolidine-2-thione-4-carboxylic acid.¹⁴⁾ Since S-1358 does not directly react with cysteine or glutathione under physiological conditions, it is reasonable to speculate that S-1358 may be transformed to transient products which are reactive with the cysteine moiety in the presence or absence of enzymes. The mechanism of formation of the thiazolidine from S-1358 is under investigation.

The methyl ester of metabolite HM, HM-1, was synthesized by reaction of NCS with L-cysteine followed by methylation with diazomethane. The reaction process could be

proposed as follows:

Major biliary products were MR, PTB and the glucuronide conjugates of metabolite A and B. MR was one of the major product in the feces but not present in the urine.

PTB was not found in both urine and feces. Although the glucuronides of A and B were detected in both bile and urine, they were not present in the feces in significant amounts. These results suggest that these metabolites were reabsorbed and eliminated in the urine or further biotransformed to other metabolites.

Acknowledgments. The authors wish to thank Mr. S. Tanaka of this laboratory for advice and providing the reference compounds, Mr. H. Koda of this laboratory for technical assistance, Messrs. N. Mikami and M. Miyakado of this laboratory for NMR measurement and Mr. S. Hishida of Hitachi Ltd. for accurate mass measurement. The authors thank Sumitomo Chemical Co. Ltd. for permission to publish this paper.

REFERENCES

- 1) T. Kato, S. Tanaka, S. Yamamoto, Y. Kawase and M. Ueda, *Ann. Phytopath. Soc. Japan*, **41**, 1 (1975).
- 2) T. Kato, S. Tanaka, M. Ueda and Y. Kawase, *Agr. Biol. Chem.*, **38**, 2377 (1974).
- 3) T. Kato, S. Tanaka, M. Ueda and Y. Kawase, *ibid.*, **39**, 169 (1975).
- 4) T. Kadota, unpublished.
- 5) M. Hazue, unpublished.
- 6) S. Tanaka, unpublished.
- 7) J. Miyamoto, Y. Sato, K. Yamamoto, M. Endo and S. Suzuki, *Agr. Biol. Chem.*, **32**, 628 (1968).

- 8) S. Ullberg, *Proc. Second U.N. Intern. Confer. Peaceful Uses At. Energy*, **24**, 248 (1958).
 - 9) J. Miyamoto, T. Nishida and K. Ueda, *Pest. Biochem. Physiol.*, **1**, 293 (1971).
 - 10) H. Ohkawa and M. Eto, *Agr. Biol. Chem.*, **33**, 443 (1969).
 - 11) R. J. Lukens, S. Rich and J. G. Horsfall, *Phytopathology*, **55**, 658 (1965); R. J. Lukens and H. D. Sisler, *ibid.*, **48**, 235 (1958); R. J. Lukens and H. D. Sisler, *Science*, **127**, 650 (1958).
 - 12) H. Seidler, H. Hartig, W. Schnaak and R. Engst, *Die Nahrung*, **15**, 177 (1971); J. R. Debaun, J. B. Miaullis, J. Knarr, A. Mihailovski and J. J. Menn, *Xenobiotica*, **4**, 101 (1974).
 - 13) D. V. Parke, "The Biochemistry of Foreign Compounds," Pergamon Press, London, 1968, p.97.
 - 14) H. M. Dekhuijzen, J. W. Vonk and A. K. Sijpesteijn, "Pesticide Terminal Residues," ed. by A. S. Tahori, Butterworths, London, 1971, p. 233.
-