Original Article

Metabolism of (*R*)- and (*S*)-Isomers of (*E*)-1-(2,4-Dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol (S-3308L) in Rats

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Metabolic fates of (*R*)- and (*S*)-(*E*)-1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ols, designated as (*R*)-*E* and (*S*)-*E*, labeled with ^{14}C at the benzene or triazole ring were examined after oral administration to male and female rats at dose levels of 5 and 125 mg/kg. There was no remarkable difference between enantiomers in the ^{14}C -excretion into the feces and urine, ^{14}C tissue residues on the 7th day after administration, and amounts of metabolites in the feces and urine. However, biotransformation and excretion rates were faster with (*S*)-*E* *in vivo* than with (*R*)-*E*, and also biotransforming rates by the hepatic microsomal fraction of rats were faster with (*S*)-*E* than with (*R*)-*E*. Males excreted more ^{14}C into the feces than females. A major metabolite, a carboxylic acid derivative of the 4-methyl group of (*R*)-*E* or (*S*)-*E*, was mainly excreted into feces in both sexes, but its excretion into the urine was in larger amounts in females than in males. 1-(2,4-Dichlorophenyl)-2-(1,2,4-triazol-1-yl)propan-1,3-diols were found in small amounts in the feces, and 1,2,4-triazole in the feces, urine and skin with hair. A successive 14-day pretreatment with unlabeled S-3308L, a mixture of (*R*)-*E* and (*S*)-*E* (*ca.* 8/2), did not affect the metabolism of (*R*)-*E* and (*S*)-*E* in rats.

INTRODUCTION

S-3308L is a vinyl triazole derivative, which has antimicrobial activities derived from its ability to inhibit biosynthesis of ergosterol.¹⁻⁴⁾ Its major ingredients are (*R*)- and (*S*)-(*E*)-1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ols, designated as (*R*)-*E* and (*S*)-*E*,* respectively. Their isomers, (*R*)-*Z* and (*S*)-*Z*, are the minor ingredients (about 3%).

Metabolic fates of racemic (*RS*)-*E* and (*RS*)-*Z* in male rats have already been examined using ^{14}C -labeled compounds in the previous

studies,⁵⁾ which revealed the following facts: 1) ^{14}C was excreted rapidly and almost completely into the feces and urine, 2) ^{14}C residue levels in the tissues were extremely low, 3) the main metabolic pathway was oxidation of the 4-methyl group, and 4) metabolites underwent enterohepatic circulation. To evaluate the safety of S-3308L, the comparison of metabolic fates of (*R*)-*E* and (*S*)-*E* in rats becomes necessary, since the composition of S-3308L is not (*R*)-*E*/*(S)*-*E*=5/5 but about 8/2. The comparison of metabolic fate between the optical isomers and (*RS*)-*E* is also of interest.

This is to report the studies on the absorption, distribution, excretion and biotransformation of (*R*)-*E* and (*S*)-*E* in male and female rats using ^{14}C -labeled compounds. The effect of repeated oral administration of S-3308L on the

* In the previous papers these isomers were designated as *R*(—) and *S*(+),⁴⁾ or (*R*)-8AE and (*S*)-8AE,⁵⁾ respectively.

metabolism of (R)-E and (S)-E are also discussed. The results obtained are compared with those of racemic (RS)-E.

MATERIALS AND METHODS

1. Chemicals

S-3308L (chemical purity 97.3%, $E/Z=96.8/3.2$, $R/S=82.9/17.1$), (R)-E (chemical purity 98.6%, $E/Z=100/0$, $R/S=99.8/0.2$) and (S)-E (chemical purity 100%, $E/Z=100/0$, $R/S=0.6/99.4$) were prepared in our research center.¹⁻³⁾ 1,2,4-Triazole was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). (R)-E and (S)-E labeled at either the triazolyl group [$\text{Tr-}^{14}\text{C}$ -(R)-E and $\text{Tr-}^{14}\text{C}$ -(S)-E] or the phenyl group [$\text{Ph-}^{14}\text{C}$ -(R)-E and $\text{Ph-}^{14}\text{C}$ -(S)-E] were prepared by Nakatsuka *et al.*⁶⁾ The specific activities of $\text{Tr-}^{14}\text{C}$ -(R)-E and $\text{Tr-}^{14}\text{C}$ -(S)-E were 25.7 mCi/mmol and those of $\text{Ph-}^{14}\text{C}$ -(R)-E and $\text{Ph-}^{14}\text{C}$ -(S)-E 23.4 mCi/mmol. Their radiochemical and optical purities were more than 99%.

2. Excretion Balance Study

SD rats were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan) and acclimatized for about one week before administration of chemicals in an air-conditioned room at a temperature of $25\pm 2^\circ\text{C}$ and a humidity of $55\pm 10\%$. Rats were given CE-2 laboratory chow (Clea Japan, Inc.) and water *ad libitum*. Rats treated with ^{14}C -labeled compounds dissolved in corn oil at a rate of 5 ml/kg were housed in Metabolica CO-2® (Sugiyamagen Iriki Co., Ltd., Tokyo, Japan) to collect their feces and urine separately. On the 7th day, rats were sacrificed by bleeding, and

their tissues and organs were dissected out.

Rats were treated with chemicals in the following manners: *Experiment 1.* Five male and five female rats of seven-week old were dosed with ^{14}C -labeled (R)-E or (S)-E orally at rates of 5 and 125 mg/kg. *Experiment 2.* Five male and five female rats of five-week old were dosed with S-3308L orally at a rate of 5 mg/kg/day for successive 14 days, and on the next day of the last pretreatment they were dosed with $\text{Tr-}^{14}\text{C}$ -(R)-E or $\text{Tr-}^{14}\text{C}$ -(S)-E at a rate of 5 mg/kg.

For higher dosage, the labeled compounds were diluted 25 times with the corresponding unlabeled compounds.

3. Bile Study

The bile study was carried out using eight- or nine-week old male and female SD rats as described previously.^{5,7)}

4. Measurement of Radioactivity

Radioactivity in the feces, urine, bile, tissues and silica gel regions was quantified by liquid scintillation counting (LSC) as described previously.⁵⁾

5. Thin-layer Chromatography (TLC)

Silica gel 60F₂₅₄ chromatoplates (Merck, Darmstadt, FRG) were used with solvent systems A to F, shown in Table 1. Unlabeled standards were detected by viewing under UV light. Radioactive metabolites were located by a TLC Linear Analyser LB283 (Berthold, FRG) or autoradiography using Kodak SB-5 films.

Table 1 R_f values of DTP-diols, their acetates and triazole.

		R_f value on TLC ^{a)}					
		A	B	C	D	E	F
DTP-diol(A)		0.00	0.00	—	—	0.30	0.32
DTP-diol(B)		0.00	0.00	—	—	0.21	0.20
DTP-diol(A)	monoacetate	—	—	—	—	—	0.42
DTP-diol(B)	monoacetate	—	—	—	—	—	0.25
Triazole		0.05	0.11	0.01	0.07	0.50	—

^{a)} Solvent systems: A, toluene/acetonitrile/methanol (20/4/1, v/v); B, toluene/ethyl acetate/acetic acid (3/6/1, v/v); C, chloroform/acetone (9/1, v/v); D, chloroform/acetic acid (5/1, v/v); E, ethyl acetate/ethanol/water/acetic acid (10/4/1/1, v/v); F, ethyl acetate/ethanol (3/2, v/v).

Fig. 1 Proposed metabolic pathways of (R)-E and (S)-E in rats.

6. Identification and Quantification of Metabolites

Metabolites in the feces, urine and bile were separated by TLC, identified by TLC co-chromatography with authentic standards, and quantified by LSC of the scraped silica gel as described previously.⁵⁾ Chemical structure and abbreviation of each metabolite are shown in Fig. 1.

Triazole was isolated from the urine by extraction with ethyl ether, followed by purification of the extracts using TLC with solvent systems A, B, E and toluene/ethyl acetate/acetic acid/2-propanol (8/12/3/5, v/v), and identified by TLC cochromatography with an authentic standard.

DTP-diol (A) and DTP-diol (B) whose structure remained as unknown were purified as described below and identified by using a JEOL-JNM-FX-100 NMR spectrometer and a Hitachi M-80 mass spectrometer. Fifteen male rats of seven-week old orally received 500 mg/kg of Tr-¹⁴C-(R)-E with specific activity of 51.4 μ Ci/mmol 3 or 4 times a week (total amount: 4.35 g) and their feces were collected. An aqueous suspension of the feces was homog-

enized by a Multi-Blender-Mill (Nihon Seiki, Tokyo, Japan) and the homogenate was extracted with methanol. After concentration the methanol extract was applied on an Amberlite XAD-2 column (95 mm i.d. $\times$ 260 mm) and the column was washed with water. Most of the radioactivity was eluted with methanol. The methanol eluate was concentrated and adjusted to pH 2 by adding 2 N HCl and the aqueous solution was washed with ethyl ether. The aqueous layer was neutralized with sodium bicarbonate and lyophilized. The residue dissolved in a small volume of methanol was applied on a silica gel 60 column (70–230 mesh, Merck, 25 mm i.d. $\times$ 600 mm). Two of the main metabolites eluted by 800 ml of ethyl acetate/ethanol=3/2 (v/v) and 500 ml of ethyl acetate/ethanol/acetone=3/2/2 (v/v) were further purified by TLC using solvent systems E and F (See Table 1). The isolated DTP-diol (A) and DTP-diol (B) amounted to 13.3 and 8.8 mg, respectively.

Metabolites in the skin and hair were extracted 4 times with 10 fold volume of methanol by sonication and decantation. After quantification of radiocarbon in the extract and

the residual tissue, metabolites in the extract were analyzed by TLC.

7. Distribution of Metabolites in Tissues

Seven-week old male SD rats were orally dosed with Tr-¹⁴C-(*R*)-*E* or Tr-¹⁴C-(*S*)-*E* at a rate of 5 mg/kg. Three rats each were sacrificed 0.5, 1, 2, 4, 8, 16, 24, 48 and 72 hr after treatment. The amount of ¹⁴C distributed to the liver, kidney, blood, adrenal and skin were measured, and the metabolites in the liver, kidney and blood were quantified.

8. In Vitro Metabolism Study

Seven-week old male and female SD rats were decapitated and their livers were dissected out. The hepatic microsomal fraction was obtained as described previously.⁸⁾ The reaction mixtures containing a 0, 1, 2, 4, 8 or 12 μ M substrate [Tr-¹⁴C-(*R*)-8AE or Tr-¹⁴C-(*S*)-8AE], 3 mM NADPH, a microsomal fraction equivalent to 210 mg fresh tissue and a 50 mM potassium phosphate buffer of pH 7.4 in a total volume of 0.42 ml were incubated aerobically for 15 min at 37°C. Reaction was initiated by adding the substrate in 10 μ l of ethanol using a microliter syringe and terminated by adding 4 ml of ice-cold methanol.

Radioactivity was recovered nearly 100% in the supernatant solution after centrifugation at 1500 $\times g$ for 10 min. The metabolites were separated by TLC. Biotransforming activities were calculated from the amount of the substrate metabolized. Values of the kinetic constants were calculated from the Lineweaver-Burk diagrams as previously reported.⁸⁾

RESULTS

1. Elimination

Table 2 summarizes the cumulative ¹⁴C excretion into the feces and urine for 7 days. Total recoveries of radioactivity in male and female rats with both (*R*)-*E* and (*S*)-*E* were 95–102%. At a dose level of 5 mg/kg in Expt. 1, males excreted 80–87% of dosed ¹⁴C into the feces and 14–19% into the urine, while females excreted 54–68% into the feces and 27–41% into the urine. Males excreted more radio-carbon into the feces than females. By increasing a dose to 125 mg/kg, the fecal ¹⁴C excretion slightly decreased in males dosed with (*R*)-*E* and females dosed with (*S*)-*E* but remained almost unchanged in others. The ¹⁴C excretion into the excreta was quite similar in rats which had received S-3308L at 5 mg/kg/day for successive 14 days prior to the ad-

Table 2 Cumulative ¹⁴C excretion in rats dosed with ¹⁴C-labeled (*R*)-*E* or (*S*)-*E* within 7 days post oral administration.

Sex	Isomer	Excreta	Amount (% of dosed ¹⁴ C)			
			Expt. 1		Expt. 2	
			5 mg/kg		125 mg/kg	
			Tr- ¹⁴ C	Ph- ¹⁴ C	Tr- ¹⁴ C	Tr- ¹⁴ C
Male	(R)	Feces	80.3 $\pm$ 2.0	81.0 $\pm$ 2.1	72.3 $\pm$ 2.3	80.5 $\pm$ 3.5
		Urine	16.8 $\pm$ 1.6	18.9 $\pm$ 2.3	27.5 $\pm$ 2.5	19.8 $\pm$ 3.5
		Total	97.1 $\pm$ 0.9	99.9 $\pm$ 0.3	99.8 $\pm$ 0.5	100.3 $\pm$ 0.3
	(S)	Feces	81.9 $\pm$ 2.7	87.2 $\pm$ 1.1	80.2 $\pm$ 2.0	84.4 $\pm$ 1.7
		Urine	16.0 $\pm$ 2.0	13.7 $\pm$ 1.1	19.9 $\pm$ 1.9	16.4 $\pm$ 1.7
		Total	97.9 $\pm$ 1.2	100.9 $\pm$ 1.2	100.1 $\pm$ 0.2	100.8 $\pm$ 0.2
	(R)	Feces	59.2 $\pm$ 3.4	54.3 $\pm$ 1.9	52.1 $\pm$ 4.0	58.9 $\pm$ 2.5
		Urine	38.3 $\pm$ 3.5	41.2 $\pm$ 2.5	46.3 $\pm$ 4.3	41.3 $\pm$ 2.4
		Total	97.5 $\pm$ 0.4	95.5 $\pm$ 1.0	98.4 $\pm$ 0.6	100.2 $\pm$ 0.4
Female	(S)	Feces	67.8 $\pm$ 1.8	68.1 $\pm$ 4.0	57.0 $\pm$ 2.4	75.2 $\pm$ 1.2
		Urine	27.4 $\pm$ 1.9	28.6 $\pm$ 4.0	44.6 $\pm$ 2.9	26.5 $\pm$ 1.2
		Total	95.2 $\pm$ 0.3	96.7 $\pm$ 0.9	101.6 $\pm$ 0.5	101.7 $\pm$ 0.4

Values are means $\pm$ S.E. of five animals.

ministration of ^{14}C -labeled compounds (Expt. 2) and in rats which had not (Expt. 1). Female rats dosed with (*S*)-*E* excreted a larger amount of ^{14}C into the feces than rats dosed with (*R*)-*E*.

2. Tissue Residues

Tissue residues were very low in all groups of male and female rats (Expts. 1 and 2) dosed with (*R*)-*E* and (*S*)-*E*. Skin with hair had the highest levels, but the levels were below 30 ng of parent compound equivalents/g tissue (ppb) in rats given 5 mg/kg and 1125 ppb in the rats receiving 125 mg/kg. Most of the radiocarbon distributed to skin and hair was extracted with methanol, and a major metabolite in the methanol extract was identified as triazole (75% of ^{14}C distributed to the skin and hair). ^{14}C -Levels were similar in the tissues of rats dosed with (*R*)-*E* or (*S*)-*E* and rats dosed with (*RS*)-*E*, which the previous report described.⁵⁾

3. Identification of Metabolites in Excreta

Two polar metabolites, which had not been identified in the previous study,⁵⁾ were isolated from the feces of rats dosed with 500 mg/kg of (*R*)-*E* and identified by spectroscopy. To confirm the chemical structures, these metabolites were treated with acetic anhydride-pyridine overnight at room temperature, and the monoacetates obtained were analyzed. Based on the spectral data shown below, the metabolites were identified as diastereomers of 1-(2, 4-dichlorophenyl)-2-(1, 2, 4-triazol-1-yl)propan-1, 3-diol [designated as DTP-diol (A) and DTP-diol (B)].

DTP-diol (A) ^{13}C -NMR $\delta^{\text{CD}_3\text{OD}}$ ppm: 151.7, 145.9, 137.2, 134.7, 133.2, 131.5, 129.7, 127.8, 65.3, 63.5 and 62.2; ^1H -NMR $\delta^{\text{CD}_3\text{O}_2\text{CO}}$ ppm: 4.0–4.5 (2 H, m), 5.2–5.3 (2 H, m), 7.15 (1 H, dd, $J=8.5$, 2.2), 7.25 (1 H, d, $J=2.2$), 7.62 (1 H, s), 7.76 (1 H, d, $J=8.5$) and 8.26 (1 H, s); EI-MS m/z : 232, 200, 165 (base peak) and 69. *DTP-diol (A) monoacetate* ^1H -NMR $\delta^{\text{CD}_3\text{O}_2\text{CO}}$ ppm: 1.88 (3 H, s), 4.64 (1 H, dd, $J=9.5$, 3), 4.75 (1 H, dd, $J=9.5$, 6), 5.05 (1 H, d, $J=10.3$), 5.33 (1 H, ddd, $J=10.3$, 6, 3), 7.13 (1 H, dd, $J=8.4$, 2.2), 7.23 (1 H, d, $J=2.2$), 7.63 (1 H, s), 7.70 (1 H, d, $J=8.4$) and 8.24 (1 H, s); EI-MS m/z : 232, 200, 165 (base peak) and 69. *DTP-diol (B)* ^{13}C -NMR $\delta^{\text{CD}_3\text{OD}}$ ppm: 151.9, 146.6, 137.3, 135.2, 133.2, 131.6, 130.2, 128.4,

64.9, 62.3 and 62.0; ^1H -NMR $\delta^{\text{CD}_3\text{O}_2\text{CO}}$ ppm: 3.6–3.8 (2 H, m), 5.08 (1 H, d, $J=8.8$), 5.2–5.6 (1 H, m), 7.32 (1 H, dd, $J=8.5$, 2.2), 7.45 (1 H, d, $J=2.2$), 7.87 (1 H, s), 8.02 (1 H, d, $J=8.5$) and 8.52 (1 H, s); EI-MS m/z : 232, 200, 165 (base peak) and 69. *DTP-diol (B) monoacetate* ^1H -NMR $\delta^{\text{CD}_3\text{O}_2\text{CO}}$ ppm: 1.84 (3 H, s), 4.39 (1 H, d, $J=7.8$), 4.40 (1 H, d, $J=4.9$), 5.03 (1 H, d, $J=8.3$), 5.50 (1 H, ddd, $J=8.3$, 7.8, 4.9), 7.37 (1 H, dd, $J=8.3$, 2.2), 7.47 (1 H, d, $J=2.2$), 7.83 (1 H, s), 8.03 (1 H, d, $J=8.3$) and 8.44 (1 H, s); EI-MS m/z : 165 (base peak) and 69.

4. Quantification of Metabolites in Excreta

Since rats dosed with ^{14}C labeled (*R*)-*E* or (*S*)-*E* excreted more than 94% of radiocarbon of the dose within 2 or 3 days after administration, the metabolites in their feces and urine pooled for 2 or 3 days were quantified. The results are summarized in Tables 3 and 4.

In male rats dosed with 5 mg/kg of (*R*)-*E* (Expt. 1), a carboxylic acid derivative, 4-COOH, was a major metabolite in feces (47–56% of the dose) and urine (7–9% of the dose). Unmetabolized (*R*)-*E* and its alcohol derivative, 5-OH, were excreted only in the feces, amounting to 1–5% and 2–5%, respectively. Metabolites hydroxylated at the benzene ring, 3-OH- ϕ , (4-OH-5-Cl)- ϕ and (3-OH-4-SCH₃)- ϕ , accounted for only small amounts. DTP-acids (both *E* and *Z* forms), which lack the *tert*-butyl group, amounted to 0.2% in the feces and 4–6% in the urine. DTP-diols [(A) and (B)] (4–8%) were excreted in the feces. Larger amounts of unmetabolized (*R*)-*E* and DTP-acids were found in rats given 125 mg/kg than in rats given 5 mg/kg, while smaller amounts of 4-COOH were in rats given 125 mg/kg. Although the amount of unmetabolized (*R*)-*E* slightly decreased by pretreatment with S-3308L (Expt. 2), no significant change was observed in the metabolism of (*R*)-*E* compared to Expt. 1. Triazole amounted to 1% in the feces and 3–8% in the urine. The total amount of metabolites identified was 82–85% of dosed ^{14}C in male rats.

In Expt. 1 similar metabolites were found in the excreta of male and female rats, and their amounts were almost comparable as well. The major metabolite, 4-COOH, amounted to

Table 3 Quantification of metabolites in feces and urine after single oral administration of ^{14}C -labeled (R)-E.

Metabolite	Amount ^{a)} (% of dosed ^{14}C)					
	Expt. 1			Expt. 2		
	Triazoly- ^{14}C 5 mg/kg		Phenyl- ^{14}C 5 mg/kg	Triazoly- ^{14}C 125 mg/kg		Triazoly- ^{14}C 5 mg/kg
	Feces	Urine	Feces	Urine	Feces	Urine
Male^{b)}						
(R)-E	4.2±1.9	<0.1	5.3±1.5	<0.1	7.9±1.6	<0.1
5-OH	3.0±0.6	<0.1	4.6±1.5	<0.1	5.6±0.6	<0.1
4-COOH	52.2±1.7	7.2±0.7	46.6±3.6	8.9±1.4	31.2±4.7	4.9±1.2
DTP-alcohol (Z)	0.2±0.0	<0.1	0.2±0.1	<0.1	0.3±0.0	0.2±0.1
DTP-acids (E and Z)	1.6±0.6	4.3±0.6	1.4±0.1	5.5±0.4	0.9±0.4	10.5±1.2
3-OH- ϕ	0.8±0.2	0.1±0.0	1.0±0.3		0.2±0.0	<0.1
(4-OH-5-Cl)- ϕ			<0.1		0.6±0.1	0.1±0.0
(3-OH-4-SCH ₃)- ϕ	0.4±0.1	<0.1	0.4±0.1	<0.1	0.1±0.1	<0.1
DTP-diol (A)	2.6±0.4	<0.1	4.1±0.9	<0.1	7.0±2.1	<0.1
DTP-diol (B)	1.9±0.3	<0.1	3.5±1.0	<0.1	5.9±1.9	<0.1
Triazole	1.0±0.2	2.9±0.4			0.9±0.1	7.5±0.9
Others extracted ^{c)}	4.6±0.3	1.5±0.3	5.2±1.0	3.9±0.5	5.5±0.8	3.4±0.4
Others unextracted ^{c)}	4.1±0.4		4.9±0.2		3.6±0.2	
Total	76.6±3.0	16.0±1.4	77.2±2.0	18.3±2.2	69.7±2.3	26.6±2.4
Female^{d)}						
(R)-E	7.5±2.2	<0.1	3.8±1.0	<0.1	6.3±2.3	<0.1
5-OH	11.9±0.6	<0.1	6.9±1.1	<0.1	2.7±0.6	0.2±0.1
4-COOH	21.0±1.7	16.9±2.4	25.3±3.2	22.2±1.7	22.0±3.5	20.4±4.5
DTP-alcohol (Z)	0.3±0.1	<0.1	0.1±0.1	<0.1	0.1±0.1	0.1±0.1
DTP-acids (E and Z)	1.9±0.8	8.8±1.8	0.6±0.3	8.0±1.5	0.7±0.3	7.5±2.5
3-OH- ϕ	1.6±0.1	0.2±0.0	1.2±0.3	0.3±0.1	1.4±0.4	1.0±0.3
(4-OH-5-Cl)- ϕ	0.2±0.1	<0.1	0.2±0.0		0.4±0.1	0.2±0.1
(3-OH-4-SCH ₃)- ϕ	0.2±0.1	<0.1	<0.1	<0.1	0.1±0.1	<0.1
DTP-diol (A)	2.7±1.0	<0.1	4.4±0.9	<0.1	3.4±2.0	<0.1
DTP-diol (B)	2.4±0.8	<0.1	3.5±0.7	<0.1	2.7±1.7	<0.1
Triazole	1.1±0.4	5.8±0.8			1.8±0.7	9.7±0.1
Others extracted ^{c)}	4.5±0.5	5.8±0.7	3.4±0.4	10.1±0.7	5.3±1.2	6.1±0.7
Others unextracted ^{c)}	2.7±0.3		3.8±0.2		3.1±0.4	
Total	58.0±3.3	37.5±3.4	53.2±2.1	40.6±2.5	50.0±4.3	45.2±4.2
					57.9±2.5	40.7±2.4

^{a)} Values are means±S.E. of five animals.^{b)} Urine and feces excreted within two days after administration were analyzed except those of rats dosed at a rate of 125 mg/kg (three days).^{c)} Three times with methanol/water (4/1, v/v).^{d)} Urine and feces excreted within three days after administration were analyzed.

Table 4 Quantification of metabolites in feces and urine after single oral administration of ^{14}C -labeled (S)-E.

Metabolite	Amount ^{a)} (% of dosed ^{14}C)					
	Expt. 1			Expt. 2		
	Triazolyl- ^{14}C 5 mg/kg		Phenyl- ^{14}C 5 mg/kg	Triazolyl- ^{14}C 125 mg/kg		Triazolyl- ^{14}C 5 mg/kg
	Feces	Urine	Feces	Urine	Feces	Urine
Male^{b)}						
(S)-E	4.3±1.3	<0.1	8.1±2.4	<0.1	2.7±0.8	0.1±0.0
5-OH	5.7±1.1	<0.1	10.4±1.2	<0.1	7.6±1.3	<0.1
4-COOH	47.3±1.3	4.0±0.9	41.2±2.2	3.9±0.4	31.2±5.2	2.3±0.5
DTP-alcohol (Z)	0.2±0.1	<0.1	0.2±0.1	<0.1	0.5±0.1	0.2±0.0
DTP-acids (E and Z)	0.7±0.1	4.5±0.5	1.1±0.2	5.2±0.7	0.6±0.1	8.1±1.3
3-OH- ϕ	<0.1	<0.1	1.3±0.3	<0.1	<0.1	<0.1
(4-OH-5-Cl)- ϕ	0.6±0.1	<0.1	1.6±0.3	<0.1	0.4±0.1	<0.1
(3-OH-4-SCH ₃)- ϕ	1.2±0.2	<0.1	1.9±0.4	<0.1	0.3±0.1	<0.1
DTP-diol (A)	3.1±0.3	<0.1	1.9±0.4	<0.1	10.4±3.6	<0.1
DTP-diol (B)	2.4±0.3	<0.1	1.9±0.3	<0.1	10.3±3.4	<0.1
Triazole	1.7±0.1	4.4±0.7			1.9±0.3	7.1±0.5
Others extracted ^{c)}	5.1±0.3	2.4±0.3	8.0±0.7	4.3±0.4	7.9±0.4	1.8±0.4
Others unextracted ^{c)}	6.9±0.4		7.3±0.3		4.9±0.3	
Total	79.2±2.7	15.3±1.9	83.0±0.5	13.4±1.1	78.7±1.8	19.6±2.0
Female^{d)}						
(S)-E	7.4±2.4	<0.1	3.3±1.1	<0.1	2.1±0.4	<0.1
5-OH	14.1±1.8	<0.1	11.4±2.0	<0.1	6.8±0.4	0.1±0.1
4-COOH	21.5±3.1	12.3±1.7	25.4±2.8	15.2±3.8	24.5±3.8	27.5±7.6
DTP-alcohol (Z)	0.4±0.1	<0.1	0.3±0.2	0.1±0.0	0.4±0.2	0.3±0.2
DTP-acids (E and Z)	1.0±0.2	7.4±1.0	<0.1	8.1±1.0	0.5±0.1	10.4±4.1
3-OH- ϕ	<0.1	<0.1	0.4±0.1	<0.1	<0.1	<0.1
(4-OH-5-Cl)- ϕ	0.3±0.1	<0.1	1.0±0.4	0.2±0.1	0.2±0.0	<0.1
(3-OH-4-SCH ₃)- ϕ	1.1±0.5	0.2±0.1	6.1±1.8	<0.1	0.2±0.1	0.1±0.0
DTP-diol (A)	3.0±0.7	<0.1	4.9±1.5	<0.1	5.4±2.7	<0.1
DTP-diol (B)	2.7±0.5	<0.1			5.0±2.6	<0.1
Triazole	0.5±0.1	2.3±0.1	7.2±0.7	4.7±0.5	0.7±0.2	2.7±0.1
Others extracted ^{c)}	8.3±0.9	4.6±0.5	7.2±0.5		4.7±0.4	3.0±0.9
Others unextracted ^{c)}	6.3±0.4				4.0±0.7	
Total	66.6±1.9	26.8±1.9	67.3±4.3	28.3±4.0	54.5±2.8	44.1±3.0
						74.7±1.3
						26.1±1.2

a) Values are means±S.E. of five animals.

b) Urine and feces excreted within two days after administration were analyzed except those of rats dosed at a rate of 125 mg/kg (three days).

c) Three times with methanol/water (4/1, v/v).

d) Urine and feces excreted within three days after administration were analyzed.

21–26% in the feces and 17–22% in the urine in female rats; indicating that the urinary excretion of 4-COOH was larger in females than in males. Neither increased doses nor a pretreatment with S-3308L produced any remarkable changes in females (Expt. 2). The metabolites identified amounted to 77–83% of dosed ^{14}C .

After identifying metabolites of (S)-E corresponded to 74–87% of the dosed ^{14}C , metabolites were considered to be similar in amounts between (S)-E and (R)-E.

5. Bile Studies

After single oral administration (p.o. treatment) of Tr- ^{14}C -labeled (R)-E or (S)-E to bile duct-cannulated rats at a rate of 5 mg/kg, ^{14}C was excreted mainly into the bile, where 61–82% of the dose was found in 48 hr (Table 5). Radioactivity was also found in the urine, feces and intestinal contents 48 hr after ad-

ministration, although the amount was relatively small. The biliary ^{14}C -excretion within 6 hr after p.o. treatment was larger with (S)-E (22–31%) than with (R)-E (7–9%). When intraduodenally (i.d.) treated with the bile collected from other rats orally treated with Tr- ^{14}C -labeled (R)-E or (S)-E at 5 mg/kg, the bile duct-cannulated rats excreted ^{14}C mainly into the bile, showing that most metabolites in the bile underwent enterohepatic circulation.

Amounts of metabolites in the bile and urine of bile duct-cannulated rats are shown in Tables 6 and 7. 4-COOH and 5-OH glucuronide were major metabolites in the bile, while 4-COOH and DTP-acids were in the urine. Female rats excreted a larger amount of 5-OH glucuronide and a smaller amount of 4-COOH than male rats.

6. Distribution of Metabolites in Tissues

After single oral administration of Tr- ^{14}C -

Table 5 Cumulative ^{14}C excretion into the bile, urine and feces of bile duct-cannulated rats after treatment with Tr- ^{14}C -(R)-E or Tr- ^{14}C -(S)-E orally at a rate of 5 mg/kg (p.o.) or with the collected bile intraduodenally (i.d.).

Isomer	Treatment	Excreta	Amount ^{a)} (% of dosed ^{14}C)					
			Male			Female		
			0–6 hr	0–24 hr	0–48 hr	0–6 hr	0–24 hr	0–48 hr
(R)-E	p.o.	Bile	9.2±9.0	57.0±8.8	73.8±5.2	6.7±1.3	41.7±4.9	61.4±4.7
		Urine		4.9±2.0	7.8±3.4		6.1±0.8	8.9±1.2
		Feces		5.3±4.6	8.7±5.7		13.5±6.7	16.2±5.1
		Intestinal contents ^{b)}			1.1±1.4			3.3±2.3
	i.d.	Bile	47.3±12.5	76.5±3.8	81.8±0.2	17.0±3.6	52.8±6.5	60.9±0.8
		Urine		6.8±2.5	7.9±2.5		17.2±0.5	21.8±1.1
		Feces		8.0±4.9	12.7±4.4		15.4±10.0	23.5±2.7
		Intestinal contents ^{b)}			2.1±2.6			1.3±0.6
(S)-E	p.o.	Bile	31.2±9.8	63.8±1.9	66.7±1.7	22.0±1.2	67.6±4.7	82.4±4.2
		Urine		8.8±4.8	10.1±5.2		8.0±4.5	15.6±7.7
		Feces		17.2±4.0	21.6±3.9		1.2±1.1	4.4±1.0
		Intestinal contents ^{b)}			0.2±0.1			2.2±2.0
	i.d.	Bile	31.1±5.7	67.2±11.4	71.9±8.4	25.3±2.1	77.7±3.0	83.9±8.6
		Urine		11.0±9.2	13.3±11.1		4.0±0.2	5.5±0.2
		Feces		13.6±11.4	17.7±8.9		7.3±4.9	11.2±8.2
		Intestinal contents ^{b)}			1.3±0.8			5.9±2.2

^{a)} Values are means±S.D. of three males and two females.

^{b)} 48 hr after treatment.

Table 6 Quantification of metabolites in the bile and urine of bile duct-cannulated rats within 24 hr after oral (p.o.) treatment with Tr-¹⁴C-(*R*)-*E* or with the collected bile intra-duodenally (i.d.).

Sex	Metabolite	Amount (% of dosed ¹⁴ C)			
		p.o.		i.d.	
		Bile	Urine	Bile	Urine
Male	(<i>R</i>)- <i>E</i>	<0.1	<0.1	<1	<1
	5-OH free	0.1±0.0	<0.1	<1	<1
	glucuronide	18.9±2.8	<0.1	22±1	<1
	4-COOH	23.0±4.8	1.7±1.1	41±3	3±2
	DTP-alcohol (<i>Z</i>)	<0.1	<0.1	<1	<1
	DTP-acids (<i>E</i> and <i>Z</i>)	<0.1	2.2±0.5	<1	2±0
	Others	15.0±2.9	1.0±0.5	14±1	2±0
	Total	57.0±8.8	4.9±2.0	77±4	7±3
Female	(<i>R</i>)- <i>E</i>	<0.1	<0.1	<1	<1
	5-OH free	0.2±0.1	<0.1	<1	<1
	glucuronide	27.1±4.4	0.4±0.2	25±8	<1
	4-COOH	5.6±2.2	1.9±1.2	9±1	6±0
	DTP-alcohol (<i>Z</i>)	<0.1	<0.1	<1	<1
	DTP-acids (<i>E</i> and <i>Z</i>)	<0.1	2.9±1.5	<1	8±0
	Others	8.8±2.3	0.9±0.2	19±3	3±0
	Total	41.7±4.9	6.1±0.8	53±7	17±1

Values are means±S.D. of three rats except for i.d. dosed females (two).

Table 7 Quantification of metabolites in the bile and urine of bile duct-cannulated rats within 24 hr after oral (p.o.) treatment with Tr-¹⁴C-(*S*)-*E* or with the collected bile intra-duodenally (i.d.).

Sex	Metabolite	Amount (% of dosed ¹⁴ C)			
		p.o.		i.d.	
		Bile	Urine	Bile	Urine
Male	(<i>S</i>)- <i>E</i>	<0.1	<0.1	<1	<1
	5-OH free	0.6±0.2	<0.1	<1	<1
	glucuronide	19.5±3.8	<0.1	18±2	2±2
	4-COOH	12.9±2.5	1.7±1.5	21±3	2±2
	DTP-alcohol (<i>Z</i>)	<0.1	0.1±0.1	<1	<1
	DTP-acids (<i>E</i> and <i>Z</i>)	<0.1	2.8±0.2	<1	3±2
	Others	30.8±2.2	4.2±3.3	28±7	4±4
	Total	63.8±1.9	8.8±4.8	67±11	11±9
Female	(<i>S</i>)- <i>E</i>	<0.1	<0.1	<1	<1
	5-OH free	0.8±0.1	0.1±0.1	<1	<1
	glucuronide	37.9±4.0	1.7±2.0	42±4	<1
	4-COOH	6.5±0.4	1.5±0.5	12±0	1±0
	DTP-alcohol (<i>Z</i>)	<0.1	<0.1	<1	<1
	DTP-acids (<i>E</i> and <i>Z</i>)	<0.1	3.5±0.9	<1	2±0
	Others	22.4±1.3	1.2±1.0	24±1	1±0
	Total	67.6±4.7	8.0±4.5	78±3	4±0

Values are means±S.D. of three males and two females.

Table 8 ^{14}C -Concentrations in the tissues of rats after single oral administration of $\text{Tr-}^{14}\text{C-(R)-E}$ or $\text{Tr-}^{14}\text{C-(S)-E}$ at a rate of 5 mg/kg.

Tissue	Isomer	^{14}C -Concentration ^{a)} (μg parent compound equivalent/g tissue)									
		Time after administration (hr)									
		0.5	1	2	4	8	16	24	48	72	
Liver	(R)	5.70 $\pm$ 0.48	6.55 $\pm$ 0.05	6.29 $\pm$ 1.03	6.01 $\pm$ 1.09	7.17 $\pm$ 0.34	2.36 $\pm$ 0.57	0.95 $\pm$ 0.30	0.48 $\pm$ 0.16	0.16 $\pm$ 0.06	
	(S)	5.57 $\pm$ 0.47	4.99 $\pm$ 0.66	7.44 $\pm$ 0.30	3.74 $\pm$ 0.43	4.10 $\pm$ 0.45	1.15 $\pm$ 0.42	1.02 $\pm$ 0.35	0.62 $\pm$ 0.18	0.15 $\pm$ 0.05	
Kidney	(R)	1.45 $\pm$ 0.23	2.22 $\pm$ 0.30	2.62 $\pm$ 0.12	3.40 $\pm$ 0.61	3.37 $\pm$ 0.26	1.11 $\pm$ 0.31	0.40 $\pm$ 0.18	0.23 $\pm$ 0.05	0.10 $\pm$ 0.04	
	(S)	1.25 $\pm$ 0.08	1.42 $\pm$ 0.17	2.50 $\pm$ 0.53	2.11 $\pm$ 0.34	1.46 $\pm$ 0.15	0.55 $\pm$ 0.16	0.63 $\pm$ 0.21	0.35 $\pm$ 0.11	0.08 $\pm$ 0.03	
Blood	(R)	0.25 $\pm$ 0.04	0.49 $\pm$ 0.08	0.57 $\pm$ 0.02	0.66 $\pm$ 0.10	0.70 $\pm$ 0.07	0.35 $\pm$ 0.08	0.26 $\pm$ 0.05	0.07 $\pm$ 0.02	0.04 $\pm$ 0.01	
	(S)	0.15 $\pm$ 0.02	0.18 $\pm$ 0.02	0.36 $\pm$ 0.06	0.33 $\pm$ 0.04	0.32 $\pm$ 0.02	0.21 $\pm$ 0.05	0.26 $\pm$ 0.03	0.10 $\pm$ 0.03	0.03 $\pm$ 0.00	
Adrenal	(R)	1.93 $\pm$ 0.09	2.26 $\pm$ 0.02 ^{b)}	2.58 $\pm$ 0.32	2.42 $\pm$ 0.72	2.18 $\pm$ 0.09	0.57 $\pm$ 0.29 ^{b)}	0.09 $\pm$ 0.07	<0.05	<0.05	
	(S)	1.25 $\pm$ 0.23	2.16 $\pm$ 0.36	4.59 $\pm$ 1.16 ^{b)}	2.16 $\pm$ 0.42	1.26 $\pm$ 0.25	0.21 $\pm$ 0.05	0.16 $\pm$ 0.05	0.06 $\pm$ 0.03	<0.05	
Skin & hair	(R)	0.28 $\pm$ 0.08	0.61 $\pm$ 0.09	0.84 $\pm$ 0.10	0.74 $\pm$ 0.15	0.66 $\pm$ 0.03	0.34 $\pm$ 0.05	0.18 $\pm$ 0.04	0.18 $\pm$ 0.02	0.18 $\pm$ 0.03	
	(S)	0.16 $\pm$ 0.02	0.29 $\pm$ 0.08	0.52 $\pm$ 0.11	0.37 $\pm$ 0.09	0.30 $\pm$ 0.01	0.18 $\pm$ 0.02	0.20 $\pm$ 0.02	0.17 $\pm$ 0.02	0.17 $\pm$ 0.01	
Skin	(R)	0.30 $\pm$ 0.12	0.83 $\pm$ 0.07	0.92 $\pm$ 0.22	1.01 $\pm$ 0.48	0.97 $\pm$ 0.21	0.35 $\pm$ 0.12	0.15 $\pm$ 0.07	0.08 $\pm$ 0.02	0.05 $\pm$ 0.02	
	(S)	0.18 $\pm$ 0.02	0.30 $\pm$ 0.05	0.85 $\pm$ 0.45	0.49 $\pm$ 0.16	0.39 $\pm$ 0.05	0.20 $\pm$ 0.07	0.22 $\pm$ 0.08	0.08 $\pm$ 0.03	0.04 $\pm$ 0.02	
Hair	(R)	0.06 $\pm$ 0.02	0.16 $\pm$ 0.09	0.08 $\pm$ 0.01	0.08 $\pm$ 0.03	0.16 $\pm$ 0.06	0.30 $\pm$ 0.16	0.29 $\pm$ 0.03	0.54 $\pm$ 0.07	0.91 $\pm$ 0.19	
	(S)	0.02 $\pm$ 0.01	0.10 $\pm$ 0.06	0.07 $\pm$ 0.01	0.08 $\pm$ 0.02	0.12 $\pm$ 0.04	0.20 $\pm$ 0.10	0.56 $\pm$ 0.40	0.56 $\pm$ 0.07	0.76 $\pm$ 0.06	

a) Values are means $\pm$ S.E. of three animals.b) Values are means $\pm$ S.D. of two animals.

Table 9 Concentrations of undegraded (*R*)-*E* or (*S*)-*E* in the liver, kidney and blood of rats after single oral administration of Tr-¹⁴C-(*R*)-*E* or Tr-¹⁴C-(*S*)-*E* at a rate of 5 mg/kg.

Tissue	Isomer	Concentration ($\mu\text{g/g}$ tissue)								
		Time after administration (hr)								
		0.5	1	2	4	8	16	24	48	72
Liver	(<i>R</i>)- <i>E</i>	4.51	4.56	4.73	3.16	3.17	0.56	0.15	0.05	0.03
	(<i>S</i>)- <i>E</i>	4.22	3.56	5.00	2.08	1.18	0.12	0.18	0.13	<0.01
Kidney	(<i>R</i>)- <i>E</i>	0.70	1.01	1.19	1.09	0.91	0.11	<0.01	<0.01	<0.01
	(<i>S</i>)- <i>E</i>	0.54	0.60	1.05	0.48	0.21	0.02	<0.01	<0.01	<0.01
Blood	(<i>R</i>)- <i>E</i>	0.09	0.11	0.16	0.14	0.04	0.02	<0.01	<0.01	<0.01
	(<i>S</i>)- <i>E</i>	0.06	0.09	0.17	0.04	0.01	0.01	0.01	<0.01	<0.01

Values are means of three animals.

(*R*)-*E*, ¹⁴C-concentrations in the liver ranged between 5.70 and 7.17 μg (*R*)-*E* equivalents/g tissue (ppm) during a 0.5- to 8-hr period and decreased thereafter (Table 8). In rats dosed with (*S*)-*E*, ¹⁴C-concentrations in the liver reached a maximal level of 7.44 ppm 2 hr after administration and decreased thereafter. In rats dosed with (*S*)-*E*, ¹⁴C-concentrations in the liver were about one-half of those in rats dosed with (*R*)-*E* 4 to 16 hr after administration. A similar tendency was observed in other tissues although ¹⁴C-concentrations remained much lower. ¹⁴C-Concentrations in other tissues reached maximal levels 2 to 8 hr after administration of (*R*)-*E* and 2 hr after administration of (*S*)-*E*.

Concentrations of unmetabolized (*R*)-*E* or (*S*)-*E* were examined in the liver, kidney and blood, where ¹⁴C-concentrations were relatively high (Table 9). Concentration in the tissues reached maximum 2 hr after administration of (*S*)-*E* as well as (*R*)-*E*. Levels of radioactivity in the tissues remained relatively high up to 4–8 hr with (*R*)-*E*, but dropped rapidly with (*S*)-*E*. Undegraded compounds in the liver, kidney and blood disappeared at biological half life of 3.6, 3.2 and 2.2 hr with (*R*)-*E* and 2.7, 2.5 and 1.5 hr with (*S*)-*E*, respectively.

Individual ¹⁴C levels in the skin and hair were examined, since the residue levels of ¹⁴C in the skin with hair were the highest among the tissues examined on the 7th day posttreatment. ¹⁴C-Levels in the skin changed in parallel with those in the blood, decreasing rapidly to 0.04–0.05 ppm 72 hr after administration, while ¹⁴C-

levels in the hair increased with time, reaching 0.76–0.91 ppm 72 hr after administration.

7. *In Vitro* Metabolism Study

Figure 2 and Table 10 show the results of the *in vitro* metabolism study on (*R*)-*E* and (*S*)-*E*. The biotransformation rate of (*S*)-*E* by hepatic microsomal fractions of male and female rats with NADPH was faster than that of (*R*)-*E*. The V_{max} and K_m values with (*S*)-*E* were approximately 5 times higher than those with (*R*)-*E*. As shown in Table 9, the highest level of undegraded (*R*)-*E* or (*S*)-*E* in the liver is

Fig. 2 Biotransformations of (*R*)-*E* and (*S*)-*E* by rat liver microsomes with NADPH.

○ and △: Biotransformation of (*R*)-*E* by microsomes of male and female rats, respectively, ● and ▲: Biotransformation of (*S*)-*E* by microsomes of male and female rats, respectively.

Table 10 Biotransformation of (R)-E and (S)-E by rat liver microsomes with NADPH.

Substrate	K_m (μM)	V_{max} (pmole/min/mg protein)	Activity ^{a)}
(R)-E	5.1	1.56	1.18
(S)-E	31.4	7.81	2.64

^{a)} Activity was calculated from the linear regression least-square analysis of Lineweaver-Burk plots where substrate concentration was 16 μM .

approximately 5 ppm, which can be calculated to be ca. 16 μM . In the *in vitro* study, (S)-E at 16 μM gave twofold higher reaction velocity than (R)-E (Table 10). The reaction velocity with (R)-E and (S)-E was 6 to 8-fold higher in male rats than in female rats (Fig. 2).

DISCUSSION

By identifying metabolites which amounted to 74–87% of the dose, the metabolic pathway for (S)-E in rats was proposed to be identical with that for (R)-E since the metabolites excreted were similar (Fig. 1). The ^{14}C elimination patterns and tissue residues of ^{14}C were also similar between (S)-E and (R)-E with some fluctuations. However, significant differences between them were observed in metabolic fates during the early period after single oral administration. Firstly, the amount of biliary ^{14}C excretion during the first 6 hr after administration was about threefold larger with

(S)-E than with (R)-E (Table 7). Secondly, concentrations of undegraded compounds and their metabolites in tissues reached almost the same levels with both (S)-E and (R)-E 2 hr after administration, but decreased much faster with (S)-E than with (R)-E (Tables 8 and 9). (S)-E has a shorter biological half life (alpha phase) than (R)-E in the liver, kidney and blood. Finally, the biotransformation rate of (S)-E *in vitro* was faster than that of (R)-E (Fig. 2). These facts suggest that the rates of biotransformation and excretion *in vivo* are more rapid with (S)-E than with (R)-E, although the biotransformation occurs at the same sites.

Parameters of the metabolic fate of (R)-E and (S)-E are compared with those of (RS)-E⁵⁾ to examine the metabolic interaction of (S) isomer with (R) isomer. As shown in Table 11, there are no remarkable differences among (R)-E, (S)-E and (RS)-E in the ^{14}C -excretion, ^{14}C -residue levels in tissues, excreted metabolites and their amounts, suggesting that there occurs no substantial metabolic interaction between (R) and (S) isomers.

After administration of ^{14}C -labeled (R)-E or (S)-E, male rats excreted more radiocarbon into the feces than female rats. The fecal excretion in male and female rats amounted to 72–87% and 52–75% of the dose, respectively (Table 2). The analysis of metabolites in the excreta indicated that the sex difference observed in the ^{14}C elimination was mainly attributable to the fact that female rats ex-

Table 11 Metabolic parameters after p.o. treatment with Tr- ^{14}C -labeled (R)-E, (S)-E or (RS)-E to male rats at a rate of 5 mg/kg.

	(R)-E	(S)-E	(RS)-E ^{a)}
^{14}C elimination within 7 days (% of dosed ^{14}C)			
Feces/Urine	80.3/16.8	81.9/16.0	78.9/19.2
^{14}C residue levels on the 7th day (ng parent compd. eq./g tissue)			
Liver	7 $\pm$ 0	8 $\pm$ 1	17 $\pm$ 2
Kidney	5 $\pm$ 0	<1	6 $\pm$ 1
Skin with hair	12 $\pm$ 2	7 $\pm$ 1	11 $\pm$ 1
Amounts of metabolites excreted (% of dosed ^{14}C)			
Methyl group hydroxylated	62.4	57.0	50.4
Phenyl group hydroxylated	1.3	1.8	1.5
DTP derivatives ^{b)}	10.6	6.4	11.4

^{a)} See Ref. 5).

^{b)} DTP-alcohol (Z), DTP-acids (E and Z) and DTP-diols.

creted the major metabolite, 4-COOH, in a smaller amount into the feces and in a larger amount into the urine than male rats (Tables 3 and 4). It is known that the hydroxylation activity of xenobiotics is generally weaker in females than in males.⁹⁾ In the case of (*R*)-*E* or (*S*)-*E*, females are considered to generate 5-OH, the precursor of 4-COOH, in a less amount than males. Females excreted the major metabolite, 4-COOH, into the bile in a smaller amount than males, and excreted another major metabolite, 5-OH glucuronide, in a much larger amount than males (Tables 6 and 7). The generation of a large amount of 5-OH glucuronide in females would result in excretion of large amounts of 5-OH, 4-COOH and some other metabolites into the urine.

Biliary excretion rate of ¹⁴C was not greatly different between males and females (Table 5). However, biotransformation of (*R*)-*E* or (*S*)-*E* by rat liver microsomes with NADPH was much slower in females than in males (Fig. 2). The 5-OH, a major metabolite in female rats, seemed to be rapidly biotransformed *in vivo* into glucuronide, which was secreted into the bile as rapidly as 4-COOH was formed in male rats.

Most radiocarbon distributed to the skin and hair was 1,2,4-triazole. Time course changes of the radiocarbon distributed to the skin with hair (Table 8) indicated that 1,2,4-triazole was secreted from the skin and distributed to the hair. Based on the bioassay using *Candida albicans*, ketoconazole, an anti-mycotic drug having an imidazole group, is reported to be distributed to the hair of rats and guinea-pigs and retained for a relatively long period.^{10,11)} However, in the case of S-3308L, the distributed compound was not the parent compound but 1,2,4-triazole. The mechanism of triazole formation is assumed to be identical with that of dehalogenation catalyzed by cytochrome *P*-450 in liver microsomes,¹²⁻¹⁸⁾ since triazole could be generated from (*R*)-*E* by aerobic incubation with hepatic microsomes only in the presence of NADPH at 37°C. The fate of 1,2,4-[3(5)-¹⁴C]triazole in rats has already been examined by Weber *et al.*¹⁹⁾ Following oral administration, radioactivity was absorbed nearly 100% from the gastrointestinal tract, widely distributed to the

whole body, and excreted 92-94% of the dose into the urine and 3-5% into the feces within 48 hr.¹⁹⁾ More than 90% of the ¹⁴C eliminated into the urine was identified as unchanged triazole.²⁰⁾ Some of above observations are consistent with our finding that 1,2,4-triazole was excreted mainly in the urine.

In this study, DTP-diols were found as newly identified metabolites, which are considered to be generated from DTP-alcohol (*Z*).

Pretreatment with S-3308L at a rate of 5 mg/kg/day for successive 14 days (Expt. 2) did not produce any remarkable changes in the ¹⁴C-excretion profile (Table 2), ¹⁴C-tissue residues, and amounts of metabolites in the excreta (Table 3). These findings suggest that neither (*R*)-*E* nor (*S*)-*E* nor their metabolites are likely to accumulate or persist in organs and tissues in significant amounts by repeated oral administration of S-3308L.

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要 約

(E)-1-(2,4-Dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol (S-3308L) の (R) および (S) 異性体のラットにおける代謝

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(R)- および (S)-(*E*)-1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol (以下 (R)-*E* および (S)-*E* と略す) のベンゼン環またはトリアゾール環 ^{14}C 標識体を 5 および 125 mg/kg の割合で雌雄ラットに経口投与し, 体内動態を調べた。糞および尿への ^{14}C 排泄, 投与後 7 日目の ^{14}C 組織残留, および糞・尿中の代謝物量には両異性体間の顕著な差はなかった。しかし, (S)-*E* の代謝変換および排泄速度 (*in vivo*) は (R)-*E* に比べ速く, ラット肝ミクロゾーム分画による *in vitro* 代謝速度も (R)-*E* より速かった。雄は雌に比べ ^{14}C を糞中に多く排泄した。(R)-*E* や (S)-*E* の主要な代謝物は, 4-メチル基が酸化されたカルボン酸誘導体であり, おもに糞中に排泄されたが, 雌では雄に比べこの代謝物が尿中に多く排泄された。糞中に少量の 1-(2,4-dichlorophenyl)-2-(1,2,4-triazol-1-yl)propan-1,3-diols を, 糞・尿および皮膚・毛に少量の 1,2,4-triazole を見いだした。(R)-*E* および (S)-*E* の代謝は, S-3308L を 14 日間連続して 5 mg/kg/日 の割合で投与したラットにおいても変化しなかった。