Original Article

Metabolism of (S),(E)-1-(*p*-Chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol (Uniconazole) in Rats

Naohiko ISOBE, Naoko KOBAYASHI, Shigemi ITO,
Iwao NAKATSUKA, Hideo KANEKO and Akira YOSHITAKE

*Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd.,
Konohana-ku, Osaka 554, Japan*

(Received October 11, 1990)

On single oral administration of (S),(E)-1-(*p*-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol [designated as (S)-*E*], a main ingredient of uniconazole, labeled with ^{14}C at the triazole ring at dose levels of 1 and 200 mg/kg to male and female rats, radiocarbon was excreted into urine and feces nearly 100% within 7 days. The urinary and fecal excretion accounted for 43–67% and 34–55% of the dose, respectively. Males excreted more radioactivity into feces than females. ^{14}C -Concentration reached a peak in tissues 1 to 8 hr after administration. Adrenal and liver showed relatively higher peak concentrations of 4.10–4.57 and 2.34–2.59 μg (S)-*E* equivalents/g tissue decreasing with half-lives of 8–11 and 5–6 hr, respectively. ^{14}C -Levels in tissues were generally low on the 7th day after administration. Urinary and fecal metabolites amounting to 83–91% of the dosed ^{14}C were identified, and metabolic pathways for (S)-*E* were proposed. Recovery of unchanged (S)-*E* in feces was 7 to 13% of the dose. A main biotransformation was oxidation of the methyl group to form hydroxymethyl and carboxy metabolites, which amounted to 58–75% of the total dose. Slight sex-related differences were observed in amounts of excreted metabolites: females excreted a larger amount of the carboxy metabolite and a smaller amount of 1,2,4-triazole into urine than males. Although main metabolic reactions of (S)-*E* were similar to those of (R)-(E)-1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol, diniconazole, there was a difference in the excretion route of the main carboxy metabolites. Rats receiving Tr- ^{14}C -(S)-*E* excreted more ^{14}C into urine than those receiving ^{14}C -diniconazole. It is considered that ^{14}C in the former was excreted into urine more easily than that in the latter because the octanol-water partition coefficient ($P_{o/w}$) of the carboxy metabolite from (S)-*E* is smaller than that from diniconazole.

INTRODUCTION

(E)-1-(*p*-Chlorophenyl)-4, 4-dimethyl-2-(1, 2, 4-triazol-1-yl)-1-penten-3-ol (uniconazole), a vinyl triazole derivative, has been developed as a growth regulator of plants.^{1–3)} Uniconazole has two optical isomers due to a chiral carbon at the C-3 position. (S)-Isomer, designated as (S)-*E*, of the enantiomers is an active ingredient.

This report deals with the metabolic fate of the (S)-*E* isomer labeled with ^{14}C at the triazole in rats.

MATERIALS AND METHODS

1. Chemicals

(S)-*E* labeled with ^{14}C at the triazole group [Tr- ^{14}C -(S)-*E*, 1.34 GBq (36.1 mCi)/mmol] was prepared in our laboratory by the method of Nakatsuka *et al.*⁴⁾ The radiochemical purity was more than 99% on TLC. The specific radioactivity was adjusted appropriately by mixing the ^{14}C labeled material with unlabeled (S)-*E*.

The following unlabeled authentic compounds were prepared in our laboratory.⁵⁾ (Z)-

Isomer of (S)-*E* [designated as (S)-*Z*], (E)- and (Z)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1, 2, 4-triazol-1-yl)-1-penten-3-one [designated as KE and KZ, respectively], 1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-pentan-3-ol [designated as SA], (E)- and (Z)-5-(4-chlorophenyl)-2, 2-dimethyl-4-(1, 2, 4-triazol-1-yl)-4-penten-1,3-diol [designated as CH₂OH-E and CH₂OH-Z, respectively], (E)- and (Z)-5-(4-chlorophenyl)-2, 2-dimethyl-4-(1, 2, 4-triazol-1-yl)-4-pentenoic acid [designated as COOH-E and COOH-Z, respectively], (E)- and (Z)-alpha-(1,2,4-triazol-1-yl)-4-chlorocinnamic acid [designated as CC acids (*E* and *Z*)], (E)-1-(4-chloro-2-hydroxyphenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol [designated as 2-OH-E], (E)-1-(4-chloro-3-hydroxyphenyl)-4, 4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol [designated as 3-OH-E] and (E)-1-(3-chloro-4-hydroxyphenyl)-4, 4-dimethyl-(1, 2, 4-triazol-1-yl)-1-penten-3-ol [designated as 4-OH-E].

1,2,4-[3,5-¹⁴C]Triazole with a specific activity of 1.96 GBq (53 mCi)/mmol was purchased from Amersham, UK. Triazolyl ¹⁴C-labeled COOH-E [Tr-¹⁴C-COOH, 1.34 GBq/mmol] was purified from urine of rats orally treated with Tr-¹⁴C-(S)-*E* (1.34 GBq/mmol) at

1 mg/kg. Triazolyl ¹⁴C-labeled CH₂OH-E [Tr-¹⁴C-CH₂OH-E, 1.34 GBq/mmol] was prepared by beta-glucuronidase hydrolysis of the glucuronide in bile collected from the rat orally treated with Tr-¹⁴C-(S)-*E* (1.34 GBq/mmol) at 1 mg/kg.

Triazolyl-¹⁴C labeled (R)-(*E*)-1-(2,4-dichlorophenyl)-4, 4-dimethyl-2-(1, 2, 4-triazol-1-yl)-1-penten-3-ol [¹⁴C-diniconazole, 2.16 GBq (58.4 mCi)/mmol] was prepared in our laboratory.⁴⁾ Hydroxymethyl and carboxylic acid derivatives of ¹⁴C-diniconazole [designated as ¹⁴C-CH₂OH-diniconazole and ¹⁴C-COOH-diniconazole, respectively] were prepared by a method similar to the preparation of Tr-¹⁴C-CH₂OH-E and Tr-¹⁴C-COOH-E.

2. TLC

Precoated silica gel 60 F₂₅₄ chromatoplates (200×200 mm, 0.25 mm thickness, Merck, FRG) were used for qualitative and quantitative analyses of metabolites with solvent system A to G shown in Table 1. Unlabeled standards were detected under UV light. Radioactive metabolites were detected by a TLC Linear Analyzer LB283 (Berthold, FRG) or by autoradiography using Kodak SB-5 films.

Table 1 *R_f* values of authentic standards on TLC.

Compound	<i>R_f</i> value in solvent system ^{a)}						
	A	B	C	D	E	F	G
(S)- <i>E</i>	0.34	0.52	0.89	0.27	0.57	0.15	0.48
(S)- <i>Z</i>	0.37	0.53	—	0.36	—	—	—
KE	0.43	0.54	—	0.29	—	—	—
KZ	0.50	0.55	—	0.47	—	—	—
SA	0.35	—	—	—	—	0.24	0.57
CH ₂ OH-E	0.13	0.42	0.82	—	—	0.05	0.20
CH ₂ OH-Z	0.14	—	0.82	—	—	—	—
COOH-E	0.03	0.46	0.79	—	0.32	—	—
COOH-Z	—	—	0.81	—	0.34	—	—
CC acids (<i>E</i> and <i>Z</i>)	0.02	0.14	0.39	—	—	—	—
2-OH-E	0.18	0.48	0.89	—	—	—	—
3-OH-E	0.16	0.47	0.87	—	—	—	—
4-OH-E	0.17	0.49	0.85	—	—	—	—
Triazole	0.05	0.11	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, ethyl acetate/ethanol/water/acetic acid (10/4/1/1, v/v); D, diethyl ether; E, chloroform/acetic acid (5/1, v/v); F, benzene/ether (1/1, v/v); G, chloroform/methanol (15/1, v/v).

3. Excretion Balance Study

Six-week-old Sprague-Dawley rats were purchased from Charles River Japan (Kanagawa, Japan) and acclimatized for one week. Five male and five female rats orally received 1 or 200 mg/kg of Tr- ^{14}C -(S)-E [1.34 GBq (36.1 mCi) or 6.73 MBq (0.182 mCi)/mmol, respectively]. The treated animals were housed in Metabolica CO-2® (Sugiyamagen Iriki Co., Ltd., Tokyo, Japan) to collect urine, feces and expired air separately after 1, 2, 3, 5 and 7 days. On the 7th day, the rats were sacrificed by bleeding, and their tissues and organs were dissected out to analyze total radioactivity. The following tissues were collected: adrenal, blood, bone, brain, fat, gastrointestinal tract (stomach, small intestine, caecum, large intestine), heart, kidney, liver, lung, muscle, pancreas, spleen, testis, ovary, uterus, skin with hair and residual carcass.

4. Identification of Metabolites

Metabolites in the feces were extracted as follows: Feces were homogenized with a threefold volume of water with a Waring blender. Aliquots (1X) of the fecal homogenates were shaken with a fourfold volume (4X) of methanol and centrifuged at $4000\times g$ for 10 min. ^{14}C -Metabolites were extracted twice by shaking of the precipitates with a 5X volume of methanol and centrifugation. The supernatants were combined and concentrated. Metabolites in the liver, kidney and blood were extracted three times with a fourfold volume of methanol after homogenizing the tissues with a Polytron® type PT10-35 (KINEMATICA, Switzerland). The methanol extracts were concentrated. Metabolites in the fecal extracts, urine samples and tissues extracts were identified by TLC co-chromatography with authentic standards in solvent systems A to C.

5. Radioanalysis

Radioactivity in expired air trapped in 2.5 N NaOH solution was quantified with a Tri-Carb 460CD liquid scintillation spectrometer (Packard, U.S.A.), which gave dpm by the external standard method after adding an aliquot (2 ml) of the NaOH solution to a 20 ml of Aquasol-2® (New England Nuclear, U.S.A.)-water mixture

(1/1, v/v).

Radioactivity in organosoluble fractions, urine and silica gel scraped from TLC plates was quantified by liquid scintillation counting (LSC) after adding an appropriate part to a 10 ml of Emulsifier Scintillator 299™ (Packard).

Radioactivity in tissue samples (50 to 300 mg) and feces was quantified by a combustion method.⁶⁾

6. Distribution of ^{14}C -(S)-E and Its Metabolites in Tissues

Seven-week-old S.D. male and female rats were orally treated with Tr- ^{14}C -(S)-E at a rate of 1 mg/kg [134 MBq (3.61 mCi)/mmol]. Groups each consisting of three rats were sacrificed by bleeding 1, 2, 4, 8, 24 and 72 hr after treatment, and tissues shown in Table 4 were dissected out. Heparinized blood was centrifuged at $1500\times g$ for 10 min to get a

Fig. 1 Cumulative ^{14}C -excretion of rats after single oral administration of Tr- ^{14}C -(S)-E.

Male: A, 1 mg/kg; B, 200 mg/kg. Female: C, 1 mg/kg; D, 200 mg/kg.

□, total ^{14}C ; ○, ^{14}C in urine; ●, ^{14}C in feces; and ▲, ^{14}C in expired air.

Points and bars represent the mean values and standard deviations of five animals, respectively.

plasma fraction.

7. Octanol-Water Partition Coefficients ($P_{o/w}$)

Each of Tr- ^{14}C -(S)-E, Tr- ^{14}C -CH₂OH-E, Tr- ^{14}C -COOH-E, ^{14}C -diniconazole, ^{14}C -CH₂OH-diniconazole or ^{14}C -COOH-diniconazole was dissolved in 7 ml of octanol at a concentration of 14.3 μM . An aliquot (3 ml) of the octanol solutions was added to 3 ml of water and shaken for 1 min. Radioactivity in the organic and aqueous layers was measured, and partition coefficients ($P_{o/w}$) were calculated as follows.

$$P_{o/w} = (\text{Concentration of a test material in octanol phase}) / (\text{Concentration of the test material in water phase})$$

RESULTS

1. ^{14}C -Elimination

Cumulative ^{14}C -excretion into feces and urine is summarized in Fig. 1. With the low dose of 1 mg/kg, 73–80% of the dosed ^{14}C was eliminated within 24 hr after treatment. ^{14}C -Recovery within 7 days was 98–100% of the dose for both sexes. In the low dose groups, males excreted 55% of the dosed ^{14}C into feces

Table 2 ^{14}C -Tissue residues in rats 7 days after single oral administration of Tr- ^{14}C -(S)-E.

Dosage (mg/kg)	^{14}C concentration			
	Male		Female	
	1	200	1	200
Tissue	[ng (S)-E equivalent/g tissue]			
Adrenal	<3	<800	4 ^{a)}	<500
Blood	<1	<200	<1	<200
Bone	<1	<100	<1	<100
Brain	<1	<100	<1	<100
Fat	<2	<300	<1	<300
Gastrointestinal tract				
Stomach	<1	<100	<1	<100
Small intestine	<1	200 ^{a)}	<1	<100
Caecum	<1	200 $\pm$ 50 ^{b)}	1 ^{a)}	<100
Large intestine	<1	200 ^{a)}	<1	<100
Heart	<1	<200	<1	<200
Kidney	<1	<100	<1	<100
Liver	2 $\pm$ 0.7	200 $\pm$ 10	1 $\pm$ 0.3 ^{c)}	<100
Lung	<1	<100	1 ^{a)}	<100
Muscle	<1	<100	<1	<100
Pancreas	<1	<100	<1	<200
Spleen	<1	<100	<1	<100
Testis	<1	<100		
Ovary			<2	<300
Uterus			<1	
Skin with hair	5 $\pm$ 2.2	1800 $\pm$ 800	3 $\pm$ 2.0 ^{d)}	700 $\pm$ 270
Carcass	2 $\pm$ 0.3	400 $\pm$ 50	3 $\pm$ 0.3	400 $\pm$ 70
Contents of gastrointestinal tract [ng (S)-E eq./g contents]				
Stomach	<1	<100	<1	<100
Small intestine	<1	400 $\pm$ 180 ^{e)}	<1	<100
Caecum	<1	400 $\pm$ 90	2 ^{a)}	200 ^{a)}
Large intestine	<1	300 $\pm$ 90	<1	<100

Data represent means $\pm$ S.D. of 5 animals, but values below the detection limit were excluded;

^{a)} the value of 1 rat, ^{b)} the means $\pm$ S.D. of 2 rats, ^{c)} the means $\pm$ S.D. of 3 rats, and ^{d)} the means $\pm$ S.D. of 4 rats.

and 43% into urine, while females excreted a smaller amount (42%) into feces and a larger amount (58%) into urine. High dosage (200 mg/kg) led to a notable delay in the ^{14}C -excretion and a slight decrease in fecal ^{14}C -excretion within 24 hr after treatment for both sexes. ^{14}C -Recovery in excreta within 7 days, however, reached 100% of the dose, and the proportion of ^{14}C -recovery in feces to that in urine became similar to the proportion with the low dose.

2. ^{14}C -Tissue Residues

^{14}C -Tissue residues were generally very small in all groups of male and female rats on the 7th day after administration (Table 2). The ^{14}C -residues in tissues except skin with hair were less than 4 and 800 ng of (S)-E equivalents/g tissue(ppb) for the low and high dose groups, respectively. Skin with hair

showed the highest levels of 3–5 ppb (low dose) and 700–1800 ppb (high dose). Most of the radiocarbon in skin with hair was bound on the hair. Eighty percent of the radiocarbon distributed to the hair was identified as 1,2,4-triazole by TLC analysis. The triazole seems to be excreted with sebum.

No marked sex-related difference was observed.

3. Amounts of Metabolites in Excreta

Results are summarized in Table 3. In male rats of the low dose group, a relatively small amount of unmetabolized (S)-E was excreted into feces (7% of the dose). COOH-E, a carboxylic acid derivative, was a major metabolite in feces (31%) and urine (22%). CH_2OH -E, an alcohol derivative, was excreted mainly into feces (5%). 4-OH-E, a metabolite with 4-hydroxyphenyl, was excreted into

Table 3 Amounts of metabolites in feces and urine after single oral administration of Tr- ^{14}C -(S)-E.

Sex Metabolite	Amount ^{a)} (% of dosed ^{14}C)			
	1 mg/kg		200 mg/kg	
	Feces	Urine	Feces	Urine
Male ^{b)}				
(S)-E	6.9 ± 3.01	N.D.	12.5 ± 4.06	N.D.
CH_2OH -E	5.0 ± 2.88	0.1 ± 0.05	8.3 ± 1.86	0.3 ± 0.08
COOH-E	31.0 ± 0.75	22.2 ± 4.17	11.9 ± 3.87	39.9 ± 7.94
CC acids (E & Z)	N.D.	3.2 ± 0.63	N.D.	3.2 ± 0.82
4-OH-E	0.8 ± 0.26	N.D.	1.0 ± 0.30	N.D.
Triazole	1.0 ± 0.29	13.1 ± 1.94	0.5 ± 0.14	11.7 ± 1.00
Others extracted ^{c)}	6.1 ± 0.86	2.9 ± 0.61	3.2 ± 0.65	2.2 ± 0.28
Unextractable ^{c)}	2.9 ± 0.17		1.4 ± 0.25	
Total	53.6 ± 6.76	41.6 ± 6.98	38.9 ± 9.02	57.4 ± 8.55
Female ^{b)}				
(S)-E	7.1 ± 5.09	N.D.	12.3 ± 6.59	0.1 ± 0.02
CH_2OH -E	13.6 ± 8.65	0.2 ± 0.11	7.7 ± 1.53	0.4 ± 0.18
COOH-E	14.0 ± 1.54	47.6 ± 14.38	8.6 ± 2.08	56.6 ± 4.31
CC acids (E & Z)	0.1 ± 0.05	2.2 ± 0.42	0.1 ± 0.04	1.1 ± 0.13
4-OH-E	1.2 ± 0.57	0.2 ± 0.13	0.6 ± 0.47	0.2 ± 0.12
Triazole	0.2 ± 0.05	2.9 ± 0.49	0.2 ± 0.04	3.2 ± 0.13
Others extracted ^{c)}	2.8 ± 0.69	3.2 ± 1.12	2.2 ± 0.25	4.0 ± 0.44
Unextractable ^{c)}	1.4 ± 0.17		0.9 ± 0.12	
Total	40.5 ± 13.69	56.3 ± 15.83	32.6 ± 4.91	65.6 ± 4.91

^{a)} Data represent means ± S.D. of 5 animals. ^{b)} Urine and feces excreted within two days after administration were analyzed except for high dose group (three days). ^{c)} Three times with methanol/water (4/1, v/v). N.D.: not detected by autoradiography.

feces (0.8%). 4-OH-E is considered to have been generated by a kind of NIH shift. Other metabolites oxidized at the benzene ring such as 2-OH-E and 3-OH-E were not detected in the excreta. CC acids (both *E* and *Z* forms), which lack the *tert*-butyl group, were excreted into urine (3%). Triazole was mainly excreted into urine (13%).

As compared to males of the low dose group, males of the high dose group excreted larger amounts of unmetabolized (*S*)-*E* (12%) and CH₂OH-E (8%), but a smaller amount of COOH-E (12%) in feces. On the contrary, the urinary excretion of COOH-E was larger (40%) in the high dose group. The high dose group excreted comparatively larger amounts of 4-OH-E and triazole than the low dose group.

The profile of metabolites in excreta of female rats was similar to that in male rats. The major metabolite COOH-E was excreted mainly into urine (48% at the low dose and 57% at the high dose).

The metabolism of (*S*)-*E* can be classified into four categories as shown in Table 4. There was no significant difference in the metabolism between the low and high dose groups, but a sex-related difference was observed only in the formation of triazole (male: 12–14%, female, 3%).

Total amounts of identified metabolites were

Table 4 Metabolic transformation of (*S*)-*E* in rats after oral administration.

Metabolism	% of dosed ¹⁴ C			
	1 mg/kg		200 mg/kg	
	Male	Female	Male	Female
Oxidation				
at 4-methyl	58.3	75.4	60.4	73.3
at 4-C of phenyl	0.8	1.4	1.0	0.8
Formation of				
CC acids (<i>E</i> and <i>Z</i>)	3.2	2.3	3.2	1.2
Triazole	13.2	3.1	12.2	3.4

Data were taken from Table 3.

83–89% and 89–91% of the dose in male and female rats, respectively. Based on the above results, proposed metabolic pathways for (*S*)-*E* in rats are illustrated in Fig. 2.

4. Distribution of ¹⁴C to Tissues

¹⁴C-Concentrations in tissues of rats orally given 1 mg/kg of ¹⁴C-(*S*)-*E* are shown in Table 5. ¹⁴C-Concentration reached a peak in tissues except muscle (male) and hair (female) 1 to 4 hr after administration. Adrenal and liver showed peak levels as high as 4.10–4.57 and 2.59–2.34 μg (*S*)-*E* equivalents/g tissue

Fig. 2 Proposed metabolic pathways for uniconazole in rats.

Table 5 ^{14}C -Concentration in tissues of rats dosed with Tr- ^{14}C -(S)-E at a rate of 1 mg/kg.

Tissue	^{14}C -Concentration (ng (S)-E equivalent/g tissue)					
	Time after administration (hr)					
	1	2	4	8	24	72
Male						
Adrenal	2420 $\pm$ 440	4100 $\pm$ 388	3200 $\pm$ 111	2790 $\pm$ 454	570 $\pm$ 112	<40
Blood	100 $\pm$ 10	110 $\pm$ 15	120 $\pm$ 7	110 $\pm$ 24	70 $\pm$ 4	10 $\pm$ 5
Plasma	130 $\pm$ 13	140 $\pm$ 28	150 $\pm$ 8	130 $\pm$ 35	80 $\pm$ 6	10 $\pm$ 1 ^{b)}
Bone	80 $\pm$ 14	70 $\pm$ 7	90 $\pm$ 13	80 $\pm$ 9	40 $\pm$ 2	<10
Brain	190 $\pm$ 36	150 $\pm$ 4	150 $\pm$ 21	120 $\pm$ 12	50 $\pm$ 3	<10
Fat	260 $\pm$ 14	320 $\pm$ 67	600 $\pm$ 91	500 $\pm$ 67	20 $\pm$ 2	<10
Heart	200 $\pm$ 36	160 $\pm$ 4	160 $\pm$ 18	130 $\pm$ 19	70 $\pm$ 25	<10
Kidney	510 $\pm$ 100	480 $\pm$ 70	570 $\pm$ 69	400 $\pm$ 122	130 $\pm$ 15	10 $\pm$ 2 ^{b)}
Liver	2360 $\pm$ 168	2590 $\pm$ 230	2220 $\pm$ 226	1630 $\pm$ 68	180 $\pm$ 58	20 $\pm$ 5
Lung	390 $\pm$ 52	350 $\pm$ 39	310 $\pm$ 66	270 $\pm$ 22	70 $\pm$ 9	<10
Muscle	100 $\pm$ 20	90 $\pm$ 5	100 $\pm$ 12	110 $\pm$ 23	50 $\pm$ 5	<10
Pancreas	240 $\pm$ 51	200 $\pm$ 30	280 $\pm$ 149	150 $\pm$ 22	60 $\pm$ 12	<10
Spleen	130 $\pm$ 12	120 $\pm$ 15	120 $\pm$ 13	110 $\pm$ 12	60 $\pm$ 6	<10
Thyroid ^{c)}	210	200	180	150	70	<30
Testis	190 $\pm$ 21	190 $\pm$ 29	190 $\pm$ 15	160 $\pm$ 24	70 $\pm$ 2	<10
Skin	160 $\pm$ 29	150 $\pm$ 2	160 $\pm$ 11	120 $\pm$ 30	50 $\pm$ 2	<10
Hair	30 $\pm$ 9	30 $\pm$ 9	40 $\pm$ 8	30 $\pm$ 13	40 $\pm$ 10	140 $\pm$ 24
Female						
Adrenal	3870 $\pm$ 795	2530 $\pm$ 2128	4570 $\pm$ 93	2890 $\pm$ 1067	1370 $\pm$ 923	<40
Blood	100 $\pm$ 23	110 $\pm$ 21	120 $\pm$ 15	100 $\pm$ 23	30 $\pm$ 9	<10
Plasma	130 $\pm$ 19	140 $\pm$ 25	140 $\pm$ 19	120 $\pm$ 29	40 $\pm$ 13	<10
Bone	140 $\pm$ 57	140 $\pm$ 7	150 $\pm$ 19	80 $\pm$ 48	15 ^{a)}	<10
Brain	370 $\pm$ 100	350 $\pm$ 51	350 $\pm$ 19	200 $\pm$ 140	10 $\pm$ 8	<10
Fat	550 $\pm$ 49	1100 $\pm$ 75	1830 $\pm$ 34	1040 $\pm$ 745	70 $\pm$ 53	<20
Heart	410 $\pm$ 90	350 $\pm$ 38	350 $\pm$ 24	220 $\pm$ 152	20 $\pm$ 12	<10
Kidney	530 $\pm$ 86	620 $\pm$ 74	620 $\pm$ 27	490 $\pm$ 103	90 $\pm$ 47	<10
Liver	1850 $\pm$ 244	2340 $\pm$ 234	1890 $\pm$ 84	1250 $\pm$ 530	180 $\pm$ 109	<10
Lung	530 $\pm$ 83	610 $\pm$ 77	500 $\pm$ 56	400 $\pm$ 126	40 $\pm$ 29	<10
Muscle	150 $\pm$ 54	210 $\pm$ 62	170 $\pm$ 26	110 $\pm$ 63	10 $\pm$ 4 ^{b)}	<10
Pancreas	540 $\pm$ 161	440 $\pm$ 5	480 $\pm$ 20	260 $\pm$ 202	20 $\pm$ 13	<10
Spleen	240 $\pm$ 71	210 $\pm$ 33	200 $\pm$ 10	130 $\pm$ 97	20 $\pm$ 7	<10
Thyroid ^{c)}	500	800	380	230	<30	<40
Ovary	400 $\pm$ 92	460 $\pm$ 110	390 $\pm$ 29	370 $\pm$ 337	70 ^{a)}	<20
Uterus	200 $\pm$ 45	220 $\pm$ 56	180 $\pm$ 3	170 $\pm$ 132	20 $\pm$ 10	<10
Skin	250 $\pm$ 30	340 $\pm$ 92	430 $\pm$ 162	240 $\pm$ 152	10 $\pm$ 11	<10
Hair	60 $\pm$ 44	40 $\pm$ 10	40 $\pm$ 8	80 $\pm$ 41	50 $\pm$ 16	40 $\pm$ 16

Data represent mean values $\pm$ S.D. of three animals, but values below the detection limit were excluded; ^{a)} the value of 1 rat, ^{b)} the mean values $\pm$ S.D. of 2 rats, ^{c)} the value of three rats.

(ppm), respectively, followed by fat (0.60–1.83 ppm) and kidney (0.57–0.62 ppm). The levels in adrenal, liver and kidney decreased with half-lives of 5 to 11 hr (Table 6), reaching 0.57–1.37, 0.18 and 0.09–0.13 ppm, respectively, 24 hr after treatment. ^{14}C -Levels in

tissues at 72 hr were less than 0.04 ppm, except in hair (0.04–0.14 ppm). The peak ^{14}C -concentration in fat of females (1.83 ppm) was three times higher than that of males (0.60 ppm), but no other significant sex-related differences were observed.

Table 6 $T_{1/2}$ of ^{14}C in tissues.

Tissue	$T_{1/2}$ (hr) ^{a)}	
	Male	Female
Adrenal	8	11
Kidney	10	7
Liver	5	6

a) 4-24 hr post treatment.

5. Metabolites in Blood, Kidney and Liver

Recovery of ^{14}C from whole blood, kidney and liver by methanol extraction was 77-99%. Concentrations of metabolites in whole blood, kidney and liver are summarized in Tables 7 and 8. (S)-E, $\text{CH}_2\text{OH-E}$, COOH-E and triazole were found in all tissues examined. (S)-E concentration reached a peak 1-2 hr after administration in liver and kidney, being 1.73-

1.88 and 0.20-0.41 ppm, respectively. (S)-E concentration was somewhat lower in males than in females. Major metabolites in both sexes were $\text{CH}_2\text{OH-E}$ and COOH-E in liver, and COOH-E in kidney. Triazole was a major metabolite in blood of males but a minor one in females.

6. Octanol-Water Partition Coefficients ($P_{o/w}$)

As shown in Table 9, $\log P_{o/w}$ values of $\text{Tr-}^{14}\text{C-(S)-E}$, $\text{Tr-}^{14}\text{C-CH}_2\text{OH-E}$ and $\text{Tr-}^{14}\text{C-COOH-E}$ were smaller than those of $^{14}\text{C-diniconazole}$ and corresponding $^{14}\text{C-CH}_2\text{OH}$ or $^{14}\text{C-COOH-derivatives}$.

DISCUSSION

Most of (S)-E was metabolically transformed by oxidation of the methyl group in rats. Males excreted a slightly larger amount of

Table 7 Concentrations of metabolites in tissues of male rats.

Tissue	Concentration (μg (S)-E equivalent/g tissue)					
	Time after administration (hr)					
	1	2	4	8	24	72
Blood						
(S)-E	0.01	<0.01	0.02	0.01	N.D.	N.D.
$\text{CH}_2\text{OH-E}$	<0.01	<0.01	<0.01	<0.01	N.D.	N.D.
COOH-E	0.03	<0.01	0.03	0.02	N.D.	N.D.
Triazole	0.04	0.04	0.04	0.05	0.05	N.D.
Others extracted	0.01	0.06	0.02	0.02	<0.01	N.D.
Unextractable	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Total	0.10	0.11	0.12	0.11	0.07	0.01
Kidney						
(S)-E	0.20	0.16	<0.13	<0.07	<0.07	N.D.
$\text{CH}_2\text{OH-E}$	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
COOH-E	0.21	0.20	0.31	0.17	<0.07	N.D.
Triazole	<0.09	<0.08	<0.13	0.08	<0.07	N.D.
Others extracted	<0.09	<0.08	<0.13	<0.07	<0.07	N.D.
Unextractable	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Total	0.51	0.48	0.57	0.40	0.13	0.01
Liver						
(S)-E	1.73	1.69	1.23	0.81	0.01	<0.01
$\text{CH}_2\text{OH-E}$	0.13	0.18	0.24	0.21	0.02	N.D.
COOH-E	0.14	0.18	0.22	0.16	0.03	N.D.
Triazole	<0.05	<0.07	0.08	0.10	0.06	<0.01
Others extracted	0.29	0.44	0.43	0.32	0.05	N.D.
Unextractable	0.02	0.03	0.03	0.03	0.02	<0.01
Total	2.36	2.59	2.22	1.63	0.18	0.02

N.D.: not detected.

Table 8 Concentrations of metabolites in tissues of female rats.

Tissue Metabolite	Concentration (μg (S)-E equivalent/g tissue)					
	Time after administration (hr)					
	1	2	4	8	24	72
Blood						
(S)-E	0.07	0.07	0.07	0.04	0.01	N.D.
CH ₂ OH-E	<0.01	<0.04	0.01	<0.02	<0.01	N.D.
COOH-E	<0.01	<0.04	0.02	0.02	0.01	N.D.
Triazole	N.D.	N.D.	<0.01	<0.02	<0.01	N.D.
Others extracted	<0.01	<0.04	<0.01	0.01	<0.01	N.D.
Unextractable	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Total	0.10	0.11	0.12	0.10	0.03	<0.01
Kidney						
(S)-E	0.41	0.38	0.37	0.20	<0.05	N.D.
CH ₂ OH-E	N.D.	<0.05	<0.08	<0.08	N.D.	N.D.
COOH-E	<0.10	0.15	0.12	0.17	0.05	N.D.
Triazole	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Others extracted	<0.10	0.06	0.09	0.08	<0.05	N.D.
Unextractable	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Total	0.53	0.62	0.62	0.49	0.09	<0.01
Liver						
(S)-E	1.51	1.88	1.32	0.74	<0.04	<0.01
CH ₂ OH-E	0.11	0.16	0.24	0.22	0.08	N.D.
COOH-E	<0.08	0.09	0.12	0.13	<0.04	N.D.
Triazole	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Other extracted	0.15	0.19	0.19	0.13	0.04	N.D.
Unextractable	0.02	0.02	0.03	0.02	0.01	<0.01
Total	1.85	2.34	1.89	1.25	0.18	<0.01

N.D.: not detected.

Table 9 Octanol-water partition coefficients of (S)-E, diniconazole and their metabolites.

	Log $P_{o/w}$	
	(S)-E	Diniconazole ^{a)}
Parent compound	2.82 $\pm$ 0.002	3.22 $\pm$ 0.038
CH ₂ OH derivative	1.64 $\pm$ 0.002	1.74 $\pm$ 0.088
COOH derivative	1.45 $\pm$ 0.056	1.61 $\pm$ 0.014

Figures represent mean values $\pm$ S.D. of three experiments.^{a)} (R)-E isomer.

triazole into urine than females, but there was no marked difference in (S)-E metabolism between males and females.

Certain sex-related differences were observed in ¹⁴C-elimination after single oral administration of Tr-¹⁴C-(S)-E. Males excreted a larger

amount of ¹⁴C into feces than females. Analysis of metabolites in the excreta revealed that the sex difference in ¹⁴C-elimination was mainly attributable to the fact that males excreted a larger amount of COOH-E, a major metabolite, into feces than females (Table 10). Similar results have been observed in the metabolism of (E)-1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1, 2, 4-triazol-1-yl)-1-penten-3-ol, diniconazole,⁷⁾ an analog of (S)-E.

Table 10 shows the urinary excretion of ¹⁴C and COOH derivatives in rats receiving Tr-¹⁴C-(S)-E or ¹⁴C diniconazole. Rats dosed with Tr-¹⁴C-(S)-E tended to excrete ¹⁴C in a larger amount (43–67%) than rats dosed with ¹⁴C-diniconazole (16–46%). This difference was due to the difference in excretion route of Tr-¹⁴C-COOH and ¹⁴C-COOH-diniconazole, major metabolites. Tr-¹⁴C-COOH was excreted into

Table 10 Urinary and fecal excretion of ^{14}C and COOH derivatives after single oral administration of ^{14}C -(S)-E and ^{14}C -labeled diniconazole.

Sex	Compound	Labeled position ^{a)}	Dose (mg/kg)	Amount (% of the dose)			
				Urinary excretion		Fecal excretion	
				Total ¹⁴ C ^{b)}	COOH derivative ^{c)}	Total ¹⁴ C ^{b)}	COOH derivative
Male							
(S)-E	Tr	1	42.7± 7.1	22.2± 4.2	55.0± 6.6	31.0± 0.8	
			200	59.2± 9.0	39.9± 7.9	40.9± 9.3	11.9± 3.9
Diniconazole ^{d)}	Tr	5	16.8± 1.6	7.2± 0.7	80.3± 2.0	52.2± 1.7	
	Ph	5	18.9± 2.3	8.9± 1.4	81.0± 2.1	46.6± 3.6	
	Tr	125	27.5± 2.5	4.9± 1.2	80.5± 3.5	31.2± 4.7	
Female							
(S)-E	Tr	1	57.7±15.3	47.6±14.4	42.3±14.6	14.0± 1.5	
		200	66.5± 4.9	56.6± 4.3	33.7± 5.0	8.6± 2.1	
Diniconazole ^{d)}	Tr	5	38.3± 3.5	16.9± 2.4	59.2± 3.4	21.1± 1.7	
		Ph	5	41.2± 2.5	22.2± 1.7	54.3± 1.9	25.3± 3.2
	Tr	125	46.3± 4.3	20.4± 4.5	52.1± 4.0	22.0± 3.5	

Figures represent mean values $\pm$ S.D. of five rats. ^{a)} Tr: triazolyl, Ph: phenyl. ^{b)} 0-7 days after administration. ^{c)} 0-2 or 0-3 days after administration. ^{d)} Data were taken from Ref. 7), (R)-E isomer.

urine in larger amount than ^{14}C -COOH-diniconazole. The log $P_{o/w}$ values of Tr- ^{14}C -COOH and ^{14}C -COOH-diniconazole were 1.45 and 1.61, respectively. The higher water solubility of Tr- ^{14}C -COOH may be the cause of its larger excretion into urine.

ACKNOWLEDGMENTS

We wish to thank Mrs. Etsuko Kato and Miss Tomoko Takayama for their skillful technical supports.

REFERENCES

- 1) Y. Funaki, Y. Ishiguri, T. Kato & S. Tanaka: *J. Pesticide Sci.* **9**, 229 (1984)
- 2) K. Izumi, I. Yamaguchi, A. Wada, H. Oshio & N. Takahashi: *Plant Cell Physiol.* **25**, 611 (1984)
- 3) K. Izumi, Y. Kamiya, A. Sakurai, H. Oshio & N. Takahashi: *Plant Cell Physiol.* **26**, 821 (1985)
- 4) I. Nakatsuka, H. Kanamaru, T. Kamada, K. Kawahara & A. Yoshitake: *J. Labelled Compd. Radiopharm.* **23**, 637 (1986)
- 5) K. Furuzawa, S. Tanaka, N. Mikami, T. Matsuda & J. Miyamoto: Manuscript in preparation.

- 6) N. Isobe, M. Matsuo & J. Miyamoto: *J. Pesticide Sci.* **8**, 451 (1983)
- 7) N. Isobe, S. Ito, A. Yoshitake, M. Matsuo & J. Miyamoto: *J. Pesticide Sci.* **12**, 421 (1987)

要 約

(S), (E)-1-(p-Chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol (Uniconazole) のラットにおける代謝

磯部直彦, 小林直子, 井藤重美
中塚 巖, 金子秀雄, 吉武 彬

Uniconazole の主成分である (S), (E)-1-(p-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol [以下 (S)-E と略す] のトリアゾール環 ^{14}C 標識体を1または200 mg/kg の割合で雌雄ラットに1回経口投与すると, 放射能は投与後7日以内にほぼ100%が尿および糞中に排泄された。尿および糞中排泄率はそれぞれ43~67% および34~55%であった。雄は雌より放射能を糞中に多く排泄した。組織中 ^{14}C 濃度は投与後1~8時間に最高となった。副腎と肝には他に比べ高濃度の ^{14}C が分布した。最高濃度はそれぞれ4.10~4.57 および2.34~2.59 μg (S)-E 相当量/g 組織であり, それぞれ半減期8~11 および5~6 時間で減少した。投与した ^{14}C

の 83~91% に相当する尿および糞中代謝物を同定し、(S)-E の代謝経路を推定した。投与した化合物の 7~13% は未変化のまま糞中に排泄された。主要な代謝はメチル基の酸化であり、生成したヒドロキシメチルおよびカルボキシ体が全排泄量の 58~75% を占めた。雌は雄に比べカルボキシ体を多量、トリアゾールを少量排泄した。(S)-E の主要代謝経路は、(R)-(E)-1-(2,4-dichloro-

phenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol [diniconazole] と同様であったが、主要代謝物であるカルボキシ体の排泄経路には差を認めた。(S)-E のカルボキシ体は、diniconazole のカルボキシ体に比べオクタノール-水分配係数が小さく、尿中に容易に排泄されると考えられた。