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Metabolism of Procymidone Derivatives in Female Rats

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PCM-CH_2OH [*N*-(3,5-dichlorophenyl)-1-hydroxymethyl-2-methylcyclopropane-1,2-dicarboximide] and PA-CH_2OH [2-carboxyl-*N*-(3,5-dichlorophenyl)-1-hydroxymethyl-2-methylcyclopropane-1-carboxamide] are metabolites of the fungicide procymidone [*N*-(3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide] in rat. The distribution and metabolism of PCM-CH_2OH and PA-CH_2OH were here clarified by analyzing plasma and tissues (liver, kidney, heart, lung, spleen and ovary) of female rats after single subcutaneous administration of [phenyl-^{14}C]PCM-CH_2OH and [phenyl-^{14}C]PA-CH_2OH at 62.5 mg/kg, respectively. In both rats dosed with PCM-CH_2OH and PA-CH_2OH, the radioactivity was similarly distributed into plasma and tissues, and PA-CH_2OH was detected as the main metabolite in plasma, whereas PCM-CH_2OH predominated in tissues except for kidney at 1 h after administration of PA-CH_2OH. Furthermore, the cyclization ratio [PCM-CH_2OH/(PCM-CH_2OH + PA-CH_2OH)] increased in tissues of PA-CH_2OH dosed rats with passage of time. Both procymidone and PCM-CH_2OH have convertible conformations (closed and open ring forms), so influence of pH conditions to their conversion was examined. Both compounds demonstrated closed rings under acidic conditions, and open rings under alkaline conditions. Generally, intracellar pH is kept at approximately neutral, and extracellular pH is kept at 0.6−0.7 units higher in all the animal species, so that our *in vitro* results supported *in vivo* findings.

KEYWORDS: Metabolism; rat; distribution; conversion; procymidone; PCM-CH_2OH; PA-CH_2OH

INTRODUCTION

Procymidone is a fungicide for use with grapes. In previous *in vivo* rat and mouse metabolism studies, PCM-NH-COOH, PCM-CH_2OH, and PA-CH_2OH were identified as major metabolites(*1*, *2*). The concentrations of radioactivity in blood, kidney and liver of rats were higher than those in mice 24 h after administration. The main metabolites in blood and tissues of rats were PCM-CH_2OH and PA-CH_2OH, though the metabolites were not so much seen in excreta. Thus PCM-CH_2OH and PA-CH_2OH are important to understand the kinetics of procymidone in rats. However, analysis of chemical profiles is complicated by convertible conformations (closed and open ring forms) of metabolites. Previously, it was predicted that procymidone and PCM-CH_2OH transformed to PCM-NH-COOH and PA-CH_2OH in equilibrium reactions, respectively (**Figure 1**).

To confirm whether PCM-CH_2OH and PA-CH_2OH convert each other in rat, distribution and metabolism in plasma and tissues were examined after subcutaneous administration of [phenyl-^{14}C]-PCM-CH_2OH and [phenyl-^{14}C]PA-CH_2OH to female rats at 62.5 mg/kg. On the other hand, to clarify the influence of pH to conversion of procymidone and PCM-CH_2OH, an *in vitro* study was conducted.

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MATERIALS AND METHODS

Chemicals. Procymidone [*N*-(3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide] labeled uniformly in the phenyl group with a specific activity of 15.6 MBq/mg, [phenyl-^{14}C]procymidone [radiochemical purity 98.0% and chemical purity 98.4% (UV 205 nm)] was synthesized by Amersham Biosciences UK Limited (Cardiff, South Wales) and stored under refrigeration.

PCM-CH_2OH [*N*-(3,5-dichlorophenyl)-1-hydroxymethyl-2-methylcyclopropane-1,2-dicarboximide] labeled uniformly in the phenyl group with a specific activity of 3.25 MBq/mg, [phenyl-^{14}C]PCM-CH_2OH [radiochemical purity 98.9% and chemical purity 98.2% (UV 254 nm)] was synthesized in our laboratory and stored in a refrigerator.

Unlabeled PCM-CH_2OH [chemical purity 100.0% (UV 254 nm)] was synthesized in KNC Laboratories Co., Ltd. (Hyogo, Japan).

Four authentic metabolite standards synthesized in our laboratory were used for the identification of metabolites: PCM-NH-COOH (chemical purity 98.6%), PCM-COOH (chemical purity 94.7%), PA-CH_2OH (chemical purity 98.8%) and PA-COOH (chemical purity 96.9%).

Other chemicals were reagent grade unless otherwise noted in the text.

Thin Layer Chromatography. Precoated silica gel 60 F_{254} TLC plates (20 × 20 cm, 0.25 thickness, Merck, Germany) were employed, with chloroform/ethanol/acetic acid [19/1/2 (v/v/v)] as solvent system. Radioactive compounds on TLC plates were detected by autoradiography using imaging plates (Fuji Photo Film, Tokyo, Japan). They were contacted with TLC plates at room temperature and then processed with a fluorescent image analyzer (FLA-5000, Fuji Photo Film). Authentic standards on TLC plates were detected under UV light.

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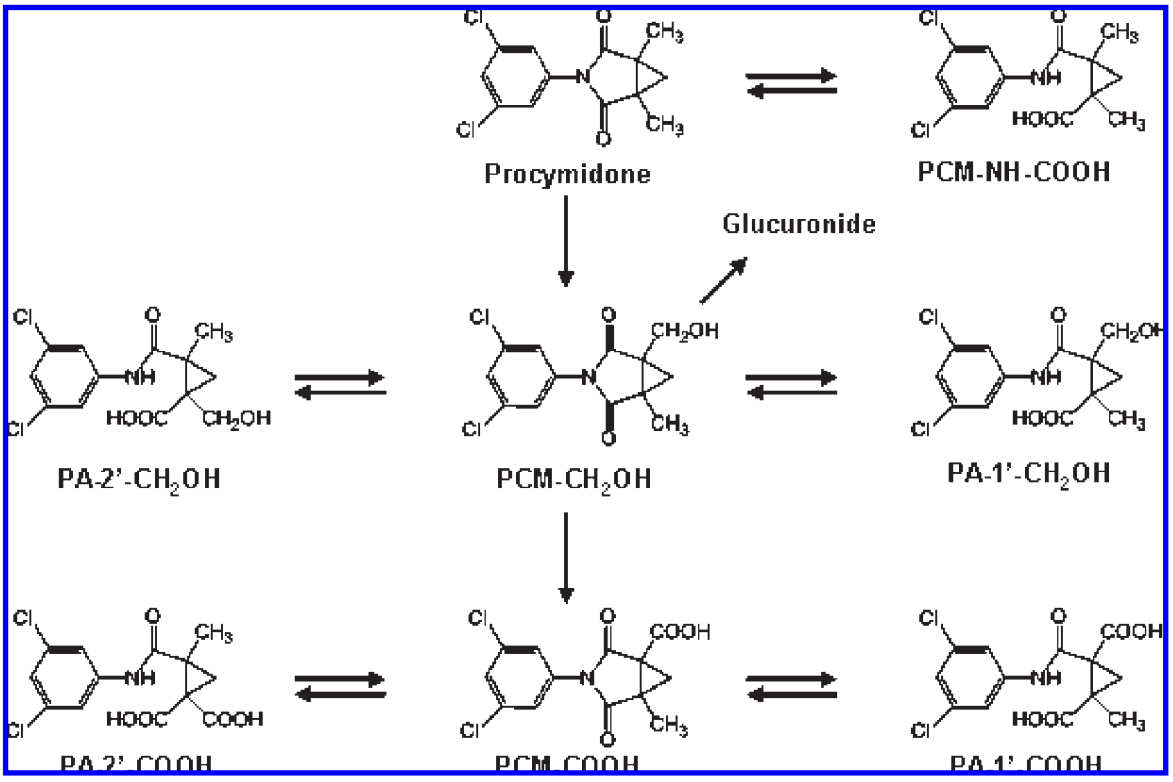

Figure 1. Metabolic pathway of procymidone and equilibrium relation of the metabolites (*1*).

Measurement of Radioactivity. Radioactivity in organosoluble fractions like dosing formulations, plasma, and extracts of plasma and tissues was quantified with a Tri-Carb 2500TR liquid scintillation analyzer (PerkinElmer Inc., CA, USA). Precipitates of plasma and tissues after extraction were taken separately to a combust-cone (PerkinElmer Inc.), dried, treated in a sample combustion apparatus (sample oxidizer: system 307, PerkinElmer Inc.), and measured for radioactivity by LSC.

Animals. Charles River Japan Inc. (Shiga, Japan) derived-CD (Sprague−Dawley strain, IGS) female rats at the age of 6−7 weeks were housed in polypropylene cages (290 × 340 × 170 mm, Clea Japan Inc., Tokyo, Japan) on sawdust bedding (White Flake, Charles River Japan Inc.) at five or fewer animals/cage. The in-life portion of the study was conducted under the following environmental conditions: room temperature, 21−25 °C; relative humidity, 40−70%; ventilation, 10 air exchanges per hour; and artificial lighting from 8:00 a.m. to 8:00 pm. Animals had free access to diet (CRF-1, Oriental Yeast, Tokyo, Japan) and filtered water (filtration device type: PTS-3, 5D-C-1, AMF, Tokyo, Japan) throughout the study. Rats dosed with the ^{14}C-labeled compound were housed in aluminum cages (W × H × D: 224 × 200 × 419 mm, Yamato Scientific Co., Ltd., Tokyo, Japan) at three animals per cage for each time point until sacrifice.

Preparation of Dosing Formulations and Administration. The dose level was set to make similar exposure of procymidone at 100 mg/kg to rats. Subcutaneous administration was selected as the dosing route to prevent the test substances from suffering low pH of gastric juice in the stomach.

The dose level of [phenyl-^{14}C]PCM-CH_2OH was set at 62.5 mg/3.70 MBq/kg in 2 mL/kg of corn oil. The dosing formulation was prepared as follows. [Phenyl-^{14}C]PCM-CH_2OH and PCM-CH_2OH in methanol solution were transferred to mortars and the solvent was evaporated to dryness under the gentle stream of nitrogen gas. Then the compound was ground with a pestle and dissolved in corn oil. Aliquots of the dosing formulation were diluted with acetone to measure the concentration. Three rats (body weights: 175.3−182.8 g) were administered subcutaneously with an injection syringe. The radiochemical purity of [phenyl-^{14}C]PCM-CH_2OH in the dosing formulation was confirmed by TLC analysis on the day of administration.

The dose level of [phenyl-^{14}C]PA-CH_2OH was also set at 62.5 mg/3.7 MBq/kg in 2 mL/kg of corn oil. The ^{14}C-labeled test substance was synthesized from [phenyl-^{14}C]PCM-CH_2OH after dilution with unlabeled PCM-CH_2OH to adjust for the appropriate specific radioactivity. The dosing formulation was prepared as follows. Methanol solutions of [phenyl-^{14}C]PCM-CH_2OH and unlabeled PCM-CH_2OH were mixed in a flask and evaporated to dryness under a gentle stream of nitrogen gas. The diluted [phenyl-^{14}C]PCM-CH_2OH was dissolved in 10% ammonia solution (28%)/methanol (v/v) solution and left to stand at room temperature for approximately 16 h to give [phenyl-^{14}C]PA-CH_2OH. The obtained [phenyl-^{14}C]PA-CH_2OH solution was transferred to a mortar, the solvent was evaporated, and then the residue was ground with a pestle and dissolved in corn oil. An aliquot of the dosing formulation was diluted with acetone for measurement of the concentration. Three rats (body weights: 175.4−196.8 g) at each time point were administered subcutaneously with an injection syringe. The radiochemical purity of [phenyl-^{14}C]PA-CH_2OH in the dosing formulation was confirmed by TLC analysis on the day of administration.

Table 1. Radioactivity Concentrations (μg equiv of PA-CH_2OH/g) in Plasma and Tissues of Female Rats after a Single Subcutaneous Administration of [Phenyl-^{14}C]PA-CH_2OH at 62.5 mg/kg[a]

tissue	1 h	2 h	4 h
plasma	30.1 ± 6.5	32.8 ± 3.3	32.9 ± 4.3
liver	34.8 ± 9.6	32.7 ± 2.1	33.0 ± 6.0
kidney	51.2 ± 11.4	39.7 ± 3.9	41.3 ± 4.1
heart	14.6 ± 3.3	16.9 ± 1.7	16.4 ± 2.1
lung	15.8 ± 2.7	19.2 ± 0.8	18.1 ± 1.3
spleen	9.6 ± 2.5	10.6 ± 1.3	11.1 ± 2.3
ovary	14.1	16.6	16.6

[a] Each value represents the mean ± SD of 3 rats except for ovary (pooled sample of 3 rats).

Collection of Plasma and Tissues. Blood was collected from the abdominal aorta using a heparinized injection syringe under ether anesthesia at 6 h (PCM-CH_2OH), and 1, 2, and 4 h (PA-CH_2OH) after administration, and then liver, kidneys, heart, lungs, spleen and ovaries were dissected. Blood was centrifuged at 3,000 rpm at 4 °C for 15 min to separate the plasma. Samples were stored in a freezer until analysis of metabolites.

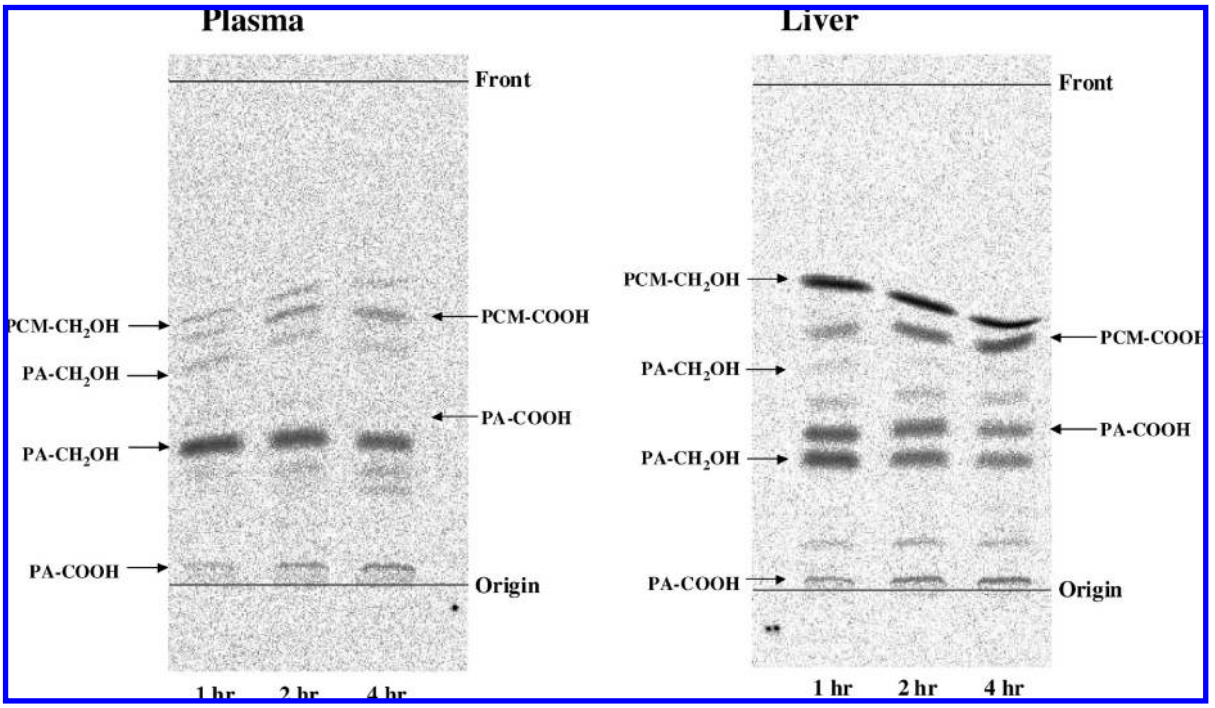

Figure 2. Autoradiography of TLC plates with metabolites in plasma and liver of female rats at 6 h after a single subcutaneous administration of [phenyl-^{14}C]PA-CH_2OH at 62.5 mg/kg.

Table 2. Concentrations (μg equiv of PA-CH_2OH/g) of Metabolites in Plasma and Tissues of Female Rats after a Single Subcutaneous Administration of [Phenyl-^{14}C]PA-CH_2OH at 62.5 mg/kg[a]

	plasma			liver			kidney			heart		
metabolite	1 h	2 h	4 h	1 h	2 h	4 h	1 h	2 h	4 h	1 h	2 h	4 h
PCM-CH_2OH	0.8	0.9	0.6	14.3	15.9	16.4	11.7	12.0	12.7	9.3	11.9	11.2
PCM-COOH	0.7	1.8	2.7	1.1	2.7	5.1	3.6	6.6	10.4	0.2	0.4	0.8
PA-CH_2OH[b]	17.5	15.0	12.8	5.7	3.2	2.0	18.0	7.7	5.4	3.0	2.3	2.1
PA-COOH[c]	1.0	2.5	2.9	4.6	4.1	2.9	4.7	3.8	3.8	0.2	0.2	0.2
others[d]	3.4	3.3	4.2	0.5	0.7	0.6	0.9	1.4	1.3	0.3	0.4	0.4
unextractable	6.7	9.3	9.6	8.6	6.2	6.1	12.4	8.2	7.8	1.6	1.7	1.7
total	30.1	32.8	32.9	34.8	32.7	33.0	51.2	39.7	41.3	14.6	16.9	16.4

	lung			spleen			ovary		
metabolite	1 h	2 h	4 h	1 h	2 h	4 h	1 h	2 h	4 h
PCM-CH_2OH	10.0	10.7	10.1	5.7	6.4	6.6	9.0	10.9	10.6
PCM-COOH	0.7	1.9	2.1	0.3	0.8	1.4	0.5	1.5	2.0
PA-CH_2OH[b]	2.7	2.5	2.0	0.6	0.5	0.4	1.3	0.9	0.5
PA-COOH[c]	0.2	0.6	0.7	1.4	1.0	0.7	1.9	1.3	0.7
others[d]	0.4	1.0	0.9	0.5	1.1	0.9	0.0	0.1	0.3
unextractable	1.8	2.4	2.3	1.1	0.9	1.2	1.4	1.9	2.5
total	15.8	19.2	18.1	9.6	10.6	11.1	14.1	16.6	16.6

[a] Data were obtained from pooled sample of 3 rats. [b] Sum of PA-1′-CH_2OH and PA-2′-CH_2OH. [c] Sum of PA-1′-COOH and PA-2′-COOH. [d] Sum of the amount of seven fractions (unknown metabolites).

Extraction of Plasma and Tissues. Plasma samples (200 mg) were extracted with 4-fold acetonitrile. Tissue samples (liver and kidney: 1 g, heart, lung and spleen: whole) were homogenized and extracted with a 5-fold v/w of acetonitrile. To confirm the recovery, radioactivity in the supernatant and precipitate was measured by LSC. The supernatant was evaporated with nitrogen gas and dissolved in 100 μL of acetonitrile/water (1/1, v/v) for TLC analysis.

Chemical Transformation. HCl solution (pH 2.0), 0.1 M sodium acetate buffer (pH 4.0), 0.1 M sodium phosphate buffer (pH 6.8), 0.1 M sodium phosphate buffer (pH 7.4), 0.05 M Tris-HCl buffer (pH 8.0), and sodium hydroxide solution (pH 11.0) were prepared as buffered solutions according to general methods.

A 10 μM acetonitrile solution (276,000 dpm/μL) of [phenyl-^{14}C]-procymidone and an 88 μM acetonitrile solution (512,000 dpm/μL) of PCM-CH_2OH were prepared as substrate solutions. Reactions and analyses were conducted as follows. Fifty microliters of substrate solution was added to 500 μL of buffered solution in a glass vial and incubated at room temperature for 16 h. The reaction mixtures were then analyzed directly by TLC. Each radioactive compound was identified by cochromatography with authentic standards.

RESULTS

Radioactivity Concentrations and Metabolism in Plasma and Tissues. Radioactivity concentrations in plasma and tissues of female rats at 1, 2, and 4 h after single subcutaneous administration of [phenyl-^{14}C]PA-CH_2OH at 62.5 mg/kg are shown in **Table 1**. The radioactivity concentrations in plasma and liver

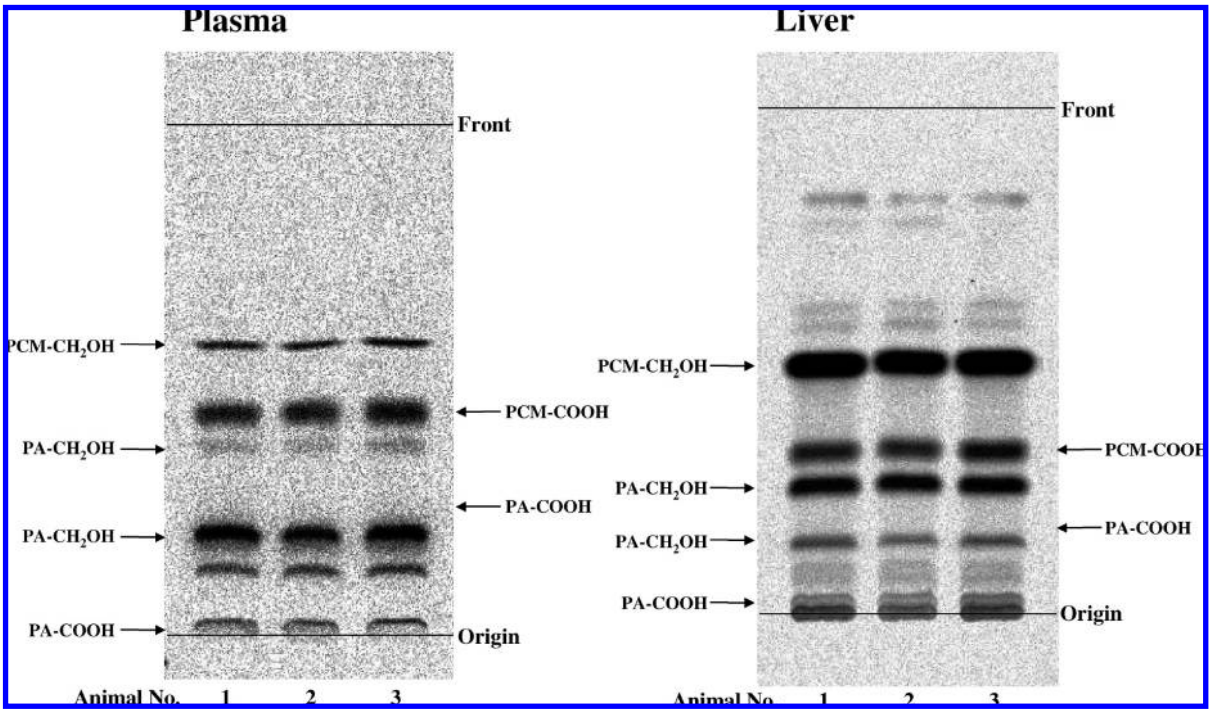

Figure 3. Autoradiography of TLC plates with metabolites in plasma and liver of female rats after a single subcutaneous administration of [phenyl-^{14}C]PCM-CH_2OH at 62.5 mg/kg.

were similar and stable during the study period after administration. The radioactivity concentrations in kidney were 1.2−1.7 times higher than those in plasma at all time points after administration, and showed a tendency to decrease. The radioactivity concentrations in other tissues (heart, lung, spleen and ovary) were less than those in plasma at all time points after administration.

In analysis of the metabolites, PCM-CH_2OH, PCM-COOH, PA-CH_2OH and PA-COOH were identified by TLC cochromatography. Typical TLC autoradiograms are shown in **Figure 2**. Concentrations of metabolites in plasma and tissues are summarized in **Table 2**.

In plasma at 1 h after administration of [phenyl-^{14}C]PA-CH_2OH, PA-CH_2OH was mainly detected (17.5 μg equiv/g). Until 4 h after administration, PA-CH_2OH decreased and PA-COOH increased with time. However, in liver at 1 h after administration, PCM-CH_2OH was mainly detected (14.3 μg equiv/g), followed by PA-CH_2OH (5.7 μg equiv/g) and PA-COOH (4.6 μg equiv/g). Until 4 h after administration, PA-CH_2OH and PA-COOH decreased, and PCM-CH_2OH and PCM-COOH increased with time. On the other hand, in kidney at 1 h after administration, PA-CH_2OH was mainly detected (18.0 μg equiv/g), followed by PCM-CH_2OH (11.7 μg equiv/g). Until 4 h after administration, PA-CH_2OH and PA-COOH decreased, and PCM-CH_2OH and PCM-COOH increased with time. In other tissues, PCM-CH_2OH was mainly detected (heart, 9.3−11.9 μg equiv/g; lung, 10.0−10.7 equiv/g; spleen, 5.7−6.6 equiv/g; ovary, 9.0−10.9 μg equiv/g) during study periods. The cyclization ratio [PCM-CH_2-OH/(PCM-CH_2OH + PA-CH_2OH)] was calculated for plasma and tissues (**Table 3**). And the summed concentrations of PCM-CH_2OH and PA-CH_2OH, and PCM-COOH and PA-COOH in blood, liver and kidney are shown in Figure **4**.

Radioactivity concentrations in plasma and tissues of female rats after a single subcutaneous administration of [phenyl-^{14}C]-PCM-CH_2OH at 62.5 mg/kg are shown in **Table 4**. The radioactivity in plasma was similar to that of PA-CH_2OH dosed rats, though the radioactivity in tissues of PCM-CH_2OH dosed rats was higher than that of PA-CH_2OH dosed rats.

Table 3. Cyclization Ratios [PCM-CH_2OH/(PCM-CH_2OH + PA-CH_2OH)] in Plasma and Tissues of Female Rats after a Single Subcutaneous Administration of [Phenyl-^{14}C]PA-CH_2OH at 62.5 mg/kg

	ratio [PCM-CH_2OH/(PCM-CH_2OH + PA-CH_2OH)]						
time after administration (h)	plasma	liver	kidney	spleen	lung	heart	ovary
1	0.04	0.72	0.40	0.90	0.78	0.75	0.88
2	0.06	0.83	0.61	0.93	0.81	0.84	0.92
4	0.04	0.89	0.70	0.94	0.83	0.84	0.95

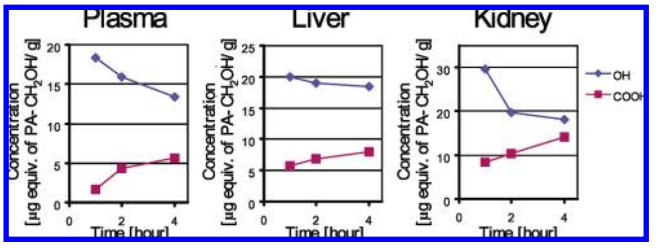

Figure 4. Concentrations of OH (PCM-CH_2OH + PA-CH_2OH) and COOH (PCM-COOH + PA-COOH) in blood, kidney and liver of female rats after a single subcutaneous administration of [phenyl-^{14}C]PA-CH_2OH at 62.5 mg/kg.

In analysis of the metabolites, PCM-CH_2OH, PCM-COOH, PA-CH_2OH and PA-COOH were identified by TLC cochromatography. Typical TLC autoradiograms are shown in **Figure 3**. Concentrations of metabolites in plasma and tissues are shown in **Table 4**.

In plasma, PA-CH_2OH was mainly detected (12.9 μg equiv/g), followed by PCM-COOH (7.3 μg equiv/g). However, in all tissues, unchanged PCM-CH_2OH was mainly detected, though PCM-COOH was also detected as one of the main metabolites in kidney (15.1 μg equiv/g). The cyclization ratio [PCM-CH_2-OH/(PCM-CH_2OH + PA-CH_2OH)] was calculated for plasma and tissues of rats after administration of PCM-CH_2OH (**Table 5**).

Table 4. Concentrations (μg equiv of PCM-CH_2OH/g) of Metabolites in Plasma and Tissues of Female Rats 6 h after a Single Subcutaneous Administration of [Phenyl-^{14}C]PCM-CH_2OH at 62.5 mg/kg[a]

metabolite	plasma	liver	kidney	heart	lung	spleen	ovary
PCM-CH_2OH	2.9 ± 0.6	33.8 ± 7.0	20.9 ± 3.2	18.0 ± 3.3	16.8 ± 3.5	11.0 ± 2.2	16.8
PCM-COOH	7.3 ± 1.2	3.0 ± 0.6	15.1 ± 5.6	1.3 ± 0.1	4.2 ± 0.4	0.5 ± 0.1	1.8
PA-CH_2OH[b]	12.9 ± 3.0	6.0 ± 0.4	6.2 ± 1.2	3.3 ± 0.2	3.2 ± 0.2	2.3 ± 0.1	3.1
PA-COOH[c]	2.3 ± 0.2	1.4 ± 0.3	2.8 ± 0.9	0.3 ± 0.1	0.6 ± 0.1	0.2 ± 0.0	0.5
others[d]	3.1 ± 0.3	1.4 ± 0.1	2.6 ± 0.9	0.4 ± 0.0	0.7 ± 0.1	0.3 ± 0.0	0.6
unextractable	1.6 ± 0.2	5.0 ± 0.9	6.8 ± 1.7	2.6 ± 0.4	2.8 ± 0.5	1.4 ± 0.2	1.4
total	30.1 ± 4.8	50.6 ± 9.1	54.4 ± 12.1	26.0 ± 3.8	28.2 ± 4.6	15.6 ± 2.6	24.2

[a] Each value represents the mean ± SD of 3 rats except for ovary (pooled sample of 3 rats). [b] Sum of PA-1′-CH_2OH and PA-2′-CH_2OH. [c] Sum of PA-1′-COOH and PA-2′-COOH. [d] Sum of the amount of four fractions (unknown metabolites).

Table 5. Cyclization Ratios [PCM-CH_2OH/(PCM-CH_2OH + PA-CH_2OH)] in Plasma and Tissues of Female Rats 6 h after a Single Subcutaneous Administration of [Phenyl-^{14}C]PCM-CH_2OH at 62.5 mg/kg

plasma	liver	kidney	spleen	lung	heart	ovary
0.18	0.85	0.77	0.85	0.84	0.83	0.84

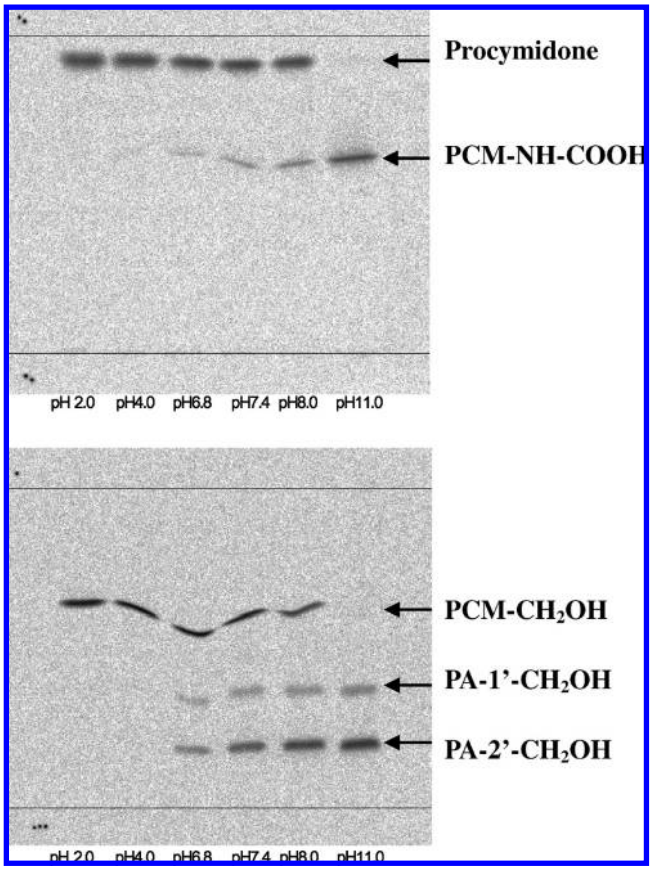

Figure 5. Autoradiography of TLC plates for characterization of the equilibrium between procymidone and PCM-NH-COOH, and between PCM-CH_2OH and PA-CH_2OH.

Chemical Transformation. The reaction mixtures of procymidone and PCM-CH_2OH were analyzed by TLC (**Figures 5**) and ratios of procymidone to PCM-NH-COOH and PCM-CH_2OH to PA-CH_2OH were calculated, respectively. Procymidone and PCM-CH_2OH were transformed to PCM-NH-COOH and PA-CH_2OH, depending on pH conditions, respectively. Under acidic conditions (less than pH 7), procymidone and PCM-CH_2OH were stable. On the other hand, under alkali conditions (more than pH 8), procymidone and PCM-CH_2OH were transformed to PCM-NH-COOH and PA-CH_2OH, respectively (**Figure 6**).

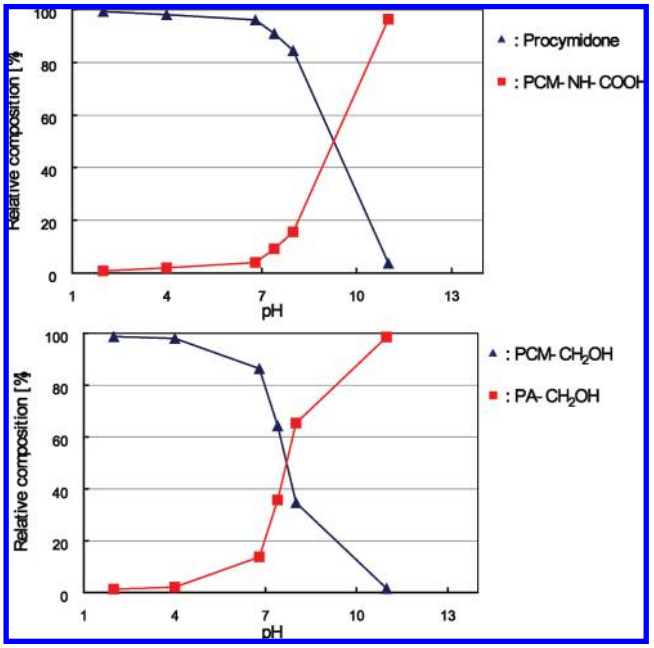

Figure 6. Conversion of procymidone and PCM-CH_2OH according to the pH conditions.

DISCUSSION

In the previous *in vivo* rat and mouse metabolism studies of procymidone, PCM-NH-COOH, PCM-CH_2OH, and PA-CH_2OH were identified as metabolites(*1*, *2*). In this study, radioactivity in plasma and tissues of rats dosed with [phenyl-^{14}C]-PCM-CH_2OH and [phenyl-^{14}C]PA-CH_2OH was similarly distributed with similar profiles of metabolites. In both PCM-CH_2OH and PA-CH_2OH dosed rats, PA-CH_2OH was detected as the main metabolite in plasma, and PCM-CH_2OH was detected as the main metabolite in all tissues, except for kidney at 1 h after administration of PA-CH_2OH. Furthermore, in tissues of PA-CH_2OH dosed rats, the cyclization ratio [PCM-CH_2OH/(PCM-CH_2OH + PA-CH_2OH)] increased with time, and finally became almost equal to the cyclization ratio of PCM-CH_2OH dosed rats. These findings indicate that (1) the dosed cyclic compound (PCM-CH_2OH) was transformed to the related product arising from imide cleavage (PA-CH_2OH) in blood, and then (2) the product (PA-CH_2OH) circulating in blood was transformed to the related cyclic compound (PCM-CH_2OH) gradually after transfer into tissues. Therefore, conversion between PCM-CH_2OH and PA-CH_2OH clearly occurs in rat.

According to comparison of radioactivity concentrations and relative compositions of closed and open ring conformations in blood, kidney and liver of rats 2 h after a single oral administration of [phenyl-^{14}C]procymidone at 100 mg/kg in a previous study

Table 6. Radioactivity Concentrations (μg equiv of procymidone/g) and Relative Compositions[a] of Procymidone and PCM-NH-COOH and of PCM-CH_2OH and PA-CH_2OH in Blood, Kidney and Liver of Rats 2 h after a Single Oral Administration of [Phenyl-^{14}C]procymidone at 100 mg/kg

compound	blood	kidney	liver
procymidone	1.2 (17.6)	16.1 (98.0)	37.7 (99.3)
PCM-NH-COOH	5.5 (82.4)	0.3 (2.0)	0.3 (0.7)
total	6.7 (100.0)	16.4 (100.0)	38.0 (100.0)
PCM-CH_2OH	0.6 (25.2)	5.2 (88.7)	6.1 (96.7)
PA-CH_2OH[b]	1.8 (74.8)	0.7 (11.3)	0.2 (3.3)
total	2.5 (100.0)	5.9 (100.0)	6.3 (100.0)

[a] Relative composition (%) is given in parentheses. [b] Sum of PA-1′-CH_2OH and PA-2′-CH_2OH.

(*2*), both procymidone and its metabolite PCM-CH_2OH formed the closed ring in kidney and liver, but they formed the open ring in blood (**Table 6**). It was proposed that absorbed procymidone in liver remained the closed ring, and transformed into the open ring in blood, then back to the closed ring in tissues. PCM-CH_2OH generated in liver similarly remained the closed ring, and transformed into the open ring in blood, then back to the closed ring in tissues. The results of the present study support this hypothesis.

According to the result of *in vitro* study to clarify pH dependent transformation of procymidone to PCM-NH-COOH and PCM-CH_2OH to PA-CH_2OH, under acidic conditions (less than pH 7.0), procymidone and PCM-CH_2OH were present as closed ring forms, on the other hand, under alkali conditions (more than pH 7.4), open ring conformations increased.

Generally, intracellular pH is approximately kept neutral and the extracellular pH is 0.6−0.7 unit higher in all animal species, including humans (*3*−*5*). The results for this *in vitro* chemical equilibrium might thus explain the *in vivo* findings, though the pH of tissue extracts was not determined. Thus we conclude that the pH condition is one of the factors that cause conversion between closed and open ring forms *in vivo*.

According to the comparison of radioactivity concentration and relative composition between PCM-COOH and PA-COOH, metabolites in blood and tissues of rats after administration of PCM-CH_2OH and PA-CH_2OH, we further conclude that PCM-COOH and PA-COOH are in equilibrium. The ratios of their concentrations showed a similar tendency to those of PCM-CH_2OH and PA-CH_2OH. The concentrations of the sum of PCM-COOH and PA-COOH increased in line with decrease of the sum of PCM-CH_2OH and PA-CH_2OH in blood, liver and kidney of rats after administration of PA-CH_2OH. This reflects metabolism in the procymidone dosed rat, and the generated metabolites (PCM-COOH and PA-COOH) have been found in urine of rats after administration of procymidone (*1*, *2*). Dosed PCM-CH_2OH and PA-CH_2OH are metabolized to PCM-COOH and PA-COOH, distributed around rat body and finally excreted into urine.

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