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Metabolism of Pyridalyl in Rats: Excretion, Distribution, and Biotransformation of Dichloropropenyl-Labeled Pyridalyl

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Metabolism of pyridalyl [2,6-dichloro-4-(3,3-dichloroallyloxy)phenyl 3-[5-(trifluoromethyl)-2-pyridyloxy]-propyl ether] labeled at position 2 of the dichloropropenyl group with ^{14}C was investigated after single oral administration to male and female rats at 5 and 500 mg/kg. Absorbed ^{14}C was excreted into feces (68−79%), urine (8−14%), and expired air (6−10%) in all of the groups. Regarding ^{14}C-tissue residues on the seventh day after administration, fat showed the highest levels at 0.98−2.34 ppm and 219−221 ppm with the low and high doses, respectively. ^{14}C-Residues in other tissues accounted for 0.03−0.32 ppm at the low dose and 3−70 ppm at the high dose. The percentages of the ^{14}C-residue in fat were 1.50−3.16% of the dose, and those of muscle and hair and skin were also relatively high, accounting for 0.49−1.20%. Total ^{14}C-residues in the whole body were 2.95−6.80% of the dose. Fecal metabolites in male rats treated with 500 mg/kg pyridalyl were purified by a combination of chromatographic techniques, and chemical structures of 8 metabolites were identified by NMR and MS spectrometry. The biotransformation reactions in rats were proposed to be as follows: (1) epoxidation of the double bond in the dichloropropenyl group followed by hydration, dehydrochlorination, decarboxylation, and/or mercapturic acid conjugation; (2) CO_2 formation after O-dealkylation of pyridalyl and its metabolites; (3) hydroxylation of C3 in the pyridyl ring; (4) O-dealkylation of the pyridyloxy and the trimethylene groups; (5) dehydrochlorination and hydration in the dichloropropenyl group.

KEYWORDS: Metabolism; pyridalyl; identification; rats; NMR; MS

INTRODUCTION

Pyridalyl [2,6-dichloro-4-(3,3-dichloroallyloxy)phenyl 3-[5-(trifluoromethyl)-2-pyridyloxy]propyl ether, S-1812] is a new class of insecticide for *Lepidoptera* and *Thysanoptera* (*1*, *2*). It has the dichloropropenyl, dichlorophenyl and pyridyl groups in its structure, and does not share structural similarity with other insecticides. Previously conducted toxicity studies showed low acute toxicity, no oncogenicity and mutagenicity, and no teratogenicity (*1*, *2*).

In a previous study, metabolism of [dichlorophenyl-^{14}C]-pyridalyl in rats was investigated in conjunction with toxicological studies for safety evaluation (*3*). After the administration of [dichlorophenyl-^{14}C]pyridalyl to rats at 5 mg/kg, the radiocarbon was rapidly absorbed, and the ^{14}C-concentrations in blood, thus, reached maxima after 2−10 h. In the low dose group, relatively high levels were observed in the liver, fat, adrenals, and spleen. However, ^{14}C was rapidly excreted mainly into feces (> 99% of the dose), and total ^{14}C-residues in tissues at 7 days were below 1.3−2.3% of the dose. S-1812-DP (resulting from O-dealkylation) was the major metabolite.

The biotransformation of the dichloropropenyl group is considered to be important since its rapid metabolism seems to be strongly related to the toxicity of pyridalyl (*3*). However, biotransformation of the dichloropropenyl group could not be fully understood from the previous study since large amounts of metabolites were only identified with [dichlorophenyl-^{14}C]-pyridalyl, and examples derived from [dichloropropenyl-2-^{14}C]-pyridalyl could not be investigated. For the present article, therefore, we used [dichloropropenyl-2-^{14}C]pyridalyl, and the biotransformation of the dichloropropenyl group was more completely assessed by identification of metabolites present in only small amounts. A comparison of metabolic profiles between [dichloropropenyl-2-^{14}C]pyridalyl and [dichlorophenyl-^{14}C]-pyridalyl was conducted to facilitate this first investigation of biotransformation at the dichloropropenyl group.

The present article, thus, deals with the metabolism (^{14}C-excretion into feces, urine, expired air, ^{14}C-concentrations in tissues, and amounts of metabolites in excreta) of pyridalyl in rats.

MATERIALS AND METHODS

Chemicals. [Dichloropropenyl-2-^{14}C]pyridalyl (specific activity: 4.12 MBq/mg) and unlabeled pyridalyl (purity: 99.7% for the metabolism study and 94.2% for the metabolite identification study) were synthesized

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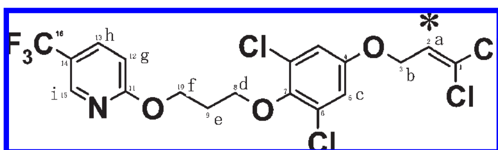

Figure 1. Chemical structure of pyridalyl. Proton and carbon numbers were shown for the determination of chemical structures (see Identification of Metabolites).

in our laboratory (**Figure 1**). Pyridalyl ^1H NMR (CD_3OD, 270 MHz, ppm): δ6.29 (1Ha, t, J = 6.2 Hz), 4.66 (2Hb,m), 6.97 (2Hc,m), 4.15 (2Hd, t, J = 5.6 Hz), 2.30 (2He, m), 4.66 (2Hf, m), 6.97 (1Hg, m), 7.94 (1Hh, dd, J = 8.5, 2.3 Hz), 8.49 (1Hi, d, J = 2.3 Hz). ^{13}C NMR: (CD_3OD, 67.5 MHz, ppm) δ126(C2), 64(C3), 117(C5), 131(C7), 71(C9), 31(C10), 67(C11), 112(C13), 137(C14), 146(C16). FAB-MS m/z: 490[M + H]$^+$. Three authentic metabolite standards, 3,3-dichloroacrolein, 3,3-dichloropropenal, and 3,3-dichloroacrylic acid, were also synthesized in our laboratory. [Dichlorophenyl-^{14}C]DCHM and [dichlorophenyl-^{14}C]-S-1812-Py-OH isolated in a previous study were used as metabolite standards (*3*). Other chemicals were of reagent grade.

Chromatographic Procedures. Metabolite analyses and isolation of metabolites were conducted by thin-layer chromatography (TLC) using precoated silica gel 60 F_{254} chromatoplates (20 × 20 cm, 0.25 or 0.5 mm layer thickness, Merck, Darmstadt, Germany). The solvent systems were hexane/toluene/acetic acid (A, 3:15:2, v/v/v, developed twice), ethyl acetate/ethanol/water (B, 4:2:1, v/v/v), and acetone/water (C, 5:1, v/v). Radioactive spots on TLC plates were detected by placing them in contact with imaging plates (Fuji Photo Film, Tokyo, Japan), followed by analysis with BAS2000 Bioimage Analyzer (Fuji Photo Film). High-performance liquid chromatography (HPLC) was carried out with a system consisting of an L-6200 HPLC intelligent pump (Hitachi, Tokyo, Japan), an L-4000 UV detector (Hitachi), an LB 507A radioactivity monitor (Berthold, Calmbacher, Germany), and an Integrator (805 data station, Millipore, or C-R7A, Shimazu, Kyoto, Japan) fitted with an ODS column (either a YMC-Pack, 20 mm i.d. × 250 mm, YMC Co., Kyoto, Japan (Column A), or a Cosmosil packed column 5C18, 4.6 mm i.d. × 250 mm, Nacalai tesque, Kyoto, Japan (Column B)). The mobile phases were methanol/water, (a, 80:20, v/v), (b, 77:23, v/v), (c, 70:30, v/v), (d, 65:35, v/v), (e, 50:50, v/v), and acetonitrile/water, (f, 60:40, v/v), (g, 20:80, v/v) for Column A, with a flow rate of 1 mL/min, and acetonitrile (h) for Column B, with a flow rate of 3 mL/min.

Radioanalysis. The radioactivity in urine and organosoluble fractions was quantified by liquid scintillation counting (LSC) of disintegrations per minute (dpm) by the external standard method. Samples (100−300 mg) of fecal homogenates, unextractable fecal residues, and tissues were combusted with a sample oxidizer (PerkinElmer, Waltham, Massachusetts, USA) prior to LSC after air-drying (combustion-LSC method). Metabolites in feces after TLC analysis were quantified by scraping-LSC methods. Radioactive bands were scraped and analyzed by LSC. The radioactivity in other areas was summed up as others. Metabolites in urine after TLC analysis were quantified with BAS2000 Bioimage Analyzer.

Spectroscopic and Spectrometric Analysis. Purified metabolites were identified by nuclear magnetic resonance (NMR) analysis with Unity 400 plus (Varian, CA) operating at 400 MHz for ^1H and 100 MHz for ^{13}C at room temperature with methanol-d_4 (99.5%, Merck, Germany) as a solvent. The chemical structures of metabolites were determined with ^1H NMR, ^{13}C NMR, H-H COSY, HMQC, and HMBC spectra. FAB-MS spectra were recorded on JMS-SX102A (JEOL, Tokyo, Japan) with a matrix of glycerol or nitrobenzyl alcohol. HRMS was also obtained with JMS-SX102A.

Treatment of Animals. Male and female Crj:CD(SD) rats at the age of 6 weeks were purchased from Charles River Japan, Inc. (Kanagawa, Japan) and maintained under constant environmental conditions: room temperature, 20−26 °C; relative humidity, 55 ± 10%; ventilation, 10 air exchanges per hour; and artificial lightning from 8:00am to 8:00 pm. Animals had free access to pelleted diet and water throughout the study. The animals after dosing were housed in metabolism cages to allow the separate collection of urine and feces. All animal experiments were conducted in accordance with the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan).

Preparation of Dosing Solutions. [Dichloropropenyl-2-^{14}C]pyridalyl was purified by preparative TLC developed with hexane/diethyl ether (20:1, v/v) prior to use. The labeled compound was radiochemically diluted with unlabeled pyridalyl with a specific activity of about 1.86 and 0.0185 to 0.203 MBq/mg for low and high doses, respectively. The test compound was dissolved in corn oil to give 5 or 500 mg/mL in the dosing solution. The radiochemical purity of the ^{14}C-labeled compound in the dosing solution was analyzed and quantified by TLC and was more than 98.7% in all the dose groups.

^{14}C-Excretion Study. Three male and three female Crj:CD(SD) rats at the age of 7 weeks old were given a single oral dose of [dichloropropenyl-2-^{14}C]pyridalyl at 5 and 500 mg/5 mL corn oil/kg by intragastric intubation. Urine and feces from each rat were collected at 6 h (urine only), 1, 2, 3, 5, and 7 days after administration of the ^{14}C-labeled compound. Expired air was passed through an alkaline trap containing 10% NaOH solution (about 200 mL) for trapping CO_2 gas and collected at 1, 2, 3, 5, and 7 days after administration. Following the collection of feces and urine, each metabolism cage was washed with water to recover the remaining ^{14}C (cage-wash). Radioactivity in the cage-wash samples was included in the urinary excretion. The 0−1 day and 1−2 day feces of each rat were homogenized with an approximate 2-fold volume of acetone, and the homogenates were centrifuged at 2000*g* for 10 min. The supernatants were obtained by decantation, and the residues were further extracted twice and three times with acetone and methanol/water (9:1, v/v), respectively. Radioactivity in supernatants and unextractable residues was analyzed by LSC and combustion, respectively. Feces collected from 3 to 7 days were homogenized with water and combusted for the radioassay. Each 10% NaOH solution for collecting expired air was radioassayed by LSC.

^{14}C-Tissue Residue Study. Rats used in the ^{14}C-excretion study were euthanized by bleeding from the abdominal artery under anesthesia at 7 days after administration, and the tissues were removed for the radioassay. The residual carcasses were minced with a meat chopper. Duplicate aliquots of blood were combusted for the radioassay, and the remaining blood was separated into blood cells and plasma by centrifugation at 2000*g* for 10 min. Each tissue sample was subjected to combustion analysis for the determination of ^{14}C-levels. The percentage of the ^{14}C-distribution to tissues was calculated on the basis of the weight of each tissue actually dissected out, but the weight of blood, bone, fat, muscle, and skin was calculated from the reported percentages of tissue weight (*4*).

Metabolite Analysis. The 0−2 day fecal extracts of each rat were combined and analyzed by TLC using solvent systems A and B with authentic metabolite standards, 3,3-dichloroacrolein, 3,3-dichloropropenal, 3,3-dichloroacrylic acid, [dichlorophenyl-^{14}C]DCHM, and [dichlorophenyl-^{14}C]S-1812-Py-OH. Polar metabolites retained near the origin on TLC plates after developing in solvent system A were well separated using solvent system B. Fecal metabolites on the TLC plates were quantified by the scraping-LSC method. The 0−2 day urine samples of each rat were combined and subjected to TLC analysis using solvent system C. Urinary metabolites on the TLC plate were quantified with BAS2000 Bioimage Analyzer. CO_2 in expired air was measured by LSC and identified by $BaCl_2$ precipitation analysis. One milliliter of 3 M $BaCl_2$ solution was added to 1 mL of each 0−1 or 1−2 day trapping solution. The mixture was shaken and centrifuged at 3000*g* for 5 min. Supernatants were then radioassayed by LSC.

Identification of Metabolites. To collect sufficient amounts of unknown metabolites in feces, a single oral dose of pyridalyl was given to 5 male and 5 female rats at approximately 500 mg/kg/5 mL by intragastric intubation. Feces were collected from rats within 3 days after administration of ^{14}C-labeled dosing solution.

A flow diagram of the purification procedures is given in **Figure 2**. Feces of each rat were combined and homogenized with a 2-fold volume of methanol/water (9:1, v/v). The homogenate was centrifuged at 3000*g* for 10 min, the supernatant was collected by decantation, and the residue was shaken twice with methanol/water (9:1, v/v). The supernatants were then combined and concentrated in vacuo. Fecal extracts were applied to XAD-2 (Amberlite, Organo, Tokyo) column chromatography, and the metabolites were eluted with 2 × 1 L of water, 1.5 L of methanol, and 1.5 L of acetone. The water, methanol, and acetone fractions were designated as

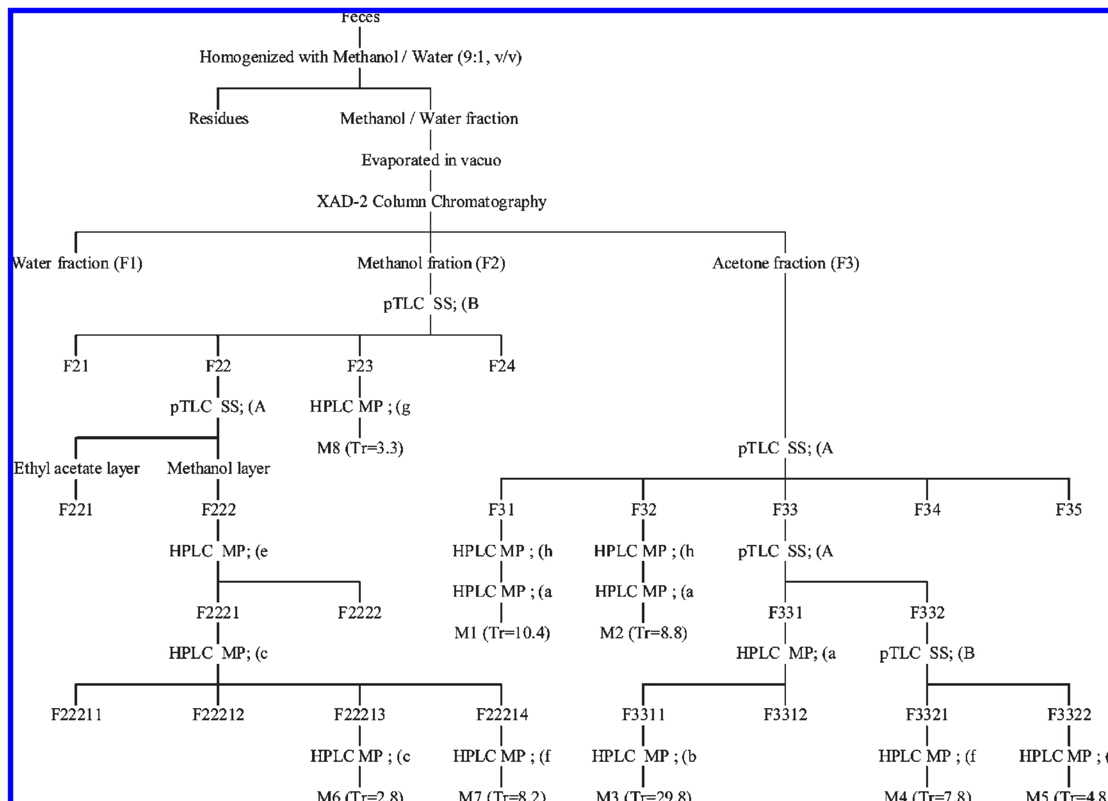

Figure 2. Flow diagram of the purification procedures for fecal metabolites. Abbreviations: SS, solvent system; MP, mobile phase; Tr, retention time (min).

Table 1. TLC Rf Values for Pyridalyl and Its Metabolites

	metabolite	chemical name	Rf values with solvent systems[a] A	B
	pyridalyl	2,6-dichloro-4-(3,3-dichloroallyloxy)phenyl 3-[5-(trifluoromethyl)-2-pyridyloxy]propyl ether	0.87	0.95
M1	S-1812-DH	2,6-dichloro-4-(3-chloroprop-2-ynyloxy)phenyl 3-[5-(trifluoromethyl)-2-pyridyloxy]propyl ether	0.86	0.95
M2	S-1812-Py-OH	2-[3-[2,6-dichloro-4-[(3,3-dichloroallyloxy)phenoxy]propoxy]-5-(trifluoromethyl)-3-pyridinol	0.59	0.95
M3	DCHM	3-[2,6-dichloro-4-[(3,3-dichloroprop-2-propenyl)oxy]phenol	0.52	0.91
M4	HPHM	3-[2,6-dichloro-4-(3,3-dichloroprop-2-enyloxy)phenoxy]propanol	0.39	0.92
M5	S-1812-PA	3-[3,5-dichloro-4-[3-(5-trifluoromethyl-2-pyridyloxy)propoxy]phenoxy]propionic acid	0.38	0.79
M6	S-1812-AA	2-[3,5-dichloro-4-[3-(5-trifluoromethyl-2-pyridyloxy)propoxy]phenoxy]acetic acid	0.26	0.61
M7	S-1812-PA-OH	3-[3,5-dichloro-4-[3-(5-trifluoromethyl-2-pyridyloxy)propoxy]phenoxy]-2-hydroxypropionic acid	0.11	0.48
M8	S-1812-PA-MA	3-[3,5-dichloro-4-[3-(5-trifluoromethyl-2-pyridyloxy)propoxy]phenoxy]-2-(*N*-acetylcysteinyl)propionic acid	0.03	0.44

[a] Solvent systems: (A) hexane/toluene/acetic acid (3:15:2, v/v/v, developed twice) and (B) ethyl acetate/ethanol/water (4:2:1, v/v/v).

F1, F2, and F3, respectively. F2 and F3 were applied to preparative TLC and preparative HPLC, as shown in **Figure 2**.

Finally, 8 metabolites (designated as M1 to M8) were purified by HPLC. The structures of the 8 metabolites isolated were identified by NMR spectroscopic and MS spectrometric analyses or TLC cochromatography. TLC Rf values for these metabolites are shown in **Table 1**, and HPLC retention times are shown in **Figure 2**.

RESULTS

^{14}C-Excretion. Data for ^{14}C-Excretion into feces, urine, and expired air during 7 days after single oral administration of [dichloropropenyl-2-^{14}C]pyridalyl are shown in **Figure 3**.

^{14}C was rapidly excreted into feces, urine, and expired air in all of the groups and within 2 days was > 80% of the dose. Total ^{14}C-recoveries within 7 days after administration were 92.6% (urine, 13.6%; feces, 70.4%; and expired air, 8.6%) in males and 96.4% (urine, 10.9%; feces, 79.3%; and expired air, 6.2%) in females with the low dose, and 86.7% (urine, 8.1%; feces, 72.5%; and expired air, 6.1%) in males and 89.8% (urine, 11.6%; feces, 67.9%; and expired air, 10.3%) in females with the high dose. ^{14}C-Elimination profiles were similar in both sexes.

^{14}C-Tissue Residues. Data for ^{14}C-tissue residues on the seventh day after the administration of the ^{14}C-labeled compound are shown in **Table 2**. In male and female rats, fat showed the highest ^{14}C-residue levels of 2.34 and 0.98 ppm, respectively, with the low dose, and 219 and 221 ppm, respectively, with the high dose. ^{14}C-Residues in other tissues accounted for 0.03−0.32 ppm with the low dose and 3−70 ppm with the high dose. The percentages ranged from 1.50−3.16% of the dose in fat, and figures for the muscle and hair and skin were also relatively high, accounting for 0.49−1.20%. The total ^{14}C-residues in the whole body was 2.95−6.80% of the dose.

Metabolites in Feces and Urine. Table 3 shows the amounts (% of the dosed ^{14}C) of metabolites in urine, feces, and expired air.

When 0−2 day pooled fecal extracts after the administration of [dichloropropenyl-2-^{14}C]pyridalyl were subjected to TLC analyses using solvent systems A and B, at least 12 metabolites were detected.

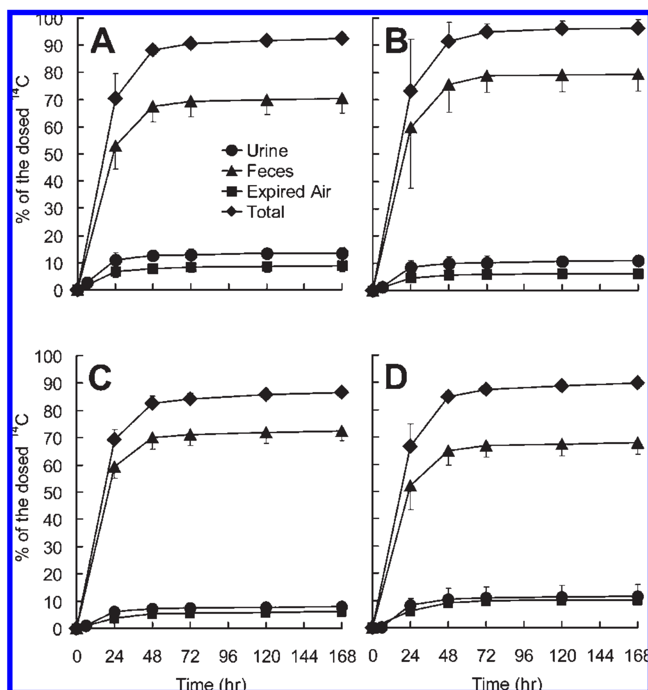

Figure 3. Cumulative ^{14}C-excretion into urine, feces, and expired air within 7 days after single oral administration of [dichloropropenyl-^{14}C]pyridalyl to rats. **A**, male (5 mg/kg); **B**, female (5 mg/kg); **C**, male (500 mg/kg); and **D**, female (500 mg/kg). The results show the mean values ± standard deviations for three rats.

The parent compound was the major form in feces, accounting for 44.5−59.6% of the dose. Each of the metabolites accounted for below 2.4% of the dose in all groups. Six fecal metabolites, M2, M3, M4, M6, M7, and M8, were detected at levels of 0.1−1.4% of the dose, but the amounts of M1 and M5 were trace. The ether-cleaved metabolites, 3,3-dichloroacrolein, 3,3-dichloropropenal, and 3-dichloroacrylic acid, were not detected.

When 0−2 day pooled urine was subjected to TLC using solvent system C, the parent compound was not detected in the urine, but at least 10 unidentified metabolites were present. Each metabolite accounted for 2.1% or less of the dose. There were no metabolites matching authentic standards.

When 0−1 and 1−2 day trapping solutions were subjected to $BaCl_2$ precipitation analysis, the radioactivity in supernatants after the addition of $BaCl_2$ accounted for $< 0.5\%$ of the dose. CO_2 was considered to be a major metabolite in the trapping solution, accounting for 5.3−9.2% of the dose.

Identification of Isolated Metabolites. The isolated metabolites were purified and identified by NMR spectroscopic and MS spectrometric analyses. ^{1}H NMR data and chemical structures are shown in **Table 4** and **Figure 4**, respectively.

M1. The chemical structure of M1 was proposed from ^{1}H NMR, ^{13}C NMR, and HMQC spectra. The signals in ^{13}C NMR (CD_3OD, 100 MHz) of M1 were assigned as follows: δ58 (C3), 117 (C5), 131 (C7), 71 (C9), 31 (C10), 65 (C11), 113 (C13), 137 (C14), and 146 ppm (C16). ^{1}H NMR and ^{13}C NMR data showed the disappearance of one proton on C2 of the dichloropropenyl group. The signals of 6.29 ppm (Ha) and 126 ppm (C2) in the ^{1}H NMR and ^{13}C NMR spectra of pyridalyl, respectively, disappeared. The FAB-MS spectrum showed a molecular ion peak at m/z 454 $[M + H]^+$ with isotope peaks of chlorine atoms, which was 36 lower than that of pyridalyl (m/z 490 $[M + H]^+$). These results showed the disappearance of HCl from the dichloropropenyl group of pyridalyl. On the basis of these results, M1 was identified as S-1812-DH.

Table 2. ^{14}C-Concentrations in Tissues of Rats 7 Days after Single Oral Administration of [Dichloropropenyl-2-^{14}C]pyridalyl at 5 or 500 mg/kg[a]

	μg equivalents of pyridalyl/g wet tissue (ppm)			
	5 mg/kg		500 mg/kg	
tissue	male	female	male	female
adrenal	0.27 ± 0.08	0.15 ± 0.10	28 ± 8	38 ± 5
blood	0.07 ± 0.01	0.03 ± 0.01	5 ± 1	5 ± 1
blood cells	0.09 ± 0.02	0.04 ± 0.01	7 ± 2	7 ± 2
plasma	0.03 ± 0.01	0.02 ± 0.00	3 ± 1	3 ± 0
bone	0.11 ± 0.02	0.05 ± 0.02	8 ± 1	11 ± 3
bone marrow	0.09 ± 0.02	0.06 ± 0.02	16 ± 3	10 ± 3
brain	0.08 ± 0.03	0.04 ± 0.01	5 ± 1	9 ± 3
cecum	0.15 ± 0.08	0.08 ± 0.05	10 ± 3	14 ± 3
fat	2.34 ± 0.68	0.98 ± 0.63	219 ± 39	221 ± 20
hair and skin	0.32 ± 0.15	0.16 ± 0.04	70 ± 47	53 ± 28
heart	0.10 ± 0.02	0.06 ± 0.02	8 ± 1	11 ± 1
kidney	0.27 ± 0.07	0.21 ± 0.05	23 ± 4	29 ± 2
large intestine	0.14 ± 0.03	0.07 ± 0.02	9 ± 2	13 ± 5
liver	0.19 ± 0.03	0.13 ± 0.02	21 ± 1	24 ± 3
lung	0.11 ± 0.02	0.07 ± 0.02	12 ± 3	13 ± 1
mandibular gland	0.10 ± 0.02	0.05 ± 0.01	10 ± 2	10 ± 1
muscle	0.09 ± 0.03	0.05 ± 0.00	10 ± 6	7 ± 1
ovary	NA	0.08 ± 0.01	NA	24 ± 4
pancreas	0.20 ± 0.10	0.11 ± 0.06	18 ± 1	20 ± 3
pituitary	0.16 ± 0.06	0.06 ± 0.02	12 ± 4	10 ± 2
small intestine	0.17 ± 0.06	0.09 ± 0.06	15 ± 8	18 ± 12
spinal cord	0.14 ± 0.05	0.07 ± 0.03	10 ± 1	15 ± 5
spleen	0.12 ± 0.01	0.07 ± 0.01	9 ± 2	11 ± 1
stomach	0.12 ± 0.01	0.08 ± 0.03	22 ± 12	17 ± 4
testis	0.07 ± 0.02	NA	5 ± 1	NA
thymus	0.11 ± 0.04	0.07 ± 0.03	11 ± 4	16 ± 9
thyroid	0.17 ± 0.06	0.09 ± 0.02	30 ± 7	30 ± 12
uterus	NA	0.05 ± 0.02	NA	13 ± 3
carcass	0.27 ± 0.07	0.15 ± 0.05	42 ± 6	32 ± 4

[a] Figures show the mean values ± standard deviations for three rats. NA: Not applicable.

Table 3. Amounts of Metabolites in the Urine, Feces, and Expired Air of Rats within 2 Days after Single Oral Administration of [Dichloropropenyl-2-^{14}C]-pyridalyl at 5 mg/kg or 500 mg/kg

	amount (% of the dosed ^{14}C)			
	5 mg/kg		500 mg/kg	
metabolite	male	female	male	female
urine				
others	<2.1	<2.0	<1.3	<1.8
total	12.7	10.0	7.2	10.6
feces				
pyridalyl	44.5	59.6	57.5	54.8
M2 (S-1812-Py-OH)	1.4	0.9	0.2	0.2
M3 (DCHM)	0.3	0.2	0.2	0.1
M4 (HPHM)	1.2	1.0	1.1	1.1
M6 (S-1812-AA)	1.0	0.8	0.5	0.4
M7 (S-1812-PA-OH)	1.4	0.9	0.5	0.6
M8 (S-1812-PA-MA)	0.5	0.4	0.4	0.5
others	<2.4	<1.2	<1.4	<0.7
unextractable	11.4	8.9	5.2	4.3
total	67.6	75.7	70.1	65.1
expired air				
CO_2	7.4	5.7	5.3	9.2
total	87.6	91.3	82.6	84.9

M2. The ^{1}H NMR spectrum and the TLC Rf value of M2 were essentially the same as those of [dichlorophenyl-^{14}C]S-1812-Py-OH. On the basis of these results, this metabolite was considered

Table 4. ^{1}H-NMR Analysis Data for Pyridalyl and Isolated Metabolites[a]

	S-1812-DH (M1)		HPHM (M4)		S-1812-PA (M5)		S-1812-AA (M6)		S-1812-PA-OH (M7)		S-1812-PA-MA (M8)	
proton no.	δ(CD$_3$OD)	J(Hz)	δ(CD$_3$OD)	J(Hz)	δ(CD$_3$OD)	J(Hz)	δ(CD$_3$OD)	J(Hz)	δ(CD$_3$OD)	J(Hz)	δ(CD$_3$OD)	J(Hz)
Ha	-----		6.32 (1H, t)	6.4	2.63 (2H, t)	6.6	-----		4.16 (1H, m)		3.62 (1H, m)	
Hb	4.70 (2H, m)		4.59 (2H, m)		4.20 (2H, t)	6.6	4.57 (2H, s)		4.16 (1H, m)		4.16 (1H, m)	
									4.24 (1H, m)		4.34 (1H, m)	
Hc	7.06 (2H, m)		7.03 (2H, s)		6.99 (2H, m)		7.00 (2H, m)		7.04 (2H, s)		7.03 (2H, s)	
Hd	4.18 (2H, t)	5.8	4.10 (2H, t)	6.4	4.16 (2H, t)	5.8	4.17 (2H, t)	6	4.16 (2H, t)	6	4.16 (2H, m)	
He	2.33 (2H, m)		2.07 (2H, m)		2.31 (2H, m)		2.32 (2H, m)		2.32 (2H, m)		2.31 (2H, m)	
Hf	4.70 (2H, m)		3.85 (2H, t)	6.4	4.68 (2H, m)	6.4	4.68 (2H, m)	6.4	4.68 (2H, t)	6.4	4.68 (2H, t)	6.2
Hg	7.06 (1H, m)		-----		6.99 (1H, m)		7.00 (1H, m)		6.99 (1H, d)	8.8	6.99 (1H, d)	8.8
Hh	7.97 (1H, ddd)	8.8, 2.4, 0.4	-----		7.96 (1H, d)	8.8	7.97 (1H, dd)	8.8, 2.4	7.95 (1H, dd)	8.8, 2.4	7.97 (1H, dd)	8.8, 2.6
Hi	8.51 (1H, m)		-----		8.51 (1H, s)		8.51 (1H, s)		8.51 (1H, s)		8.52 (1H, s)	
					mercapturic acid moiety							
CH_3CO-	-----		-----		-----				-----		2.04 (3H, s)	
-CH_2CH-	-----		-----		-----				-----		3.17 (1H, m)	
											3.30 (1H, m)	
-CH$_2$C*H*-	-----		-----		-----				-----		4.49 (1H, m)	

[a] s, singlet; d, doublet; dd, double−doublet; ddd, double−double−doublet; t, triplet; m, multiplet; -----, none.

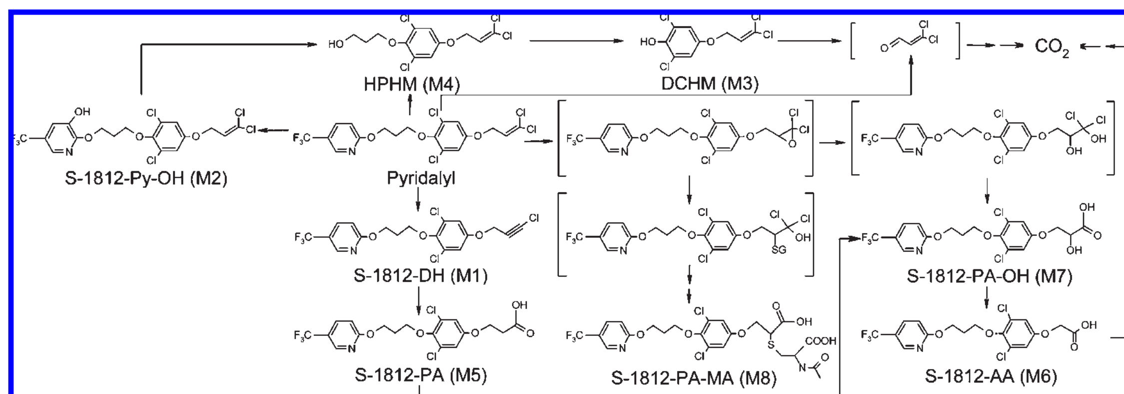

Figure 4. Proposed metabolic pathways of pyridalyl in rats.

to have undergone hydroxylation at position-3 of the pyridyl ring of pyridalyl and was identified as S-1812-Py-OH.

M3. The ^{1}H NMR spectrum and the TLC Rf value of M3 were essentially the same as those of [dichlorophenyl-^{14}C]DCHM. On the basis of these results, this metabolite was considered to be DCHM, formed by ether cleavage of pyridalyl between the trimethylene and the dichlorophenylene groups.

M4. The ^{1}H NMR spectrum showed the disappearance of the pyridine ring of pyridalyl. One-proton signals at 6.97, 7.94, and 8.49 ppm disappeared on ^{1}H NMR. The FAB-MS spectrum showed a molecular ion peak at m/z 345 $[M + H]^+$ with isotope peaks of chlorine atoms. On the basis of these results, M4 was identified as HPHM.

M5. The ^{1}H NMR and H-H COSY spectra showed the addition of one proton to the methylidyne group in the dichloropropenyl group. The signal at 6.29 ppm (Ha) on the ^{1}H NMR spectrum of pyridalyl disappeared, and the two-proton signal on C3 in the dichloropropenyl group of pyridalyl was shifted to a higher magnetic field as a 2 proton triplet signal at 4.20 ppm (Hb) on ^{1}H NMR. A two-proton triplet signal coupled with the 4.20 ppm signal (Hb) on H-H COSY spectrum was observed at 2.63 ppm (Ha) on ^{1}H NMR. FAB-MS spectrum (negative mode) showed a molecular ion peak at m/z 452 $[M - H]^-$ with isotope peaks of chlorine atoms. On the basis of these results, M5 was identified as S-1812-PA.

M6. The chemical structure of M6 was proposed from ^{1}H NMR, ^{13}C NMR, HMQC, and HMBC spectra. The signals in ^{13}C NMR (CD$_3$OD, 100 MHz) of M6 were assigned as follows; δ73 (C3), 117 (C5), 131 (C7), 71 (C9), 31 (C10), and 65 (C11). The ^{1}H NMR and H-H COSY spectra showed the disappearance of the one proton on C2 in the dichloropropenyl group. The signals at 6.29 (Ha) and 4.66 ppm (Hb) on the ^{1}H NMR spectrum of pyridalyl disappeared, and the two-proton singlet signal at 4.57 ppm (Hb) appeared at higher magnetic fields in the ^{1}H NMR spectrum. The FAB-MS spectrum (negative mode) showed a molecular ion peak at m/z 438 $[M - H]^-$ with isotope peaks of chlorine atoms. On the basis of these results, M6 was identified as S-1812-AA. HRMS showed an error of molecular weight calculated as −3.5 mmu (−8.1 ppm), and the molecular mass matched that of M6.

M7. The ^{1}H NMR and H-H COSY spectra showed the addition of a hydroxyl group to C2 in the dichloropropenyl group. The signals at 6.29 (Ha) and 4.66 ppm (Hb) on the ^{1}H NMR spectrum of pyridalyl disappeared, and three one-proton signals, at 4.16 (Ha), 4.16 (Hb), and 4.24 ppm (Hb), appeared and were coupled with each other at higher magnetic fields on H-H COSY. The FAB-MS spectrum (negative mode) showed a molecular ion peak and a fragment peak at m/z 468 $[M - H]^-$ and 380 $[M - CH_2CH(OH)COOH]^-$ with isotope peaks of chlorine atoms. On the basis of these results, M7 was identified as S-1812-PA-OH. HRMS showed an error of molecular weight calculated as −0.8 mmu (−1.8 ppm), and the molecular mass matched that of M7.

M8. The ^{1}H NMR and H-H COSY spectra showed the addition of a mercapturic acid group to C2 in the dichloropropenyl

group. A three-proton singlet signal at 2.04 ppm and proton multiplet signals at 3.17, 3.30, and 4.49 ppm appeared as mercapturic acid protons, the three multiplet signals being coupled to each other on H-H COSY. The signals at 6.29 (Ha) and 4.66 ppm (Hb) on the ^{1}H NMR spectrum of pyridalyl disappeared, and one-proton signals at 3.62 (Ha), 4.16 (Hb), and 4.34 ppm (Hb) appeared and were coupled to each other at higher magnetic fields on H-H COSY. The FAB-MS spectrum (negative mode) showed a molecular ion peak and a fragment peak at m/z 635 $[M + Na - 2H]^-$ and 380$[M - CH_2CH(C_5H_8O_3NS)COOH]^-$ with isotope peaks of chlorine atoms. On the basis of these results, M8 was identified as S-1812-PA-MA. HRMS showed an error of molecular weight calculated as 5.1 mmu (8.3 ppm), and the molecular mass matched that of M8.

DISCUSSION

The present study revealed the radiocarbon to be rapidly absorbed after single oral administration of [dichloropropenyl-2-^{14}C]pyridalyl to male and female rats at 5 or 500 mg/kg and then excreted into feces, urine, and expired air at 68−79%, 8−14% and 6−10%, respectively. Relatively high amounts of ^{14}C-residues were observed in fat, ranging from 0.98−2.34 ppm and 219−221 ppm with the low and high doses, respectively. ^{14}C-Residues in other tissues were 0.03−0.32 ppm and 3−70 ppm for the low and high doses, respectively. The percentage of ^{14}C-residues in fat was 1.50−3.16% of the dose, and that of muscle and hair and skin was also relatively high, accounting for 0.49−1.20%. No marked sex-related differences were observed in ^{14}C-elimination, ^{14}C-distribution, and metabolite profile.

The high ^{14}C concentration observed in fat was in line with findings for [dichlorophenyl-^{14}C]pyridalyl and can be considered to be derived from the parent compound because of the high lipophilic property of pyridalyl (log P = 8.1) as described in a previous report (*3*).

However, the ^{14}C-residual profile of tissues other than fat was different and was about 10 times higher than that with [dichlorophenyl-^{14}C]pyridalyl (*3*). This difference was considered to be caused by differences in metabolites. The higher residue with [dichloropropenyl-2-^{14}C]pyridalyl might be due to the incorporation of ^{14}C-natural products formed from pyridalyl. [Dichloropropenyl-2-^{14}C]pyridalyl is metabolized to form S-1812-AA, whose carboxymethoxy group can be cleaved to form S-1812-DP (3,5-dichloro-4-(3-(5-trifluoromethyl-2-pyridyloxy)propoxy)-phenol) and ^{14}C-glyoxylic acid. The ^{14}C-glyoxylic acid can be converted to ^{14}C-serine or ^{14}C-threonine by serine hydroxymethyltransferase or threonine aldolase, and ^{14}C-serine then further converted to ^{14}C-glucose or ^{14}C-fatty acids via the formation of ^{14}C-pyruvic acid (*5*). ^{14}C-Amino acids, ^{14}C-glucose, or ^{14}C-fatty acids are able to be incorporated into protein, polysaccharide, or lipids (*6*). Therefore, this provides an explanation of why the ^{14}C-concentration in each tissue was larger than that with [dichlorophenyl-^{14}C]pyridalyl.

The excretion profile of [dichloropropenyl-2-^{14}C]pyridalyl was here found to be different from that of [dichlorophenyl-^{14}C]-pyridalyl (*3*). About 14−23% of urinary and expired air excretion was observed with [dichloropropenyl-2-^{14}C]pyridalyl, while [dichlorophenyl-^{14}C]pyridalyl was excreted mainly into feces (>99%). This difference can be well explained by the difference in metabolites between each labeled compound. The major metabolite with [dichlorophenyl-^{14}C]pyridalyl, S-1812-DP, was excreted mainly into feces, while those with [dichloropropenyl-2-^{14}C]pyridalyl, CO_2, and many other kinds of urinary metabolites were excreted into expired air and urine. These results indicate that the difference was caused by the metabolites formed after cleavage of the ether linkage between the dichloropropenyl group and the dichlorophenyl ring.

Biotransformation of the dichloropropenyl group was proposed by identification of the small amounts (<2% of the dose) of metabolites. Pyridalyl could be metabolized by epoxidation of double bonds followed by hydration, decarboxylation, and glutathione conjugation, accompanied by dechlorination, which was proposed earlier by cleavage reactions of the C−C double bond of trichloroethylene (*7*), stilbene (*8*), pregnenolone (*9*), and OPB-20454 (*10*) after the formation of ketol. These reactions readily occur; therefore, pyridalyl would be excreted rapidly. Though metabolism of the 3,3-dichloroallyloxy group has not been reported yet, the transformation would be expected to be similar to that of the chlorinated organic compounds previously reported.

Ether-cleaved metabolites of pyridalyl, 3,3-dichloroacrolein, 3,3-dichloropropenal, and 3,3-dichloroacrylic acid were not detected, while CO_2 and many kinds of urinary metabolites were detected as metabolites derived from the dichloropropenyl group. Generally, α-β-unsaturated aldehydes react with DNA (*11*, *12*) or protein (*13*), and some of them have mutagenicity with Michael addition to DNA. However, the mutagenicity of 3,3-dichloroacrolein is small, at only 1/144 the level of its isomer, 2,3-dichloroacrolein (*14*), whose strong mutagenicity is caused by a chlorine atom at the α position. Since there is no such substitute group in 3,3-dichloroacrolein (*14*), 3,3-dichloroacrolein has no carcinogenicity (*15*), in contrast to the marked effects of 2,3-dichloroacrolein. Furthermore, it is considered that formation of ether-cleaved metabolites was very small or that these metabolites were themselves metabolized rapidly since these metabolites were not detected in this study. These results are consistent with no carcinogenicity of pyridalyl.

On the basis of the identification of 8 metabolites in this study, pyridalyl is extensively metabolized. The biotransformation reactions in rats are proposed to be as follows: (1) epoxidation of the double bond in the dichloropropenyl group followed by hydration, dehydrochlorination, decarboxylation, and/or mercapturic acid conjugation; (2) CO_2 formation after O-dealkylation of pyridalyl and its metabolites; (3) hydroxylation of C3 in the pyridyl ring; (4) O-dealkylation of the pyridyloxy and trimethylene groups; and (5) dehydrochlorination and hydration in the dichloropropenyl group.

On the basis of these observations, the proposed metabolic pathways of pyridalyl in rats are detailed in **Figure 4**.

ABBREVIATIONS USED

S-1812-DP, 3,5-dichloro-4-(3-(5-trifluoromethyl-2-pyridyloxy)-propoxy)phenol; NMR, nuclear magnetic resonance; COSY, correlation spectroscopy; HMQC, heteronuclear multiple quantum coherence; HMBC, heteronuclear multiple-bond connectivity; MS, mass spectrometry; FAB, fast atom bombardment; HRMS, high resolution mass spectrometer; TLC, thin-layer chromatgraphy; HPLC, high performance liquid chromatography; LSC, liquid scintillation counting.

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