Metabolic Fate of Cinmethylin in Rats

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The metabolic fate of [*phenyl*-^{14}C]cinmethylin (1), a novel cineole herbicide, in laboratory rats following a single oral dose of 15 and 450 mg/kg was examined. The major route of elimination was via urinary excretion, and approximately 75–85% of the administered radioactivity was eliminated during the initial 48 h postdosing. No $^{14}CO_2$ or other radioactive volatile material was detected in the respired air. A complex degradation pattern of 1 was observed in the animal excreta. In addition to the undegraded 1 (recovered only in the fecal excreta), at least 10 metabolites were isolated and identified from the urinary and fecal excreta as both organic-extractable and conjugated products. The proposed metabolic pathways of 1 involved hydroxylation and oxidation at the benzyl and cineole portions of the parent molecule, conjugation (with glucuronic acid and glycine), and ether cleavage. Pharmacokinetic patterns indicated the rapid disposition of 1 and its metabolites from the treated animals. No toxicological relevant level of residues was detected in tissues other than the liver.

INTRODUCTION

Cinmethylin (1), the proposed common name for CINCH herbicide [7-oxabicyclo[2.2.1]heptane, 1-methyl-4-(1-methylethyl)-2-[(2-methylphenyl)methoxy]-, *exo*-], is a novel cineole herbicide invented by Shell Development Co. The exo diastereomer of this compound exhibited broad-spectrum preemergent herbicidal activity against various grassy weeds in soybeans, peanuts, and cotton (Peterson et al., 1983). The acute mammalian toxicity of cinmethylin is low. The oral and dermal LD_{50} values are 4.5 g/kg (rat) and greater than 2 g/kg (rabbit), respectively. The elimination, tissue distribution, metabolism, and the pharmacokinetic properties of cinmethylin and its metabolites in rats following single oral doses of 15 and 450 mg/kg were examined. Major objectives of this report are to describe (1) the primary route and rate of elimination of the administered dose, (2) characterization and identification of significant metabolites, (3) tissue residue distribution profile, and (4) assessment of the pharmacokinetic parameters of [^{14}C]cinmethylin and its metabolites in the treated animals.

EXPERIMENTAL SECTION

Radiosynthesis. [U-*phenyl*-^{14}C]Cinmethylin (1) was synthesized by the condensation of 2-*exo*-hydroxy-1,4-cineole [**2**, *exo*-1-methyl-4-(1-methylethyl)-7-oxabicyclo-[2.2.1]heptan-2-ol] with 2-methyl [U-*phenyl*-^{14}C]benzyl chloride.

2 was prepared by the addition of 1.95 g of vanadium(IV) bis(2,4-pentanedionate) oxide and 0.75 g of triethylamine to a stirring solution of 150 g (0.97 mol) of (±)-terpinen-4-ol (**3**, SCM Specialty Chemicals) in 500 mL of toluene. The solution was heated to reflux and treated with 90% *tert*-butyl hydroperoxide (112 g, 1.12 mol) in toluene (100 mL) and stirred for 1 h. The cooled mixture was washed with 5% sodium carbonate (150 mL) and water (150 mL) and dried by azeotropic distillation of toluene (ca. 100 mL). The resulting solution of crude *cis*-3,4-epoxy-4-methyl-1-(1-methylethyl)cyclohexanol (**4**; Ohloff and Uhde, 1965) was cooled to 30 °C, treated with 96% sulfuric acid (0.54 mL) in tetrahydrofuran (6 mL), and stirred for 20 h. The reaction mixture was washed with water (150 mL), 5% sodium carbonate (150 mL), and water (150 mL). Calcium hydroxide (2 g) was added as stabilizer, and the reaction mixture was distilled at 100 mm at a kettle temperature of 165 °C. Crystallization from hexane at −20 °C yielded 88 g (53%, >98% pure), mp 58–60 °C. Recrystallization from hexane yielded a final product of **2**: mp 60–62.5 °C; MS (EI) *m/z* (% RA) 170 (M^+, 5), 153 (6), 137 (4), 127 (7), 112 (19), 71 (20), 43 (100).

To 43 mg (1.8 mmol) of freshly activated sodium hydride in dry dimethylformamide was added 292 mg (1.7 mmol) of **2**. The reaction mixture was stirred for 1 h at 20 °C and then maintained at 80 °C for 30 min. After cooling to room temperature, 243 mg (1.7 mmol) of 2-methyl-[U-*phenyl*-^{14}C]benzyl chloride (New England Nuclear, sp act. 9.1 mCi/mmol) in dimethylformamide was added dropwise. This mixture was stirred at 20 °C for 1 h and then at 50 °C for 0.5 h. To isolate the product, 3 mL of water was added to the reaction mixture followed by adjustment to pH 7 and extraction with hexane to yield 368 mg (79%) of **1**, radiochemical purity ca. 80%. [^{14}C]Cinmethylin was purified by repeated preparative TLC using toluene–ethyl acetate (4:1) and heptane–dioxane (4:1) as developing solvents. The final yield of **1** was 182 mg (0.66 mmol, 39%) and had radiochemical purity and specific activity of ca. 98.5% and 7.7 mCi/mmol, respectively: MS (EI) *m/z* (% RA) 274 (M^+, 8), 216 (5), 169 (20), 154 (18), 133 (10), 123 (47), 107 (42), 105 (100), 93 (24), 83 (20), 71 (35), 55 (15), 43 (95).

Synthetic Standards. The schematic diagram for the synthesis of the various reference standards is outlined in Figure 1. Analytical data (elemental analysis and infrared, nuclear magnetic resonance, and mass spectroscopies) are consistent with the structures shown. Melting and boiling points are not corrected.

***exo*-2-[[2-(Chloromethyl)phenyl]methoxy]-1-methyl-4-(1-methylethyl)-7-oxabicyclo[2.2.1]heptane (5).** A solution of **2** (23.8 g, 0.14 mol) in toluene (160 mL) and powdered sodium hydroxide (12 g) was refluxed under a Stark–Dean trap until no more water was removed. The solution was cooled to 25 °C and α,α-dichloro-*o*-xylene (25 g, 0.143 mol) was added. The mixture was stirred at reflux for 3.5 h, cooled, washed with water, and concentrated to give ca. 50:50 mixture of the mono- and disubstitution products along with the unreacted dichloroxylene. Silica gel column chromatography (hexane–ethyl acetate) gave **5** as the second emerging component. Crystallization (hexane) gave **5** in 30% yield: mp 53–54 °C; MS (EI) *m/z* (% RA) 265 ($M^+ - C_3H_7$, <1), 250 (1), 169 (2), 139 (24), 91 (20), 71 (21) and 43 (100).

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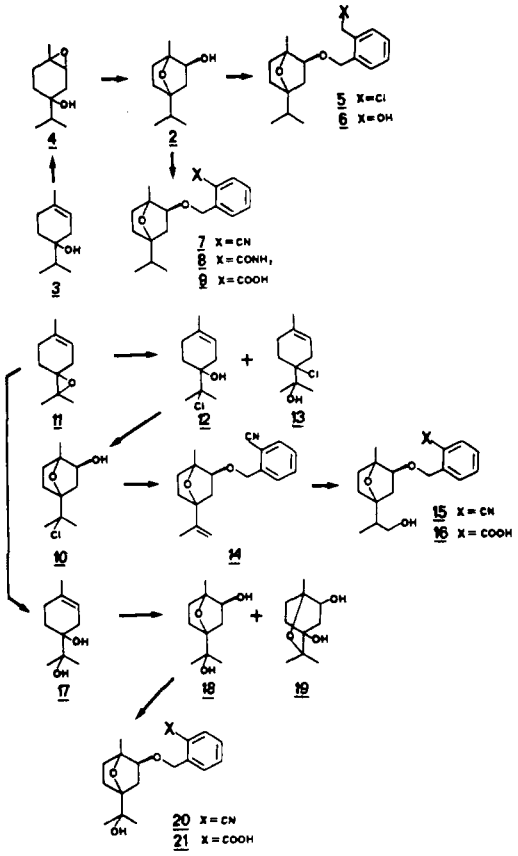

Figure 1. Reaction sequences for the synthesis of cinmethylin (1) metabolites.

***exo*-2-[[[1-Methyl-4-(1-methylethyl)-7-oxabicyclo-[2.2.1]hept-2-yl]oxy]methyl]benzenemethanol (6).** A solution of **5** (5.4 g, 0.0175 mol) in dioxane (60 mL) was mixed with a solution of sodium hydroxide (1.2 g, 0.03 mol) in water (60 mL) and refluxed for 8 h. The mixture was diluted with water (350 mL) and made weakly basic by treatment with hydrochloric acid–sodium bicarbonate, followed by extraction with ether. Chilling the concentrated ether solution to −50 °C gave **6** as a white solid: 4.1 g (80%); mp 71–73 °C. Anal. Calcd: C, 74.5; H, 9.0. Found: C, 74.8; H, 9.3. MS (EI) *m/z* (% RA) 272 ($M^+ - H_2O$, <1), 247 (<1), 232 (2), 171 (17), 169 (6), 123 (33), 121 (12), 91 (59), 77 (38), 43 (100); IR (KBr) 3422 (OH) cm^{-1}.

***exo*-2-[[[1-Methyl-4-(1-methylethyl)-7-oxabicyclo-[2.2.1]hept-2-yl]oxy]methyl]benzenecarbonitrile (7).** A suspension of hexane washed sodium hydride (1.08 g, 0.045 mol) in *N,N*-dimethylacetamide (10 mL) was added to a stirred solution of **2** (6.8 g, 0.04 mol) in the same solvent (40 mL). After stirring at ambient temperature (1 h) and at 70 °C (0.5 h), the mixture was cooled and *o*-cyanobenzyl bromide (8.6 g, 0.044 mol) was added in portions over 7 min. The mixture was stirred at ambient temperature (1 h) and 80 °C (1 h), then cooled, poured into water (400 mL), and extracted with methylene chloride (200 mL, 2 × 100 mL). Combined extracts were washed with water (400 mL), dried (magnesium sulfate), and concentrated to give a red oil. Claisen distillation gave crude **7**: 5.8 g; bp 140–148 °C (0.15 mm); 93% pure by gas liquid chromatography (GLC). Anal. Calcd: C, 75.8; H, 8.1; N, 4.9. Found: C, 75.6; H, 8.0; N, 5.6. IR (CH_2Cl_2) 2230 (CN) cm^{-1}; MS (EI) *m/z* (% RA) 285 (M^+, 1), 267 (<1), 242 (1), 227 (8), 169 (7), 116 (49), 71 (33), 43 (100).

***exo*-2-[[[1-Methyl-4-(1-methylethyl)-7-oxabicyclo-[2.2.1]hept-2-yl]oxy]methyl]benzenecarboxamide (8).** A mixture of **7** (4.1 g, 0.0144 mol) in ethanol (50 mL) and sodium hydroxide (0.70 g, 0.018 mol) in water (15 mL) was refluxed (20 h), cooled and diluted with water (100 mL), acidified (6 N hydrochloric acid, 5 mL), and extracted with methylene chloride (3 × 75 mL). Organic extracts were washed with water (50 mL), dried (magnesium sulfate), and concentrated to give 4.5 g of yellow oil. Claisen distillation gave crude **8**: 3.5 g; bp 180–184 °C (0.2 mm); 90% pure by GLC. Crystallization of a sample from ether gave pure **8** as a white solid; mp 121–124 °C. Anal. Calcd: C, 71.3; H, 8.2; N, 4.6. Found: C, 71.4; H, 8.3; N, 4.4. MS (EI) *m/z* (% RA) 303 (M^+, 1), 260 (<1), 169 (12), 134 (100), 116 (18), 107 (14), 71 (15), 43 (95); IR (KBr) 3330, 3150 (NH), 1680 (C=O) cm^{-1}.

***exo*-2-[[[1-Methyl-4-(1-methylethyl)-7-oxabicyclo-[2.2.1]hept-2-yl]oxy]methyl]benzoic Acid (9).** A mixture of crude **8** (1.9 g, 0.0063 mol), sodium hydroxide (2.5 g, 0.063 mol), and ethylene glycol (25 mL) was stirred under nitrogen at 120 °C for 6 h, cooled, diluted with water (50 mL), acidified (6 N hydrochloric acid, 12 mL), and extracted with methylene chloride (3 × 75 mL). Organic extracts were washed with water (2 × 50 mL), dried (magnesium sulfate), and concentrated to a yellow oil. Crystallization from ether pentane gave **9**: 0.5 g; mp 96–108 °C. Anal. Calcd: C, 71.1; H, 7.9. Found: C, 72.2; H, 8.0. MS (EI) *m/z* (% RA) 304 (M^+, 4), 285 (17), 246 (32), 169 (7), 135 (81), 77 (29), 71 (29), 43 (100).

***exo*-4-(1-Chloro-1-methylethyl)-1-methyl-7-oxabicyclo[2.2.1]heptan-2-ol (10).** A stirred solution of terpinolene 4(8)-epoxide (**11**; Leffingwell, 1970) (15.2 g, 0.1 mol) in chloroform (100 mL) was treated at −15 °C with 0.3 N hydrogen chloride in chloroform (400 mL) and then stirred at 3–5 °C for 1.5 h. The product (20 g), after the removal of solvent, was distilled through a Bantam-ware Vigreux column to collect a center fraction (14 g; bp 70–71 °C (0.5 mm)) consisting primarily of **12** [1-(1-chloro-1-methylethyl)-4-methyl-3-cyclohexen-1-ol] with a small amount of the isomeric chlorohydrin **13**. A pure sample of **12** was obtained by silica gel column chromatography (2% tetrahydrofuran–hexane). Anal. Calcd: C, 63.7; H, 9.0. Found: 63.6; H, 9.3. MS (EI) *m/z* (% RA) 188 (M^+, 3), 170 (12), 152 (7), 111 (99), 93 (100), 83 (38), 69 (57), 55 (45), 43 (53); IR (CH_2Cl_2) 3560 (OH) cm^{-1}.

The distilled crude **12** (10.2 g, 0.054 mol) and vanadium(IV) bis(2,4-pentanedionate) oxide (0.5 g) in methylene chloride (100 mL) were heated to near reflux and treated with 90% *tert*-butyl hydroperoxide (5.9 g, 0.059 mol). After a very exothermic reaction, the mixture was refluxed for 2 h cooled, and dried (magnesium sulfate), and toluenesulfonic acid (0.36 g) in glyme (4 mL) was added. After the reaction mixture was maintained for 18 h at ambient temperature, sodium acetate (3 g) was added and the mixture was filtered and concentrated to a red oil (13.1 g). This residue was dissolved in ether, diluted with pentane, and chilled to give 3.7 g of solid **10**, mp 104–107 °C. Anal. Calcd for $C_{10}H_{17}ClO_2$: C, 58.7; H, 8.3. Found: C, 58.9; H, 8.4. MS (EI) *m/z* (% RA) 204 (M^+, 1), 187 (<1), 168 (6), 150 (6), 107 (36), 43 (100); IR (KBr) 3280 (OH) cm^{-1}.

***exo*-2-[[[1-Methyl-4-(1-methylethenyl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy]methyl]benzenecarbonitrile (14).** A solution of **10** (18 g, 0.088 mol) in 150 mL of dry *N,N*-dimethylacetamide was treated under nitrogen with sodium hydride (2.2 g, 0.092 mol) and stirred for 24 h. The resulting mixture was cooled in ice and treated rapidly with *o*-cyanobenzyl bromide (17.2 g, 0.088 mol). After the mixture was stirred at 25 °C for 1 h and 50–60 °C for 0.5 h, additional sodium hydride (2.2 g) was added and the

mixture was heated at 80–85 °C for 0.5 h. The reaction mixture was poured into water, extracted with methylene chloride, washed, concentrated, and Claisen distilled at 0.1 mm to a kettle temperature of 180 °C. The product **14** was collected at a head temperature of 145–150 °C; 10.1 g; 95% pure by GLC. Anal. Calcd: C, 76.3; H, 7.5. Found: C, 75.7; H, 7.8. MS (EI) m/z (% RA) 283 (M^+, 1), 265 (<1), 240 (<1), 225 (1), 167 (2), 116 (42), 107 (36), 92 (19), 69 (23), 43 (100); IR (GC) 2240 (CN), 1000–1200 (COC), 3086 (C=C) cm^{-1}.

***exo*-2-[[[4-(2-Hydroxy-1-methylethyl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy]methyl]benzenecarbonitrile (15).** A chilled solution of **14** (10.8 g, 0.08 mol) in dry tetrahydrofuran (50 mL) was treated all at once with borane dimethyl sulfide complex (1.3 mL, 0.013 mol) and then stirred 0.5 h at 0 °C, 0.5 h at 25 °C, and 0.5 h at reflux. The mixture was treated at <30 °C with ethanol (12 mL), 3 N sodium hydroxide solution (4.4 mL), and 30% hydrogen peroxide (4.6 g) and then refluxed for 0.5 h. The resulting mixture was poured into water and extracted with ether (3 × 50 mL), and the extracts were washed with 6 N hydrochloric acid and water, dried (magnesium sulfate), and concentrated. The resulting oil was purified by silica gel column chromatography (hexane–tetrahydrofuran–ethyl acetate) and distilled (80 °C (0.1 mm)) to give **15** (4.7 g (40%)) as an amber oil.

***exo*-2-[[[4-(2-Hydroxy-1-methylethyl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy]methyl]benzoic Acid (16).** A mixture of **15** (4.7 g, 0.0156 mol), ethylene glycol (55 mL), water (15 mL), and sodium hydroxide (4 g, 0.1 mol) was refluxed for 4.5 h, cooled, poured into water, and extracted with methylene chloride. The aqueous phase was acidified (6 N hydrochloric acid, 20 mL) and extracted twice with methylene chloride. The organic extract was washed with water, dried (magnesium sulfate), and distilled (80 °C (0.1 mm)) to give **16**: 3.4 g (68%); ^1H and ^{13}C NMR, ca. 50:50 mixture of diastereomers; MS (EI) m/z (% RA) 321 (M^+, 1), 302 (1), 185 (3), 168 (20), 153 (25), 135 (86), 110 (100), 43 (85); IR (CH_2Cl_2) 3600–2400 (OH, NH), 1694 (C=O), 1000–1200 (COC) cm^{-1}.

1-Hydroxy-α,α,4-trimethyl-3-cyclohexene-1-methanol (17). A mixture of terpinolene 4(8)-epoxide (**11**; 26 g, 0.17 mol) and 1% aqueous sulfuric acid (250 g) was stirred at ambient temperature (20 h) and then extracted with methylene chloride (4 × 100 mL). The combined extracts were washed with water (100 mL), dried (magnesium sulfate), concentrated, and Claisen distilled to collect the diol as an almost colorless oil: 21.2 g (78%); bp 78–81 °C (0.15 mm). Anal. Calcd: C, 70.6; H, 10.6. Found: C, 70.7; H, 10.6. MS (EI) m/z (% RA) 170 (M^+, <1), 155 (1), 152 (9), 137 (7), 111 (99), 93 (95), 59 (86), 43 (100).

***exo*-3-Hydroxy-α,α,4-trimethyl-7-oxabicyclo[2.2.1]-heptane-1-methanol (18).** A stirred solution of the diol **17** (46.9 g, 0.276 mol) in methylene chloride (400 mL) was treated with vanadium(IV) bis(2,4-pentanedionate) oxide (1.5 g) followed by *tert*-butyl hydroperoxide (29.0 g of 90%, 0.29 mol). When the exothermic reaction subsided, the mixture was refluxed (1 h), cooled, dried (magnesium sulfate), and filtered. The filtrate was treated with *p*-toluenesulfonic acid (1.75 g) in glyme (22 mL) over 15 min. After an additional 1 h at ambient temperature, the mixture was washed with dilute potassium carbonate solution, dried, and distilled (70 °C (0.2 mm)) to give crude **18** and **19** (43.5 g) in a ratio of 4:7. Crystallization from chilled acetone (50 mL) gave crude **18**: 11.3 g; mp 134–146 °C; 90% **18** and 10% **19** (GLC). Recrystallization from methylene chloride–pentane gave pure **18**, mp 141–146 °C. Anal. Calcd: C, 64.5; H, 9.7. Found: C, 64.4; H, 9.8. MS (EI) m/z (% RA) 186 (M^+, <1), 168 (1), 153 (7), 150 (2), 109 (11), 107 (24), 59 (46), 43 (100).

***exo*-2-[[[4-(1-Hydroxy-1-methylethyl)-1-methyl-7-oxabicyclo[2.2.1]hept-4-yl]oxy]methyl]benzenecarbonitrile (20).** Sodium hydride (1.25 g, 0.052 mol) was added in portions under nitrogen to a solution of crude **18** (9.3 g, 0.05 mol) in *N*,*N*-dimethylacetamide (100 mL) and the mixture stirred at ambient temperature for 2 h. After cooling in an ice bath, *o*-cyanobenzyl bromide (9.8 g, 0.050 mol) was added, giving a mild exothermic reaction. The mixture was stirred at ambient temperature (0.5 h), poured into water, and extracted with methylene chloride. The organic phase was washed with water, dried (magnesium sulfate), and concentrated (90 °C (0.1 mm)) to give crude **20**, 13.1 g. Kugelrohr distillation gave a center fraction (11.6 g) that upon crystallization from ether gave pure **20**: 8.9 g; mp 101–103 °C. Anal. Calcd: C, 71.7; H, 7.7. Found: C, 71.6; H, 7.5. MS (EI) m/z (% RA) 283 (M^+, <1), 268 (1), 243 (1), 185 (2), 167 (2), 116 (40), 17 (22), 59 (16), 43 (100); IR (KBr) 3501 (OH), 2224 (CN), 1000–1200 (COC) cm^{-1}.

***exo*-2-[[[4-(1-Hydroxy-1-methylethyl)-1-methyl-7-oxabicyclo[2.2.1]hept-4-yl]oxy]methyl]benzoic Acid (21).** A mixture of **20** (7.5 g, 0.025 mol) in ethylene glycol (80 mL) and sodium hydroxide (6 g, 0.15 mol) in water (20 mL) was refluxed (4 h) under a slow nitrogen sweep. The mixture was cooled, poured into water, and extracted with methylene chloride. The aqueous phase was acidified (6 N hydrochloric acid, 30 mL) to Congo Red and extracted twice with methylene chloride. The extracts were washed with water, dried (magnesium sulfate), and concentrated to give crude acid, 7.3 g. Crystallization from chilled ether gave pure **21**: 5.2 g; mp 144–148 °C. Anal. Calcd: C, 67.5; H, 7.5. Found: C, 67.9; H, 7.5. MS (EI) m/z (% RA) 302 ($M^+ - H_2O$, 1), 287 (8), 244 (26), 135 (86), 122 (70), 107 (53), 59 (31), 43 (100); IR (KBr) 3455 (OH), 1709 (C=O) cm^{-1}.

Phthalic acid (**22**) and *o*-toluic acid (**23**) were obtained from Aldrich Chemical Co. *o*-(Hydroxymethyl)benzoic acid lactone (**24**) was obtained from Pfaltz & Bauer, Inc. *o*-(Hydroxymethyl)benzoic acid (**25**) was prepared by solvolysis of **24** (0.1 mol) with sodium hydroxide (0.15 mol)–ethanol. Final product **25** (mp 118–119 °C) was recovered by ether extraction following acidification (6 N hydrochloric acid, 0.13 mol). MS (EI) m/z (% RA) 152 (M^+, 10), 134 (22), 123 (30), 105 (100), 89 (8), 79 (25), 77 (82), 63 (12), 51 (35).

Treatment of Animals. Male and female Fischer-344 albino rats (7 weeks old, 175–200 g each) were obtained from Simonsen's Laboratories, Gilroy, CA. Animals were fasted for 16 h and then administered a single oral dose of [^{14}C]cinmethylin. Two dosage levels, 15 and 450 mg/kg, were administered. The final specific activities of the [^{14}C]cinmethylin for the low and high dose studies were 4.7 and 0.16 mCi/mg, respectively. The labeled compound was formulated in propylene glycol. Control animals were treated with the carrier solvent only at the rate of approximately 2 mL/kg. Each treatment group consisted of five male and five female test animals. Treated animals were held individually in Nalgene plastic metabolism chambers (Sybron/Nalge Co.) that allowed the daily separate collection of urine and feces. Preliminary data had shown that no [^{14}C]carbon dioxide was detected in the respired air when the treated animals were maintained in all glass metabolism cages (Stanford Model MC 3000, Stanford Glass Blowing Co.). Animals were given free access to food (Purina rodent chow) and water. Under the experimental conditions of this study, there were no ob-

servable toxicological effects in the control and the [^{14}C]cinmethylin-treated animals.

Animals were sacrificed after elimination of 95% of the administered dose was reached or 7 days after treatment, whichever occurred first. The following tissues were collected: blood, lung, heart, liver, kidney, fat (inquinal), gonad, muscle (from hind leg), bone, brain, and spleen.

Additional treatment groups were used to determine the whole blood residue kinetics, and animals were sacrificed 48 h postdosing.

Analysis of Urinary Excreta. Individual daily urinary excreta from the control and treatment animals were adjusted to the final volume of 25 mL with 0.01 M phosphate buffer (pH 7.4). Triplicated 0.1-mL aliquots were sampled, and the total daily [^{14}C] residues were quantitatively analyzed by LSC.

For the quantitative and qualitative characterizations of the eliminated urinary metabolites, the pH of the combined 48-h urine excreta for each individual test animal was adjusted to pH 3 with 6 N hydrochloric acid and extracted three times with equal volumes of ethyl acetate. The organic extract was dried over anhydrous magnesium sulfate, concentrated, and analyzed by two-dimensional TLC.

Water-soluble conjugates remaining in the aqueous phase after the initial ethyl acetate extraction were analyzed after sequential enzyme and acid hydrolysis. Enzyme hydrolysis was carried out at pH 5, 35 °C for 12 h with β-glucuronidase enzyme (Sigma Chemical Co.). Following enzyme hydrolysis, acid hydrolysis was carried out at pH 1, 90 °C for 4 h. Radiolabeled materials released after enzyme and acid hydrolysis were recovered by solvent extraction and analyzed by two-dimensional TLC. Radioactivity remaining in the aqueous urinary excreta after the initial organic solvent extraction and enzyme and acid hydrolysis was considered as unextractable material and was not further qualitatively characterized.

Analysis of Urinary Excreta. Individual daily urinary excreta from the control and treatment animals were adjusted to the final volume of 25 mL with 0.01 M phosphate buffer (pH 7.4). Triplicated 0.1-mL aliquots were sampled, and the total daily [^{14}C] residues were quantitatively analyzed by LSC.

The combined 48-h fecal excreta for each individual test animal was extracted three times with 30 mL of acetone. The combined acetone extract was concentrated to approximately 15 mL, and the final volume was readjusted to 30 mL with 0.01 M phosphate buffer, pH 7.4. Solid fecal material, after the initial acetone extraction, was further extracted twice with 30 mL of aqueous pH 7.4 buffer solution. [^{14}C] residues remaining with the solid fecal materials after the acetone and aqueous buffer extractions were considered as unextractable residues, and their chemical nature was not further characterized.

The concentrated acetone extract and the aqueous buffered fecal extract (after adjustment to pH 3) were combined and partitioned with three 40-mL portions of ethyl acetate. Organic extracts were dried over anhydrous magnesium sulfate, concentrated, and analyzed by two-dimensional TLC.

The aqueous phase of the fecal extract, after the initial ethyl acetate solvent partitioning, was subjected to the sequential enzyme and acid hydrolysis as described above. Radiolabeled materials released after enzyme and acid hydrolysis were recovered by solvent extraction and analyzed by two-dimensional TLC.

Determination of Whole Blood Residues Kinetics. Whole blood samples (70 μL), anticoagulated with 5 USP units of ammonium heparin, were collected from the lateral tail vein of each test animal (restrained in a plastic tube) in microcapillary tubes attached to a disposable 25-gauge needle. This procedure was rapid and kept stress to the test animal to a minimum (Stearns and Lee, 1984). Each animal served as its own control.

Blood samples were transferred immediately to scintillation vials that contained 1.0 mL of the NCS tissue solubilizer (Amersham). Hydrogen peroxide (30%, 0.1 mL) and acetic acid (25 μL) were added to the solubilized blood samples for the purpose of bleaching the released hemoglobin and to reduce quenching due to chemiluminescence. The above mixture was allowed to stand at 4 °C in darkness for 12 h before the addition of 15 mL of Aquasol-2 counting solution (New England Nuclear). Scintillation samples were stored under refrigeration for at least 48 h prior to LSC quantitation.

Characterization of Liver Residues. The chemical nature and the distribution pattern of [^{14}C]cinmethylin equivalent residues in the male and female liver tissues 48 h after single oral 15-mg/kg dosing were examined.

The combined male, or female, liver tissues were homogenized in 200 mL of 0.01 M phosphate buffer, pH 7.4. Tissue debris (membranes, connective tissues, etc.) were removed by centrifugation. The proteinaceous materials in the liver homogenate were precipitated by the addition of 5% trichloroacetic acid. This mixture was allowed to stand overnight at room temperature. Precipitated protein was separated by centrifugation and dissolved in the tissue solubilizer prior to LSC quantitation.

Organic-extractable liver residues were examined by TLC after being recovered by the repeated partitioning of the aqueous liver homogenate with equal volumes of chloroform. Radioactivity remaining in the aqueous phase after chloroform extraction was subjected to acid hydrolysis at pH 1, 85 °C for 2 h. Released [^{14}C] residues were recovered by chloroform extraction and analyzed by TLC.

Chromatography and Radioassay. Radioactivity was quantitated in 15 mL of Aquasol-2 scintillation solution in Packard Models 2660 or 300 liquid scintillation systems. Radioactive areas of the TLC plate, after solvent development and autoradiography, were removed by scraping and analyzed in an Aquasol-2–water (11:4) gel system. Radioactive residues associated with the fecal excreta and tissues were analyzed by combusting subsamples (approximately 100 mg) in a Packard Model 306B sample oxidizer. Background and combustion efficiency were determined by using control animals and calibrated [^{14}C]cinmethylin solution as the internal standard. Oxidizer counting solution included Carbo-Sorb and Permafluor V (Packard Instrument Co.), 10:12 mixture. All LSC quantitations were corrected for combustion efficiency and quenching. Excreted radiocarbon was expressed as percent of the administered dose, and tissue residues are given as parts per million (ppm) [^{14}C]cinmethylin equivalents on the basis of tissue wet weight.

The [^{14}C] residues recovered from the urine and feces were analyzed by two-dimensional TLC (silica gel F-254, 0.25 mm, E. Merck). The TLC R_f values of cinmethylin and reference standards in several solvent systems are presented in Table I. The [^{14}C] residues on the TLC plate were visualized by autoradiography on Kodak SB-5 single-coated X-ray film.

Radio–gas–liquid chromatography (RGLC) was carried out on a Varian 1440 gas–liquid chromatograph equipped with a flame-ionization detector and a Packard Model 894 gas-proportional counter. The column used was a 1 m ×

Table I. R_f Values of Cinmethylin (1) and Its Degradation Products on Silica Gel F-254 Plates Using Three Solvent Systems[a]

	R_f		
	system 1	system 2	system 3
cinmethylin (1)	0.69	0.87	0.94
o-toluic acid (23)	0.43	0.68	0.80
α-hydroxycinmethylin (6)	0.43	0.60	0.77
α-carboxycinmethylin (9)	0.46	0.64	0.82
8-hydroxy-α-carboxycinmethylin (21)	0.36	0.51	0.38
9-hydroxy-α-carboxycinmethylin (16)	0.34	0.41	0.32
o-(hydroxymethyl)benzoic acid (25)	0.29	0.32	0.30
o-(hydroxymethyl)benzoic acid lactone (24)	0.55	0.45	0.79
phthalic acid (22)	0.18	0.15	0.10

[a] Key: solvent system 1, toluene–2-propanol–acetic acid (150:20:1.5); solvent system 2, hexane–2-propanol–acetic acid (120:30:1); solvent system 3, ethyl acetate–hexane–chloroform–acetic acid (100:50:5:1.5).

and helium flow rates were 210, 30, and 30 mL/min, respectively.

GC mass spectroscopy was carried out on a Finnigan 1020 and an HP-5992B mass spectrometer.

RESULTS AND DISCUSSION

Elimination Rates. [^{14}C]Carbon dioxide or volatile metabolites were not detected in the respired air of the treated animal following a single oral dose of [^{14}C]cinmethylin. A majority of the administered radioactivity was eliminated in the urine and feces during the initial 48 h postdosing. Urinary excretion was the major route of elimination and accounted for approximately 60% of the administered dose. Elimination data are summarized in Table II. There was no apparent quantitative difference between the elimination rates of the male and female test animals when administered low (15 mg/kg) or high (450 mg/kg) dosages.

Distribution Profile of Urinary Degradation Products. Undegraded [^{14}C]cinmethylin was not detected in the urinary excreta. Ten degradation products were detected (Figure 2). Summaries of the metabolite distribution data are presented in Table III. The primary metabolic pathway of [^{14}C]cinmethylin involved oxidation at the benzyl and cineole portion of the parent molecule, followed by conjugation with glucuronic acid or glycine. Cleavage of the ether linkage was also observed. Metabolic products were characterized as organic extractable, and conjugates were recovered after enzyme (β-glucuronidase) and acid hydrolysis. No attempt was carried out to elucidate the chemical nature of the intact conjugates.

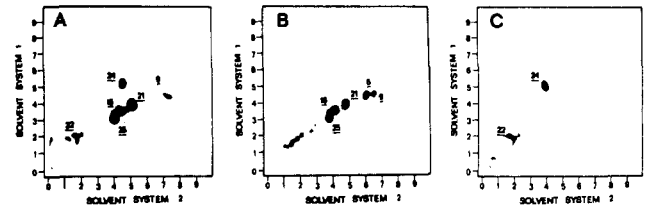

Figure 2. Two-dimensional thin-layer autoradiograms: (A) organic-extractable urinary metabolites from a male rate, the low-dose treatment group; (B) conjugates recovered after enzyme hydrolysis from a female rat, the low-dose treatment group; (C) conjugates recovered after acid hydrolysis from a female rat, the low-dose treatment group.

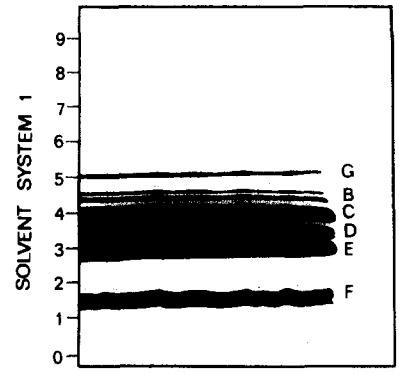

Figure 3. Preparative TLC autoradiogram of the organic-extractable metabolites from the combined male urine, the low-dose treatment group. Radioactive band A is assigned to the undegraded [^{14}C]cinmethylin which was recovered only in the fecal excreta. [^{14}C]Cinmethylin was not detected in the urine.

Separation of urinary metabolites was carried out by preparative TLC using solvent system 1 as the initial developing solvent (Figure 3). Isolated metabolites required additional TLC separation in other solvent system combinations prior to being examined by chromatographic and spectroscopic techniques. Confirmation of structure was obtained by the direct comparison with an authentic standard.

Preparative TLC using solvent systems 2 and 3 separated three major products from the radioactive band B (see Figure 3). o-Toluic acid (**23**), detected in the high-dose study only, was identified by GC–MS directly [MS (EI) m/z (% RA) 136 (M^+, 72), 119 (22), 118 (100), 91 (92), 90 (90), 89 (30), 77 (30), 63 (40)] and after trimethylsilylation using BSTFA reagent [MS (EI) m/z (% RA) 208 (M^+, 35), 194 (100), 149 (40), 119 (85), 91 (43)]. α-Hydroxycinmethylin (**6**) was identified by GC–MS directly and also after trimethylsilylation [MS (EI) m/z (% RA) 362 (M^+ <1), 272 (3), 229 (5), 193 (45), 169 (10), 153 (10), 123 (40),

Table II. Summary of the Daily Elimination Rate of [^{14}C]Cinmethylin Equivalent Radioactivity in the Urinary and Fecal Excreta

	percent of administered radioactivity[a]							
	15 mg/kg				450 mg/kg			
	male		female		male		female	
day	urine	feces	urine	feces	urine	feces	urine	feces
1	47.2 ± 3.4	28.8 ± 8.7	51.1 ± 3.2	22.7 ± 5.3	44.5 ± 4.1	20.4 ± 6.8	52.5 ± 4.6	16.3 ± 5.1
2	3.3 ± 1.6	10.5 ± 4.8	4.4 ± 0.8	10.6 ± 2.3	3.4 ± 0.8	8.4 ± 1.8	7.9 ± 2.3	8.6 ± 1.2
3	0.7 ± 0.4	2.6 ± 1.5	1.3 ± 0.3	2.9 ± 0.8	0.9 ± 0.3	1.7 ± 0.4	1.2 ± 0.3	2.2 ± 0.6
4			0.4 ± 0.1	1.1 ± 0.3	0.4 ± 0.1	0.7 ± 0.2	0.4 ± 0.1	0.5 ± 0.1
5					0.1 ± 0.1	0.2 ± 0.1	0.2	0.2 ± 0.1
6					0.1	0.1	0.1	0.1
7					0.1 ± 0.1	0.1 ± 0.1	0.1	0.1 ± 0.1
total	51.2 ± 4.1	41.9 ± 3.0	57.2 ± 4.1	37.3 ± 3.5	49.5 ± 4.5	31.6 ± 4.6	62.4 ± 3.2	28.0 ± 3.3

[a] Mean ± standard deviation (number of animals, 5).

Table III. Distribution of [^{14}C]Cinmethylin Urinary Degradation Products in the Urine of the Male and Female Test Animals following a Low (15 mg/kg) and High (450 mg/kg) Oral Dose Administration

	percent of administered dose			
	15 mg/kg		450 mg/kg	
	male	female	male	female
organic extractable	45.6[a]	25.6	10.2	8.5
6		2.2	0.5	0.3
9	0.8	0.4	0.4	1.3
21	16.9	14.5	5.9	3.9
16	7.0	6.5	2.3	1.5
25	8.9		0.5	0.5
24	6.4	1.0	0.2	0.3
22	3.3			
28			0.3	0.4
conjugates[b]	4.9	30.2	38.6	53.2
6		1.2	0.8	1.2
9			0.6	0.9
21		5.7	7.4	8.7
16		4.7	4.8	3.8
25	1.0	3.8	4.4	1.9
24		3.3	6.3	11.2
22	0.8	4.4		
28			2.5	1.2
unextractable	2.2	3.5	9.7	21.2
total	50.5	55.8	48.8	61.7

[a] Average of the five test animals. [b] Conjugates recovered after enzyme and acid hydrolysis.

107 (35), 73 (55), 43 (100)]. α-Carboxycinmethylin (**9**) was identified by GC–MS directly and also after trimethylsilylation [MS (EI) m/z (% RA) 376 (M^+, 2), 333 (<1), 286 (10), 223 (10), 207 (60), 169 (15), 153 (5), 133 (32), 123 (23), 107 (20), 90 (18), 73 (65), 43 (100)].

8-Hydroxy-α-carboxycinmethylin (**21**) was the major product recovered from radioactive band C. **21** required trimethylsilylation prior to GC–MS analysis. Its mass spectrum is consistent with that of the Me_3Si derivative of the authentic standard [MS (EI) m/z (% RA) 464 (M^+, <1), 257 (2), 223 (3), 207 (100), 167 (3), 131 (25), 90 (2), 73 (45), 43 (15)].

By monitoring the fragment ion m/z 167 (hydroxylated cineole ring structure) or 169 (intact cineole ring structure), the ion chromatograms of the Me_3Si derivative of radioactive band D showed the presence of three related components. The EI mass spectra of the Me_3Si derivatives of two of the three components were identical and were consistent with the authentic standard of the two corresponding diastereomers of 9- and 10-hydroxy-α-carboxycinmethylin (**16**) [MS (EI) m/z (% RA) 464 (M^+, <1), 374 (2), 257 (10), 223 (25), 207 (100), 167 (35), 133 (30), 103 (25), 90 (5), 73 (65)]. The fragment ion at m/z 167 [attained from m/z 257–90 (silyl alcohol)] was an indication of a hydroxy group at the isopropyl moiety of the cineole portion of the molecule. The molecular ion of the Me_3Si derivative of the third component was also at m/z 464 (<1). Fragment ions at m/z (% RA) 257 (8), 207 (100), 169 (15), 90 (2), and 73 (35) suggested this metabolite contained a carboxylic acid moiety in the aromatic portion and a hydroxyl group in the cineole ring of the molecule. A fragment ion at m/z 169 instead of m/z 167 indicated the hydroxyl group is not in the isopropyl moiety but rather in the cineole ring. There is insufficient data to elucidate the precise location of the cineole hydroxyl moiety in the third component. These three metabolites were combined and designated as **16** throughout this study.

o-(Hydroxymethyl)benzoic acid lactone (**24**) was the major component in the radioactive band G. The EI mass spectrum of this compound [MS (EI) m/z (% RA) 134 (M^+, 45), 105 (100), 77 (48), 51 (20)] is consistent with that of the authentic standard.

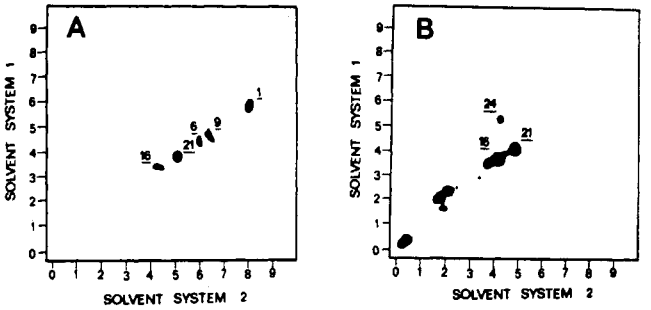

Figure 4. Two-dimensional TLC autoradiograms: (A) organic-extractable fecal metabolites from a male rat, the high-dose treatment group; (B) conjugates recovered after enzyme hydrolysis from a male rat, the high-dose treatment group.

A two-dimensional TLC autoradiogram of the isolated radioactive band E, after the initial preparative TLC separation (see Figure 3), showed the rapid transformation of this metabolite to **24**. Extensive conversion to **24** was also observed during direct GC–MS analysis. The Me_3Si derivative of this metabolite [MS (EI) m/z (% RA) 296 (M^+, 30), 281 (28), 206 (100), 177 (20), 163 (18), 147 (90), 90 (15), 73 (85)] is consistent with the Me_3Si derivative of an o-(hydroxymethyl)benzoic acid (**25**) authentic standard. Previous examples of the cyclization or lactonization of the γ-hydroxycarboxylic acid metabolites, as potential artifacts during sample workup, had been reported in the metabolism studies with the pyrethroid insecticides permethrin (Gaugham et al., 1977) and fenvalerate (Ohkawa et al., 1979).

In the low-dose study, phthalic acid (**22**) was the major component identified in the radioactive band F. The EI mass spectra of the Me_3Si derivative of the isolated metabolite and the authentic standard are consistent [MS (EI) m/z (% RA) 310 (M^+, <1), 295 (9), 147 (82), 73 (100), 45 (30)].

In the high-dose study, N-(2-methylbenzoyl)glycine (**28**), the glycine conjugate of o-toluic acid (**23**), was the major component isolated from radioactive band F. **28** was identified by GC–MS after trimethylsilylation [Me_3Si derivative MS (EI) m/z (% RA) 265 (M^+, 35), 250 (20), 220 (18), 206 (40), 175 (15), 119 (100), 91 (40), 73 (40); $(Me_3Si)_2$ derivative MS (EI) m/z (% RA) 337 (M^+, 15), 322 (45), 290 (20), 265 (10), 250 (5), 220 (25), 206 (15), 119 (100), 91 (45), 73 (40)]. A sample of the isolated **28** was also analyzed directly by fast atom bombardment mass spectrometry (FAB-MS) on the VGZAB2F mass spectrometer. The FAB mass spectrum showed the presence of two major ions m/z 119 (2-methylbenzoyl fragment) and m/z 194 (M + H) that are consistent with the proposed structure of **28**. The elemental composition of the M + H ion was found to be N, O_3, C_{10}, H_{12} (exact mass: found, 194.0800; calcd, 194.0817).

A majority of the identified urinary metabolites were detected as both organic-extractable (Figure 2A) and as water-soluble conjugates recovered after enzyme hydrolysis (Figure 2B). o-(Hydroxymethyl)benzoic acid lactone (**24**), the decomposition product of o-(hydroxymethyl)benzoic acid (**25**), and phthalic acid (**22**) were the predominant products recovered from the urine excreta (from the low-dose study) after acid hydrolysis (Figure 2C). **24** was the only product observed in the high-dose study.

Distribution Profile of Fecal Degradation Products. The distribution profile of [^{14}C]cinmethylin fecal metabolites is illustrated in Figure 4. Summaries of the metabolite distribution data are presented in Table IV. In

Table IV. Distribution of [^{14}C]Cinmethylin Fecal Degradation Products in the Feces of the Male and Female Test Animals following a Low- (15 mg/kg) and High- (450 mg/kg) Dose Administration

	percent of administered dose			
	15 mg/kg		450 mg/kg	
	male	female	male	female
organic extractable	16.7[a]	14.3	17.2	14.6
1	3.8	2.2	2.6	7.4
9	1.4	0.9	1.8	1.7
6	5.3	2.6	2.7	1.7
21	2.9	2.5	4.3	1.4
16	2.0	2.4	3.5	1.3
others[b]	1.3	1.9	2.3	1.2
conjugates	11.2	6.7	9.3	9.7
21	2.7	1.2	2.1	1.4
16	2.6	1.5	1.6	1.6
22	1.4	1.0	1.2	1.6
other[b,c]	4.5	3.0	4.4	5.1
solid unextractable residues	11.3	11.3	4.0	2.7
total	39.2	32.3	30.5	27.0

[a] Average of the five test animals. [b] Including radioactivity associated with the origin of the TLC plate and minor products. [c] Including unextractable radioactivity in the aqueous phase after enzyme and acid hydrolysis.

Table V. Summary of the [^{14}C] Residue Level in the Various Organ Tissues

	[^{14}C]cinmethylin equiv, ppm			
	15 mg/kg		450 mg/kg	
	male	female	male	female
blood	0.17 ± 0.01[a]	0.10 ± 0.01	5.26 ± 0.86	4.99 ± 0.41
lung	<0.04	<0.04	1.84 ± 0.39	1.34 ± 0.14
heart	<0.04	<0.04	<1.20	<1.20
liver	0.97 ± 0.13	0.48 ± 0.06	9.19 ± 1.75	10.37 ± 2.33
kidney	0.12 ± 0.01	0.08 ± 0.01	1.93 ± 0.28	2.18 ± 0.23
fat	0.06 ± 0.03	0.09 ± 0.03	<1.20	2.00 ± 0.32
gonad	<0.04	<0.04	<1.20	<1.20
muscle	<0.04	<0.04	<1.20	<1.20
bone	<0.04	<0.04	<1.20	<1.20
brain	<0.04	<0.04	<1.20	<1.20
spleen	0.06 ± 0.01	0.05 ± 0.01	2.06 ± 0.46	3.16 ± 0.39

[a] Mean ± standard deviation (number of animals, 5).

addition to the undegraded [^{14}C]cinmethylin, six major degradation products were detected as organic-extractable and as conjugate products. These had been isolated and identified previously as urinary metabolites. [^{14}C] residues remaining in the solid fecal materials after acetone and aqueous extraction were considered as unextractable bound residues.

Tissue Residue Distribution Profile. The distribution of [^{14}C]cinmethylin equivalent residues (ppm) in the various organ tissues is summarized in Table V. The levels of [^{14}C] residues observed in most of the tissues examined were either slightly above or below the limit of detection (0.04 and 1.20 ppm for the low- and high-dose study, respectively). Based on tissue to blood residue ratio, a significant level of residues was detected only in the liver. An apparent twofold difference in residue level was observed between the male and female test animals in the low-dose study; however, this difference was not evident in the high-dose study.

Characterization of Liver [^{14}C] Residues. The combined male, or female, liver homogenates (48 h postdosing) contained approximately 1 and 0.5 ppm, respectively, of the total [^{14}]cinmethylin equivalent residues. Distribution data presented in Table VI showed approximately 50 and 40% of the total [^{14}C] residues that could be recovered by the organic solvent extraction and in the tissue debris/precipitates, respectively. Further methanol washing of the tissue pellets did not release detectable radioactivity.

Table VI. Chemical Nature and Distribution Pattern of [^{14}C]Cinmethylin Equivalent Residues in the Male and Female Rat Livers 48 h after a Single Oral Dose of 15 mg/kg

	percent of recd radioact	
	male	female
tissue homogenate	81.7	69.6
organic extractable	56.6	38.5
6	1.8	8.3
21	10.8	4.6
16	38.3	13.4
23	1.6	2.8
others[a]	4.1	9.4
water soluble	6.7	11.8
protein precipitate	18.4	19.3
tissue debris	18.3	30.4
total [^{14}C] residues, ppm	2.13	1.89

[a] Minor degradation products.

Table VII. Pharmacokinetic Parameters of [^{14}C]Cinmethylin following a Single Oral Dose Treatment[a]

dose, mg/kg	sex	C_p, μg/mL	elimin k_e, h^{-1}	abs k_a, h^{-1}	half-life $t_{1/2}$, h
15	M	1.5 ± 0.1	0.062 ± 0.009		11
	F	2.0 ± 0.3	0.051 ± 0.019		14
450	M	63 ± 13	0.065 ± 0.016	0.46 ± 0.11	11
	F	76 ± 39	0.077 ± 0.030	0.30 ± 0.13	9

[a] $C_t = C_p[e^{-k_e t} - e^{-k_a t}]$, where C_t = concentration of [^{14}C]cinmethylin equivalent residues at time t; C_p = hypothetical concentration at time 0; k_e = overall elimination rate constant; k_a = absorption rate constant.

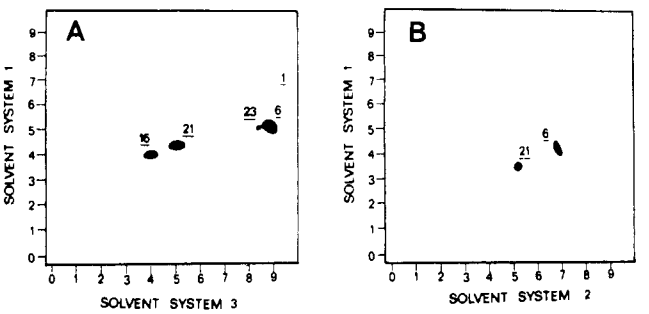

Figure 5. Two-dimensional TLC autoradiograms: (A) organic-extractable liver metabolites from the female rats, 48 h following a low-dose treatment; (B) organic-extractable metabolites in the plasma of the female rats 1 h following a low-dose treatment.

TLC analysis of the organic-extractable residues did not show any significant qualitative or quantitative differences between the male and female test animals. A trace of the undegraded [^{14}C]cinmethylin was detected (less than 1% of the total liver residue); **6**, **21**, and **16** were recovered as the major liver metabolites (Figure 5A). *o*-Toluic acid (**23**) was observed as a minor component.

Pharmacokinetic Assessment. [^{14}C]Cinmethylin was rapidly eliminated and extensively metabolized following oral administration. Pharmacokinetic parameters, which described the disposition of [^{14}C]cinmethylin, were calculated by the one-compartment model and are presented in Table VII. Semilogarithmic plots of the total [^{14}C] residue concentration against time are shown in Figure 6. Except for the low-dose female treatment group, there is an apparent excellent fit of the blood residue data by the one compartment model.

The absorption of [^{14}C]cinmethylin was rapid and reached peak concentration, 1.5–2 ppm for the low-dose study and 36–39 ppm for the high-dose study, within the

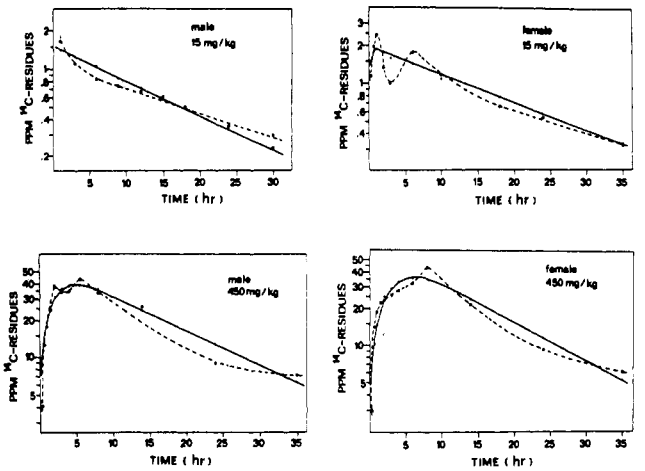

Figure 6. Semilogarithmic graphs of the whole blood residues time course in the male and female rats following the low- and high-dose treatment: ---, experimental data; —, best fit curve.

first and 4–6 h postdosing, respectively. Because of the unexpected rapid absorption in the low-dose study, insufficient blood samples were obtained during the initial absorption phase to allow an accurate calculation of the absorption rate constant (k_a). However, the calculated k_a values for the male/female test animals from the high-dose study were 0.38 ± 0.13 and 0.46 ± 0.11 h^{-1}, respectively. The estimated absorption half-lives were approximately 1.5 h postdosing.

Bile recycling occurred in the low-dose female treatment group as evidenced by the presence of the irregularly space peak in the declining part of the whole blood residue curve. There is no major difference in the overall disposition pattern between the male and female test animals in the high-dose study. The blood residue/time plots between the two sexes were consistent. The overall elimination rate constants (k_e) between the low- and the high-dose studies were comparable, thus indicating that the disposition of [^{14}C]cinmethylin was linear within these two dosage levels. The calculated C_p (1.8 vs. 65 ppm) reflected the 30-fold difference between the high- and low-dose study (15 vs. 450 mg/kg).

Characterization of Whole Blood [^{14}C] Residues. An experiment was carried out to characterize the chemical nature of [^{14}C]cinmethylin equivalent residues in the whole blood sample. Two female test animals were administered a single oral dose of [^{14}C]cinmethylin (15 mg/kg) and were sacrificed 1 and 12 h postdosing. Total [^{14}C] residues in the 1- and 12-h whole blood samples were approximately 2.1 and 1.1 ppm, respectively. A majority of the [^{14}C] residues, 75% of the recovered radioactivity, was found to be associated with the plasma fraction (Table VIII). The binding characteristics of the [^{14}C] residues to the plasma proteins were further evaluated by the protein precipitation procedure using 2% trichloroacetic acid. It is apparent that the binding of [^{14}C]cinmethylin equivalent residues to the plasma protein was not extensive and accounted for less than 30% of the recovered radioactivity.

A majority of the [^{14}C] residues in the plasma after protein precipitation could be recovered by the ethyl acetate solvent extraction. In addition to a trace of [^{14}C]cinmethylin (1), approximately 5% of the total whole blood residue, a TLC autoradiogram presented in Figure 5B showed **6** and **21** as major metabolic products. The formation and the detection of these products and the absence of a significant amount of cinmethylin in the circulation system at 1 h postdosing further indicated the rapid and extensive metabolism of [^{14}C]cinmethylin.

Table VIII. Fractionation and Distribution of [^{14}C]Cinmethylin Equivalent Residues in the Female Whole Blood Samples 1 and 12 h following a Low-Dose Treatment

	percent recd radioact	
	1 h	12 h[a]
red blood cell	28.4	24.8
plasma	71.6	75.2
unbound	54.3	47.5
6	20.4	
21	10.9	
others[b]	26.8	
bound	17.3	27.7

[a] Qualitative examination of the 12-h blood samples was not conducted. [b] Including minor metabolites and radioactivity remaining in the aqueous fraction after solvent extraction.

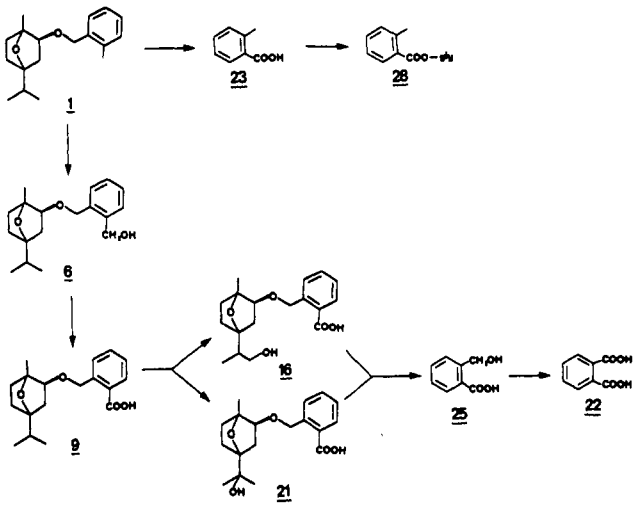

Figure 7. Proposed primary metabolic pathways of [^{14}C]cinmethylin (1) in rats following an oral dose administration.

CONCLUSION

Whole blood residue kinetics and elimination rate data showed the rapid disposition of [^{14}C]cinmethylin (1), a novel cineole compound, in laboratory rats. The extensive metabolic degradation of 1 was illustrated by the complexity of degradation products recovered in the excreta. A proposed metabolic scheme for cinmethylin is presented in Figure 7. The primary metabolic pathway involved hydroxylation and oxidation at the 2-methylbenzyl moiety to yield **6** and **9**, which were subsequently hydroxylated at the cineole portion of the molecule to yield **16** and **21**. Hydroxylation of the isopropyl moiety of 1 is consistent with the metabolism of other structurally related compounds such as *p*-cymeme (Walde et al., 1983) and 1,8-cineole (Southwell and Flynn, 1980). Hydroxylation on the cineole ring also occurred, but the precise location of the hydroxyl group could not be determined. Aromatic ring hydroxylation products were not detected at a significant level. The metabolism of 1 was rapid, since **6** and **21** were the major products recovered in the blood sample as early as 1 h postdosing.

Ether cleavage, a secondary metabolic reaction, occurred with the parent molecule (1) to yield *o*-toluic acid (**23**), which subsequently conjugated with an amino acid (glycine, **28**). This reaction predominated when test animals were under the high-dose treatment.

Ether cleavage also occurred on the major hydroxylated/oxidized metabolic products (**6**, **9**, **16**, **21**) to yield *o*-(hydroxymethyl)benzoic acid (**25**) and phthalic acid (**22**), which were subsequently conjugated with glucuronic acid. This reaction predominated when test animals were under

2 mm i.d. glass column packed with 3% OV-101 on 80/100-mesh Supelcoport (Supelco Co.). The air, hydrogen, the high-dose treatment. A dose-related difference showed the majority of the urinary metabolites were recovered as conjugated products under the high-dose treatment.

A significant sex-related metabolic difference was observed under the low-dose treatment. A majority of the urinary metabolites from the female rats were conjugated products, 30% of the administered dose, compared to the 5% observed in the male rats. A similar sex-related difference was also observed in the whole blood residue/time plot, which showed the contribution of biliary recirculation in the disposition of cinmethylin in the female test animals.

ACKNOWLEDGMENT

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Registry No. (±)-**1**, 87818-31-3; (±)-[U-*phenyl*-^{14}C]-**1**, 99827-45-9; (±)-**2**, 87172-89-2; (±)-**3**, 28219-82-1; (±)-**4**, 96612-51-0; (±)-**5**, 99765-52-3; (±)-**6**, 99765-53-4; (±)-**7**, 87819-11-2; (±)-**8**, 87819-12-3; (±)-**9**, 87819-14-5; **10**, 99783-11-6; (±)-**11**, 54605-40-2; (±)-**12**, 87128-93-6; (±)-**13**, 99765-54-5; (±)-**14**, 99765-55-6; **15**, 99765-56-7; (±)-**16** (isomer 1), 99765-57-8; (±)-**16** (isomer 2), 99827-44-8; (±)-**17**, 54649-49-9; (±)-**18**, 87129-26-8; **19**, 99765-58-9; (±)-**20**, 99765-59-0; **21**, 99765-60-3; **22**, 88-99-3; **23**, 118-90-1; **24**, 87-41-2; **25**, 612-20-4; **28**, 42013-20-7; [U-*phenyl*-^{14}C]-$MeC_6H_4CH_2Cl$-*o*, 99765-61-4; *o*-$MeC_6H_4CHCl_2$, 60973-59-3; *o*-$NCC_6H_4CH_2Br$, 22115-41-9.

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Fate of [^{15}N]Glycine in Peat as Determined by ^{13}C and ^{15}N CP-MAS NMR Spectroscopy

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Peat samples, nonsterile, sterilized by γ irradiation or autoclaving, were incubated with [^{15}N]glycine for a period of 6 months. The ^{13}C NMR data showed the established trend of increased humification with decreasing particle size and that autoclaving had significantly disturbed the humification–particle size distribution. The ^{15}N CP-MAS NMR spectra showed the presence of [^{15}N]glycine in all fractions after incubation. $^{15}NH_4^+$, a result of either biological or chemical deamination, was one of the main products in the nonsterile peat series. The ^{15}N spectra also showed resonances corresponding to amine, secondary amide, and pyrrole-type nitrogen and the presence of glycine derivatives and melanoidins. The results presented give the first spectroscopic evidence of the possible involvement of the Maillard reaction in the humification process.

INTRODUCTION

Recent studies (Benzing-Purdie and Ripmeester, 1983; Benzing-Purdie et al., 1983) based on ^{13}C and ^{15}N CP-MAS NMR have shown that natural humic substances resemble very closely melanoidins, the brown, high molecular weight nitrogenous polymers formed upon reaction of carbohydrates and amino acids. These studies lent further support to Maillard's original hypothesis; that this reaction may be at the origin of humic substances in soils (Maillard, 1916).

The objective of the present study is to determine the extent to which this reaction occurs in highly organic peat soils. Natural humic substances take a very long time to form and normally only a very small proportion of any substrate remains after attack by soil organisms. Testing Maillard's hypothesis in a soil environment within a manageable time span therefore requires sterile soil conditions. Unfortunately, all methods of sterilization lead to physical and chemical alterations in the soil (Ramsay and Bawden, 1983). After any sterilization treatment, be it chemical, steam sterilization or γ irradiation, the treated soil will differ from the original. Autoclaving of peat not only damages the physical microenvironment but, due to a combination of high temperature and low pH, results in hydrolysis of organic polymers, particularly polysaccharides, liberating the more labile sugars, e.g. pentoses, which are very reactive in the Maillard reaction. Its one advantage is that it completely destroys enzymatic activity. γ irradiation has been reported to cause the minimum disturbance to the system (Ramsay and Bawden, 1983), although it has been shown to release some nutrients (Brown, 1981; Lynch, 1982; Powlson and Jenkinson, 1976) and certain metabolic activities linked to dead cells and residual extracellular enzymes may still be present. Advantage was taken of the known reactivity of glycine in the Maillard reaction and the data available on the different forms of C and N produced in the reaction of this amino acid with carbohydrates (Benzing-Purdie and Ripmeester,

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