Metabolic Fate of Cinmethylin in Goat

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The metabolic fate of [*phenyl*-^{14}C]cinmethylin (1), a novel cineole herbicide, in a lactating goat was examined. The test animal was administered four consecutive daily doses equivalent to approximately 100 ppm cinmethylin in the daily diet. The animal was sacrificed 6 h after the last dosing. A rapid and extensive metabolism of cinmethylin was observed. The major route of elimination was via urine: 40% of the administered dose and feces (20%). [^{14}C]Carbon dioxide or radioactive material in the respired air and residual radioactivity in the digestive tract were not monitored. A complex degradation pattern in the excreta and liver tissue was observed. In addition to the undegraded cinmethylin, recovered only in the feces, at least 25 metabolites were isolated and identified as both organic-extractable and conjugated products. They were classified as mono-, di-, trihydroxylated, dehydrated, carboxylated, methoxylated, and ether linkage cleavage products. The level of ^{14}C residues in the milk and tissues was low and combined to account for less than 1% of the administered radioactivity.

Cinmethylin (1), the 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 developed by Du Pont. 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 with an oral and dermal LD_{50} of 4.5 g/kg in rats. The metabolic fate of [*phenyl*-^{14}C]cinmethylin in laboratory rats following a single oral dose has been reported (Lee et al., 1986, 1988). A rapid elimination and the extensive metabolism of the administered dose were observed. At least 10 metabolites were identified that resulted from hydroxylation and/or oxidation at the benzyl and cineole portions of the parent molecule or the cleavage of the ether linkage. In order to further the understanding of the comparative metabolism of this novel compound, this report describes the metabolic fate of cinmethylin in the goat following oral dosing.

EXPERIMENTAL SECTION

Test Materials and Reference Standards. Radiolabeled [*phenyl*-^{14}C]cinmethylin and appropriate reference standards were synthesized at the Biological Sciences Research Center, Shell Agricultural Chemical Co. The specific activity and radiochemical purity of the test material were 4.1 μCi/mg and greater than 98%, respectively.

Animal Treatment. One female Alpine goat (42 kg) was administered with four consecutive daily doses of [^{14}C]cinmethylin after each morning milking via oral capsule intubation. Each gelatin capsule contained 204 mg of [^{14}C]cinmethylin. The treated animal was exposed to an equivalent of 4.9 mg/kg of [^{14}C]cinmethylin or approximately 102 ppm test material in the daily diet. This exaggerated dose level represents greater than 1000-fold the maximum dietary intake of cinmethylin residues by the ruminant animals. The dosed animal was maintained in an elevated metal metabolism stall that allowed for the separate collection of urine and feces. Radioactivity in the respired air was not monitored in this study. Feed ration (1 kg) consisted of 0.4 kg of grain and 0.6 kg of roughage and was offered in the morning and afternoon. The amount of leftover ration was weighed and replaced with fresh diet prior to the next feeding. Water and feed were provided ad libitum. Milk samples were collected twice daily, and the urine and feces were sampled after the morning milking.

The test animal was sacrificed 6 h after the final dose. Samples of the following were collected: (1) blood, (2) mesenteric fat, (3) subcutaneous fat, (4) muscle (front and hind leg), (5) liver, and (6) kidney. All samples (milk, excreta, tissues) were placed in plastic jars and frozen (−20 °C) prior to preparation for radioassay.

Analysis of Urine. The combined urine sample was adjusted to pH 3 with 6 N hydrochloric acid and partitioned three times with 250 mL of chloroform. Water-soluble conjugates were subjected to enzyme and acid hydrolysis. Enzyme hydrolysis was carried out at pH 5, 35 °C, for 16 h with β-glucuronidase enzyme (Sigma). Radioactivity released after enzyme hydrolysis was recovered by partitioning of the aqueous phase (after adjustment to pH 3 with HCl) three times with equal volumes of chloroform. Organic extracts were dried over anhydrous magnesium sulfate, concentrated, and analyzed by two-dimensional TLC.

Radioactivity remaining in the aqueous phase after enzyme hydrolysis was subjected to further acid treatment at pH 1, 80 °C, for 2 h. Radioactivity released by the acid hydrolysis was recovered by chloroform solvent partitioning as described above and analyzed by two-dimensional TLC.

Radioactivity remaining in the final aqueous phase after the initial chloroform extraction and enzyme and acid hydrolysis were considered as unextractable, polar water-soluble material, and the chemical nature of this material was not further characterized.

Analysis of Feces. The combined fecal materials were subjected to a continuous ether Soxhlet extraction for 24 h. Solid fecal material after the initial ether extraction was further extracted three times with 100 mL of acetone. The combined ether and acetone extracts were dried over anhydrous magnesium sulfate, concentrated, and analyzed directly by two-dimensional TLC. ^{14}C residues remaining with the solid materials after solvent extractions (ether and acetone) were considered as unextractable residues, and their chemical nature was not further characterized.

Extraction of Cinmethylin Residues in the Liver Tissue. Twenty-five grams of the liver tissue was 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 partitioning was subjected to acid hydrolysis at pH 1, 85 °C, for 2 h. Released ^{14}C residues were recovered by chloroform partitioning and analyzed by TLC.

Chromatography and Radioassay. Radioactivity was quantitated in 15 mL of Aquasol-2 scintillation solution in a

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Packard Model 300 liquid scintillation system. Radioactive areas of the TLC plate, after solvent development and autoradiography, were removed by scraping and analyzed in an Aquasol-2–water (11 mL:4 mL) 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. The oxidizer counting solution included Carbo-Sorb and Permafluor V (Packard Instrument Co.), 10 mL:12 mL mixture. All LSC quantitations were corrected for counting efficiency and quenching.

Excreted radioactivity 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, feces, and liver tissue were analyzed by two-dimensional TLC (silica gel F-254, 0.25 mm, E. Merck) and were visualized by autoradiography on Kodak SB-5 single-coated X-ray film.

Gas–liquid radiochromatography (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 × 2 mm (i.d.) glass column packed with 3% OV-101 on 80/100-mesh Supelcoport (Supelco Co.). The air, hydrogen, and helium flow rates were 210, 30, and 30 mL/min, respectively. The helium and propane quench gas flow rates for the gas-proportional counter were 120 and 15 mL/min, respectively.

Capillary gas–liquid chromatography was carried out on a 30 m × 0.25 mm (i.d.) fused silica SE-54 WCOT capillary column in a Varian 3700 gas–liquid chromatograph equipped with a flame ionization detector. Temperature-programmed and isothermal analyses were carried out at injector and detector temperatures of 245 and 320 °C, respectively. The helium carrier gas and nitrogen makeup gas flow rates through the detector were 3 and 36 mL/min, respectively. On-column split ratio was controlled at approximately 10:1.

GC–mass spectroscopy was carried out on Finnigan Models 1020 and 4500 mass spectrometers. Analyses were also carried out on a HP-5992B mass spectrometer, and fast-atom bombardment spectra were obtained on a VGZAB2F mass spectrometer.

RESULTS AND DISCUSSION

Distribution of Metabolites in Urine. Urine contained approximately 40% of the administered radioactivity. A majority of the eliminated radioactivity was recovered by the initial chloroform partitioning (36%) and water-soluble conjugates recovered after enzyme (38%) and acid (6%) treatments. Approximately 20% of the urinary ^{14}C residues remained in the aqueous phase after the above treatments and was considered to be unextractable, water-soluble polar residues and not further analyzed.

Preparative Separation and Characterization of Urinary Metabolites. The extensive metabolism of cinmethylin in the goat was evident by the detection of more than 25 metabolites (Figure 1). Similar compounds were recovered in the urine and feces as both organic-extractable and conjugated products. They were isolated and characterized by a combination of TLC, autoradiography, gas–liquid radiochromatography, GLC, and GC–mass spectroscopy. When an authentic standard was available, the structure of the isolated metabolite was confirmed by direct spectral comparison and cochromatography.

The initial preparative TLC separation of the urinary metabolites was carried out with the toluene–IPA–HOAc solvent system (1, Table I). The area of the preparative TLC plate corresponding to the radioactive band was removed, and radioactive materials were eluted from the silica gel with ethyl acetate as the eluting solvent. A majority of the isolated products from the initial preparative TLC separation required additional cleanup and further chromatographic separation prior to structural analysis. To facilitate the discussion, metabolites, in

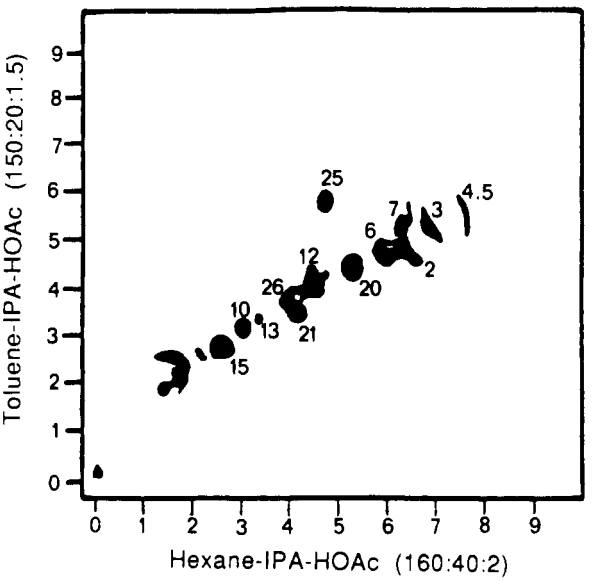

Figure 1. Thin-layer autoradiogram of the organic-extractable residues recovered in the goat urine after oral administration of [*phenyl*-^{14}C]cinmethylin.

general, were classified as mono-, di-, and trihydroxylated, acidic, methoxylated, and ether cleavage products. They were arbitrarily designated by the following alphabetical system to indicate the position of biotransformation. For example, *exo*-2-[[[1-methyl-4-(1-methylethyl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy]methyl]benzenemethanol (**2**) was monohydroxylated at the A-position and *exo*-2-[[[4-(2-hydroxy-1-methylethyl)-1-methyl-7-oxabicyclo[2.2.1]-hept-2-yl]oxy]methyl]benzenemethanol (**10**) was hydroxylated at the A- and F-positions.

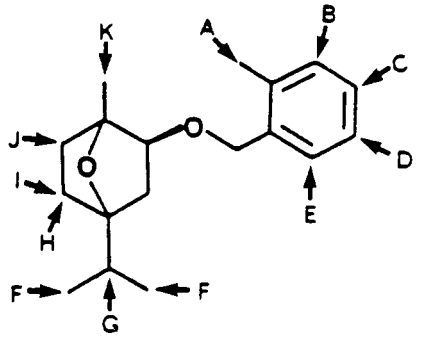

Table I summarizes the chemical structures, preparative separation, characterization, and identification of all the primary urinary metabolites.

Characterization of Urinary Monohydroxylated Products. Eight primary monohydroxylated cinmethylin metabolites were isolated from the organic-extractable fraction and also after enzyme hydrolysis. Monohydroxylation occurred at both the aromatic ring [A (**2**), B (**3**), C (**4**), D (**5**)] and the cineole portion of the parent molecule [F (**6**), J (**7**)].

Two additional monohydroxylated dehydrated products were also detected [A′ (**8**), F′ (**9**)].

Characterization of Urinary Dihydroxylated Products. Seven primary dihydroxylated cinmethylin metabolites were isolated from the organic-extractable fraction and also after enzyme hydrolysis. Six of these products had one hydroxyl moiety at either the 2-methylbenzyl or on the aromatic ring and the second hydroxyl moiety at the cineole portion of the parent molecule [A/F (**10**), A/F′ (**11**), A/G (**12**), A/J (**13**), B/G (**14**), D/G (**15**)]. One product contained two hydroxyl groups on the

cineole portion of the parent molecule [F/G (16)].

Characterization of Urinary Trihydroxylated Products. Three trihydroxylated metabolites were isolated after enzyme hydrolysis. Each contained a single hydroxyl group on the aromatic portion and two hydroxyl groups on the cineole portion of the parent molecule [A/G/J (17), A/G/F (18), C/G/J (19)].

Characterization of Urinary Acidic Products. Four carboxylated acidic cinmethylin metabolites were isolated from the organic-extractable fraction. A trace amount was also detected after enzyme hydrolysis. All of these metabolites contained a carboxyl group at the 2-methylbenzyl (A) moiety [A*/G (20), A*/F, two diastereomers (21), A*/J (22), A*/G/J (23)].

Characterization of Urinary Methoxylated Products. Three metabolites that contained a methoxyl moiety on the aromatic ring of the intact parent molecule were isolated. Two of these methoxylated products appeared to have two hydroxyl groups, and the third product contained three hydroxyl groups.

The two dihydroxylated products appeared to be isomers, and their structures were postulated based on mass spectral interpretation. The TMS derivatives of these two products were virtually identical. The locations of the aromatic hydroxyl and methoxyl groups are uncertain since many isomeric combinations are possible. However, it is unlikely that the hydroxyl and the methoxyl groups are adjacent. The location of the hydroxyl group on the cineole portion of the parent molecule is proposed at the G-position based on mass spectral comparison with **27** [a major plant metabolite of cinmethylin (unpublished data)].

The third product appears to contain two hydroxyl groups on the aromatic portion and one hydroxyl group on the cineole portion of the parent molecule. Mass spectral data suggested the presence of a hydroxyl group at the isopropyl group; however, the location of the aromatic hydroxyl groups is uncertain.

Characterization of Other Products in Urine. Benzoic acid (**24**) and *o*-(hydroxylmethyl)benzoic acid lactone (**25**) were isolated from the goat urine.

The source of [^{14}C]benzoic acid as a metabolite of cinmethylin was uncertain; however, it has been detected as a minor impurity in the ^{14}C test material. In the rat metabolism study, *o*-(hydroxymethyl)benzoic acid lactone had been demonstrated as a decomposition product of *o*-(hydroxymethyl)benzoic acid (**26**) (Lee et al., 1986) during sample preparation and analysis. The lactone was detected as the major radiolabeled product in the goat urine after acid hydrolysis.

A summary of the distribution pattern of the major goat urine metabolites is presented in Table II. Compounds **2** (A) and **12** (A/G) were the major metabolites, and each accounted for greater than 10% of the recovered urinary radioactivity.

Distribution and Preparative Separation of Metabolites in Feces. The amount of radioactivity in the feces accounted for approximately 20% of the total administered dose. The ether-extractable residues accounted for 54% of the total fecal radioactivity. Less than 2% of the radioactivity could be released by acetone extraction of the solid fecal materials after the initial ether Soxhlet extraction. In addition to the undegraded cinmethylin (**1**), at least 15 primary degradation products were detected and their distribution profile is summarized in Table III. Mono-, di-, and trihydroxylated products were observed.

Extractable fecal products were separated by silica gel column chromatography using a stepwise gradient elution of ethyl acetate in hexane. Further cleanup and separation of the elution fractions from the column by preparative TLC were also carried out prior to structural characterization.

Characterization and Identification of Metabolites in Feces. Metabolites isolated from feces were characterized and identified by a combination of chromatographic (TLC, GLC) and mass spectral analyses. Proposed structures were consistent with authentic standards (when available) or with the isolated urinary products described in the earlier sections.

Nearly all the mono- and dihydroxylated fecal metabolites had been detected in the urine. Seven monohydroxylated products were identified [A (**2**), B (**3**), C (**4**), D (**5**), F (**6**), G (**27**), J (**7**)]. In addition, four dihydroxylated products [A/G (**12**), C/G (**28**), D/G (**15**), A/F (**10**)], two hydroxylated dehydrated products [A′ (**8**), F′ (**9**)], and two trihydroxylated products [A/G/J (**17**), C/G/J (**19**)] were also detected. In general, the qualitative distribution profile of the fecal metabolites is similar to that observed in the urine. Additional products from feces (not observed in the urine) were G (**27**) and C/G (**28**). Quantitatively, the relative amounts of the monohydroxylated products were much higher in the feces than in the urine. Compounds **2** (A), **6** (F), and **12** (A/G) were the major fecal metabolites, and each accounted for greater than 10% of the recovered fecal radioactivity.

Characterization of Cinmethylin Residues in the Liver. In this goat metabolism study, the test animal was administered an exaggerated dose level equivalent to 1000× the maximum dietary exposure. The level of ^{14}C residues in the milk and various tissues (muscle and fat) were low (<0.5 ppm), except in liver and kidney tissues which were approximately 3 ppm [^{14}C]cinmethylin equivalent. Total tissue residues represented less than 1% of the administered radioactive dose.

Fractionation data showed the majority of the radioactivity in the liver tissue was associated with the proteinaceous materials (62%) after TCA precipitation. Washing of the protein precipitate with hot acetone resulted in the release of an additional 30% of the total radioactivity. Because of the highly contaminated nature of the acetone extract with lipid and other biological components, no conclusive chromatographic characterization of the acetone extract could be achieved. No further qualitative examination of the protein-bound ^{14}C residues was carried out.

The tissue aqueous homogenate, after the removal of proteinaceous materials, contained 38% of the total liver radioactivity. A subsequent fractionation study showed the organic-extractable fraction contained 9% of the liver ^{14}C residues, 3% of conjugates recovered after β-glucuronidase hydrolysis, 1% after acid hydrolysis, and 26% in the final aqueous phase.

TLC was used as the primary means to characterize the chemical nature of the liver residues. More than 20 metabolites were observed. The amount of undegraded [^{14}C]cinmethylin detected was minimal and accounted for less than 1% of the total liver residues. *o*-(Hydroxymethyl)benzoic acid lactone (**25**) was the major product detected in the organic-extractable fraction, and a monohydroxylated product on the G-position (**27**) was the major product recovered after enzyme and acid hydrolysis.

CONCLUSION

The diversity in the metabolism of cinmethylin in the goat was evident by the detection of greater than 25 metabolites in the urine and feces. The primary metabolic pathway involved hydroxylation at the 2-methylbenzyl moiety to yield monohydroxylated metabolite(s) followed by subsequent hydroxylation, mainly at the cineole portion

Table I. Chemical Structures, Preparative TLC Separation, and Identification of Cinmethylin Metabolites in Goat Urine and Feces

compound	TLC (R_f)[d]	mass spectral data [m/z (% RA)]
cinmethylin (1)[a]	1 (0.69), 2 (0.87)	(EI) 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)
		Monohydroxylated Products
2 (A)[a]	1 (0.52), 3 (0.90), 4 (0.18)	(EI) 290 (M^+, <1), 232 (3), 171 (23), 169 (7), 123 (32), 121 (18), 119 (17), 107 (29), 93 (40), 91 (28), 77 (25), 71 (22), 43 (100)
3 (B)[a]	1 (0.52), 3 (0.90), 4 (0.21)	(EI) 290 (M^+, 6), 169 (8), 154 (5), 151 (5), 123 (28), 121 (46), 111 (8), 109 (13), 107 (32), 93 (19), 91 (20), 83 (15), 77 (23), 71 (18), 55 (18), 43 (100)
4 (C)[a]	1 (0.52), 3 (0.95), 2 (0.57), 4 (0.27), 5 (0.30)	(EI) 290 (M^+, 4), 169 (15), 123 (19), 122 (23), 121 (100), 109 (11), 107 (18), 91 (27), 83 (12), 77 (13), 71 (19), 55 (16), 43 (83)
5 (D)[a]	1 (0.52), 3 (0.95), 2 (0.57), 4 (0.27), 5 (0.35)	(EI) 290 (M^+, 19), 169 (9), 123 (34), 122 (18), 121 (61), 120 (12), 111 (11), 109 (15), 107 (38), 93 (18), 91 (17), 77 (23), 71 (26), 55 (28), 43 (100)
6 (F)[a]	1 (0.52), 3 (0.66), 5 (0.27)	(EI) 290 (M^+, <1), 185 (3), 169 (5), 168 (21), 167 (18), 121 (13), 110 (18), 109 (28), 107 (18), 105 (75), 79 (20), 77 (16), 43 (100)
7 (J)	1 (0.52), 3 (0.82), 5 (0.31)	(EI) 290 (M^+, <1), 185 (4), 167 (26), 124 (19), 121 (12), 105 (100), 91 (9), 83 (9), 79 (15), 77 (12), 55 (11), 63 (62)
8 (A′)[a,b]	1 (0.52), 3 (0.90), 4 (0.21)	(EI) 167 (5), 151 (9), 150 (31), 149 (10), 121 (24), 119 (18), 109 (18), 107 (39), 93 (42), 91 (43), 77 (30), 69 (21), 43 (100)
9 (F′)[b]	1 (0.52), 3 (0.82), 5 (0.27)	(EI) 270 (<1), 167 (3), 166 (13), 165 (24), 147 (10), 121 (6), 119 (6), 107 (8), 105 (100), 91 (9), 79 (24), 77 (17), 67 (6), 55 (8), 43 (76) (EI, Me_3Si) 360 (M^+ <1), 302 (5), 255 (3), 237 (5), 170 (26), 147 (13), 105 (100), 79 (17), 77 (12), 75 (16), 73 (33), 43 (45)
27 (G)[a]	1 (0.46), 4 (0.14), 5 (0.29)	(EI) 290 (M^+, <1), 272 (1), 257 (1), 232 (4), 217 (2), 167 (5), 140 (12), 139 (10), 122 (24), 121 (22), 105 (100), 97 (18), 79 (18), 77 (15), 71 (17), 59 (18), 43 (74)
		Dihydroxylated Products
10 (A/F)[a]	1 (0.29), 3 (0.24), 1 (0.29), 9 (0.41)	(FAB) 307 ($M + H^+$, 72), 169 (42), 139 (60), 121 (100), 105 (54), 91 (30), 81 (18) [EI, $(Me_3Si)_2$] 360 (2), 257 (6), 193 (30), 192 (47), 167 (23), 119 (27), 104 (26), 103 (29), 75 (27), 73 (100), 43 (41)
11 (A/F′)[b]	1 (0.29), 3 (0.29), 3 (0.46), 8 (0.20)	(CI) 305 ($M + H^+$, 45), 199 (54), 185 (65), 167 (59), 159 (43), 121 (57), 105 (53), 85 (100) (EI, Me_3Si) 376 (M^+, <1), 358 (<1), 255 (4), 193 (44), 192 (32), 165 (37), 119 (48), 75 (40), 73 (100), 43 (45)
12 (A/G)[a]	1 (0.39), 3 (0.39), 6 (0.74)	(CI) 307 ($M + H^+$, 27), 289 (14), 169 (65), 151 (50), 133 (70), 121 (100) [EI, $(Me_3Si)_2$] 360 (2), 257 (2), 193 (89), 192 (31), 131 (29), 119 (27), 75 (29), 73 (100), 43 (42)
13 (A/J)	1 (0.31), 3 (0.29), 9 (0.50), 8 (0.16)	[EI, $(Me_3Si)_2$] 360 (2), 257 (5), 193 (100), 192 (14), 169 (18), 167 (8), 119 (32), 75 (27), 73 (87), 71 (10), 43 (38)
14 (B/G)	1 (0.46), 3 (0.65), 6 (0.43), 9 (0.49)	(EI, Me_3Si) 378 (M^+, 14), 320 (14), 238 (21), 194 (30), 193 (87), 192 (21), 177 (17), 149 (12), 139 (19), 122 (13), 121 (24), 109 (19), 107 (28), 97 (14), 75 (17), 73 (63), 71 (13), 59 (26), 43 (100) [EI, $(Me_3Si)_2$] 450 (M^+, 2), 360 (12), 194 (23), 193 (100), 177 (11), 143 (13), 131 (25), 107 (21), 75 (19), 73 (65), 71 (4), 43 (33)
15 (D/G)	1 (0.46), 3 (0.58), 6 (0.37), 9 (0.58)	[EI, $(Me_3Si)_2$] 450 (M^+, 3), 360 (7), 194 (22), 193 (100), 143 (11), 139 (11), 131 (22), 107 (19), 75 (21), 73 (88), 43 (28)
16 (F/G)	1 (0.39), 3 (0.36), 6 (0.23), 3 (0.36)	(CI), 307 ($M + H^+$, 42), 289 (9), 271 (2), 227 (3), 203 (11), 199 (39), 185 (30), 183 (40), 173 (4), 167 (20), 163 (12), 155 (13), 147 (10), 138 (17), 127 (11), 121 (18), 113 (20), 105 (100), 97 (18), 90 (10), 85 (35) [EI, $(Me_3Si)_2$] 347 (1), 257 (2), 219 (3), 105 (100), 75 (8), 73 (32), 43 (10)
28 (C/G)	3 (0.43), 4 (0.62)	(EI, Me_3Si) 378 (M^+, 1), 209 (4), 193 (100), 185 (12), 167 (13), 109 (10), 107 (14), 73 (43), 59 (8), 43 (37) [EI, $(Me_3Si)_2$] 450 (M^+, <1), 374 (<1), 360 (<1), 257 (3), 193 (100), 167 (100), 131 (18), 75 (11), 73 (50), 43 (14)
		Trihydroxylated Products
17 (A/G/J)	3 (0.05), 4 (0.14)	[EI, $(Me_3Si)_3$] 448 (<1), 345 (4), 315 (14), 225 (14), 219 (9), 193 (86), 192 (21), 147 (11), 119 (25), 75 (20), 73 (100), 43 (22)
18 (A/G/F)	3 (0.05), 4 (0.20)	(CI) 323 ($M + H^+$, 17), 193 (72), 180 (100) [EI, $(Me_3Si)_3$] 448 (2), 433 (6), 311 (5), 194 (12), 193 (62), 192 (12), 147 (5), 143 (6), 131 (62), 119 (21), 104 (7), 75 (19), 73 (100), 43 (17)
19 (C/G/J)	3 (0.05), 4 (0.25)	[EI, $(Me_3Si)_3$] 448 (4), 345 (7), 255 (7), 194 (20), 193 (100), 165 (7), 131 (53), 75 (13), 73 (78), 43 (13)
		Acidic Products
20 (A*/G)[a,c]	1 (0.46), 3 (0.39), 6 (0.24), 8 (0.51)	[EI, $(Me_3Si)_2$] 406 (<1), 374 (2), 359 (2), 257 (3), 207 (100), 167 (4), 133 (16), 131 (18), 75 (23), 73 (87), 43 (28)
21 (A*/F)[a,c]	1 (0.39), 2 (0.34), 3 (0.34), 2 (0.34), 6 (0.18), 8 (0.38)	[EI, $(Me_3Si)_2$] 464 (M^+, 2), 374 (5), 257 (8), 222 (15), 207 (64), 167 (26), 133 (31), 131 (2), 75 (24), 73 (100), 43 (32)
22 (A*/J)[c]	1 (0.39), 2 (0.36), 3 (0.35), 6 (0.24), 3 (0.35)	[EI, $(Me_3Si)_2$] 464 (M^+, 8), 330 (3), 257 (6), 241 (5), 240 (6), 225 (7), 223 (5), 207 (98), 167 (9), 133 (14), 131 (2), 124 (16), 75 (25), 73 (100), 43 (43)
23 (A*/G/J)[c]	1 (2×, 0.25), 6 (0.80)	[EI, $(Me_3Si)_2$] 462 (2), 447 (10), 345 (2), 255 (1), 207 (100), 133 (12), 131 (35), 75 (14), 73 (82)

Table I (Continued)

compound	TLC (R_f)[d]	mass spectral data [m/z (% RA)]
		Other Products
24 (benzoic acid)[a]	1 (0.71)	(EI) 122 (M^+, 92), 105 (100), 77 (70), 51 (32), 50 (19)
25 (*o*-(hydroxymethyl)-benzoic acid lactone)	1 (0.55), 2 (0.45), 3 (0.79)	(EI) 134 (M^+, 42), 133 (13), 105 (100), 77 (72), 51 (18), 50 (16)
26 (*o*-(hydroxymethyl)-benzoic acid)[a]	1 (0.29), 1 (0.32)	(EI) 134 (M^+, 42), 133 (13), 105 (100), 77 (72), 51 (18), 50 (16) [EI, $(Me_3Si)_2$] 296 (M^+, 30), 281 (28), 206 (100), 177 (20), 163 (18), 147 (90), 90 (15), 73 (85)

[a] Authentic standard is available. [b] Dehydro compounds at the F-position designated by prime. [c] Carboxylated compounds designated by asterisk. [d] Solvent systems: (1) toluene–2-propanol–HOAc (150:20:1.5); (2) hexane–2-propanol–HOAc (120:30:1); (3) ethyl acetate–hexane–chloroform–HOAc (100:50:5:1.5); (4) toluene–ethyl acetate (17:3); (5) heptane–dioxane (3:1); (6) toluene–ethyl acetate–HOAc (100:100:1); (7) heptane–dioxane–HOAc (100:100:1); (8) toluene–ethyl acetate (1:1); (9) heptane–dioxane (1:1).

Table II. Distribution of Metabolites in the Goat Urine after Oral Dose Administration of [*phenyl*-^{14}C]Cinmethylin

compound	% of total urinary radioactivity		
	organic extractable	enzyme hydrolysis	acid hydrolysis
2 (A)[a]	7.4	7.0	–[b]
3 (B), **4** (C), **5** (D), **6** (F), **7** (J)	1.8	2.6	–
12 (A/G)	2.7	7.4	–
20 (A*G)	3.9	–	–
21 (A*/F)	2.6	–	–
13 (A/J)	0.5	1.1	–
16 (F/G)	0.8	3.3	–
10 (A/F)	2.1	4.1	–
15 (D/G)	3.0	–	–
17 (A/G/J)	–	4.4	–
18 (A/G/F)	–	3.7	–
25	0.6	–	3.9
others[c]	10.3	4.0	2.0
total	35.7	37.6	5.9

[a] Designated position(s) of biotransformation. [b] At trace level or not detected. [c] Includes minor and other unidentified products.

Table III. Distribution of Metabolites in the Goat Feces after Oral Dose Administration of [*phenyl*-^{14}C]Cinmethylin

compound	% of total extractable fecal radioactivity	compound	% of total extractable fecal radioactivity
1 (cinmethylin)	4.6	**27** (G)	6.8
2 (A)	29.4	**8** (A′)	3.8
3 (B)	1.0	**12** (A/G)	13.7
4 (C)	0.8	**10** (A/F)	2.8
5 (D)	1.7	**18** (A/G/J)	1.6
6[a] (F)	19.8	others[b]	14.0

[a] Including **7** (J) and **9** (F′) as minor components. [b] Including radioactivity associated with the origin of the TLC plate and minor unidentified products.

of the parent molecule (F- and G-positions) to yield di- and trihydroxylated products. A majority of these products were excreted as glucuronide conjugates. Once hydroxylated at the G-position of the isopropyl moiety (tertiary carbon), a dehydration reaction could occur yielding dehydrated products such as **8** and **9**. These metabolic pathways are consistent with other monoterpenoid compounds such as (±)-α-pinene, (−)-β-pinene, and (−)-*cis*-pinane, etc. (Ishida et al., 1981). Ether cleavage is considered as a secondary metabolic reaction for cinmethylin in the goat. The lack of total [^{14}C]cinmethylin equivalent residues detected in the milk, fat, and meat and the multitude of metabolic products recovered in the excreta support the conclusion that cinmethylin residues do not accumulate and are extensively metabolized in food-producing animals.

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