Environmental Degradation of the Miticide Cycloprate (Hexadecyl Cyclopropanecarboxylate). 1. Rat Metabolism

Gary B. Quistad,* Luana E. Staiger, and David A. Schooley

When rats were given a single oral dose (21 mg/kg) of [*carboxyl*-^{14}C]cycloprate (hexadecyl cyclopropanecarboxylate), two-thirds of the applied dose was excreted within 1 day. After 4 days 67 and 15% of the applied ^{14}C label had been excreted in the urine and feces, respectively, but 18% still remained in the tissues. Analysis of the tissue-bound radioactivity revealed that 80% of the residual ^{14}C was associated with fatty acids and two-thirds of all the tissue radiolabel was contributed by three ω-cyclopropyl fatty acids which were identified as 11-cyclopropylundecanoic acid, 13-cyclopropyltridecanoic acid, and 15-cyclopropylpentadecanoic acid. Cycloprate contributed only 1–3% of the total ^{14}C label found in tissues. The major excretory metabolite in feces was cyclopropanecarboxylic acid, while in urine *N*-(cyclopropylcarbonyl)glycine represented 85% of the 1-day urinary radiolabel (or 46% of the applied dose). Rats analyzed 60 days after dosage at 21 mg/kg still contained 5% of the applied ^{14}C label, but reduction of the dose rate to 1 mg/kg lowered the percentage of ^{14}C residue in tissues by half after 4 days.

A novel class of miticides containing the cyclopropane moiety has been shown to be selective for phytophagous mites (Staal et al., 1975; Nelson and Show, 1975; Henrick et al., 1976). A particularly active compound was hexadecyl cyclopropanecarboxylate (cycloprate, ZR-856, trademark Zardex) which was administered to rats in this study in order to assess its metabolic degradation.

In planning metabolic studies for cycloprate we decided to radiolabel the carboxyl moiety of the cyclopropanecarboxylate since hydrolysis of cycloprate would give hexadecanol which is a known natural product (Schoenheimer and Hilgetag, 1934) in various mammals including man, and its metabolic fate in rats has already been studied (Blomstrand and Rumpf, 1954). It has been shown (Wood and Reiser, 1965; Chung, 1966) that when a cyclopropane ring occurs in a nonterminal position in fatty acids, the ring is stable to metabolic breakdown in intact rats or rat liver mitochondria. The metabolism of cyclopropanecarboxylic acid (CPCA) itself was studied in vitro by Duncombe and Rising (1968) who showed apparent conversion of CPCA to ω-cyclopropyl fatty acids by rat adipose tissue and liver, but they were unable to determine the exact chemical structures of the metabolites. CPCA is also metabolized to 4-hydroxybutyrate (via a carnitine ester) by fungi (Schiller and Chung, 1970; Guilbert and Chung, 1974), but such a conversion has not been demonstrated in other organisms.

EXPERIMENTAL SECTION

Synthetic Procedures. Cyclopropyl bromide [Aldrich, purity claimed: 99%; purity found: 92% by gas–liquid chromatography (GLC)] was distilled through a glass bead packed column. A constant boiling midfraction (99.9% pure by GLC) was converted to the Grignard reagent in ether and carbonated with $^{14}CO_2$ (54.1 mCi/mmol) in 88% radiochemical yield after isolation (carbonation performed by Dr. John C. Leak, ICN).

[*carboxyl*-^{14}C]Cycloprate was prepared by B. J. Bergot via Fischer esterification of the labeled acid with carefully purified hexadecanol. The resultant ester (i.e., cycloprate; sp act. 54.1 mCi/mmol) showed radiochemical purity of >99% as determined by high-resolution liquid chromatography (HRLC). Unlabeled cycloprate of 97.6% chemical purity was used to dilute the radioactive sample to desired specific activities.

11-Cyclopropylundecanoic acid [14(11cPr):0], 13-cyclopropyltridecanoic acid [16(13cPr):0], and 15-cyclopropylpentadecanoic acid [18(15cPr):0] were prepared by the Chemical Research Department at Zoecon (Henrick et al., 1976).

N-(Cyclopropylcarbonyl)glycine was prepared by adding cyclopropylcarbonyl chloride to glycine in 10% NaOH. The crude conjugate was converted to its *p*-phenylphenacyl ester and purified by TLC.

Radioassay. Radioactivity was determined by liquid scintillation counting, total combustion (after lyophilization), and radiochromatogram scanning as described previously (Quistad et al., 1974).

Balance Studies. Four male Simonsen albino rats (Simonsen Laboratories, Gilroy, Calif.) weighing 247–308 g were dosed orally with a corn oil solution (0.5 mL) of [*carboxyl*-^{14}C]cycloprate (1.26 mCi/mmol, 23.7 ± 2.5 μCi, 21.3 ± 0.6 mg/kg). This dose yielded no visible toxic effects, and the rats were apparently healthy during the 4-day test period as evidenced by normal food and water consumption. Immediately after dosing, rats were placed in all-glass metabolism chambers (Stanford Glassblowing Laboratories, Palo Alto, Calif.) which were modified for continuous collection of separated urine, feces, and expired CO_2. Four days after the single oral dose the animals were sacrificed by cervical transsection, and the tissues were removed, weighed, and frozen. There were no obvious pathological abnormalities at time of sacrifice.

In a second study, two male rats were dosed with [^{14}C]cycloprate (22 mg/kg, 1.26 mCi/mmol, 21 μCi), but urine and feces were analyzed for 60 days prior to sacrifice for tissue analysis. In a third study, male and female rats (two each) were dosed with [^{14}C]cycloprate at 1 mg/kg (54.1 mCi/mmol, 43 μCi) and maintained 4 days.

Structural Identification of ω-Cyclopropyl Fatty Acids and *N*-(Cyclopropylcarbonyl)glycine. In order to accumulate sufficient mass for structure elucidation of the radioactive fatty acids found in rat tissues, a rat was given a single high oral dose of cycloprate (738 mg/kg, 0.89 mCi/mmol). After 4 days the rat was sacrificed and the liver was removed for homogenization in $CHCl_3$. The extracted lipid (70 mg) was saponified (1 M KOH in ~90% ethanol, 75 °C, 1 h), esterified with diazomethane, and purified by TLC (two 20 × 20 cm plates, 1 mm silica

Zoecon Corporation Research Laboratory, Palo Alto, California 94304.

0021-8561/78/1426-0064$01.00/0

gel GF). When the fatty acid methyl ester zone was further purified by TLC on silver nitrate impregnated silica gel plates (Quistad et al., 1975), the radioactivity migrated with saturated fatty acid methyl esters.

The saturated ester zone (16.2 mg) was subjected to reversed-phase HRLC [two 8-mg injections via 100-μL loop; Waters Associates M-6000 pump, R401 differential refractometer detector, and μBondapak C_{18} column (30 × 0.4 cm) eluted at 1.6 mL/min with methanol–water (85:15)]. Radioactive bands in the regions of authentic standards of 16(13cPr):0 and 18(15cPr):0 were rechromatographed separately under the same conditions. Final purification of 16(13cPr):0 and 18(15cPr):0 was achieved by normal-phase HRLC [same pump and detector, with a Zorbax-SIL column (22 × 0.79 cm, du Pont) eluted at 3.0 mL/min using ether–hexane (0.5:95.5)]. Mass spectra of the metabolites were obtained by coupled gas–liquid chromatography–mass spectroscopy (GLC–MS) as described previously (Schooley et al., 1975). The following mass spectra for the two most abundant radioactive bands were identical with synthetic standards: for 16(13cPr):0, *m/e* (rel intensity), 268 (<1), 237 (7), 236 (5), 197 (6), 194 (9), 152 (12), 124 (20), 98 (47), 97 (60), 96 (47), 87 (68), 74 (100); for 18(15cPr):0, 296 (<1), 265 (11), 264 (15), 237 (6), 222 (13), 152 (17), 110 (34), 98 (53), 97 (59), 96 (55), 87 (71), 74 (100).

N-(Cyclopropylcarbonyl)glycine (CPCA-gly) was isolated from the 1-day urine of the rat dosed at 738 mg/kg. An aliquot of the urine (0.1% of total) was derivatized to its *p*-phenylphenacyl ester (vide infra) and partially purified by TLC (silica gel, ether). The *p*-phenylphenacyl ester was purified further by HRLC [pneumatic amplifier pump (Haskel Engineering), loop injector (Valco), ultraviolet absorbance detector (Chromatronix Model 230), and a Zorbax-SIL column (22 × 0.79 cm) eluted with ether–pentane, 70:30]. A direct-inlet mass spectrum of the *p*-phenylphenacyl ester of the urinary metabolite matched an authentic synthetic standard: *m/e* (rel intensity) 337 (7), 196 (7), 194 (4), 182 (23), 181 (59), 153 (16), 152 (10), 85 (3), and 69 (13). Saponification of this major urinary metabolite gave CPCA (quantitative yield) which was characterized by HRLC as its *p*-phenylphenacyl ester.

O-(Cyclopropylcarbonyl)carnitine (CPCA-carnitine) was isolated from urine and the carcass by TLC ($CHCl_3$–CH_3OH–58% NH_4OH, 50:30:8), and the structural assignment was confirmed by HRLC (for details, see Quistad et al., 1978).

Extraction of Metabolites from Tissues and Feces. For detailed analysis of radiolabeled residues, tissues were pooled from several rats: fat and liver (three animals each) and carcass remains (two rats). Tissue groups were extracted with $CHCl_3$ (3×) and methanol (3×) with a Virtis homogenizer. Preliminary TLC (silica gel GF, hexane–ether, 6:1) allowed separation of the crude $CHCl_3$ extract into zones corresponding to cycloprate and natural products (i.e., triglycerides, cholesteryl ester, etc; see Christie, 1973, for natural product analysis by TLC) which were eluted from the silica for further processing. Fatty acids were liberated from these natural products by saponification (1 M KOH in ~90% ethanol, 75 °C, 1 h) and then esterified with diazomethane prior to analysis by HRLC with a μBondapak C_{18} reversed-phase column as described above. The final quantification of 14(11cPr):0, 16(13cPr):0, and 18(15cPr):0 was obtained from HRLC as was the verification of cycloprate residues. CPCA bound to glycerides ($CHCl_3$ extract) and polar lipids (methanol extract) was characterized by saponification, acidification, extraction, and derivatization as the *p*-phenylphenacyl ester (for extract biomass <10 mg, 25 mg of α-bromo-*p*-phenylacetophenone, 300 mg of $KHCO_3$, 2 mL of dimethylformamide, room temperature, 1 h). Metabolite radioactivity was coincident with an authentic standard of the *p*-phenylphenacyl ester of CPCA when examined on μBondapak C_{18} (eluted with methanol–water, 70:30). Radiolabel bound as more polar products (i.e., methanol extract and residual solids) was released by saponification prior to characterization of the derivatized fatty acids (as methyl esters) by reversed-phase HRLC.

Table I. Radioactivity Balance for Four Male Rats Dosed at 21 mg/kg with [*carboxyl*-^{14}C]Cycloprate

	% applied dose				
	Days posttreatment				
	1	2	3	4	Total
Urine	53.7	9.2	2.8	1.2	66.9 ± 3.4[a]
Feces	13.2	0.8	0.3	0.2	14.5 ± 5.8
$^{14}CO_2$					<0.14
Tissues				17.9	17.9 ± 2.8
Total recovery					99.3 ± 3.2

[a] Mean ± standard deviation.

Examination of rat muscle was similar to that described for the tissues above; however, a single lipid fraction was obtained by pooling muscle extracts ($CHCl_3$–methanol, 2:1) from three rats. Radiolabel associated with natural products was again determined by TLC and the cycloprate zone was analyzed by reversed-phase HRLC for quantitation. The ω-cyclopropyl fatty acids were quantitated by HRLC after saponification and methylation of an aliquot of the $CHCl_3$–methanol extract.

Fecal radioactivity was easily extractable with methanol for preliminary examination by TLC (silica gel GF, hexane–ether–acetic acid, 60:10:1) which allowed isolation of cycloprate for structural verification by reversed-phase HRLC. Care is necessary when handling minute quantities of CPCA since it is reasonably volatile and readily disappears during evaporation at reduced pressure.

RESULTS AND DISCUSSION

When male albino rats were given a single oral dose of [*carboxyl*-^{14}C]cycloprate (24 μCi, 21 mg/kg), collection of urine and feces initially showed rapid excretion of radiolabel with 67% of the applied ^{14}C being eliminated after 1 day (Table I). Urine was the preferred route of radiolabel elimination, containing two-thirds of the applied dose after 4 days. Feces contained only 15% of the applied ^{14}C over the same time period (Figure 1). Expiration of $^{14}CO_2$ was negligible (<0.1% applied dose), indicating stability of the ^{14}C carboxyl to biological decarboxylation. During the study, progressively smaller amounts of ^{14}C were excreted so that 17.9 ± 2.8% of the applied dose remained in the rats after 4 days with a total recovery of 99.3 ± 3.2% for the radiolabel.

Excreted Metabolites. The feces served as a major excretory route for only 0–24 h during which time 13% of the applied dose was excreted (Table II). Most fecal radiolabel was unmetabolized cycloprate, indicating that about 10% of the applied dose was unabsorbed. The predominant fecal metabolite was unconjugated CPCA (1.3% applied dose).

Urine was the major route of elimination of metabolites (54% of the applied dose in one day). Urinary products were highly polar (only 2% ether extractable), and a single major product was identified as the glycine conjugate of CPCA [i.e., *N*-(cyclopropylcarbonyl)glycine, CPCA-gly] which represented 85% of the ^{14}C residue in 1-day urine. Thus, at least 46% of the applied radiolabel was excreted as this glycine conjugate. The complexity of the urinary

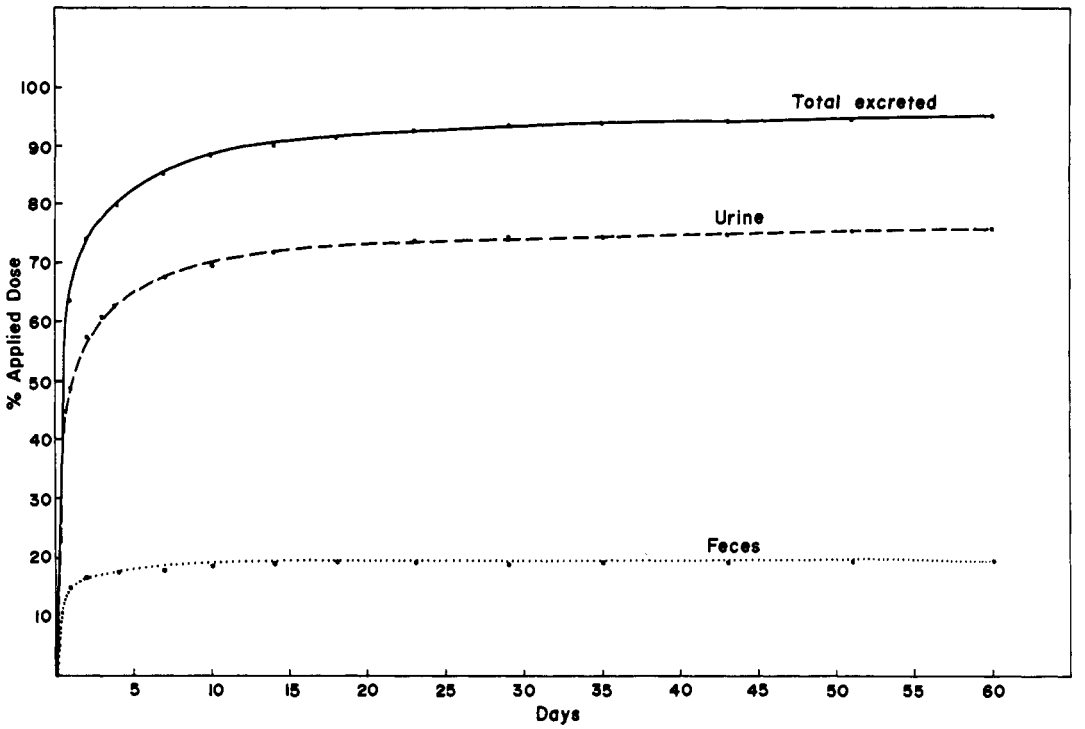


Figure 1. Excretion of radiolabeled residues from rats given a single oral dose (21 mg/kg) of [*carboxyl*-^{14}C]cycloprate.

Table II. Metabolites from [*carboxyl*-^{14}C]Cycloprate in Excrement

Feces (0–24 h, 13.2% applied dose)	% total ^{14}C in feces
CH_3OH extract	98.2
1. CPCA (free)	10.2
2. Cycloprate	83.0
Total identified	93.2
Urine (0–24 h, 53.7% applied dose)	**% total ^{14}C in urine**
Ether extractable	2.0
CPCA (free)	<0.2
Aqueous soluble	98.0
CPCA-gly	85.1
CPCA-carnitine	2

metabolites increased after the first day since *N*-(cyclopropylcarbonyl)glycine was only 9% of the ^{14}C residue in urine pooled from 15–18 days posttreatment. Neither free CPCA nor cycloprate were detectable in urine. However, *O*-(cyclopropylcarbonyl)carnitine (CPCA-carnitine) was also present (2% urinary ^{14}C), but in substantially reduced quantities compared to that found in dogs (Quistad et al., 1978).

Identification of ω-Cyclopropyl Fatty Acids. Preliminary TLC analysis of several tissues readily showed that most residual radiolabel was in fatty acid fractions. In order to characterize chemically the suspected ω-cyclopropyl fatty acid metabolites (cf. Duncombe and Rising, 1968) in tissues (Table III), a single rat was treated with a high dose of cycloprate (0.5 mCi, 738 mg/kg). The increased dose produced higher metabolite residues which were then rigorously separated from the comparatively enormous mass of natural fatty acid congeners by HRLC (Scheme I). In this way several micrograms of 13-cyclopropyltridecanoic acid 16(13cPr):0 and 15-cyclopropylpentadecanoic acid 18(15cPr):0 were isolated from the liver for structural verification by GLC–MS. The presence of 11-cyclopropylundecanoic acid 14(11cPr):0 was strongly implicated by coincidence of a smaller radiolabeled region with authentic standard. However, 14-(11cPr):0 was not isolated for spectral characterization.

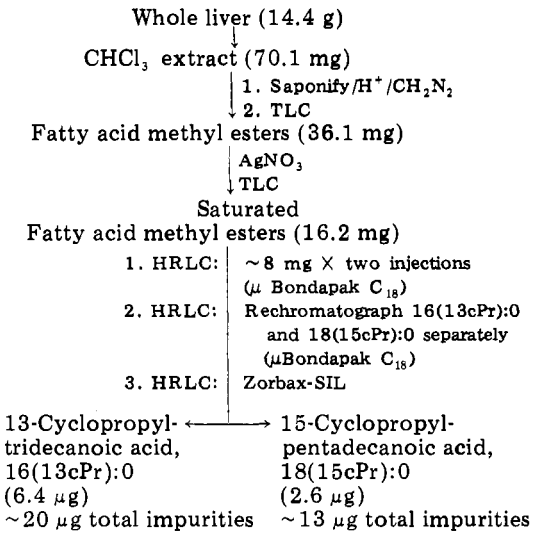
Quantitation of Tissue Metabolites. Analysis of the radiolabeled residues in various tissues was accomplished

Table III. Distribution of Radioactivity in Tissues after 4 Days for Rats Dosed at 21 mg/kg with [*carboxyl*-^{14}C]Cycloprate

	Wet weight, g	Total ^{14}C dpm × 10^{-3}	% applied dose	ppm equiv of cycloprate
Muscle (leg and pectoral)[a]	17.615	351	0.70	2.20
Stomach and intestines	33.45	602	1.15	1.99
Liver	12.92	180	0.35	1.52 ± 0.21[b]
Kidney	2.51	69.3	0.13	3.06
Lung	1.46	39.0	0.07	3.00
Testes	2.95	33.9	0.07	1.28
Fat (epididymal)	2.52	307	0.59	13.8 ± 2.5
Fat (abdominal)	1.72	201	0.39	13.0 ± 2.1
Brain	1.69	49.0	0.10	3.28
Blood	5.0	13.8	0.02	0.30
Hide[a]	3.56	240	0.45	7.76
Spleen	0.59	6.8	0.02	1.30
Heart	1.04	17.8	0.04	1.90
Carcass remains	188	7190	13.9	4.31
Total	278	9280	17.9	3.74

[a] Only part of tissue analyzed. [b] Standard deviation.

Scheme I. Purification of 16(13cPr):0 and 18(15cPr):0 Metabolites from Rat Liver



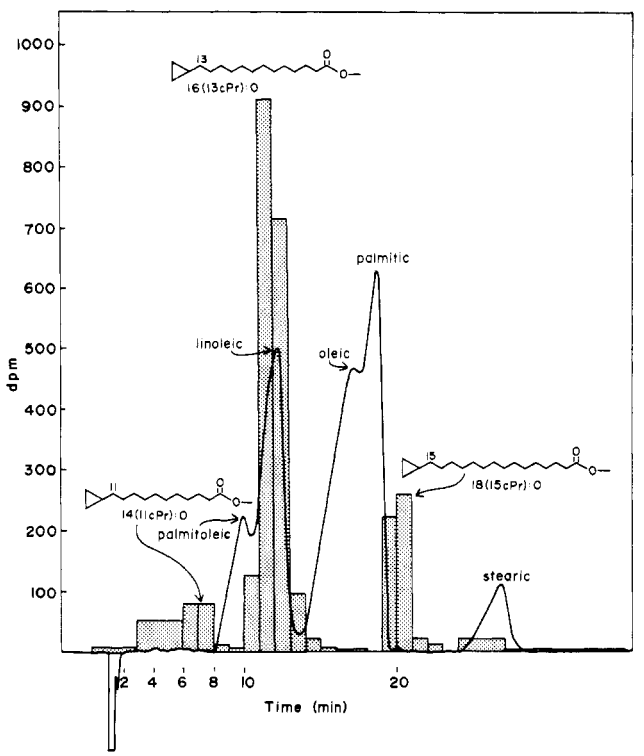


Figure 2. Typical HRLC separation of ω-cyclopropyl fatty acids (hatched bars) from natural fatty acids (solid line): Waters M-6000 pump; μBondapak C_{18} column; methanol–water, 87.5:12.5; refractive index detector.

by reversed-phase HRLC of fatty acid methyl esters arising after derivatization of fractionated natural products from those tissues. A typical radiochromatogram is shown in Figure 2. The abnormal peak shapes ("leading") could be caused by column overloading or marginal sample solubility in the elution solvent.

After selective organ removal, the carcass remains still contained three-fourths of all tissue radioactivity so this residue was thoroughly examined. Table IV shows that ^{14}C label was found in all fractions containing normal fatty acids. The majority of the radiolabel was in the triglyceride fraction (54% total carcass ^{14}C), but lesser amounts were associated with cholesteryl esters (9%), free fatty acids (2%), and fatty acid esters (3%, probably isolation artifacts). About 80% of all the residual ^{14}C in the carcass

Table IV. Metabolites from [*carboxyl*-^{14}C] Cycloprate in Rat Carcass Remains after Tissues and Organs Removed (4 Days after Dosage)

Carcass remains (13.9% applied dose)	% total ^{14}C in carcass (ppm)
$CHCl_3$ Extract	71.8
1. Triacylglycerol	53.9
Fatty acids	48.3
14(11cPr):0	3.7
16(13cPr):0	30.6
18(15cPr):0	6.0
2. Cholesteryl esters	8.8
Fatty acids	5.6
14(11cPr):0	0.07
16(13cPr):0	2.5
18(15cPr):0	2.2
3. Free fatty acids	1.9
14(11cPr):0	0.14
16(13cPr):0	1.0
18(15cPr):0	0.43
4. Fatty acid esters	3.1
14(11cPr):0	0.22
16(13cPr):0	1.4
18(15cPr):0	0.36
5. Cycloprate	1.1 (0.047)
6. CPCA (free)	<1
7. CPCA (released by saponification)	2.3
CH_3OH Extract	12.5
Saponification products	11.1
Fatty acids	7.6
14(11cPr):0	0.17
16(13cPr):0	2.8
18(15cPr):0	1.6
CPCA	2.1
Residue solids	14.7
Saponification products	13.0
Fatty acids	13.0
14(11cPr):0	0.87
16(13cPr):0	10.4
18(15cPr):0	1.5
Total identified	71.5

remains was in fatty acid fractions. Three ω-cyclopropyl fatty acids [i.e., 14(11cPr):0, 16(13cPr):0, and 18(15cPr):0] represented two-thirds of all the ^{14}C in carcass remains and were present in a ratio of 0.4:4:1. Thus, almost half the residual radiolabel was 16(13cPr):0. The carcass remains contained no free CPCA (<1%), but lipid bound CPCA represented 4% of the total carcass residue. Based on gross amounts of radiolabel, the carcass remains bore 4.31 ppm *equivalents* of cycloprate, but chemical analysis gave only 0.047 ppm (or 1.1% of the total ^{14}C in carcass remains). Hence, tissue residues expressed as ppm equivalents of cycloprate (Table III) are greatly misleading since cycloprate was consistently a minor ^{14}C residue in all tissues examined.

Analysis of fat and muscle (Table V) gave results consistent with the trend of small amounts of residual cycloprate (3% total tissue ^{14}C) and more significant quantities of ω-cyclopropyl fatty acids. 14(11cPr):0, 16(13cPr):0, and 18(15cPr):0 represented 79 and 90% of the total radiolabel in fat and muscle. Again 16(13cPr):0 was the single most abundant residue in these tissues (63–65% total ^{14}C residue) and it was predominantly bound as mixed triglycerides.

The liver had the lowest residue of cycloprate (0.06 ppm) of any tissue examined in detail which probably indicates its facility to promote degradation. 14(11cPr):0, 16(13cPr):0, and 18(15cPr):0 contributed 58% of the total ^{14}C residue in liver (Table VI).

Excretion of Tissue-Bound Radiolabel. When rats dosed at 22 mg/kg were maintained 60 days prior to sacrifice, the distribution of radioactivity in tissues was

Table V. Metabolites from [*carboxyl*-^{14}C] Cycloprate in Fat and Muscle (4 Days after Dosage)

Fat (epididymal and abdominal)	% total ^{14}C in fat
$CHCl_3$ extract	98.7
1. Triacylglycerol	88.5
14(11cPr):0	4.2
16(13cPr):0	62.8
18(15cPr):0	11.9
2. Cycloprate	3.0 (0.39 ppm)
Total identified	81.9
Muscle (pectoral and leg)	**% total ^{14}C in muscle**
$CHCl_3$-CH_3OH Extract	95.8
1. Triacylglycerol	66.3
2. Cholesteryl esters	0.4
3. Other glycerides and free fatty acids	30.6
4. Cycloprate	2.7 (0.045 ppm)
14(11cPr):0	7.3
16(13cPr):0	64.6
18(15cPr):0	18.0
Total identified	92.0

Table VI. Metabolites from [*carboxyl*-^{14}C] Cycloprate in Liver (4 Days after Dosage)

Liver (0.35% applied dose)	% total ^{14}C in Liver
$CHCl_3$ extract	46.8
1. Triacylglycerol	17.2
14(11cPr):0	0.4
16(13cPr):0	12.0
18(15cPr):0	3.0
2. Free fatty acids	23.4
14(11cPr):0	0.7
16(13cPr):0	14.2
18(15cPr):0	7.3
3. Fatty acid esters	0.7
16(13cPr):0	0.4
18(15cPr):0	0.3
4. Cycloprate	0.06 (0.001 ppm)
CH_3OH extract	34.3
Fatty acid methyl esters (after saponification and methylation)	26.4
14(11cPr):0	0.5
16(13cPr):0	7.1
18(15cPr):0	12.5
Total identified	58.5

Table VII. Distribution of Radioactivity in Tissues for Rats Dosed at 21-22 mg/kg with Cycloprate

	% applied dose	
	4 day[a]	60 day[b]
Hide	0.13[c]	0.05[c]
Muscle (rear leg)	0.04[c]	0.04[c]
Brain	0.10	0.05
Fat (brown)		0.03
Fat (perirenal)	0.39	0.30
Fat (epididymal)	0.59	0.45
Carcass remains	13.9	4.45
Total unexcreted ^{14}C	17.9	5.37

[a] Average for four rats. [b] Average for two rats. [c] Per gram wet weight.

used to calculate approximate turnover rates for the ω-cyclopropyl fatty acids (Table VII). The turnover rate for these unusual fatty acids in the *carcass* (first $t_{1/2}$ = 10 days, second $t_{1/2}$ = 42 days) is substantially in agreement with the turnover rate of natural fatty acids. The initial half-life of collective natural fatty acids in the rat carcass has been determined independently as 8 days (White et al., 1964), 9 days (Bernhard and Bullet, 1943), and 16–20 days (Pihl et al., 1950).

Stein and Stein (1965) reported that in vivo metabolism in epididymal fat pads (of rats) which had been radiolabeled with [^{3}H]palmitic and [^{14}C]linoleic acids gave half-lives for these acids of 163 and 187 days, respectively. These data indicate that the half-life of labeled fatty acids is shorter if determined for the whole carcass than if individual fat depots are considered. Thus, it appears that the carcass fat does not form a single metabolic pool, but rather is compartmentalized with a locational dependency for half-life determination.

Table VIII. Comparative Metabolism of [^{14}C] Cycloprate by Male and Female Rats (1 mg/kg Dose)

	% applied dose	
	Male	Female
Urine	72	61
CPCA-gly[a]	61[b]	46
CPCA-carnitine[a]	2[b]	11
Feces	21	16
Carcass	9.4	13.0
Extractable ($CHCl_3$-CH_3OH)	6.2	11.4
ω-cyclopropyl fatty acids (total transesterified)	4.4	10.0
14(11cPr):0	0.7	0.5
16(13cPr):0	2.3	5.8
18(15cPr):0	0.9	1.7
CPCA-carnitine	1.4	0.6
Residual Solids	3.2	1.7
Total Recovery	101	90

[a] Calculated for 4 days using percent in 1-day urine only. [b] Calculated using percent in urine at 21 mg/kg dosage.

Our data also indicate a heterogeneous, location-dependent turnover rate for ω-cyclopropyl fatty acids in the rat. The half-life of these unnatural fatty acids in epididymal fat pads and abdominal fat was ca. 120 days. It is evident from Table VII that elimination of radiolabel from the carcass as a whole was more rapid than from selected tissues such as fat. After 60 days 70% of the 4-day ^{14}C residue was excreted from the carcass while only 24% had been displaced from fat. The profile of ω-cyclopropyl fatty acids in the carcass after 60 days was essentially the same as at 4 days (i.e., 16(13cPr):0 to 18(15cPr):0 = 4:1).

Variable Dosage and Sexual Similarities. All of the discussion to this point has focused on data from dosage at ca. 21 mg/kg. Reduction of the single oral dose from 21 mg/kg to 1 mg/kg lowered the ^{14}C residue as a relative percent of applied dose in the carcass by one-half (or the absolute amount of ^{14}C residue by 40-fold; cf. Table I with Table VIII), although the relative proportion of metabolites was similar (i.e., 14(11cPr):0 to 16(13cPr):0 to 18(15cPr):0 = 0.7:2.5:1.0). Thus, the abundance of ω-cyclopropyl fatty acids in the rat carcass is dose dependent and lower doses of cycloprate result in a smaller net percent retention of these acids in tissues. Excluding considerations of multiple doses, one would expect by extrapolation of these data that as actual field residue quantities of cycloprate are approached, the relative abundance of ω-cyclopropyl fatty acids in tissues should be greatly diminished.

A comparison of the metabolism of cycloprate by male and female rats is given in Table VIII. This work constitutes the first definitive characterization of CPCA-carnitine in rats, although Duncombe and Rising (1972) suggested its presence from TLC analysis when CPCA was incubated with rat liver mitochondria in vitro. There was a tendency for slightly greater retention of ^{14}C in the female carcass and substantially greater excretion of CPCA-carnitine in urine of females. However, considering that only two rats were examined in each case, these differences may not be statistically significant. Thus, the most noticeable feature of the sexual comparison is the

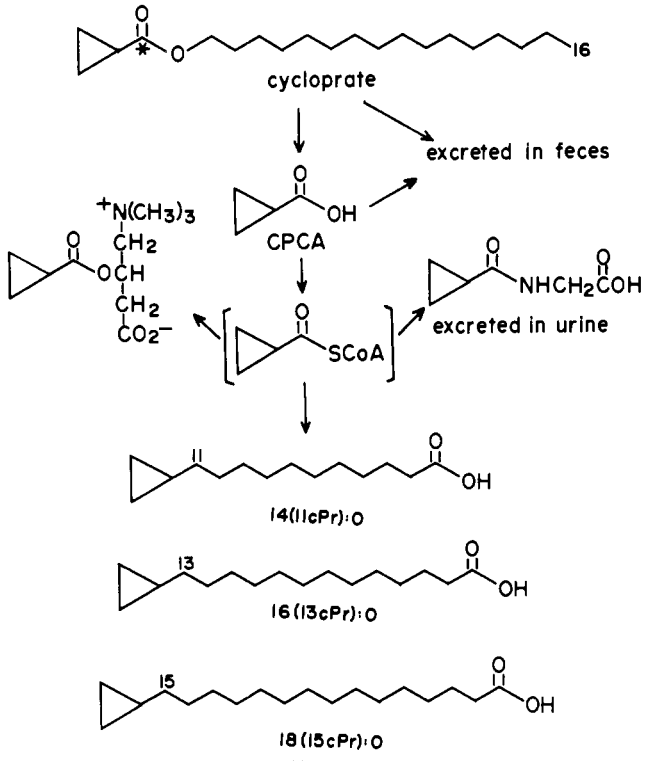


Figure 3. Metabolism of [^{14}C]cycloprate by rats.

overall similarity of male and female degradation of cycloprate.

CONCLUSIONS

No evidence was found for metabolites of CPCA with opened cyclopropane rings (cf. fungal metabolism, Guilbert and Chung, 1974). Moderate amounts of radiolabeled residue were found in rats 4 days after a single oral treatment with cycloprate. Only 1–3% of the radiolabeled residue could be attributed to cycloprate while ω-cyclopropyl fatty acids comprised about 80% of the total tissue residue. These cyclopropyl fatty acids apparently arise by chain elongation of the carboxyl group of CPCA in a manner similar to normal fatty acid biosynthesis. This process results in a series of homologous saturated fatty acids bearing a terminal cyclopropyl group. The analogous unsaturated ω-cyclopropyl fatty acids were not detectable (<10% total long-chain cyclopropyl acids) although unsaturates are relatively abundant from cycloprate metabolism by plants (Quistad et al., 1978). Two-thirds of the rat tissue ^{14}C residue was identified as 14(11cPr):0, 16(13cPr):0, and 18(15cPr):0 while 16(13cPr):0 alone represented about half of the total tissue radiolabel or about 9% of the applied dose. The metabolic fate of cycloprate in rats is summarized in Figure 3.

Mammals are confronted with many natural alicyclic fatty acids (Figure 4). For example, 11-cyclohexylundecanoic acid is a constituent of butterfat, sheep perirenal fat, and ruminant bacteria (Hansen, 1967). Chaulmoogric acid [13-(2-cyclopenten-1-yl)tridecanoic acid] is the major ingredient in chaulmoogra oil which has been used for centuries in leprosy "treatment" (Spener and Mangold, 1974). Since cyclopropane fatty acids (such as lactobacillic) are abundant in certain ubiquitous bacteria in ruminants and to a lesser extent in the intestinal flora of monogastric animals (Christie, 1970), certain mammals have a constant natural exposure to the cyclopropane fatty acids with no apparent detrimental effects. In fact, the metabolic degradation potential for such cyclopropane acids is evidenced by the excretion of 15 mg/day of 3,4-methylenehexanedioic acid in human urine (Lindstedt et al., 1974). Similar metabolism of sterculic acid (cotton seed and *Sterculia* oil) has been reported for rats (Eisele et al., 1977). Alkyl-substituted CPCA even occurs naturally in the rumen tissue of sheep (Body, 1972) and a carboxamide of CPCA (rubesamide) is found in the root bark of certain plants (e.g., *Fagara rubescens*, citrus family) used by natives in Ghana as a decoction to cure toothaches (Dadson and Minta, 1976). An amino-CPCA is also a natural constituent in apples, pears, and cowberries (Burroughs, 1957).

Incorporation of a xenobiotic acid into pathways of fatty acid biosynthesis is of considerable novelty. To our knowledge no pesticide metabolite has ever been reported to undergo chain-elongation reactions in mammals, although such reactions have been encountered in plants. Linscott and Hagin (1970) found that 2,4-dichlorophenoxyacetic acid (applied as the methyl ester) was partially converted by alfalfa to 2,4-dichlorophenoxybutanoic and

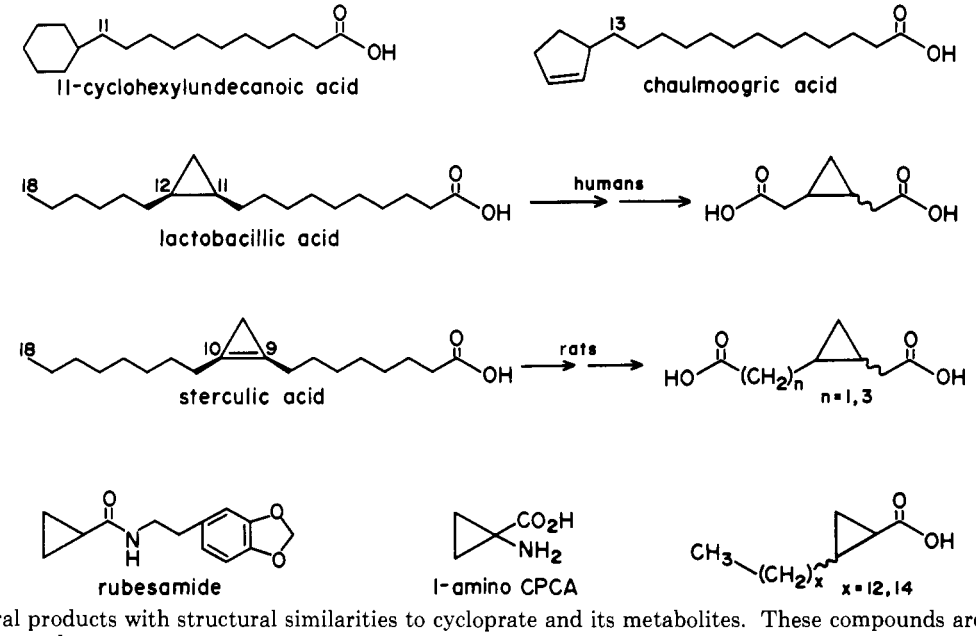


Figure 4. Natural products with structural similarities to cycloprate and its metabolites. These compounds are isolated from (or consumed by) mammals.

-hexanoic acids (addition of one and two acetates, respectively), while 2,4-dichlorophenoxybutanoic acid was metabolized by alfalfa to 2,4-dichlorophenoxyhexanoic (one acetate added) and -decanoic acids (three acetates, Linscott et al., 1968). In contrast, CPCA can form metabolite acids resulting from addition of up to seven acetates in rats (this work) or eight acetates in plants (Quistad et al., 1978). Thus, these results suggest that such chain-elongation reactions may play a relatively widespread role, and investigation of animal tissues for similar chain-elongated products of other acidic metabolites, especially substituted cyclopropanecarboxylic acids derived from pyrethroids, could be of considerable importance.

It should be emphasized that the ω-cyclopropyl fatty acids described herein were only minor products in the free state. Instead, they are predominantly present as a unique class of *nonpolar conjugates* (mostly glycerides) which are more slowly eliminated because of their lipophilicity (cf. Quistad et al., 1976). Since these unusual long-chain cyclopropyl acids are essentially identical in physical properties with natural fatty acids, it is readily apparent that with current analytical technology it would be extremely difficult to analyze for trace amounts of these metabolites which are found in the matrix of a relatively enormous biomass unless metabolites were radiolabeled (impractical for field residue samples).

ACKNOWLEDGMENT

We thank L. L. Dunham for mass spectral analysis and B. A. Garcia and W. Willy for synthesis of authentic cyclopropyl acids. We also acknowledge the helpful advice of T. L. Burkoth.

LITERATURE CITED

Bernhard, K., Bullet, F., *Helv. Chim. Acta* **26**, 75, 1185 (1943).
Blomstrand, R., Rumpf, J. A., *Acta Physiol. Scand.* **32**, 374 (1954).
Body, D. R., *FEBS Lett.* **27**, 5 (1972).
Burroughs, L. F., *Nature (London)* **179**, 360 (1957).
Christie, W. W., "Topics in Lipid Chemistry", Vol. I, Gunstone, F. E., Ed., Wiley, New York, N.Y., 1970, p 40.
Christie, W. W., "Lipid Analysis", Pergamon Press, Oxford, 1973, p 152.
Chung, A. E., *Biochim. Biophys. Acta* **116**, 205 (1966).
Dadson, B. A., Minta, A., *J. Chem. Soc., Perkin Trans 1*, 146 (1976).
Duncombe, W. G., Rising, T. J., *Biochem. J.* **109**, 449 (1968).
Duncombe, W. G., Rising, T. J., *Biochem. Pharmacol.* **21**, 1075 (1972).
Eisele, T. A., Nixon, J. E., Pawlowski, N. E., Sinnhuber, R. O., *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **36**, 1156 (1977).
Guilbert, C. C., Chung, A. E., *J. Biol. Chem.* **249**, 1026 (1974).
Hansen, R. P., *Chem. Ind. (N.Y.)*, 1640 (1967).
Henrick, C. A., Willy, W. E., Staal, G. B., Ludvik, G. F., *J. Agric. Food Chem.* **24**, 1023 (1976).
Linscott, D. L., Hagin, R. D., *Weed Sci.* **18**, 197 (1970).
Linscott, D. L., Hagin, R. D., Dawson, J. E., *J. Agric. Food Chem.* **16**, 844 (1968).
Lindsedt, S., Steen, G., Wahl, E., *Clin. Chim. Acta* **53**, 143 (1974).
Nelson, R. D., Show, E. D., *J. Econ. Entomol.* **68**, 261 (1975).
Pihl, A., Bloch, K., Anker, H. S., *J. Biol. Chem.* **183**, 441 (1950).
Quistad, G. B., Staiger, L. E., Schooley, D. A., *J. Agric. Food Chem.* **22**, 582 (1974).
Quistad, G. B., Staiger, L. E., Schooley, D. A., *J. Agric. Food Chem.* **23**, 750 (1975).
Quistad, G. B., Staiger, L. E., Schooley, D. A., *J. Agric. Food Chem.* **24**, 644 (1976).
Quistad, G. B., Staiger, L. E., Schooley, D. A., *J. Agric. Food Chem.* following papers in this issue.
Schiller, J. G., Chung, A. E., *J. Biol. Chem.* **245**, 5857 (1970).
Schoenheimer, R., Hilgetag, G., *J. Biol. Chem.* **105**, 73 (1934).
Schooley, D. A., Bergot, B. J., Dunham, L. L., Siddall, J. B., *J. Agric. Food Chem.* **23**, 293 (1975).
Spener, F., Mangold, H. K., *Biochemistry* **13**, 2241 (1974).
Staal, G. B., Ludvik, G. F., Nassar, S. G., Henrick, C. A., Willy, W. E., *J. Econ. Entomol.* **68**, 91 (1975).
Stein, Y., Stein, O., "Handbook of Physiology. Section 5: Adipose Tissue", Renold, A. E., Cahill, G. B., Ed., American Physiological Society, Washington D.C., 1965, p 431.
White, A., Handler, P., Smith, E. L., "Principles of Biochemistry", McGraw-Hill, New York, N.Y., 1964, p 438.
Wood, R., Reiser, R., *J. Am. Oil Chem. Soc.* **42**, 315 (1965).

Received for review May 16, 1977. Accepted July 25, 1977. Contribution No. 56 from Zoecon Research Laboratory. Preliminary results from this work were presented at the 173rd National Meeting of the American Chemical Society, Division of Pesticide Chemistry, New Orleans, La., March 1977.

Environmental Degradation of the Miticide Cycloprate. 2. Metabolism by Apples and Oranges

Gary B. Quistad,* Luana E. Staiger, and David A. Schooley

The degradation of cycloprate (hexadecyl cyclopropanecarboxylate) was studied on the foliage and fruit of dwarf apple and orange trees. The single major metabolite on both fruit and foliage was cyclopropanecarboxylic acid (up to 38% applied dose) with ω-cyclopropyl fatty acids also abundant (up to 14% applied dose). These metabolites were found predominantly as polar conjugates, being relatively unimportant as free acids. The mixture of ω-cyclopropyl fatty acids was resolved into 15-cyclopropylpentadecanoic, 15-cyclopropylpentadecenoic, and, apparently, 17-cyclopropylheptadecenoic acids which contributed up to 8, 4, and 0.3% of the applied dose, respectively. Cycloprate applied to the surface of fruit does not penetrate the outer surface and neither cycloprate nor cyclopropanecarboxylic acid translocated into fruit from treated leaves in significant levels (<0.4%).

Cycloprate (hexadecyl cyclopropanecarboxylate, ZR-856, trademark Zardex) is a new miticide (Staal et al., 1975; Nelson and Show, 1975; Henrick et al., 1976). As part of a comprehensive investigation of the environmental fate of cycloprate we now report its degradation by apples and oranges.

Zoecon Corporation Research Laboratory, Palo Alto, California 94304.

EXPERIMENTAL SECTION

Treatment. Leaves and fruit were treated at approximately 1 kg/ha (10 μg/cm^2) by painting via sable hair brush with an emulsion of [*carboxyl*-^{14}C]cycloprate (Quistad et al., 1978). The emulsion was prepared by

0021-8561/78/1426-0070$01.00/0