Pyrethroid Metabolism: Microsomal Oxidase Metabolites of (S)-Bioallethrin and the Six Natural Pyrethrins

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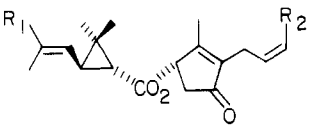
Metabolism of the chrysanthemates (S)-bioallethrin, cinerin I, jasmolin I, and pyrethrin I by NADPH-dependent oxidases of mouse liver microsomes yields 13–18 metabolites in each case oxidized at the methyl, methylene, and alkenyl substituents to form alcohols, aldehydes, carboxylic acids, epoxides, and dihydrodiols. Rat microsomes are more specific than mouse microsomes in hydroxylating the (E)-methyl substituent of the 2-methylpropenyl moiety compared with other molecular sites. Metabolites in the urine of allethrin-treated rats include compounds modified in both the 2-methylpropenyl and allyl moieties as free carboxylic acids and glucuronides. The pyrethrates cinerin II, jasmolin II, and pyrethrin II undergo microsomal hydrolysis of the methoxycarbonyl group and oxidation of the butenyl, pentenyl, and pentadienyl substituents to alcohols, epoxides, and dihydrodiols. Metabolites of these chrysanthemates and pyrethrates are tentatively identified by chemical ionization mass spectrometry following treatment with diazomethane or diazoethane and bis(trimethylsilyl)acetamide and separation by high-resolution gas chromatography with hydrogen as the carrier gas.

The six natural pyrethrins in pyrethrum extract and their synthetic analogue (S)-bioallethrin (i.e., the rethrins) are important insecticides for control of household and stored products pests (Casida, 1973). The metabolic fates



(S)-bioallethrin (**A**): $R_1 = CH_3$, $R_2 = H$

pyrethrin	chrysanthemate (**I**)	pyrethrate (**II**)
cinerins (**C**)	**CI**: $R_1 = CH_3$, $R_2 = CH_3$	**CII**: $R_1 = CO_2CH_3$, $R_2 = CH_3$
jasmolins (**J**)	**JI**: $R_1 = CH_3$, $R_2 = CH_2CH_3$	**JII**: $R_1 = CO_2CH_3$, $R_2 = CH_2CH_3$
pyrethrins (**P**)	**PI**: $R_1 = CH_3$, $R_2 = CH{=}CH_2$	**PII**: $R_1 = CO_2CH_3$, $R_2 = CH{=}CH_2$

of **A** and **PI**, partially defined in microsomal oxidase systems and in rats, involve multiple sites of oxidation with little or no cyclopropanecarboxylate hydrolysis (Casida et al., 1971; Elliott et al., 1972). Comparable information is not available on the other pyrethrum constituents except for their relative ease of oxidation in microsomal oxidase systems (Soderlund and Casida, 1977a). With molecules as complex as the rethrins it is difficult to predict the effects of relatively small structural modifications (e.g., $R_1 = CH_3$, CO_2CH_3; $R_2 = H$, CH_3, CH_2CH_3, $CH{=}CH_2$) on their rates and sites of biodegradation. In further evaluating the comparative fate of the rethrins, it is important to use analytical methods applicable to all of the relevant compounds. The pyrethrum constituents and **A** are conveniently analyzed by high-resolution gas chromatography (HRGC) with an electron-capture detector (ECD) or HRGC–chemical ionization (CI) mass spectrometry (MS). These methods are applied here to the rethrin metabolites, after appropriate derivatizations. This study compares the metabolic fate of **A** and the six natural pyrethrins in mouse and rat liver microsomal oxidase systems and of **A** in rats.

MATERIALS AND METHODS

Structures and Abbreviations for Chemicals. Structures and designations for most of the compounds discussed are given in Figures 1 and 2. The abbreviations ol, al, and acid refer to alcohols, aldehydes, and carboxylic acids, respectively. Trimethylsilyl ethers are designated as TMS, ethyl esters as Et, epoxides as epoxy, diols from hydrolysis of epoxides as dihydrodiol, and their TMS derivatives as dihydro(TMS)$_2$. Thus, **A**-10-TMS is the TMS derivative of the alcohol **A**-10-ol and **CI**-8′,9′-dihydro(TMS)$_2$ is the bis(trimethylsilyl) derivative of the dihydrodiol formed by hydrolysis of **CI**-8′,9′-epoxy. Designations such as 5/6-ol and 6′/10′/11′-ol indicate that the available information does not differentiate among the specified positions. "**PI**" and "**PII**" refer to rearranged products shown in Figures 1 and 2, respectively.

Gas Chromatography and Chemical Ionization Mass Spectrometry. HRGC employed an SPB5 fused silica capillary column (Supelco Inc., Bellafonte, PA) and a split/splitless (1 min) injection at 220 °C. HRGC-ECD with argon/methane (40 mL/min) as the makeup gas was performed with the Hewlett-Packard Model 5840A instrument. HRGC–CI-MS involved the Hewlett-Packard 5985 GC/MS system with the Hewlett-Packard 59870C RTE-A data system. Methane (0.9 Torr) was the reactant gas, and the source temperature was 130 °C. The GC conditions used are given in Table I. The retention times (R_ts) of the parent rethrins (Table I) are used to calculate relative R_ts for the related derivatives (Table II).

Spectroscopy. Nuclear magnetic resonance (NMR) spectroscopy was carried out with a Bruker WM 300 instrument (300 MHz for ^1H and 75 MHz for ^{13}C) and an ASPECT 3000 computer for samples dissolved in chloroform-*d*.

Chemicals. **A** was obtained from McLaughlin Gormley King Co. (Minneapolis, MN) and (1*R*)-*cis*-permethrin from FMC Corp. (Princeton, NJ). The six pyrethrins were isolated from purified pyrethrum extract (72% pyrethrins) (McLaughlin Gormley King Co.). Preparative thin-layer chromatography (TLC) on silica gel (*n*-hexane/acetone, 4:1) separated chrysanthe-

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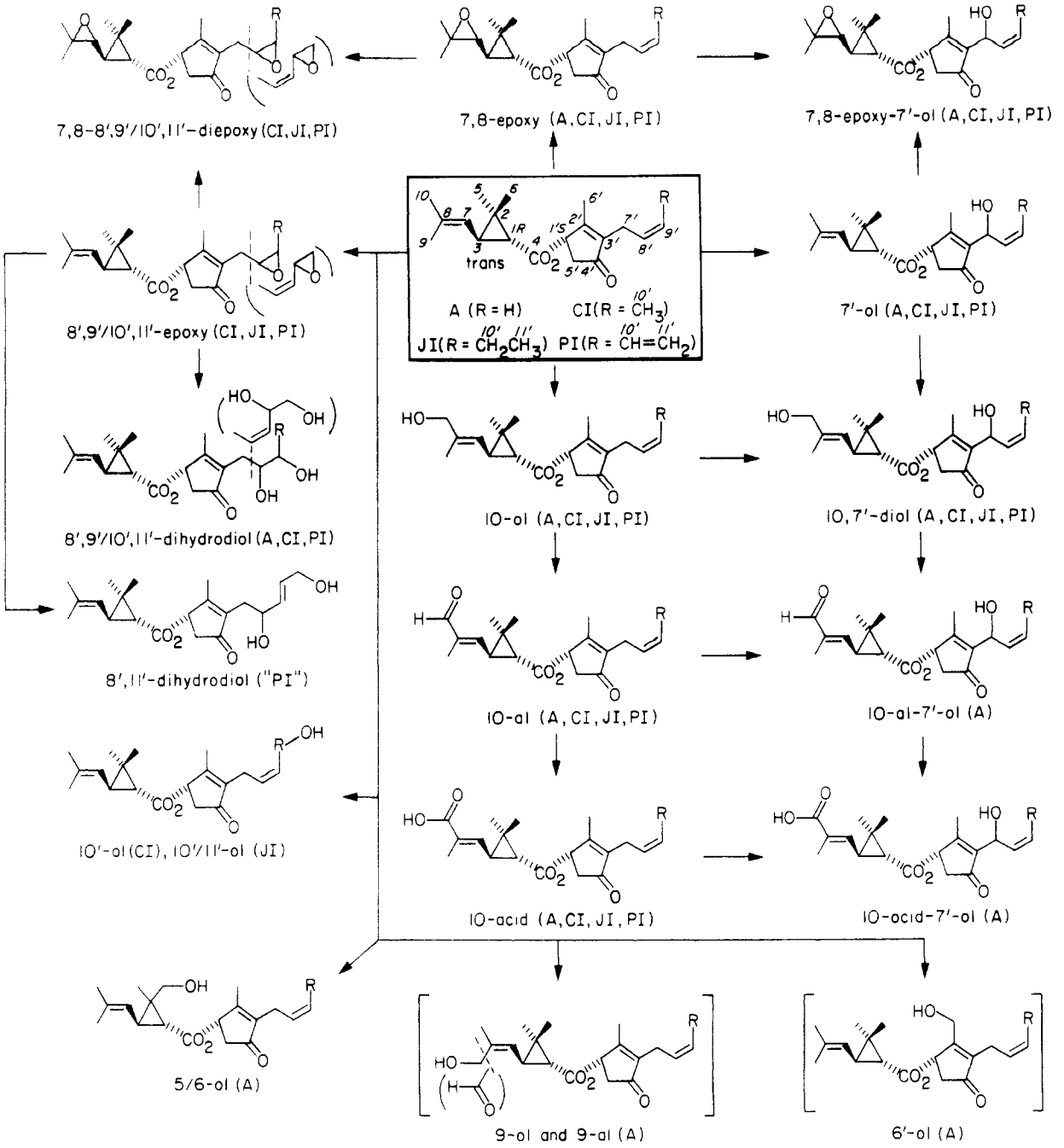


Figure 1. Partial metabolic pathways for (*S*)-bioallethrin (**A**), cinerin I (**CI**), jasmolin I (**JI**), and pyrethrin I (**PI**) in mouse and rat liver microsomal oxidase systems and of (*S*)-bioallethrin in rats in vivo. Additional metabolites not designated by structures arise from other combinations of modifications in the acid and alcohol moieties. Brackets designate compounds tentatively identified from SeO_2 oxidation and indicated but not established as metabolites.

mates (R_f 0.49) and pyrethrates (R_f 0.28). High-performance liquid chromatography (HPLC) on a Macherey-Nagel Nucleosil 5 NO_2 column (10 mm (i.d.) × 25 cm) (Rainin Instrument Co., Inc., Woburn, MA) was used with 5% tetrahydrofuran (THF) in *n*-hexane (2 mL/min) to separate **JI**, **CI**, and **PI** [R_t (min) 18.6, 20.0 and 23.2, respectively] and with 10% THF in *n*-hexane (2 mL/min) to separate **JII**, **CII**, and **PII** [R_t (min) 20.9, 22.6, and 25.8, respectively] (Ando et al., 1986; McEldowney and Menary, 1988). The *E*-8'-isomers of **JI**, **CI**, and **PI** were prepared by irradiation for 1 h of the individual natural *Z*-8'-isomers in degassed *n*-hexane with ultraviolet light at 300 nm and purification by HPLC as above with 5% THF in *n*-hexane [R_t (min) for *E* isomers of 20.4 for **JI**, 21.4 for **CI**, and 24.9 for **PI**]. In each photoisomerization reaction there was retention of the (1*R*)-*trans*-acid and (1'*S*)-alcohol configurations. The identity of each pyrethrum constituent and of the photoisomers was confirmed by NMR (Bramwell et al., 1969; Crombie et al., 1975; Ando and Casida, 1983). Purities for the rethrins used in the metabolism studies were established by HRGC-ECD as >96% for the pyrethrins and their photoisomers and >98% for **A**. Phenyl saligenin cyclic phosphonate (PSCP), a potent esterase inhibitor (Casida et al., 1961), was prepared by C. J. Palmer of this laboratory.

Two diastereomers (1:1) of **A**-7,8-epoxy were obtained in 90–100% yield by treatment of **A** (1 mmol) with equimolar 3-chloroperoxybenzoic acid in dichloromethane (10 mL) for 1 h at 25 °C with product separation by preparative TLC on silica gel (*n*-hexane/diethyl ether, 2:1) and characterization by NMR (supplementary Table I) and HRGC–MS (Tables II and III). The higher R_f isomer (0.26) with the slightly smaller GC R_t is tentatively assigned the 7*S* configuration, and the lower R_f isomer (0.19) with the slightly larger GC R_t is assigned the 7*R* configuration. This is based on examination of space-filling models that show that rotation around the C_3–C_7 bond is more hindered for the 7*R* isomer (with H_3 and H_7 in a predominantly anti conformation) than for the 7*S* isomer. Thus, the larger

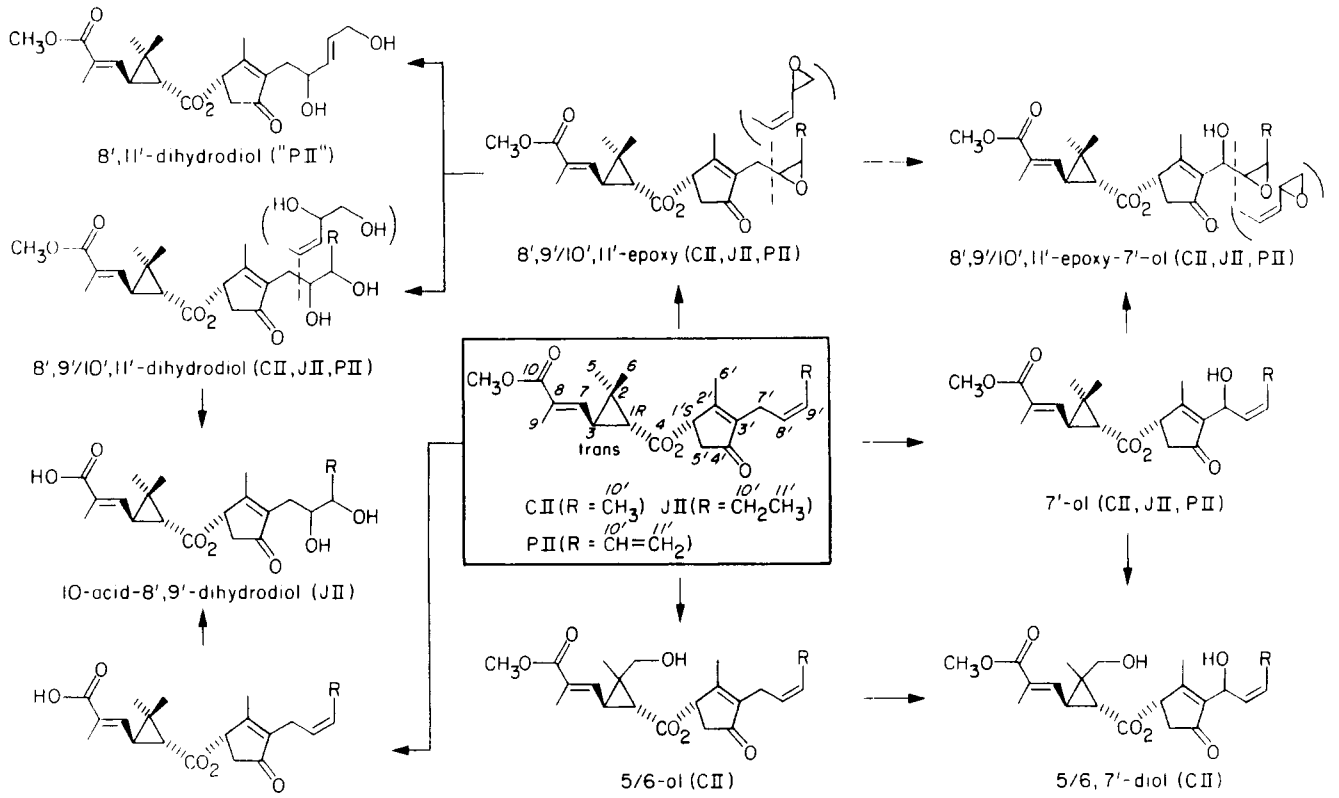


Figure 2. Partial metabolic pathways for cinerin II (**CII**), jasmolin II (**JII**), and pyrethrin II (**PII**) in mouse liver microsomal oxidase systems. Although not observed, **PII**-10',11'-epoxy is included as a likely intermediate.

Table I. Gas Chromatographic Retention Times of (*S*)-Bioallethrin and the Six Natural Pyrethrins

	HRGC retention time,[a] min		
rethrin	H_2 ECD[b]	H_2 CI-MS[c]	He CI-MS[d]
A	5.30	9.35	9.90
CI	6.02(5.85)[e]	11.21(10.74)[e]	10.70
JI	6.58(6.41)[e]	12.59(12.13)[e]	11.39
PI	6.80(6.64)[e]	13.21(12.80)[e]	
CII	9.16	18.06	14.40
JII	10.20	19.71	15.50
PII	10.60	20.47	

[a] R_t values (min) for (1*R*)-*cis*-permethrin used as internal standard: 10.80 in H_2 ECD; 21.24 in H_2 CI-MS; 17.46 in He CI-MS. [b] Hydrogen as carrier gas (8 psi, 50 cm/s). SPB5 (15 m × 0.32 mm (i.d.), 0.25-μm film) capillary column. Temperature program of 90 °C for 2 min, 30 °C/min to 180 °C, and finally 3 °C/min to 240 °C. A modified temperature program (90 °C for 2 min, 30 °C/min to 150 °C, and finally 2 °C/min to 200 °C) was used in one special study comparing the metabolism of coincubated **A**, the natural chrysanthemates, and their *E*-8'-isomers to give R_t values (min) of 6.74, 8.36, 8.78, 9.87, 10.31, 10.47, and 10.91 for **A**, (*E*)-8'-**CI**, **CI**, (*E*)-8'-**JI**, **JI**, (*E*)-8'-**PI**, and **PI**, respectively. [c] Hydrogen as carrier gas (30 psi, 80 cm/s). SPB5 (30 m × 0.25 mm (i.d.), 0.25-μm film) capillary column. Temperature program of 90 °C for 2 min, 30 °C/min to 180 °C, and finally 3 °C/min to 240 °C. [d] Helium as carrier gas (15 psi, 30 cm/s). SPB5 capillary column as in *c*. Temperature program of 90 °C for 2 min, 30 °C/min to 240 °C, and finally 3 °C/min to 280 °C. **PI** and **PII** give broad peaks due to isomerization on the column. [e] *E*-8'-isomer.

J_{3-7} coupling is expected (based on the Karplus relationship) for the 7*R* isomer (J = 8.0 Hz) than for the 7*S* isomer (J = 3.8 Hz).

A-10-ol and **A**-10-al were each obtained in ~30% yield on treatment of **A** (1 mmol) with equimolar SeO_2 in dioxane/water (10:1) (5 mL) under reflux (1 h). They were isolated by preparative TLC on silica gel (*n*-hexane/diethyl ether, 1:1; R_f 0.15 for **A**-10-ol and 0.27 for **A**-10-al) and characterized by NMR (supplementary Table I) and HRGC–MS (Tables II and III). The oxidation occurs almost exclusively at the 10-position (Matsui and Yamada, 1963; Crombie et al., 1970).

A-7'-ol was synthesized from (*S*)-allethrolone (Roussel-Uclaf, Paris, France). This alcohol moiety was acetylated with acetic anhydride in pyridine and treated with SeO_2 under the conditions described above for oxidation of **A**. From the oxidized mixture, preparative TLC (*n*-hexane/acetone, 2:1; R_f 0.44) separated the derivative of allethronyl acetate with a hydroxyl group at the 7'-position in ~20% yield. After protection of the hydroxyl group with dihydropyran, the acetoxyl group was hydrolyzed with K_2CO_3 in methanol to obtain the alcohol with the tetrahydropyranyl (THP) ether at the 7'-position, which was coupled with (1*R*)-*trans*-chrysanthemoyl chloride in benzene. The THP ether was removed with *p*-toluenesulfonic acid in ethanol (Ando and Casida, 1983) to give a mixture of two diastereomers (1:1) of **A**-7'-ol (~20% overall yield from 7'-hydroxyallethronyl acetate). The two diastereomers were separated by HPLC with the Nucleosil 5 NO_2 column [*n*-hexane/THF, 4:1; R_t (min) 25.4, 28.1] and analyzed by NMR and HRGC–MS (Tables II and III). The isomer eluting first on HPLC is also the one eluting first on GC (Table II). NMR data of the two diastereomers reveal the 7'-position of the hydroxyl group but are not suitable to assign its configuration (supplementary Table I).

A-8',9'-epoxy (50 mg), prepared from (*S*)-allethrolone by the method of Ando et al. (1983), was stirred in methanol/water (2:1) (3 mL) with one drop of H_2SO_4 at 25 °C, resulting in hydrolysis of the epoxy derivative in 1 h. **A**-8',9'-dihydrodiol was recovered (yield ~60%) on addition of saturated aqueous $NaHCO_3$ (5 mL) and extraction with ethyl acetate. The epoxy and dihydrodiol derivatives are mixtures of two diastereomers (8'*S* and 8'*R* isomers) in ca. 1:1 ratio. Since HPLC on the NO_2 column was not sufficient for separating the isomers [**A**-8',9'-epoxy; *n*-hexane/THF, 9:1; R_t (min) 26.5, 26.9. **A**-8',9'-dihydrodiol; *n*-hexane/THF, 1:1; R_t (min) 60.0, 62.8], they were analyzed by NMR (supplementary Table I) and utilized as standards for the metabolic studies without separation.

Treatment of **A**-10-ol, **A**-7'-ol, and **A**-8',9'-dihydrodiol with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) in acetonitrile for 1 h at 80 °C gave **A**-10-TMS, **A**-7'-TMS, and **A**-8',9'-dihydro-$(TMS)_2$, respectively, characterized by HRGC–MS (Tables II and III).

Table II. Relative Gas Chromatographic Retention Times of Rethrin Metabolites and Their GC Derivatives

designation	retention time relative to parent rethrin,[a] min						
	A	**CI**	**JI**	**PI**	**CII**	**JII**	**PII**
parent rethrin	1.00	1.00	1.00	1.00	1.00	1.00	1.00
10-Et					1.06	1.07	1.04
7,8-epoxy (*R* isomer)	1.11	1.11	1.11	1.09			
7′-TMS(a,b)[b]	1.20, 1.25	1.14, 1.17	1.09, 1.10	1.08, 1.10	1.09, 1.13	1.07, 1.09	nd,[c] 1.04
8′,9′/10′,11′-epoxy[d,e]	nd	1.25	1.24	1.15	1.19	1.18	nd
7,8-8′,9′/10′,11′-diepoxy[d]	nd	1.39	1.37	1.28			
7,8-epoxy-7′-TMS(a,b)	1.36, 1.39	1.26, 1.30[f]	nd, 1.24	nd, 1.21			
8′,9′/10′,11′-epoxy-7′-TMS(a,b)	nd	nd	nd	nd, 1.26	1.27, 1.29	1.24, 1.29	1.11, 1.16
10-al	1.46	1.43	1.41	1.38			
10-TMS	1.63	1.56	1.52	1.51			
TMS	1.50,[g] 1.71	1.49, 1.57	1.38, 1.44	nd	1.24,[g] 1.33, 1.39	nd	nd
$(TMS)_2$	1.91,[h] 1.93[h]	1.65, 1.71,[h] 1.75,[h] 2.01	1.56, 1.65[h]	1.60[h]	1.34, 1.42, 1.55	1.18, 1.27, 1.29	1.44
dihydro$(TMS)_2$[i]	1.96	1.61	nd	1.44, 1.73	1.40	1.36 (1.42)	1.27, 1.45

[a] HRGC(H_2)–CI-MS system described in Table I. [b] Relative R_t 1.24 and 1.26 for **A**-7′-ol (a) and (b), respectively (Supplemental Figure 2). [c] Not detected. [d] 8′,9′-Epoxy for **CI**, **CII**, **JI**, and **JII** and 10′,11′-epoxy for **PI**. [e] Relative R_t 1.35 for synthetic **A**-8′,9′-epoxy. [f] Relative R_t 1.63 for the related **CI**-7,8-epoxy-6′/10′-TMS. [g] Probably the 5/6-TMS derivative. Likely positions for other TMS derivatives are 6′ for **A**, 6′/10′ for **CI** and **CII**, and 6′/10′/11′ for **JI**. [h] Individual or unresolved 10,7′-diastereomers. Each of the other $(TMS)_2$ derivatives is modified in both the acid (probably position 10 of chrysanthemates and 5/6 of pyrethrates) and alcohol moieties. [i] The 8′,9′-dihydro$(TMS)_2$ derivatives of **A**, **CI**, **CII**, and **JII**, the 8′,11′-dihydro$(TMS)_2$ derivatives of "**PI**" and "**PII**" (smaller R_t), and the 10′,11′-dihydro$(TMS)_2$ derivatives of **PI** and **PII** (larger R_t). The corresponding 7,8-epoxy derivatives are detected at relative R_t 1.57 and 1.84 for "**PI**"-7,8-epoxy-8′,11′-dihydro$(TMS)_2$ and **PI**-7,8-epoxy-10′,11′-dihydro$(TMS)_2$, respectively. The number in parentheses for **JII** is the ethyl ester from enzymatic hydrolysis and treatment with diazoethane. Relative R_t 1.82 for **A**-8′,9′-dihydrodiol.

Table III. Partial CI-MS Data (*m/z* and Relative Intensity) for (*S*)-Bioallethrin (A) and Its Metabolites and Their GC Derivatives

	ions characteristic for											
	ester				acid moiety				alcohol moiety			
designation[a]	$[MH]^+$	$[M+C_2H_5]^+$	$[MH-CH_4]^+$	$[MH-X^bOH]^+$	$[RC(OH)_2]^+$[c]	$[RCO]^+$	$[R]^+$	$[RC(OH)_2-(TMS)OH]^+$	$[R'+C_2H_4]^+$	$[R']^+$	$[R'H_2-(TMS)OH]^+$	$[R'-(TMS)OH]^+$
A	303 (70)	331 (8)			169 (32)	151 (42)	123 (25)		163 (30)	135 (100)		
					Metabolites							
A-10-al[d]	317 (60)	345 (12)			183 (55)	165 (35)	137 (70)		163 (12)	135 (100)		
A-7,8-epoxy[d,e]	319 (8)	347 (4)		301 (20)	185 (15)	167 (75)	139 (20)		163 (18)	135 (100)		
A-8′,9′-epoxy[f]	319 (100)	347 (8)		301 (1)	169 (38)	151 (73)	123 (23)		179 (14)	151 (73)		
A-10-ol[d]	319 (6)	347 (4)		301 (20)		167 (75)	139 (20)		163 (20)	135 (100)		
A-7′-ol[d,e]	319 (<2)	347 (4)		301 (20)	169 (50)	151 (60)	123 (60)			151 (60)		
A-10-Et	361 (50)	389 (8)		315 (20)	227 (100)	209 (20)	181 (45)		163 (55)	135 (100)		
					Trimethylsilyl Ethers of Alcohols							
A-10-TMS	391 (8)	419 (6)	375 (8)	301 (22)	257 (12)	239 (4)		167 (80)	163 (20)	135 (100)		
A-9-TMS[g]	391 (4)	419 (5)	375 (4)	301 (26)	257 (15)	239 (6)		167 (100)	163 (20)	135 (100)		
A-5/6-TMS[g]	391 (2)	419 (2)	375 (4)	301 (6)	257 (6)	239 (6)		167 (40)	163 (12)	135 (100)		
A-5′-TMS[g]	391 (4)	419 (4)	375 (20)	301 (75)	169 (75)	151 (100)	123 (75)		251 (14)	223 (80)	135 (75)	133 (80)
A-6′-TMS[g]	391 (36)	419 (8)	375 (10)	301 (6)	169 (50)	151 (100)	123 (55)		251 (6)	223 (42)	135 (30)	133 (15)
A-7′-TMS[d,e]	391 (5)	419 (10)	375 (45)	301 (100)	169 (22)	151 (26)	123 (22)		251 (14)	223 (45)	135 (25)	133 (60)
A-10-al-7′-TMS	405 (2)	433 (10)	389 (18)	315 (100)	183 (20)	165 (30)	137 (50)		251 (6)	223 (32)	135 (70)	133 (75)
A-10-Et-7′-TMS	449 (2)	477 (4)	433 (8)	359 (55)	227 (100)	209 (40)	181 (50)		251 (6)	223 (30)	135 (70)	133 (65)
A-7,8-epoxy-7′-TMS[e]	407 (2)	435 (4)	391 (12)	317 (40)	185 (8)	167 (80)	139 (30)		251 (10)	223 (30)	135 (50)	133 (100)
A-10,7′-$(TMS)_2$[e]	479 (4)	507 (8)	463 (40)	389 (100)	257 (22)	239 (50)	211 (6)	167 (50)	251 (18)	223 (60)	135 (35)	133 (88)
A-8′,9′-dihydro-$(TMS)_2$[d]	481 (70)	509 (8)	465 (60)	391 (100)	169 (50)	151 (70)	123 (50)				225 (18)	223 (36)
A-10-Et-8′,9′-dihydro-$(TMS)_2$	539 (28)	567 (4)	523 (15)	449 (2)	227 (100)	209 (35)	181 (74)		341 (2)	313 (6)	225 (28)	223 (40)

[a] GC properties are shown in Tables I and II, Figures 4 and 5, and Supplementary Figures 1 and 2. [b] X = H for epoxides and alcohols, C_2H_5 for ethyl esters, and TMS for trimethylsilyl ethers. [c] An additional $[RC(OC_2H_5)OH]^+$ fragment ion is present in most MS with an intensity of 10–20% relative to $[RC(OH)_2]^+$. [d] MS of standards essentially identical with those of metabolites. [e] Two diastereomers have very similar MS. [f] Synthetic standard not detected as metabolite. [g] MS for compounds tentatively designated **A**-5/6-TMS, **A**-9-TMS, **A**-5′-TMS, and **A**-6′-TMS are from products formed by SeO_2 oxidation followed by BSA derivatization. The 5/6-ol, 9-ol, and 6′-ol but not the 5′-ol appear to be trace microsomal metabolites.

Formation and Analysis of Microsomal Metabolites. The substrate (0.1 μmol) was incubated with twice-washed mouse or rat liver microsomes (Johnston et al., 1989) (0.1, 0.3, 1.0, or 3.0 mg of protein) and NADPH (0 or 2.4 μmol) in phosphate buffer (0.1 M, pH 7.4, 2 mL) with shaking for 1 h at 37 °C. An internal standard (0.1 μmol of (1*R*)-*cis*-permethrin or 0.025 μmol of **A**) in ethanol (10 μL) was then added, and the mixture was saturated with NaCl and extracted with ethyl acetate (2 mL). The aqueous phase was acidified with HCl (2 N) to pH 2–3 and extracted again with ethyl acetate (2 mL). After the combined organic extract was dried with $MgSO_4$, the organosoluble metabolites were treated with diazomethane or diazoethane (generated from 1-methyl- or 1-ethyl-3-nitro-1-nitrosoguanidine) in diethyl ether (2 h, 25 °C) to obtain the esters, and then after concentration under a stream of nitrogen the residue was treated with a 10% acetonitrile solution (25 μL) of BSA (0.5 h, 80 °C). Before and following each derivatization step, the solutions were analyzed by HRGC-ECD injecting an equivalent of 0.01–0.1 nmol

Table IV. Partial CI-MS Data (m/z and Relative Intensity) for Pyrethrin I and Its Metabolites and Their GC Derivatives

	ions characteristic for											
	ester				acid moiety				alcohol moiety			
designation[a]	$[MH]^+$	$[M + C_2H_5]^+$	$[MH - CH_4]^+$	$[MH - X^bOH]^+$	$[RC(OH)_2]^{+c}$	$[RCO]^+$	$[R]^+$	$[RC(OH)_2 - (TMS)OH]^+$	$[R' + C_2H_4]^+$	$[R']^+$	$[R'H_2 - (TMS)OH]^+$	$[R' - (TMS)OH]^+$
Pyrethrins												
PI	329 (70)	357 (9)			169 (25)	151 (25)	123 (25)		189 (20)	161 (100)		
PII[d]	373 (80)	401 (10)		341 (3)	213 (40)	195 (12)	167 (12)		189 (30)	161 (100)		
Metabolites												
PII[e]	373 (30)	401 (4)		341 (<2)	213 (40)	195 (22)	167 (30)		189 (30)	161 (100)		
PI-10-al	343 (40)	371 (6)			183 (40)	165 (60)	137 (50)		189 (36)	161 (100)		
PI-7,8-epoxy	345 (6)	373 (4)		327 (8)	185 (10)	167 (50)	139 (40)		189 (14)	161 (100)		
PI-10′,11′-epoxy	345 (40)	373 (6)			169 (50)	151 (50)	123 (70)			177 (40)		
PI-7,8-10′,11′-diepoxy	361 (18)	389 (6)		343 (4)		167 (100)	139 (70)			177 (90)		
Trimethylsilyl Ethers of Alcohols												
PI-10-TMS	417 (4)	445 (2)	401 (2)	327 (8)	257 (4)	239 (6)		167 (40)	189 (10)	161 (100)		
PI-7′-TMS[f]	417 (28)	445 (6)	401 (32)	327 (36)	169 (75)	151 (70)	123 (95)		277 (8)	249 (20)		
PI-7,8-epoxy-7′-TMS	433 (2)	461 (<2)	417 (8)	343 (20)		167 (100)	139 (70)		277 (4)	249 (20)	161 (40)	159 (90)
PI-10′,11′-epoxy-7′-TMS[g]	433 (60)	461 (20)	417 (60)	343 (80)	169 (28)	151 (30)	123 (30)		293 (8)	265 (22)	177 (10)	175 (22)
PI-10,7′-$(TMS)_2$	505 (<2)	533 (<2)	489 (6)	415 (10)	257 (4)	239 (16)		167 (80)		249 (20)	161 (70)	159 (55)
"**PI**"-8′,11′-dihydro$(TMS)_2$	507 (18)	535 (2)	491 (16)	417 (12)	169 (70)	151 (60)	123 (100)		367 (2)	339 (6)	251 (12)	249 (32)
PI-10′,11′-dihydro$(TMS)_2$	507 (4)	535 (4)	491 (20)	417 (70)	169 (100)	151 (80)	123 (85)		367 (2)	339 (6)	251 (12)	249 (60)
"**PI**"-7,8-epoxy-8′,11′-dihydro$(TMS)_2$[g]	523 (100)	551 (20)	507 (95)	433 (65)	185 (5)	167 (80)	139 (20)			339 (30)	251 (33)	249 (90)
PI-7,8-epoxy-10′,11′-dihydro$(TMS)_2$	523 (4)	551 (8)	507 (30)	433 (70)	185 (5)	167 (60)	139 (10)			339 (15)	251 (20)	249 (100)

[a] GC properties are given in Tables I and II and Figure 6. [b] X = H for epoxides and alcohols, CH_3 for **PII**, and TMS for trimethylsilyl ethers. [c] An additional $[RC(OC_2H_5)OH]^+$ fragment ion is present in most MS with an intensity of 10–20% relative to $[RC(OH)_2]^+$. [d] Standard from pyrethrum extract. [e] MS obtained from trace level of product from enzymatic oxidation followed by methylation with diazomethane. Relative R_t identical with that of **PII**. [f] Two diastereomers with similar MS. [g] MS obtained at slightly different instrumental conditions favoring higher intensities for larger m/z.

of internal standard in ethyl acetate (1–4 μL). Changes in chromatographic behavior on derivatization were used to interpret newly introduced functional groups of the metabolites. Finally, the extracts were concentrated to 30 μL and analyzed by HRGC–CI-MS. The amount of substrate recovered was determined by HRGC–ECD relative to the internal standard. Recoveries of the chrysanthemates were 100 ± 10% at 0–3 mg of microsomal protein in the absence of NADPH. The extent of metabolism of the chrysanthemates was therefore calculated from the loss of substrate with NADPH.

One special study compared the microsomal metabolism of the pyrethrates in the presence and the absence of PSCP. The esterase inhibitor (0 or 0.04 μmol) in ethanol (10 μL) was added to the microsomes in buffer with preincubation for 5 min at 37 °C before adding the substrate and NADPH and proceeding as above. The extent of pyrethrate metabolism was determined as substrate loss for incubations with microsomes compared with no microsomes. Another investigation compared the extent of metabolism of the *E*-8′-versus the *Z*-8′-isomers of **CI**, **JI**, and **PI** incubated as a mixture with **A** each at 0.014 μmol/incubation with 0.3 and 1.0 mg of mouse microsomal protein and NADPH. The incubation and analysis methods were the same as above except for a modified temperature program (Table I).

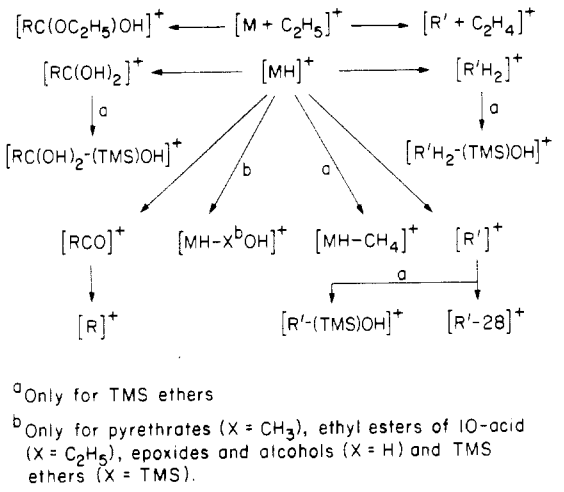
Formation and Analysis of Urinary Metabolites. Male albino rats were treated with **A** orally by stomach tube (250 mg/kg followed after 6 h with 500 mg/kg) or intraperitoneally (12.5 mg/kg followed after 6 h with 25 mg/kg) with methyl sulfoxide (99.9%; Aldrich Chemical Co., Milwaukee, WIS) as the carrier vehicle. None of the treatments produced any signs of poisoning. Urine collected 0–6 h after the second treatments (and comparable control urine) were frozen and later analyzed for free and conjugated urinary metabolites. Deconjugation involved incubating (16 h, 37 °C) 0.1 mL of urine in phosphate buffer (0.4 M, pH 4.5, 1.9 mL) either with β-glucuronidase (0 or 63 units, Type 1 bacterial; Sigma Chemical Co., St. Louis, MO) alone and with D-saccharic acid 1,4-lactone (1.0 mg; Sigma; to inhibit β-glucuronidase activity; Capel et al., 1974) or with arylsulfatase (0 or 12 units; Type V; Sigma). The deconjugation mixtures were analyzed as outlined above for microsomal incubations.

RESULTS

Analysis of Rethrins. *High-Resolution Gas Chromatography.* Table I gives the GC R_t values for the seven parent rethrins and for the photoisomers of the chrysanthemates in three different HRGC systems. Hydrogen was employed instead of helium as the carrier gas in analyzing the pyrethrins since with helium the higher required column temperature of above 240 °C results in thermal isomerization of **PI** and **PII** [discussed by Elliott (1964)]. ECD is particularly sensitive and selective for the rethrins, thereby providing a method to analyze subnanogram amounts of the parent compounds and their derivatives in the present study.

Chemical Ionization Mass Spectrometry (Tables III and IV; Supplementary Tables II–IV). Electron impact MS of the rethrins shows weak molecular ions and the primary fragments result from fission at the ester linkage (King and Paisley, 1969; Pattenden et al., 1973). CI-MS is preferred since protonation by CH_5^+ and addition of reagent ions at the carbonyl oxygens result in intense quasi-molecular ions $[MH]^+$ (relative intensity 70–100%) and ion adducts $[M + C_2H_5]^+$ and $[M + C_3H_5]^+$. An interpretation of the CI-MS fragmentation pathways of the rethrins [RC(O)OR′] is given in Figure 3 [see also Munson and Field (1966) and Herman and Harrison (1981)]. Ion series from the acid moiety are $[R + 74]^+$, $[R + 46]^+$, $[R + 28]^+$, and $[R]^+$ whereas those from the alcohol moiety are $[R' + 28]^+$, $[R' + 2]^+$, $[R']^+$, and $[R' - 28]^+$. Pyrethrates show some loss of CH_3OH.

Examination of the authentic derivatives of **A** [10-al, 7,8-epoxy, 10-ol, 10-TMS, 7′-ol, 7′-TMS, 8′,9′-epoxy, 8′,9′-dihydro$(TMS)_2$] established the changes in CI-MS fragmentation patterns with known structural modifications (Figure 3; Table III). The aldehyde shows CI-MS similar to the parent rethrin with fragments characteristic for the acid moiety shifted by addition of 14 mass units. The 7,8-epoxides but not the 8′,9′-epoxides show loss of H_2O on CI-MS; the position of epoxidation is indicated by incorporation of oxygen into the fragments of the acid or alcohol moiety, respectively. The hydroxy derivatives give weak $[MH]^+$ signals, while loss of H_2O predominates. The position of modification in the acid or alcohol moiety is indicated by changes in the fragment series originating from their respective portion of the parent



[a] Only for TMS ethers

[b] Only for pyrethrates (X = CH_3), ethyl esters of 10-acid (X = C_2H_5), epoxides and alcohols (X = H) and TMS ethers (X = TMS).

Figure 3. Proposed partial CI-MS fragmentation pathways for the rethrins, RC(O)OR′, and their derivatives. Additional ion (not tabulated) is $[M + C_3H_5]^+$.

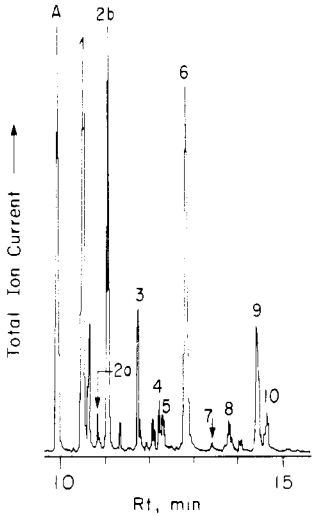


Figure 4. HRGC(He)–MS chromatogram of (*S*)-bioallethrin and some of its mouse liver microsomal (1.0 mg of protein) oxidase metabolites after treatment with diazoethane and BSA. Metabolites are characterized or tentatively identified as indicated in the text. Peaks: A = (*S*)-bioallethrin; 1 = **A**-7,8-epoxy; 2a,b = **A**-7′-TMS(a,b); 3 = **A**-7,8-epoxy-7′-TMS(b); 4 = **A**-10-al; 5 = **A**-5/6-TMS; 6 = **A**-10-TMS; 7 = **A**-10-Et; 8 = **A**-10-al-7′-TMS; 9 = **A**-10,7′-$(TMS)_2$; 10 = **A**-8′,9′-dihydro-$(TMS)_2$. Peaks 1 and 5 contain the indicated metabolite incompletely resolved from endogenous materials. Some of the unidentified peaks are normal microsomal components. **A**-10-Et-7′-TMS as a derivative of a urinary metabolite of **A** chromatographs immediately after peak 8.

molecule. The TMS derivatives lose CH_4 and (TMS)OH as neutral species, resulting in $[MH - 16]^+$ and $[MH - 90]^+$ fragments, respectively. The presence of the $[RC(OH)_2 - (TMS)OH]^+$ ion or of the $[R'H_2 - (TMS)OH]^+$ and $[R' - (TMS)OH]^+$ ions establishes the site of the TMS on the acid or alcohol moiety, respectively.

Metabolites of **A** and the six natural pyrethrins and their GC derivatives not available as synthetic standards can be tentatively identified by the presence of ions characteristic for the intact esters and of ion series unique for the modified or unmodified acid and alcohol moieties and by the loss of CH_4 and (TMS)OH in the case of trimethylsilyl ethers. The same criteria can be used for metabolites modified at two sites in the molecule.

Selenium Dioxide Oxidation of (*S*)-Bioallethrin. Treatment of **A** with SeO_2 gives primarily **A**-10-ol and **A**-10-al. Additional minor products become evident when the two principal compounds are mostly removed by TLC and the remaining portion is derivatized with BSA and analyzed by HRGC–MS (Table III; Supplementary Figure 1). One minor product is considered to be **A**-9-ol since its TMS derivative gives a slightly smaller R_t value and the same CI-MS characteristics as **A**-10-TMS. The TMS derivatives of two additional minor products, tentatively designated as **A**-5-TMS and **A**-6-TMS, show an unmodified allethrolone moiety but hydroxylation in the acid portion. Three other TMS derivatives are modified only in the allethrolone moiety. Two of these compounds, designated together as **A**-5′-TMS, give the same CI-MS characteristics appropriate for a diastereomeric pair. The third, indicated as **A**-6′-TMS, is a single product of different CI-MS properties. These tentative assignments of the trimethylsilyl ethers are consistent with a more intense fragment originating from loss of (TMS)OH for the TMS derivatives of the secondary alcohols [**A**-5′-TMS and **A**-7′-TMS (not detected as SeO_2 product in the fractions analyzed)] than of the primary alcohols (**A**-9-TMS, **A**-10-TMS, **A**-5/6-TMS, **A**-6′-TMS). Although not illustrated, additional SeO_2 products are observed with oxidation in both the acid moiety (e.g., 10-al and 10-ol) and the alcohol moiety.

Microsomal Metabolism of Rethrins. The chrysanthemates undergo significant metabolism in the mouse or rat liver microsomal incubations only when these systems are fortified with NADPH. Mouse microsomes are more effective than equivalent levels of rat microsomes in metabolizing each of **A**, **CI**, **JI**, and **PI**, which differ little in their relative extent of metabolism (supplementary Table V). In a direct comparison as cosubstrates, the *E*-8′-photoisomers of **CI**, **JI**, and **PI** are metabolized slightly faster than the corresponding natural *Z*-8′-isomers. Mouse microsomal metabolism of the pyrethrates proceeds to some extent without NADPH. Preincubation of microsomes with PSCP reduces the extent of metabolism in both the presence and the absence of NADPH.

HRGC–MS analysis was normally made for incubations with 1 mg of microsomal protein to maximize formation of metabolites modified primarily at only one molecular site and to minimize endogenous interfering peaks.

Microsomal Metabolites of the Chrysanthemates. *(S)-Bioallethrin.* **A** yields more than 10 NADPH-dependent microsomal metabolites when the incubation extracts are examined by HRGC–MS and HRGC–ECD following sequential treatment with diazoethane and BSA (Table II; Figures 4 and 5; supplementary Figure 2). Two of these metabolites do not change their chromatographic properties on treatment with the derivatizing agents and are identified by their R_ts as **A**-7,8-epoxy (7*R* isomer) and **A**-10-al. Eight of the metabolites shift slightly in R_t values on treatment with BSA, and one is detectable only on treatment with diazoethane; so they are considered to be alcohols and a carboxylic acid, respectively (supplementary Figure 2).

Chemical structures of the microsomal oxidase metabolites of **A** are established or suggested by HRGC–MS following derivatization (Tables II and III; Figure 4). Five of the **A** metabolites are identified by having the same GC and CI-MS properties as the standards, i.e. **A**-7,8-epoxy, -10-al, -10-ol, and -7′-ol(a,b) and their derivatives **A**-10-TMS and -7′-TMS(a,b) (Table III). Only the longer R_t 7,8-epoxide (7*R* isomer) is detected as a metabolite in

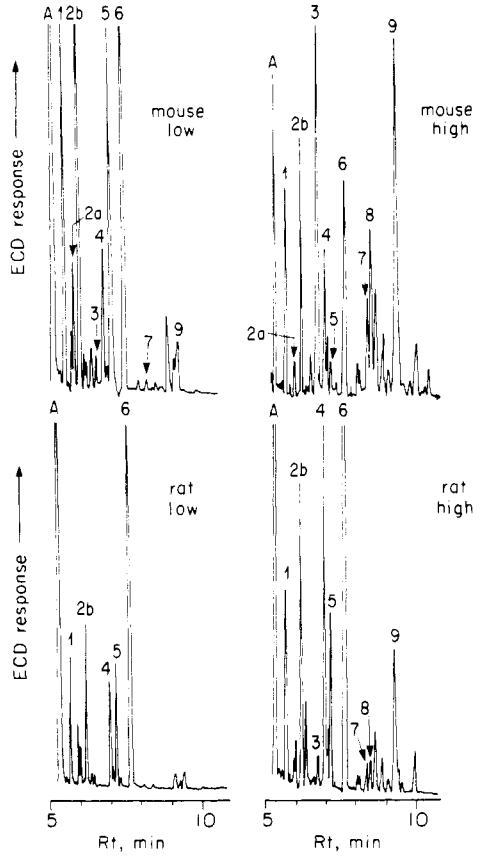


Figure 5. HRGC-ECD chromatogram of (*S*)-bioallethrin and some of its mouse and rat liver microsomal oxidase metabolites at different protein levels (0.3 and 3 mg) after treatment with diazoethane and BSA. Metabolites are characterized or tentatively identified as indicated in the text. Peak assignments as in Figure 4. The 7*S* isomer of A-7,8-epoxy appears as a shoulder immediately prior to the 7*R* isomer (peak 1) with rat but not mouse microsomes. Some of the unidentified peaks are normal microsomal components.

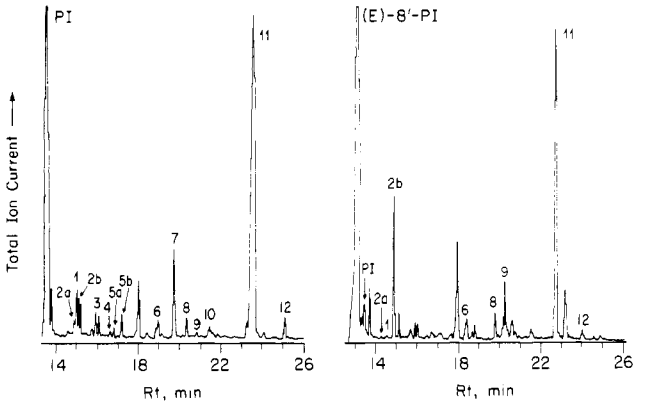


Figure 6. HRGC(H_2)–MS chromatograms of pyrethrin I and its *E*-8′-photoisomer and some of their mouse liver microsomal (1.0 mg of protein) oxidase metabolites after treatment with diazomethane and BSA. Metabolites are characterized or tentatively identified as indicated in the text assuming no isomerization except as an intermediate step in hydrolysis of the 10′,11′-epoxy compounds. Peaks: **PI** = pyrethrin I and (*E*)-8′-**PI** = *E*-8′-isomer of **PI**; 1 = 7,8-epoxy; 2a,b = 7′-TMS(a,b); 3 = 10′,11′-epoxy; 4 = 7,8-epoxy-7′-TMS; 5a,b = 10′,11′-epoxy-7′-TMS(a,b); 6 = 10-al; 7 = "**PI**"-8′,11′-dihydro-$(TMS)_2$; 8 = 10-TMS; 9 = **PII** and (*E*)-8′-**PII**; 10 = "**PI**"-7,8-epoxy-8′,11′-dihydro$(TMS)_2$; 11 = 10′,11′-dihydro$(TMS)_2$; 12 = 7,8-epoxy-10′,11′-dihydro-$(TMS)_2$. Some of the unidentified peaks are normal microsomal components.

mouse microsomes, but both are detected in rat microsomes (Figure 5). **A**-10-Et is identified following diazoethane treatment by its fragmentation pattern. The longer R_t diastereomer **A**-7′-ol(b) (analyzed as its TMS derivative) is preferentially formed by mouse and rat microsomes. **A**-5/6-ol analyzed as **A**-5/6-TMS is a minor metabolite in both mouse and rat microsomes. Although not illustrated, compounds with GC–MS characteristics appropriate for **A**-9-ol and **A**-6′-ol (as **A**-9-TMS and **A**-6′-TMS) appear in trace amounts with mouse microsomes. **A**-5′-TMS is not detected by HRGC–MS. **A**-8′,9′-dihydro$(TMS)_2$ but not its 7,8-isomer is detected as a metabolite following derivatization.

Several metabolites are detected involving more than one metabolic modification, and their structures are confirmed by experiments with the primary metabolites used as substrates. The 7*R* isomer of **A**-7,8-epoxy is the more stable diastereomer when they are individually used as substrates in microsomal incubations (supplementary Table V). Each 7,8-epoxy diastereomer, as a substrate, yields preferentially the longer R_t 7′-ol metabolite [analyzed as **A**-7,8-epoxy-7′-TMS(b)], which is also detected with **A**-7′-ol as the substrate. This is confirmed by finding that **A**-10-al yields two major metabolites on incubation with microsomes, one giving **A**-10-Et on reaction with diazoethane and the other forming **A**-10-al-7′-TMS on reaction with BSA. The corresponding acid, analyzed as its ethyl ester, i.e., **A**-10-Et-7′-TMS, is not detected in the microsomal preparation. With **A**-10-ol as the substrate the two major metabolites are **A**-10-al and, following BSA treatment, **A**-10,7′-$(TMS)_2$(a,b), which is also formed with **A**-7′-ol(a,b) as the substrate. No diastereomer difference is observed in the extent of metabolism of the **A**-7′-ol diastereomers (supplementary Table V).

Natural Chrysanthemates. **CI, JI,** and **PI** provide several NADPH-dependent metabolites analogous in GC properties, following derivatization, to those obtained from **A** (Table II). In each case a carboxylic acid metabolite is tentatively identified as **CI**-, **JI**-, and **PI**-10-acid by treatment with diazomethane and cochromatography with the corresponding pyrethrate (**CII, JII, PII**). Many of the major metabolites of **CI, JI,** and **PI** are interpretable based on their CI-MS characteristics compared to those of the **A** metabolites (Figure 6; Table IV; supplementary Tables II and III). Analogous metabolites and derivatives in the **CI, JI,** and **PI** series versus **A** include the following: 10-al; 7,8-epoxy; 10-TMS; 7′-TMS; 6′-TMS; 7,8-epoxy-7′-TMS; 10,7′-$(TMS)_2$; 8′,9′-dihydro-$(TMS)_2$. New metabolites from the chrysanthemates without analogy from **A** result from the extended alcohol side chain in each series, i.e., the 8′,9′- or 10′,11′-epoxide (and the 7,8-8′,9′- or 7,8-10′,11′-diepoxide), and from specific modifications for each compound, i.e., 10′-TMS and 7,8-epoxy-6′/10′-TMS from **CI**, 6′/10′/11′-TMS from **JI**, and 8′,11′-dihydro$(TMS)_2$, 10′,11′-dihydro$(TMS)_2$, and the corresponding 7,8-epoxy-dihydro$(TMS)_2$ derivatives from **PI**.

Mouse vs Rat Microsomes. The major metabolites of **A** in mouse microsomes involve oxidation at the 10-methyl and 7′-methylene substituents and at the 7,8 double bond, and there is extensive formation of metabolites combining these sites of attack (Figure 5). Rat microsomes appear to be more selective for oxidation of **A** at the 10-methyl substituent compared with other sites, yielding smaller amounts of metabolites oxidized at two molecular positions (Figure 5). With **CI** and **JI** the additional methyl and methylene groups are hydroxylated to a comparable extent. With **PI** the major site of attack with both species is the 10′,11′ double bond, forming the 10′,11′-epoxide and after hydrolysis the dihydrodiols (Figure 6).

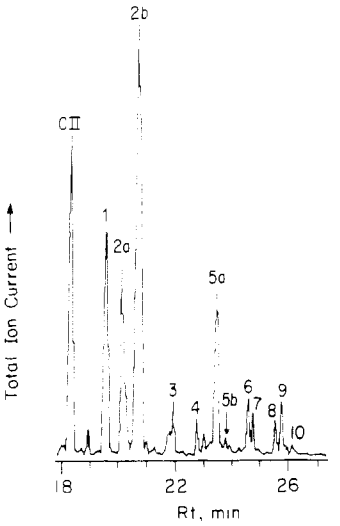


Figure 7. HRGC(H_2)–MS chromatogram of cinerin II and some of its mouse liver microsomal (1.0 mg of protein) oxidase metabolites after treatment with diazoethane and BSA. Metabolites are characterized or tentatively identified as indicated in the text. Peaks: **CII** = cinerin II; 1 = **CII**-10-Et; 2a,b = **CII**-7′-TMS(a,b); 3 = **CII**-8′,9′-epoxy; 4 = **CII**-5/6-TMS; 5a,b = **CII**-8′,9′-epoxy-7′-TMS(a,b); 6 and 8 = **CII**-6′/10′-TMS; 7 = **CII**-5/6,7′-$(TMS)_2$; 9 = **CII**-8′,9′-dihydro$(TMS)_2$; 10 = **CII**-$(TMS)_2$. Some of the unidentified peaks are normal microsomal components.

Microsomal Metabolites of the Pyrethrates. The principal and almost the only microsomal metabolite from each of **CII**, **JII**, and **PII** in the absence of NADPH is identified as the corresponding 10-acid based on conversion to the 10-Et derivative on treatment with diazoethane. With PSCP there is less loss of the parent compound and less formation of the corresponding carboxylic acids (supplementary Table V). The metabolites detected with each substrate in the presence of NADPH are the same with or without PSCP. Metabolites of **CII**, **JII**, and **PII** (Table III; Figures 2 and 7; supplementary Table IV) analogous to those in the chrysanthemates series are 10-acid, 8′,9′-epoxy (from **CII** and **JII**), 7′-ol, 5/6-ol (from **CII**), and various dihydrodiols including the 8′,9′-isomer (from **CII** and **JII**) and the 8′,11′- and 10′,11′-isomers (from **PII**). New metabolites are the 5/6,7′-diol (from **CII**), 8′,9′-epoxy-7′-ol (from **CII** and **JII**), 10′,11′-epoxy-7′-ol (from **PII**), and 10-acid-8′,9′-dihydrodiol (from **JII**).

Urinary Metabolites of (*S*)-Bioallethrin. The urinary metabolites from both oral and intraperitoneal treatments, on direct extraction and derivatization with diazoethane and BSA, yield three distinct HRGC peaks identified by CI-MS (Table III) as **A**-10-Et, **A**-10-Et-7′-TMS, and **A**-10-Et-8′,9′-dihydro$(TMS)_2$ (R_t larger than other **A** metabolites in Table II). **A**-10-Et is also evident in microsomal reactions, but the other microsomal metabolites and parent **A** itself are not detected in the urine. The three metabolites detected appear in at least 2-fold higher amounts on treatment of the urine with β-glucuronidase (but not with β-glucuronidase in the presence of the inhibitor D-saccharic acid 1,4-lactone), indicating that they each exist in part as glucuronide conjugates. Sulfatase treatment gave no indication of any sulfate conjugates of these metabolites of **A**.

DISCUSSION

The sites of metabolic attack on the chrysanthemates and the metabolites observed for each compound are shown in Figure 1. (*S*)-Bioallethrin was examined most extensively as the simplest compound within the series. The most facile oxidation occurs at the 10-position to give **A**-10-ol, -10-al, and -10-acid followed by the allylic methylene group to give **A**-7′-ol with preference for one diastereomer. Oxidative attack at multiple sites is evident directly from **A** and also from intermediary metabolites; i.e., **A**-10-ol and **A**-10-al undergo further oxidations to give **A**-10,7′-diol and **A**-10-al-7′-ol, both of which are also formed from **A**-7′-ol. Epoxidation gives specifically (in mouse) or preferentially (in rat) a single isomer of **A**-7,8-epoxy. The major epoxyallethrin isomer formed metabolically is tentatively assigned the 7*R* configuration. **A**-7,8-epoxy-7′-ol is formed from both **A**-7,8-epoxy and **A**-7′-ol. The 8′,9′-dihydrodiol is probably formed via the 8′,9′-epoxy intermediate. Minor metabolites or those only tentatively identified are **A**-5/6-ol, **A**-9-ol, **A**-9-al, and **A**-6′-ol from oxidation at other methyl groups.

Enzymatic reactions occurring in the microsomal oxidase system appear to closely mimic those observed in vivo. Metabolites previously noted in the urine of **A**-treated rats are **A**-10-ol, **A**-10-acid, **A**-5/6-ol-10-acid, **A**-10-acid-7′-ol, and **A**-10-acid-8′,9′-dihydrodiol (Elliott et al., 1972); the second, fourth, and fifth of these oxidation products are also excreted as glucuronides based on the present study.

The naturally occurring chrysanthemates (**CI**, **JI**, **PI**) undergo metabolic reactions in mouse and rat liver microsomal oxidase systems anticipated from the pathways discussed above with **A** (Figure 1). Thus, major sites of oxidation are the 10-,10′-, and 11′-methyls, the 7′- and 10′-methylene groups, the 7,8- and 8′,9′-double bonds, and particularly the 10′,11′-double bond of **PI**. Epoxidation of these double bonds yields 7,8-epoxy, 8′,9′-epoxy, and 10′,11′-epoxy derivatives. Epoxides from the alcohol moiety metabolites readily form dihydrodiols, i.e., the 8′,9′-dihydrodiol from **CI**-8′,9′-epoxy and the 10′,11′- and 8′,11′-dihydrodiols from **PI**-10′,11′-epoxy, which are the major metabolites of **PI** (Elliott et al., 1972). In addition, several of the metabolites are formed by oxidation at both the acid and alcohol moieties.

The pyrethrates (**CII**, **JII**, **PII**) (Figure 2) give one major metabolite in common with the corresponding chrysanthemates as expected since oxidation at the 10-position of the chrysanthemates yields the same carboxylic acids formed on hydrolysis of the pyrethrates. Esteratic hydrolysis of the pyrethrates may involve both A and B esterases because it is only partially inhibited by PSCP. The acid moiety of the pyrethrates is resistant to oxidation except at a 5/6-methyl substituent whereas the alcohol moieties undergo the oxidation reactions anticipated from the findings on the chrysanthemates. The resistance of the acid moiety of the pyrethrates to oxidation may contribute to the formation of 8′,9′- or 10′,11′-epoxy-7′-ol metabolites observed with the pyrethrates but not with the chrysanthemates.

Rat microsomal oxidases are more selective than mouse microsomal oxidases in hydroxylating the chrysanthemates at the 10-position versus oxidation at the 7,8 double bond and 7′-position. Rat and mouse microsomal oxidases also differ in their stereospecificity for metabolism of the resmethrin isomers (Ueda et al., 1975; Soderlund and Casida, 1977b).

The methodology used in the present investigation is applicable to other pyrethroids and xenobiotics in general. In studies of an isobutyldienamide insecticide this GC–MS approach without metabolite standards led to the same conclusions as HPLC analysis with authentic metabolites from synthesis (Horsham et al., 1989; Johnston

et al., 1989). The rethrins are particularly appropriate for the present type of GC–MS study because of their ease of metabolic attack at multiple sites. When compared with other pyrethroids on a preliminary basis, the rethrins were metabolized more rapidly than the permethrin isomers and particularly than biphenthrin or ethofenprox.

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Supplementary Material Available: Partial ^{1}H and ^{13}C NMR data for **A**, **A**-10-ol, **A**-10-al, the diastereomers of **A**-7,8-epoxy, **A**-7′-ol, **A**-8′,9′-epoxy, and **A**-8′,9′-dihydrodiol, partial CI-MS data for **CI**, **CII**, **JI**, **JII**, and **PII** and their microsomal metabolites and GC derivatives thereof, quantitative data on the extent of metabolism of **A**, diastereomers of **A**-7,8-epoxy, **A**-10-al, **A**-10-ol, **A**-7′-ol, and the six natural pyrethrins in mouse and/or rat liver microsomal systems in the presence and absence of NADPH and/or PSCP, HRGC(He)–MS chromatogram of **A** and some of its SeO_2 oxidation products after derivatization with BSA, and HRGC-ECD chromatogram of **A** and some of its mouse liver microsomal oxidase metabolites before and after treatment with diazoethane and BSA (7 pages). Ordering information is given on any current masthead page.

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