Epoxy and Hydroxy Derivatives of (S)-Bioallethrin and Pyrethrins I and II: Synthesis and Metabolism†

Tetsu Ando,‡ Robert F. Toia,* and John E. Casida

Pesticide Chemistry and Toxicology Laboratory, Department of Entomological Sciences, University of California, Berkeley, California 94720

The 2-methylpropenyl group of (S)-bioallethrin (**A**) and the pentadienyl group of pyrethrin II (**PII**) are selectively oxidized by *m*-chloroperoxybenzoic acid in dichloromethane to yield the 7,8-epoxide (**1**) from **A** and a mixture of the 8′,9′- and 10′,11′-epoxides (**7** and **8**) from **PII**. These epoxides are hydrated in aqueous acid to the corresponding diols and other hydroxy derivatives produced by opening of the cyclopropyl ring or migration of the adjacent double bond. The epoxy and hydroxy derivatives are identified by two-dimensional NMR techniques. Mouse liver enzymes do not detectably hydrate epoxide **1** but quickly hydrate epoxides **7** and **8** without migration of the double bond. HPLC analyses of the microsomal metabolites of pyrethrins I and II identify the 10′,11′-diols as major metabolites and the 8′,9′-diols as minor products.

INTRODUCTION

The natural pyrethrins I and II (**PI** and **PII**) and their synthetic analogue (S)-bioallethrin (**A**) have olefinic side chains in both their acid and alcohol moieties (Figure 1). Olefins are generally metabolized by epoxidation and then hydration. These rethrins are, therefore, expected to be converted to epoxy and dihydroxy derivatives in both insects and mammals. Despite detailed investigations of the metabolic fates of **PI**, **PII**, and **A** (Elliott et al., 1971, 1972; Class et al., 1990), there are still some structural uncertainties pertaining to the epoxides and the diols not only as metabolites but also as products of model chemical reactions.

This study reexamines the chemical and metabolic epoxidation and subsequent hydration reactions of the 2-methylpropenyl side chain of **A** and the pentadienyl moiety of **PI** and **PII**. Epoxide and diol standards, prepared by oxidation of the olefins with *m*-chloroperoxybenzoic acid (MCPBA) and acid-catalyzed hydration of the resulting epoxides, are compared with the relevant metabolites generated from mouse liver microsome incubations.

MATERIALS AND METHODS

Chromatography. Column chromatography utilized silica gel 60 (70–230-mesh ASTM; Merck, Darmstadt, Germany) with a solvent gradient of *n*-hexane to acetone. TLC and HPLC methods were as described previously (Ando et al., 1990). Semiquantitative determinations of pyrethroids and their derivatives were performed by integration of the HPLC peak areas determined at 235 nm.

Spectroscopy. NMR spectra were recorded as previously reported (Ando et al., 1990). Chemical ionization GC/MS was accomplished with a Hewlett-Packard 5985 GC/MS system fitted with a SPB5 (Supelco Inc., Bellefonte, PA) (for derivatives of **PI** and **PII**) or a DB5 (J&W Scientific, Folsom, CA) (for derivatives of **A**) fused silica capillary column (30 m × 0.25 mm i.d., 0.25-μm film). Data were processed with the Hewlett-Packard 59872C RTE-A data system. The source temperature was 130 °C, with methane (0.9 Torr) as the ionizing gas. The GC was temperature programmed from 90 °C for 2 min, 30 °C/min to 180 °C, and finally 3 °C/min to 240 °C. Hydrogen (30 psi, 80 cm/s) was the carrier gas.

Chemicals. The numbering system for the chrysanthemates is shown in Figure 1. Pyrethroid **A** and concentrated pyrethrum extract (72% pyrethrins) were supplied by McLaughlin Gormley King Co. (Minneapolis, MN). Column chromatography of the pyrethrum extract (5 g) on silica gel (100 g) separated the chrysanthemates (1.7 g; eluting with 10% acetone in *n*-hexane) from the pyrethrates (1.5 g; eluting with 15% acetone in *n*-hexane). The chrysanthemates were a mixture of **PI** (71%), cinerin I (**CI**, 23%), and jasmolin I (**JI**, 6%), and the pyrethrates were a mixture of **PII**, cinerin II (**CII**), and jasmolin II (**JII**) in a similar ratio. Preparative HPLC was used to isolate **PI** from the chrysanthemate mixture (5% tetrahydrofuran in *n*-hexane) and **PII** from the pyrethrate mixture (10% tetrahydrofuran in *n*-hexane). 7,8-Epoxy-**A** (**1**) and the individual diastereomers (**1a** and **1b**) were obtained as previously described (Class et al., 1990).

Acid-Catalyzed Hydration of 7,8-Epoxy-(S)-bioallethrin (1). A suspension of isomer **1a** (0.31 mmol, 100 mg) in dioxane (2 mL) and aqueous 0.5 N H_2SO_4 (4 mL) was stirred for 2 h at room temperature during which time the suspension changed to a solution. Recovery of the hydroxy derivatives (**2**–**4**) involved addition of saturated aqueous $NaHCO_3$ (2 mL) and extraction with ethyl acetate. After purification by preparative TLC, each component was analyzed by NMR (Tables I and II) and GC/MS (Table III) (Figure 2). This acid-catalyzed hydration procedure was repeated with **1b**.

As a comparison standard, the 7,8-dihydroxy derivative (**5**) of methyl *trans*-chrysanthemate was synthesized via the ketol (**6**) (Figure 3) (Matsui et al., 1963).

Synthesis of 8′,9′-Epoxypyrethrin II (7) and 10′,11′-Epoxypyrethrin II (8). The pyrethrate mixture (600 mg) in dichloromethane (10 mL) was treated with MCPBA (1.5 mmol, 260 mg) for 3 h at room temperature. After the usual workup, a mixture of epoxy derivatives of pyrethrates (420 mg) [composed of **7** (41%), **8** (17%), the 8′,9′-epoxide of **CII** (34%), and the 8′,9′-epoxide of **JII** (8%) as indicated by HPLC analysis] was obtained in the 20% acetone in *n*-hexane fraction from a silica gel (35 g) column. Further fractionation was achieved with a Chromatotron Model 8924 (Harrison Research, Palo Alto, CA) instrument using a 2-mm silica gel plate, 6% acetone in *n*-hexane as solvent, and a UV lamp for detection, giving **7** mixed with the 8′,9′-epoxide of **JII** (180 mg), **8** (60 mg), and the 8′,9′-epoxide of **CII** (120 mg); TLC R_f values (20% acetone in *n*-hexane) were 0.36, 0.29, and 0.32, respectively. The diastereomers of epoxide **7** were readily obtained pure from the above fraction by semipreparative HPLC (Table IV).

Acid-Catalyzed Hydration of Epoxides 7 and 8 (Figure 4). As described for **1**, epoxide **7** (60 mg) was hydrated in a

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‡ Permanent address: Department of Plant Protection, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183, Japan.

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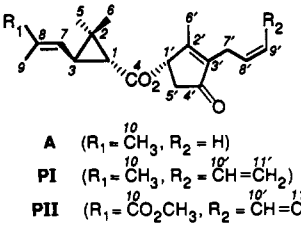

A (R_1 = $^{10}CH_3$, R_2 = H)

PI (R_1 = $^{10}CH_3$, R_2 = $^{10'}CH=^{11'}CH_2$)

PII (R_1 = $^{10}CO_2CH_3$, R_2 = $^{10'}CH=^{11'}CH_2$)

Figure 1. Structures of (*S*)-bioallethrin (**A**), pyrethrin I (**PI**), and pyrethrin II (**PII**) showing the numbering system used in this paper.

mixed solvent of dioxane and 0.5 N H_2SO_4 to yield 8′,9′-dihydroxypyrethrin II (**9**, 46 mg, 77% yield) after preparative TLC (40% acetone in *n*-hexane, R_f 0.24). Epoxide **8** (60 mg) was hydrated on similar treatment to yield a mixture (40 mg) of 10′,11′-dihydroxypyrethrin II (**10**, 67%) and the 8′,11′-dihydroxy derivative (**11**, 33%). **10** and **11** showed the same R_f value (0.12) on TLC as above, but they were separated by HPLC (Table IV). The two diastereomers of **11** were separated also by HPLC, but those of **10** were not separable. Treatment of each of the diols (**9**–**11**) with bis(trimethylsilyl)acetamide (BSA) in acetonitrile for 1 h at 80 °C gave their respective bis(trimethylsilyl) ether (i.e., TMS) derivatives for GC/MS measurements. Epoxide **7** (10 mg) was also subjected to acid-catalyzed methanolysis (1.5 mL containing H_2SO_4, 40 mg) giving, after preparative TLC (40% acetone in *n*-hexane, R_f 0.40), the monomethyl ether (**12**, 4 mg). Acetylation of the methyl ether (**12**) with acetic anhydride in pyridine gave the acetate (**13**) (40% acetone in *n*-hexane, R_f 0.52) in excellent yield.

Synthesis of Epoxy and Dihydroxy Derivatives of Pyrethrin I (14–18). The chrysanthemate mixture (1 g) and K_2CO_3 (1 g) in methanol (40 mL) were stirred for 1 day at room temperature. After the reaction mixture was concentrated to 10 mL, the residue was poured into water (20 mL) and the crude products were recovered by extraction with ethyl acetate. Silica gel column chromatography (30% acetone in *n*-hexane) gave a mixture (240 mg) of pyrethrolone (75%), cinerolone (22%), and jasmolone (3%). This mixture was treated with MCPBA (240 mg) in dichloromethane (10 mL) in the normal manner and the epoxy derivatives (130 mg) were isolated from the product mixture by preparative TLC (40% acetone in *n*-hexane). These were then coupled with (1*R*)-*trans*-chrysanthemoyl chloride (100 mg) in benzene (2 mL) containing pyridine (80 μL) to give an epoxy ester mixture (150 mg) which, after purification by preparative TLC (15% acetone in *n*-hexane), consisted of the diastereomeric pairs of the 8′,9′-epoxide of **PI** (**14**, 48%), the 10′,11′-epoxide of **PI** (**15**, 11%), the 8′,9′-epoxide of **CI** (36%), and the 8′,9′-epoxide of **JI** (5%). Each component of the mixture was separable by HPLC except for the diastereomers of **15** and pure samples of **15**, and the two diastereomers of **14** were obtained by this method (Table IV). Epoxides **14** and **15** were hydrated by using the same acid conditions described for **1**, above, yielding the 8′,9′-diol of **PI** (**16**) and a mixture of the 10′,11′-diol (**17**) and 8′,11′-diol of **PI** (**18**), respectively, each of which was purified by preparative HPLC (Figure 4) (Table IV). The NMR data for the alcohol moieties of these chrysanthemates (**14**–**18**) closely approximate those for the corresponding pyrethrates (**7**–**11**). The diols (**16**–**18**) were converted to their TMS ether derivatives by BSA and analyzed by GC/MS.

Formation of Enzymatic Metabolites. The substrate (0.1 μmol) was incubated with mouse liver microsomes (washed twice by resuspension in buffer and resedimentation; 1 mg of protein) or mouse liver cytosol (2 mg of protein) and NADPH (0 or 2.4 μmol) in phosphate buffer (0.1 M, pH 7.4, 2 mL) with shaking at 37 °C unless otherwise specified for 1 h (Johnston et al., 1989). The mixture was saturated with NaCl, extracted with ethyl acetate (2 mL, three times), and dried with Na_2SO_4. After concentration, the residue was dissolved in the HPLC solvent (50% dioxane in *n*-hexane, 100 μL) and analyzed by HPLC. The epoxy and dihydroxy derivatives were chromatographed by using 20% and 50% dioxane in *n*-hexane, respectively (see Table IV).

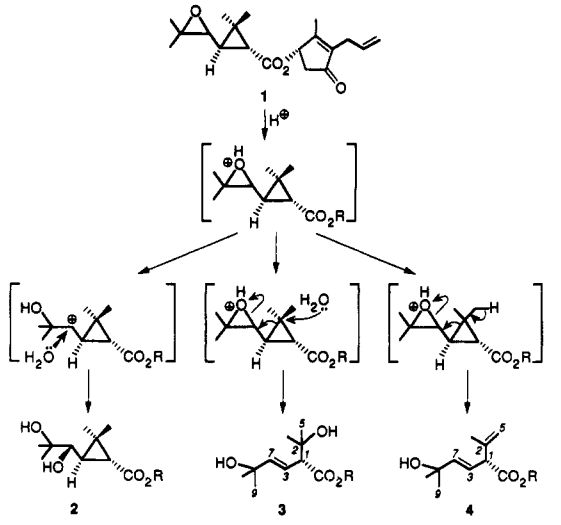

Figure 2. Acid-catalyzed hydration of 7,8-epoxy-(*S*)-bioallethrin (**1**). The same reactions are applicable to **1a** and **1b**.

RESULTS AND DISCUSSION

7,8-Epoxy-(*S*)-bioallethrin (1) and Its Acid-Catalyzed Hydration Products. Epoxide **1**, obtained as the principal product on treatment of **A** with MCPBA, is also a major microsomal metabolite of **A** (Class et al., 1990). While chemical oxidation gives two diastereomers (**1a** and **1b**) in a ca. 1:1 ratio, the microsomal system selectively produces the more polar isomer (**1b**) (Class et al., 1990), the stereochemistry of the newly introduced chiral center of which has been assigned the 7*R* configuration (Ando et al., 1990).

The hydration of epoxychrysanthemates has not been defined in detail, and only the cyclopropane ring-opened compound (analogous to **3**, discussed later) has been identified as an acid-catalyzed hydration product (Smith and Casida, 1981). On treatment of **1** with aqueous acid, each diastereomer gives three hydroxy derivatives (**2**–**4**, see Figure 2) which can be separated by a combination of TLC and HPLC methods. ^1H and ^{13}C NMR data for their acid moieties are listed in Tables I and II, respectively. Table III gives their TLC R_f values, HPLC R_t values, GC/MS data, and composition ratio in the crude product mixture as obtained from each isomer of **1**. The chromatographic behavior and $[MH]^+$ ions indicate that **2** and **3** are dihydroxy derivatives and **4** is a monohydroxy derivative of **A**.

The 7,8-diol structure for **2** is assigned by its ^1H NMR spectrum which resembles that of the parent epoxide (**1**) in that resonances characteristic of the substituted cyclopropyl functionality are retained but differs in that those for the epoxy ring are absent. Surprisingly, however, the diastereomeric epoxides **1a** and **1b** both give **2**. This requires inversion of configuration at C^7 in one case, consistent with the normally accepted major pathway for acid-catalyzed ring opening of epoxides. For the other the stereochemistry of the product requires, mechanistically, the formation of a discrete carbocation at C^7 which, presumably because of the steric bulk of the adjacent cyclopropyl group, is approached from only one face and with retention of configuration. Examination of models in light of the above observations suggests that the C^7 center in **2** has the *S* configuration. To confirm the structure of **2** and the stereochemical assignment at C^7, the 7,8-dihydroxy derivative (**5**) of methyl *trans*-chrysanthemate was synthesized via the ketol **6**, according to the method of Matsui et al. (1963) (Figure 3). NMR analysis

Table I. Partial ^1H Peak Assignments[a] of (*S*)-Bioallethrin (A), Its Derivatives with Modified Acid Moieties, and Diols of Methyl Chrysanthemate

	^1H shift, ppm, for indicated position							coupling constant, Hz	
compd	1	3	5	6	7	9	10	J_{1-3}	J_{3-7}
A	1.42	2.08	1.26	1.15	4.91	1.72	1.73	5.5	7.5
2	1.62	1.64	1.22	1.22	3.14	1.24[c]	1.27[c]	6[b]	8.5[b]
3	3.04	~5.8	1.20[d]	1.27[d]	~5.8	1.33[e]	1.34[e]	9.5[b]	15.5[b]
4	3.70	5.86	4.89,4.94	1.77	5.76	1.34	1.34	8	15.5
5a	1.46	1.54	1.22	1.29	3.10	1.22[f]	1.23[f]	5.5	10
5b	1.60	1.64	1.20	1.19	3.12	1.23[g]	1.24[g]	5.5	9

[a] Assigned by COSY, long-range COSY, and NOESY measurements. [b] Measured in pyridine-d_5. [c-g] Two methyl ^1H signals are arbitrarily assigned.

Table II. Partial ^{13}C Peak Assignments[a] of (*S*)-Bioallethrin (A), Its Derivatives with Modified Acid Moieties, and Diols of Methyl Chrysanthemate

	^{13}C shift, ppm, for indicated position									
compd	1	2	3	4	5	6	7	8	9	10
A	34.4	28.9	32.8	172.1	20.3	22.0	120.6	135.7	18.4	25.4
2	30.3	29.1	35.9	172.1	20.5	22.3	76.6	72.8	23.8[b]	26.7[b]
3	58.6	78.4	120.9	173.6	26.5[c]	28.7[c]	144.0	71.6	29.6[d]	29.8[d]
4	55.6	141.6	122.5	172.0	113.8	21.0	141.6	70.7	29.7[e]	29.8[e]
5a	33.2	26.1	33.9	172.4	21.0	21.4	78.4	73.5	23.8[f]	27.0[f]
5b	30.6	28.7	35.8	173.2	20.5	22.4	76.6	72.8	23.8[g]	26.6[g]

[a] Assigned by C-H COSY and long-range C-H COSY measurements. [b-g] Two methyl ^{13}C signals are arbitrarily assigned.

Table III. Chromatographic Behavior, GC-MS Data, and Composition Ratio of Acidic Hydration Products (2-4) from 7,8-Epoxy-(*S*)-bioallethrin (1)

		HPLC	GC-MS (chemical ionization)			composition ratio,[c] %	
compd	TLC R_f[a]	R_t,[b] min	R_t, min	MH^+ (m/z)	base ion (m/z)	from **1a**	from **1b**
2	0.57	18.1	13.62	337 [14][d]	135	25	29
3	0.52	19.0	11.46	337 [2][d]	261	65	60
4	0.67	12.8	9.87	319 [7][d]	301	10	11

[a] Silica gel chromatoplate with 40% acetone in *n*-hexane. [b] Nucleosil NO_2 column with 35% dioxane in *n*-hexane at a flow rate of 2 mL/min. [c] Calculated from the peak area obtained by HPLC analyses. [d] Relative intensity.

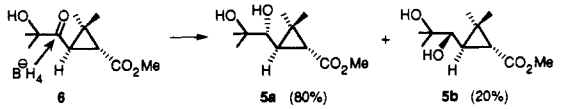

Figure 3. $NaBH_4$ reduction of a ketol derivative of methyl chrysanthemate (6).

of **5** (see Tables I and II) revealed that this sample consisted of two diastereomers (**5a** and **5b**) in a ca. 4:1 ratio. The chemical shifts for the ^1H and ^{13}C signals of the minor component (**5b**) and the acid moiety of **2** show close coincidence. Isomer **5b** was subsequently shown to be identical with the methyl ester produced by hydrolysis of the allethronyl ester (**2**) with K_2CO_3 and methylation with diazomethane. The main component (**5a**) from reduction of the ketol (**6**) has the 7*R* configuration, in contrast to **2**, consistent with preferential attack by $NaBH_4$ from the *si* side of C^7. Furthermore, these configurational assignments are confirmed by NOESY experiments. The 2D NMR spectrum of a pure sample of **5a**, prepared by recrystallization of the corresponding free acid and reesterification with diazomethane, further confirms the 7*R* configuration. Specifically, NOE cross peaks are observed between H^1 and either H^9 or H^{10}, and in addition there are mutual NOE correlations between H^1, H^6, and H^7. The diol (**2**) shows NOE peaks only between H^1, H^6, and H^7 consistent with the 7*S* configuration.

The NMR spectrum of diol **3**, the major component, shows two newly introduced olefinic resonances whose mutual coupling indicates them to be vicinal. Further, one of them shows an additional coupling to a methine proton (Table I). This is consistent with the analogous cyclopropyl ring-opened structure identified from hydration of the epoxy derivative of tetramethrin (Smith and Casida, 1981). Formation of **3** can be rationalized from attack by water at C^2 of the cyclopropyl group, cleavage of the C^2–C^3 bond, and opening of the epoxy ring. Although the configuration at C^1 is retained during this process, the sample of **3** derived from **1a** and **1b** actually includes ca. 30% and 10%, respectively, of an isomerized product, which cannot be separated even by HPLC. Since the proportion of this isomer does not change on long-term acid treatment (1 day), the isomerization may occur during opening of the epoxy ring. However, at this time, the mechanism by which this occurs is unclear. The *E* configuration of the double bond is indicated by the magnitude of the coupling (15.5 Hz) of the olefinic protons.

The third discrete product (**4**) also shows two olefinic proton signals (J = 15.5 Hz) but, in addition, a newly introduced vinyl methyl group and a vinylidine proton pair appear at the expense of the cyclopropyl methyl resonances (Table I). These data are again in accord with a cyclopropyl ring-opened product, but with loss of a proton rather than the involvement of water. Consistent with this interpretation, **4** retains the 1*R* configuration, as shown, albeit derived from **1a** or **1b**. A plausible mechanistic rationale for the formation of the products is presented in Figure 2. However, it should be noted that other interpretations are possible and it is not known whether **3** and **4** are formed via a carbocation or a concerted process.

Enzymatic Hydration of 7,8-Epoxy-(*S*)-bioallethrin (1). Earlier studies (Smith and Casida, 1981; Class et al., 1990) failed to demonstrate any hydrated derivatives of the epoxychrysanthemate moiety as microsomal metabolites of chrysanthemates or epoxychrysanthemates. The present investigation, therefore, used **2**–**4** as authentic standards for direct HPLC comparisons with the ethyl acetate extractable metabolites of **1** following its incubation with liver microsomes or cytosol. On incubation without NADPH, loss of the substrate is 20–30% with microsomes and 50–60% with cytosol, whereas in the presence of NADPH 80% of **1a** and 55% of **1b** are metabolized by the microsome incubations. However, on HPLC analysis no

Table IV. HPLC R_t Values and GC-MS Data of Pyrethrins I and II (PI, PII) and Their Derivatives with Modified Alcohol Moieties

	pyrethrates						chrysanthemates				
	HPLC R_t,[a] min		GC[b]–MS (chemical ionization)				HPLC R_t,[a] min		GC[b]–MS (chemical ionization)		
compd	I	II	R_t, min	MH^+ (m/z)	base ion (m/z)		I	II	R_t, min	MH^+ (m/z)	base ion (m/z)
pyrethrins											
PII	14.3	7.3	20.79 [1.00][c]	373 [100][d]	213	**PI**	12.2	8.3	13.19 [1.00][c]	329 [51][d]	161
epoxides											
7a	20.0	7.9	22.08 [1.06]	389 [22]	179	**14a**	15.4	8.8	15.30 [1.16]	345 [20]	151
7b	20.6	7.9	22.68 [1.09]	389 [52]	179	**14b**	15.9	8.8	15.46 [1.17]	345 [32]	177
8[e]	27.2	8.5	25.08 [1.21]	389 [18]	177	**15**[e]	19.4	9.4	16.35 [1.24]	345 [12]	177
diols[f]											
9a		11.5	26.56 [1.28]	551 [28]	213	**16a**		12.0	19.20 [1.46]	507 [39]	169
9b		11.9	26.93 [1.30]	551 [22]	213	**16b**		12.4	19.58 [1.48]	507 [32]	169
10[e]		21.8	30.60 [1.47]	551 [2]	213	**17**[e]		21.4	23.28 [1.76]	507 [6]	169
11a		18.7	28.91 [1.39]	551 [4]	213	**18a**		18.8	21.44 [1.63]	507 [8]	169
11b		19.3	29.35 [1.41]	551 [2]	213	**18b**		19.4	21.96 [1.66]	507 [6]	169

[a] Chromatography on a Nucleosil NO_2 column with 20% dioxane (I) or 50% dioxane (II) in *n*-hexane at a flow rate of 2.5 mL/min for pyrethrates and 2.0 mL/min for chrysanthemates. [b] Chromatography on an SPB5 fused silica capillary column with hydrogen (80 cm/s) as the carrier gas. Temperature program of 90 °C for 2 min, 30 °C/min to 180 °C, and 3 °C/min to 240 °C. [c] Relative R_t. [d] Relative intensity (%). [e] Two diastereomers were not separated. [f] Analyzed as bis-TMS derivatives by GC–MS.

Table V. Partial ^1H Peak Assignments of Pyrethrin II (PII) and Its Derivatives with Modified Alcohol Moieties

	^1H shift, ppm, for indicated position[a]						coupling constant, Hz						
compd	7′	8′	9′	10′	11′*c*	11′*t*	$J_{7'-7'}$	$J_{7'-8'}$	$J_{8'-9'}$	$J_{9'-10'}$	$J_{10'-11'c}$	$J_{10'-11't}$	$J_{11'c-11't}$
PII	3.13	5.35	6.04	6.77	5.19	5.24		7.5	11	11	11	17	1.0
7a	2.40, 2.54	3.26	3.45	5.83	5.42	5.51	14.5	7.5	4.5	6.5	10.5	17.5	1.5
7b	2.40, 2.55	3.25	3.46	5.84	5.43	5.52	14.5	7.5	4.5	6.5	10.5	17.5	1.5
8[b]	3.18, 3.20	5.64 5.65	5.10	3.77	3.04	2.67		7.5	10.5	9	4.5	2.5	5.5
9a	2.47, 2.55	3.62	3.92	5.89	5.29	5.39	14.5	8, 4.5	5[c]	6	10.5	17	1.5
9b	2.45, 2.56	3.63	3.91	5.89	5.29	5.38	14.5	8, 4.5	5[c]	6	10.5	17	1.5
10[b]	2.97, 3.25 2.99	5.37 5.40	5.55	4.73	~3.6		14	9.5, 6	10	8.5	*d*		
11a	2.47, 2.55	4.32	5.74	5.88	4.15		14	7.5, 4.5	*d*	15.5	5.5		
11b	2.47, 2.56	4.34	5.74	5.87	4.14		14	7.5, 4.5	*d*	15.5	5.5		

[a] The vinylidene protons cis and trans to $H^{10'}$ are designated 11′*c* and 11′*t*, respectively. [b] Mixture of two diastereomers. [c] Measured as the TMS derivative. [d] Coupling constants not determined.

Table VI. Partial ^{13}C Peak Assignments of Pyrethrin II (PII) and Its Derivatives with Modified Alcohol Moieties

	^{13}C shift, ppm, for indicated position										
compd	1′	2′	3′	4′	5′	6′	7′	8′	9′	10′	11′
PII	73.4	164.9	142.2	203.4	42.0	14.1	21.7	126.7	130.4	131.5	118.3
7a	73.4	166.8	140.1	203.6	42.0	14.4	22.3	56.7	57.2	131.9	120.7
7b	73.4	166.7	140.2	203.6	41.9	14.4	22.2	56.6	57.2	131.9	120.7
8[a]	73.3	165.1	141.7	203.4	41.9	14.1	21.8	131.0 131.1	128.8	47.8	48.6
9[a]	73.4 73.5	168.0	140.7	206.2	41.9	14.3	27.8	72.5 72.7	75.1	137.07 137.13	117.5
10[a]	73.1	166.1	141.2	204.9	42.1	14.1	22.4	128.2 128.4	130.4 130.5	67.6	66.1
11[a]	73.5	167.8	140.6	205.6	41.9	14.4	31.7	70.4 70.5	133.1	129.8 130.0	62.7

[a] Mixture of two diastereomers with all resonances coincident except as indicated.

peak corresponding to **2**, **3**, or **4** is observed in the extracts. The substrate loss may be caused by hydrolysis of the ester bond or secondary oxidation in the incubations of microsomes with NADPH. Conversion of a trisubstituted epoxide to the corresponding diol is known in the metabolism of juvenile hormones (White, 1972; Ajami and Riddiford, 1973) and a synthetic mimic (Gill et al., 1974). Epoxychrysanthemate **1** can be hydrated in aqueous acid but not under any of the conditions examined by using mouse liver enzymes. This interesting stability against epoxide hydratase action may reflect steric hindrance, enzyme specificity, or conjugation of the epoxide with the cyclopropyl ring.

MCPBA Oxidation of Pyrethrin II (PII). MCPBA specifically functionalizes the pentadienyl substituent of **PII** to give two monoepoxy derivatives, the 8′,9′-epoxide (**7**) and the 10′,11′-epoxide (**8**) in a ca. 7:2 ratio. These two epoxides show different chromatographic behaviors with terminal epoxide **8** being the more polar (Table IV). Their chemical structures were determined by NMR analyses (Tables V and VI), particularly by the COSY spectra. Starting from the $H^{7'}$ signals, each proton signal of the epoxypentenyl side chain is easily assigned, thus confirming the placement of the epoxy ring. Their NMR data also indicate that each epoxide is a mixture of two diastereomers in a ca. 1:1 ratio; thus, MCPBA oxidation takes place from either face of the double bond with equal facility.

Acid-Catalyzed Hydration of 8′,9′-Epoxypyrethrin II (7) and 10′,11′-Epoxypyrethrin II (8). 8′,9′-Epoxide **7** in aqueous acid gives 8′,9′-dihydroxypyrethrin II (**9**) specifically, with a trace (2%) of the 8′,11′-dihydroxy

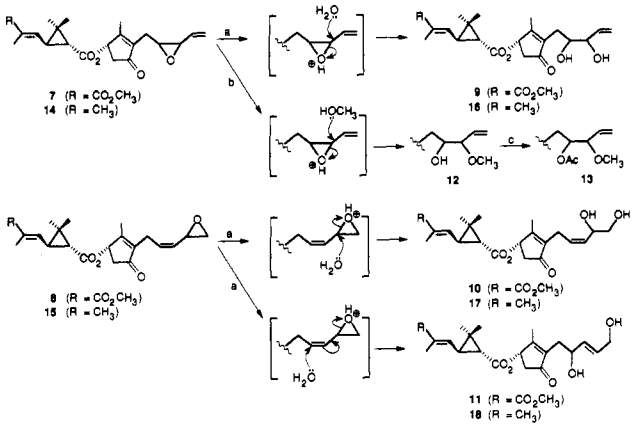

Figure 4. Acid-catalyzed hydration of 8′,9′-epoxypyrethrin II (7), 10′,11′-epoxypyrethrin II (8), and the corresponding pyrethrin I derivatives (14 and 15) and acetylation of a methylation product (12). Conditions: (a) acid-catalyzed hydration in a mixture of water and dioxane (2:1); (b) acid methanolysis; (c) acetylation with acetic anhydride in pyridine.

derivative (11) resulting from migration of the 10′,11′ double bond. In contrast, the hydration products of the 10′,11′-epoxide (8) consist of the 10′,11′-dihydroxy and 8′,11′-dihydroxy derivatives (10 and 11) in a ca. 2:1 ratio (Figure 4). NMR data (Tables V and VI) for these diols indicate the positions of the hydroxy groups and the configurations of the double bonds; i.e., 9 and 10 retain the 10′,11′ and 8′,9′ double bonds with the *Z* configuration, respectively, while 11 has a 9′,10′ double bond with the *E*-configuration. Starting from protonation of the epoxy oxygen of 8, diol 10 may be produced by epoxy ring opening and hydration at $C^{10'}$ and diol 11 by epoxy ring opening at $C^{10'}$ and hydration at $C^{8'}$. By acid methanolysis, 7 gives a monomethyl ether (12), the structure of which is confirmed by conversion to the acetyl derivative (13) (Figure 4). The $H^{8'}$ signal of 12, which shows a cross peak with $H^{7'}$ in the COSY spectrum, moves downfield by 1.4 ppm after acetylation, while the $H^{9'}$ signal of 12, showing a cross peak with $H^{10'}$, moves only by 0.2 ppm. In the hydration of epoxides, water approaches trans to the oxygen of the epoxy ring; therefore, 9 is obtained as a threo isomer. By hydration experiments with each diastereomer of 7 purified by HPLC, it is clarified that 7a and 7b are converted to 9a and 9b, respectively.

Enzymatic Hydration of 8′,9′-Epoxypyrethrin II (7) and 10′,11′-Epoxypyrethrin II (8). While small amounts of 7 and 8 undergo hydration even in a phosphate buffer, these epoxides are readily hydrated by enzymes in both mouse liver microsomes and cytosol (Table VII). Epoxides 7a, 7b, and 8 are converted to diols 9a, 9b, and 10, respectively, without the migration of the conjugated double bond observed during acid-catalyzed hydration. Epoxide 7a is hydrated much faster than its diastereomer (7b) but to the same extent as 8. As might be expected, the enzymatic hydration is a NADPH-independent reaction. When the epoxides are incubated with microsomes and NADPH, the yield of the corresponding diols is decreased, presumably because of their further oxidative metabolism.

Metabolism of the Pentadienyl Side Chain of Pyrethrins I and II (PI and PII). In earlier work, two dihydroxy derivatives of **PII** were noted as urinary metabolites in rats treated orally with either **PI** or **PII** (Elliott et al., 1972). These were assigned by ^1H NMR analysis, after methylation, as 10′,11′-diol 10 (major component) and 8′,11′-diol 11 (minor component), which were both considered to arise from the 10′,11′-epoxide as an intermediate (Elliott et al., 1971, 1972). However, although the ^1H NMR spectrum quoted for the major metabolite coincides well with that of synthetic 10, the NMR data for the minor metabolite are not in agreement with those of synthetic 11. This earlier spectrum is instead consistent with the data for synthetic 8′,9′-diol 9 (see Table V and Elliott et al., 1972), indicating that this original assignment needs to be revised.

Table VII. Enzymatic Hydration of 8′,9′-Epoxypyrethrin II (7a) and 10′,11′-Epoxypyrethrin II (8)

substrate	enzyme	NADPH	recovery of substrate,[a] %	hydrated product,[a] %		
				9a	10	11
7a[b]	none	+	70	13	0	0
		−	75	14	0	0
	microsomes	+	5	10	0	0
		−	7	65	0	0
	cystol	+	5	74	0	0
		−	4	70	0	0
8	none	+	79	0	8	2
		−	76	0	8	2
	microsomes	+	5	0	16	0
		−	3	0	72	0
	cystol	+	7	0	68	0
		−	4	0	75	0

[a] Analyzed by HPLC with UV detection (235 nm). [b] **7b** was specifically hydrated to **9b** on incubation with microsomes (24%) or cytosol (37%) without NADPH. Recovery of the substrate was 53% and 44%, respectively.

Two diols were previously tentatively identified among the microsomal metabolites derived from each of **PI** and **PII** by GC/MS analysis as their TMS ethers following derivatization with BSA (Class et al., 1990). Relative R_t values of the major metabolites from **PI** and **PII** coincide with those of the synthetic standards of 17 and 10, respectively, all analyzed as their TMS ethers. However, the data for the minor diols from **PI** and **PII** do not agree with those from the synthetic TMS standards of 18 and 11 but are more appropriate for those of **16a** and **9a**, respectively (see Table IV and Class et al., 1990). This conclusion is supported by HPLC analysis (Table IV) of the microsomal metabolites, carried out directly without derivatization. The major components of the diol mixture are the 10′,11′ isomers (17 and 10), probably produced by hydration of the corresponding 10′,11′-epoxides (15 and 8). The minor components are the less polar isomers of the 8′,9′-diols (**16a** and **9a**), which can be made from the corresponding 8′,9′-epoxides (14 and 7). The 8′,11′-diols (18 and 11), which arise through double-bond migration, are not observed in the microsomal metabolites. Therefore, it is confirmed that both the 10′,11′ and the 8′,9′ double bonds are oxidized and hydrated enzymatically. However, whereas the microsomal enzymes oxidize the 10′,11′ double bond preferentially, MCPBA is selective toward the 8′,9′ functionality.

Only one compound with an epoxypentenyl side chain was observed, in trace amounts, by GC/MS analysis of the **PI** metabolites (Class et al., 1990). This metabolite was tentatively assigned as the 10′,11′-epoxide (15), but its GC R_t indicates that it is the 8′,9′-epoxide (14) (see Table IV and Class et al., 1990). HPLC analysis of metabolite mixtures incubated for different time periods (5, 10, 30, and 60 min) indicates traces of both diastereomers of the 8′,9′-epoxides (7 and 14). However, the 10′,11′-epoxides (8 and 15), which are likely precursors of the major diol metabolites, are not found in the reaction mixtures regardless of the incubation periods. This observation suggests that in mouse microsomes the pentadienyl epoxidation reaction is closely coupled with subsequent hydration.

Concluding Remarks. **A**, **PI**, and **PII** have many metabolically labile sites. The present investigation focuses on reactions initiated by epoxidation of the 7,8 double bond of the chrysanthemates (examined with **A**) and the 8′,9′ and 10′,11′ double bonds of the (*Z*)-pentadienyl group. It shows that in mouse microsomes metabolism of the pentadienyl side chain is quite different from that of the cyclopropyl-conjugated 2-methylpropenyl group. The allylic methyl and methylene substituents of the acid and alcohol moieties and the carbomethoxy group are also major sites of oxidative and hydrolytic metabolism, respectively. The sensitivity of these pyrethroids to cytochrome P_{450} catalyzed oxidations has two important implications. First, it limits their insecticidal potency and often requires the use of a synergist such as piperonyl butoxide as an oxidase inhibitor. Second, it ensures their ease of metabolism and lack of residual persistence in mammals.

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Registry No. **1**, 72598-26-6; **2**, 131380-53-5; **3**, 131380-54-6; **4**, 131380-55-7; **5a**, 131484-60-1; **5b**, 131484-67-8; **6**, 2259-16-7; **7** isomer 1, 131380-56-8; **7** isomer 2, 131484-68-9; **8** isomer 1, 131484-61-2; **8** isomer 2, 131380-61-5; **9** isomer 1, 131486-45-8; **9** isomer 2, 131380-62-6; **10** isomer 1, 131484-62-3; **10** isomer 2, 131484-69-0; **11** isomer 1, 131484-63-4; **11** isomer 2, 131484-70-3; **12** isomer 1, 131380-57-9; **12** isomer 2, 131484-71-4; **13** isomer 1, 131380-58-0; **13** isomer 2, 131484-72-5; **14** isomer 1, 131380-59-1; **14** isomer 2, 131484-73-6; **15** isomer 1, 131484-64-5; **15** isomer 2, 131484-74-7; **16** isomer 1, 131380-60-4; **16** isomer 2, 131484-75-8; **17** isomer 1, 131484-65-6; **17** isomer 2, 131484-76-9; **18** isomer 1, 131484-66-7; **18** isomer 2, 131484-77-0; A, 28434-00-6; PI, 121-21-1; PII, 121-29-9.