Epoxychrysanthemates: Two-Dimensional NMR Analyses and Stereochemical Assignments†

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Two-dimensional NMR analyses of two pyrethroid insecticides, (*S*)-bioallethrin and (1*R*)-*cis*-phenothrin, and their diastereomeric 7,8-epoxy derivatives resulted in complete assignments of the ^1H signals of the acid moieties by COSY, long-range COSY, and NOESY measurements. The relevant ^{13}C signals of the six compounds were then assigned by C–H COSY experiments. Subsequent analyses of long-range C–H COSY spectra established that correlations between methyl carbons and adjacent methine protons involve those in a cis rather than a trans relationship, making this technique useful in assigning methyl resonances on the cyclopropyl and epoxy ring of related pyrethroids. The known stereochemical preference of Sharpless asymmetric epoxidation was used to assign the stereochemistry of the epoxy ring for the 7,8-epoxy-10-hydroxy derivatives of these two pyrethroids. Comparison of the NMR data of the 7,8-epoxy and 7,8-epoxy-10-hydroxy derivatives confirms that the configurations at C^7 of the principal 7,8-epoxy metabolites are *R* and *S* from microsomal oxidation of *trans*- and *cis*-chrysanthemates, respectively.

INTRODUCTION

^1H and ^{13}C NMR studies of pyrethroids with natural *trans*-chrysanthemic acid and synthetic *cis*-chrysanthemic acid moieties are important for structural, stereochemical, and conformational analyses (Bramwell et al., 1969; Crombie et al., 1975; Iwaoka et al., 1981; Janes, 1977; Krishnamurthy and Casida, 1987; Nakazawa et al., 1980). In these papers, there are some contradictory assignments involving the pairs of resonances for the cyclopropyl and vinyl *gem*-dimethyl groups. On incubation with microsomal oxidases (Smith and Casida, 1981; Class et al., 1990) or photooxidation initiated by sunlight or UV irradiation (Ruzo et al., 1980, 1982; Ueda et al., 1974) the chrysanthemates are converted to the corresponding 7,8-epoxy derivatives, i.e., epoxychrysanthemates, products that contain a new asymmetric center. Determination of the absolute configuration of this chiral center would establish the stereochemical preference of microsomal oxidation.

We used two-dimensional (2D) NMR techniques to unambiguously assign the ^1H and ^{13}C signals of the acid moieties of two chrysanthemate insecticides, (1*R*,1′*S*)-*trans*-allethrin [(*S*)-bioallethrin (**A**)] and (1*R*)-*cis*-phenothrin (**Ph**), and their 7,8-epoxy derivatives (**1** and **I**) (Figure 1). Although long-range C–H COSY studies were not needed for this purpose, this method was examined in detail to clarify conflicting assignments in the literature. Finally, Sharpless asymmetric epoxidation (Katsuki and Sharpless, 1980) was examined as a means of determining the stereochemistry of the epoxy ring in microsomal metabolites.

MATERIALS AND METHODS

Chromatography. TLC utilized silica gel 60 F_{254} chromatoplates (0.25- and 0.5-mm layers) (Merck, Darmstadt, Germany). HPLC, for analysis and separation of diastereomers (Ando et al., 1986), used a Beckman 344 gradient liquid chromatograph fitted with a Machrey-Nagel Nucleosil 5 NO_2 column (10 mm i.d. × 25 cm) (Rainin Instrument Co., Inc., Woburn, MA). The eluent was monitored at 235 nm, and quantitation was by integration of the peak areas.

NMR Spectroscopy. NMR spectra were recorded with a Bruker AM-300 instrument (300 MHz for ^1H and 75 MHz for ^{13}C) and an ASPECT 3000 computer for chloroform-*d* or pyridine-d_5 solutions, containing tetramethylsilane as internal standard, in 5-mm tubes. 2D NMR spectra were acquired by using the following pulse sequences and parameters: ^1H–^1H correlation spectroscopy (COSY), T–$^1H90°$–t_1–$^1H90°$–Acq (T = 1.5 s); long-range COSY, T–$^1H90°$–Δ–t_1–$^1H90°$–Δ–Acq (T = 2.0 s, Δ = 0.2 s); nuclear Overhauser effect spectroscopy (NOESY), T–$^1H90°$–t_1–$^1H90°$–τ–$^1H90°$–Acq (T = 2.5 s, mixing delay τ = 1.0 s); C–H COSY, T–$^1H90°$–$t_1/2$–$^{13}C180°$–$t_1/2$–Δ_1–$^1H90°$,$^{13}C90°$–Δ_2–^1HBBD/Acq (T = 1.5 s, Δ = 3.3 ms, Δ_2 = 1.65 ms); long-range C–H COSY, same sequence with $\Delta_2 = \Delta_1/2$ and Δ_1 as indicated later. Homonuclear correlation spectra were acquired with a spectral width of 1901 Hz by using a 1024 × 256 data set, zero filling to 2048 × 1024, and transforming after multiplication by an unshifted sine-bell function in both dimensions. Heteronuclear correlation spectra were acquired with spectral widths of 8065 (^{13}C) and 1800 or 900 Hz (^1H) by using a 2048 (t_2) × 128 (t_1) data set, zero filling to 4096 × 256, and transforming after multiplication by an exponential function (line-broadening factor = 6 Hz) in t_2 and an unshifted sine-bell function in t_1. The number of transients acquired for each t_1 increment varied with sample concentration, but long-range C–H COSY spectra required 200 scans per increment even with concentrated samples (300 mg/mL).

Chemicals. The numbering system for the chrysanthemates and their derivatives is shown in Figure 1. Pyrethroid **A**, supplied by McLaughlin Gormley King Co. (Minneapolis, MN), was converted to its 7,8-epoxy derivative (**1**) and 10-hydroxy derivative (**2**) as previously described (Class et al., 1990). The diastereomers of **1** were separated by preparative TLC (20% acetone in *n*-hexane; **1a**, R_f 0.35; **1b**, R_f 0.28). Pyrethroid **Ph**, from Roussel-Uclaf (Paris, France), was oxidized to its diastereomeric (49:51) 7,8-epoxy derivative (**I**) in ~90% yield by treatment with equimolar *m*-chloroperoxybenzoic acid (MCPBA) in dichloromethane. The diastereomers were separated by HPLC (10% dioxane in *n*-hexane, 2.5 mL/min; **Ia**, R_t = 13.4 min; **Ib**, R_t = 16.8 min). The 10-hydroxy derivative (**II**) was obtained in ~25% yield, accompanied by the corresponding aldehyde, on treatment of **Ph** with equimolar selenium dioxide in 10% water in dioxane under re-

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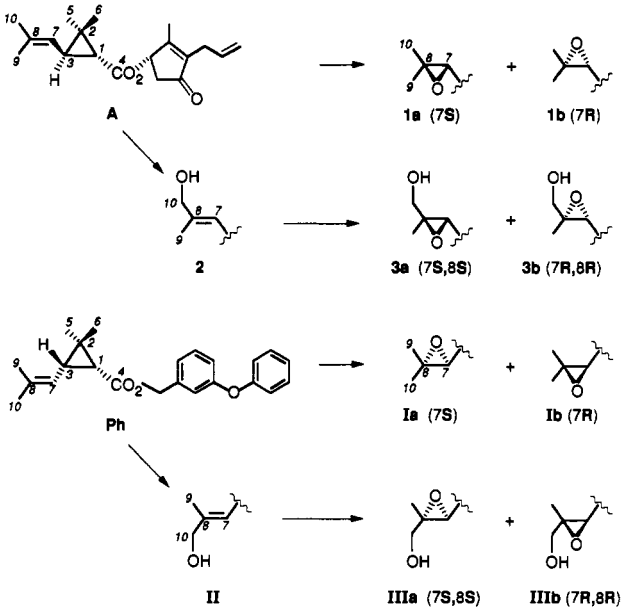

Figure 1. Structures, numbering system, and stereochemical designations for (*S*)-bioallethrin (**A**), (1*R*)-*cis*-phenothrin (**Ph**), and their 7,8-epoxy (**1a**, **1b**, **Ia**, and **Ib**), 10-hydroxy (**2** and **II**), and 7,8-epoxy-10-hydroxy (**3a**, **3b**, **IIIa**, and **IIIb**) derivatives.

flux. The desired alcohol was isolated by preparative TLC and characterized by NMR (see Tables I and II). Oxidation of this *cis*-chrysanthemate occurs almost exclusively at the 10-methyl group as observed also for oxidation of *trans*-chrysanthemate **A** (Class et al., 1990).

MCPBA Epoxidation of 10-Hydroxy-(*S*)-bioallethrin (2) and 10-Hydroxy-(1*R*)-*cis*-phenothrin (II). Compound **2** (0.16 mmol, 51 mg) or **II** (0.23 mmol, 80 mg) in dichloromethane (1.5 mL) was treated with equimolar MCPBA for 2 h at room temperature. The reaction mixtures were washed with 1 N hydrochloric acid and aqueous sodium bicarbonate solutions, dried with sodium sulfate, and fractionated by TLC, yielding diastereomeric epoxides **3** (40 mg, 75% yield) or **III** (54 mg, 71% yield). HPLC separated the two isomers of **3** (35% dioxane in *n*-hexane, 2 mL/min; **3a**, R_t = 15.3 min; **3b**, R_t = 16.2 min) and **III** (25% dioxane in *n*-hexane, 2.5 mL/min; **IIIa**, R_t = 16.9 min; **IIIb**, R_t = 18.8 min). Products of each reaction were analyzed by HPLC, and the ratio of the two diastereomers was calculated. NMR data for the epoxides are given in Tables I and II.

Sharpless Epoxidation of 10-Hydroxy-(*S*)-bioallethrin (2) and 10-Hydroxy-(1*R*)-*cis*-phenothrin (II). Epoxidation was carried out according to the method of Katsuki and Sharpless (1980). Thus, dichloromethane (5 mL, distilled from calcium hydride) was cooled in a dry ice/carbon tetrachloride bath; titanium(IV) tetraisopropoxide [Ti(O*i*Pr)$_4$] (0.18 mmol, 51 mg), (+)-diisopropyl tartrate (DIPT) (0.18 mmol, 42 mg), and **2** (0.16 mmol, 51 mg) were added sequentially under nitrogen gas. After 10 min, *tert*-butyl hydroperoxide (TBHP) (0.36 mmol, 120 μL of 3 M solution in toluene) was added, and the mixture was stirred for 4 h in the cooling bath and stored overnight at −20 °C. After the usual workup, the product mixture was analyzed by HPLC. The major component (**3a**) was isolated by preparative HPLC (22 mg, 41% yield) and its structure confirmed by NMR. The procedure was repeated with (−)-DIPT, and the structure of the major component (**3b**) (19 mg, 36% yield) was confirmed by NMR. Sharpless epoxidations were repeated for **II** (0.2 mmol, 70 mg), again with both (+)- and (−)-DIPT, and product mixtures analyzed by HPLC.

RESULTS AND DISCUSSION

^{1}H NMR Assignments of the Acid Moieties of A, Ph, and Their 7,8-Epoxy Derivatives (Table I). All compounds were examined in chloroform-*d*; **1a**, **1b**, **Ph**, and **Ib** were also examined in pyridine-d_5, in which otherwise overlapping methyl proton signals were resolved. The H^1 and H^3 methine and the H^7 vinyl proton resonances are readily assigned from the one-dimensional spectra on the basis of their chemical shifts and coupling patterns, and these assignments are confirmed by H^1–H^3 and H^3–H^7 cross peaks in the COSY spectra.

With **A**, the pair of cyclopropyl methyl resonances can be distinguished from the vinyl pair on the basis of chemical shifts (δ 1.26, 1.15 and δ 1.73, 1.72, respectively) which reflect the deshielding effect of the double bond. Within each of these pairs, the resonances may be assigned from the NOESY spectra. Thus, the resonance of δ 1.15 that correlates with H^1 and H^7 but not H^3 is assigned to H^6. The other cyclopropylmethyl resonance at δ 1.26, showing a cross peak with H^3 but not H^1, is assigned as H^5. Similarly, H^{10} correlates with H^7, indicative of a cis relationship, and this is supported by a second correlation between H^9 and H^3 (Figure 2). Similar analyses allow the assignment of the corresponding resonances in **Ph**.

For epoxides **1** and **I**, the NOESY spectra show correlations analogous to those observed for **A** and **Ph** (see Figures 2 and 3). However, because the methyl signals of the epoxides can no longer be subdivided into pairs on the basis of chemical shifts, it is not possible to discriminate between H^5 and H^9 of **1** and between H^5 and H^{10} of **I**. This ambiguity is resolved by long-range COSY measurements. In the spectrum of **1** (Figure 3) the protons of one of the methyl groups show long-range coupling to H^3, permitting its assignment as H^5 (four-bond coupling) rather than H^9 (five-bond coupling). Similarly, for **I**, the protons of the methyl group that are correlated to H^7 are assigned to H^{10} (four-bond coupling) rather than H^5 (five bond coupling).

The ^{1}H NMR assignments of chrysanthemic acids and their esters were reported by Bramwell et al. (1969) and our assignments for the *trans*-chrysanthemate (**A**) are consistent with their data. However, our assignments of the cyclopropylmethyl signals for the *cis*-acid moiety of **Ph** are opposite to their assignments in *cis*-chrysanthemic acid; they report the H^6 signal at lower field than the H^5 signal. Because of the possibility that the relative chemical shifts of the two signals might be reversed in going from the free acid to the ester, we examined the NOESY spectrum of the free *cis*-acid. As with the ester (**Ph**), protons appearing as the higher field singlet show cross peaks with H^1 and H^3 and are therefore assigned to H^6 rather than H^5.

^{13}C NMR Assignments of the Acid Moieties of A, Ph, and Their 7,8-Epoxy Derivatives (Table II). With all of the proton signals of the acid moieties unambiguously assigned, the ^{13}C NMR assignments for carbons bearing protons can be readily elucidated from C–H COSY spectra. For **1a**, **1b**, and **Ib**, where the methyl proton signals overlap in chloroform-*d*, the C–H COSY spectra were acquired also in pyridine-d_5. Since ^{13}C chemical shifts in the present case showed little dependence on solvent, the assignments obtained in pyridine-d_5 were utilized to assign some methyl signals in spectra recorded in chloroform-*d*. Quaternary carbons (C^2, C^4, and C^8) were readily assigned, solely from chemical shift considerations.

Our assignments for the vinyl methyl (C^9 and C^{10}) and the cyclopropylmethyl (C^5 and C^6) carbons for **Ph** differ from literature reports (Janes, 1977; Krishnamurthy and Casida, 1987) but are consistent with predictions based on steric compression (Nakazawa et al., 1980). In all cases, C^9 is crowded relative to C^{10} and is expected to resonate at higher field. Similarly, in the *cis*-acid derived esters C^5 is expected to show a large upfield shift relative to C^6. Chemical shift differences of 7–14 ppm are, in fact, observed in the predicted direction.

Table I. Partial ^1H Peak Assignments of (*S*)-Bioallethrin (A), (1*R*)-*cis*-Phenothrin (Ph), and Their Derivatives with Modified Acid Moieties

		^1H shift, ppm, for indicated position							coupling constant,[a] Hz	
compd	solvent	1	3	5	6	7	9	10	J_{1-3}	J_{3-7}
A	chloroform-*d*	1.42	2.08	1.26	1.15	4.91	1.72	1.73	5.5	7.5
1a	chloroform-*d*	1.68	1.58	1.26	1.26	2.72	1.31	1.33	5.5	4
	pyridine-d_5	1.89	1.70	1.31	1.22	2.77	1.29	1.27	5.5	4
1b	chloroform-*d*	1.57	1.39	1.25	1.33	2.52	1.37	1.33	5.5	8
	pyridine-d_5	1.90	1.62	1.29	1.31	2.71	1.33	1.26	5.5	8
3a	chloroform-*d*	1.70	1.59	1.26	1.26	3.05	1.33	3.61, 3.71	5.5	4
3b	chloroform-*d*	1.59	1.44	1.27	1.34	2.88	1.37	3.63, 3.73	5.5	8.5
Ph	chloroform-*d*	1.71	1.90	1.24	1.19	5.37	1.68	1.74	8.5	8.5
	pyridine-d_5	1.88	2.03	1.36	1.11	5.69	1.66	1.70	8.5	8.5
Ia	chloroform-*d*	1.69	1.01	1.35	1.21	3.10	1.22	1.26	8.5	7.5
Ib	chloroform-*d*	1.73	1.05	1.31	1.18	3.26	1.33	1.33	8.5	7.5
	pyridine-d_5	1.83	1.21	1.35	1.08	3.49	1.30	1.27	8.5	7.5
II	chloroform-*d*	1.77	1.92	1.25	1.21	5.66	1.73	4.01	8.5	8.5
IIIa	chloroform-*d*	1.73	1.06	1.32	1.24	3.24	1.27	3.45, 3.58	8.5	7
IIIb	chloroform-*d*	1.76	1.11	1.30	1.21	3.54	1.36	3.57, 3.70	8.5	8

[a] J_{10-10} = 12.5 Hz for **3a** and **3b**, 11.5 Hz for **IIIa**, and 12 Hz for **IIIb**.

Table II. Partial ^{13}C Peak Assignments of (*S*)-Bioallethrin (A), (1*R*)-*cis*-Phenothrin (Ph), and Their Derivatives with Modified Acid Moieties

		^{13}C shift, ppm, for indicated position									
compd	solvent	1	2	3	4	5	6	7	8	9	10
A	chloroform-*d*	34.4	28.9	32.8	172.1	20.3	22.0	120.6	135.7	18.4	25.4
1a	chloroform-*d*	30.7	27.3	31.3	171.6	20.1	21.7	60.7	58.4	18.9	24.4
	pyridine-d_5	31.0	27.2	31.8	171.8	20.2	21.5	60.4	58.1	18.9	24.4
1b	chloroform-*d*	31.7	28.0	30.7	171.6	20.5	21.9	62.7	58.1	19.4	24.5
	pyridine-d_5	31.6	27.7	31.2	171.6	20.4	21.7	62.2	57.8	19.4	24.4
3a	chloroform-*d*	30.9	27.3	30.6	171.5	20.1	21.7	56.7	61.0	14.4	64.7
3b	chloroform-*d*	31.6	28.0	29.8	171.2	20.4	21.9	58.3	60.7	14.8	64.5
Ph	chloroform-*d*	31.2	26.6	32.4	170.8	14.8	28.8	118.0	134.8	18.3	25.9
	pyridine-d_5	31.3	26.7	32.6	170.8	15.0	28.4	118.9	134.7	18.3	25.9
Ia	chloroform-*d*	29.4	25.7	31.0	170.7	14.4	28.4	59.8	58.2	19.0	24.5
Ib	chloroform-*d*	29.0	25.4	31.8	170.7	14.8	28.5	58.7	58.6	19.4	24.6
	pyridine-d_5	29.0	25.5	32.0	170.8	14.8	28.1	58.5	58.1	19.4	24.6
II	chloroform-*d*	31.5	27.0	31.7	170.8	14.8	28.7	119.8	138.3	14.2	68.7
IIIa	chloroform-*d*	29.7	25.6	30.2	171.1	14.4	28.2	57.2	60.5	14.6	67.0
IIIb	chloroform-*d*	29.2	25.7	30.8	170.8	14.7	28.5	55.4	61.5	14.8	65.6

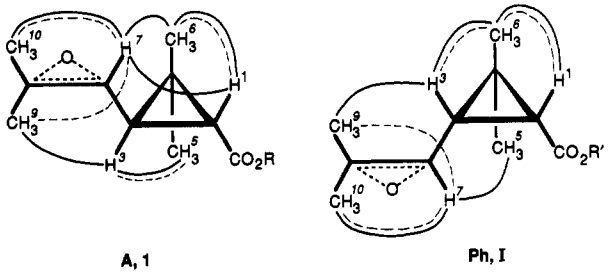

Figure 2. Correlations in NOESY (—) and long-range COSY (- - -) spectra of (*S*)-bioallethrin (**A**, 7,8 double bond) and (1*R*)-*cis*-phenothrin (**Ph**, 7,8 double bond), and their 7,8-epoxy derivatives (**1** and **I**).

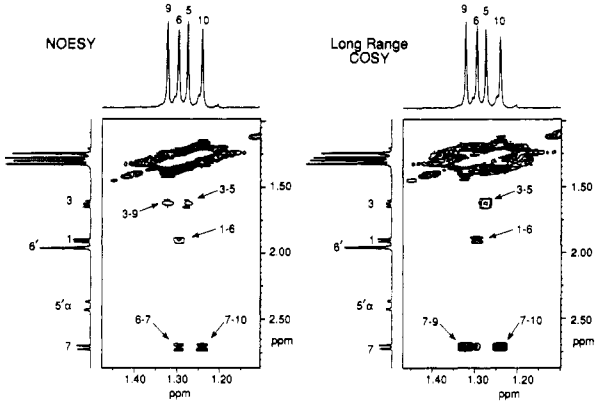

Figure 3. Partial NOESY and long-range COSY spectra of (7*R*)-7,8-epoxy-(*S*)-bioallethrin (**1b**).

Long-Range C–H COSY Analyses of A, Ph, and Their Epoxy Derivatives. Since our assignments of the ^{13}C NMR spectra of **Ph** and its epoxy derivative, based on NOESY and long-range homonuclear COSY spectra, are in conflict with literature reports based on long-range C–H COSY spectra (Krishnamurthy and Casida, 1987; Krishnamurthy et al., 1987), we further considered the long-range C–H COSY spectra of **A**, **Ph**, **1**, and **I** as a means of verifying the new assignments.

In the standard pulse sequence for heteronuclear shift correlation spectroscopy there are two delays, Δ_1 and Δ_2, which are determined by the coupling constant (J_{XH}) of interest and the number of hydrogens coupled (Derome, 1987). To optimize coherence transfer and refocusing for long-range ^{13}C–^1H couplings ($^nJ_{CH}$ < 15 Hz: Marshall, 1983), delays are used that are more than 10 times longer than those used for one-bond correlations ($^1J_{CH}$ = ca. 150 Hz). In the simple case of a methine carbon, which has only one long-range coupling to a single proton, the intensity of a cross peak is modulated by the product of four terms: the efficiency of coherence transfer ($\sin(\pi\ ^nJ\Delta_1)$), the efficiency of refocusing ($\sin(\pi\ ^nJ\Delta_2)$), the generation of antiphase magnetization with respect to $^1J_{CH}$ during the Δ_2 delay ($\cos(\pi\ ^1J\Delta_2)$), and the exponential loss of signal due to transverse (T_2) relaxation during the Δ_1 and Δ_2 delays (Krishnamurthy and Nunlist, 1988). If ^nJ is equal to $1/(2\Delta_1)$, the first term will be maximized; similarly, if ^nJ is equal to $1/(2\Delta_2)$, the second term will be maximized. The third term will vary rapidly with Δ_2 since

Table III. Cross Peaks in Long-Range C–H COSY Spectra[a] of (*S*)-Bioallethrin (A) in Chloroform-*d* and Its Epoxy Derivative (1) in Pyridine-d_5

	A				1a (7*S* isomer)			1b (7*R* isomer)
Δ_1, ms:	33.4	65	80	90	65	80	90	80
$^nJ_{CH}$, Hz:	15	7.7	6.3	5.6	7.7	6.3	5.6	6.3
C^1	H^5, H^6	H^5, H^6	H^5, H^6	H^5, H^6	H^5, H^6, (H^7)[b]	H^5, H^6, (H^7)	H^5, H^6	(H^3), H^5, H^6
C^2	H^5, H^6	H^5, H^6	(H^1), H^5, H^6	H^5, H^6	H^5, H^6	H^5, H^6	H^5, H^6	H^5, H^6
C^3	H^5, H^6	H^5, H^6	(H^1), H^5, H^6	(H^1), H^5, H^6, (H^7)	H^5, H^6, H^7	H^5, H^6, (H^7)	(H^1), H^5, H^6, (H^7)	(H^1), H^5, H^6, (H^7)
C^5	H^6	H^6	(H^3), H^6	(H^3), H^6	H^6	(H^3), H^6	H^6	H^3, H^6
C^6	H^5	H^5	H^1, H^5	(H^1), H^5	H^1, H^5	H^1, H^5	(H^1), H^5	H^1, H^5
C^7	(H^9), H^{10}	H^9, H^{10}	H^9, H^{10}	(H^1), H^9, H^{10}	H^9, H^{10}	H^1, H^9, H^{10}	H^9, H^{10}	(H^1), H^9, H^{10}
C^8	H^9, H^{10}	H^9, H^{10}	H^9, H^{10}	H^9, H^{10}	H^9, H^{10}	H^9, H^{10}	H^9, H^{10}	H^9, H^{10}
C^9	H^7, (H^{10})	(H^7), H^{10}	(H^7), H^{10}	H^{10}	H^{10}	H^{10}	H^{10}	H^{10}
C^{10}	(H^7), (H^9)	H^7, H^9	H^7, H^9	H^9	(H^7), H^9	(H^7), H^9	H^9	H^7, H^9

[a] Pulse sequence (T–^{1}H90°–$t_1/2$–^{13}C180°–$t_1/2$–Δ_1–^{1}H90°, ^{13}C90°–Δ_2–^{1}HBBD/Acq, T = 1.5 s, $\Delta_2 = \Delta_1/2$). [b] Parentheses indicate weak long-range correlation.

Table IV. Cross Peaks in Long-Range C–H COSY Spectra[a] of (1*R*)-*cis*-Phenothrin (Ph) in Pyridine-d_5 and Its Epoxy Derivative (I)[b] in Chloroform-*d*

	Ph				I			
Δ_1, ms:	33.4	65	80	90	33.4	65	80	90
$^nJ_{CH}$, Hz:	15	7.7	6.3	5.6	15	7.7	6.3	5.6
C^1	H^5, H^6	H^5, H^6	(H^5),[c] H^6	(H^3), H^5, H^6	H^5, H^6	H^5, H^6	(H^5), H^6	H^5, H^6
C^2	H^5, H^6	(H^1), H^5, H^6, (H^7)	(H^1), (H^3), H^5, H^6, (H^7)	(H^3), H^5, H^6	H^5, H^6	(H^1), (H^3), H^5, H^6	H^5, H^6	H^5, H^6
C^3	H^5, H^6	H^5, H^6	H^5, H^6	(H^1), H^5, H^6	H^5, H^6	H^5, H^6, H^7	(H^5), H^6	H^5, H^6, (H^7)
C^5	H^6	H^6	(H^1), (H^3), H^6	H^6	H^6	H^6	H^6	H^6
C^6	H^5	H^1, H^3, H^5	H^1, H^3, H^5	(H^1), H^5	(H^1), (H^3), H^5	H^1, H^3, H^5	H^1, H^3, H^5	H^5
C^7	H^9, H^{10}	H^9, H^{10}	H^9, H^{10}	H^9, H^{10}	(H^3), H^9, H^{10}	H^9, H^{10}	(H^3), H^9, H^{10}	(H^1), (H^3), H^9, H^{10}
C^8	H^9, H^{10}	(H^3), H^9, H^{10}	(H^3), H^9, H^{10}	(H^3), H^9, H^{10}	H^9, H^{10}	H^9, H^{10}	H^9, H^{10}	H^9, H^{10}
C^9	H^7, H^{10}	H^7, H^{10}	(H^7), H^{10}	H^{10}	H^{10}	H^{10}	H^{10}	H^{10}
C^{10}	(H^7), H^9	H^7, H^9	H^7, H^9	(H^9)	H^9	H^7, H^9	(H^7), H^9	H^9

[a] Pulse sequence (T–^{1}H90°–$t_1/2$–^{13}C180°–$t_1/2$–Δ_1–^{1}H90°, ^{13}C90°–Δ_2–^{1}HBBD/Acq, T = 1.5 s, $\Delta_2 - \Delta_1/2$). [b] Mixture of two diastereomers (**Ia** and **Ib**). [c] Parentheses indicate weak long-range correlation.

$^1J \gg 1/(2\Delta_2)$, introducing a more or less random variation that is very sensitive to the exact value of Δ_2. The final term means that long-range couplings with small ^nJ values will be very difficult to detect due to the large Δ_1 and Δ_2 values required to maximize the first two terms. Thus, one can, in principle, determine the relative size of long-range C–H couplings by repeating the experiment with different values of Δ_1 and Δ_2, but the results must be interpreted with care.

For the long-range C–H COSY of chrysanthemates and epoxychrysanthemates, four separate experiments were performed by using different Δ_1 and Δ_2 values (Δ_1 = 33.4, 65, 80, and 90 ms, corresponding to $^nJ_{CH}$ = 15, 7.7, 6.3, and 5.6 Hz; $\Delta_2 = \Delta_1/2$). Table III summarizes the cross peaks observed in the long-range C–H COSY spectra of the compounds with a 1,3-trans configuration at the cyclopropyl ring (**A** and **1**), and Table IV shows the data for the corresponding compounds with the 1,3-cis configuration (**Ph** and **I**). Although each spectrum shows many cross peaks from long-range coupling (two-bond or three-bond coupling) with the protons of the four methyl groups, of particular interest for stereochemical assignments are the cross peaks observed for the cyclopropylmethyl carbons (C^5 and C^6) with the cyclopropylmethine protons (H^1 and H^3). The spectra of **A** and **1** show C^6–H^1 and C^5–H^3 cross peaks, while the spectra of **Ph** and **I** show C^6–H^1 and C^6–H^3 correlations. Each of these represents a cis relationship of the relevant nuclei with respect to the cyclopropyl ring. Cross peaks between methyl carbons and protons in a trans relationship are observed in only one spectrum, and in this case the corresponding cis correlations are much stronger. This indicates that the three-bond carbon–proton couplings for nuclei in a cis orientation with respect to the cyclopropyl ring are larger than the corresponding $^3J_{CH}$ values from a trans relationship. All of these cross peaks are weak, but they are clearly observable with large numbers of transients and concentrated samples by using a Δ_1 delay of 80 ms.

Although the available literature data on $^3J_{CH}$ are limited compared with those on $^3J_{HH}$, the $^3J_{CH}$ is predicted to be about 0.5–0.7 times the corresponding $^3J_{HH}$ value for a structure where proton replaces carbon in the same configuration (Marshall, 1983; Breitmaier and Voelter, 1987). By use of this rule, average $^3J_{HH}$ values of 8–10 Hz for cyclopropyl protons in a cis relationship and 4–6 Hz in a trans relationship (Gordon and Ford, 1972; Jackman and Sternhell, 1969; see also Table I) correspond to predicted values of 4–7 Hz for the cis $^3J_{CH}$ and 2–4 Hz for the trans $^3J_{CH}$. This is consistent with our results which indicate that long-range correlations between cis-related (relative to the ring) cyclopropylmethyl carbons (C^5 and C^6) and methine protons reach optimal intensity when $1/(2\Delta_1)$ equals 6.3 Hz and that trans $^3J_{CH}$ values are apparently so small that in most cases cross peaks are not observed.

A similar comparison can be made for the epoxy ring protons and carbons of epoxychrysanthemates **1** and **I**. Average $^3J_{HH}$ values of 3–5 Hz for protons in a cis relationship and 2–3.5 Hz for protons in a trans relationship on an epoxy ring lead to predicted $^3J_{CH}$ values of 1.5–3.5 for cis and 1–2.5 for trans. This is consistent with our observation that C^9–H^7 (trans) cross peaks are not observed while C^{10}–H^7 (cis) cross peaks are observed but only with longer Δ_1 delays (Tables III and IV). Likewise, average values of 12–18 (trans) and 6–12 Hz (cis) for $^3J_{HH}$ in olefins correspond to predicted $^3J_{CH}$ values of 6–12.5 (trans) and 3–8.5 Hz (cis). This difference is clearly evident from the cross peaks observed between the vinylmethyl carbons (C^9 and C^{10}) and the olefinic proton (H^7) of **A** and **Ph**, which show an optimum $1/(2\Delta_1)$ value of 15 Hz for the trans (C^9–H^7) cross peak and an optimum value of 7.7 or 6.3 Hz for the cis (C^{10}–H^7) cross peak (Tables III and IV).

The configuration of the cyclopropyl ring is very important to chemical and biological studies of rethrins and their derivatives. Our results indicate that the configuration can be determined from long-range C–H COSY spectra with appropriate delays. The complete ^1H and ^{13}C NMR assignments for **Ph** (Krishnamurthy and Casida, 1987) and epoxycyphenothrin (Krishnamurthy et al., 1987), with a 1,3-cis configuration, have been reported by using only C–H COSY and long-range C–H COSY methods. In those studies it was assumed that long-range C–H COSY spectra should only give cross peaks between trans vicinal nuclei on the cyclopropyl ring. This led to an incorrect conclusion, and their assignments for the cyclopropylmethyl groups must now be reversed. When the close analogy between $^3J_{CH}$ and $^3J_{HH}$ values is applied to average values of ^1H–^1H coupling constants for the specific ring system in question, however, the correct signal assignments can be accomplished by their method. Similarly, C–H COSY and long-range C–H COSY spectra of **A** and **1**, with 1,3-trans configurations, are sufficient to assign all of their proton and carbon signals.

Stereochemistry of 7,8-Epoxy- and 7,8-Epoxy-10-hydroxy-(*S*)-bioallethrin (1 and 3, Respectively). 7,8-Epoxy-(*S*)-bioallethrin (**1**) is one of the main products of **A** either in mouse microsomal oxidase systems (Class et al., 1990) or in thin films exposed to sunlight (Ruzo et al., 1980). While oxidation of **A** with MCPBA gives **1** as a mixture of two diastereomers in a ratio of ca. 1:1, only the more polar isomer **1b** is detected as a microsomal metabolite. The configuration of the epoxy ring can be tentatively assigned from the ^1H NMR spectrum on the basis of the relative values of $J_{3\text{-}7}$ and the relative chemical shifts of H^1 and H^6 for the two diastereomers. If one assumes that rotation about the C^3–C^7 bond favors a conformation in which the maximum distance is maintained between bulky groups on each of the three-membered rings, the H^3–C^3–C^7–H^7 dihedral angle can be estimated for each isomer. For 1,3-*trans*-epoxychrysanthemates **1a** and **1b**, rotation about the C^3–C^7 bond is primarily restricted by interaction of the C^6 and C^9 methyl groups. Examination of models and energy minimization calculations indicate that maximum separation of these groups (C^2–C^3–C^7–C^8 dihedral angle of 180°) corresponds to H^3–C^3–C^7–H^7 dihedral angles of nearly 180° for the (7*R*)-epoxide and significantly less than 180° for the (7*S*)-epoxide. The Karplus–Conroy correlation for these dihedral angles allows one to assign the 7*R* stereochemistry to the isomer with the nearly maximal $^3J_{3\text{-}7}$ value (**1b**, 8 Hz) and the 7*S* stereochemistry to the isomer with a smaller ^3J value (**1a**, 4 Hz). Furthermore, this conformation places the epoxy oxygen on the H^1 side of the cyclopropyl ring for the 7*S* isomer and on the H^6 side for the 7*R* isomer. This is consistent with the relative chemical shifts of H^1 and H^6 in the diastereomers (**1a** and **1b**) if one assumes that proximity of the epoxy oxygen leads to a downfield shift. Thus, the 7*R* configuration has been assigned to the metabolite **1b** (Class et al., 1990).

Although NOESY experiments proved effective for assigning the configuration at C^7 of the 7,8-dihydroxy derivatives of **A** (Ando et al., 1990), we were unable to confirm the stereochemical assignment of the 7,8-epoxy derivatives (**1a** and **1b**) by this method. An alternative approach involved the 10-hydroxy derivative of **1**, which, in principle, could be stereospecifically epoxidized to either diastereomer by using the Sharpless procedure (Katsuki and Sharpless, 1980). Stereochemical assignments for **3a** and **3b** could then be extended to **1a** and **1b** if comparison of their NMR spectra permits unambiguous correlation.

Table V. 7,8-Epoxide Diastereomers Formed on Sharpless and MCPBA Epoxidation of 10-Hydroxy-(*S*)-bioallethrin (2) and 10-Hydroxy-(1*R*)-*cis*-phenothrin (II)

		diastereomer, %	
reactant	epoxidation condition	7*S*,8*S*	7*R*,8*R*
2		**3a**	**3b**
	TBHP/Ti(O*i*Pr)$_4$		
	(+)-DIPT	88	12
	(−)-DIPT	14	86
	MCPBA	52	48
II		**IIIa**	**IIIb**
	TBHP/Ti(O*i*Pr)$_4$		
	(+)-DIPT	55	45
	(−)-DIPT	29	71
	MCPBA	32	68

This approach proved to be fruitful. TBHP oxidation of **2** with (+)-DIPT gave a predominance of the less polar epoxide (**3a**), while reaction with (−)-DIPT gave primarily the more polar epoxide (**3b**) (Table V). Spectral assignments for these products were made by using the same 2D NMR techniques previously described for the 7,8-epoxy derivatives (Tables I and II). With the exception of the H^{10} resonances, the data for **3a** and **3b** are in close accord with those for **1a** and **1b**, respectively. These observations, in conjunction with the empirically expected result that (+)-DIPT should give the 7*S*,8*S* isomer and (−)-DIPT the 7*R*,8*R* isomer, allow assignment of the configurations of C^7 for the diastereomers **1a** and **1b** as *S* and *R*, respectively. Thus, the assignment of microsomal metabolite **1b** as 7*R* is confirmed. By way of comparison, oxidation of **2** with MCPBA is nonstereoselective and gives the diastereomeric 7,8-epoxy-10-hydroxy derivatives of **A** (**3**) in a ca. 1:1 ratio (Table V).

Stereochemistry of 7,8-Epoxy- and 7,8-Epoxy-10-hydroxy-(1*R*)-*cis*-phenothrin (I and III, Respectively). The approach analogous to that described above was less successful when applied to **II**: the Sharpless procedure displayed reduced stereoselectivity, particularly with (+)-DIPT (Table V). Apparently the increased steric hindrance in the vicinity of the olefin from the cis-oriented ester functionality impedes the formation of the specific complex necessary for stereoselectivity. Nevertheless, on the basis of the trends in stereoselectivity, the configurations of the less polar isomer (**IIIa**) and the more polar isomer (**IIIb**) can be assigned as 7*S*,8*S* and 7*R*,8*R*, respectively. Interestingly, the achiral oxidant MCPBA shows some selectivity in the epoxidation of **II**, and the major product (**IIIb**) is formed by the attack of MCPBA from the *re* face of C^7. Stereoselective MCPBA epoxidation has been noted previously with a chiral allylic alcohol containing an adjacent ether substituent and was rationalized as a cooperative directing effect involving two hydrogen bonds: one from the hydroxyl group and one to the ether oxygen (Johnson and Kishi, 1979). Since **II** possesses 1,3-cis stereochemistry, it is possible to draw a similar transition state for **II**, as shown in Figure 4, utilizing the allylic alcohol and either of the ester oxygen atoms. The alternative transition state in which MCPBA approaches C^7 from the *si* face is prohibited by the steric bulk of C^5.

NMR data for **Ia** correspond well with those of **IIIa** (Tables I and II), indicating that these two less polar isomers have the 7*S* configuration. Also, the close correspondence of the NMR data of **Ib** and **IIIb** indicates that the two more polar isomers have the 7*R* configuration. (1*R*)-cis-Cyphenothrin, which has the same acid moiety as **Ph**, was stereoselectivity oxidized by rat liver microsomes to yield the less polar isomer of the 7,8-epoxy

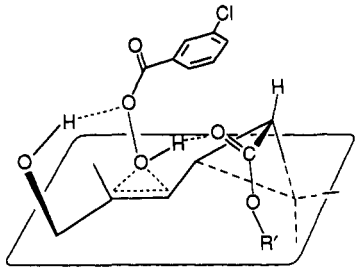

Figure 4. Proposed transition state of MCPBA oxidation of 10-hydroxy-(1*R*)-*cis*-phenothrin (**II**) based on a cooperative effect between the hydroxy group and ester carbonyl oxygen.

derivative (Krishnamurthy et al., 1987). Since this isomer and the less polar isomer of **I** show very similar NMR spectra with respect to the acid moiety (see Tables I and II and Krishnamurthy et al., 1987), the C^7 center in this metabolite now can be assigned the *S* configuration, indicating that oxygen approaches from the *si* face of the 7,8-double bond in this enzymatic epoxidation.

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