Resolution of Deltamethrin, Permethrin, and Cypermethrin Enantiomers by High-Performance Liquid Chromatography with Diode-Laser Polarimetric Detection

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Abstract

A normal-phase liquid chromatographic method is used to resolve the stereoisomers of the pyrethroid insecticides deltamethrin, cypermethrin, and permethrin by using two detectors: an ultraviolet (UV) spectrometer and a diode-laser polarimeter. In a mixture of enantiomers and diastereomers such as those resulting from the chemical structure of the pyrethroid insecticides with several asymmetric carbons, the UV spectrometer allows the detection of the diastereomers, whereas the diode-laser polarimeter permits the identification of the enantiomers. In this work, two enantiomers of deltamethrin, all four enantiomers of permethrin, and the four diastereomeric forms of cypermethrin are resolved without physical separation.

Introduction

Pyrethroids (deltamethrin, cypermethrin, and permethrin) are gaining increasing importance as insecticides in agriculture, forestry, horticulture, public health, and household usage. Deltamethrin ([*S*]-α-cyano-3-phenoxybenzyl[1*R*]-*cis*-3-[2,2-dibromovinyl]-2,2-dimethylcyclopropane-carboxylate) is a synthetic pyrethroid insecticide. It is an optically active compound that contains three asymmetric carbon atoms, so eight stereoisomers are possible. The enantiomer with the configuration 1R,3S,SαCN is a potent insecticide, whereas the optical antipode 1S,3R,RαCN is inactive (Figure 1). Cypermethrin ([*RS*]-α-cyano-3-phenoxybenzyl[1*RS*,3*RS*;1*RS*,3*SR*]-3-[2,2-dichlorovinyl]-2,2-dimethylcyclopropanecarboxylate) is a synthetic pyrethroid insecticide. The presence of three chiral centers results in eight possible stereoisomers—four pairs of enantiomers, each member of which has a diastereomeric relationship with each member of the other enantiomeric pairs (Figure 1). Permethrin (3-phenoxybenzyl[1*RS*,3*RS*;1*RS*,3*SR*]-3-[2,2-dichloroethenyl]-2,2-dimethylcyclopropanecarboxilate) is a synthetic pyrethroid insecticide possessing asymmetric centers at C-1 and C-3 of the cyclopropane ring; four stereoisomers are possible—two sets of enantiomers, each member of which is diastereomeric to each member of the other enantiomeric pair (Figure 1). It is well-known that the insecticidal activity is primarily associated with the (1R,3S)-*cis* and (1R,3R)-*trans* isomers. The *cis–trans* isomers ratio is about 40:60 (1).

Numerous methods have been proposed for the determination of the pyrethroid insecticides deltamethrin, cypermethrin, and permethrin by liquid chromatography (LC) (2–4) and gas chromatography (GC) (5–11) without the separation of isomers (12,13). Pyrethroid diastereomer and enantiomer separation into individual stereoisomers is of interest because its toxicity strongly depends on molecular shapes. However, little work has been directed to the separation of pyrethroid diastereomers and enantiomers into individual stereoisomers (14–18).

The resolution of racemates and the identification of optical antipodes is delicate and requires specific methods such as polarimetry or chiral phase high-performance liquid chromatography (HPLC). The separation and identification of deltamethrin isomers by LC has been previously (14,15) carried out using a chiral column with photometric and polarimetric detection. The separation and identification of cypermethrin stereoisomers by LC has been accomplished using a chiral column with photometric detection (16,17), and the separation and identification of stereoisomers of permethrin has been principally studied by GC (18).

Ideally, any analytical technique used for the analysis of pyrethroids must be capable of differentiating among these structural analogs. HPLC with chiral supports has allowed the separation of racemates. The specifity of these types of columns with respect to the substances to be studied has led to numerous theoretical studies on the separation mechanisms, thus permitting a better understanding and optimization of these separations (19–21). UV or fluorescence detection is currently used in this type of analysis. In contrast with the usual detection methods, polarimetric detection provides significantly different qualitative information. Currently conventional detectors cannot confirm the identity of the isomers as they elute. Optical-activity-based detectors can give information on the elution order of the antipodes. Additionally, UV and polarimetric detectors combined in series makes chromatographic enantiomer separation unnecessary because the UV detector signal is proportional to the total amount of enantiomer present, whereas the polarimetric detector signal is

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dependent on the ratio of enantiomers present.

Two distinct advantages favor HPLC against the batch approach for enantiomeric determinations based on polarimetric detection: easy automatization and elimination of potential interferences, chemical and optical, by virtue of the column selectivity. The characterization of enantiomers with a polarimetric detector allows the identification and quantitation (22–24) of the optical antipodes.

Unfortunately, conventional polarimeters do not have the requisite sensitivity to permit their application to trace determinations. However, the laser-based polarimetric system does possess the ability to provide specific rotation information on material present with microgram or lower quantities of sample (25,26). It is not the objective of this work to determine specific rotations for eluting materials. However, accurate and precise measurements of specific rotations can be made by calibrating the chromatographic peak height against the standard rotation induced by a Faraday coil.

This work describes a normal-phase LC method on an achiral column for the identification of stereoisomers of the pyrethroid insecticides deltamethrin, permethrin, and cypermethrin with photometric and diode-laser polarimetric detection. The method described here permits resolution without physical separation of two enantiomers of deltamethrin, all four enantiomers of permethrin, and the four diastereomeric forms of cypermethrin. The *cis*- diastereomers of cypermethrin were resolved from each other and from the *trans*- diastereomers but were not resolved from the individual enantiomers, probably because of the low rotatory power used in the solvent.

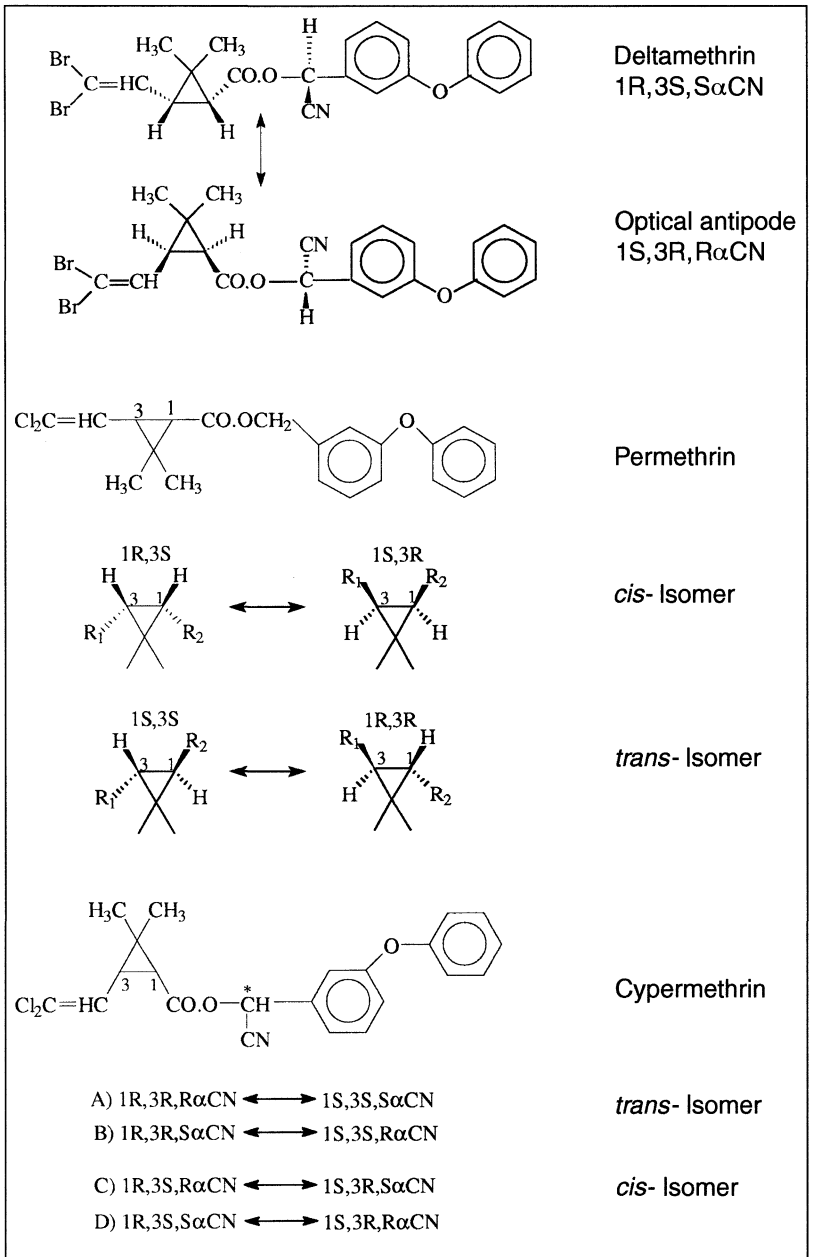

Figure 1. Structures of the pyrethroid insecticides.

Experimental

Standards and reagents

Deltamethrin and cypermethrin were obtained from Dr. S. Ehrenstorfer (Augsburg, Germany), and permethrin was obtained from Riedel-de Haën (Seelze, Germany). HPLC-grade 2-propanol, tetrahydrofuran, ethanol, dichlorometane, benzene, and hexane were purchased from Lichrosolv Merck (Darmstadt, Germany).

Stock standard solutions of deltamethrin, cypermethrin, and permethrin (10 g/L) were prepared by dissolving the compounds in benzene and protecting them from light. Working standard solutions were prepared by dilution with benzene. The solvents used as mobile phases were filtered through 0.2-µm nylon membrane filters.

Chromatographic systems

The measurements were performed with a Merck-Hitachi (Darmstadt, Germany) LC consisting of an L-6200 pump, an AS-4000 autosampler, an L-4250 UV–visible detector, and a D-6000 interface. Integration was carried out with a PC/AT computer, and instrumental parameters were controlled by Hitachi-Merck HM software.

A ChiralMonitor 2000 optical rotation detector (Applied Chromatography Systems, Macclesfield, England) was placed in series with and after the UV–visible detector and equipped with a collimated laser diode providing up to 30 mW of light at 830 nm, a flow cell path length of 0.48 dm, and a volume of 73 µL. The polarimetric detection system has been described in detail elsewhere (27). The instrumental parameters were controlled by Picolog Software (Picotechnology, Cambridge, UK), and the calculation of the area (negative and positive peaks), peak height, and retention time was performed with Lab-Cal LC software (Galactic, Salem, NH). The Picolog program has been described in detail elsewhere (28).

LC operating conditions

The stereoisomers deltamethrin, cypermethrin, and permethrin were analyzed using a Lichrospher Si60 column (25 cm × 4-mm i.d.; 10-µm particle size) from Merck (Darmstadt, Germany). The mobile phase composition was hexane–benzene (50:50, v/v) at a flow rate of 1 mL/min. Photometric (absorbance wavelength, 280 nm) and diode-laser polarimetric detection were used.

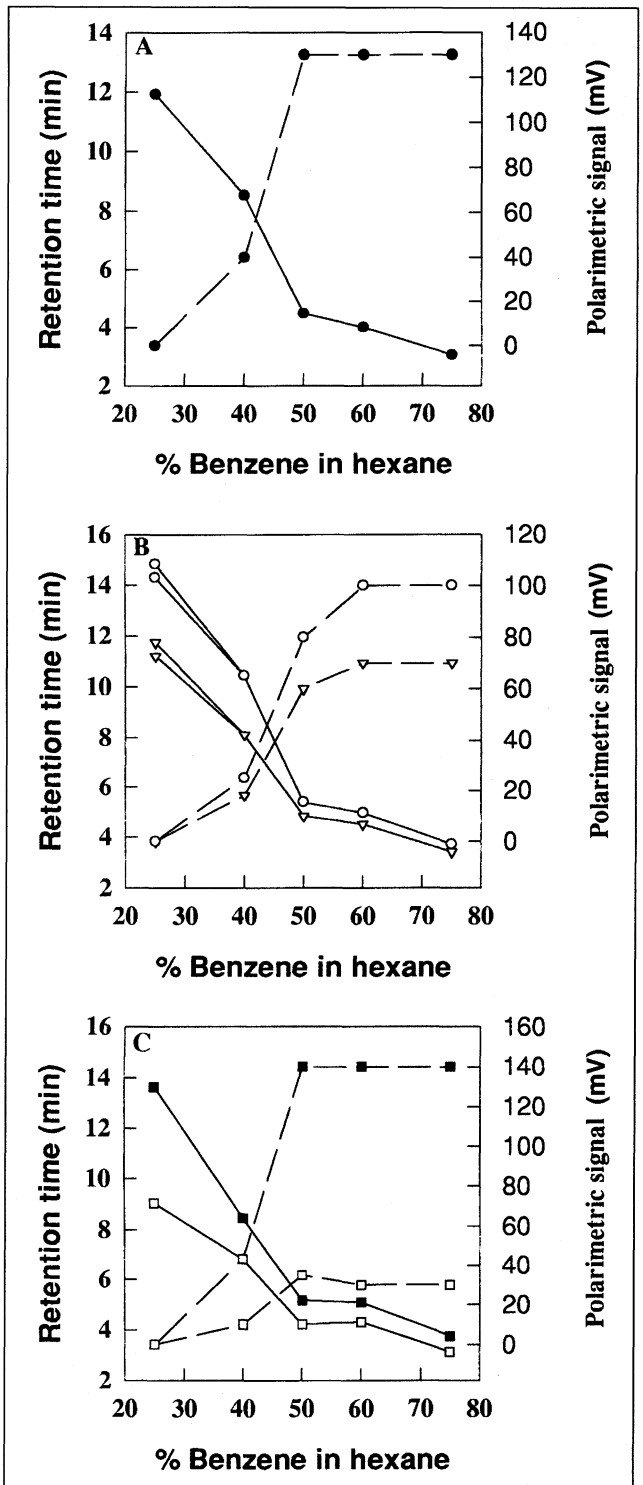

Figure 2. Effect of the benzene percentage in hexane on retention times (—) and polarimetric signals (---) of deltamethrin (A), cypermethrin (B), and permethrin (C). O and ∇ correspond to the *trans*- and *cis*-diastereoisomers of cypermethrin, respectively, and ■ and □ respectively correspond to the *trans*- and *cis*- diastereoisomers of permethrin.

Results and Discussion

Optimization of chromatographic conditions

The utilization of the diode-laser polarimeter and photometric detector in HPLC can eliminate possible interferences by other components or by other instrumental effects because of the unique selectivity of this device. The signals from the polarimetric detector have a primary problem; specifically, the injection peak is very big and sensitive to variations in pressure due to pump pulsations and thermal variation in the mobile phase. Thus the analyte peak must be separated from the injection peak for several minutes and the mobile phase must be previously mixed.

The selection of the mobile phase is important because of the solvent influence in the magnitude of optical rotation of the pyrethroids. The apolar solvent currently used in the separation and identification of the pyrethroids is hexane, in which the pyrethroids present negligible optical rotation but retention times are large enough to favor a good separation. The addition of polar solvents to the mobile phase such as methanol, ethanol, or 2-propanol increase the magnitude of rotation but shorten the retention times. The higher optical rotation of pyrethroids is obtained in benzene; however, a percentage higher than 60% shortens the retention times. Thus, several benzene–hexane mixtures were assayed as mobile phases in order to perform the separation and obtain good polarimetric signals. The use of the reversed stationary phase (Lichrospher RP18 column) with methanol, ethanol, 2-propanol, or water as the mobile phase resulted in poorer separation and a weak optical rotation of deltamethrin, permethrin, and cypermethrin.

Thus the diastereomers and enantiomers of deltamethrin, permethrin, and cypermethrin were analyzed using a normal stationary phase (Lichrospher Si60 column) and benzene–hexane as a mobile phase. The addition of more polar solvents like ethanol, 2-propanol, tetrahydrofuran, dichloromethane, acetonitrile, and 1,4-dioxane to the mobile phase (benzene–hexane) decreased the retention time of the pyrethroid so that the analytes' retention times were close to the injection peak.

Figure 2 shows the variations of the retention time and polarimeter signal of deltamethrin, permethrin, and cypermethrin with different percentages of benzene in hexane. As a trend, increasing the percentage of benzene in hexane caused a decrease in retention time and an increase in the polarimeter signal of the pyrethroids. As a result, 50% benzene in hexane was selected because at this percentage, the polarimetric signal was high enough and overlap between injection and analyte peaks did not occur.

The chromatograms of the pyrethroids studied under optimum conditions of mobile and stationary phases are depicted in Figure 3. The chromatogram of deltamethrin (Figure 3A) presents a chromatographic peak in the UV detector and two chromatographic peaks in the polarimeter

matching the two enantiomers; the configuration of the enantiomers was 1R,3S,SαCN(-) and 1S,3R,RαCN(+).

The chromatogram of permethrin (Figure 3C) presents two chromatographic peaks (UV detector) corresponding to two diastereomers (the configurations of the *cis-* and *trans-* diastereomers were 1RS,3SR and 1RS,3RS, respectively) and four chromatographic peaks (polarimeter) for the four enantiomers (the configurations of the diastereomers were 1R,3S[+] and 1S,3R[-] for the *cis*-diastereomer and 1R,3R[+] and 1S,3S[-] for the *trans*-diastereomer).

The chromatogram of cypermethrin (Figure 3B) shows two chromatographic peaks (UV detector) corresponding to the *cis-* and *trans-* diastereomers; the configurations of the *cis-* and *trans-* diastereomers were 1RS,3SR,RSαCN and 1RS,3RS,RSαCN, respectively. The polarimetric signal gave four chromatographic peaks corresponding to the *cis-* and *trans-* diastereomers; the configurations of the *cis-* and *trans-* diastereomers were 1RS,3SR,SαCN(+); 1RS,3SR,RαCN(-); 1RS,3RS,SαCN(+); and 1RS,3RS,RαCN(-), respectively.

The elution order of cypermethrin diastereomers on the silica phase met expectations because the carbonyl oxygen was more protected against hydrogen binding with the silanol groups in the *cis-* isomers, which were more lipophilic than the *trans-* isomers (29). The polarimetric peaks were attributed arbitrarily to one of the two possible forms because the isolated standards corresponding to each form were not available.

Table I indicates the retention times of the deltamethrin enantiomers and cypermethrin and permethrin diastereomers with respect to UV detection and diode-laser polarimetric signal as well as the corresponding relative standard deviations (RSDs).

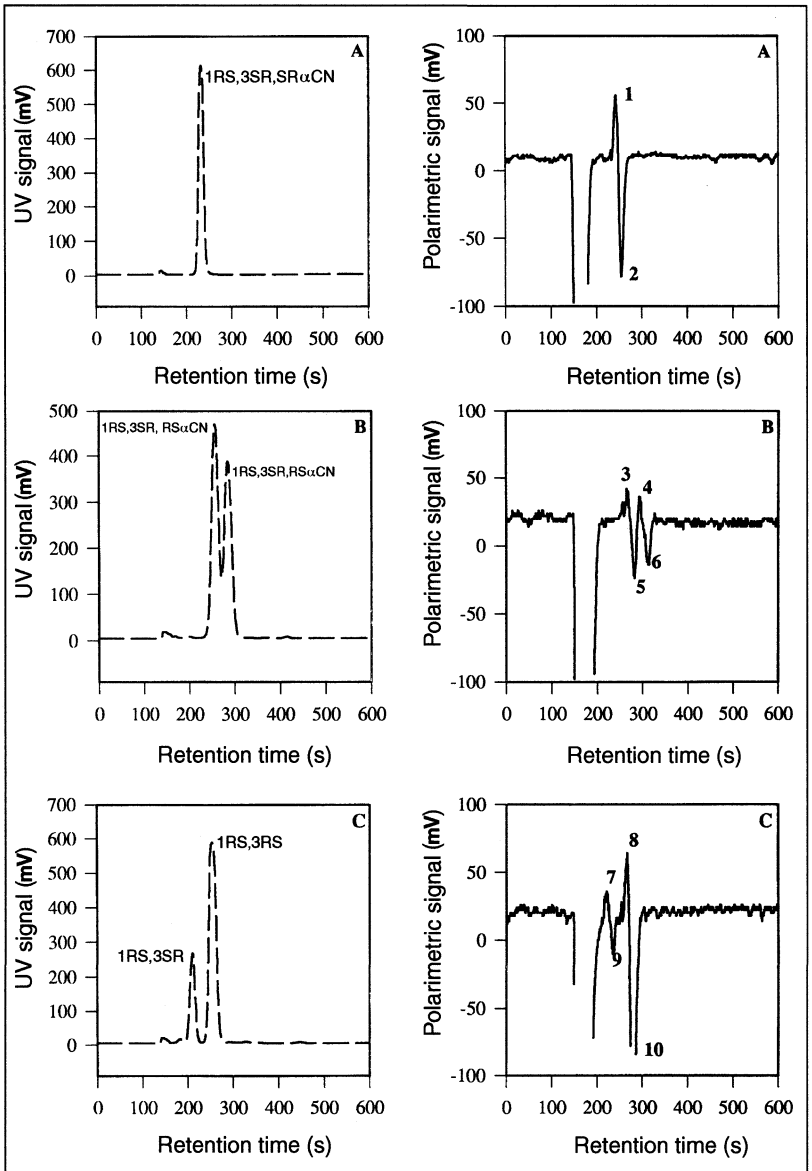

Figure 3. Chromatogram of the pyrethroids deltamethrin (280 µg) (A), cypermethrin (400 µg) (B), and permethrin (440 µg) (C). Photometric (---) and polarimetric (—) detection. Chromatographic conditions are described in the Experimental section (*LC operating conditions*). Peaks: 1, 1S,3R,RαCN; 2, 1R,3S,SαCN; 3, 1RS,3SR,SαCN; 4, 1RS,3SR,RαCN; 5, 1RS,3RS,SαCN; 6, 1RS,3RS,RαCN; 7, 1R,3S; 8, 1S,3R; 9, 1R,3R; 10, 1S,3S.

Enantiomeric determination using UV and polarimeter detectors in series

The response linearity of the polarimeter has been investigated for the diastereomers of deltamethrin, cypermethrin, and permethrin. The least squares line for each diastereomer of the pyrethroids studied in the range of 70–800 µg for deltamethrin and 200–1000 µg for cypermethrin and permethrin are shown in Table II. The detection limits (signal-to-noise ratio, 3) of deltamethrin, permethrin, and cypermethrin were 34, 91, and 112 µg, respectively.

Figure 4 shows the linearity of the two detectors in series (UV and polarimeter) for deltamethrin, cypermethrin, and permethrin; correlation coefficients were 0.99 or higher in each case. The linearity between two detectors eliminated any contribution to uncertainty from preparation of solutions or injections.

Table I and Figure 4 show that identification of the deltamethrin, cypermethrin, and permethrin stereoisomers may be established without the need for a physical separation with the aid of a diode-laser polarimetric detector and a careful selection of the mobile phase regarding both aspects of these kinds of compounds (e.g., separation and polarimetric signal).

Conclusion

Resolution of deltamethrin, permethrin, and cypermethrin enantiomers using polarimetric detection in LC has been shown. This technique has been used both as a selective detection and as a method for determining enantiomeric purity when coupled with an achiral detector such as a photometer.

Table I. Retention Times of the Pyrethroid Insecticides in the UV Spectrometer and Diode-Laser Polarimeter

Pyrethroid insecticides	UV detection			Polarimetric detection		
	Retention time (min)	RSD* (%)	Stereoisomeric forms	Retention time (min)	RSD* (%)	Diastereomeric forms
Deltamethrin	4.72	4.41	1RS,3SR,SRαCN	4.93	4.30	1S,3R,RαCN
				5.17	4.37	1R,3S,SαCN
Cypermethrin	4.92	1.93	*cis*- isomers	3.06	1.67	1RS,3SR,SαCN
				5.41	1.01	1RS,3SR,RαCN
	5.62	2.65	*trans*- isomers			
				5.74	2.29	1RS,3RS,SαCN
				6.10	2.68	1RS,3RS,RαCN
Permethrin	3.63	0.98	*cis*- isomers	3.76	1.99	1R,3S
				4.03	1.36	1S,3R
	4.39	2.14	*trans*- isomers			
				4.52	2.71	1R,3R
				4.85	1.01	1S,3S

* Eight replicates.

Table II. Least Square Fit for the Diastereomers of the Pyrethroids in the UV and Diode-Laser Polarimeter Detectors

Pyrethroid insecticides	Stereoisomeric forms	UV detection	Diastereoisomeric forms	Polarimetric detection
Deltamethrin	1RS,3SR,SRαCN	Area = 23563 + 404*C** r = 0.9974	1S3R,RαCN	Area = –1.84 + 0.12*C** r = 0.9944
			1R3S,SαCN	Area = 12.57 – 0.30*C** r = 0.9910
Cypermethrin	*cis*- isomers	Area = 35554 + 170*C** r = 0.9982	1RS3SR,SαCN	Area = 2.32 + 0.02*C** r = 0.9920
			1RS3SR,RαCN	Area = –2.11 – 0.02*C** r = 0.9980
	trans- isomers	Area = 37782 + 124*C** r = 0.9980	1RS3RS,SαCN	Area = 4.71 + 0.01*C** r = 0.9900
			1RS3RS,RαCN	Area = –2.34 – 0.02*C** r = 0.9990
Permethrin	*cis*- isomers	Area = 3930 + 304*C** r = 0.9980	1R,3S	Area = –3.40 + 0.03*C** r = 0.9921
			1S,3R	Area = –3.02 – 0.02*C** r = 0.9900
	trans- isomers	Area = 49595 + 304*C** r = 0.9975	1R,3R	Area = –2.80 + 0.05*C** r = 0.9915
			1S,3S	Area = 22.26 – 0.31*C** r = 0.9925

* C = amount (μg).

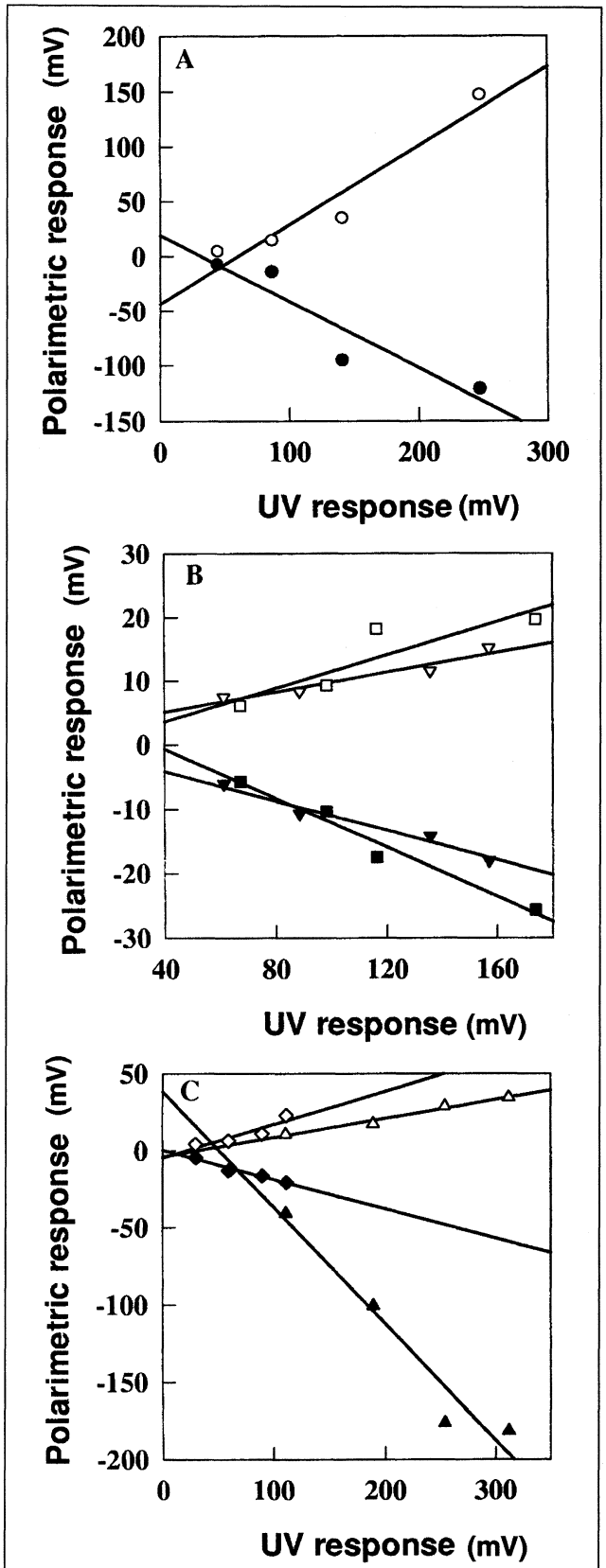

Figure 4. Linearity series showing UV response (abscissa) versus polarimetric response (ordinate). Deltamethrin (A), cypermethrin (B), and permethrin (C) stereoisomers. (○) 1S,3R,RαCN and (●) 1R,3S,SαCN deltamethrin enantiomers. (□) 1RS,3SR,SαCN, (■) 1RS,3SR,RαCN, (▽) 1RS,3RS,SαCN, and (▼) 1RS,3RS,RαCN cypermethrin diastereomers. (◊) 1R,3S, (◆) 1S,3R, (Δ) 1R,3R and (▲) 1S,3S permethrin diastereomers.

Acknowledgments

We thank the Comisión Interministerial de Ciencia y Tecnología (Projects PB93-1006 and BIO94-0548).

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Manuscript accepted November 17, 1997.