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CHIRAL-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF ENANTIOMERS OF PYRETHROID INSECTICIDE ESTERS DERIVED FROM α-CYANO-3-PHENOXYBENZYL ALCOHOL

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SUMMARY

The two enantiomers of fenpropanate and the four enantiomers of fenvalerate were separated on a commercially available NH_2-bonded column containing (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine as chiral phase. This column also separated three of the four possible enantiomer pairs of cypermethrin isomers and two of the four pairs of BAY FCR 1272 isomers. Conditions for these separations and an application to the analysis of cypermethrin residues in soil are described. The chromatographic behavior of the diastereomers of these materials and other pyrethroid insecticides on silica and NH_2-bonded columns was also examined.

INTRODUCTION

The toxicity of the various enantiomers of the pyrethroid insecticides is known to vary greatly. The toxicity of materials synthesized from 2,2-dimethylcyclopropanecarboxylic acid (permethrin, cypermethrin, BAY FCR 1272, fenpropanate and deltamethrin) and from substituted α-phenylbutyric acids (fenvalerate and flucythrinate) is strongly dependent on the configuration of the chiral carbon adjacent to the carboxyl group. Permethrin enantiomers having the *R* configuration at this carbon are about 25 times more toxic to houseflies than those with the *S* configuration[1]. Fenvalerate enantiomers having the *S* configuration at this carbon (spatially equivalent to the *R* configuration in the dimethylcyclopropanecarboxylic acids) are 10–100 times more toxic to houseflies than those with the *R* configuration[2]. The toxicity of those insecticides having a chiral cyano-substituted benzylic carbon present in the 3-phenoxybenzyl alcohol portion of the molecule (all of the materials discussed except permethrin) is also affected by the configuration of this chiral carbon, with the *S* configuration being the more toxic to houseflies by a factor of 20–100[3]. To assess and compare meaningfully the overall toxicological properties of the mixtures of enantiomers present in the formulated insecticides or residual deposits, it is necessary to be able to determine the amounts of the individual enantiomers present.

The separation of enantiomers is usually accomplished by one of two general

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methods: (1) the separation of diastereomers formed by reaction with a chiral reagent or (2) separation by physical interaction with an added chiral material. The first method has been applied by Murano and co-workers[4–6] and Chapman and Harris[7] to the separation of diastereomeric esters of the cyclopropanecarboxylic acids derived from a number of pyrethroid insecticides. There are two difficulties with this type of separation. First, the molecule should be functionally capable of reacting with the chiral reagent directly. These pyrethroid esters do not possess a suitable functional group so it is first necessary to hydrolyze them to the constituent acids. For pyrethroids containing a chiral α-cyanobenzyl alcohol portion (most materials except permethrin) access to information on the isomer composition of the alcohol portion is lost at this step. This will occur even if the alcohol is recovered because it will have epimerized during the hydrolysis due to the ease of formation of the anion on the benzylic carbon adjacent the cyano group[8]. Second, the acid enantiomers must be stable under the conditions required for diastereomer formation. Fortuitously, the cyclopropanecarboxylic acids produced by hydrolysis of many of the pyrethroid insecticides do not epimerize under the alkaline conditions used for hydrolysis even though they have a potentially ionizable hydrogen on the chiral C-1 carbon. However, the substituted butyric acid enantiomers produced by the hydrolysis of fenvalerate and flucythrinate are not configurationally stable under alkaline conditions. This problem has been overcome in the case of fenvalerate by using catalytic hydrogenolysis to cleave the ester without epimerizing the acid[9]. Direct separation of the materials would avoid these problems.

The number of direct separations of mixtures of enantiomers has increased dramatically in the last few years with the concurrent development of high-performance liquid chromatography (HPLC) and the surface-modified silicas commonly used in this technique. The investigations of direct separation by Pirkle and co-workers, first using the interaction between stationary electron-rich α-aryl carbinols and a variety of chiral molecules containing an electron-deficient function[10] followed by the reverse separation of chiral α-aryl carbinols on a stationary phase of a chiral electron-deficient amino acid derivative[11] are of particular interest. The structural similarity between these α-aryl carbinols and the electron-rich α-aryl nitrile portions of the pyrethroids suggested that this chiral phase might provide some separation of the pyrethroid enantiomers. I wish to describe our results on the direct separation of enantiomers of fenpropanate, cypermethirn, BAY FCR 1272 and fenvalerate by HPLC on a commercially available chiral-phase column. Application of the technique to the determination of the enantiomer composition of cypermethrin residues in soil is illustrated and the chromatographic properties of the diastereomers of these and other pyrethroids on silica and NH_2-bonded phases are described.

MATERIALS AND METHODS

The HPLC system consisted of a Constametric IIG pump [Laboratory Data Control (LDC) Division of Milton Roy, Riviera Beach, FL, U.S.A.], a Rheodyne 7120 valve with a 20 μl loop (Rheodyne, Berkeley, CA, U.S.A.) and a variable-wavelength Spectromonitor III UV detector (LDC) operated at 220 nm. Columns used were 25 cm × 4.2 mm I.D. packed with 8-μm Spherisorb silica (LDC), 5-μm NH_2-bonded Spherisorb (Applied Science Division of Milton Roy, State College, PA,

U.S.A.) and 5-μm NH_2-bonded Spherisorb containing (*R*)-N-(3,5-dinitrobenzoyl)-phenylglycine (Pirkle type 1A) (Regis, Morton Grove, IL, U.S.A.). They were operated at 1 ml/min for all the separations described. The NH_2-bonded column was protonated with acetic acid and washed free of excess acid before determining the retention times listed in Table I. Poor chromatographic behavior was observed for cypermethrin, BAY FGC 1272, fenvalerate and flucythrinate on the NH_2-bonded column in the free base form with the solvent system used.

Hexane and isopropanol were HPLC grade (Caledon Labs., Georgetown, Canada). Mixtures of 0.1% isopropanol in hexane were used for all separations described. For operation at high sensitivity it was necessary to keep the solvent in an inert atmosphere. To accomplish this, 100 ml of hexane, contained in a reservoir surrounded by a plastic bag, was degassed by passing nitrogen through the liquid for 15 min. The nitrogen was passed through hexane in a small gas washing trap before entering the solvent to absorb contaminants from the nitrogen and "saturate" the gas with hexane to minimize evaporation. To the solvent in the reservoir was added 1 ml of isopropanol–hexane (10:90) and degassing was continued for *ca.* 1 min. The gas outlet was then removed from the solvent reservoir but left inside the plastic bag which was secured tightly about the tubes entering the reservoir. As the concentration of solute in the eluate was low, generally the solvent was recycled continuously from the detector to the reservoir except when larger concentrations or unknown, early-eluting components in soil extracts were encountered.

(*RS*)-*cis,trans*-Permethrin (93.9%) was supplied by Chipman (Stoney Creek, Canada). The individual (1*R*)(3*R*)-, (1*S*)(3*S*)-, (1*R*)(3*S*)- and (1*S*)(3*R*)-isomers of permethrin were from samples synthesized by Dr. W. Taylor (Agriculture Canada Research Station, Lethbridge, Canada). Fenpropanate (99%), cypermethrin (96%) and fenvalerate (97%) were provided by Shell Research (Woodstock Agricultural Research Centre, Sittingbourne, Great Britain). BAY FCR 1272 [cyfluthrin, (*RS*)-α-cyano-4-fluoro-3-phenoxybenzyl (*RS*)-*cis,trans*-3-(2,2-dichlorovinyl-2,2-dimethylcyclopropanecarboxylate] (87%) was supplied by Chemagro (Mississauga, Canada). Flucythrinate [(*S*)-α-cyano-3-phenoxybenzyl (*RS*)-2-(4-difluoromethoxyphenyl)-3-methylbutyrate] (86%) was from Cyanamid Canada (Willowdale, Canada). Deltamethrin [decamethrin, (*S*)-α-cyano-3-phenoxybenzyl (1*R*)(3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate] and its *R*-α-cyano isomer were supplied by Hoechst Canada (Regina, Canada) and both were analytical standard materials.

The extracts of soil containing cypermethrin were from an experiment similar to that described previously[12]. The hexane extracts were obtained by extraction of the soil with acetone, dilution of the acetone extract with water followed by extraction of the water-diluted acetone extract with hexane. An aliquot of the hexane equivalent to 10 g of soil was reduced to a small volume by evaporation on a rotary evaporator. The residue was treated with 10 ml of HPLC-grade hexane and the evaporation and addition was repeated three times to remove residual acetone. The residue in a small volume of hexane was transferred to a silica Sep-Pak cartridge (Waters Scientific, Mississauga, Canada) and eluted with 20 ml of isopropanol–hexane (1:99). The hexane transfer solvent and the eluting solvent were all collected, concentrated by evaporation and diluted to a known volume (5 or 10 ml) before injection into the HPLC system.

RESULTS AND DISCUSSION

The separation of the enantiomers of the various pyrethroids that can be achieved on a commercially available chiral-phase column[13] is shown in Fig. 1. The configurational assignments and retention times relative to *cis*-permethrin are given in Table I. It was anticipated that a chiral recognition mechanism would most likely involve the α-carbon of the benzyl ester portion of these molecules. The fact that the enantiomers of permethrin, which lack this function, were not separated while those of cypermethrin, which differs structurally from permethrin only in the presence of an α-cyano group, were separated indicates that interaction(s) involving this carbon are largely responsible for the separation observed.

The configurational assignments at the α-carbon are based on the elution order for *R* and *S* fenpropanate from the chiral-phase HPLC column (Fig. 1A). The early eluting enantiomer was found to be less toxic to houseflies than the later eluting one and on the basis of the previously established structure–activity relationship for this

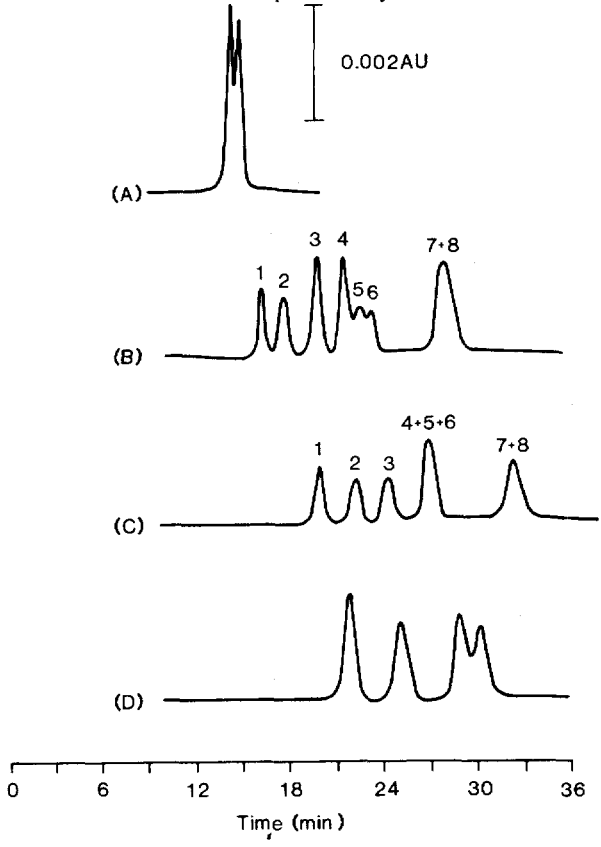

Fig. 1. Separation of enantiomers of pyrethroid insecticides on Pirkle Type 1A chiral-phase HPLC-column. (A) Fenpropanate (0.22 μg). (B) Cypermethrin (0.30 μg). Peaks: 1 = (1*R*)(3*R*)(α*R*); 2 = (1*S*)(3*S*)-(α*S*); 3 = (1*R*)(3*S*)(α*R*); 4 = (1*S*)(3*R*)(α*S*); 5 = (1*S*)(3*S*)(α*R*); 6 = (1*R*)(3*R*)(α*S*); 7 + 8 = (1*S*)(3*R*)(α*R*) + (1*R*)(3*S*)(α*S*). (C) BAY FCR 1272 (cyfluthrin) (0.22 μg). Peaks: 1 = (1*R*)(3*R*)(α*R*); 2 = (1*S*)(3*S*)(α*S*); 3 = (1*R*)(3*S*)(α*R*); 4 + 5 + 6 = (1*S*)(3*R*)(α*S*) + (1*S*)(3*S*)(α*R*) + (1*R*)(3*R*)(α*S*); 7 + 8 = (1*S*)(3*R*)(α*R*) + (1*R*)(3*S*)(α*S*). (D) Fenvalerate (0.24 μg).

series of α-cyano-3-phenoxybenzyl esters[2] the early eluting enantiomer is assigned the *R* configuration. The absolute configuration at the α-carbon has been determined for both deltamethrin[14] and a cypermethrin isomer (NRDC 167)[15] by X-ray diffraction. It is assumed that the interaction(s) required for separation will be similar for all these structurally related materials and that the *R* enantiomer is the first eluted for all α-*RS* pairs.

The configuration assigned to C-1 and C-3 of the cyclopropanecarboxylic acid-containing materials (permethrin, cypermethrin and BAY FCR 1272) are known from the method of synthesis and from the configurations at these carbons in deltamethrin. All that is required is to assign the correct structure to the components separated by HPLC. The elution order of the two diastereomeric pairs of permethrin isomers, (1*R*)(3*R*) + (1*S*)(3*S*) and (1*R*)(3*S*) + (1*S*)(3*R*), more commonly known as *cis* and *trans*, respectively, from a silica column was first reported by Kikta and Shierling[16]. This order was confirmed on our silica column with samples of the pure enantiomers and it remained the same on both the NH_2-bonded and chiral-phase columns. On C_{18}-bonded columns the reverse elution order has been observed[16]. The configurations at the cyclopropyl carbons of the four diastereomers of cypermethrin separated on a silica column [Hypersil (Shandon, Cambridge, Great Britain) were assigned by Crawford *et al.*[17]. The elution of the *cis* isomers prior to the *trans* isomers also was observed in this study on the silica column, *e.g.*, compare Fig. 2, A-1 and B-1, combined with the fact that the *trans* isomers are less persistent in moist soil[12,18–20]. The elution order of the two *cis* and two *trans* isomers is assumed to remain that observed by Crawford *et al.*[17]. Samples of the pure diastereomers of known configuration were not available for study but a sample of each diastereomer was obtained from chromatography of technical cypermethrin on the silica column and these were used to establish the retention times on the NH_2-bonded column where overlap of one *cis* and one *trans* diastereomer was observed. On the chiral-phase column the diastereomers were again separated (as well as being separated into individual enantiomers) but the elution order was further changed (see Fig. 1B) to *cis* (components 1 and 2), *trans* (components 3 and 4), *cis* (components 5 and 6) and *trans* (components 7 + 8). The (1*S*)(3*R*)(α*R*) and (1*R*)(3*S*)(α*S*) enantiomers (components 7 and 8) were not separated. To the best of our knowledge, data on the HPLC behavior of BAY FCR 1272 has not been published. We observed separation of the four possible diastereomers on the silica column and the configurations are assigned solely on the basis that the chromatographic behavior would be expected to be similar to cypermethrin. A sample of each diastereomer was obtained from HPLC on silica and, as with cypermethrin, overlap of one *cis* and one *trans* diastereomer was observed when they were chromatographed on the NH_2-bonded column. The diastereomers were again separated on the chiral-phase column (see Fig. 1C) but their elution order was changed to first (*cis*), third (*trans*), second (*cis*) and fourth (*trans*) from the order observed on the silica column as was the case for cypermethrin. The *cis* enantiomers, (1*S*)(3*S*)(α*R*) and (1*R*)(3*R*)(α*S*) (components 5 and 6) and the *trans* enantiomers (1*S*)(3*R*)(α*R*) and (1*R*)(3*S*)(α*S*) (components 7 and 8) were not separated by the chromatographic conditions used. This coupled with the fact that the (1*S*)(3*R*)(α*S*) enantiomer (component 4) overlapped with the unseparated *cis* enantiomers limits the usefulness of the separation for analysis of technical mixtures.

The HPLC separation of the diastereomers of fenvalerate was reported by

TABLE I

RETENTION TIMES OF ENANTIOMERS AND DIASTEREOMERS OF SOME PYRETHROID INSECTICIDES ON THREE HPLC COLUMNS

Material	*Configuration*				*Relative retention time*		
	C-1(2)	*C-3*	*C-α*	*C-1/C-3*	*Silica*	*NH_2*	*Chiral phase*
Permethrin	*R*	*R*	–	*cis*	1.00(3 min)	1.00 (4 min)	1.00 (4.5 min)
	S	*S*	–	*cis*	1.00	1.00	1.00
	R	*S*	–	*trans*	1.40	1.15	1.35
	S	*R*	–	*trans*	1.40	1.15	1.35
Cypermethrin	*R*	*R*	*R*	*cis*	1.70	1.48	3.42
	S	*S*	*S*	*cis*	1.70	1.48	3.74
	S	*S*	*R*	*cis*	1.90	1.74	4.77
	R	*R*	*S*	*cis*	1.90	1.74	4.94
	R	*S*	*R*	*trans*	2.20	1.74	4.19
	S	*R*	*S*	*trans*	2.20	1.74	4.58
	S	*R*	*R*	*trans*	2.45	1.93	5.94
	R	*S*	*S*	*trans*	2.45	1.93	5.94
BAY FCR 1272	*R*	*R*	*R*	*cis*	2.15	1.56	4.19
(Cyfluthrin)	*S*	*S*	*S*	*cis*	2.15	1.56	4.71
	S	*S*	*R*	*cis*	2.35	2.15	5.74
	R	*R*	*S*	*cis*	2.35	2.15	5.74
	R	*S*	*R*	*trans*	2.90	2.15	5.16
	S	*R*	*S*	*trans*	2.90	2.15	5.74
	S	*R*	*R*	*trans*	3.10	2.44	6.90
	R	*S*	*S*	*trans*	3.10	2.44	6.90
Fenpropanate	–	–	*R*	–	1.73	1.37	3.10
	–	–	*S*	–	1.73	1.37	3.23
Deltamethrin isomer	*R*	*R*	*R*	*cis*	1.61	2.00	4.43
Deltamethrin	*R*	*R*	*S*	*cis*	1.85	2.34	6.05
Fenvalerate	*S*	–	*R*	–	2.10	1.81	4.71
	R	–	*S*	–	2.10	1.81	5.42
	R	–	*R*	–	2.30	2.04	6.26
	S	–	*S*	–	2.30	2.04	6.52
Flucythrinate	*S*	–	*R*	–	3.65	3.11	8.71
	S	–	*S*	–	4.10	3.48	11.74

Mourot *et al.*[22] on a silica column (LiChrosorb Si-60) but no structural assignment was made. On gas–liquid chromatographic (GLC) columns of Dexsil 300, OV-101, QF-1 and XE-60 the (2*R*)(α*S*) + (2*S*)(α*R*) isomer pair was observed to elute before the (2*R*)(α*R*) + (2*S*)(α*S*) pair[23–26]. Samples of the two diastereomers separated by HPLC on silica in our laboratory were found to elute in the same order as on these GLC columns. The same elution order was observed with the NH_2-bonded and chiral-phase columns (see Fig. 1D). As in the case of the cyclopropyl-based compounds, the α*R* enantiomer is assumed to elute first on the chiral-phase column based on the behavior of *R* fenpropanate.

Chromatographic data on deltamethrin, its α*R* isomer and a new relative of fenvalerate, flucythrinate, are included in Table I for all three columns. These materials are simple diastereomers and no particular benefit is expected from the use of the chiral phase column.

As in the case of our previous work on pyrethroid isomer separation[7], we were

particularly interested in a practical method for observing changes in isomer composition at residue levels in soils. The chromatograms reproduced in Fig. 2 show the application of the separations described for the silica (A-1,B-1,C-1) and chiral phase (A-2,B-2,C-2) columns to the analysis of the isomers of cypermethrin in soil samples immediately following treatment and after about 50% of the initial application of 10 ppm had disappeared from air-dry soil and soil containing 5% water. The simple clean-up on the silica Sep-Pak cartridge was sufficient to remove all interfering components from the extract equivalent to 10 g of soil. Recovery of cypermethrin from this chromatography was quantitative at the 50–100 μg level. The chromatography on the silica column (see Fig. 2) clearly shows the more rapid disappearance of the *trans* isomers (the third and fourth components in A-1–C-1) in the soil containing 5% water. The chromatography on the chiral phase column shows that the (1*S*)(3*R*)(α*S*) enantiomer (the fourth component in A-2–C-2) is degraded much more rapidly than the other enantiomers in this soil. The retention times in Fig. 2 are considerably longer than in Fig. 1 for the "same" solvent system. The chromatography is sensitive to the isopropanol concentration in the carrier solvent and a slight change can pro-

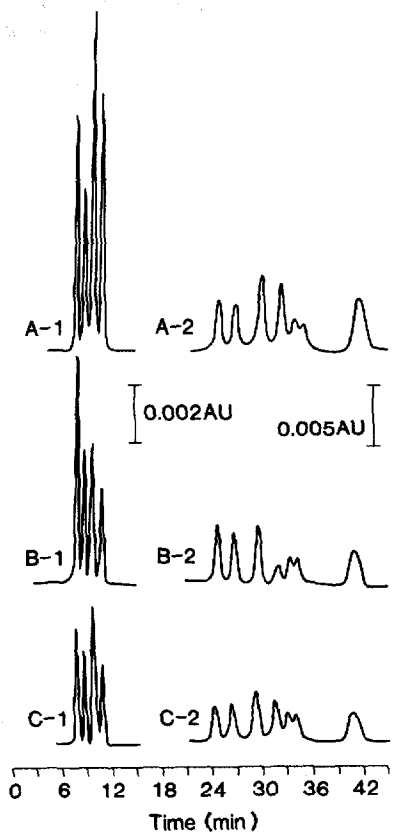

Fig. 2. Analyses of cypermethrin isomers in soil (A-1–C-1 on Spherisorb silica and A-2–C-2 on Pirkle Type 1A chiral phase. See Fig. 1B for enantiomer identification). A, Day 0 (0.20 μg; soil extract equivalent to 20 mg). B, Day 10, 5% moisture (0.15 μg; soil extract equivalent to 40 mg). C, Week 2, air dry (0.15 μg; soil extract equivalent to 40 mg).

duce the effect observed. Despite the sensitivity of isopropanol concentration, the retention times were stable in each solvent mixture prepared provided care was taken to prevent evaporation and the retention times were always within the range shown in Figs. 1 and 2.

CONCLUSION

HPLC on a chiral phase has been shown to be a useful method of separating many enantiomers of the common pyrethroid insecticides. The method provides the advantages expected of a direct resolution with no loss of information due to destruction of chiral centers and no fear of isomerization during a chemical reaction. Being based on HPLC, the sensitivity of the method is limited to that of current detection technology. Detection limits on the chiral phase column are naturally reduced by the distribution of the total response over a greater number of components.

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