JOURNAL OF CHROMATOGRAPHY A

ELSEVIER

Journal of Chromatography A, 777 (1997) 363–369

Short communication

Separation and analysis of the diastereomers and enantiomers of cypermethrin and related compounds

Darren P. Edwards[a,*], Martyn G. Ford[b]

[a]*School of Chemistry, Physics and Radiography, University of Portsmouth, St. Michael's Building, White Swan Road, Portsmouth PO1 2DT, UK*

[b]*School of Biological Sciences, University of Portsmouth, King Henry Building, King Henry I Street, Portsmouth PO1 2DY, UK*

Received 6 February 1996; received in revised form 25 March 1997; accepted 25 March 1997

Abstract

The diastereomers of cypermethrin and permethrin have been resolved on an irregular silica column. Complete separation of three of the four enantiomeric pairs of cypermethrin was achieved with reasonable retention times (<50 min) by direct injection of the separated diastereomers onto a cellulose based chiral HPLC column. The order of elution was established; structures were confirmed by NMR and comparison with the retention times of authentic enantiomeric standards. For the unseparated pair, improved resolution was achieved with a slightly longer retention time (80 min). Injection of α-cypermethrin onto the chiral system under the same conditions gave excellent separation of the two enantiomers. © 1997 Elsevier Science B.V.

Keywords: Diastereomer separation; Enantiomer separation; Cypermethrin; Permethrin; Pesticides; Pyrethroids

1. Introduction

The pyrethroid insecticides show both geometrical and optical isomerism. Furthermore, the toxicity of each isomer depends on its absolute configuration [1]. Cypermethrin is a synthetic pyrethroid with three chiral centres, and, therefore, eight optical isomers: permethrin has two chiral centres and four optical isomers (Fig. 1). Pairs of enantiomers represent diastereomers with different chemical, physical and toxicological properties. The configuration of the cyclopropyl ring, for example, has a marked effect on the toxicity of cypermethrin, with 1*R*-*cis* isomers being the most toxic [2]. The benzylic carbon atom should subtend the α-cyano group in the (*S*)-configuration for maximum potency [3].

Previous work has shown that the diastereomers of

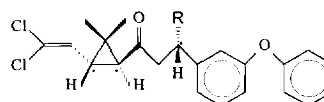

Fig. 1. The structure of cypermethrin and some related pyrethroid insecticides.

*Corresponding author. Present address: Covance Laboratories Ltd., Physical Chemistry Department, Otley Road, Harrogate, North Yorkshire HG3 1PY, UK.

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PII S0021-9673(97)00382-8

cypermethrin can be separated by gas–liquid chromatography (GLC), [4] and both normal- [5,6] and reversed-phase [7] high-performance liquid chromatography (HPLC). To separate the enantiomers two main methods can be applied: (i) bonding the analyte with another chiral molecule to form diastereomers which can then be separated by the usual methods (e.g. HPLC or GLC), or (ii) chiral HPLC or GLC. However, the first method has associated problems [6], and for preparative work only chiral HPLC is suitable. The separation of the eight enantiomers of cypermethrin has been tried on a variety of systems [8–10]. However, most of these methods suffer from partial resolution of the isomers making preparation of pure materials difficult. Where almost complete separation has been achieved, two chiral columns are required, with an associated increase in the cost of separation [8].

We wish to describe a modified, relatively low-cost procedure for resolving the diastereomers of cypermethrin and permethrin. The individual enantiomers of each diastereomer of cypermethrin have then been separated using a cellulose-based stationary phase, giving greatly improved separation of all but one pair of isomers. The same system gave excellent separation of the enantiomers of α-cypermethrin (containing two of the most active isomers of cypermethrin), comparable with that found when using a Pirkle-type column [8].

The separations described are suitable for the preparation of the diastereomers of cypermethrin and permethrin, and the enantiomers of cypermethrin and α-cypermethrin.

2. Materials and methods

The HPLC system used consisted of a Constametric IIIG pump (LDC/Milton Roy), used to deliver the solvent. Samples were injected into the solvent flow by means of a Rheodyne injection valve (Model 7125, Jones Chromatography), with a 20 μl sample loop. Detection was achieved using a SpectroMonitor III variable-wavelength detector (LDC/Milton Roy) set at a wavelength of 280 nm. Automated collection of separated fractions was carried out using a Eluant Collector (Pye Unicam) set to switch to collection at approximately 10% of the total peak absorbance. Chromatograms were plotted using either a chart recorder (Servogor 460, Recorderlab Services) or computing integrator (Spectra-Physics, SP4200).

For separation of the diastereomers, a column (stainless steel, 250×4.6 mm) packed with Partisil silica (10 μm particle size) was used (Jones Chromatography). Separation was also tried using columns of similar dimensions packed with Spherisorb or Hypersil silica, but both were found to give poorer separation of the isomers. Separation of the optical isomers was carried out using another column (again 250×4.6 mm) packed with Daicel Chiralcel OD material (Phenomenex). This column packing consists of a cellulose tris-3,5-dimethylphenylcarbamate derivative bonded to 10 μm silica particles and only mobile phases containing 0–100% *n*-hexane in 2-propanol and 0–100% *n*-hexane in ethanol were recommended by the column manufacturer to avoid damaging the packing. To protect the analytical column, a pre-column (50×4.6 mm) packed with the same material was used.

n-Hexane, 2-propanol, diethyl ether, tetrahydrofuran (THF) and chloroform were all HPLC grade (Aldrich). For normal-phase chromatography, with silica-based stationary phases the solvents were used 'as is' with no pre-treatment. For use with the Chiralcel filled column, the solvents were pre-dried overnight by adding ca. 10% (w/w) of molecular sieve (type 3A, 4 mm beads, Aldrich). The dried solvents were vacuum filtered just prior to use, and while in use were vented through a column of silica gel.

Cypermethrin {[(*R*,*S*)-α-cyano-3-phenoxybenzyl (*R*,*S*)-*cis*,*trans*-2,2-dimethyl-3 - (2,2-dichlorovinyl)-cyclopropanecarboxylate]}, 95.6% and α-cypermethrin {[(*R*,*S*)-α-cyano-3-phenoxybenzyl (*R*,*S*)-*cis*-2,2-dimethyl-3 - (2,2-dichlorovinyl)cyclopropane-carboxylate]} were kindly donated by Cyanamid UK. The individual enantiomers of cypermethrin were kindly donated from Wellcome Research.

Permethrin {[3-phenoxybenzyl (*R*,*S*)-*cis*,*trans*-2,2-dimethyl-3 - (2,2-dichlorovinyl)cyclopropanecarboxylate]}, 99.5% was supplied by ICI Agrochemicals. All compounds were used as received.

3. Results and discussion

3.1. Separation of the diastereomers of cypermethrin

The separation of the four diastereomers of cypermethrin on a Partisil silica column using four different mobile phases is shown in Fig. 2 (20 μl of 500 μg/ml solution injected). The fastest baseline separation of all four isomers was achieved using a mobile phase of hexane–chloroform–2-propanol (200:1:6) (Fig. 2iv). Two mobile phases (hexane–chloroform–2-propanol (200.2:0.1, Fig. 2i) and hexane–THF–diethyl ether (200:0.5:6, Fig. 2ii) showed incomplete separation of the four isomers (not baseline resolved). By decreasing the modifiers to each of these mobile phases (either chloroform–2-propanol or THF–diethyl ether), separation could be achieved with increased retention time. Preparative separation of the four isomers using the mobile phases described in Fig. 2iv was very sensitive to changing conditions such as temperature and to the relative proportions of the components in the mobile phase. Hexane–chloroform–diethyl ether (200:1:6, Fig. 2iii) was less sensitive to these factors (even though diethyl ether is more volatile). In light of this, and the fact that the diastereomers were very evenly baseline separated, it was decided to use a mobile phase of hexane–chloroform–diethyl ether (200:1:6). It is interesting to note that although the order of resolution was not changed for all of the mobile phases tried, the separation of particular pairs of isomers was altered by changing the mobile phase. By having four methods for separation of the diastereomers, potential impurities that arise from the analysis of 'real' samples such as plant tissue extracts, which produce components that interfere with the peaks of interest on one system, can be separated using another mobile phase.

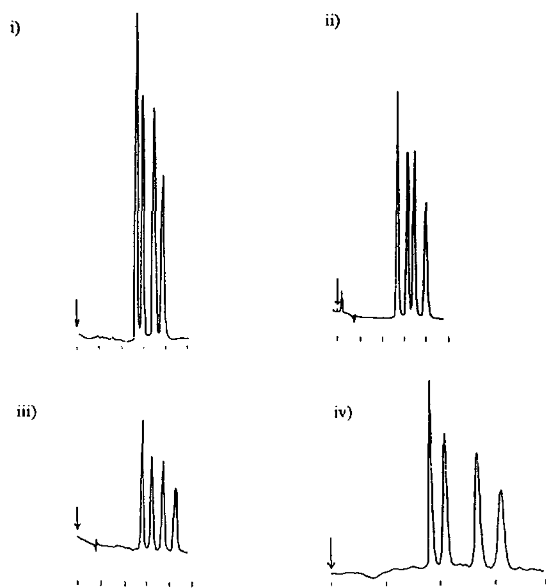

Fig. 2. Separation of the diastereomers of cypermethrin by a Partisil column (10 μm particle size, 250×4.6 mm) using various mobile phases. (Flow-rate 1.0 ml/min, 281 nm detection.) (Each baseline marker represents 5 min elution time. Arrow indicates injection of sample). (i) Hexane–chloroform–2-propanol (200:0.2:0.1). (ii) Hexane–THF–diethyl ether (200:0.5:6). (iii) Hexane–chloroform–diethyl ether (200:1:6). (iv) Hexane–chloroform–2-propanol (200:1:6).

By collecting the fractions of ca. 80 injections of cypermethrin standard (10 mg/ml, 20 μl injected) and evaporating the resultant solution to dryness (vacuum desiccator, no applied heat) ca. 4 mg of each diastereomer was prepared. Even at this high concentration the chromatography was excellent, all isomers being well separated. The stability was also excellent, with little variation (ca. 50 s for the last isomer) in retention time between batches of mobile phase and over time (ca. 40 h total injection time, carried out over several days). Although the solvents used for the preparation of the mobile phase were used 'as received', no obvious variation in the chromatographic performance was observed, even though several different batches of the solvents were used. Pre-drying the solvents over molecular sieve had no obvious effect on the chromatography.

The diastereomers were labelled according to their elution time. Thus, the first isomer eluted was known as isomer I, the second II etc. Each diastereomer was analyzed by proton-NMR, and two distinct types of spectra were obtained (Table 1). Isomers I and II had virtually identical spectra, as did III and IV. The *J* coupling constant for the proton on the cyclopropyl carbon atom adjacent to the carbonyl group was found to be 8.1 Hz for isomers I and II, and 5.1 Hz for isomers III and IV. The signal from this proton was split by the hydrogen on the carbon atom next to

Table 1
Peak assignments from the proton NMR spectra of the four diastereomers of cypermethrin

Chemical shift/ppm	Multiplicity	Integration (approximate)	Identity (see Fig. 1)
1.2–1.3	Singlet or doublet	Variable	Methyl-group protons on cyclopropyl ring
1.6	Broad singlet	Variable	Unknown
1.9 (for isomers I and II) 1.7 (for isomers III and IV)	Doublet	1	Proton attached to cyclopro-pane ring adjacent to carbonyl group
2.2 (for isomers I and II) 2.3 (for isomers III and IV)	Triplet	1	Second proton attached directly to cyclopropane ring
6.1 (for isomers I and II) 5.6 (for isomers III and IV)	Doublet	1	Proton attached to double bond
6.3 (for isomers I and II) 6.4 (for isomers III and IV)	Singlet	1	Proton adjacent cyano-group
7.0–7.8	Multiplet	9	Aromatic protons

it, and the interaction would be expected to be stronger if the ring protons were *cis* rather than *trans* to each other, as the atoms would be closer together. Thus, for isomers I and II the protons must be *cis* configured (i.e. on the same side of the ring). The assignment has been confirmed by the Nuclear Overhausen Enhancement of the proton signal for this pair of isomers [11].

The proton NMR spectra show no significant differences between isomers I and II, and III and IV, suggesting the alkyl proton and cyano group adjacent to the benzene ring are in similar environments, and that each pair of isomers are enantiomers.

3.2. Separation of the diastereomers of permethrin

Permethrin standard solution (20 μl of 500 μg/ml solution) injected onto the same system as used to separate the isomers of cypermethrin gave good separation of its two geometrical isomers (Fig. 3). The retention time of 6–8 min was very much less than that of cypermethrin, although the peaks were still very nearly baseline resolved.

3.3. Separation of the enantiomers of cypermethrin

A chromatogram of an injection of cypermethrin standard (10 μl of 200 μg/ml solution) on the Chiralcel OD column is shown in Fig. 4. From the chromatogram it can be seen that not one of the isomers was baseline resolved, even with a run time of ca. 40 min. Separation could not be achieved either by increasing the elution time (by decreasing the amount of 2-propanol) or by using ethanol instead of 2-propanol as the mobile phase modifier.

Injection on to the chiral HPLC system of the diastereomers separated as described above was much more successful. Chromatograms of each diastereomer are shown in Fig. 5. All of the dia-

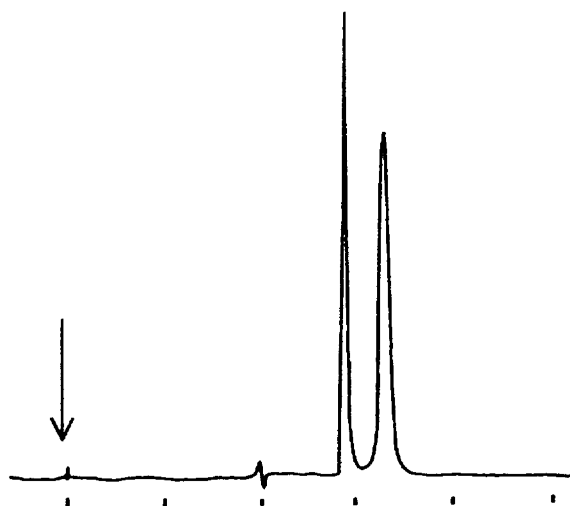

Fig. 3. Separation of the diastereomers of permethrin by a Partisil column (10 μm particle size, 250×4.6 mm) using a hexane–chloroform–diethyl ether (200:1:6) mobile phase. (Flow-rate 1.0 ml/min, 281 nm detection.) (Each baseline marker represents 2 min elution time. Arrow indicates injection of sample).

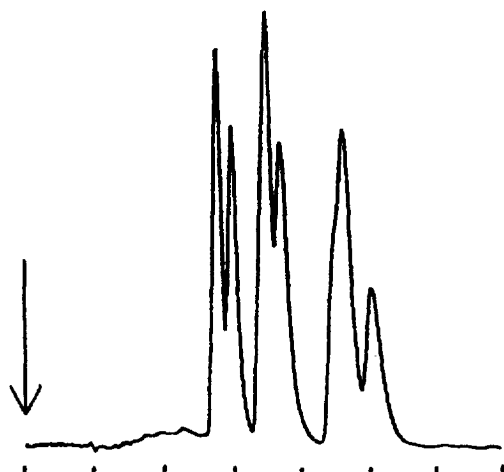

Fig. 4. Separation of the enantiomers of cypermethrin using a Chiralcel OD column 250×4.6 mm), 0.5 ml/min, 10 μl injected of a 200 μg/ml solution of the test substance (all isomers) in mobile phase (hexane–2-propanol, 250:5). (Each baseline marker represents 10 min elution time. Arrow indicates injection of sample).

stereomers showed excellent separation into their corresponding enantiomeric pairs except isomer III. This showed excellent separation into their corresponding enantiomeric pairs except isomer III. This showed incomplete resolution after an elution time of ca. 40 min, with a mobile phase of hexane–2-propanol (250:5). However, the two enantiomers of III could be baseline resolved using a mobile phase of hexane–2-propanol (250:1), although the peaks were somewhat broad (Fig. 5v). The first eluting enantiomer of isomer II showed two closely eluting interferent peaks (Fig. 5ii). These eluted with the isomer if the retention time was shortened. The origin or identity of these peaks is not known at present.

By injecting individual enantiomers onto the chiral HPLC system, the retention times of each were found and the enantiomeric make-up of each diastereomer deduced. The results of this are shown in Table 2.

3.4. Separation of the enantiomers of permethrin

A chromatogram of an injection of permethrin standard (10 μl of 200 μg/ml solution) on the

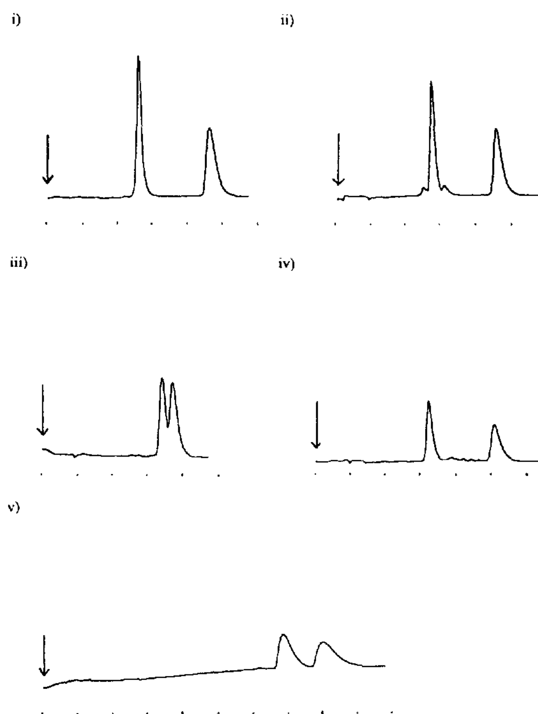

Fig. 5. Separation of the enantiomers from each of the diastereomers of cypermethrin isolated using the method described in Fig. 1iii. Chiralcel OD column, (250×4.6 mm), 0.5 ml/min, 10 μl injected of a 200 μg/ml solution in mobile phase (hexane–2-propanol, 250:5). (i) Isomer I; (ii) isomer II; (iii) isomer III; (iv) isomer IV; (v) isomer III chromatographic under the same conditions, but using a mobile phase of hexane–2-propanol (250:1). (Each baseline marker represents 10 min elution time. Arrow indicates injection of sample.)

Chiralcel OD column is shown in Fig. 6i. Although two of the four isomers are almost baseline separated using a mobile phase of hexane–2-propanol (250:5), the other two co-elute. The separation of these two isomers was not achieved by increasing the retention time (decreasing the amount of 2-propanol added).

3.5. Separation of the enantiomers of α-cypermethrin

A chromatogram of an injection of α-cypermethrin standard (10 μl of 200 μg/ml solution) on the Chiralcel OD column is shown in Fig. 6ii. The two enantiomers were easily separated using the mobile phase of hexane–2-propanol (250:5).

Table 2
Elution order of the diastereomers and enantiomers of cypermethrin, and their absolute optical configurations

Diastereomer	Approximate retention time of diastereomer on non-chiral HPLC system	Absolute configuration of enantiomers making up each diastereomer	Elution order on chiral HPLC system
I	14	1*R* 3*R* α*R*	1
		1*S* 3*S* α*S*	6
II	16	1*R* 3*R* α*S*	2
		1*S* 3*S* α*R*	7
III	19	1*R* 3*S* α*R*	3[a]
		1*S* 3*R* α*S*	5
IV	22	1*S* 3*R* α*R*	4[a]
		1*R* 3*S* α*S*	8

[a] These two isomers were found to swap positions with only slight modifications to the 2-propanol concentration in the mobile phase.

4. Conclusions

A reasonably fast (<30 min), robust and relatively inexpensive method has been developed for separating the diastereomers of the insecticide cypermethrin. A partisil silica column and a mobile phase of hexane–chloroform–diethyl ether (200:1:6) was used for the analysis, and to prepare each diastereomer by fraction collection. This method can also be used to separate the geometrical isomers of permethrin, with an elution time of less than 10 min. For cypermethrin, four different mobile phases have been shown to give different selectivity towards the isomers, but not enough to change the elution order.

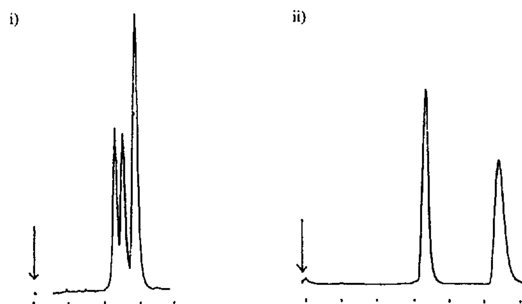

Fig. 6. Separation of the enantiomers of (i) permethrin and (ii) α-cypermethrin on Chiralcel OD column, (250×4.6 mm), 0.5 ml/min, 10 μl injected of a 200 μg/ml solution in mobile phase (hexane–2-propanol, 250:5). (Each baseline marker represents 10 min elution time. Arrow indicates injection of sample.)

These methods may provide useful alternatives if co-eluting peaks are found in extracts of biological materials.

The enantiomeric pair of each diastereomer prepared by the method shown above have been separated by a Chiralcel OD column using a mobile phase of hexane–2-propanol (250:5). Three of the diastereomers of cypermethrin (I, II and IV) showed clear baseline separation of their enantiomeric pairs, with a retention time of under 50 min. One diastereomer, however, showed incomplete resolution of its enantiomers (isomer III) using the above system. These isomers could, however, be separated using a mobile phase of hexane–2-propanol (250:1), at the expense of a much greater elution time (ca. 70 min). This method would be suitable for preparing the enantiomers of cypermethrin by fraction collection.

The chiral column used was unsuitable for the analysis of the four enantiomers of permethrin, but showed excellent separation of those of α-cypermethrin.

No attempt was made to couple the normal-phase column with the chiral column. This was principally due to the fact that only two solvent mixtures (2-propanol–hexane or ethanol–hexane) were recommended by the manufacturer for use with the chiral column. It may, however, be possible to achieve direct separation of the enantiomers of cypermethrin by coupling the achiral and chiral columns using a modified mobile phase based upon that used for the achiral separations.

Acknowledgments

We wish to acknowledge the assistance of Mrs. S. Lucas for supplying some of the HPLC equipment, and Dr. N. Hoare for his assistance with obtaining standards and interpreting the NMR spectra.

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