Diastereoselective and Enantioselective Chromatography of the Pyrethroid Insecticides Allethrin and Cypermethrin

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Summary
Liquid and gas chromatographic separations of the Pyrethroid insecticides allethrin and cypermethrin have been investigated with various achiral and chiral stationary phases. Diastereomeric and enantiomeric selectivity was observed for cypermethrin on a Pirkle-type chiral LC stationary phase, but very strong interactions and therefore long retention times prevented the separation of allethrin on this phase. Trans-allethrin isomers were separated on a chiral β-cyclodextrin RP-HPLC column while cypermethrin showed some difficulties on this phase due to isomerization. Diastereomeric but no enantiomeric selectivity by GC was achieved for cypermethrin with an apolar DB 5 capillary. GC separation of the diastereomers was used to study the selective photodegradation of cypermethrin isomers after forestry applications. Chiral β-cyclodextrin-based GC phases showed some enantioselectivity for cis- and trans-allethrin isomers. A separation of the eight isomers into six partially resolved peaks was achieved by GC with a coupled column consisting of chiral permethylated β-cyclodextrin and DB 1701 as stationary phases. This combination was used to characterize allethrin formulations intended for indoor use and to investigate allethrin products formed by ozonolysis of thin films of the insecticide.

Introduction
Natural pyrethrins and their structural analogs, the Pyrethroids (e.g. allethrin, tetramethrin, permethrin, cyphenothrin, cypermethrin, cyfluthrin and deltamethrin) are gaining increasing importance as insecticides in agriculture, forestry, horticulture, public health and household usage. The toxicity and the metabolic detoxification of the active compounds in insects and in non-target organisms strongly depend on molecular shape as do all biological processes involving membranes, receptors or enzymes [1-3].

Pyrethroids are synthesized, tested, marketed and used either as a single, most active isomer (e.g. (S)-bioallethrin, deltamethrin, (S)-fenvalerate) or as isomeric mixtures containing two (e.g. bioallethrin, cyphenothrin, alphacypermethrin), four (e.g. tetramethrin and permethrin) or eight (e.g. allethrin, cypermethrin and cyfluthrin) different stereoisomers, depending on the number of chiral centers in the molecules and the synthesis route [4, 5]. Thus chromatographic separations of the diastereomers and enantiomers into individual stereoisomers provide either defined compounds (via micropreparation) or a means for thorough examination of biological activity and enzymatic metabolism. Furthermore biotic or abiotic transformation yields products with either the identical, reduced or increased number of asymmetric C atoms depending on the kind and the site of alteration [6-8].

We have used liquid and gas-liquid chromatography to investigate diastereomeric and enantiomeric selectivities for allethrin and cypermethrin (Figure 1) on various achiral and chiral separation systems.

Experimental

Chemicals
Allethrin (8 isomers, CAS-No. [584-79-2]), bioallethrin ([1R,trans,αS and αR]-allethrin isomers, CAS-No. [584-79-2]), (S)-bioallethrin ([1R,trans,αS]-allethrin isomer, CAS-No. [28434-00-0]), cypermethrin (8 isomers, CAS-No. [52315-07-8]), alphacypermethrin ([1R,cis,αS]- and [1S,cis,αR]-cypermethrin isomers, CAS-No. [67375-30-8]) and permethrin (4 isomers, CAS-No. [52643-53-1]) were obtained from Riedel de Haën (Seelze, Germany) or from Dr. Ehrenstorfer (Augsburg, Germany). [1R, trans]-permethrin was a gift from J. E. Casida (PCTL, UC Berkeley, CA, USA). The carboxaldehyde formed from (S)-bioallethrin via ozonolysis was isolated and characterized by
Figure 1

(S)-bioallethrin (a) and [1R,trans,cis]-allethrin (b) as present in bioallethrin; [1R,cis,trans]-cypermethrin (c) and [1S,cis,cis]-cypermethrin (d) as present in alphacypermethrin.

GC-MS and NMR [9]. Solvents were HPLC-grade from Rathburn (Walkerburn, Scotland), Merck (Darmstadt, Germany), or Nanograde from Promochem (Wesel, Germany).

Liquid Chromatography

Liquid chromatography (HPLC) was performed using a Knauer Model 64 HPLC Pump and a Gynkotech SP-4 Spectrophotometer with variable wavelength. Detection was carried out at 220 nm. Flow rates were always 1 mL/min.

Achiral normal-phase separations (NP-HPLC) were carried out on unmodified silica gel (LiChrospher Si 100, 5 µm, 120 × 4 mm i.d. column, Merck, Darmstadt, Germany) and on NO₂-modified silica gel (Nucleosil 5 NO₂, 5 µm, 120 × 4 mm i.d. column, Macherey-Nagel, Düren, Germany).

Chiral normal phase (NP-CSP-) HPLC was performed on silica gel modified with L-tartaric acid and L-dinitrobenzylphenylethylamine (Chiral-2, 5 µm, 250 × 4 mm i.d. column, Macherey-Nagel) (Pirkle-type column).

Achiral reversed-phase (RP-) HPLC was done on C18-modified silica gel (LiChrosorb RP 18, 5 µm, 120 × 4 mm i.d. column, Merck).

For chiral reversed-phase (RP-CSP-) HPLC separations silica gel modified with β-cyclodextrin (Cyclobond I, 5 µm, 250 × 4.6 mm i.d. column, Astech, Whippany, NJ, USA) was used.

Gas Chromatography

Gas chromatography (GC) employed a Hewlett-Packard 5890 Series II GC equipped with autosampler, on-column and split/splitless injectors, and an electron capture (ECD) detector. For automated analysis a Hewlett-Packard 3365 Chemstation was used.

Achiral and chiral GC separations were performed by split or on-column injections employing H₂ as carrier gas and various temperature programs (TP) or isothermal conditions on the following fused silica capillaries:

- DB 5 (5 % phenyl methyl polysiloxane), 25 m length, 0.25 mm i.d., 0.1 µm film (J & W, Folsom, CA, USA).
- SB-biphenyl-30 (30 % biphenyl methyl polysiloxane), 25 m length, 0.32 mm i.d., 0.25 µm film (Lee Scientific, Salt Lake City, UT, USA).
- DB 1701 (14 % cyanopropyl phenyl methyl polysiloxane), 30 m length, 0.25 mm i.d., 0.15 µm film (J & W); SP 2331 (100 % cyanopropyl polysiloxane), 60 m length, 0.32 mm i.d., 0.20 µm film (Supelco, Bellefonte, PA, USA).
- CDX-B (permethylated β-cyclodextrin in 14 % cyanopropyl phenyl methyl polysiloxane), 30 m length, 0.25 mm i.d., 0.25 µm film (J & W).
- Lipodex C (heptakis-(2,3,6-tri-O-pentyl)-β-cyclodextrin), 25 m length, 0.25 mm i.d. (Macherey-Nagel).
- Lipodex D (heptakis-(2,6-di-O-pentyl-3-O-acetyl)-β-cyclodextrin), 25 m length, 0.25 mm i.d. (Macherey-Nagel). The CDX-B and the DB 1701 column were coupled using glass fittings (Chrompack, Middlebourg, The Netherlands) to give a 60 m, 0.25 mm i.d., 0.15–0.25 µm film column.

Procedures

Assignment. Assignment of separated HPLC and GC peaks involved, where possible, chromatography of individual isomers (i.e. (S)-bioallethrin, bioallethrin, alphacypermethrin) and/or fractionation of HPLC peaks and GC-ECD on the DB 5 column (cypermethrin) and the DB 1701 column (allethrin) [10].

Assignment of cis- and trans-configuration to isomers of cypermethrin separated by NP-HPLC was done by fractionation, ester cleavage, methylation of the 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid with diazomethane and GC-ECD on a DB 5 capillary [1R,trans]-Permethrin and permethrin were used as model compounds to establish the order of GC elution of the cis and trans methyl esters [10].

Alphacypermethrin isomerized when dissolved in methanol or acetonitrile for RP-HPLC analysis. No isomerization was observed using a methanol/buffer mixture (various buffers, pH 3–6.5).

Further assignments of single cypermethrin isomers are based on [11] and [12].