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Note

Liquid chromatographic determination and resolution of the enantiomers of the acid moieties of pyrethroid insecticides as their (–)-1-(1-phenyl)ethylamide derivatives

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Most synthetic pyrethroid insecticides have from one to three chiral centers. Typical commercial samples are unresolved and therefore consist of mixtures of from two to eight optical and geometrical isomers, the biological activities of which range from highly toxic to virtually non-toxic[1,2]. Pyrethroid isomers also differ considerably in their rates and preferred pathways of biodegradation[3–5]. Thus, the study of the biological activity and the biological and environmental fate of pyrethroid isomer mixtures may not adequately describe the behavior of the toxic components, and methods for both the determination of pyrethroid isomer composition and the preparation of optically pure samples are clearly needed.

Several gas–liquid chromatographic (GLC) procedures for the determination of the optical isomers of chiral pyrethroid acid moieties have been reported. Most of these involve cleavage of the pyrethroid ester and derivatization of the acid moiety with optically active alcohols[6–9] or amines[10], followed by GLC separation of the resulting diastereomeric esters or amides. A recent method[11,12] involves the separation of amides prepared from optically inactive amines on optically active stationary phases. Although high-resolution liquid chromatographic (HRLC) methods have been reported for the analysis of pyrethroids[13–16], no HRLC method has been described for the determination of the optical isomers of pyrethroid acids.

Methods are also required for the optical resolution of pyrethroid acids as intermediates in the synthesis of optically pure esters. Pyrethroid acids are generally resolved on a preparative scale by fractional crystallization of diastereomeric salts prepared from racemic acids and optically active amines[17–19]. Although these methods are satisfactory for large-scale preparations, fractional crystallization is not suited to situations where only small amounts of racemic acid (*e.g.* radiolabeled preparations) are available. The HRLC separation of acid derivatives that could be readily converted into the optically pure free acids would provide a useful micro-scale alternative to conventional optical resolution procedures.

HRLC analysis of optically active arylethylamides has been used to determine the optical purity of several chiral terpenoid acids[20–22]. In this paper we report the

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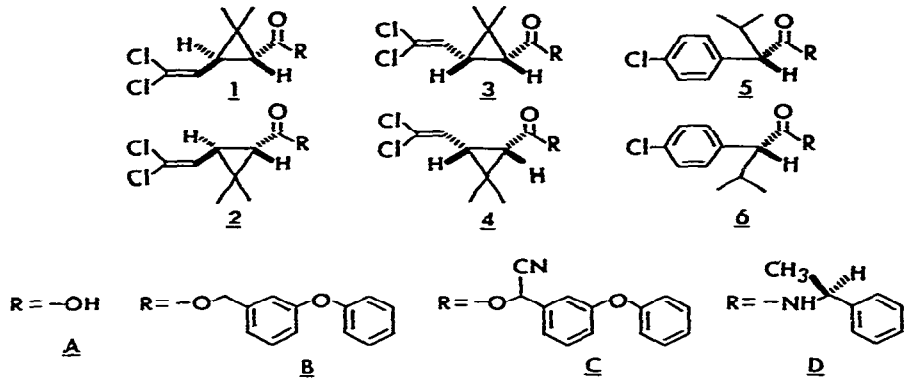

HRLC determination and small-scale preparative resolution of the four isomers of 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (DCVA; 1A–4A), the acid moiety of the pyrethroid insecticides permethrin (1B–4B) and cypermethrin (1C–4C), and the enantiomers of 2-(4-chlorophenyl)-3-methylbutyric acid (CMBA; 5A–6A), the acid moiety of the pyrethroid insecticide fenvalerate (5C–6C), as their (–)-1-(1-phenyl)ethylamide derivatives (1D–6D).

EXPERIMENTAL

Acid moieties of pyrethroids

Samples of a mixture of all four DCVA isomers and of the separated 1*RS,trans* (1A+2A) and 1*RS,cis* (3A+4A) racemates were provided by R. A. Robinson, FMC Corporation, Middleport, NY, U.S.A. Samples of optically pure 1*S* enantiomers (2A and 4A) prepared by fractional crystallization[18], were provided by M. Elliott, Roth-

TABLE I

HRLC AND TLC PARAMETERS FOR THE SEPARATION OF THE DIASTEREOMERIC (–)-1-(1-PHENYL)ETHYLAMIDES OF DCVA AND CMBA

Acid moiety	*Absolute configuration*	*HRLC parameters*-***					*TLC R_F****	
		V_1'	k'	N	α	R_s	*A*	*B*
DCVA	1*S,cis*	26	1.51	3810			0.27	0.56
					1.56	4.24		
	1*R,cis*	40.4	2.35	3320			0.22	0.51
	1*S,trans*	53.2	3.09	2203			0.23	0.45
					1.44	2.58		
	1*R,trans*	76.4	4.44	973			0.19	0.40
CMBA	*R*	35.2	2.05	2750			0.38	0.35
					1.64	4.07		
	S	58.0	3.37	1745			0.32	0.31

* See Experimental section for analytical conditions.

** V_1' = Corrected retention volume (ml); k' = capacity ratio; N = number of theoretical plates; α = selectivity ratio; R_s = resolution factor.

*** TLC solvent systems: A = carbon tetrachloride–diethyl ether (9:1); B = hexane–diethyl ether (1:1).

amsted Experimental Station, Harpenden, Great Britain. Racemic CMBA (5A +6A) was purchased from Frinton Labs., Vineland, NJ, U.S.A., and was recrystallized three times from hexane–benzene (10:1, v/v) prior to use. Optically pure 5A and 6A were obtained by fractional crystallization[19].

Preparation of diastereomeric derivatives

DCVA and CMBA samples (10–50 mg) were converted into their acid chlorides using an excess of $SOCl_2$ (Aldrich, Milwaukee, WI, U.S.A.) at 50–55°C for 2 h. Unchanged $SOCl_2$ was removed by rotary evaporation *in vacuo*. Derivatives were prepared by treating the crude acid chlorides with excesses of (−)menthol, (−)-2-octanol, or (−)-1-(1-phenyl)ethylamine (all from Aldrich) in 1–2 ml of dry benzene containing 30 μl of dry pyridine at 70–80°C for 0.5 h and then at room temperature for 16 h. The resulting esters or amides were purified by preparative thin-layer chromatography (TLC) on silica (Merck silica gel 60 F_{254} chromatoplates, 0.5 mm gel thickness, E. M. Labs., Elmsford, NY, U.S.A.) prior to HRLC analysis. Appropriate TLC solvent systems and R_F values for the amide derivatives are given in Table I.

Liquid chromatography

The liquid chromatograph consisted of an Altex Model 110A pump, a Valco CV-UHP loop injector and an LDC Model 1202 variable-wavelength UV detector operated at 235–240 nm. Separations were performed on a 25 × 1.02 cm I.D. column slurry-packed with LiChrosorb SI-60 (E. M. Labs.) at 3500 p.s.i. and eluted with various mixtures of hexane and diethyl ether, depending on the derivative analyzed, at a flow-rate of 4 ml/min.

Regeneration of free acids from derivatives

The (−)-1-(1-phenyl)ethylamides of the resolved acids (*ca.* 20 mg) recovered from HRLC were hydrolyzed in 6 *M* hydrochloric acid (5 ml) at 90–100°C for 6 h (DCVA) or in 50% aqueous sulfuric acid (5 ml) at 50°C for 6 h (CMBA). The reaction mixture was extracted with diethyl ether (3 × 2 ml), and the organic fractions combined and concentrated *in vacuo*. The residue was dissolved in dilute aqueous sodium hydroxide (pH 10), extracted with diethyl ether (2 × 2 ml), acidified to pH 2 (hydrochloric acid), and re-extracted with diethyl ether (2 × 3 ml) to recover the resolved acid free of any amine impurity (yield from amide, 75–90%). The resolved acids were converted into their acid chlorides as described above for preparation of (−)-1-(1-phenyl)ethylamides to confirm their optical purity.

RESULTS

HRLC separation of derivatized DCVA isomers

The (−)-1-(1-phenyl)ethylamides of the four DCVA isomers were resolved completely and rapidly on silica upon elution with 25% ether–hexane (Fig. 1a). This small-scale preparative column exhibited adequate efficiency and excellent resolution for this separation (Table I). Separate experiments with the *trans* (1A+2A) and *cis* (3A+4A) enantiomer pairs showed that the amides of *cis*-substituted acids eluted first. Analysis of the amides of authentic samples of 2A and 4A established that the 1*S* enantiomer of each pair eluted first, thus allowing the unambiguous assignment of

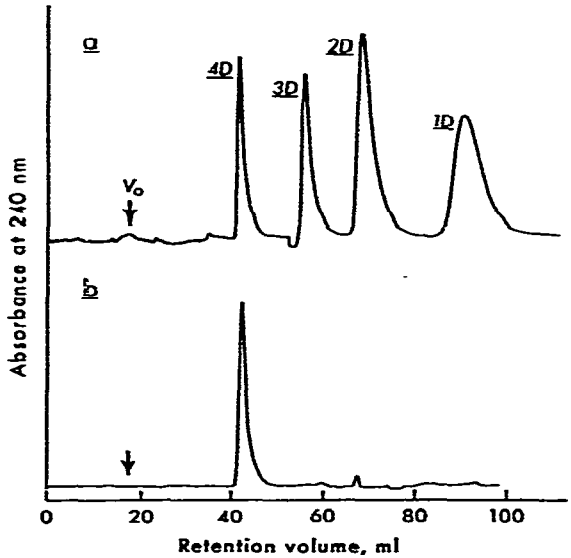

Fig. 1. (a) HRLC separation of diastereomeric amides 1D–4D; see Experimental section for conditions; (b) HRLC analysis of amide 4D after recovery from the amide mixture, acid hydrolysis to give free DCVA (4A) and re-preparation of the amide.

absolute configuration to each separated derivative. Minimum detectable levels at optimum detector sensitivity (A_{240}, 0.01 a.u.f.s.) were 0.1 and 0.5 μg for the amides of 2A and 4A, respectively.

In preliminary studies, we also explored the use of menthyl and 2-octyl ester derivatives for the resolution of the DCVA isomers by HRLC. These derivatives proved much more difficult to separate than the amides, requiring low solvent strengths ($\leq 1\%$ diethyl ether in hexane) and long analysis times for the achievement of partial resolution.

HRLC separation of enantiomeric CMBA amides

We extended the use of (−)-1-(1-phenyl)ethylamide derivatives to explore the resolution of the enantiomers of CMBA, a pyrethroid acid moiety lacking the cyclopropane ring and having only one chiral center. The diastereomeric amides were well resolved in the same chromatographic system used for the DCVA amides (Fig. 2a). Analysis of the amide prepared from the authentic *R* enantiomer (6A) established that this enantiomer eluted first. The efficiency and degree of resolution for this separation were similar to those observed for the amides of 3A and 4A (Table I). The minimum detectable of the amide of 5A at optimum detector sensitivity (A_{237}, 0.01 a.u.f.s.) was approximately 0.5 μg.

TLC separation of amide derivatives

We routinely used TLC for preliminary clean-up of amide derivatives and for the selection of candidate HRLC mobile phases. These procedures identified two solvent systems capable of separating the amides of *cis*-DCVA, *trans*-DCVA and

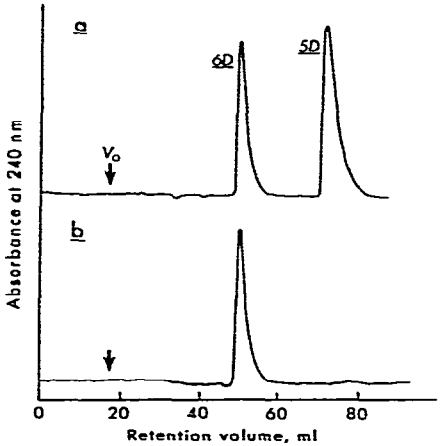

Fig. 2. (a) HRLC separation of diastereomeric amides 5D and 6D; see Experimental section for conditions; (b) HRLC analysis of amide 6D after recovery from the amide mixture, acid hydrolysis to give free CMBA (6A) and re-preparation of the amide.

CMBA on both analytical and preparative layers (Table I). The degree of resolution observed for TLC was considerably lower than that obtained by HRLC, so that rechromatography of each initial fraction was required to obtain optically pure material.

Regeneration of optically pure acids from amide derivatives

The chromatographically resolved DCVA amides were hydrolyzed in 6 *M* hydrochloric acid, and the free acids were recovered directly from the reaction mixtures by organic extraction. Re-preparation of the amide derivatives of the resolved acids, followed by HRLC analysis, demonstrated that neither chiral center underwent significant epimerization under the conditions required for hydrolysis (Fig. 1b). Attempts to hydrolyze the DCVA amides under basic conditions were unsuccessful, resulting in all cases in complex product mixtures and low yields of the recovered optically pure acids.

The CMBA amides were not hydrolyzed under the same conditions used for the DCVA derivatives, but the free CMBA enantiomers were obtained from hydrolysis in 50% aqueous sulfuric acid. Sulfuric acid-catalyzed hydrolysis at 50°C gave the optically pure acid without epimerization, as determined by re-preparation and chromatographic analysis of the amide derivative (Fig. 2b). However, higher temperatures (60–80°C) resulted in 15–30% epimerization as determined by analysis of the re-prepared amide derivatives.

DISCUSSION

Our results extend the usefulness of optically active arylethylamines as derivatizing agents for the HRLC separation of enantiomeric carboxylic acids to the optical and geometrical isomers of the acid moieties of pyrethroid insecticides. We found (−)-1-(1-phenyl)ethylamine, readily available from commercial sources at a

high degree of optical purity, to be a suitable derivatizing agent for the resolution of pyrethroid acids. In particular, these amide derivatives proved to be more readily separable than the menthyl and 2-octyl derivatives under normal-phase HRLC conditions.

We determined that optically pure pyrethroid acids could be regenerated by acid hydrolysis from the chromatographically isolated amide derivatives in satisfactory yields without epimerization. This finding suggests that the HRLC or TLC separation of amide derivatives may be an efficient method for the micro-scale preparative resolution of pyrethroid acid moieties. Chromatographic resolution may be particularly applicable for radiolabeled preparations necessary for metabolism, mode of action and environmental degradation studies. Previous approaches have required optical resolution of labeled pyrethroid acid moieties by conventional techniques[23,24] or the radiosynthesis of a single isomer from an unlabeled chiral intermediate[25]. Chromatographic micro-scale resolution may offer access to a wide variety of acid-labeled optically pure pyrethroids presently available only as racemates. This technique may also prove useful in the preparation of unlabeled optically pure pyrethroids in instances where the availability of racemic acid is limited.

Because of our primary interest in preparative resolution, we did not optimize chromatographic conditions for enantiomer composition determinations at high sensitivity. Nevertheless, we were able to detect sub-microgram quantities of the amides using essentially preparative chromatographic conditions. It is likely that the use of more efficient, smaller-diameter analytical columns and an amide derivative having a higher molecular extinction coefficient[22] would result in a several-fold increase in the sensitivity of this method. With analytical scale optimization, this HRLC method for the determination of pyrethroid isomer composition may prove to be a useful alternative to the GLC methods[6–12] available.

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