Capillary gas chromatographic separation of some diastereomeric amides from carbonyldiimidazole-mediated microgram-scale derivatizations of the acid moiety of permethrin insecticide ☆

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ABSTRACT

Mixtures from microgram-scale derivatizations of reference samples of (1R,3S)-*cis*-, (1S,3R)-*cis*-, (1R,3R)-*trans*- and (1S,3S)-*trans*-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane carboxylic acid with carbonyldiimidazole and seven chiral amines were investigated by achiral phase capillary gas chromatography (GC) with a flame ionization detector. The desired diastereomeric amides were identified by GC–mass spectrometry. Several combinations of derivatives and GC liquid phases were useful for resolving either pair of enantiomeric acids. Baseline separation of the four acids was achieved with (R)(−)- or (S)(+)-amphetamine derivatives and with a polar GC column of DB-WAX. The enantiomeric composition of the permethrin amides determined by GC was in agreement with the enantiomeric composition of the permethrin acids determined by polarimetry.

INTRODUCTION

Permethrin, a synthetic pyrethroid insecticide possessing asymmetric centers at C-1 and C-3 of the cyclopropane ring, is a mixture of four stereoisomeric esters (**1a–4a**, Fig. 1). It is well known that the insecticidal activity is primarily associated with the (1R,3S)-*cis* and (1R,3R)-*trans* isomers, **1a** and **3a**. Metabolic studies have shown that ester hydrolysis occurs to various extents in plant and animal species [1]. Thus, the chiral carboxylic acids (**1b–4b**) and their water-soluble conjugates are important metabolites of permethrin.

To study stereoselective processes involved in the synthesis and degradation of permethrin, isomer-specific approaches are needed to sepa-

rate trace amounts of **1b–4b**. Separations by achiral phase gas chromatography (GC) with diastereomeric esters derived from (+)-2-octanol [2] and (−)-menthol [3] and by chiral phase GC with esters of achiral alcohols [4] have previously been reported. Diastereomeric amide derivatives, prepared from the acid chlorides of **1b–4b** and optically active α-methylbenzenemethaneamine [α-methylbenzylamine or 1-(1-phenyl)ethylamine], have also been resolved by high-performance liquid chromatography (HPLC) [5].

In analytical derivatizations of antiinflammatory 2-arylpropionic acids with the chiral amines leucinamide [6,7], α-methylbenzylamine [8–12] and α-methylbenzeneethanamine (amphetamine) [13,14], a popular amide bond-forming strategy has involved the use of carbonyldiimidazole (CDI). Some of these procedures were applied to the stereospecific HPLC [6,7,9–11] and GC [8,12–14] determination of the R and S enantio-

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Fig. 1. Structures of the isomers of permethrin and of the derivatives under investigation.

EXPERIMENTAL

Chemicals and reagents

Reference samples of **1b–4b** were purchased from Raylo Chemicals Ltd. (Edmonton, Canada). Comparison of $[\alpha]_D$ values to those found in the literature [18] showed that **1b–3b** were approximately 100% optically pure. The sample of **4b** gave $[\alpha]_D = -22.3$ ($c = 2.1\%$, ethanol) whereas the literature [18] $[\alpha]_D = -34.6$ ($c = 1.9\%$, ethanol). This corresponds to 64.5% optical purity or 82% **4b** (18% **3b**).

$(R)(-)$-Amphetamine sulfate, $(S)(+)$-amphetamine sulfate and $(S)(+)$-methamphetamine hydrochloride were gifts from Health and Welfare Canada. $(R)(+)$-α-Methylbenzylamine and $(S)(-)$-2-methylbutylamine were purchased from Aldrich, whereas the N-methyl derivatives of these amines were prepared by a literature procedure [19] using ethyl chloroformate and lithium aluminum hydride and characterized by mass spectrometry (MS). CDI was purchased from Aldrich and Sigma and stored under vacuum in a desiccator. Organic solvents were OmniSolv glass-distilled grade (supplied by BDH, Edmonton, Canada). Water was distilled and further purified by a Barnstead NANOpure II system. All glassware was sonicated in methanol and oven-dried prior to use.

Stock solutions of CDI (1 mg/ml) and the chiral amines (0.7–1.2 mg/ml; equivalent to 800 nmol of the free base/ml) were prepared fresh daily in methylene chloride (water for amphetamine and methamphetamine salts). Test solutions of **1b–4b** (100 μg/ml) were prepared weekly in methylene chloride and stored at 4°C. Prior to derivatizations with amphetamine or methamphetamine, the free bases were formed (100×13 mm glass tube equipped with a PTFE-lined screw cap) by diluting 100 μl of the aqueous stock solution with water (900 μl) and adding 1 M sodium hydroxide (100 μl). After mixing for 10 min on an electronic tube shaker (Vibrax Model VXR with VX attachment), the mixture was extracted (Vibrax) four times with

mers of the carboxylic acids in biological samples. The capillary GC separation of other chiral carboxylic acids as their diastereomeric α-methylbenzylamides has also been reported [15]. Furthermore, CDI has previously been utilized in pyrethroid chemistry, for example in the preparative-scale coupling of $(1R,3R)$-*trans*-chrysanthemic acid with $(R)(+)$-1-(α-naphthyl)ethylamine [16].

Using CDI as the coupling reagent and reference samples of **1b–4b**, which were previously employed for preparing the stereoisomers of permethrin [17], we have investigated the microgram-scale derivatization of these carboxylic acids with $(R)(-)$-amphetamine, $(S)(+)$-amphetamine, $(R)(+)$-α-methylbenzylamine, $(S)(-)$-2-methylbutylamine, N-methyl $(S)(+)$-amphetamine (methamphetamine), N-methyl $(R)(+)$-α-methylbenzylamine and N-methyl $(S)(-)$-2-methylbutylamine. The capillary GC properties of the 28 diastereomeric amides (**1–4**, c–i; Fig. 1) have been examined on three common achiral liquid phases with a flame ionization detector. The novel 1-methyl-2-phenylethyl-amides derived from amphetamine were found

methylene chloride (1 ml) and the solvent was removed with a Savant evaporator (Model SVC-100H). The residue was transferred to the reaction vial in methylene chloride (200 μl).

Derivatizations

CDI (100 μl of stock solution, 100 μg, 616 nmol) and one of **1b–4b** (100 μl of test solutions, 10 μg, 47.8 nmol) were added to a Pierce Reacti-Vial (45 × 13 mm) equipped with a PTFE-coated micro stir bar (10 × 3 mm) and a PTFE-lined screw cap (septum type). The mixture was magnetically stirred (Reacti-Therm III module) under argon gas for 15 min at room temperature (21–23°C), then the amine (800 nmol) in methylene chloride (100 or 200 μl) was added and stirring was continued at room temperature for 1 h. The reaction mixture was evaporated to dryness (Savant). Methyl *tert.*-butyl ether (500 μl) and 1 *M* hydrochloric acid (500 μl) were added followed by a 2-min shake (Vibrax) and a 2-min centrifugation. The organic phase was transferred to a glass tube (100 × 13 mm) and washed with 500 μl of a saturated solution of sodium borate (prepared from $Na_2B_4O_7 \cdot 10H_2O$, Fisher), then with water (500 μl), centrifuging after each wash. The organic phase in a glass tube (100 × 13 mm) was evaporated (Savant), the residue was dissolved in methylene chloride (100 μl), vortexed (VWR vortex mixer) and 1–4-μl portions were removed (Hamilton 701 syringe) for capillary GC and GC–MS analyses.

Instrumentation and chromatographic conditions

Hewlett-Packard 5830 and 5890 gas chromatographs equipped with flame ionization detectors were used. The injector and detector temperatures were 225 and 250°C, respectively. Helium was used as the carrier gas with inlet pressures adjusted to give linear flow velocities for hexane of 28–29 cm/s at 35°C. J&W fused-silica capillary columns of DB-1 (30 m × 0.25 mm I.D.; film thickness 0.25 μm), DB-5 (30 m × 0.32 mm I.D.) and DB-WAX (30 m × 0.24 mm I.D.) were used. Samples were injected splitless (30 s) with column temperature programming (DB-1, DB-5) from 50 to 140°C at 25°C/min, at 3°C/min to 215°C and at 15°C/min

to 265°C. With DB-WAX, the rate was 25°C/min from 50 to 140°C and 3°C/min to 250°C.

Mass spectra were obtained on a Hewlett-Packard 5985B GC–MS instrument under electron-impact (70 eV) and chemical-ionization conditions. Isobutane (0.5 Torr; 1 Torr = 133.322 Pa) served as the reagent gas for chemical-ionization mass spectrometry. The transfer lines were maintained at 250°C and the ion source at 200°C (electron impact) or 180°C (chemical ionization). Capillary columns of HP-1 (25 m × 0.2 mm I.D.) from Hewlett-Packard and SPB-1 (30 m × 0.32 mm I.D.) from Supelco were used. The samples were injected splitless (30 s) with helium as the carrier gas.

RESULTS AND DISCUSSION

Derivatization experiments were initially carried out with the (1R,3S)-*cis* enantiomer **1b** by reacting this optically active acid with excess CDI and (R)(−)-amphetamine. Our procedure is based, in part, on a publication of Singh *et al.* [13] who reported the derivatization of several 2-arylpropionic acids with these reagents in methylene chloride or chloroform. Although the reported conditions suggested a 2-h stir at 65–85°C [13,14], **1b** (and the other permethrin acids) could be derivatized in methylene chloride at room temperature (1 h).

Assuming that both chiral reagents were optically pure and that racemization did not occur during the reaction, the anticipated derivative 3-(2,2-dichloroethenyl)-2,2-dimethyl-N-(1-methyl-2-phenylethyl) cyclopropanecarboxamide (**1c**) will possess the *R*, *S* and *R* stereochemistry at C-1, C-3 and in the amine moiety, respectively. Therefore, **1c** is designated as the *RSR* diastereomer.

GC–MS analysis of a derivatized mixture gave a mass chromatogram shown in Fig. 2. Under electron-impact conditions, the component at 22.7 min showed a pair of weak ions at *m/z* 325 and 327 corresponding to the molecular ions for **1c** ($C_{17}H_{21}Cl_2NO^{+\cdot}$). Prominent fragment ions at *m/z* 91 (base peak), 119 and 162, originating from the amphetamine portion of the molecule, were observed. The chemical-ionization mass
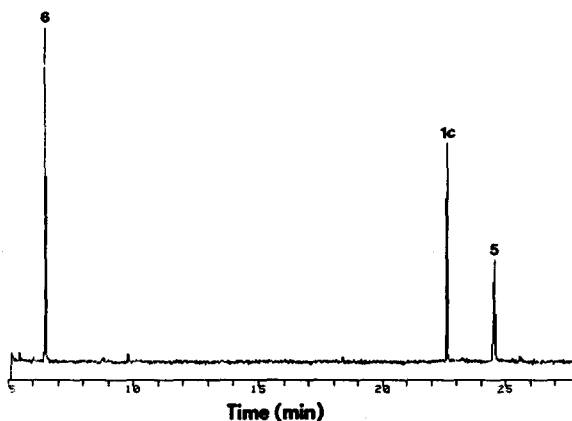
Fig. 2. Total ion mass chromatogram (70 eV) of a mixture obtained from derivatization of (1R,3S)-cis acid **1b** with CDI and (R)(−)-amphetamine. The HP-1 column was temperature programmed from 50 to 140°C at 25°C/min, at 5°C/min to 215°C, then at 15°C/min to 265°C. Peaks: **1c** = amide; **5** = urea; **6** = isocyanate.

Fig. 3. Reactions of CDI with carboxylic acids and primary amines. (a) Amide formation; (b) urea and isocyanate formation. IM = Imidazole.

spectrum gave prominent quasimolecular ions for **1c**, at m/z 326 and 328.

Derivatization of **1b** with (S)(+)-amphetamine gave a mass chromatogram that was similar to Fig. 2 except that the desired **1d** (RSS) eluted before **1c**, at 22.4 min. The mass spectra of **1c** and **1d** were nearly identical.

Fig. 2 shows that a second major component of the derivatization reaction eluted from the capillary column at 24.5 min. The molecular mass (296) and fragment ions (m/z 205, 91, 44) suggested that this product was the symmetrically disubstituted methylphenylethyl derivative of urea, $C_6H_5CH_2CH(CH_3)NHCONHCH(CH_3)$-$CH_2C_6H_5$ (**5**). Ureas are formed by the reaction of amines with CDI [20], and this pathway would be expected to compete with the desired amide bond-forming reaction (Fig. 3). Furthermore, intermediate imidazole-N-carboxamides can dissociate to an isocyanate and imidazole [20]. Indeed, a third major component eluting at 6.5 min (Fig. 2) gave a molecular ion at m/z 161 corresponding to $C_6H_5CH_2CH(CH_3)N = C = O$ (**6**) in addition to fragment ions at m/z 91 and 70. The urea (**5**) and isocyanate (**6**) were also found in control derivatizations without **1b**.

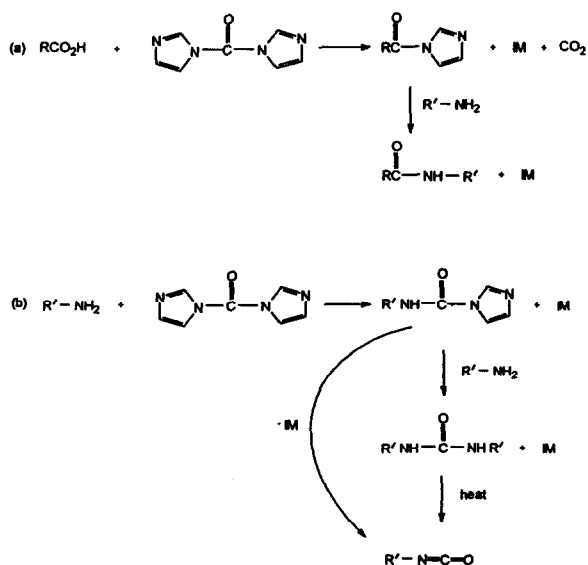It is also possible that **6** represents a thermal degradation product since ureas are capable of

decomposing to isocyanates during gas chromatography [21].

GC analyses with DB-1 indicated that samples of **1c** (RSR) contained a small amount of a compound with the same retention time as **1d** (RSS). Naturally, **1d** would also be formed if (S)(+)-amphetamine was a contaminant in the sample of (R)(−)-amphetamine. This minor isomeric component (1–2% from GC peak area ratios) could also be **2c** (SRR) if **1b** was contaminated with **2b** because **1d** and **2c** are optical isomers and have identical retention times on achiral columns.

Samples of **1d**, prepared from **1b** and (S)(+)-amphetamine, were found by GC to contain **1c** (or possibly **2d**) but to the extent of 5%. This discrepancy could be attributed to small differences in the optical purities of the amine samples, (R)(−)-amphetamine being purer than (S)(+)-amphetamine. Indeed, derivatization of the (1S,3R)-cis acid **2b** with CDI and (R)(−)-amphetamine gave **2c** (SRR) and **2d** (or **1c**) in a ratio of 98 to 2 whereas experiments with (S)(+)-amphetamine gave a ratio of **2d** to **2c** (or **1d**) of 95 to 5. These results also indicated that the derivatizations proceeded without racemiza-

tion at C-1 or C-3 and that both of the cis acids were of high (98–100%) optical purity.

Derivatization of **1b** with CDI and $(\bar{S})(+)$-amphetamine was studied in detail. Using the integrated peak areas of **1d** from capillary GC analysis of the samples with a DB-1 column, enhancements in derivatization yield could not be demonstrated by heating the reaction mixture, as suggested [13]. Furthermore, decreased amounts of CDI (15–30 μg) resulted in a 4–7-fold reduction in area counts compared with the standard 100 μg addition. Urea (**5**) was always found in the derivatized samples, regardless of conditions, but the relative amount detected by GC varied between experiments. Typically, a peak area ratio of **1d** to **5** of 0.3 was observed.

Two other chiral primary amines, $(R)(+)$-α-methylbenzylamine and $(S)(-)$-2-methylbutylamine, were coupled to **1b** and **2b** using CDI. Under the standard derivatization conditions, these amines gave the expected amides as determined by GC–MS. On DB-1 and SPB-1 capillary columns, the urea from α-methylbenzylamine, $C_6H_5CH(CH_3)NHCONHCH(CH_3)$-$C_6H_5$ (**7**), eluted after **1e** and **2e** whereas the urea from 2-methylbutylamine, CH_3CH_2CH-$(CH_3)CH_2NHCONHCH_2CH(CH_3)CH_2CH_3$(**8**), eluted before **1f** and **2f**. In the mixture from α-methylbenzylamine, an early-eluting component in the mass chromatogram corresponded to $C_6H_5CH(CH_3)N = C = O$ (**9**) but the isocyanate from 2-methylbutylamine was not found.

Derivatization of the $(1R,3R)$-trans acid **3b** with $(R)(-)$-amphetamine, $(S)(+)$-amphetamine, $(R)(+)$-α-methylbenzylamine and (S)-$(-)$-2-methylbutylamine gave the trans amides (**3c**, **3d**, **3e**, **3f**). Based on GC retention times with DB-1, the ureas (**5**, **7**, **8**) and isocyanates (**6**, **9**) were also formed. About 2% of **3d** (RRS) or **4c** (SSR) were detected in the reaction of **3b** with $(R)(-)$-amphetamine, which confirmed the high (98–100%) optical purity of **3b**. The impure $(1S,3S)$-trans acid **4b** on derivatization with $(R)(-)$-amphetamine gave **4c** and **3c** (RRR) in a ratio of 82 to 18, the same ratio calculated from the optical purity of **4b** determined by polarimetry.

Although the ureas encountered in these de-

rivatization reactions could be separated from the pyrethroid amides on non-polar liquid phases, it was of considerable interest to suppress their formation. Some interesting approaches to this problem have been described [22]. We rationalized (Fig. 3) that secondary amines might not react with CDI for steric reasons so the N-methylamines of $(S)(+)$-amphetamine, $(R)(+)$-α-methylbenzylamine and $(S)(-)$-2-methylbutylamine were investigated. Using the same stoichiometry and conditions as before, **1b–4b** were derivatized with these amines and the products were examined by capillary GC and GC–MS. These reactions gave the desired tertiary amides (**1–4, g–i**) but none of the N-methylureas were found as side products. A mass chromatogram from the derivatization of **1b** with $(S)(+)$-methamphetamine is shown (Fig. 4).

The identity of the diastereomeric amides was confirmed by chemical-ionization mass spectrometry. All of the derivatives gave prominent quasimolecular ions during GC–MS analysis. The $(1R,3S)$-cis derivatives **1c–1i** were selected for electron-impact mass spectrometry to illus-
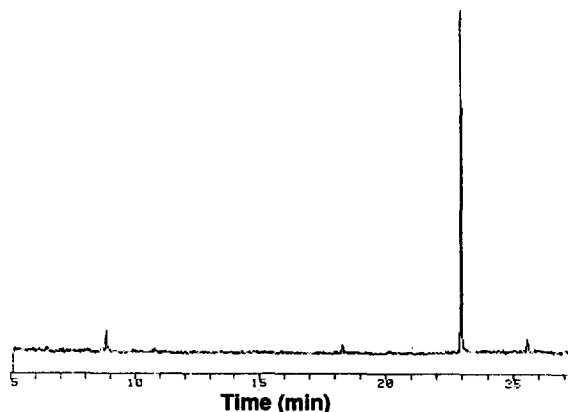

Fig. 4. Total ion mass chromatogram (70 eV) from the derivatization of $(1R,3S)$-cis acid **1b** with CDI and $(S)(+)$-methamphetamine. The HP-1 column was programmed as in Fig. 2. The peak at 23.0 min is amide **1g**. Minor peaks at 8.8, 18.3 and 25.6 min were present in control extracts without the derivatization reagents.

trate characteristic fragmentation patterns for these amides.

Besides weak molecular ions, **1c–1i** gave fragment ions (5–10% relative abundance) at m/z 163, which corresponded to the $C_7H_9Cl_2^{+ \cdot}$ ion from bond cleavage α to the carbonyl group. Rupture of this same bond, but with charge retention on the amide fragment, resulted in

other diagnostic ions at m/z 176 (**1g**), 162 (**1c, 1d, 1h**), 128 (**1i**) and 114 (**1f**). The corresponding ion for **1e** at m/z 148 was very weak (1% of the base peak at m/z 105). Prominent ions were found that corresponded to N-alkyl bond cleavage and subsequent decomposition of the alkylaryl (or alkyl) portions. This resulted in strong ions (35–100% relative abundance) at

TABLE I

SEPARATION OF AMIDE DERIVATIVES OF PERMETHRIN ACIDS ON THREE CAPILLARY COLUMNS

Compound	Molecular mass[a]	Stereo-chemistry[b]	DB-1		DB-5		DB-WAX	
			Retention time (min)	α^c	Retention time (min)	α^c	Retention time (min)	α^c
1c (see Fig. 1)	325	RSR	30.92		30.10		49.66	
2c		SRR	30.34	1.019	29.50	1.020	48.81	1.017
3c		RRR	31.34		30.50		51.82	
4c		SSR	30.92	1.014	30.10	1.013	50.81	1.020
1d	325	RSS	30.34		29.52		48.82	
2d		SRS	30.92	1.019	30.10	1.020	49.67	1.017
3d		RRS	30.92		30.10		50.82	
4d		SSS	31.34	1.014	30.50	1.013	51.82	1.020
1e	311	RSR	32.44		28.22		49.03	
2e		SRR	32.18	1.008	27.84	1.014	48.43	1.012
3e		RRR	32.75		28.68		51.13	
4e		SSR	32.75	1.000	28.68	1.000	51.35	1.004
1f	277	RSS	23.16		18.64		32.64	
2f		SRS	23.12	1.002	18.65	1.001	32.63	1.000
3f		RRS	23.83		19.21		34.07	
4f		SSS	23.76	1.003	19.26	1.003	34.15	1.002
1g	339	RSS	34.37		30.97		45.45	
2g		SRS	34.42	1.001	31.03	1.002	45.65	1.004
3g		RRS	34.72		31.40		47.71	
4g		SSS	34.47	1.007	31.10	1.010	46.69	1.022
1h	325	RSR	33.65		29.99		44.09	
2h		SRR	33.52	1.004	29.76	1.008	43.82	1.006
3h		RRR	33.84		30.32		45.77	
4h		SSR	33.83	1.000	30.32	1.000	45.84	1.002
1i	291	RSS	23.96		19.18		26.85	
2i		SRS	24.06	1.004	19.21	1.002	26.89	1.001
3i		RRS	24.55		19.72		28.50	
4i		SSS	24.54	1.000	19.77	1.002	28.50	1.000

[a] Based on Cl = 35. M + 1 ions (100% relative abundance) were observed for each compound during isobutane chemical-ionization GC–MS analysis.

[b] The absolute stereochemistry at C-1, C-3 and in the amine moiety, respectively.

[c] α is the chromatographic separation factor, obtained from the indicated retention times.

m/z 119 and 91 (**1c**, **1d**, **1g**), at m/z 105 (**1e**, **1h**) and at m/z 71, 43 and 41 (**1f**, **1i**).

Samples of the derivatives were examined on capillary columns of differing polarities using the splitless injection technique (Table I). For the resolution of either pair of enantiomeric acids, the amphetamine derivatives showed the best chromatographic separation factors ($\alpha = 1.013$–1.020) on the three columns.

Only DB-WAX was adequate for the separation of the amphetamine derivatives because either **1c** and **4c** or **2d** and **3d** coeluted on DB-1 and DB-5. The separation achieved on DB-1 and DB-WAX is illustrated (Fig. 5).

The $(R)(+)$-α-methylbenzylamine derivatives showed relatively good separation of the *cis* isomers **1e** and **2e** ($\alpha = 1.008$–1.014) but the *trans* isomers **3e** and **4e**, which had longer retention times, were poorly separated on the three capillary columns. Derivatives prepared with 2-methylbutylamine (**1f**–**4f**) showed very small α values (1.000–1.003) although the *cis* and *trans* isomers were well separated.

In derivatizations with N-methylamines, the $(S)(+)$-methamphetamine derivatives were only separated on DB-WAX but the α value for separation of **1g** and **2g** was less than the corresponding derivatives from $(R)(-)$- and $(S)(+)$-amphetamine. The **2g** and **4g** stereoisomers nearly coeluted on DB-1 and DB-5. Use of N-methyl $(R)(+)$-α-methylbenzylamine resulted in

some separation of **1h** and **2h** ($\alpha = 1.004$–1.008) but **3h** and **4h**, like the *trans* isomers from $(R)(+)$-α-methylbenzylamine, were not completely resolved on these capillary columns. Derivatives from N-methyl $(S)(-)$-2-methylbutylamine (**1i**–**4i**) yielded very small α values also.

The techniques described here provide new approaches for derivatizing the permethrin acids with chiral amines and for estimating the enantiomeric composition of microgram quantities of these acids by achiral phase capillary GC. Although direct comparisons with previously reported separations of diastereomeric octyl and menthyl esters by packed column GC is difficult, the α values derived from the capillary column of DB-WAX appear to be smaller than those obtained with liquid phases of QF-1 [2] or OV-210 [3]. Samples of the diastereomeric amides described here were only studied on three common liquid phases. Other achiral capillary GC columns may give better separations. Introduction of a nitrogen atom raises interesting possibilities for the GC detection of these acids with a nitrogen-selective detector, which might be preferred by some laboratories to electron-capture or GC–MS techniques. We hope that this technology will be useful in the development of sensitive, enantiospecific assays for these metabolites, for example in the monitoring of **1b**–**4b** in urine samples following exposure to permethrin [23,24] or cypermethrin [25–27].
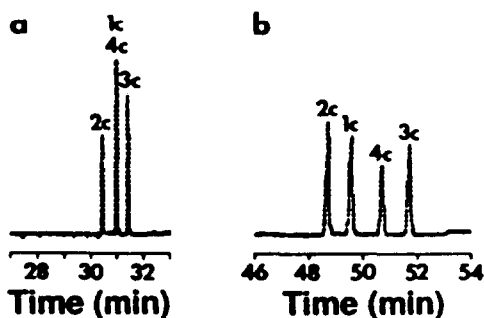
ACKNOWLEDGEMENTS

Fig. 5. Capillary gas chromatograms illustrating the separation of diastereomeric amides **1c**–**4c** on (a) DB-1 and (b) DB-WAX. The four permethrin acids (2.5 μg each of **1b**–**4b**) were derivatized with CDI and $(R)(-)$-amphetamine at room temperature. See the Experimental section for chromatographic conditions. HP 5890 chromatograph, HP 3396A integrator, attenuation 0.

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