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DETERMINATION OF (*R*)- AND (*S*)-EPIMERS AT C-1 IN RESIDUAL AMOUNTS OF (±)-*cis,trans*-PERMETHRIN AND CYPERMETHRIN BY GAS–LIQUID CHROMATOGRAPHY*

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SUMMARY

Treatment of (1*R*)*cis*-, (1*S*)*trans*- and (1*R*)*trans*-permethrin with sodium *l*-menthylate at room temperature produced the *l*-menthyl esters of the corresponding 2,2-dimethyl-3-(2,2-dichlorovinyl)cyclopropanecarboxylic acids without epimerization at C-1 or C-3. These three esters, or the mixture of four esters produced by similar treatment of (±)-*cis,trans*-permethrin were readily separated by gas–liquid chromatography on OV-210. Minimum detectable amounts of each ester were *ca.* 0.1 ng using a pulsed ^{63}Ni electron-capture detector. The *l*-menthyl esters corresponding to the permethrin isomers could also be prepared in high yield and without epimerization by alkaline hydrolysis at room temperature to the respective cyclopropanecarboxylic acids, crown-ether-catalyzed conversion to the benzyl esters and subsequent room-temperature trans-esterification of this ester with sodium *l*-menthylate. Using the longer procedure, cypermethrin could be converted into the same four-component mixture of *l*-menthyl esters that was obtain from permethrin. The application of these procedures to the determination of the individual (*R*)- and (*S*)-epimers (at C-1) in residual amounts of the parent pyrethroids is discussed.

INTRODUCTION

The significance of total residues of the pyrethroid insecticides permethrin (1b–4b) and cypermethrin (1c–4c) is difficult to evaluate because they are mixtures of isomers with different toxicological properties. In general, high insecticidal activity is associated with the (*R*)-configuration of the chiral cyclopropyl carbon, C-1, adjacent to the carboxyl group [2 (b and c); 4 (b and c)], whereas the enantiomer having the opposite configuration [1 (b and c); 3 (b and c)] is only slightly toxic[1,2]. In addition, enantiomers of cypermethrin differing in the configuration at C-α of the *m*-phenoxybenzyl alcohol portion of the molecules also differ in toxicity[2,3]. The synthetic methods used commercially to prepare these two insecticides do not involve chiral reagents, and equal quantities of the (*R*)- and (*S*)-configuration at C-1 and C-α are produced but degradation by enzymes or other biologically active systems in the

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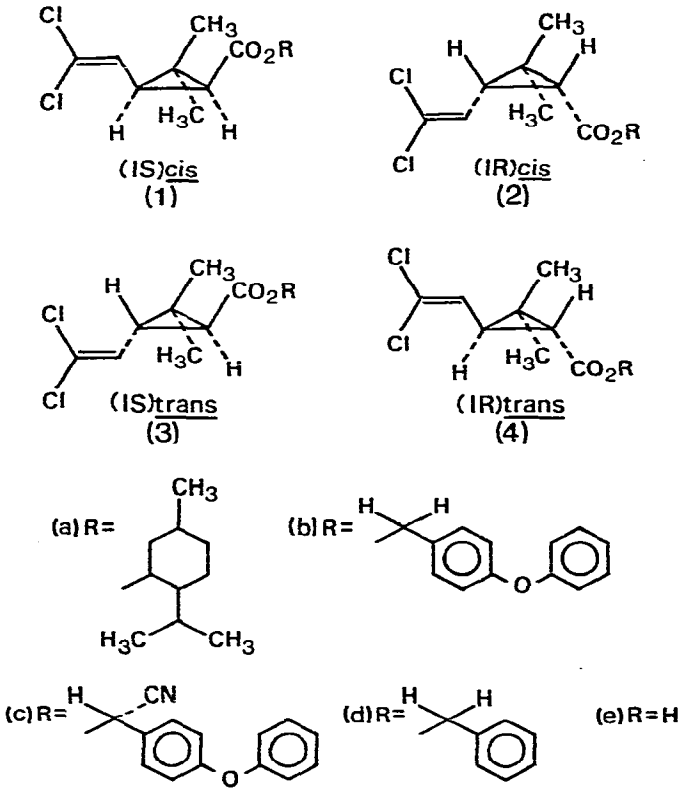
environment will involve natural chiral reagents, which may preferentially degrade one enantiomer[4,5]. A quantitative determination of each of the optical isomers is required to describe fully the degradation of commercial mixtures or to assess the importance of residues.



Gas–liquid chromatography (GLC) has proven to be a valuable quantitative technique for both the direct separation of enantiomers on chiral stationary phases and their indirect separation on non-chiral phases after conversion into diastereoisomers; short reviews have been included in several publications[6,7,8]. The separation of diastereoisomers has been successfully applied to the C-1 enantiomers of chrysanthemic acid[9], a number of pyrethroids containing this acid[8] and, more recently, to the 2,2-dimethyl-3-(2,2-dichlorovinyl)cyclopropanecarboxylic acids (1e–4e) derived from permethrin at the level of 30–60 mg[10]. We wish to describe procedures for the quantitative preparation of the four *l*-menthyl 2,2-dimethyl-3-(2,2-dichlorovinyl)-cyclopropanecarboxylates (1a–4a) from permethrin or cypermethrin at the microgram level, and the separation and analysis of these esters by GLC with electron-capture detection to provide quantitative determination for the four optical isomers of permethrin and four of the eight possible optical isomers of cypermethrin at levels down to 0.05 ppm in soil.

MATERIALS AND METHODS

Instrumentation, chemicals and reagents

A Tracor MT 220 gas chromatograph fitted with a U-shaped glass column (60 cm × 4 mm I.D.) packed with 2.5% of OV-210 on Varaport 30 (100–120 mesh) and equipped with a ^{63}Ni electron-capture detector was used for analysis of the *l*-menthyl esters. The first 7 cm of the column were left unpacked to provide a glass liner for the injection port (maintained at 200°); glass wool was not used at the injector end of the column. Argon–methane (95:5) at *ca.* 70 ml/min was used as carrier gas. The detector was maintained at 290°, with a 55-V polarizing voltage applied at a 4-μsec pulse width and 240-μsec pulse rate. The detector signal was fed to a strip-chart recorder (sensitivity of 255 mm/mV) via an electrometer operating at 1.6 nA/mV. The column was maintained at 125°, and, under these conditions, the retention times of *l*-menthyl (1*S*)*cis*-, (1*R*)*cis*-, (1*S*)*trans*- and (1*R*)*trans*-2,2-dimethyl-3-(2,2-dichlorovinyl)cyclopropanecarboxylates were 4.9, 5.3, 6.5 and 7.6 min, respectively. Typical sensitivity is shown in Fig. 1. Under these conditions, the *cis*-benzyl esters (1d and 2d) were eluted in 4.7 min, and their *trans*-analogues (3d and 4d) in 6.0 min. In preliminary experiments, analyses for permethrin or cypermethrin were carried out as described previously[11].

Permethrin [3-phenoxybenzyl (±)-*cis,trans*-2,2-dimethyl-3-(2,2-dichlorovinyl)-cyclopropanecarboxylate] (93.9%) was supplied by Chipman (Stoney Creek, Canada). Cypermethrin [(±)-α-cyano-3-phenoxybenzyl (±)-*cis,trans*-2,2-dimethyl-3-(2,2-dichlorovinyl)cyclopropanecarboxylate] (96%) was provided by Shell Research (Woodstock Agricultural Research Centre, Sittingbourne, Great Britain). The samples of (1*R*)-*cis*- (2b), (1*S*)*trans*- (3b) and (1*R*)*trans*-permethrin (4b) were from the laboratory of Dr. M. Elliott (Department of Insecticides and Fungicides, Rothamsted Experimental Station, Harpenden, Great Britain). Benzyl bromide, *l*-menthol and the crown ether, dicyclohexyl-18-crown-6, were from Aldrich (Milwaukee, Wisc., U.S.A.).

Hexane (Code No. 641100, Shell Canada, Burlington, Canada), benzene (ACS reagent, Fisher Chemical Co., Don Mills, Canada) and chloroform (ACS reagent, Caledon Labs., Georgetown, Canada) were distilled in glass in our laboratory, the benzene and hexane from potassium permanganate and sodium–lead alloy (dri-Na, Baker) and the chloroform from permanganate only. Benzene used in the preparation of sodium *l*-menthylate solutions was redistilled from sodium. Absolute ethanol was from Consolidated Alcohols (Toronto, Canada) and was used as received. Silica gel (100–200 mesh, Grade 923) (Fisher) containing 4.2% of moisture was used as received. The glass chromatography columns were of 1.5 cm I.D. and were fitted with a coarse fritted-glass disc at the bottom and a ground-glass joint at the top to permit attachment of a reservoir.

Solutions of dicyclohexyl-18-crown-6 (0.02 *M*) and benzyl bromide (0.2 *M*) were prepared by dissolving 0.37 g and 2.4 ml, respectively, of chemical in 50 and 100 ml of benzene; benzyl bromide solution was prepared freshly each month. The ethanolic 5% potassium hydroxide used was prepared by dissolving 5 g of 85% pellets in 5 ml of water and diluting with 95 ml of absolute ethanol.

The 0.01 and 0.05 *M* solutions of sodium *l*-menthylate in benzene were prepared by treating 13.4 g of *l*-menthol dissolved in 50 ml of anhydrous benzene with 0.15 and 0.60 g of finely cut sodium, respectively, and refluxing the mixture until the

sodium was dissolved (*ca.* 24 h), while maintaining anhydrous conditions. Before cooling, the apparatus was purged with nitrogen, and the solution was allowed to cool to room temperature in this atmosphere. For storage, the reagent was transferred to a glass container that was closed by a PTFE-lined cap after being purged with nitrogen. The solutions of sodium *l*-menthylate in benzene were susceptible to hydrolysis and (particularly the more concentrated solutions) to oxidation. Oxidation was minimized by allowing the prepared reagent to cool in a relatively inert atmosphere and by storing it under nitrogen in a tightly closed container. With these precautions, the more concentrated solution was usable for several days. Samples that had undergone considerable oxidation produced unacceptable levels of interference in the chromatograms. The more dilute solution was apparently more susceptible to hydrolysis, which was observed as a gradual loss of activity in trans-esterification; its useful life was several weeks (depending on the frequency of opening the container). Reagent-blank and standard solutions should be processed with the unknowns to permit assessment of these problems should they arise.

The effects of benzene, chloroform and dicylclohexyl-18-crown-6 on human physiological processes have not been fully examined, and appropriate care should be taken to keep exposure to the absolute minimum until these effects have been carefully evaluated.

Hydrolysis of permethrin and cypermethrin and recovery of the 2,2-dimethyl-3-(2,2-dichlorovinyl)cyclopropanecarboxylic acids (1e–4e)

Permethrin or cypermethrin (up to 200 μg) in 2–3 ml of ethanol, or the pyrethroid-containing benzene–hexane (80:20) fraction from Florisil chromatography of a soil extract as described previously for crops[12] and solvent-exchanged three times to a similar volume of ethanol, was treated with 10 ml of ethanolic 5% potassium hydroxide in a 250-ml flask and allowed to stand overnight (*ca.* 16 h) at room temperature. The solvent was then evaporated to dryness at 52° on a rotary evaporator under vacuum (the large flask contains any spattering of the solid residue). The residue was rinsed with hexane (2 × 10 ml), which was transferred to a 60-ml separating funnel. The rinsed residue was dissolved in 10 ml of water, and the aqueous solution was rinsed into the separating funnel with water (2 × 5 ml). The aqueous phase was extracted with the hexane to remove neutral material, and the hexane was discarded. The aqueous phase was then acidified (pH *ca.* 2) with 1 ml of concentrated hydrochloric acid, returned to the separating funnel with water rinses (bringing the volume to *ca.* 35 ml), saturated with sodium chloride (10–12 g) and extracted with chloroform (4 × 25 ml), each extract being dried by passing it through anhydrous sodium sulphate in a funnel; a further two rinses of the sodium sulphate with chloroform completed extraction of the cyclopronanecarboxylic acids.

Crown-ether-catalyzed formation of the benzyl esters (1d–4d) of 2,2-dimethyl-3-(2,2-dichlorovinyl)cyclopropanecarboxylic acids

The chloroform extract from the hydrolysis was evaporated to 2–3 ml and solvent-exchanged three times with 15-ml portions of benzene, which was finally evaporated to 2–3 ml. This residue was transferred quantitatively with rinsing (final volume 5–7 ml) to a culture tube (200 × 25 mm) with a PTFE-lined cap. To this, 1 ml of benzyl bromide reagent, 1 ml of dicyclohexyl-18-crown-6 reagent, 0.3 g of

potassium bicarbonate and a small glass-covered magnetic stirring-bar were added. The tube was capped tightly and heated for 1 h in a water bath at 80° on a magnetic stirrer providing vigorous stirring. After cooling, the mixture was rinsed quantitatively onto a chromatographic column packed with 2 g of anhydrous sodium sulphate and 5 g of silica gel and topped with another 2 g of sodium sulphate. Sufficient benzene was used in the rinsing to provide *ca.* 40 ml of eluate, which was evaporated to *ca.* 2 ml and then made up to volume in a 10-ml volumetric flask.

Trans-esterification of benzyl 2,2-dimethyl-3-(2,2-dichlorovinyl)cyclopropanecarboxylates and permethrin with sodium l-menthylate

A 3-ml portion of the benzene solution of the benzyl esters was treated with 1 ml of the 0.05 *M* sodium *l*-menthylate in a culture tube (120 × 5 mm) with a PTFE-lined cap, and the mixture was allowed to stand for 2 h at room temperature. Water (4 ml) was added, and the mixture was shaken vigorously, then centrifuged on a clinical centrifuge to speed the separation. The aqueous phase was removed with a disposable pipette, and the benzene was dried by adding anhydrous sodium sulphate before the solution was used for analysis.

Permethrin standards or permethrin in a benzene–hexane (80:20) solution from soil extracts cleaned up by columnn chromatography on Florisil as mentioned earlier for cypermethrin (volume up to 5 ml) were treated with 1 ml of 0.01 *M* sodium *l*-menthylate reagent for 2 h at room temperature and worked up as described above for the benzyl ester trans-esterification.

DISCUSSION

Of the two general techniques for the separation of enantiomers to which GLC is applicable, direct separation on a chiral stationary phase is the more desirable, especially when one of a number of chiral centres is lost in the preparation of the diastereoisomeric derivative required for application of the second technique. Unfortunately, the low volatility of the pyrethroids, the limited thermal stability of most chiral GLC phases and the apparently general requirement for a capillary column make application of the direct technique difficult. For this reason, we directed our efforts to the development of a GLC method that would at least determine the amounts of C-1 epimers present in residues of permethrin and cypermethrin based on the separation of diastereoisomeric esters of the constituent 2,2-dimethyl-3-(2,2-dichlorovinyl)cyclopropanecarboxylic acids.

The formation of disastereoisomeric esters of these acids with *l*-menthol[13] and *d*- and *l*-2-octanol[10] has been reported. Also, as previously reported[7], we found that ester yields were not quantitative when microgram amounts of the acids were converted into the acid chloride with thionyl chloride and subsequently treated with an alcohol, (in our work, *l*-menthol) as described by Elliott *et al.*[13]. Esterification of the acids at the microgram level by treatment with thionyl chloride and *l*-menthol simultaneously as described[7] produced high yields of the esters, but numerous interfering components were present from the reagents when the reaction mixture was analyzed by GLC using an electron-capture detector. These components could not be removed easily (by, *e.g.*, washing with water or Florisil chromatography), and their presence would not permit analysis at the levels required.

The trans-esterification of esters with solutions of alkali-metal alkoxides is a well-known reaction. The treatment of (1*R*)*cis*-, (1*S*)*trans*- and (1*R*)*trans*-permethrin and (±)-*cis*,*trans*-permethrin with 0.01 *M* sodium *l*-menthylate in benzene or benzene–hexane mixtures at room temperature rapidly converted the permethrin isomers into the corresponding *l*-menthyl esters. Most of the parent *cis*-isomers (the least reactive) had disappeared after 1 h and none could be detected after 2 h when the hexane extract of the reaction mixture was analyzed for permethrin. That the isomers were not racemized during this treatment with anhydrous alkali is shown by the fact that a single product was formed from each isomer (see Fig. 1, A, B and C). Murano reported that no racemization of the chrysanthemic acids occurred on refluxing in aqueous alkali[9]. The stability of these enolizable materials under the alkaline conditions is presumably due to the existence of an energy barrier to racemization in the cyclopropyl carbanion[14]. The sensitivity of the electron-capture detector to these esters and the absence of interfering responses from the reagent is also shown by Fig. 1. Fig. 2 shows typical results from the analysis of permethrin-treated soil. Although some of the cyclopropanecarboxylic acids potentially present will be recovered from the soil by the extraction procedure used, they will not interfere with the analysis, as they are unreactive in the trans-esterification reaction.

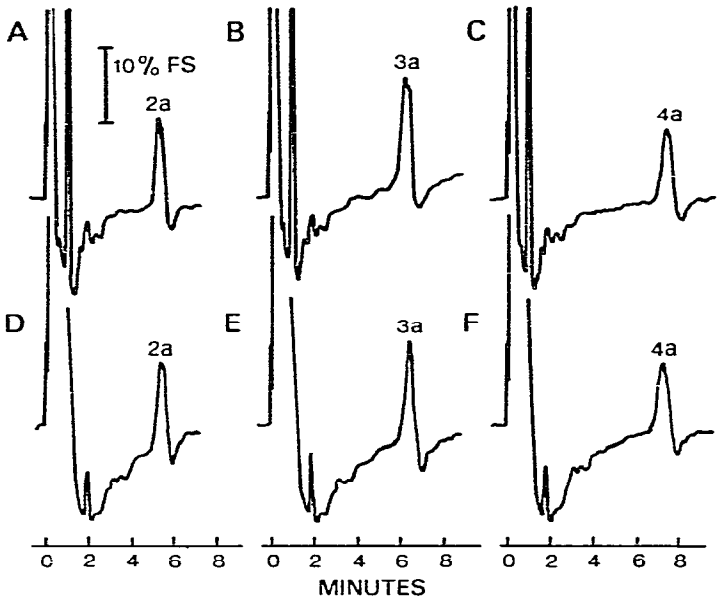


Fig. 1. Gas chromatograms of *l*-menthyl 2,2-dimethyl-3-(2,2-dichlorovinyl)cyclopropanecarboxylate. A, B and C, from direct trans-esterification of permethrin isomers; D, E and F, from hydrolysis, benzyl ester formation and trans-esterification of permethrin isomers. [2a = (1*R*)*cis*; 3a = (1*S*)-*trans*; 4a = (1*R*)*trans*]. Responses are equivalent to 0.7, 0.6 and 0.6 ng of the original permethrin isomer, respectively.

Cypermethrin disappeared rapidly in 0.01 *M* sodium *l*-menthylate, but the *l*-menthyl esters of the 2,2-dimethyl-3-(2,2-dichlorovinyl)cyclopropanecarboxylic acids were not formed. The failure of this reaction, presumably because of competing reactions of the α-cyano group, necessitated development of alternative procedures

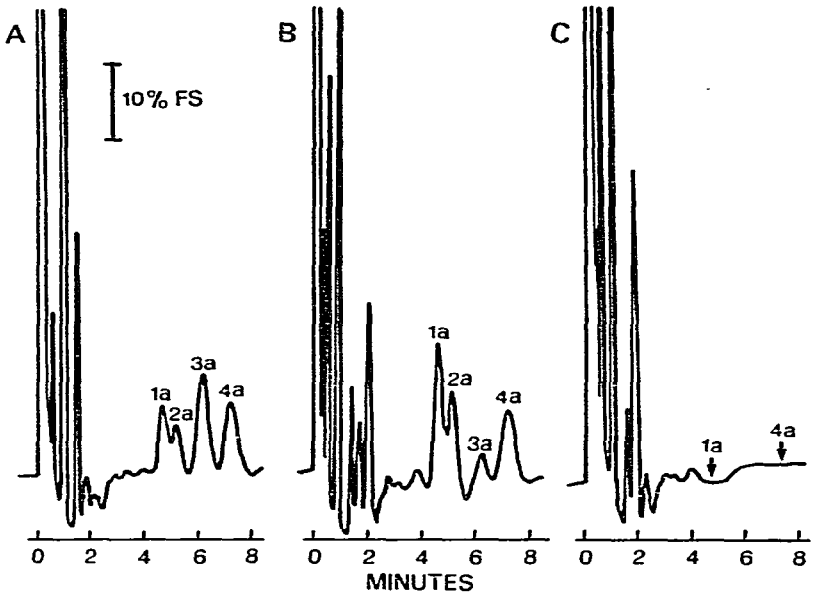


Fig. 2. Gas chromatograms of *l*-menthyl esters derived from permethrin residues in soil by direct trans-esterification. A, Immediately after treatment (2.8 ng of permethrin; 0.3 mg of soil); B, after 32 weeks (2.8 ng of permethrin; 1.6 mg of soil); C, control (1.6 mg of soil).

for determining the isomeric composition of residual cypermethrin. The hydrolysis of cypermethrin (and permethrin) was found to be complete after 16 h at room temperature in 5% potassium hydroxide in 95% ethanol, and good yields of the cyclopropanecarboxylic acids were recovered as determined by GLC analysis of methyl or trimethylsilyl esters. To make use of the relatively interference-free trans-estification procedure used for permethrin, a simple method of esterifying the recovered acids was required. Methyl esters were readily prepared, but trans-esterification proceeded too slowly with 0.01 *M* sodium *l*-menthylate at room temperature to be of practical value, and heating produced intolerable interference. The more reactive benzyl esters, prepared by the reaction of the acids with benzyl bromide in the presence of potassium bicarbonate and catalytic amounts of dicyclohexyl-18-crown-6[15], were found to be completely trans-esterified within 2 h on treatment with 0.05 *M* sodium *l*-menthylate in benzene at room temperature, so providing the required alternative route to the *l*-menthyl esters. Conversion of (1*R*)*cis*-, (1*S*)*trans*-, (1*R*)*trans*- and (±)-*cis*,*trans*-permethrin into the corresponding *l*-menthyl esters using the three-step procedure proceeded without fractionation or racemization. The yields of esters were 95–100% of those observed in the single-step trans-esterification, and there was no complication of the chromatograms by components in the reagents. The results are shown in Fig. 1 (D, E and F). Separate experiments also demonstrated that the isomeric cyclopropanecarboxylic acids were stable for at least 4 days at room temperature and for at least 4 h at reflux temperature in the ethanolic potassium hydroxide used. The crown-ether-catalyzed benzyl ester formation was found to proceed at room temperature, with vigorous sturring, but only 17% of the *cis*- and 25% of the *trans*-isomers had reacted after 1 h. Thus, room-temperature reaction was considered to be impractical for our purposes. For the analysis of cypermethrin isomer ratios in soil using this

procedure, it was necessary to remove any of the corresponding cyclopropanecarboxylic acids from the extract before starting the analysis. The Florisil chromatography procedure originally reported for crops[12] and used in the preliminary clean-up of soil extracts removed at least 50 μg of the acids from a hexane extract. Typical analyses of cypermethrin-treated soil are shown in Fig. 3.

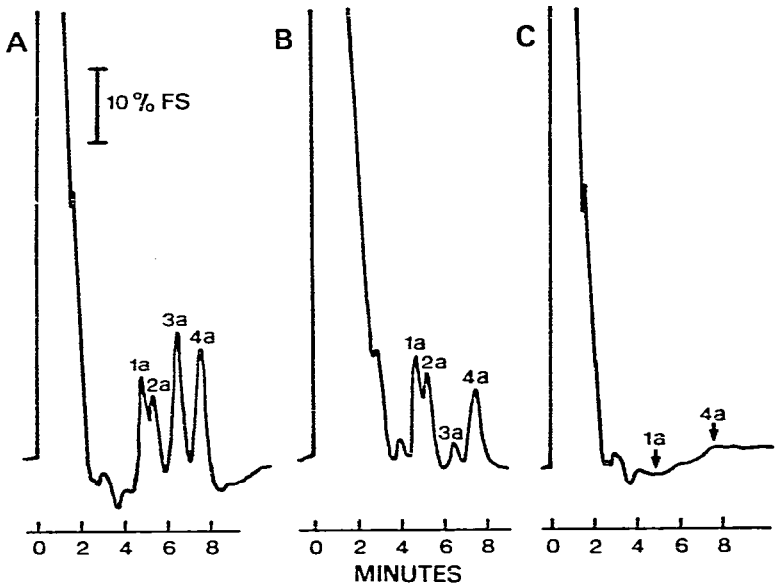


Fig. 3. Gas chromatograms of *l*-menthyl esters derived from cypermethrin residues in soil by the three-step procedure. A, Immediately after treatment (10 ng of cypermethrin; 1.5 mg of soil); B, after 6 weeks (6.4 ng of cypermethrin, 3.0 mg of soil); C, control (1.5 mg of soil).

Murano[9] investigated the GLC separation of diastereoisomeric esters of chrysanthemic acid and concluded that QF-1 gave the best separation. The *l*-menthyl (1*R*) and (1*S*)*cis*-chrysanthemates could not be separated in this study, and the optically active 2-octyl esters were used in subsequent work. The *l*-menthyl 2,2-dimethyl-3-(2,2-dichlorovinyl)cyclopropanecarboxylates were sufficiently well separated on 60-cm columns containing 2.5% of OV-210 (or QF-1) to permit satisfactory analysis for our purposes. Other esters or GLC conditions were not examined.

CONCLUSION

The procedures described provide a method of analysis for the C-1 enantiomers in residual amounts of permethrin and cypermethrin; this method has been used successfully in our laboratory over the past 2 years to measure the C-1 enantiomer ratio in residues as low as 0.05 ppm in soil. No attempt was made to use the procedure for fenvalerate, as the constituent 2-(4-chlorophenyl)-3-methylbutyric acids should racemize readily under the conditions used. Further investigation of the separation of chiral pyrethroid isomers is in progress.

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