Journal of Chromatography, 356 (1986) 123–134
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 384

SEPARATION OF PYRETHROID ENANTIOMERS BY CHIRAL HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

GEORGE R. CAYLEY* and BRUCE W. SIMPSON*
Rothamsted Experimental Station, Harpenden, Hertfordshire AL5 2JQ (U.K.)
(Received December 2nd, 1985)

SUMMARY

The separation of enantiomers of pyrethroid insecticides has been systematically studied using a commercially available Pirkle type 1-A chiral-phase high-performance liquid chromatography column. Useful resolution was obtained for compounds with a variety of acid and alcohol moieties, and containing one to four chiral centres. The chromatographic behaviour of the diastereomers of some of these insecticides on a cyano-bonded column was also examined.

INTRODUCTION

In recent years there has been a rapid increase in the number of chiral pesticides reaching the market place. In many of these the individual enantiomers have widely differing biological activity[1,2] and in the future it is likely that there will be legislative requirements for the analysis of individual enantiomers during the registration of formulations, testing for label compliance and in the collection of residue data for setting maximum residue limits. For a full understanding of structure–activity relationships there is a need to examine individual enantiomers and to be confident of their purity, and similarly for degradation studies it is essential to be able to monitor the active isomers. Where compounds contain several chiral centres differences in synthetic methods can affect the isomer ratio[3]. It is therefore essential to determine the amount of each isomer present before embarking on trials to evaluate the biological performance of different formulations. For all of these reasons chiral analysis is likely to acquire increasing importance, especially in a field as stereochemically complex as the pyrethroids.

Currently pyrethroids are generally analysed by gas–liquid chromatography (GLC) or high-performance liquid chromatography (HPLC). Using packed GLC columns chromatographic conditions are even chosen to avoid isomer separation to enable easy determination of total pyrethroids, *e.g.* in spray deposits on plants[4], although some compounds may be separated into diastereomeric pairs[3]. Changes in

* Present address: Department of Primary Industries, Agricultural Chemistry Branch, Indooroopilly, Queensland, 4068, Australia.

0021-9673/86/$03.50

column stationary phases can enable separation of geometrical isomers[5] but this approach is not always successful even when using capillary columns[6]. In contrast diastereomeric pairs are often easily separated by normal-phase HPLC on silica[7] or cyano-bonded columns[8] and may be partially resolved on amine columns[9]. Under reversed-phase conditions on octadecyl columns generally poorer separations were obtained[10].

Attempts have been made to separate individual enantiomers of fenvalerate by GLC following derivatization with (1*R*,2*S*,5*R*)-(−)-menthol[11] but epimerisation may occur during this procedure. Direct analysis of isomers by chiral-phase HPLC offers more promise. Okamoto *et al.*[12] have resolved the four isomers of phenothrin on a chiral polymer column whilst Chapman[9] and Papadopoulou-Mourkidou[13] have separated isomers of pyrethroids based on α-cyano-3-phenoxybenzyl alcohol using Pirkle type 1-A columns[14]. More recently Doi *et al.*[5] have obtained separations for pyrethroids with no α-cyano group in the alcohol moiety using Pirkle type columns.

We have made a further and more systematic study of the separation of pyrethroid isomers by chiral-phase HPLC and in this paper we report some of the results obtained for the separation of pyrethroids with different combinations of chiral centres and a range of alcohol and acid moieties.

MATERIALS AND METHODS

Covalently bonded Pirkle type 1-A stationary phase was prepared by the method of Pirkle and Finn[15] and a "reverse Pirkle" ionic phase was prepared by the method of Pirkle *et al.*[15] but starting with *S*- rather than *R*-phenylglycine. Other covalently bonded stationary phases such as the N-α-methylbenzyl-N′-propylurea were synthesized but although showing promise for the analysis of other compounds were not ideal for pyrethroid analyses and so most effort was concentrated on the Pirkle columns. A Regis Pirkle type 1-A ionic column and Spherisorb 5 μm CN packing were obtained from Phase Separations (Deeside, U.K.). 250 × 4.6 mm I.D. columns were slurry packed at 7000 p.s.i. and connected to an LDC Analyst liquid chromatograph with a variable wavelength UV detector operated at 240 or 280 nm. Distol hexane and A.R. propan-2-ol (Fisons, Loughborough, U.K.) were used in varying ratios (see the figures and Results for details) at flow-rates of 1–2 ml min^{-1}.

α-Cyano-3-phenoxybenzyl 3-(1,2-dibromo-2,2-dichloroethyl)-2,2-dimethylcyclopropanecarboxylate as a 16-isomer mixture was prepared by the method of Ackerman *et al.*[16]. Other pyrethroids (Table I and Fig. 1) were kindly provided by I.C.I. Plant Protection, Roussel-Uclaf, Shell Research, Sumitomo and Wellcome Pesticides. All other chemicals were purchased from Aldrich (Gillingham, U.K.).

RESULTS AND DISCUSSION

The classification of pyrethroid structures using the Cahn-Ingold-Prelog nomenclature[17] is complicated and can be confusing to the non-expert as biologically active compounds with essentially stereochemically identical configuration, but with different side chain substituents, can have an inversion in designation at the C-3 position in the cyclopropane ring. In this paper we have adopted the nomenclature suggested by Elliott *et al.*[18] in which the CIP designation is used for Cl combined

TABLE I

PYRETHROIDS USED

Isomer type	*Common name*	*Chemical name*	*Isomers*	*No.*
I	Fenpropathrin	(*RS*)-α-Cyano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate	(α*RS*)	2
II	Resmethrin	5-Benzyl-3-furylmethyl (1*RS*)-*cis,trans*-2,2-dimethyl-3-(methylprop-1-enyl)-cyclopropanecarboxylate	(1*RS*)*cis,trans*	4
	Bioresmethrin	5-Benzyl-3-furylmethyl (1*R*)-*trans*-2,2-dimethyl-3-(methylprop-1-enyl)-cyclopropanecarboxylate	(1*R*)*trans*	1
	Permethrin	3-Phenoxybenzyl (1*RS*)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylate	(1*RS*)*cis,trans*	4
	Phenothrin	3-Phenoxybenzyl (1*RS*)-*cis,trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate	(1*RS*)*cis,trans*	4
III	Fenvalerate	(*RS*)-α-Cyano-3-phenoxybenzyl (*RS*)-2-(4-chlorophenyl)-3-methylbutyrate	(*RS*)(*RS*)	4
	Fluvalinate	(*RS*)-α-Cyano-3-phenoxybenzyl *N*-(2-chloro-α,α,α-trifluoro-*p*-tolyl)-D-valinate	(*RS*)	2
IV	Allethrin	(*RS*)-3-Allyl-2-methyl-4-oxocyclopent-2-enyl (1*RS*)-*cis,trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate	(*RS*)(1*RS*)*cis,trans*	8
	Bioallethrin	(*RS*)-3-Allyl-2-methyl-4-oxocyclopent-2-enyl (1*R*)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate	(*RS*)(1*R*)*trans*	2
	Cypermethrin	(*RS*)-α-Cyano-3-phenoxybenzyl (1*RS*)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	(*RS*)(1*RS*)*cis,trans*	8
	Fastac® WL 85871	(*R*)(1*S*)*cis* + (*S*)(1*R*)*cis* isomers of α-cyano-3-phenoxybenzyl 3-(2,2-dichlorovinyl)-1,1-dimethyl-cyclopropanecarboxylate	(*R*)(1*S*)*cis* + (*S*)(1*R*)*cis*	2
	Karate® PP 321	(*R*)(1*S*)*cis* + (*S*)(1*R*)*cis* isomers of α-cyano-3-phenoxybenzyl (*Z*)-3-(2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethylcyclopropanecarboxylate	(*R*)(1*S*)*cis* + (*S*)(1*R*)*cis*	2
	Deltamethrin	(*S*)-α-Cyano-3-phenoxybenzyl (1*R*)-*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate	(*S*)(1*R*)*cis*	1
V		(*RS*)-α-Cyano-3-phenoxybenzyl (1*RS*)-*cis,trans*-3-[1(*RS*),2-dibromo-2,2-dichloroethyl]-2,2-dimethylcyclopropanecarboxylate	(*RS*)(1*RS*)*cis,trans*(1′*RS*)	16

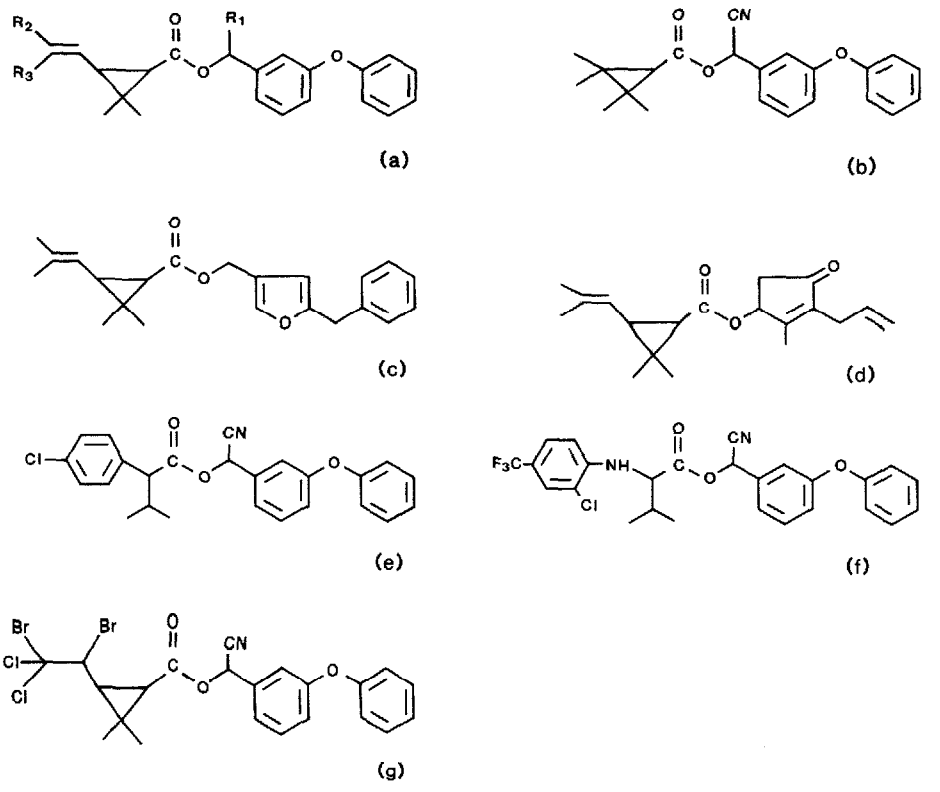

Fig. 1. Pyrethroid structures. (a) $R_1 = H$, $R_2 = R_3 = Cl$ permethrin; $R_1 = CN$, $R_2 = R_3 = Cl$ cypermethrin; $R_1 = CN$, $R_2 = R_3 = Br$ deltamethrin; $R_1 = CN$, $R_2 = CF_3$, $R_3 = Cl$ cyhalothrin; (b) fenpropathrin; (c) resmethrin; (d) allethrin; (e) fenvalerate; (f) fluvalinate; (g) α-cyano-3-phenoxybenzyl-3-(1,2-dibromo-2,2-dichloroethyl)-2,2,dimethylcyclopropanecarboxylate.

with a designation of the relative stereochemistry (*cis* and *trans*) about the cyclopropane ring. Thus permethrin is described as having (1*RS*)-*cis* and (1*RS*)-*trans* isomers. In addition, a scheme of classification of isomer types proposed by Janes and Waterhouse (personal communication) and based on the number and position of the chiral centres is used (see Table II).

Pyrethroid isomers are often readily separated into pairs of enantiomers under normal-phase HPLC conditions[7,8]. Although silica columns have normally been used for this purpose the rapid equilibration of nitrile columns with the mobile phase, making them more suitable for gradient elution, and the high reproducibility of these columns makes them more suitable for quantitative pyrethroid analysis. Typical examples of the separation for fenvalerate and cypermethrin are shown in Fig. 2 using mobile phases of 1.0% and 0.25% diethyl ether in hexane, respectively. Separation for other pyrethroids can readily be achieved using small changes in the mobile phase composition or by using different polar modifiers such as propan-2-ol. The separation of diastereomers of cypermethrin on these columns is superior to that achieved on amine columns[9].

For the separation of pyrethroid enantiomers a chiral phase is necessary and the Pirkle type 1-A ionic column has proved particularly useful as can be seen from

TABLE II

CLASSIFICATION OF PYRETHROIDS

Isomer type	*Number of chiral centres*		*Total number of isomers*
	Alcohol	*Acid*	
I	1	–	2
II	–	2	4
III	1	1	4
IV	1	2	8
V	1	3	16

the following examples. The separations have not always been optimised and resolution may be improved by slight changes in the mobile phase composition, by controlling mobile phase and column temperature or by combining two columns in series.

The isomer type I compound fenpropathrin was almost completely resolved into its two isomers using 0.1% propan-2-ol in hexane as the mobile phase (Fig. 3) but reducing the propan-2-ol concentration to 0.025% did not improve resolution. For the isomer type II compounds investigated baseline resolution between the *cis* and *trans* pairs was readily obtained with the *cis* pair being eluted before the *trans*. For resmethrin (Fig. 4a) using a mobile phase of 0.05% propan-2-ol in hexane the geometrical isomers were well separated but the enantiomeric pairs less distinctly.

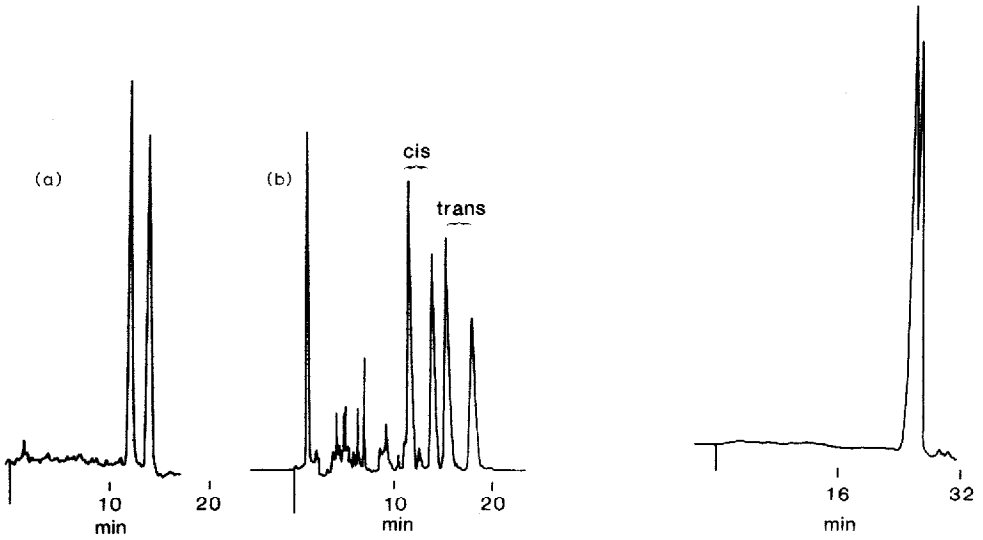

Fig. 2. Resolution of geometrical isomers of fenvalerate (a) and cypermethrin (b) on a nitrile column using mobile phases of 1% and 0.25% diethyl ether in hexane and flow-rates of 2.5 ml min^{-1} and 2 ml min^{-1}, respectively.

Fig. 3. Separation of fenpropathrin isomers using 0.1% propan-2-ol in hexane, flow-rate = 1 ml min^{-1}.

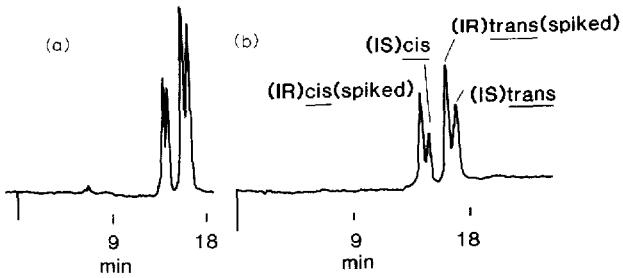

Fig. 4. (a) Separation of resmethrin isomers using 0.05% propan-2-ol in hexane, flow-rate = 1 ml min^{-1}. (b) Resmethrin spiked with bioresmethrin under the same conditions.

Addition of cismethrin[(1*R*)*cis*, NRDC 119] and bioresmethrin [(1*R*)*trans*, NRDC 107] to samples of resmethrin enabled the assignment of the four isomer peaks as in Fig. 4b. Under the conditions reported by Chapman[9] permethrin was only separated into its *cis–trans* isomers but by reducing the propan-2-ol concentration to 0.025% all four isomers were distinguished (Fig. 5a). Under these conditions it was still easy to differentiate between different formulations of permethrin and Fig. 5b shows a sample where there were approximately equal amounts of the *cis–trans* isomers compared to the 30:70 ratio in Fig. 5a. Adding (1*R*)*trans* permethrin (NRDC 147) to the four isomer mixture (Fig. 5c) and the use of a sample with a high *trans* content enabled the assignment of all components as in Fig. 5a. Replacing the chlorine atoms in the vinyl side chain with methyl groups, as in phenothrin, decreased the separation between the geometrical isomers (Fig. 6a) but had little effect on the enantiomer separation. Co-chromatography with *d*-phenothrin showed that the (1*R*) isomers

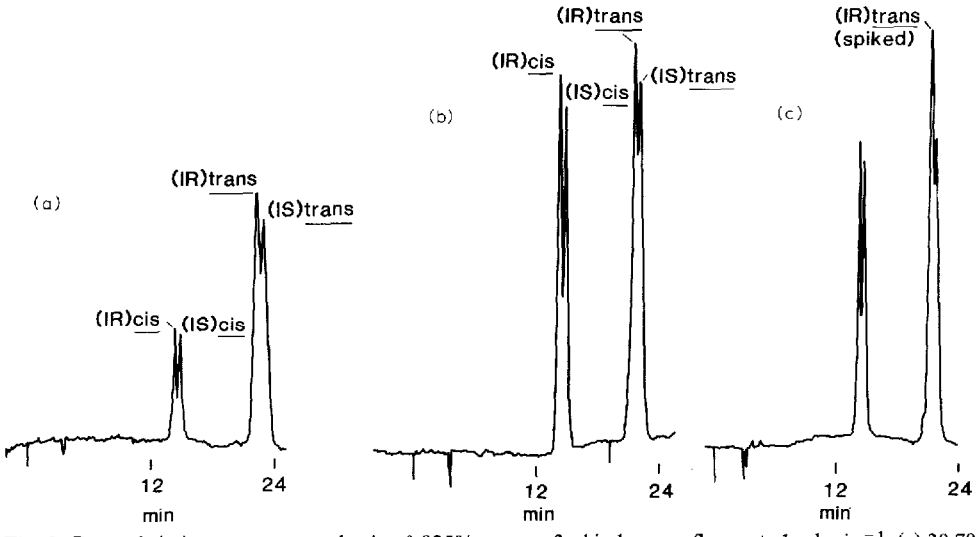

Fig. 5. Permethrin isomers separated using 0.025% propen-2-ol in hexane, flow-rate 1 ml min^{-1}. (a) 30:70 *cis/trans* ratio mixture; (b) 45:55 *cis/trans* mixture; (c) sample spiked with (1*R*)*trans* permethrin.

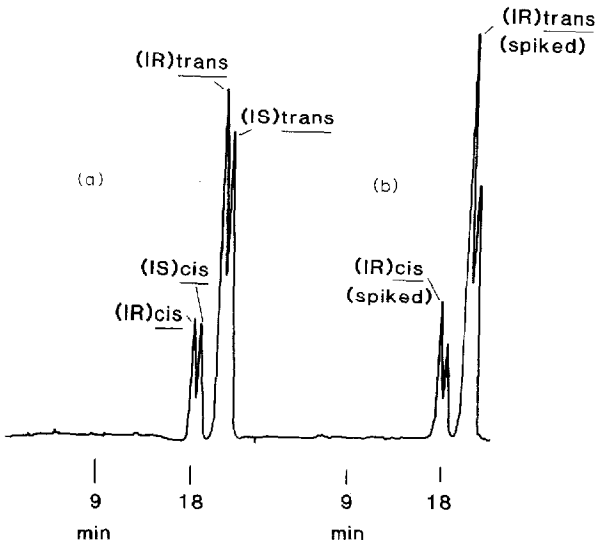

Fig. 6. (a) Separation of phenothrin isomers with 0.025% propan-2-ol, flow-rate 1 ml min^{-1}. (b) The same sample spiked with *d*-phenothrin.

were eluted before the (1*S*) (Fig. 6b) thus giving the same elution order for all the isomer-type II pyrethroids investigated. This gives a particularly useful technique to distinguish between phenothrin and *d*-phenothrin which are often confused in biological trials. Even some commercially supplied standards were found to be incorrectly labelled.

The isomer type III compound fenvalerate has previously been partially resolved[9] on an ionic Pirkle type 1-A column and in Fig. 7 we show an improved separation of the isomers using 0.1% propan-2-ol in hexane as the mobile phase. This separation was comparable to that achieved by Papadopoulou-Mourkidou[13] using a Bakerbond covalently bound chiral column.

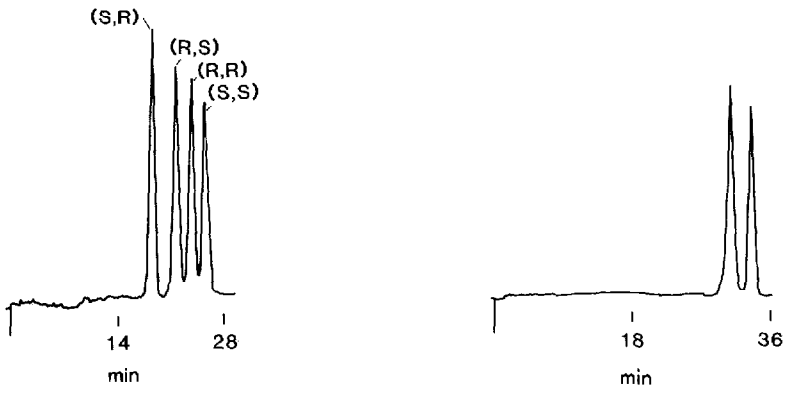

Fig. 7. Separation of fenvalerate isomers using 0.1% propan-2-ol in hexane, flow-rate 2 ml min^{-1}. Assignments according to Chapman[9].

Fig. 8. Separation of fluvalinate HR using 0.1% propan-2-ol in hexane, flow-rate 2 ml min^{-1}.

A second isomer type III compound, fluvalinate, is normally sold as the two isomer mixture synthesized from the resolved acid based on *d*-valine and the racemic α-cyano-3-phenoxybenzyl alcohol giving a diastereomeric pair. The diastereomers were well resolved using a mobile phase of 0.1% propan-2-ol in hexane (Fig. 8).

The isomer type IV compound allethrin differs from all the other pyrethroids we have investigated in that it has a chiral alcohol with no α-cyano group. The eight isomer mixture was only partially resolved with a 0.1% propan-2-ol in hexane mobile phase (Fig. 9a) but the more usually available bioallethrin, consisting of the (*S*)(1*R*)*trans* and (*R*)(1*R*)*trans* diastereomeric pair was well resolved (Fig. 9b). The earlier eluting peak of this pair was identified as the (*S*)(1*R*)*trans* isomer by comparison with *S*-bioallethrin standard.

In contrast useful separations can be obtained for other isomer type IV pyrethroids, such as cypermethrin, based on α-cyano-3-phenoxybenzyl alcohol using 0.2% propan-2-ol in hexane as the mobile phase (Fig. 10a). Decreasing the propan-2-ol concentration to 0.1% improved the resolution of the last eluting pair of enantiomers but earlier peaks started to merge (Fig. 10b) necessitating chromatographic separations under both conditions for each sample to obtain maximum information on all eight isomers. WL 85871, the (α*R*)(1*S*)*cis* and (α*S*)(1*R*)*cis* pair of isomers marketed by Shell (as Fastac®), were the fifth and sixth components to be eluted and co-injection with WL 48281 showed the (α*S*)(1*R*) *cis* isomer to be the first of this pair to be eluted (Fig. 10c). This was a reversal of the assignment made by Chapman[9] who assumed that the (α*R*) isomers were always eluted before (α*S*), as was the case with fenpropathrin. He considered that the contribution due to the α-cyano interactions dominated over all others because of his failure to resolve the permethrin enantiomers.

Resolution of the two-isomer mixture PP 321, the cyhalothrin equivalent of WL 85871, was similar to the cypermethrin analogue (Fig. 11) indicating that modifications to the vinyl side chain again have little influence on the chiral separation.

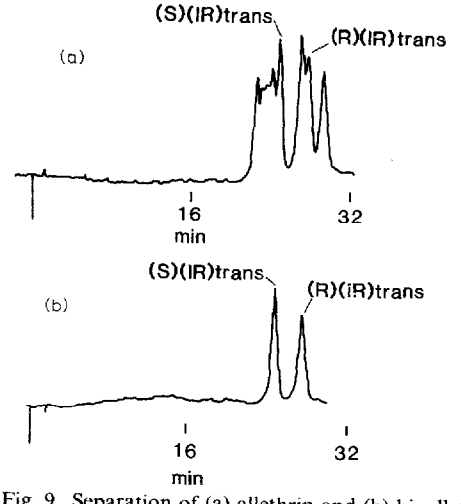

Fig. 9. Separation of (a) allethrin and (b) bioallethrin using 0.1% propan-2-ol in hexane, flow-rate 2 ml min^{-1}.

The dibromovinyl equivalent of cypermethrin is marketed only as the single isomer component (αS)(1R)*cis*, deltamethrin. This was readily separated from its diastereomer (αR)(1R)*cis* in the mixture NRDC 156 using 0.1% propan-2-ol in hexane as mobile phase with the (αR) isomer being eluted first (Fig. 12).

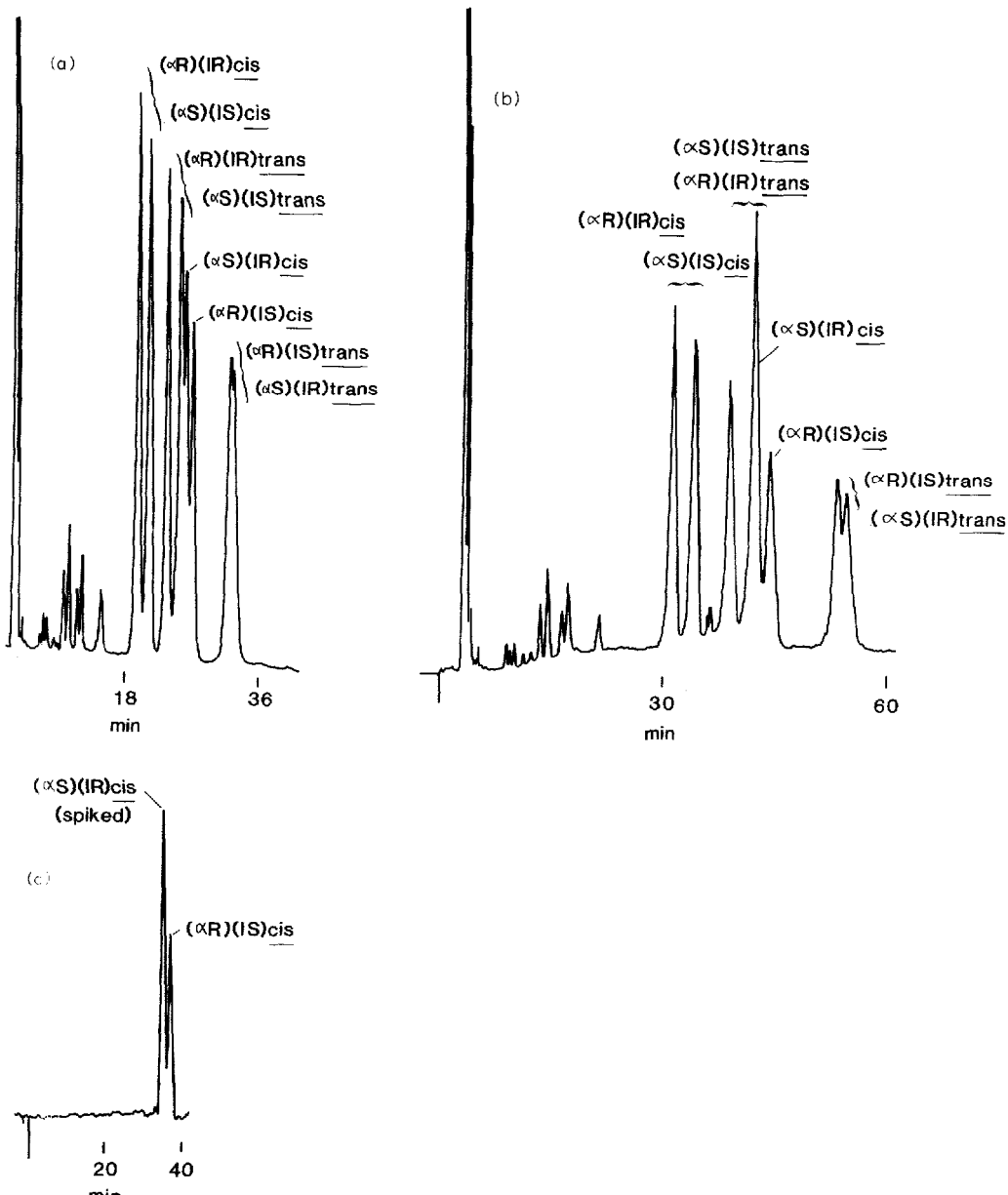

Fig. 10. Separation of cypermethrin using (a) 0.2% propan-2-ol in hexane, flow-rate 1 ml min^{-1}; (b) 0.1% propan-2-ol in hexane, flow-rate 1 ml min^{-1}; (c) WL 85871 spiked with WL 48281 using 0.1% propan-2-ol in hexane, flow-rate 1 ml min^{-1}.

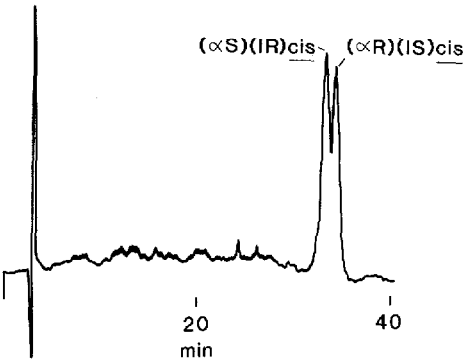

Fig. 11. Separation of cyhalothrin using 0.1% propan-2-ol in hexane, flow-rate 1 ml min^{-1}; assuming elution order the same as for WL 85871.

No isomer type V compounds are marketed as the full sixteen isomer mixture. Tralomethrin, being synthesized directly from deltamethrin has only two isomers. To get more information on an isomer type V compound cypermethrin was brominated to give a full sixteen isomer mixture. The partial resolution of this mixture achieved with a mobile phase of 0.05% propan-2-ol in hexane (Fig. 13) showed the impressive power of the Pirkle column and further modification of the mobile phase may give better resolution.

It is difficult to make predictions concerning the ease of separation of pyrethroids on the Pirkle type 1-A columns. In our experience retention of compounds and resolution of isomers was greater on the ionic than on the covalent column. Diastereomers were more easily separated than enantiomers and in all cases we investigated when the alcohol has no α-cyano group within enantiomeric pairs the (1*R*) isomer was eluted before the (1*S*) isomer. However the empirical contribution governing the elution order for esters of α-cyano-3-phenoxybenzyl alcohol were less predictable and the (1*S*) isomers can be eluted before the corresponding (1*R*) isomer as in WL 85871. In all cases where we have been able to assign the elution order unambiguously *cis* pairs of enantiomers were eluted before the corresponding *trans* pairs.

Similar results were obtained on the Pirkle and "reverse Pirkle" columns (Fig. 14) which may be useful for residue analysis where two columns of opposite chiralty may enable determination of both isomers when a non-chiral compound interferes with the analysis of one isomer.

The results presented in this paper give an indication of the type of separation of pyrethroid isomers that may be obtained on Pirkle type 1-A columns. Because of the sensitivity of the chromatography to small changes in mobile phase composition the quality of solvent may be critical in determining the separations. In other laboratories or on other columns slight modifications of mobile phase composition and of temperature may be necessary to obtain similar separations.

CONCLUSION

Chiral HPLC has been shown to be a useful technique for the analysis of a wide range of pyrethroid insecticides with a range of acid and alcohol moieties. It is

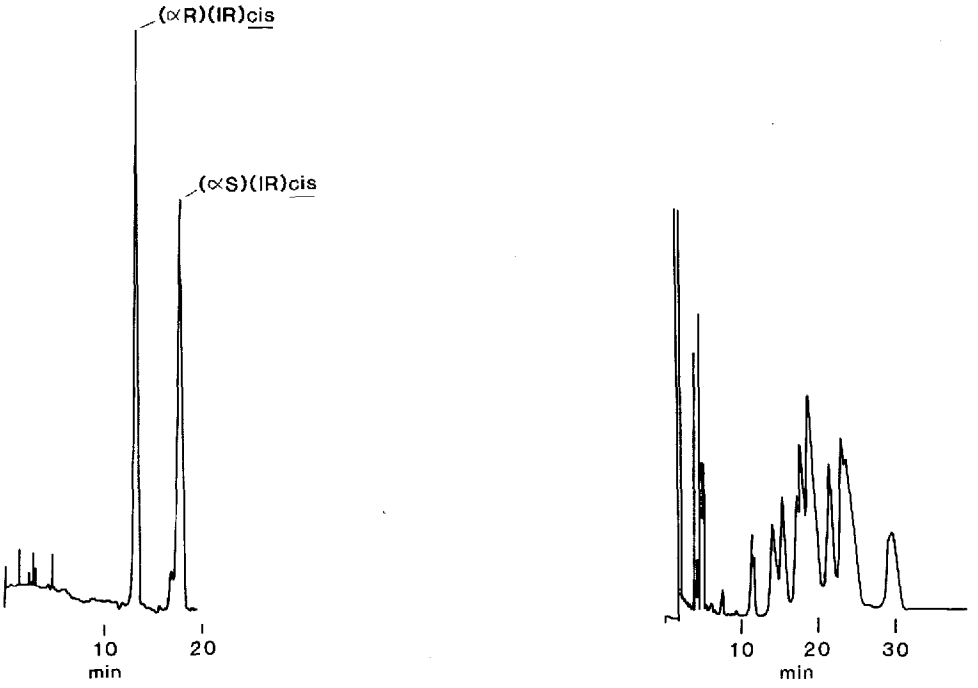

Fig. 12. Separation of NRDC 156 using 0.1% propan-2-ol in hexane, flow-rate 2 ml min^{-1}.

Fig. 13. Attempted separation of the 16 isomers of α-cyano-3-phenoxybenzyl-3-(1,2-dibromo-2,2-dichloroethyl)-2,2-dimethylcyclopropanecarboxylate using 0.05% propan-2-ol, flow-rate 2 ml min^{-1}.

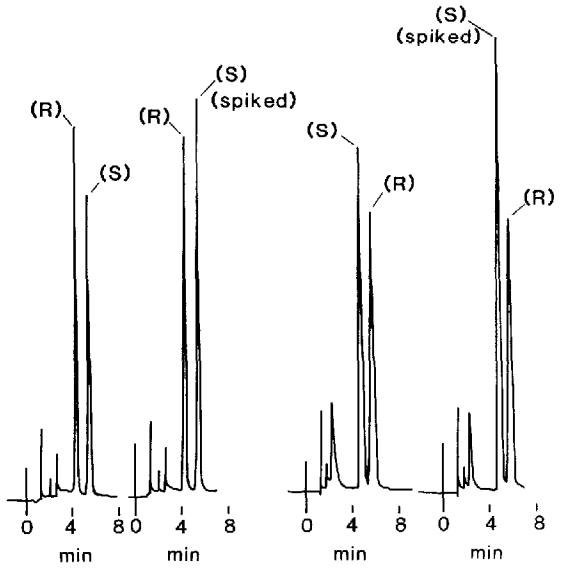

Fig. 14. Separation of 2,2,2-trifluoroanthrylethanol on Pirkle type 1-A columns using 10% propan-2-ol in hexane as mobile phase, flow-rate 2 ml min^{-1}. (a) Normal Pirkle column based on *R*(−)phenylglycine. (b) "Reverse" Pirkle column based on *S*(+)phenylglycine.

a rapid non-destructive technique in which there is little chance of epimerization during the course of analysis and is ideally suited for the analysis of technical preparations, formulations and residues.

ACKNOWLEDGEMENTS

The authors wish to thank Drs. N. F. Janes and B. P. S. Khambay for helpful discussions and the chemical companies (see Materials and Methods) who provided us with the pyrethroid insecticides.

REFERENCES

1 M. Elliott, A. W. Farnham, N. F. Janes, P. H. Needham and B. G. Pearson, *Nature (London)*, 213 (1967) 493–494.
2 M. A. Venis, *Pestic. Sci.*, 13 (1982) 309–317.
3 H. Swaine and M. J. Tandy, in G. Zweig and J. Sherma (Editors), *Analytical Methods for Pesticides and Plant Growth Regulators XIII. Synthetic Pyrethroids and Other Pesticides*, Academic Press, Orlando, FL, 1984, Ch. 6, pp. 103–120.
4 A. J. Arnold, G. R. Cayley, Y. Dunne, P. Etheridge, D. C. Griffiths, F. T. Phillips, B. J. Pye, G. C. Scott and P. R. Vojvodic, *Ann. Appl. Biol.*, 105 (1984) 353–359.
5 T. Doi, S. Sakane and M. Horiba, *J. Assoc. Off. Anal. Chem.*, 68 (1985) 911–916.
6 P. D. Bland, *J. Assoc. Off. Anal. Chem.*, 68 (1985) 592–595.
7 D. Mourot, B. Delépine, J. Boisseau and G. Gayot, *J. Chromatogr.*, 168 (1979) 277–279.
8 A. Sapiets, H. Swaine and M. J. Tandy, in G. Zweig and J. Sherma (Editors), *Analytical Methods for Pesticides and Plant Growth Regulators XIII. Synthetic Pyrethroids and Other Pesticides*, Academic Press, Orlando, FL, 1984, Ch. 2, pp. 33–51.
9 R. A. Chapman, *J. Chromatogr.*, 258 (1983) 175–182.
10 S. Lam and E. Grushka, *J. Chromatogr.*, 154 (1978) 318–320.
11 M. Horiba, H. Kitahara, S. Yamamoto and A. Murano, *Agric. Biol. Chem.*, 44 (1980) 1361–1365.
12 Y. Okamoto, S. Honda, I. Okamoto and H. Yuki, *J. Am. Chem. Soc.*, 103 (1981) 6971–6973.
13 E. Papadopoulou-Mourkidou, *Chromatographia*, 20 (1985) 376–378.
14 W. H. Pirkle, D. W. House and J. M. Finn, *J. Chromatogr.*, 192 (1980) 143–158.
15 W. H. Pirkle and J. M. Finn, *J. Org. Chem.*, 46 (1981) 2935–2938.
16 P. Ackermann, F. Bourgeois and J. Drabek, *Pestic. Sci.*, 11 (1980) 169–179.
17 R. S. Cahn, C. Ingold and V. Prelog, *Angew. Chem. Intern. Ed.*, 5 (1966) 385–415.
18 M. Elliott, N. F. Janes and D. A. Pulman, *J. Chem. Soc. Perkin I*, (1974) 2470–2474.