Journal of Chromatography, 368 (1986) 59–76
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 884

COLUMN SELECTIVITY IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF SUBSTITUTED *gem*-DIMETHYLCYCLOPROPANES*

S. L. ABIDI
U.S. Fish and Wildlife Service, National Fishery Research Laboratory, P.O. Box 65, La Crosse, WI 54602-0065 (U.S.A.)
(First received May 13th, 1986; revised manuscript received June 20th, 1986)

SUMMARY

High-performance liquid chromatographic (HPLC) separation of a series of *gem*-dimethylcyclopropanes was carried out in a variety of mobile phase and stationary phase systems. Comprehensive evaluation of column selectivity was made to identify chromatographic conditions under which various isomers could be resolved. In normal-phase HPLC on silica, retention data appeared to serve as good indicators for predicting relative polarity of the compounds in the solvent systems studied. For most cases, excellent baseline resolution of *cis–trans* mixtures was attained. The *cis* and *trans* components of chrysanthemol (CS) and ethyl chrysanthemate, however, were separated only with mobile phases of hexane and hexane–isopropanol (98:2), respectively. In reversed-phase HPLC, geometrical isomers of all but chrysanthemol were well resolved on octadecylsilica despite the poor selectivity of a propylphenylsilica column for these isomers under conditions used. The latter reversed-phase separation on propylphenylsilica succeeded only in two cases (RU-11679 and permethrin). Elution of *cis* and *trans* isomers in reversed-phase HPLC followed the same order as in normal-phase HPLC, except for the halogenated compounds where a reversal in elution order was observed. Among chiral stationary phases studied, the chiral polymeric packings, (+)-poly(triphenylmethylmethacrylate) exhibited the highest selectivity for compounds containing aromatic groups on ester moieties. The halogen substituents in permethrin and baythroid seemed to have adverse effects on the chiral recognition process. In HPLC on β-cyclodextrin-bonded silica, separations of diastereomeric (+)-*cis*- and (+)-*trans*-allethrin, and *cis*- and *trans*-chrysanthemol demonstrated unique examples of unusual selectivity of β-cyclodextrin-bonded silica for isomers that virtually remained indiscernible by all other HPLC methods investigated. The percentage of β-cyclodextrin in the bonded phase had dramatic influence on the α values of certain isomeric pairs. Increasing the percentage β-cyclodextrin in the bonded phase somewhat favored the separation of some optical antipodes.

* Presented in part at the *190th National Meeting of the American Chemical Society, Chicago, IL, U.S.A., September 8–13, 1985.*

0021-9673/86/$03.50

INTRODUCTION

Natural and synthetic cyclopropanes structurally related to insecticidal pyrethroids are biologically and chemically useful compounds. Pyrethroids insecticides have been well known for the past many decades as effective agricultural and general insecticides. Much interest in these *gem*-dimethylcyclopropane compounds has focused on the synthesis and chemical reactivity for use as potential synthetic intermediates, stereochemistry of the three-membered ring system, and structure–biological activity relationships. Because of the unique structural features associated with the presence of two asymmetric centers on the propane ring, each compound, in principle, should at least comprise four isomers: two enantiomeric pairs derived from two *cis–trans* diastereoisomers. Previous studies[1–3] showed that optical isomers of the insecticidal pyrethroids exhibited various degrees of biological activity. Generally, the (+)-*cis*-(1*R*,3*S*)- and (+)-*trans*-(1*R*,3*R*)-chrysanthemates are more toxic than their corresponding antipodes. Hence, separation of all possible isomers has been a major task in the biological evaluation of synthetic pyrethroids. In practice, pure isomers are preferred to synthetic mixtures for field efficacy studies. Cyclopropane compounds are also known to be chemically reactive and they undergo various reactions often in an unpredictable manner. The use of pure isomers as substrates facilitates elucidation of unknown products and simplifies isolation procedures. In our recent work[4], the availability of pure isomers of chrysanthemyl alcohol and related compounds was invaluable for the spectral characterization of a host of rearrangement products.

Analysis of pyrethroids by gas chromatography (GC) has been routine work in industrial quality control laboratories. In consideration of some reported operational constraints of GC instrumentation and thermal lability of some pyrethroids, the applicability of GC methodology[5–7] to general pyrethroid analysis seems to be confined to non-preparative assays of thermally stable compounds. Because of these obvious limitations with GC methods, we were interested in studying the HPLC separation of representative *gem*-dimethylcyclopropanes (Fig. 1) using several different stationary phases and aimed to expand the HPLC methods especially for preparative application. Numerous HPLC methods for the analysis of some pyrethroids have been reported in the literature[8–20]. In this paper we study the chromatographic behavior of the isomeric compounds under the influence of mobile phase and stationary phase variables and determine the relationship between the side chain structure of the cyclopropane ring system and separation factors (α).

EXPERIMENTAL

Materials

Unless stated otherwise, all compounds used were reagent-grade and solvents were used as supplied from various industrial sources without further purification. Chrysanthemol and ethyl chrysanthemate were obtained from Aldrich (Milwaukee, WI, U.S.A.); allethrin, dimethrin, phenothrin, permethrin, tetramethrin and RU-11679 (cyclopentyl analogue of dimethrin) from McLaughlin, Gormley, and King Co. (MGK) (Minneapolis, MN, U.S.A.); resmethrin from S. B. Penick and Co. (Newark, NJ, U.S.A.); and baythroid from Mobay Chemical Corp. (Kansas City,

MO, U.S.A.). (+)-*trans*-Ethyl chrysanthemate and its *trans*-racemic mixture were prepared from respective acid precursors (the optically active acid was a gift from Professor Poulter of Chemistry Department, University of Utah, Salt Lake City, UT, U.S.A.; the *trans*-racemic acid was a purified product of MGK). Thus, the potassium salt of the acid was treated with silver nitrate in ethanol to give the silver salt which was then reacted with ethyl iodide yielding the desired ethyl ester in moderate yield (60–75%). (+)-*trans*-Chrysanthemol and its *trans*-racemate were obtained by lithium aluminum hydride reduction of the corresponding esters described above. Polarimetric and spectral analysis of samples of these materials showed identical physical properties with those of authentic samples given by Professor Poulter. Alternatively, pure isomers of these and other *gem*-dimethylcyclopropane derivatives were also made available by preparative HPLC developed at the National Fishery Research Laboratory. Some of the geometrical as well as optical isomers of synthetic pyrethroids were also acquired from the suppliers listed above. HPLC solvents, organic chemicals, and inorganic reagents were products of J. T. Baker (Philipsburg, NJ, U.S.A.), Aldrich, and Alpha Products (Danvers, MA, U.S.A.), respectively.

High-performance liquid chromatography

Analytical HPLC was performed with a Varian Model 5020 liquid chromatograph comprising a multiple-wavelength ultraviolet detector (Model 110), a polarimeter (Rudolph Autopol III), a strip chart recorder (Model 9176), and a data computing system (Varian Model 4270). Four different types of stationary phases were employed. These include (i) normal-phase HPLC: silica (IBM, Danbury, CT, U.S.A.); (ii) reversed-phase HPLC; ultrasphere octadecylsilica (Beckmann, Berkeley, CA, U.S.A.) and propylphenylsilica (Analytical International, Harbor City, CA, U.S.A.); (iii) chiral-phase HPLC: Pirkle's chiral-packings [(both ionic and covalent bonded columns (Regis Chemicals, Morton Grove, IL, U.S.A.)] and Chiralpack-(+)-OT packings (Daicel Chemical Industries, Tokyo, Japan); (iv) HPLC on β-cyclodextrin(CD)-bonded silica: column (A), 3.5 mequiv. CD per g silica; column (B), 7.4 mequiv. CD per g silica (all from Advanced Separation Technologies, Whippany, NJ, U.S.A.). The column dimensions for all these packings are 25 cm $\times$ 4.6 mm I.D. (5 μm particle size). In general, mobile phases used for experiments with various stationary phase systems are as follows: (i) for the silica column, hexane and a small amount of more polar organic solvents were used; (ii) for reversed-phase- and CD-bonded silica columns, water and methanol (or acetonitrile) were used; (iii) for Pirkle's columns, hexane and isopropanol (10–20%) were used; (iv) for Chiralpack-OT-(+) column, methanol (5–10°C) was used. The latter Daicel column was equipped with a water jacketed stainless-steel condenser thermostated by means of a constant temperature circulator (FTS Model MC-4-40-2, Stoneridge, NY, U.S.A.). Details of mobile phase conditions are described in the Results and Discussion section for specific experiments under consideration. HPLC data were generated by injecting about 30–50 μg/ml samples into a column via a 10-μl loop (controlled by a Valco CV-6-UHPa-N60 injection valve) and a guard column (5 cm $\times$ 4 mm I.D.) packed with a pellicular material of 40 μm average particle size matching the stationary phase material in the analytical column. The measured capacity factors (k') were corrected for the retention in guard columns. In all analyses, the detector wavelength was set at 254 nm, except that, in some instances, the wavelength for maximum absorption,

λ_{max}, was used for measuring low levels of cyclopropane analytes. Eluents were normally pumped (30–100 atm) through a column at a flow-rate of 1 ml/min and the column temperature was maintained at ambient temperature unless otherwise specified. To avoid possible degradation of samples upon standing, all samples were freshly prepared before analyses and were stored in a freezer when not in use. Capacity factors (k') were calculated according to the general equation: $k' = (t - t_0)/t_0$, where t_0 is the retention time of the unretained solute. The void volume data were determined by injecting samples of sodium nitrate, sodium iodide, hexadecane and water as non-retained solutes in suitable column systems. Retention times were mean values of three replicate determinations.

In preparative HPLC, separations of isomers were carried out with columns (25 cm × 10 mm I.D.) packed with silica-based stationary phases described earlier. Normally, a given stationary phase was selected for separation and isolation of pure isomers of a cyclopropane compound based entirely on the results of analytical HPLC where a highest degree of component separation had been demonstrated. In a typical experiment, a mixture containing four diastereomers of baythroid was efficiently resolved by preparative HPLC on silica with a mobile phase of hexane–[tetrahydrofuran–dichloromethane–isopropanol (1:1:0.1)] (99:1) at a flow-rate of 0.5 ml/min. Aliquots of 1–3 mg were injected into the column via a 100-μl loop and the column effluents were collected into a Buchler linear automatic fraction collector (ISCO Model 328). Fractions of pure isomers were combined. Evaporation of solvents under reduced pressure afforded a sample whose structure was determined by proton and carbon-13 nuclear magnetic resonance spectrometry (NMR). The NMR spectra were recorded on a Jeol FX90-Q Fourier transform NMR spectrometer operating at 90 MHz for protons and at 22.5 MHz for carbons. Further structural confirmation was conducted by comparison with the authentic diastereomers. Isolation of other isomers in the series of the gem-dimethyl cyclopropanes was similarly accomplished by preparative HPLC under the chromatographic conditions provided by analytical HPLC. A refractive index detector (Varian Model 4300) was used in the preparative separation work.

RESULTS AND DISCUSSION

Fig. 1 presents the *trans*-structures of the compounds in the series investigated. These *gem*-dimethylcyclopropanes of interest are allethrin (AL), baythroid (BT), RU-11679 (CP) (cyclopentyl analogue of resmethrin), chrysanthemol (CS), dimethrin (DM), ethyl chrysantemate (EC), permethrin (PM), phenothrin (PN), resmethrin (RM), and tetramethrin (TM). All but CS contain the ester functionality and represent a class of compounds derived from *gem*-dimethylcyclopropane carboxylic acid. Fascinated by the conclusions[21] drawn by Elliott and co-workers on the structural requirements for pyrethroids to have potential biological activities, we were curious to know whether the structural factors play important roles in HPLC separation in a given chromatographic system and to what extent the separation of isomers is affected by the variation of structures. Further, in order to understand stationary phase effects on isomer separation, we chose a number of silica-based columns including a fairly new chiral polymer column, (+)-poly(triphenylmethylmethacrylate) for study. These column packings include silica, octadecylsilica (ODS), propylphen-

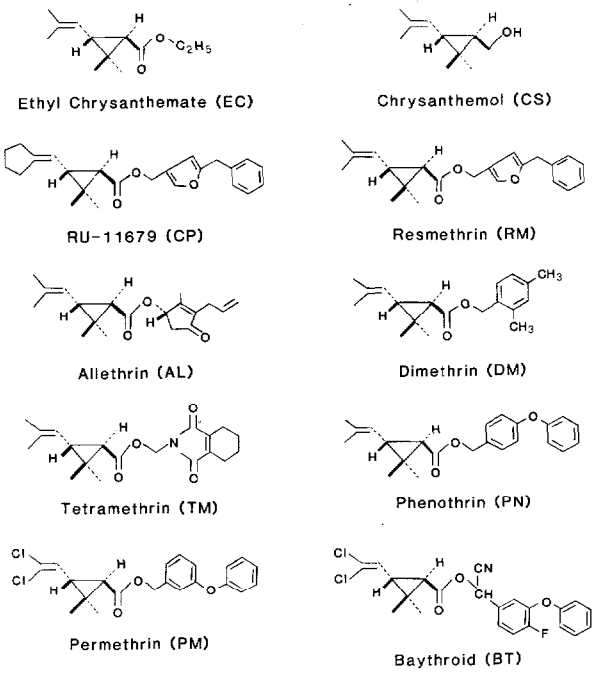

Fig. 1. Structures of *trans* isomers of the ten substituted *gem*-dimethylcyclopropanes investigated.

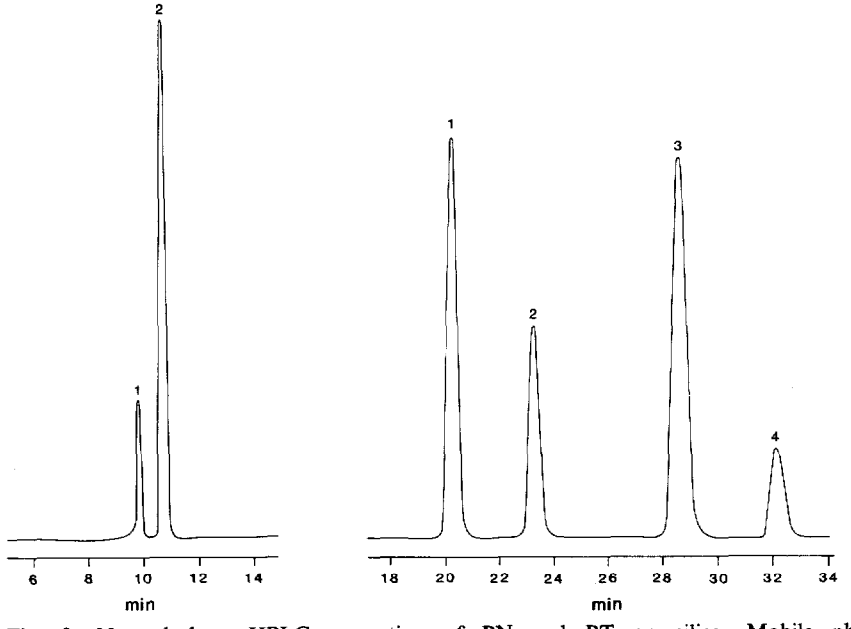

Fig. 2. Normal-phase HPLC separation of PN and BT on silica. Mobile phase: hexane–[(tetrahydrofuran–methylene chloride–isopropanol) (1:1:0.1)] (99:1). Peak identification: left, 1 = *cis*-, and 2 = *trans*-PN; right, 1 = (*R*)-*trans*-, 2 = (*S*)-*cis*-, 3 = (*R*)-*trans*-, and 4 = (*S*)-*cis*-BT. For *R* and *S* designations, see footnote to Table I.

TABLE I

NORMAL-PHASE HPLC

For abbreviations of compounds, see Results and Discussion.

Compound	*Chromatographic characteristics**											
	A			*B*			*C*			*D*		
	k' *(cis)*	*k'* *(trans)*	α	*k'* *(cis)*	*k'* *(trans)*	α	*k'* *(cis)*	*k'* *(trans)*	α	*k'* *(cis)*	*k'* *(trans)*	α
AL	2.43	3.04	1.25	26.7	41.0	1.54	19.7	29.0	1.47	12.6	16.3	1.29
BT**	2.73	2.92	1.07	38.8(*R*)	32.9(*S*)	1.18	22.3(*R*)	19.2(*S*)	1.16	9.55(*R*)	8.68(*S*)	1.10
				57.6(*R*)	50.1(*S*)	1.15	31.2(*R*)	27.5(*S*)	1.13	12.9(*R*)	11.6(*S*)	1.11
CP	2.80	2.80	1.00	15.2	17.8	1.17	10.0	11.3	1.13	8.26	8.61	1.04
CS***	1.86	1.86	1.00	2.51	2.51	1.00	2.22	2.22	1.00	2.10	2.10	1.00
DM	2.21	2.21	1.00	7.89	8.82	1.12	6.06	6.94	1.15	3.87	4.04	1.04
EC	1.83	2.06	1.13	2.42	2.42	1.00	2.31	2.31	1.00	2.21	2.21	1.00
PM	2.40	2.40	1.00	9.3	14.7	1.58	6.92	10.5	1.52	4.86	6.68	1.37
PN	2.34	2.34	1.00	11.7	13.3	1.14	8.80	9.66	1.10	5.90	6.30	1.07
RM	2.50	2.63	1.05	13.3	14.9	1.12	9.61	10.7	1.11	6.56	6.95	1.06
TM	3.90	4.15	1.06	–	–	–	–	–	–	–	–	–

* Mobile phase conditions: A, hexane–isopropanol (98:2); B, hexane–tetrahydrofuran (99:1); C, hexane–(tetrahydrofuran–methylene chloride–isopropanol [1:1:0.1]) (99:1); D, hexane–(methylene chloride–isopropanol [93:7]) (99:1); flow-rate, 1 ml/min.

** Of four diastereoisomers of BT, the cyanomethyl carbons of the earlier and later eluting *cis–trans* pairs have "*R*" and "*S*" configuration, respectively.

*** The *cis*- and *trans*-CS were resolved with a mobile phase of hexane: $k'_{trans} = 2.60, k'_{cis} = 2.91, \alpha = 1.12$.

ylsilica (PPS), Pirkle's amino acid-bonded silica (PAS)[22], Chiralpack-(+)-OT (CPOT)[23], and β-cyclodextrin (CD)-bonded silica (CDS)[24–26]. Since the molecular polarity and hydrophobicity of geometrical and diastereomers are functions of structures and depend largely on the spacial arrangement of groups around the *gem*-dimethylcyclopropane skeleton, separation of isomers by adsorption and by partition should be a plausible approach to study isomerism of these compounds with silica and ODS (or PPS) stationary phases. For separation of optical isomers, few studies have thus far been done on direct HPLC resolution of racemates of title compounds except for the widely adopted indirect methods in which auxiliary asymmetric centers are commonly introduced to the racemic mixtures to form diastereomers. The latter compounds can then be separated by repetitive fractional recrystallizations or by GC and HPLC on achiral stationary phases (or mobile phases). We chose to examine two types (PAS and CPOT) of chiral stationary phases for resolution of enantiomeric cyclopropane compounds using a direct HPLC method. If the molecular dissymmetry of a racemate can be differentiated by chiral recognition with these optically active phases in the chromatographic system, the enantiomeric solutes should be resolvable. With the recent advent of cyclodextrin methodology in chromatography, there has been a considerable success in HPLC of numerous isomeric compounds (geometrical isomers[25,26], enantiomers[27], diastereomers[28], and homologues[29]) on CDS. Application of this technique to our present study should be appropriate in view of the various modes of isomerization inherent with the structure of each cyclopropane compound under study. It was of interest to compare the results obtained with these stationary phases.

Normal-phase HPLC

The two chromatograms given in Fig. 2 represent typical normal-phase separation of *cis* and *trans* compounds on silica. Under conditions employed, the two sets of diastereomeric *cis–trans* pairs of BT were separated with great ease. As illustrated, resolution of isomeric pairs in the normal-phase system was highly efficient and in most cases excellent base-line separations were achieved. Table I compiles the capacity factors, k', and column selectivity (α) values of the ten compounds in the series. The data were obtained under four different mobile phase conditions. The results demonstrate that the magnitude of k' values appeared to serve as an indicator for predicting the relative polarity of a *gem*-dimethylcyclopropane compound and that the *trans* isomer of the isomeric pair tended to be more polar than the *cis* counterpart because of the higher k' value of the former. Under identical chromatographic conditions, when compared with PN (the 4-phenoxy and isobutenyl analogue of PM), the dichlorovinyl-substituted cyclopropane, PM, exhibited higher degrees of differential interactions (higher α values, Table I) with silica during the adsorption process. Evidently the presence of halogens (Cl and F) in both PM and BT as well as that of an allylic cyclopentenone in AL were advantageous for enhancing dipolar interactions. Correlation of the selectivity data with structures indicates that substituents other than hydrogen (as in CS) on the alcohol parts of *gem*-dimethylcyclopropane carboxylic acid ester are essential structural prerequisites for achieving adequate separation of geometrical isomers by normal-phase HPLC. It was not easy to resolve the *cis* and *trans* components of the lower-molecular-weight compounds, EC and CS. Under all HPLC conditions studied (Table I), *cis*- and *trans*-EC were separated on

silica in one instance only. In an isolate experiment, a *cis–trans* mixture of CS was resolved using 100% hexane as a mobile phase (see footnote to Table I). Although the separation of the isomeric mixture of CS met with some difficulties under the conditions chosen for studying the whole series of compounds, were were able to resolve it by GC using Carbowax 20M (5%) on Supelcoport (100–120 mesh) at 100°C. From the results in Table I, it is clear that the effect of mobile phase solvent strength on column selectivity is generally consistent with the trend of a decrease in separation factors with increasing solvent strength as commonly observed in adsorption chromatography. In the case of EC, this trend of the mobile phase effect on selectivity factors obviously does not apply. The inadequacy in solvent strength of three of the four mobile phase systems used (Table I, mobile phases B, C, and D) was apparent in the isomeric separation of TM, which failed to be eluted through the column.

Reversed-phase HPLC

Table II summarizes reversed-phase HPLC data to illustrate the mobile phase and stationary phase effects on the separation of cis-trans geometrical isomers. Much like the situation in normal-phase HPLC of the same series of compounds, excellent baseline resolution (Fig. 3) of *cis–trans* pairs was attained in most cases, though the α values were between 1.00 and 1.50 and never exceeded 2.00 under the conditions stated in the table. The *cis*- and *trans*-CS could not be resolved ($\alpha = 1.00$) under all the reversed-phase conditions tested. It is noteworthy that the *cis*- and *trans*-TM were adequately separated by reversed-phase HPLC despite our unsuccessful attempts to obtain reasonable separation by using normal-phase HPLC (Table I *vs.*

TABLE II

REVERSED-PHASE HPLC

Compound	*Chromatographic characteristics**								
	A			*B*			*C*		
	k' *(cis)*	*k'* *(trans)*	α	*k'* *(cis)*	*k'* *(trans)*	α	*k'* *(cis)*	*k'* *(trans)*	α
AL	4.68	5.32	1.14	7.11	7.65	1.08	4.08	4.08	1.00
BT**	7.41(*S*)	7.77(*R*)	1.05	10.4(*S*)	10.5(*R*)	1.01	9.72(*S*)	9.72(*R*)	1.00
	8.01(*S*)	8.73(*R*)	1.09	11.4(*S*)	11.8(*R*)	1.04	9.96(*S*)	9.96(*R*)	1.00
CP	3.6	26.1	1.11	29.1	30.9	1.06	10.2	11.7	1.15
CS	0.82	0.82	1.00	0.90	0.90	1.00	1.28	1.28	1.00
DM	15.1	16.9	1.12	19.6	21.2	1.08	7.08	7.08	1.00
EC	3.54	3.86	1.09	5.85	6.15	1.11	3.64	3.64	1.00
PM	19.5	14.9	1.31	27.9	23.1	1.21	10.9	10.2	1.07
PN	18.0	20.3	1.13	24.0	25.9	1.08	9.96	9.96	1.00
RM	13.5	15.0	1.11	17.4	18.6	1.07	8.12	8.12	1.00
TM	4.05	4.68	1.16	6.40	6.95	1.09	5.08	5.08	1.00

* Flow-rate was at 1 ml/min. ODS = Octadecylsilica, PPS = propylphenylsilica. (A) stationary phase, ODS; mobile phase, methanol–water (80:20); (B) stationary phase, ODS; mobile phase, acetonitrile–water (70:30); (C) stationary phase, PPS; mobile phase, acetonitrile–water (60:40).

** For *R* and *S* designations, see footnote to Table I.

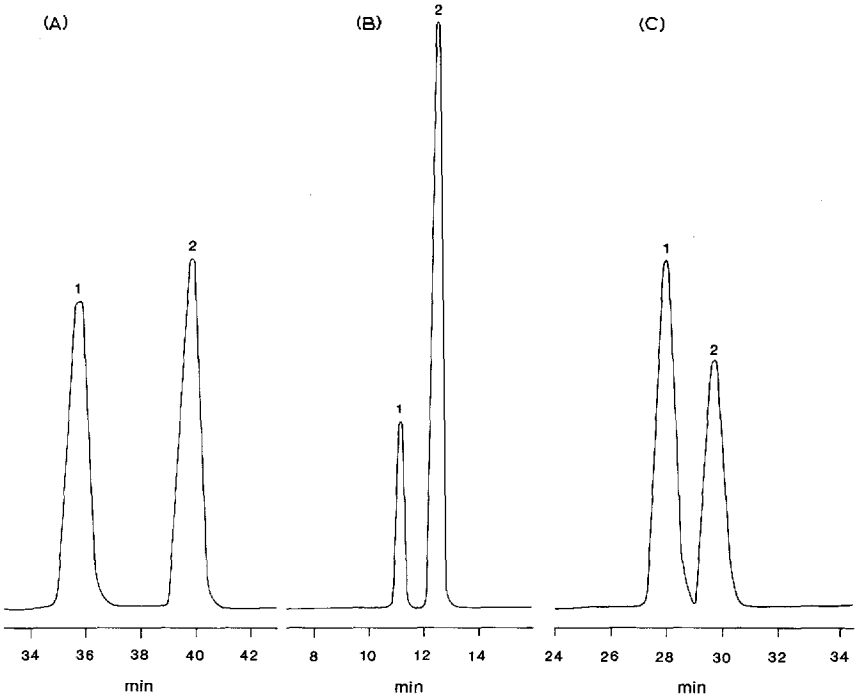

Fig. 3. Reversed-phase HPLC separation of DM, TM, and PM. (A) column: ODS; mobile phase: methanol–water (80:20); peaks: 1 = *cis*-, and 2 = *trans*-DM. (B) column: ODS; mobile phase: methanol–water (80:20); peaks: 1 = *cis*-, and 2 = *trans*-TM. (C) column: PPS; mobile phase: acetonitrile–water (60:40); peaks: 1 = *trans*-, and 2 = *cis*-PM.

Table II). The separation mechanism by hydrophobic interactions between the *gem*-dimethylcyclopropane solutes and ODS (or PPS) was best manifested in the separation of CP. Of the compounds listed in Table II, CP was most strongly retained by the reversed-phase stationary phase owing to the highest carbon content of its molecular structure. With the exception of PM, the *cis* isomers of nine other compounds in the series showed higher tendency than the *trans* counterparts to be eluted first. Comparison of elution data for normal- (Table I) and reversed-phase (Table II) HPLC revealed an unusual parallelism in the elution order of *cis–trans* pairs. We speculate that the molecular hydrophobicity in the *gem*-dimethylcyclopropane system (excluding BT and PM, the halogenated compounds) may not be correlated with molecular polarity conventionally used for predicting elution order in normal-phase adsorption chromatography. In order to explore if π–π interactions of phenyl groups contribute significantly to the solvophobic partition process, we evaluated the resolution capability of PPS (Table II) along with the ODS phase. The results in Table II show no evidence of such interactions. Of all the experiments executed under conditions indicated (Table II, see data for PPS), there were only two cases (CP and PM) that demonstrated adequate resolution of the geometrical isomers on the PPS column. An example of the reversed-phase separation of *cis*- and *trans*-PM on PPS is shown in Fig. 3C. Frequently it was desired to achieve a comparable degree of component resolution in HPLC on different stationary phases, the mobile phase for

this PPS stationary phase required a relatively lower percentage of the organic modifier than that in the ODS system in accord with the results of our previous studies[30] with a PPS column. By comparing the structures of PM and PN, it is clearly demonstrated that modifications of the side chain structures (isobutenyl *vs.* dichlorovinyl groups and 4-phenoxy *vs.* 3-phenoxy groups between the structures of PN and PM) had dramatic effects on the differential hydrophobic interactions in the chromatographic process. In relation to PN, generally higher α values were obtained for PM (Table II, $\alpha_{PM} > \alpha_{PN}$: 1.31 > 1.11; 1.21 > 1.08; 1.07 > 1.00).

Chiral-phase HPLC

In the earlier stage of this work, all attempts at resolving optical isomers of the ten *gem*-dimethylcyclopropanes on Pirkle's PAS columns were unsuccessful. In light of the chiral recognition requirements for specific interactions between the enantiomeric analytes and the PAS stationary phases, the structures of these compounds appeared to lack some of the interacting sites (π-donor, hydrogen bond donor, and hydrogen bond acceptor)[31] necessary to meet the requirements for optical resolution. Subsequently, we found that HPLC of the same series of compounds on Daicel's Chiralpack-(+)-OT (CPOT) column led to various degrees of optical resolution. Fig. 4 illustrates the separation of optical antipodes of *cis*- and *trans*-RM. Although the optical isomers of individual geometrical isomers were efficiently resolved (Fig. 4A and B), one [(+)-*cis*- and (+)-*trans*-RM)] of the *cis–trans* pairs was not resolved (Fig. 4C). The column selectivity (α) data for the series of compounds are summarized alongside their retention (k') data in Table III. The results show that the α values for resolution of enantiomers in the *trans* and *cis* series fall approximately in the order RM > PN > DM $\approx$ TM > PM, and RM > PN > PM $\approx$ TM, respectively. Since the optically active constituents of the CPOT stationary phase was (+)-poly(triphenylmethyl methacrylate)[23], chiral separation of enantiomers of subject compounds with aromatic substituents was found to be more favorable than those without aromatic groups (CS and EC), presumably due to π–π interactions of the phenyl groups on both enantiomeric analytes and the chiral CPOT phase. It was unexpected, however, to find that BT, a highly polar compound containing a fluorine on one of the phenyl rings, was not resolved into its four pairs of optical isomers. The presence of polar substituents (CN and F) might be detrimental and might weaken any possible π–π interactions between the analytes and the chiral substrate, CPOT, during chromatographic process by a chiral recognition mechanism. The selectivity data for PM (Fig. 5) and PN further demonstrate the possibilities of favorable interactions. To explain the optical resolution of TM, we believe that the conjugated carbonyl system of the tetrahydrophthalimido group may be accountable for involvement in the chiral recognition process. While resolution of the *trans* isomer of DM yielded partially resolved antipodes, the corresponding *cis* isomer remained unresolved in the HPLC system used. The different extents of optical resolution observed between *cis* and *trans* compounds are suggestive of the importance of molecular geometry for controlling chromatographic outcomes of chiral separation. Similar to Okamoto's observations[32] on the mobile phase effects on the selectivity of CPOT, our study showed that the α values were very sensitive to changes in mobile phase conditions. Thus, with hexane–isopropanol (9:1) as eluents, virtually no optical resolution (α = 1.00) was achieved in all cases attempted.

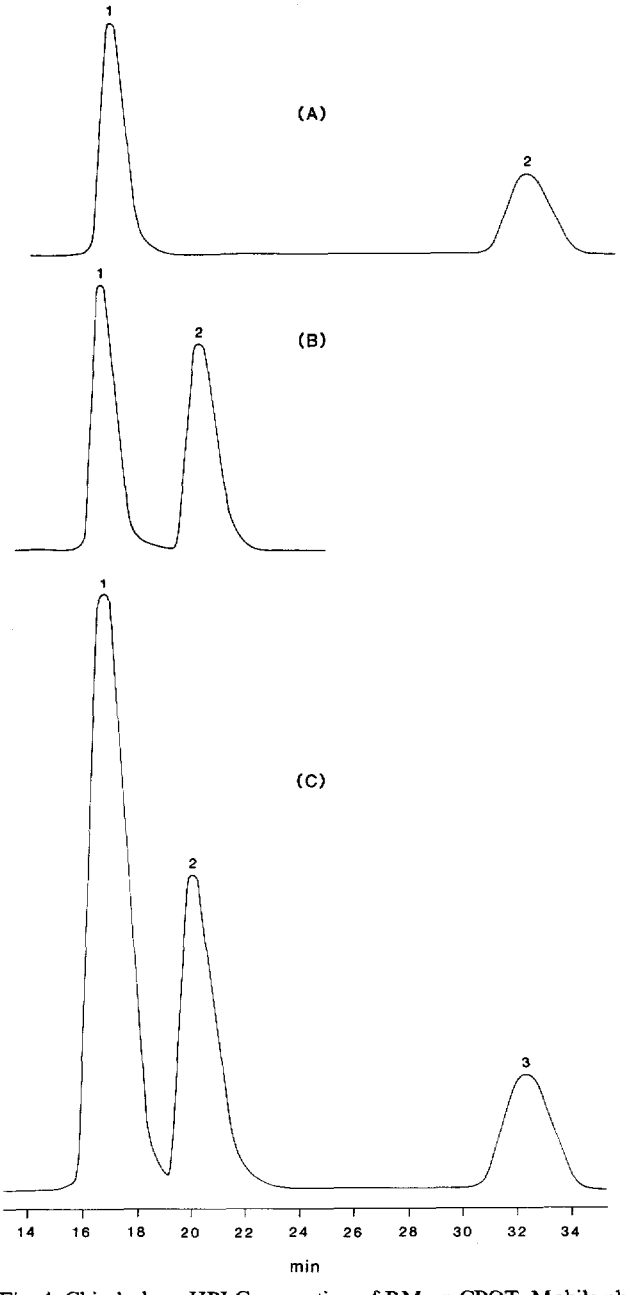

Fig. 4. Chiral-phase HPLC separation of RM on CPOT. Mobile phase: methanol, 0.5 ml/min. (A) peaks: 1 = (+)-*cis*-, and 2 = (−)-*cis*-RM. (B) peaks: 1 = (+)-*trans*-, and 2 = (−)-*trans*-RM. (C) peaks: 1 = mixture of (+)-*cis*- and (+)-*trans*-, 2 = (−)-*trans*-, and 3 = (−)-*cis*-RM.

TABLE III

CHIRAL SEPARATION ON CHIRALPAK-OT (+) (CPOT)

HPLC conditions: mobile phase, methanol at 5°C; flow-rate, 0.5 ml/min.

Compound	*Chromatographic characteristics*		
	k' *(D)**	*k'* *(L)**	α
AL (*trans*)	0.81	–	–
(*cis*)	0.81	–	–
(α)	(1.00)		
BT (*trans*)	0.84 (*R*)	0.84 (*R*)	1.00
(*cis*)	0.96 (*R*)	0.96 (*R*)	1.00
(α)	(1.14)	(1.14)	
(*trans*)	0.96 (*S*)	0.96 (*S*)	1.00
(*cis*)	1.36 (*S*)	1.36 (*S*)	1.00
(α)	(1.42)	(1.42)	
CP (*trans*)	3.80	–	–
(*cis*)	3.00	–	–
(α)	(1.27)		
CS (*trans*)	0.40	0.40	1.00
(*cis*)	0.40	0.40	1.00
(α)	(1.00)	(1.00)	
DM (*trans*)	1.08	1.23	1.14
(*cis*)	1.24	1.24	1.00
(α)	(1.15)	(1.02)	
EC (*trans*)	0.66	0.66	1.00
(*cis*)	0.66	0.66	1.00
(α)	(100)	(1.00)	
PM (*trans*)	2.82	3.08	1.09
(*cis*)	4.60	4.90	1.07
(α)	(1.63)	(1.59)	
PN (*trans*)	2.84	3.27	1.15
(*cis*)	4.30	4.88	1.13
(α)	(1.51)	(1.53)	
RM (*trans*)	2.32	3.06	1.32
(*cis*)	2.40	5.44	2.27
(α)	(1.03)	(1.78)	
TM (*trans*)	1.26	1.44	1.14
(*cis*)	1.56	1.66	1.06
(α)	(1.24)	(1.15)	

* "*D*" and "*L*" denote respective dextrorotatory (+) and levorotatory (−) optical antipodes.

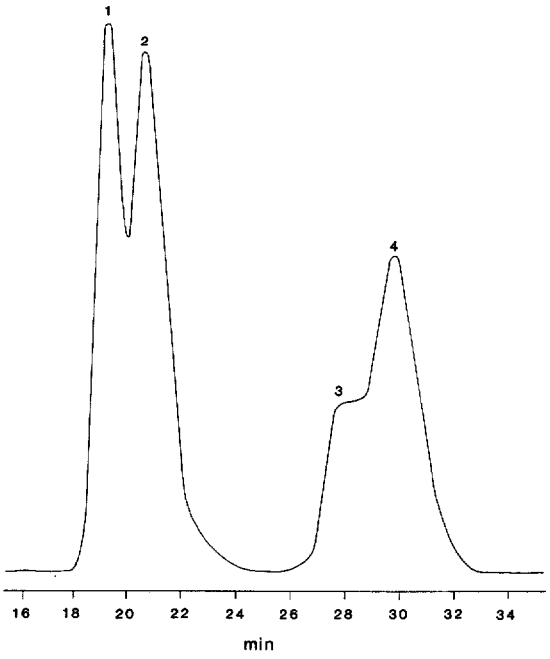

Fig. 5. Chiral-phase HPLC separation of a *cis–trans* mixture of PM on CPOT. Mobile phase conditions are same as in Fig. 4. Peak identification: 1 = (+)-*trans*-, 2 = (−)-*trans*-, 3 = (+)-*cis*-, and 4 = (−)-*cis*-PM.

HPLC on cyclodextrin-bonded silica

As mentioned in the Experimental section, two different CDS columns (Table IV, A and B) were evaluated for HPLC separation of title compounds. These stationary phases were the sole products of this type commercially available during the period when this study was undertaken. At the completion of this study, we learned that an acetylated variant of CDS has now become available and that except for some mobile phase modifications, these columns maintain nearly the same characteristics, as far as the column performance is concerned. Therefore, the results of this study may be equally suitable for HPLC on acetylated CDS, as long as mobile phases with lesser water content are used. This should eliminate the solubility problems often faced in the analyses of non-polar samples containing high levels of analytes.

It was of great interest to note the excellent separation of the diastereomers of (+)-*trans*-AL on CDS (Fig. 6 and Table IV). The separation was unique in this case, because these isomers were not distinguishable on all other stationary phases investigated. This method may be extended to the analysis of diastereomers of natural pyrethroids of similar structures. The *cis* and *trans* isomers of CS and EC were also very well resolved by HPLC on CDS column A (Table IV) using acetonitrile–water as mobile phase (Fig. 7). Using methanol–water as mobile phase, we achieved separation of four optically active components of CS on CDS column A. The components were identified as the two enantiomeric sets of *cis–trans* pairs. This is the first example of a complete optical resolution of a racemic compound in the series studied. Fig. 8 shows the effect of mobile phase composition on the retention and sepa-

TABLE IV

HPLC ON CYCLODEXTRIN-BONDED SILICA

Compound		*Chromatographic characteristics**					
		A			*B*		
		k' *(D)*	*k'* *(L)*	α	*k'* *(D)*	*k'* *(L)*	α
AL**	(*trans*)	5.08	7.16	1.41	5.40	7.74	1.43
	(*cis*)	3.51	3.51	1.00	4.03	4.65	1.15
	(α)	(1.45)	(2.04)		(1.34)	(1.66)	
BT	(*cis*)	4.71(*S*)	4.71(*S*)	1.00	5.22(*S*)	5.22(*S*)	1.00
	(*trans*)	6.45(*R*)	6.45(*R*)	1.00	5.22(*R*)	5.22(*R*)	1.00
	(α)	(1.37)	(1.37)		(1.00)	(1.00)	
	(*cis*)	6.45(*S*)	6.45(*S*)	1.00	5.22(*S*)	5.22(*S*)	1.00
	(*trans*)	7.60(*R*)	7.60(*R*)	1.00	5.22(*R*)	5.22(*R*)	1.00
	(α)	(1.18)	(1.18)		(1.00)	(1.00)	
CP	(*trans*)	28.0	28.0	1.00	28.4	28.4	1.00
	(*cis*)	19.2	19.2	1.00	22.5	22.5	1.00
	(α)	(1.46)	(1.46)		(1.26)	(1.26)	
CS	(*trans*)	3.80	5.72	1.51	3.31	3.31	1.00
	(*cis*)	6.53	7.84	1.20	3.31	3.31	1.00
	(α)	(1.72)	(1.37)		(1.00)	(1.00)	
DM	(*trans*)	17.3	17.3	1.00	15.9	15.9	1.00
	(*cis*)	17.3	17.3	1.00	15.9	15.9	1.00
	(α)	(1.00)	(1.00)		(1.00)	(1.00)	
EC	(*trans*)	8.32	8.32	1.00	10.0	10.5	1.05
	(*cis*)	3.41	3.41	1.00	3.89	3.89	1.00
	(α)	(2.44)	(2.44)		(2.57)	(2.57)	
PM	(*trans*)	12.5	12.5	1.00	11.7	11.7	1.04
	(*cis*)	12.5	12.5	1.00	12.9	12.9	1.00
	(α)	(1.00)	(1.00)		(1.15)	(1.10)	
PN	(*trans*)	19.8	19.8	1.00	17.5	17.5	1.00
	(*cis*)	14.1	14.1	1.00	17.5	17.5	1.00
	(α)	(1.40)	(1.40)		(1.00)	(1.00)	
RM	(*trans*)	18.6	18.6	1.00	19.3	22.2	1.15
	(*cis*)	17.9	17.9	1.00	18.9	18.9	1.00
	(α)	(1.04)	(1.04)		(1.02)	(1.17)	
TM	(*trans*)	15.0	16.6	1.11	17.8	20.4	1.15
	(*cis*)	5.52	5.52	1.00	10.0	10.4	1.04
	(α)	(2.17)	(3.00)		(1.78)	(1.96)	

* HPLC conditions: flow-rate 1 ml/min; (A) mobile phase, methanol–water (50:50); stationary phase, CDS containing 3.5 mequiv. CD/g silica; (B) mobile phase, methanol–water (60:40); stationary phase, CDS containing 7.4 mequiv. CD/g silica. Selectivity (α) data in parentheses are for *cis–trans* isomers.

** Diastereomers of *cis–trans* pairs of (+)-(4*S*)- and (+)-(4*R*)-AL were used. (*D*) and (*L*) in this case designate the optical rotation of the diastereomers.

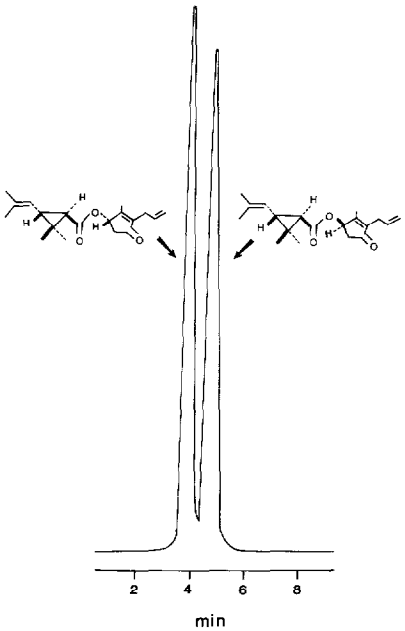

Fig. 6. HPLC separation of diastereomers of (+)-*trans*-AL on CDS (column A). Mobile phase: methanol–water (60:40). Peak identification: left, (+)-*trans*-(4R)-AL; right, (+)-*trans*-(4S)-AL.

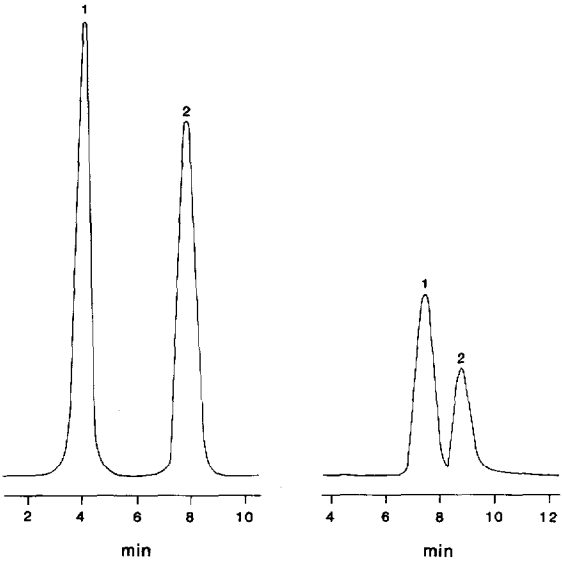

Fig. 7. HPLC separation of EC and CS on CDS (column A). Mobile phase: acetonitrile–water (30:70). Peak identification: left, 1 = *cis*-, and 2 = *trans*-EC; right, 1 = *trans*-, and 2 = *cis*-CS.

ration behavior of isomeric components of AL, CS, and EC. As reported in previous studies[25–29], methanol tended to be more efficient than acetonitrile for the separation of isomers. In general, higher α values and better resolution of component peaks were obtained with the former organic modifier in HPLC with CDS stationary phases.

When using a CDS stationary phase loaded with greater percentage of β-cyclodextrin (column B, Table IV) than column A described above, we obtained reasonable separation of optical isomers of *trans*-TM, and *trans*-RM (Fig. 9). The corresponding *cis*-racemates were either partiallly resolved or not resolved at all. It is interesting to note that this CDS column (B) was preferentially selective for enantiomeric pairs ($\alpha > 1.00$), while the α values for the separation of *cis–trans* mixtures were 1.00 in many cases (Table IV). The notable degree of chiral recognition in the case of TM may imply the probable participation of its tetrahydrophthalimido moiety in specific interactions with the chiral CDS stationary phase during the separation

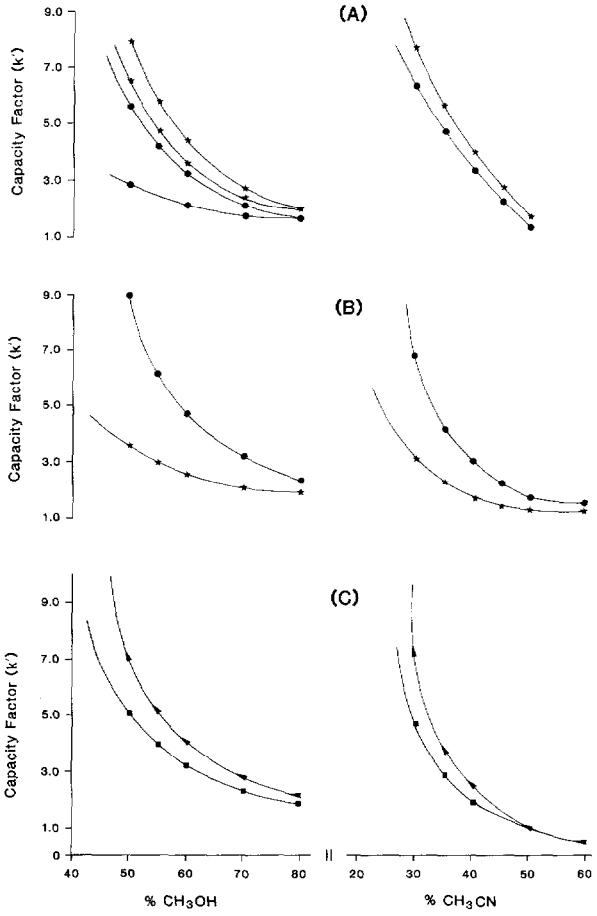

Fig. 8. Effects of mobile phase solvent compositions on capacity factors, k', of CS, EC, and (+)-*trans*-AL. Stationary phase: CDS (column A). Curve identification: (A) (★) *cis*- and (●) *trans*-CS; (B) (★) *cis*- and (●) *trans*-EC; (C) (■) (+)-*trans*-(4*R*)-, and (▲) (+)-*trans*-(4*S*)-AL.

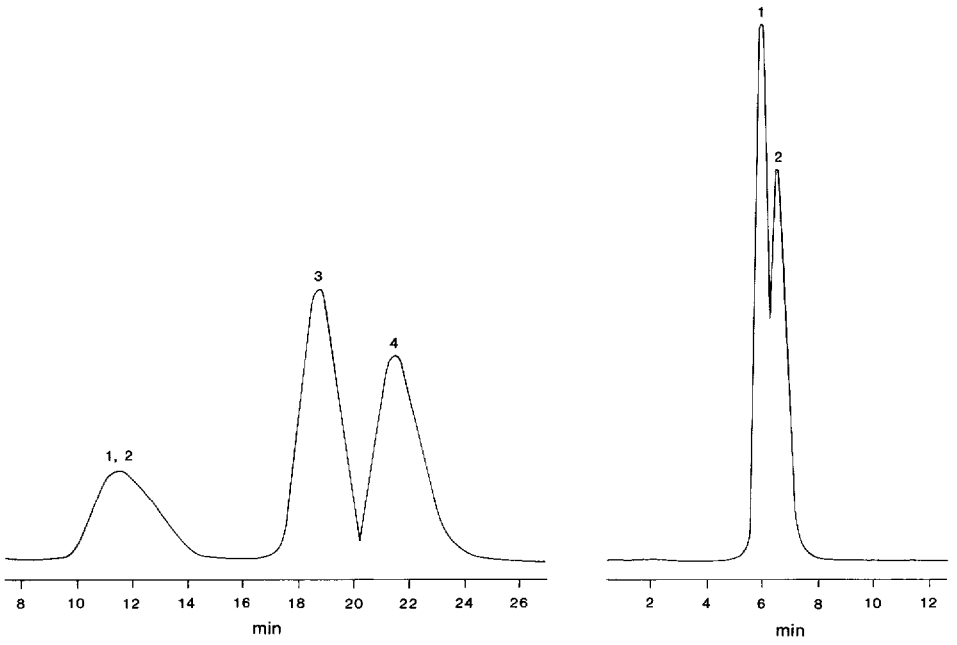

Fig. 9. HPLC separation of TM and *trans*-RM on CDS (column B). Left, mobile phase: methanol–water (60:40); peaks: 1 = (+)-*cis*-, 2 = (−)-*cis*-, 3 = (+)-*trans*-, and 4 = (−)-*trans*-TM. Right, mobile phase: acetonitrile–water (40:60); peaks: 1 = (+)-*trans*-, and 2 = (−)-*trans*-RM.

process presumably via an inclusion complex formation mechanism[33–35]. Considering the hydrophobic cavity of CD in the CDS column, one would predict longer retention times for compounds with higher carbon content. This was experimentally substantiated by the retention data in Table IV. For a few separations where *cis* and *trans* isomers were separated, the *trans* isomers tended to be eluted later from the column than the *cis* isomers, which is indicative of the formation of tighter inclusion complexes, and hence stronger hydrophobic interactions of the analytes with the CDS phase. The results are similar to those found in reversed-phase HPLC of the same structures. In contrast, *trans*-CS emerged from the CDS column prior to the *cis* isomer (Fig. 7) in a manner similar to that of other olefinic compounds reported by Armstrong and DeMond[26].

In conclusion, we have demonstrated four viable HPLC methods for separation and quantification of various isomers of ten *gem*-dimethylcyclopropanes commonly encountered in chemical and biological studies. In many ways, the methods are complementary and are potentially useful for specific applications.

REFERENCES

1 M. Elliott, *Chem. Ind. (London)*, (1969) 776–781.
2 M. Elliott, *Bull. WHO*, 44 (1971) 315.
3 F. Barlow, M. Elliott, A. W. Farnham, A. B. Hadaway, N. F. Janes, P. H. Needham and J. C. Wickham, *Pestic. Sci.*, 2 (1971) 115.
4 S. L. Abidi, unpublished results.

5 S. F. Hameed and J. G. Allen, *J. Hort. Sci.*, 51 (1976) 105.
6 M. Horiba, A. Kobayashi and A. Murano, *Agr. Biol. Chem.*, 41 (1977) 581.
7 R. A. Simonaitis, R. Cail, J. M. Zehner and R. E. Bry, *J. Ass. Offic. Anal. Chem.*, 60 (1977) 9.
8 E. J. Kikta Jr. and J. P. Shierling, *J. Chromatogr.*, 150 (1978) 229.
9 S. Lam and E. Grushka, *J. Chromatogr.*, 154 (1978) 318.
10 C. Meinard, J. C. Suglia and P. Bruneau, *J. Chromatogr.*, 176 (1979) 140.
11 C. Meinard, P. Bruneau and J. Perronnet, *J. Chromatogr.*, 349 (1985) 109.
12 D. Mourot, B. Delepine, J. Boisseau and G. Gayot, *J. Chromatogr.*, 168 (1979) 277.
13 D. Mourot, B. Delepine, J. Boisseau and G. Gayot, *J. Chromatogr.*, 173 (1979) 412.
14 U. Nehmer and N. Dimov, *J. Chromatogr.*, 288 (1984) 227.
15 R. J. Rando and Y. Y. Hammad, *J. Liq. Chromatogr.*, 8 (1985) 1869.
16 Kanth M. S. Sundaram, Cecila Seng and Johanna Broks, *J. Liq. Chromatogr.*, 8 (1985) 2607.
17 J. M. Zehner and R. A. Simonaitis, *J. Ass. Offic. Anal. Chem.*, 59 (1976) 1101.
18 E. Papadopoulou-Mourkidou, Y. Iwata and F. A. Gunther, *J. Agr. Food Chem.*, 28 (1980) 1043.
19 E. Papadopoulou-Mourkidou, Y. Iwata and F.A. Gunther, *J. Agr. Food Chem.*, 32 (1984) 800.
20 M. Jiang and D. M. Soderlund, *J. Chromatogr.*, 248 (1982) 143.
21 J. E. Casida (Editor), *Pyrethrum, The Natural Insecticides*, Academic Press, New York, 1973, pp. 88–91.
22 W. H. Pirkle, J. M. Finn, J. L. Schreiner and B. C. Hamper, *J. Amer. Chem. Soc.*, 103 (1981) 3964.
23 Y. Okamoto, S. Honda, I. Okamoto, H. Yuki, S. Murata, R. Noyori and H. Takaya, *J. Amer. Chem. Soc.*, 103 (1981) 6971.
24 K. Fujimura, T. Ueda and T. Ando, *Anal. Chem.*, 55 (1983) 446.
25 Y. Kawaguchi, M. Tanaku, M. Nakae, K. Funazo and T. Shono, *Anal. Chem.*, 55 (1983) 1852.
26 D. W. Armstrong and W. DeMond, *J. Chromatogr. Sci.*, 22 (1984) 411.
27 D. W. Armstrong, W. DeMond, A. Alak, T. Ward, W. L. Hinze and T. Riehl, *Anal. Chem.*, 57 (1985) 237.
28 D. W. Armstrong, W. DeMond, A. Alak, W. Hinze, T. Riehl and K. H. Bui, *Anal. Chem.*, 57 (1985) 234.
29 S. L. Abidi, *J. Chromatogr.*, (1986) in press.
30 S. L. Abidi, *J. Chromatogr.*, 324 (1985) 209.
31 W. H. Pirkle, T. C. Pochapsky, G. S. Mahler and R. E. Field, *J. Chromatogr.*, 348 (1985) 89.
32 Y. Okamoto, S. Honda, K. Hatada and H. Yuki, *J. Chromatogr.*, 350 (1985) 127.
33 M. L. Bender and M. Komiyama, *Cyclodextrin Chemistry*, Springer-Verlag, Berlin, 1978.
34 J. Szejtli, *Cyclodextrin and Their Inclusion Complexes*, Academiai Kiado, Budapest, 1982.
35 W. L. Hinze, *Sep. Purif. Methods*, 10 (1981) 159.