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2,6-Di-O-pentyl-3-O-trifluoroacetyl cyclodextrin liquid stationary phases for capillary gas chromatographic separation of enantiomers

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ABSTRACT

A series of liquid cyclodextrin derivatives, 2,6-di-O-pentyl-3-O-trifluoroacetyl α-, β- and γ-cyclodextrins (DP-TFA α-, β- and γ-CD), have been used as highly selective chiral stationary phases for capillary gas chromatography. More than 150 pairs of enantiomers were resolved; 120 on DP-TFA-γ-CD, which is the first reported γ-cyclodextrin stationary phase that is more widely useful than the β-cyclodextrin analogue. The enantiomers resolved include chiral alcohols, diols, polyols, amines, amino alcohols, halohydrocarbons, lactones, α-halocarboxylic acid esters, carbohydrates, epoxides, nicotine compounds, pyrans, furans and so on. Identical α values were observed for diol, amine and γ-halocarboxylic acid ester homologues, respectively. The relationship between the unusual selectivity behavior and separation mechanism is discussed.

INTRODUCTION

There is recently a rapidly increasing interest in enantiomeric separation. The significance of chirality is based on the fact that most natural organic products are asymmetric and most life processes proceed stereospecifically. It is not surprising that the stereoisomeric composition of chemical products closely related to human health, particularly pharmaceuticals, has brought increased attention of both the scientific and regulatory communities. In the pharmaceutical market today, a majority of drugs contain one or more chiral centers, and many of these are administered as the racemic mixtures[1]. Scientific studies show that the biological activity of chiral drugs is often related to stereochemistry. For many chiral drugs, further investigations are necessary to understand the activity, toxicity and metabolic pathways of the two enantiomers. Therefore, sophisticated enantiomeric separation techniques are needed not only for separating mixtures of the chiral drugs and their optically active metabolites, but also for the quality control of enantiomerically pure drugs and the development of asymmetric syntheses. The same techniques also are needed in the pesticide

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and herbicide industries because the same principles of stereochemistry hold true and the same problems exist for these chemicals. Chiral separation techniques have been used for the determination of unnatural amino acids in bacterial cell walls and in peptide antibiotics[2,3], the monitoring of amino acid purity during peptide synthesis[4,5], and the determination of enantiomer enrichment (EE). The applications of enantiomeric separations to configurational analysis[6] and archeometric investigations[7], etc. also have been reported.

Two strategies can be considered for the resolution of enantiomeric compounds by gas chromatography (GC). One method involves the conversion of the enantiomers into diastereomeric derivatives with a proper auxiliary, enantiomerically pure derivatization reagent prior to GC analysis. Disadvantages associated with the indirect approach include the requirement of an active functional group for the formation of diastereomeric derivatives, the difficulties in obtaining optically pure reagents, chiral discrimination in the reaction rates of enantiomeric compounds with the chiral derivatizing agent and the requirement of chemical and stereochemical stabilities of the derivatives under GC conditions[8,9].

Another method for the resolution of enantiomers by GC takes advantage of chiral stationary phases (CSPs) which can rapidly and reversibly form diastereomeric association complexes with chiral analytes. Successful chiral stationary phases for wall-coated capillary GC should have some characteristic properties. They need to be highly viscous even at elevated temperatures and have the proper surface tension to wet the capillary wall completely. They should be able to form rapid and reversible diastereomeric association complexes with the chiral analytes via various interactions (*e.g.*, hydrogen bonding, dispersion, dipole–dipole and steric interactions, etc.) to give reasonable chiral selectivity. Other desirable properties include high temperature stability, no racemization at elevated temperatures and low levels of bleeding.

Since Gil-Av *et al.*[10] first reported the chiral separation of N-trifluoroacetyl (TFA)-α-amino acid esters on glass capillary columns coated with N-TFA-L-isoleucine dodecyl ester or N-TFA-L-phenylalanine cyclohexyl ester, a large number of CSPs, based on peptides, amides, diamides and carbonyl bis(amino acid esters)[10] have been developed. However, most of these CSPs had lower operating temperature ranges and exhibited limited chiral selectivities so that they have not found wide applications.

Cyclodextrins (CDs) have been used successfully as chiral stationary phases in high-performance liquid chromatography (HPLC)[11–13]. They also have been used as GC stationary phases by a number of research groups but without much success because native CDs are highly crystalline solids which do not coat well and result in columns with low efficiency[14–18]. Recently, there have been reports on hydrophobic liquid CD derivatives suitable for capillary GC[19–24]. König and co-workers[20–22] have developed two types of derivatized cyclodextrin stationary phases, *i.e.* perpentylated and 2,6-di-O-pentyl-3-O-acetyl CDs. These stationary phases are relatively hydrophobic. Compounds separated on these columns include alcohols, diols, polyols, carbohydrates, halohydrocarbons, amino alcohols, hydroxy acid esters, spiroacetals, etc. Usually 40-m glass capillary columns were utilized. We have recently reported two additional types of CD stationary phases, 2,6-di-O-pentyl CDs, which are hydrophobic[23] and permethyl-O-(S)-2-hydroxypropyl CDs[24] which are hydrophilic. Racemic amines, amino alcohols, carbohydrates, lactones, furans and pyrans, epoxides,

glycidyl analogues and other compounds were separated on these columns. Fused-silica capillary columns of 10 m length were usually used. All these CD-based CSPs previously reported have rigid ring structure and a large number of chiral centers. Many racemic compounds resolved were not aromatic and cannot be separated on any known liquid chromatographic CSP.

This paper describes a new class of chiral GC stationary phases based on 2,6-di-O-pentyl-3-O-trifluoroacetyl liquid derivatives of α-, β- and γ-CDs. These stationary phases, particularly the γ-CD analogue, have extraordinary versatility and chiral selectivity. More than 150 pairs of enantiomers were resolved on wall-coated 10-m fused-silica capillary columns. The enantiomers resolved include chiral alcohols, diols, polyols, halohydrocarbons, lactones, etc. Unique retention behavior was observed for the diol, amine and α-halocarboxylic acid ester homologous series. Possible separation mechanisms and column stability are evaluated as well.

EXPERIMENTAL

Stationary phases

2,6-Di-O-pentyl cyclodextrins (DP-CDs). The synthesis of 2,6-di-O-alkyl cyclodextrins has been reported previously[25]. A 3.0-g amount of the dried CD and excess 1-bromopentane were added to 30 ml dimethyl sulfoxide (DMSO). The reaction was carried out at 60°C for 6 h. Water was then added to the reaction mixture and a waxy precipitate was obtained. The raw product was dissolved in chloroform and the solution was washed with water. Chloroform was removed under vacuum and the product was used for the next reaction without further purification. Previous results have shown that the dialkyl product formed for this reaction is actually a mixture of homologues and isomers and that it is very difficult to obtain the pure 2,6-disubstituted compound[23].

2,6-O-Dipentyl-3-O-trifluoroacetyl cyclodextrins (DP-TFA-CDs). The above material and an excess of trifluoroacetic anhydride were added to 30 ml tetrahydrofuran (THF). The mixture was boiled for 2 h then poured over ice to precipitate the product. The precipitate was washed with cold water and dissolved in chloroform. The chloroform solution was extracted three times with 5% aqueous $NaHCO_3$ and three times with water. The chloroform layer was collected and dried with anhydrous Na_2SO_4. Chloroform was allowed to evaporate in a vacuum desiccator and the final viscous liquid was dried in a vacuum over night.

Columns

Fused-silica capillary tubing (0.25 mm I.D.) was obtained from Supelco (Bellefonte, PA, U.S.A.). Untreated 10-m capillary columns were coated with DP-TFA-α-, β- and γ-CD, via the static method as previously reported[26]. The capillary was placed in a water bath at 36°C. A 0.2% (w/v) diethyl ether solution of the CSP filled the capillary. One end of the capillary was sealed and the other connected to a vacuum line. It took about 4 h to coat a 10-m column. The column efficiency was tested at 100°C by using *n*-hydrocarbons (C_{11} and C_{12}) as test solutes. Only columns that produced $\geqslant$ 3600 plates per column meter were used for this research.

Test solutions

All chemicals were obtained from Aldrich (Milwaukee, WI, U.S.A.), Sigma (St. Louis, MO, U.S.A.) or Fluka (Ronkonkoma, NY, U.S.A.). All alcohols, polyols, amines and other compounds containing hydroxyl and/or amine functionalities were derivatized using trifluoroacetic anhydride. About 1 mg of the racemic analyte was dissolved in 0.5 ml of diethyl ether and 200 μl of trifluoroacetic anhydride were added. After *ca.* 5–30 min, dry nitrogen was bubbled through the solution to remove any excess anhydride. The residue was dissolved in 0.5 ml of ether or methanol for chromatographic analysis.

Apparatus

All chromatographic measurements were performed on a Varian Model 3700 gas chromatograph equipped with a flame ionization detector. Nitrogen was used as the carrier gas and the gas velocity was *ca.* 10–15 cm/s. The injector and detector temperature were held at 200°C. A split ratio of 100:1 was used for all the columns and at all of the column temperatures. The injection volume was 0.5 μl.

RESULTS AND DISCUSSION

Enantioselective properties

Tables I and II show the enantiomeric separation data for compounds resolved

TABLE I

RETENTION AND SELECTIVITY OF RACEMIC ANALYTES ON DP-TFA-CD STATIONARY PHASES

k'_1 = Capacity factor of first-eluted enantiomer. Stationary phases: A = DP-TFA-α-CD; B = DP-TFA-β-CD; G = DP-TFA-γ-CD.

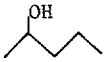
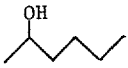
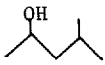
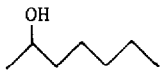
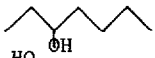
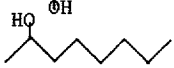
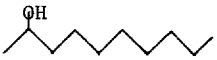
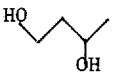
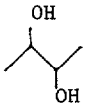
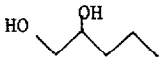
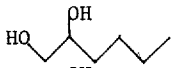
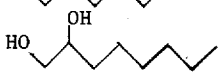
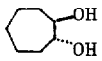
Structure	*Compound*	*Temperature (°C)*	k'_1	α	*Stationary phase*
Alcohols, diols and polyols					
	2-Butanol	40	0.69	1.22	B
		40	0.82	1.16	G
	3-Butyn-2-ol	70	0.57	1.08	B
		40	0.77	1.09	G
	2-Pentanol	40	1.31	1.26	B
		40	1.61	1.27	G
	2-Hexanol	40	3.75	1.31	G
	4-Methyl-2-pentanol	40	1.58	1.19	B
		40	2.18	1.17	G

TABLE I *(continued)*

Structure	*Compound*	*Temperature (°C)*	k'_1	α	*Stationary phase*
	2-Heptanol	40	7.85	1.25	B
		40	8.39	1.26	G
	3-Heptanol	40	4.14	1.08	G
	2-Octanol	70	2.80	1.06	B
		40	20.7	1.15	G
	2-Decanol	70	13.4	1.18	B
		40	67.1	1.22	G
	1,2-Propanediol	70	2.06	1.08	A
		70	1.67	1.18	B
	1,3-Butanediol	70	4.92	1.06	A
		70	4.86	1.14	B
		70	4.79	1.21	G
	(2*R*,3*R*)- and (2*S*, 3*S*)-butanediol	70	1.43	1.58	G
	1,2-Pentanediol	70	7.77	1.09	A
		70	3.54	1.03	B
	1,4-Pentanediol	90	5.23	1.06	B
		70	13.6	1.05	G
	1,2-Hexanediol	70	22.5	1.08	A
	1,2-Octanediol	90	10.6	1.05	B
	*trans*-1,2-Cyclohexanediol	70	12.0	1.58	G
	*trans*-1,2-Cycloheptanediol	70	19.6	1.15	G
	*trans*-1,2-Cyclooctanediol	70	36.0	1.12	G

(Continued on p. 308)

TABLE I *(continued)*

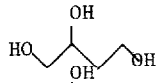
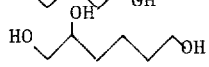
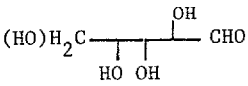
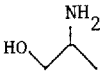
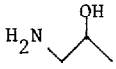
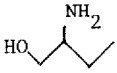
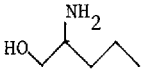
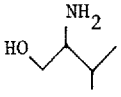
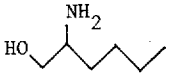
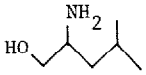
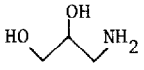
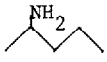
Structure	*Compound*	*Temperature (°C)*	k'_1	α	*Stationary phase*
	1,2,4-Butanetriol	110	8.10	1.02	B
	1,2,6-Hexanetriol	110	23.3	1.07	B
	Arabitol	100	20.6	1.06	B
Amino Alcohols					
	2-Amino-1-propanol	100 110 100	5.88 3.81 6.53	1.07 1.16 1.99	A B G
	1-Amino-2-propanol	100 110 100	6.25 4.76 5.71	1.14 1.03 1.20	A B G
	2-Amino-1-butanol	110 110 100	7.31 3.42 7.04	1.08 1.06 1.17	A B G
	2-Amino-1-pentanol	110 100	4.33 8.93	1.14 1.14	B G
	2-Amino-3-methyl-1-butanol	110 100	2.95 6.43	1.07 1.19	B G
	2-Amino-1-hexanol	110 110 100	15.0 7.57 10.4	1.04 1.11 1.1	A B G
	Leucinol	120 110	3.81 4.33	1.06 1.14	A B
	3-Amino-1,2-propanediol	140	6.14	1.12	B
Amines					
	2-Aminobutane	80	4.29	1.04	G
	2-Aminopentane	80	3.75	1.03	A

TABLE I *(continued)*

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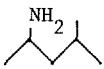
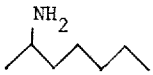
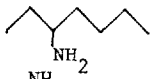
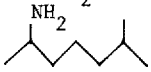
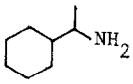
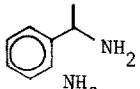
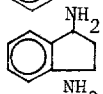
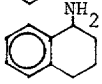
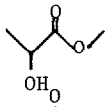
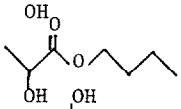
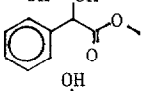
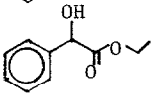
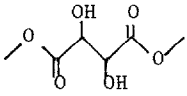
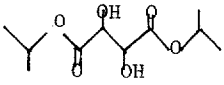
	1,3-Dimethylbutyl-amine	80	4.88	1.06	A
	2-Aminoheptane	90	10.3	1.07	A
		80	19.6	1.02	G
	3-Aminoheptane	90	7.19	1.06	A
		80	13.4	1.03	G
	1,5-Dimethylhexyl-amine	90	10.1	1.01	A
		80	28.4	1.01	G
	1-Cyclohexylethyl-amine	100	11.2	1.08	B
	1-Phenylethyl-amine	100	12.9	1.05	B
	1-Aminoindan	140	4.06	1.06	A
	1,2,3,4-Tetrahydro-1-naphthylamine	140	6.12	1.04	A
		140	8.21	1.03	G
Carboxylic acid esters					
	Lactic acid methyl ester	50	6.79	1.47	G
	Lactic acid butyl ester	60	15.0	1.05	G
	Mandelic acid methyl ester	110	4.00	1.04	B
	Mandelic acid ethyl ester	110	3.57	1.03	B
		120	4.11	1.09	G
	D,L-Tartaric acid dimethyl ester	90	2.79	1.04	B
	D,L-Tartaric acid diisopropyl ester	90	8.57	1.07	B

(Continued on p. 310)

TABLE I *(continued)*

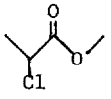
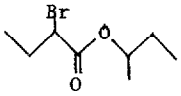
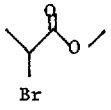
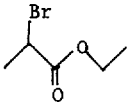
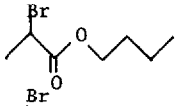
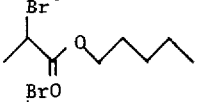
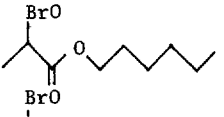
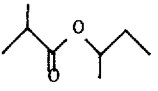
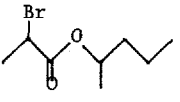
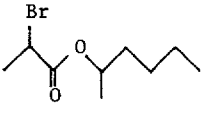
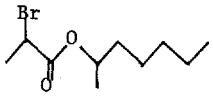
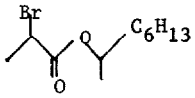
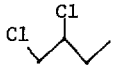
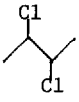
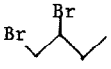
Structure	*Compound*	*Temperature (°C)*	k'_1	α	*Stationary phase*
	2-Chloropropionic acid methyl ester	60	6.25	2.69	B
		60	5.00	2.14	G
	2-Bromobutyric acid *sec.*-butyl ester[a]	80	10.0	1.22	G
			10.6	1.07	
	2-Bromopropionic acid methyl ester	80	9.38	1.12	B
		80	2.44	1.47	G
	2-Bromopropionic acid ethyl ester	80	3.85	1.14	B
		80	3.50	1.16	G
	2-Bromopropionic acid butyl ester	80	8.00	1.05	G
	2-Bromopropionic acid pentyl ester	80	15.6	1.04	G
	2-Bromopropionic acid hexyl ester	80	30.9	1.04	G
	2-Bromopropionic acid *sec.*-butyl ester[a]	60	12.0	1.25	B
			12.5	1.05	
		80	4.89	1.29	G
			5.39	1.02	
	2-Bromopropionic acid *sec.*-pentyl ester[a]	60	21.5	1.28	B
			22.9	1.12	
		80	8.00	1.24	G
			8.37	1.05	
	2-Bromopropionic acid *sec.*-hexyl ester[a]	60	45.6	1.26	B
			49.5	1.10	
		80	14.7	1.25	G
			15.1	1.07	

TABLE I *(continued)*

Structure	*Compound*	*Temperature (°C)*	k'_1	α	*Stationary phase*
	2-Bromopropionic acid *sec.*-heptyl ester[a]	90	15.1 15.4	1.16 1.04	G
	2-Bromopropionic acid *sec.*-octyl ester[a]	90	29.3 29.6	1.16 1.03	G
Halohydrocarbons					
	2-Bromo-1-chloropropane	40 40 30	4.31 4.00 7.64	1.06 1.12 1.05	A B G
	2-Chlorobutane	30 30	0.73 0.91	1.10 1.12	A G
	3-Chloro-1-butene	30 30	0.72 0.82	1.06 1.13	A G
	2-Bromobutane	30 35 30	1.65 1.74 2.44	1.38 1.04 1.09	A B G
	2-Iodobutane	40 60 30	2.92 1.31 6.36	1.04 1.24 1.06	A B G
	1,2-Dichlorobutane	60 60	2.45 0.89	1.09 1.04	B G
	(2*R*,3*R*)- and (2*S*,3*S*)-2,3-dichlorobutane	60 60	2.15 2.05	1.59 1.60	B G
	1,2-Dibromobutane	90 70 60	4.13 4.54 8.57	1.03 1.03 1.13	A B G

(Continued on p. 312)

TABLE I *(continued)*

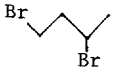
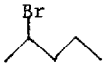
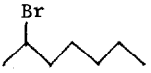
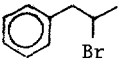
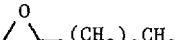
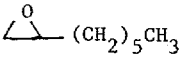
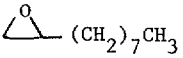
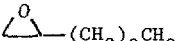
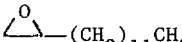
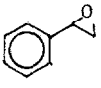
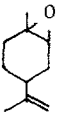
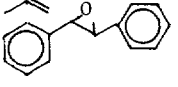
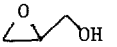
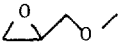
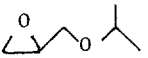
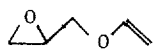
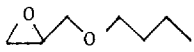
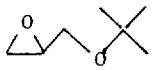
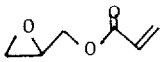
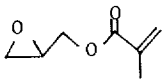
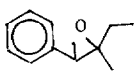
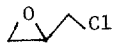
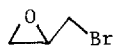
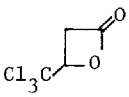
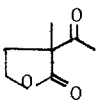
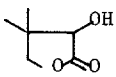
Structure	*Compound*	*Temperature (°C)*	k'_1	α	*Stationary phase*
	1,3-Dibromobutane	90	6.38	1.02	A
		70	7.38	1.05	B
		60	13.6	1.79	G
	2-Bromopentane	35	3.44	1.03	B
		30	8.27	1.32	G
	2-Bromoheptane	70	4.15	1.07	B
		50	8.57	1.18	G
	2-Bromo-1-phenyl-propane	100	6.39	1.06	G
Expoxides, glycidyl analogues and haloepihydrins					
	1,2-Epoxyhexane	50	3.31	1.02	A
		40	5.36	1.10	G
	1,2-Epoxyoctane	50	22.3	1.04	A
	1,2-Epoxydecane	90	9.88	1.02	A
	1,2-Epoxydodecane	90	108	1.02	A
	1,2-Epoxytetradecane	100	125	1.02	A
	Styrene oxide	80	5.57	1.01	B
		80	10.7	1.57	G
	Limonene oxide[a]	80	8.57	1.06	G
			8.57	1.10	
	*trans*-Stilbene oxide	140	20.0	1.02	G
	(±)-1,3-Butadiene diepoxide	80	3.93	1.11	G
	Glycidol	60	4.29	1.06	G
	Glycidyl methyl ether	40	3.33	1.04	B
		45	5.89	1.16	G
	Glycidyl isopropyl ether	40	6.00	1.04	B
		45	10.5	1.04	G

TABLE I *(continued)*

Structure	*Compound*	*Temperature (°C)*	k'_1	α	*Stationary phase*
	Allyl glycidyl ether	50	3.23	1.02	A
		45	21.6	1.42	G
	*n*-Butyl glycidyl ether	50	11.8	1.02	B
		45	23.2	1.04	G
	*tert.*-Butyl glycidyl ether	45	14.5	1.06	G
	Glycidyl acrylate	80	10.1	1.14	G
	Glycidyl methacrylate	80	10.4	1.04	G
	(2*S*,3*S*)- and (2*R*,3*R*)-2-methyl-3-phenyl-glycidol	100	13.6	1.06	G
	Epifluorohydrin	30	2.50	1.02	G
	Epichlorohydrin	60	4.82	1.20	G
	Epibromohydrin	60	4.82	1.20	G
Lactones					
	β-Butyrolactone	110	2.73	1.14	A
		70	13.7	1.62	B
		80	7.14	1.20	G
	3-Hydroxy-4,4,4-trichlorobutyric-β-lactone	120	3.50	1.11	B
		100	15.6	1.19	G
	α-Methyl-γ-butyrolactone	110	3.03	1.29	A
		110	11.5	1.07	B
	α-Acetyl-α-methyl-γ-butyrolactone	120	6.28	1.59	B
	Pantoyl lactone	120	4.25	1.04	A
		120	1.57	1.05	B

(Continued on p. 314)

TABLE I *(continued)*

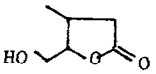
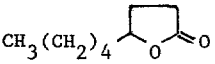
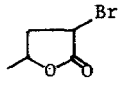
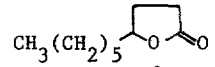
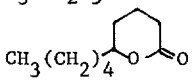
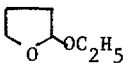
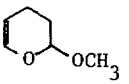
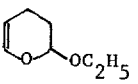
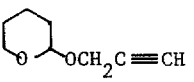
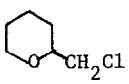
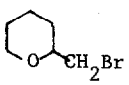
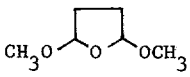
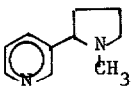
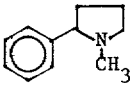
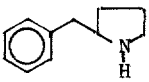
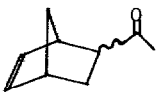
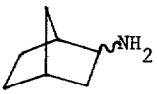
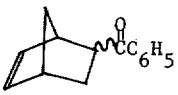
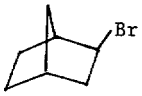
Structure	*Compound*	*Temperature (°C)*	k'_1	α	*Stationary phase*
	β,β'-Dimethyl-γ-(hydroxymethyl)-γ-butyrolactone	120	12.3	1.10	B
	γ-Nonanoiclactone	160	4.07	1.06	B
	α-Bromo-γ-valerolactone[a]	150	4.64 5.36	1.09 1.09	G
	γ-Decanolactone	140	9.24	1.05	G
	δ-Decanolactone	140	11.8	1.02	G
Furan and pyran derivatives					
	2-Ethoxytetrahydrofuran	40 40 45	3.63 3.08 3.04	1.03 1.05 1.20	A B G
	3,4-Dihydro-2-methoxy-2H-pyran	40 40 45	3.38 4.33 4.29	1.07 1.22 1.13	A B G
	3,4-Dihydro-2-ethoxy-2H-pyran	40 40 45	5.85 5.92 6.07	1.04 1.06 1.09	A B G
	Tetrahydro-2-(2-propynyloxy)-2H-pyran	80 70	3.71 8.14	1.01 1.08	B G
	2-(Chloromethyl)-tetrahydropyran	80 70	3.24 8.04	1.28 1.03	B G
	2-(Bromomethyl)-tetrahydropyran	80	6.07	1.25	B

TABLE I *(continued)*

Structure	*Compound*	*Temperature (°C)*	k'_1	α	*Stationary phase*
	3-Hydroxytetrahydropyran	50	7.14	1.18	G
	*trans*-2,5-Dimethyltetrahydrofuran	45	8.57	1.06	G
	*trans*-2,5-Dimethoxytetrahydrofuran	60	1.46	1.92	G
Nicotine compounds					
	Nicotine	160	2.14	1.13	B
	1-Methyl-2-phenylpyrrolidine	160	1.43	1.20	B
	2-Benzylpyrrolidine	140	10.2	1.06	B
Bicyclic compounds					
	*endo*- and *exo*-2-acetyl-5-norbornene[a]	110	1.73 2.45	1.03 1.00	B
		100	4.39 6.32	1.11 1.09	G
	*endo*- and *exo*-2-aminonorbornane[a]	110	4.71 6.21	1.18 1.10	B
	*endo*- and *exo*-2-benzoyl-5-norbornene[a]	120	34.6 41.8	1.00 1.04	G
	*exo*-2-Bromonorbornane	70	11.3	1.02	G

(Continued on p. 316)

TABLE I *(continued)*

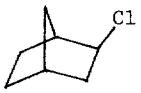
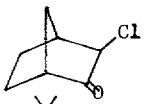
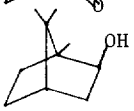
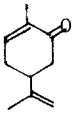
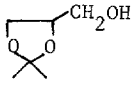
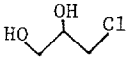
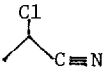
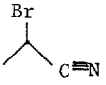
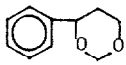
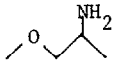
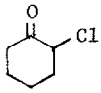
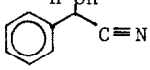
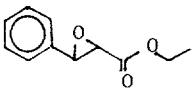
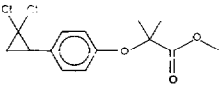
Structure	*Compound*	*Temperature (°C)*	k'_1	α	*Stationary phase*
	*exo*-2-Chloro-norbornane	70	5.79	1.01	G
	*endo*- and *exo*-3-chloro-2-norbornanone[a]	120	6.57 10.2	1.06 1.04	G
	DL-Isoborneol	70	8.68	1.05	G
Ketones					
	2-Methylcyclo-hexanone	80	5.29	1.08	G
	Carvone	90 110 100	13.6 7.05 11.4	1.04 1.09 1.01	A B G
Miscellaneous					
	3-Chloro-2-butanone	50 60	3.46 2.43	1.62 1.59	B G
	Solketal	90 60	1.57 7.86	1.04 1.07	B G
	3-chloro-1,2-propanediol	90	3.52	1.09	B
	2-Chloropropionitrile	80 70	1.93 1.65	1.07 1.06	B G
	2-Bromopropionitrile	80 70	4.07 4.13	1.14 1.06	B G
	1-Chloro-2-propanol	70	5.21	1.02	G

TABLE I *(continued)*

Structure	*Compound*	*Temperature (°C)*	k'_1	α	*Stationary phase*
	4-Phenyl-1,3-dioxane	120	7.36	1.04	G
	2-Chlorocyclo-pentanone	90 110	12.6 2.47	2.51 1.33	B G
	2-Amino-1-methoxy-propane	90 100	1.90 1.71	1.25 1.08	B G
	2-Chlorocyclo-hexanone	90 110	21.2 4.26	1.06 1.10	B G
	2-Methylpiperidin	100	5.54	1.13	G
	3-Methylpiperidin	100	5.00	1.14	G
	Mandelonitrile	100	5.21	1.10	G
	Ethyl-3-phenyl-glycidate[a]	120	16.6 23.8	1.05 1.04	G
	Ciprofibrate methyl ester	140	67.7	1.03	B

[a] Compound containing more than one chiral center.

on the α-, β- and γ-versions of DP-TFA-CD capillary GC columns. The γ-CD column displays excellent chiral selectivity to alcohols, diols, amino alcohols, α-halocarboxylic acid esters, halohydrocarbons, glycidyl analogues, lactones, bicyclic compounds, pyran and furan derivatives. Racemic amines, as well as alcohols, diols, amino alcohols, halohydrocarbons, lactones, pyran and furan derivatives were resolved on the β-CD column. The α-CD column is especially useful for the enantiomeric separations of long carbon chain epoxides (Table I). In addition, piperidines, α-halocycloketones, as well as some analytes containing nitrile functionality, were also resolved on the γ-CD columns and/or β-CD columns. Large separation factors (α) were obtained for most of the racemates, particularly alcohols, diols, lactones, α-halocarboxylic acid esters and some halohydrocarbons. (*R*)- and (*S*)-2-chloropropionic acid methyl esters (which are precursors in the synthesis of many herbicides) show an α value of 2.69 on

TABLE II

COMPARISON OF RETENTION AND SELECTIVITY OF HOMOLOGOUS RACEMIC AMINES, DIOLS AND α-HALOCARBOXYLIC ACID ESTER ANALOGUES ON DP-TFA-CD STATIONARY PHASES

k'_1 and stationary phases as in Table I.

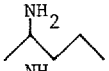
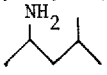
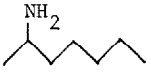
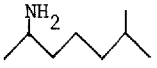
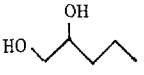
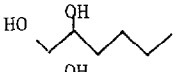
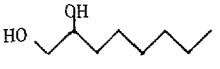
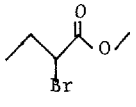
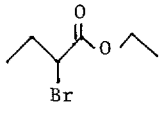
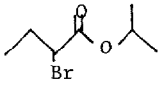
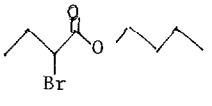
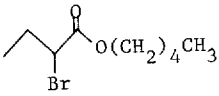
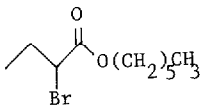
Structure	*Compound*	*Temperature* (°C)	k'_1	α	Stationary phase
	2-Aminobutane	90	1.55	1.14	B
	2-Amino-3,3-dimethylbutane	90	2.10	1.22	B
	2-Aminopentane	90	2.40	1.22	B
	1,3-Dimethylbutylamine	90	2.80	1.22	B
	2-Aminoheptane	90	8.15	1.22	B
	1,5-Dimethylhexylamine	90	12.5	1.22	B
	1,2-Propanediol	70	1.61	1.49	G
	1,2-Pentanediol	70	3.50	1.23	G
	1,2-Hexanediol	70	6.64	1.23	G
	1,2-Octanediol	70	29.3	1.23	G
	2-Bromobutyric acid methyl ester	80 80	507 6.71	1.56 1.57	B G
	2-Bromobutyric acid ethyl ester	80 80	5.25 9.93	1.29 1.16	B G
	2-Bromobutyric isopropyl ester	80	6.57	1.08	G
	2-Bromobutyric acid butyl ester	80 80	15.5 20.5	1.16 1.09	B G

TABLE II *(continued)*

Structure	*Compound*	*Temperature (°C)*	k'_1	α	*Stationary phase*
	2-Bromobutyric acid pentyl ester	80	32.3	1.16	B
		80	39.5	1.08	G
	2-Bromobutyric acid hexyl ester	80	66.4	1.16	B
		80	80.0	1.09	G

DP-TFA-β-CD (Table I), which is unusually large for a GC separation of enantiomers. Therefore, shorter columns and higher column temperatures can be used.

Typical separations are illustrated in Figs. 1–4. Optically active alcohols (Fig. 1) are found naturally in the chemical communication system of insects. For example, 2-heptanol is active as an alarm pheromone in ants. Aliphatic 1,2- and 1,3-diols (Fig. 2) are important chiral building blocks in asymmetric synthesis. All of the compounds in Figs. 1–4 lack the aromatic functionality required for most LC separations. In fact, lower enantioselectivity was observed toward some aromatic compounds. For example, although 1-indanol, 1,2,3,4-tetrahydro-1-naphthol and 1-(1-naphthyl)-ethylamine were resolved on 2,6-di-O-pentyl CD and permethyl-O-(*S*)-2-hydroxypropyl CD stationary phases as previously reported[24,25], none of these compounds was

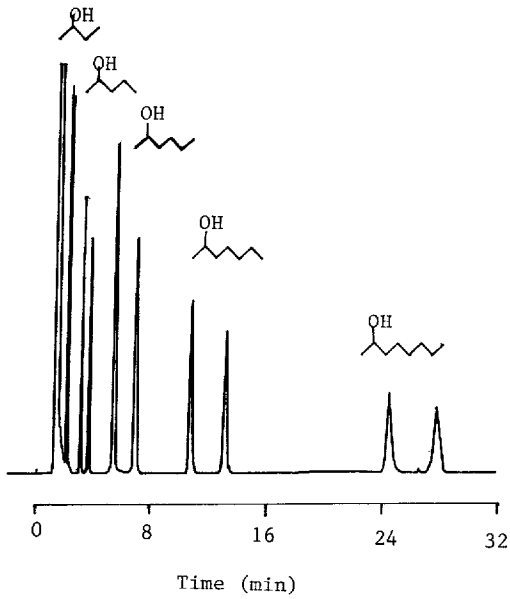


Fig. 1. Enantiomeric separation of alcohols after trifluoroacetylation. Column, 10 m fused silica with DP-TFA-γ-CD; column temperature, 40°C; carrier gas, nitrogen, 3 p.s.i.

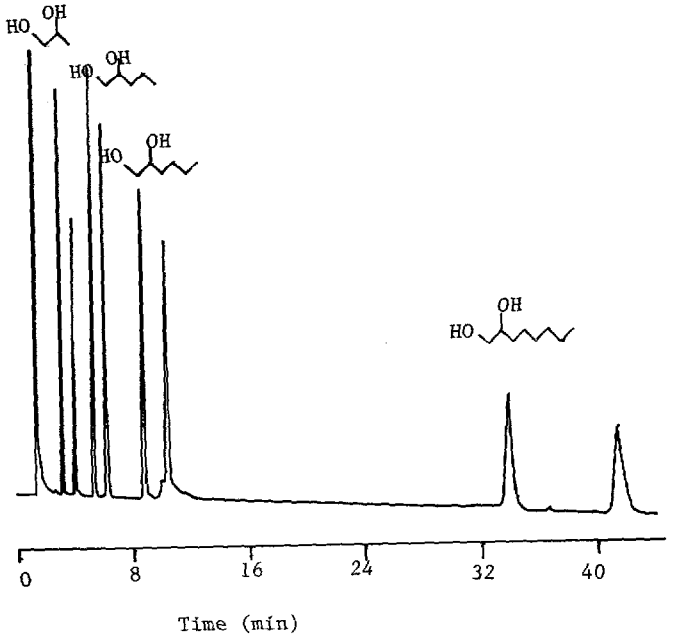


Fig. 2. Enantiomeric separation of diols after trifluoroacetylation. Column, 10 m fused silica with DP-TFA-γ-CD; column temperature, 70°C; carrier gas, nitrogen, 3 p.s.i.

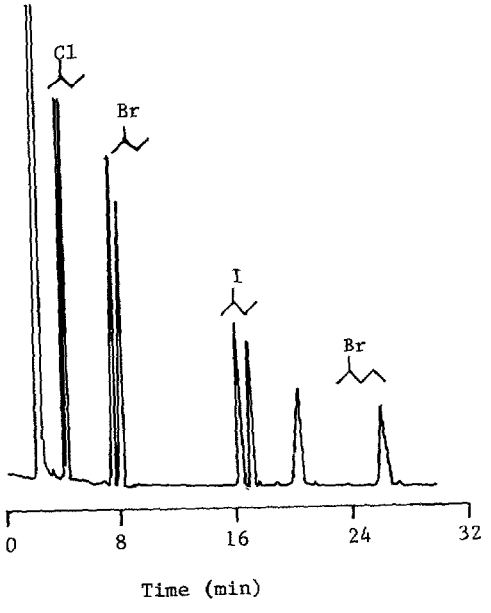


Fig. 3. Enantiomeric separation of monohalohydrocarbons. Column, 10 m fused silica with DP-TFA-γ CD; column temperature, 30°C; carrier gas, nitrogen, 3 p.s.i.

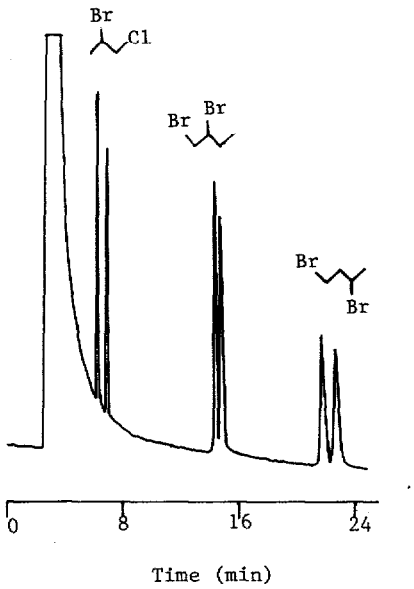


Fig. 4. Enantiomeric separation of dihalohydrocarbons. Column, 10 m fused silica with DP-TFA-β-CD; column temperature, 70°C; carrier gas, nitrogen, 3 p.s.i.

resolved on any of the DP-TFA-CD columns. Of the 150 different racemic compounds injected on the columns, 80% were resolved on the γ-CD column, 60% on the β-CD column and only 30% on the α-CD column. It appears that the DP-TFA-γ-CD column is the most widely useful of the three, although there are certain compounds that resolve only on the β-CD and α-CD analogues. To our knowledge, this is the only CD stationary phase series (in either LC or GC) thus far in which the γ-CD derivative exhibits a wider chiral selectivity and usefulness than the β-CD analogue.

Separation mechanism

There have been few mechanistic retention studies involving derivatized CD GC stationary phases. In contrast to analogous LC separations, the interactions between chiral solutes and GC CSPs are not significantly affected by mobile phase interactions. Feibush and Gil-Av[27] have suggested that association complexes via hydrogen bonding between carbonyl and amide functions were responsible for the GC chiral separation of amino acids on the dipeptide phases. Others claimed that only one significant point of attachment was involved in the formation of the diastereomeric association complex[28]. Cyclodextrins are composed of linked α-1,4-glucose units. Each glucose has three hydroxyl groups designated 2-OH, 3-OH and 6-OH. The 6-OH are primary hydroxyls and are located at the more narrow, "bottom" end of the CD torous. The 2-OH and 3-OH groups are secondary hydroxyl groups and are located at the wide end or "mouth" of the CD molecule. After 2,6-di-O-alkylation and 3-O-trifluoroacetylation, CD molecules are no longer good hydrogen bond donors as are native cyclodextrins. Also, many of the racemates resolved had no hydrogen bond donor groups and relatively poor hydrogen bond accepting

groups (*e.g.*, haloalkanes). Consequently, some of the separations shown in Tables I and II, cannot be explained by hydrogen bonding interactions. Molecules such as halohydrocarbons and lactones (that contain only hydrogen bonding acceptor groups) have permanent dipole moments. Stronger dipole–dipole interactions between analytes and DP-TFA-CD molecules are very likely. Inclusion complexation as well as dispersion, or steric interactions also may play a role in chiral recognition and enantioselective retention.

There are several different ways in which the dipole-dipole interaction may occur. The analytes may enter into the cyclodextrin's cavity via the "mouth" or the more narrow bottom of the CD, or may be adsorbed on the exterior surface of the stationary phase molecules. It is difficult to know exactly where the enantioselective interactions are occurring on the CD stationary phase. However, studies can be devised which provide circumstantial evidence as to some of the relevant interactions. For example, retention data for three homologous series on DP-TFA-β- and γ-CD are gathered in Table II. All of the compounds in this series have a common structural characteristic, *i.e.*, they all have a polar "head" and an non-polar "tail". Chromatographic measurements were carried out under identical conditions for each compound in the series. For the larger racemic homologues, identical α values are obtained regardless of the chain length or branching of the "tail" (Table II). In the amine homologous series, the smallest member has a smaller α value than the higher-molecular-weight members. In the other two series, the smallest members have larger α values than the rest (Table II). It is reasonable to assume that for these homologous series: (a) only part of the carbon chain (one to two carbons) contribute significantly to the chiral recognition because the α values of the larger homologous members are independent of carbon chain length, (b) longer carbon chains affect the retention of the molecules to the CSP (*i.e.*, the longer the chain the greater the retention) but not the enantioselectivity and (c) the orientation of the molecule can be affected by the substituent group on the CD and the size of the CD cavity. The functionality of the analytes may also play a role because other homologous series, such as epoxides, alcohols and amino alcohols did not exhibit the same behavior (Table I).

Column stability and special operational considerations

When considering column stability for GC CSPs three very different problems must be addressed: decomposition of backbone structure, racemization of chiral functionality and disruption of the coated film. All of these processes are irreversible and any one of them can render the column useless for the separation of enantiomers. It has been found[22,23] that the derivatized CD stationary phases do not racemize at temperatures up to 300°C. Also, we have found that DP-TFA-CDs have good wettability to untreated fused-silica capillary wall and that the film was stable up to 180°C. To test the stability of the film on fused-silica capillary wall, the column temperatures were held at 160, 180, 200 and 220°C for 4 h. The changes in column efficiency were monitored at 100°C using *n*-hydrocarbons as test solutes. It was found that the column efficiency dropped dramatically after the column had been used above 200°C. Droplets were observed on the capillary wall by visual inspection under a microscope. The thermal stability of 3-O-trifluoroacetyl functionality on the stationary phases is another concern because the ester linkage is susceptible to hydrolysis. We used every column continuously for 1 to 2 months. Moisture was removed from the carrier gas

by using an in-line gas purifier (Alltech, Deerfield, IL, U.S.A.). Column efficiency and selectivity were continuously monitored during all of the previously mentioned studies. Insignificant changes in the selectivity were observed. However, great care must be taken to prolong the life time of the column. For the wall-coated capillary, only split injection is advised with a split ratio of $\geqslant$ 100:1. A single splitless or on-column injection may permanently damage the column. The selection of sample solvent also is critical. The suggested sample solvent is diethyl ether, which is very volatile and inactive to derivatizing reagents such as trifluoroacetic anhydride. Solvents such as benzene and toluene are not recommended, since they may form inclusion complexes with the stationary phases and interfere with enantiomeric separation. This has occasionally been observed in our laboratory. In addition, like other common GC stationary phases, derivatized CD stationary phases are sensitive to thermal shock. As for all other wall coated GC capillaries, these columns should never be heated or cooled at a rate of more than 20°C/min.

CONCLUSION

The DP-TFA-CD, particularly the γ-CD, stationary phases exhibit wider chiral selectivity and usefulness than other previously reported CD-based GC stationary phases[19–24]. High separation factors have been observed for some racemates, which indicates that it may be possible to utilize the stationary phases in packed GC or preparative GC separations of enantiomers. The enantioselective retention data in this paper suggests a specific space-oriented dipole-dipole interaction between the analytes and stationary phase molecules. However, mechanistic studies are needed to fully understand the separation mechanism.

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