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Effect of the configuration of the substituents of derivatized β-cyclodextrin bonded phases on enantioselectivity in normal-phase liquid chromatography

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ABSTRACT

(*R*)-(−)-, (*S*)-(+)- and racemic 1-(1-naphthyl)ethylcarbamate derivatives of β-cyclodextrin (β-CD) bonded phase were used to successfully resolve a large variety of enantiomers in the normal-phase mode. The selectivity (α) and retention (k') of these new chiral packings in the normal-phase mode are somewhat analogous to a reciprocal Pirkle-type phase. Also, many of the enantiomers resolved on these new phases were not resolvable on the native β-CD phase in the reversed-phase mode or on the naphthylvaline type π-complex, hydrogen bonding phases. Comparison of the selectivity and retention of the (*R*)-, (*S*)- and racemic carbamate phases revealed that both the carbamate substituent and the cyclodextrin moieties contribute to chiral recognition. The selectivity of these phases was found to be a function not only of the configuration of the substituent on the CD but also was dependent on the number of substituents (degree of substitution). More importantly, the results indicate that enantioselectivity on these phases arises from two modes of chiral recognition. In some cases, the chiral selectivity of the one configuration of the substituent and the CD combine synergistically while the chiral selectivity of the opposite configuration of the substituent and the CD combine antagonistically. For some compounds, changing the carbamate substituent configuration resulted in a reversal of the elution order.

INTRODUCTION

Recently, it was reported that 88% of all synthetic chiral drugs are currently marketed as the racemic mixtures[1]. This is an alarming statistic considering that it is well established that optical isomers may elicit dramatically different therapeutic as well as toxic responses, *in vivo* [2,3]. In fact, it is anticipated that the U.S. Food and Drug Administration (FDA) will soon require biotoxicity as well as bioefficacy studies on not only each pure enantiomer but also the racemate before approval will be granted for marketing new chiral drugs [4]. In addition, related regulatory pressures may soon be brought to bear on the agrochemical and food and beverage industries [5].

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Most of the reported enantiomeric separations of chiral compounds are performed using high-performance liquid chromatography (HPLC) on chiral stationary phases (CSPs). Currently, it is estimated that there are more than 50 CSPs commercially available for both reversed- and normal-phase HPLC enantiomeric separations. Among the more successful of the normal-phase CSPs are the derivatized cellulosic phases developed by Okamoto *et al.* [6].

Cyclodextrin (CD)-based stationary phases have been used successfully for the enantiomeric separation of a wide variety of drugs [7], alkaloids [8,9] and other optical isomers [10]. Until recently, all reported enantiomeric separations using these CD phases have been in the reversed-phase mode [11]. The chiral recognition mechanism in the reversed-phase mode is thought to be dependent on the formation of an inclusion complex between the hydrophobic moiety of the analyte and the relatively non-polar interior of the CD cavity. In the normal-phase mode, however, the CD cavity is more likely to be occupied by the non-polar component of the mobile phase [12]. Derivatized cyclodextrins have previously been reported which extend the range of optical isomers resolved in the reversed-phase mode [13]. Recently, we reported the use of derivatized CD bonded phases that readily resolved enantiomers in the normal-phase mode [11]. In this earlier work, the reported derivatized β-CD bonded phases included acetyl and toluoyl esters as well as naphthylethyl and 2,6-dimethylphenyl carbamates. Chiral recognition was attributed to the presence of residual CD hydrogen bonding sites as well as π–π interaction and hydrogen bonding sites provided by the aromatic and carbonyl substituents on the derivatized CD. It was found that higher selectivities usually were obtained using the isocyanate-derivatized phases rather than analogous ester-linked moieties. This was thought to be the result of additional sites for hydrogen bonding and stronger dipole–dipole interactions possible with the carbamate linkages relative to the ester linkages. Although most of the new derivatized CD phases exhibited unique selectivities, analogous to the derivatized cellulosic phases, the naphthylethylisocyanate-derivatized CD, in particular, seemed to have selectivities comparable to that obtained on a Pirkle-type naphthylvaline column. The purpose of the present work was to expand upon the earlier work and to examine the effect of the configuration of the CD naphthylethyl substituent on enantioselectivity. In contrast to the native CD phases, where reversal of elution order is not always feasible, it was anticipated that because these new phases incorporate an additional stereogenic center they might afford more flexibility in this regard. Comparison of the enantioselectivities obtained on the (*R*)-, (*S*)- and racemic carbamate substituted stationary phases also provides additional insight into the chiral recognition mechanism of these new aromatic derivatized CD stationary phases.

EXPERIMENTAL

Chemicals

The structures of the solutes, which were obtained from various sources, are presented in the tables. In each case, approximately 2–5 mg of analyte was dissolved in the appropriate solvent to which was added 2–5 mg of the 3,5-dinitrophenyl derivatizing agent. In the case of the amines, amino acids and amino esters, 3,5-dinitrobenzoyl chloride was used. The alcohols were derivatized following the procedure of Pirkle *et al.* [14]. The carboxylic acids were first converted to the acid chloride using

thionyl chloride and subsequently reacted with 3,5-dinitroaniline. Thionyl chloride, sodium azide, (*R*)-, (*S*)- and racemic naphthylethylisocyanate, 3,5-dinitrobenzoyl chloride, 3,5-dinitroaniline as well as the anhydrous pyridine were obtained from Aldrich (Milwaukee, WI, U.S.A.). All other solvents were obtained from Fisher Scientific (St. Louis, MO, U.S.A.).

Preparation of the bonded sorbents

The preparation of the β-CD bonded sorbent has been described elsewhere [15]. Briefly, 4 g of the β-CD bonded sorbent was dried overnight under vacuum in drying gun using methanol and P_2O_5. The dried sorbent was placed in a three-necked round bottom flask. Anhydrous pyridine (100 ml) was added. The mixture was refluxed until all the water was removed (as an azeotrope into a Dean–Stark trap). The derivatizing reagent was added (neat) and the mixture was refluxed for 4 h. The modified sorbent was then collected on a fritted glass filter, washed with approximately 100 ml of pyridine followed by approximately 200 ml methanol and then air-dried.

The bonded sorbents were sent out for carbon analysis (Galbraith Labs., Knoxville, TN, U.S.A.). The surface concentration of CD and the degree of substitution was calculated and are reported in Table I [16]. The modified sorbents were slurry packed into 250 × 4.6 mm I.D. stainless steel columns.

TABLE I

LIST OF BONDED SORBENTS

Column designation	Substituent configuration	$\%C_{spacer}$ [a]	$\%C_{CD}$ [b]	$\%C_{TOT}$ [c]	Degree of substitution
R	*R*	2.60	4.23	7.17	6.7
S	*S*	2.60	4.23	5.81	3.5
HS	*S*	2.60	4.87	8.89	6.6
RAC	racemic	2.60	4.23	7.58	7.7

[a] %C due to the spacer used to link the CD to the silica substrate.
[b] %C due to the spacer linkage plus the CD.
[c] %C of the derivatized bonded phase, including spacer, CD and naphthylethylcarbamate.

RESULTS AND DISCUSSION

Bonding results

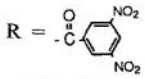
As can be seen from Table I, the high-coverage S (HS) phase had a higher overall %C loading but approximately the same degree of substitution as the R and RAC phases. This seemingly anomalous result is due to the fact that three of the phases (R, S and RAC) were prepared on the same batch of CD-bonded silica while the HS phase was prepared on a different batch of silica which has a higher initial loading of β-CD. The effects of this difference in coverage on retention and selectivity will be discussed in the subsequent sections.

Chromatographic results

The chromatographic data is summarized in Tables II–IV. The elution order, when known, is indicated as a superscript on the capacity factor. Comparisons of the

TABLE II

CHROMATOGRAPHIC DATA FOR 3,5-DINITROBENZOYL DERIVATIZED AMINES, AMINO ACID ESTERS AND AMINO ACIDS ON NAPHTHYLETHYL CARBAMOYLATED β-CD STATIONARY PHASES



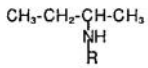
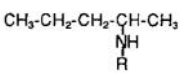
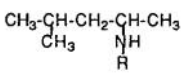
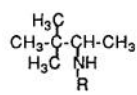
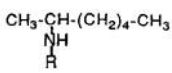
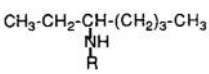
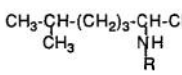
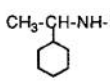
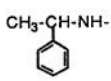
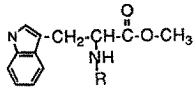
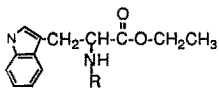
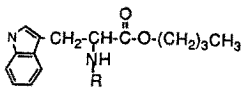
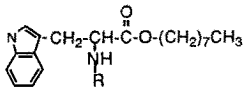
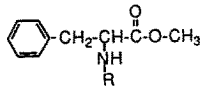
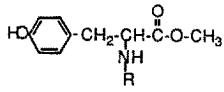
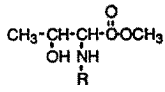
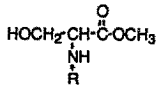
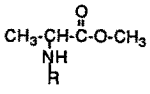
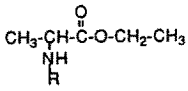
Compound	Structure	Column[a]	k'^{b}	α	R_s	Mobile phase[c]
Amines						
sec-Butylamine		R	9.14	1.09	1.5	A
		S	11.45	1.08	1.5	A
		HS	24.10	1.10	1.0	A
		RAC	17.76	1.00	–	A
1-Methylbutylamine		R	8.28	1.05	1.0	A
		S	10.65	1.03	0.7	A
		HS	20.14	1.03	0.5	A
		RAC	15.81	1.00	–	A
1,3-Dimethyl-butylamine		R	7.05	1.08	1.4	A
		S	9.43	1.04	0.8	A
		HS	18.42	1.02	0.5	A
		RAC	14.19	1.06	1.0	A
2-Amino-3,3-dimethylbutane		R	10.45	1.20	2.5	A
		S	12.00	1.09	1.5	A
		HS	22.36	1.18	1.2	A
		RAC	12.50	1.03	0.6	A
2-Aminoheptane		R	8.00	1.05	0.9	A
		S	8.54	1.08	1.5	A
		HS	16.30	1.08	1.0	A
		RAC	13.15	1.04	0.7	A
3-Aminoheptane		R	6.14	1.12	2.0	A
		S	8.32	1.18	2.6	A
		HS	15.08	1.21	1.8	A
		RAC	12.63	1.05	0.8	A
1,5-Dimethyl-hexylamine		R	6.36	1.00	–	A
		S	7.80	1.11	2.0	A
		HS	13.81	1.13	1.2	A
		RAC	11.88	1.08	1.2	A
1-Cyclohexyl-ethylamine		R	2.50^{R}	1.31	3.1	B
		S	4.91^{R}	1.17	1.6	B
		HS	7.95^{R}	1.10	0.6	B
		RAC	7.10^{R}	1.25	2.0	B
α-Methylbenzyl-amine		R	2.85^{S}	1.77	5.8	C
		S	4.56^{R}	2.03	6.4	C
		HS	8.52^{R}	2.15	4.8	C
		RAC	9.26^{R}	1.17	2.1	C

TABLE II (*continued*)

Compound	Structure	Column[a]	k'^{b}	α	R_s	Mobile phase[c]
1-(1-Naphthyl)-ethylamine		R	3.33^{S}	3.05	13.4	C
		S	5.82^{R}	3.59	14.0	C
		HS	10.71^{R}	4.28	7.9	C
		RAC	15.11^{R}	1.40	3.2	C
Amino esters						
DL-Tryptophan methyl ester		R	6.64^{L}	1.15	1.3	D
		S	11.08^{D}	1.59	3.4	D
		HS	12.69^{D}	1.80	2.2	D
		RAC	12.92^{D}	1.19	1.4	D
DL-Tryptophan ethyl ester		R	4.82^{L}	1.17	1.4	D
		S	8.09^{D}	1.68	4.0	D
		HS	12.43^{D}	1.63	2.3	D
		RAC	9.75^{D}	1.21	1.4	D
DL-Tryptophan butyl ester		R	3.45	1.25	2.1	D
		S	5.57	1.97	5.8	D
		HS	8.68	1.88	3.0	D
		RAC	6.76	1.29	2.1	D
DL-Tryptophan octyl ester		R	2.25	1.37	3.1	D
		S	4.07	2.26	6.6	D
		HS	6.10	2.09	4.2	D
		RAC	4.98	1.36	2.6	D
DL-Phenylalanine methyl ester		R	$3.95^{L,n}$	1.27	2.3	E
		S	5.99^{D}	1.44	3.8	E
		HS	11.37^{D}	1.50	2.4	E
		RAC	8.94^{D}	1.09	1.0	E
DL-Tyrosine methyl ester		R	4.17^{L}	1.29	1.9	D
		S	7.17^{D}	1.77	4.2	D
		HS	12.69^{D}	1.83	2.8	D
		RAC	9.47^{D}	1.24	1.6	D
DL-Threonine methyl ester		R	4.38^{L}	1.15	1.4	C
		S	7.26^{D}	1.24	1.5	C
		HS	10.63^{D}	1.37	1.7	C
		RAC	10.69	1.00	–	C
DL-Serine methyl ester		R	8.42^{L}	1.18	1.4	C
		S	10.89^{D}	1.23	1.4	C
		HS	17.78^{D}	1.36	1.5	C
		RAC	17.55	1.00	–	C
DL-Alanine methyl ester		R	3.38^{L}	1.41	3.7	C
		S	5.29^{D}	1.61	4.3	C
		HS	8.71^{D}	1.71	3.0	C
		RAC	8.88^{D}	1.09	0.8	C
DL-Alanine ethyl ester		R	2.38^{L}	1.42	3.6	C
		S	3.72^{D}	1.70	4.2	C
		HS	5.83^{D}	1.79	3.5	C
		RAC	6.14^{D}	1.13	1.2	C

(*Continued on p. 118*)

TABLE II (*continued*)

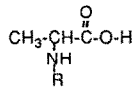
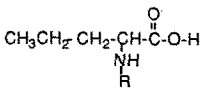
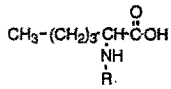
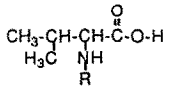
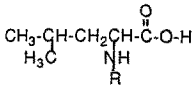
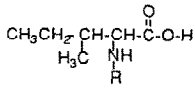
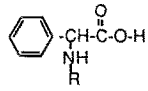
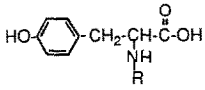
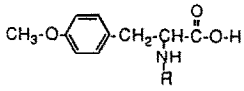
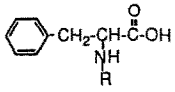
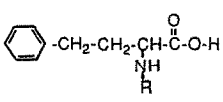
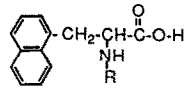
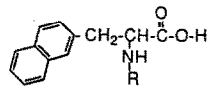
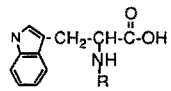
Compound	Structure	Column[a]	k'[b]	α	R_s	Mobile phase[c]
Amino acids						
DL-Alanine		R	3.80	1.00	–	F
		S	2.95[L]	1.08	1.2	F
		HS	4.01[L]	1.13	1.5	F
		RAC	4.13[L]	1.06	0.8	F
DL-Norvaline		R	2.92[D]	1.04	0.6	F
		S	2.40[L]	1.07	1.0	F
		HS	2.83[L]	1.10	0.8	F
		RAC	3.48	1.00	–	F
DL-Norleucine		R	3.11[D]	1.12	1.2	F
		S	2.49[L]	1.08	1.1	F
		HS	2.82[L]	1.14	1.2	F
		RAC	3.92	1.00	–	F
DL-Valine		R	3.16[L]	1.05	0.7	F
		S	2.70[L]	1.05	0.8	F
		HS	3.05[L]	1.11	1.0	F
		RAC	3.71[L]	1.08	1.1	F
DL-Leucine		R	2.55[D]	1.10	1.2	F
		S	2.12[L]	1.05	0.7	F
		HS	2.44[L]	1.08	0.6	F
		RAC	3.36	1.00	–	F
DL-Isoleucine		R	3.03[L]	1.10	0.8	F
		S	2.49[L]	1.11	1.5	F
		HS	2.76[L]	1.17	1.1	F
		RAC	3.61[L]	1.11	1.2	F
DL-2-Phenylglycine		R	14.49[D]	1.22	5.0	G
		S	14.28[L]	1.33	6.5	G
		HS	23.70[L]	1.43	6.5	G
		RAC	20.45[L]	1.04	1.0	G
DL-Tyrosine		R	6.91[D]	1.04	1.0	G
		S	6.25[L]	1.18	2.7	G
		HS	8.95[L]	1.31	2.8	G
		RAC	7.86[L]	1.08	1.5	G
DL-O-Methyl-tyrosine		R	8.50	1.00	–	G
		S	7.57[L]	1.13	2.3	G
		HS	12.51[L]	1.26	2.8	G
		RAC	9.67[L]	1.10	2.0	G
DL-Phenylalanine		R	7.41[L]	1.06	1.2	G
		S	7.31[L]	1.10	2.0	G
		HS	11.93[L]	1.10	1.2	G
		RAC	9.68[L]	1.11	2.1	G
DL-Homophenyl-alanine		R	7.38[D]	1.37	4.0	G
		S	8.21[D]	112	2.3	G
		HS	11.72[D]	1.12	1.1	G
		RAC	10.43[D]	1.20	2.8	G

TABLE II (*continued*)

Compound	Structure	Column[a]	k'^{b}	α	R_s	Mobile phase[c]
DL-3(1-Naphthyl)-alanine		R	8.17^{D}	1.12	2.0	G
		S	7.91^{L}	1.12	2.4	G
		HS	12.89^{L}	1.12	1.9	G
		RAC	11.31	1.00	–	G
DL-3(2-Naphthyl)-alanine		R	9.77^{D}	1.04	0.7	G
		S	9.86^{L}	1.15	2.8	G
		HS	18.43^{L}	1.15	2.0	G
		RAC	13.26^{L}	1.06	1.5	G
DL-Tryptophan		R	7.91^{D}	1.16	2.7	G
		S	7.21^{L}	1.22	3.7	G
		HS	11.77^{L}	1.19	2.1	G
		RAC	9.89	1.00	–	G

[a] Columns as designated in Table I.

[b] Capacity factor of the first eluted enantiomer; configuration indicated as a superscript, when known.

[c] Mobile phases: A = isopropanol–hexane (10:90); B = isopropanol–hexane (20:80); C = isopropanol–hexane (30:70); D = isopropanol–hexane (50:50); E = isopropanol–hexane (40:60); F = acetonitrile–ethanol–acetic acid (50:49.5:0.5); G = acetic acid–methanol (1:99).

retention, elution order, selectivity and resolution for a large number of solutes on all four columns provides some insights into the possible retention and chiral recognition mechanisms. As will be discussed, several trends emerged from the data.

Effect of loading

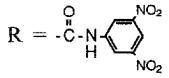
Out of the 49 compounds reported in Tables II–IV, 34 compounds had the largest capacity factors on the HS column which had the highest %C loading; the other 15 compounds (2 aromatic amines, the aliphatic amino acids, 4 out of 10 amino esters, and 3 out of 4 carboxylic acids) had the largest capacity factor on the RAC column, which had the second highest %C loading but highest degree of substitution. In the case of the amino acids, two different solvent systems were used (Table II). The aliphatic amino acids were eluted with acetic acid–acetonitrile–ethanol and had the largest capacity factor on the racemic column. Acetic acid–methanol was used as eluent for the aromatic amino acids. In this case, the largest capacity factors were obtained on the HS column. From these results, it would appear that retention is related, in a general sense at least, to %C loading but also to the degree of CD substitution and the configuration of the CD chiral substituent.

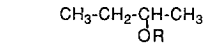
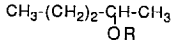
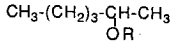
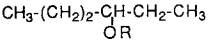
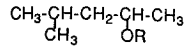
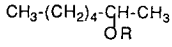
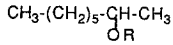
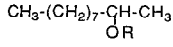
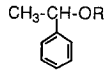
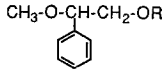
For 32 compounds, the smallest capacity factor was obtained on the R column, which had the second lowest %C loading, while 17 compounds (all of the amino acids except DL-homophenylalanine, DL-3(2-naphthyl)alanine and DL-tryptophan, aromatic alcohols, carboxylic acids) had the shortest retention on the low-coverage S column, which had the lowest number of naphthylethyl-carbamate substituents and the lowest %C loading.

TABLE III

CHROMATOGRAPHIC DATA FOR 3,5-DINITROPHENYL DERIVATIZED ALCOHOLS ON NAPHTHYLETHYL CARBAMOYLATED β-CD STATIONARY PHASES

Mobile phase: isopropanol–hexane (10:90).



Compound	Structure	Column[a]	k'^b	α	R_s
2-Butanol		R	3.63	1.0	–
		S	4.52	1.0	–
		HS	8.05	1.0	–
		RAC	6.72	1.0	–
2-Pentanol		R	3.05	1.02	0.6
		S	3.76	1.10	1.2
		HS	6.28	1.12	1.2
		RAC	5.45	1.05	0.9
2-Hexanol		R	2.70	1.04	0.7
		S	3.29	1.12	1.4
		HS	5.55	1.15	1.3
		HS	4.78	1.06	1.1
3-Hexanol		R	2.64	1.00	–
		S	3.09	1.05	1.0
		HS	5.21	1.06	0.7
		RAC	4.57	1.00	–
4-Methyl-2-pentanol		R	2.71	1.03	0.6
		S	3.24	1.22	2.3
		HS	5.11	1.25	2.4
		RAC	4.57	1.12	2.1
2-Heptanol		R	2.46	1.04	0.9
		S	3.03	1.12	1.4
		HS	5.00	1.14	1.3
		RAC	4.34	1.06	1.0
2-Octanol		R	2.27	1.05	1.1
		S	2.82	1.11	1.3
		HS	4.59	1.14	1.4
		RAC	4.01	1.05	0.8
2-Decanol		R	1.99	1.07	1.2
		S	2.43	1.10	1.2
		HS	4.01	1.12	1.2
		RAC	3.52	1.02	0.6
sec-Phenethyl alcohol		R	8.61^S	1.16	2.4
		S	6.82^R	1.32	4.5
		HS	16.49^R	1.41	2.8
		RAC	14.41^R	1.09	1.2
2-Methoxy-2-phenyl ethanol		R	9.38^R	1.05	1.0
		S	9.40^S	1.10	1.3
		HS	20.19	1.00	–
		RAC	16.28^S	1.09	0.6
1,2,3,4-Tetrahydro-naphthol		R	8.12^S	1.29	4.0
		S	6.82^R	1.26	3.3
		HS	15.39^R	1.35	2.9
		RAC	14.56	1.00	–

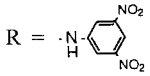
[a] Columns as designated in Table I.

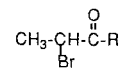
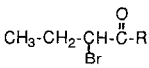
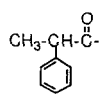
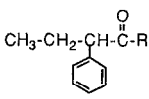
[b] Capacity factor of the first eluted enantiomer; configuration indicated as a superscript, when known.

TABLE IV

CHROMATOGRAPHIC DATA FOR 3,5-DINITROPHENYL DERIVATIZED CARBOXYLIC ACIDS ON NAPHTHYLETHYL CARBAMOYLATED β-CD STATIONARY PHASES

Mobile phase: isopropanol–hexane (20:80).



Compound	Structure	Column[a]	k'^b	α	R_S
2-Bromopropionic acid		R	4.13	1.36	3.8
		S	4.14	1.36	3.8
		HS	6.73	1.53	3.9
		RAC	7.95	1.00	–
2-Bromobutyric acid		R	3.44	1.25	2.5
		S	3.31	1.30	3.1
		HS	5.20	1.44	2.5
		RAC	6.23	1.00	–
2-Phenylpropionic acid		R	4.93	1.77	7.6
		S	4.12	2.21	8.1
		HS	7.10	2.78	7.6
		RAC	11.38	1.18	2.0
2-Phenylbutyric acid		R	4.33^R	1.63	6.1
		S	3.54^S	2.21	10.7
		HS	8.49^S	1.76	4.8
		RAC	8.38^S	1.19	1.9

[a] Columns as designated in Table I.

[b] Capacity factor of the first eluted enantiomer; configuration indicated as a superscript, when known.

As can be seen from Tables II–IV, retention and selectivity are not necessarily related. For instance, 1-methylbutylamine, 1-cyclohexylethylamine and 2-methoxy-2-phenylethanol all exhibit reduced selectivity on the HS column relative to the other columns despite larger capacity factors. This will be discussed in more detail in a subsequent section. The largest number of compounds (32) exhibited the highest selectivity on the HS column; 6 compounds had the best selectivity on the S column with an additional 2 compounds having the same, highest selectivity on both the HS and S columns. In almost all cases where the S column had the highest selectivity, the solutes were aromatic and the HS column had the second highest selectivity. It is quite probable that the larger number of CD substituents on the HS columns may crowd the chirally selective interactive sites thereby limiting enantioselectivity. Another 6 compounds had the best selectivity on the R column; 1 compound (DL-phenylalanine) had the best selectivity on the RAC column and 1 compound (DL-3(1-naphthyl) alanine) had the same selectivity on all three phases incorporating optically pure substituents (R, S, HS); 29 compounds had the lowest or no selectivity on the RAC column; 17 compounds had the lowest or no selectivity on the R column.

Commonly, when comparable enantioselectivities for a given solute were

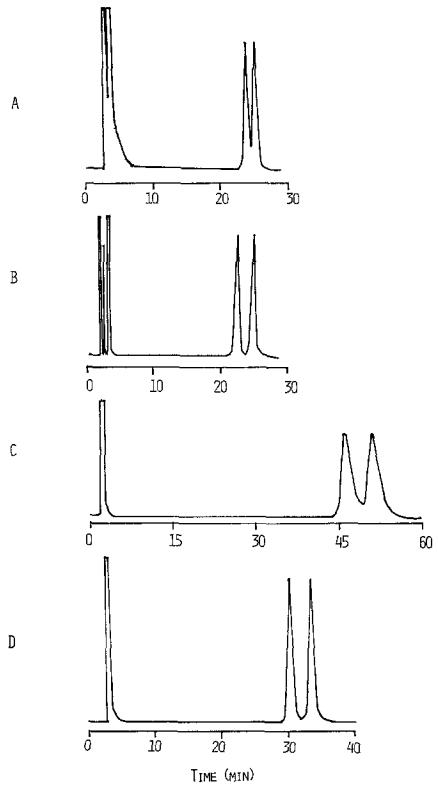


Fig. 1. Chromatograms showing the effect of %C loading on the resolution of the 3,5-dinitrobenzamide derivative of DL-phenylalanine on: (A) the R column; (B) the low-load S column; (C) the high-load S column and (D) the racemic column. Mobile phase conditions are as specified in Table II.

achieved on more than one column, better resolution (R_s) was obtained on the column with the lower %C (*e.g.*, 2-aminoheptane, DL-tryptophan ethyl ester, DL-phenylalanine, 3-hexanol) (Fig. 1).

Retention

In general, within a class of compounds, retention tended to decrease with increasing chain length (*e.g.*, *sec*-butylamine, 1-methylbutyl-amine, 2- and 3-amineheptane; the tryptophan esters; alanine methyl and ethyl esters; alanine, norvaline; 2-butanol through 2-decanol; 2-bromopropionic and 2-bromobutyric acids; 2-phenylpropionic and 2-phenylbutyric acids). This also seemed to be the case for branched compounds (*e.g.*, 1,3-dimethylbutylamine and 1,5-dimethylhexylamine; leucine, valine) although this trend was less predictable when the branched point was α to the chiral center (*e.g.*, 2-amino-3,3-dimethylbutane *vs.* 1,3-dimethylbutylamine). The reduction in capacity factor with increasing chain length may be due to the increased hydrocarbon character of the solute with increasing chain length leading to increased partitioning to the non-polar mobile phase.

Aromaticity also contributed to increased retention (*e.g.*, 1-cyclohexylethyl-

Fig. 2. Chromatogram on the low-load S column showing the enantiomeric separation of the 3,5-dinitrophenyl derivatives of: (A) 4-methyl-2-pentanol; (B) 1,2,3,4-tetrahydro-1-naphthol; (C) *sec*-butyl amine and (D) 2-phenylpropionic acid. Mobile phase: isopropanol–hexane (10:90) at 1 ml/min; UV detection at 254 nm.

amine and α-methylbenzylamine). DL-phenylalanine and DL-tyrosine differ only by the *p*-hydroxyl on the phenyl ring. In the case of the methyl esters of these amino acids, the hydroxyl increases the retention (note different mobile phase) but the opposite is true for the parent amino acids. The hydroxyl α to the stereogenic center on the serine methyl ester also leads to increased retention relative to alanine methyl ester.

In general, the capacity factors for aliphatic amines were larger than for the corresponding aliphatic alcohols (*eg. sec*-butylamine and 2-butanol; 1-methylbutylamine and 2-pentanol; 1,3-dimethylbutylamine and 4-methyl-2-pentanol; 2-aminoheptane and 2-heptanol) (Fig. 2).

Chiral recognition mechanism

As mentioned in the introduction, the retention and selectivity of these naphthylethyl-derivatized CD phases is somewhat analogous to that obtained on the reciprocal Pirkle-type phases. A principle feature of the Pirkle-type phases is the presence of an aromatic π-acid or π-base group on the bonded ligand which provides a site for π–π interactions. The substituents on the aromatic groups of the stationary phase as well as the analytes define the nature of the π–π interaction. The naphthylethyl moiety present in these derivatized CD phases is somewhat π-basic while the 3,5-dinitrophenyl substituent of the derivatized chiral analytes is somewhat π-acidic. Carbamoylation of the β-CD bonded phase not only introduces new sites for π–π interactions, but also presents opportunities for hydrogen bonding and dipole stacking with the carbamate linkage which is not present on the native β-CD phase. In addition, some of the residual hydroxyls of the β-CD are also available for chirally selective interactions.

Wainer and Alembik [17] observed that, on a Pirkle-type phase, the elution order is related to which end of the amide linkage is attached to stereogenic center. They attributed this effect to the direction of the dipole moment of the amide bond and the role that this plays in dipole–dipole interactions. In the present study, different linkages were employed in the derivatized analytes (carbamate and amide with either the nitrogen or the carbonyl attached to the dinitrophenyl ring). In particular, it is interesting to examine the elution order and selectivity for α-methylbenzylamine, *sec*-phenethyl alcohol and 2-phenyl-butyric acid. The elution order of α-methylbenzylamine (Table II) and *sec*-phenethyl alcohol (Table III) on all four columns relative to 2-phenylbutyric acid (Table IV) suggests that a mechanism similar to that proposed by Wainer and Alembik [17] is involved in chiral recognition on these derivatized CD phases. Note also that the selectivity (α) for *sec*-phenethyl alcohol is much less than those obtained for α-methylbenzylamine or 2-phenylpropionic acid. In contrast to the derivatization of the amine and carboxylic acid, derivatization of the alcohol introduces an additional atom between the stereogenic center and the 3,5-dinitrophenyl moiety. The greater distance between the stereogenic center and the π-acid moiety of the derivatized alcohol results in reduced enantioselectivity relative to the derivatized amine or carboxylic acid.

Chiral recognition on the derivatized CD phases can arise from two sources: the base CD or the chiral naphthylethyl carbamate substituents. Although the exact substitution pattern and orientation of the naphthylethyl group with respect to the CD is not known, it is likely that chiral recognition is the sum total of the interactions arising from many different chiral analyte/chiral bonded ligand associations [18]. An illustration of a possible association complex formed between a chiral analyte and the derivatized CD is shown in Fig. 3.

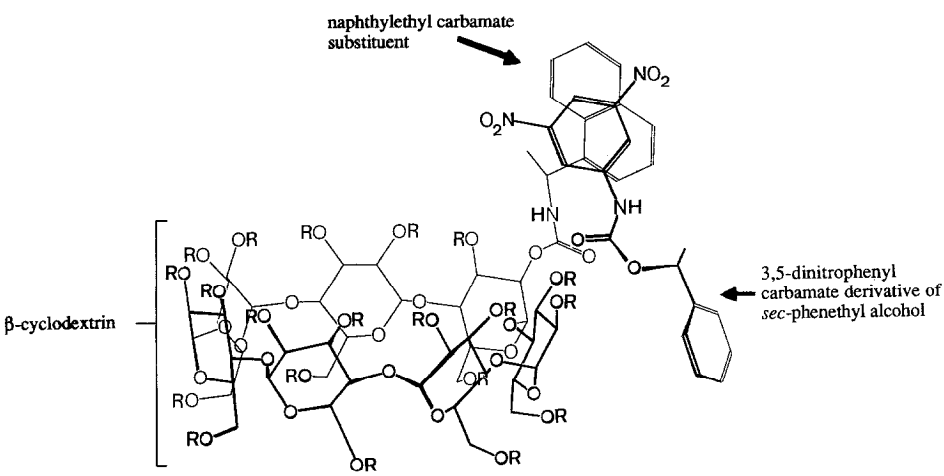


Fig. 3. Schematic illustrating likely π–π and dipole stacking interactions between the 3,5-dinitrophenylcarbamate derivative of *sec*-phenethyl alcohol and the naphthylethyl carbamoylated β-cyclodextrin stationary phase (R = H or naphthylethyl carbamate). Although only one naphthylethyl carbamate substituent is shown, the phases used in this study had 3 to 8 substituents. Other orientations include positioning the phenyl ring on the solute over or in the cyclodextrin cavity resulting in a variety of interactions which may contribute to chiral recognition.

The degree to which the CD contributes to the overall chiral recognition mechanism may be inferred from the selectivity obtained on the RAC column. At one extreme, it is possible for the chirality of the CD substituents (naphthylethylcarbamate) to totally dictate enantioselectivity with essentially no contribution from the CD (*e.g.* DL-norleucine, DL-leucine, DL-3-(1-naphthyl)-alanine, DL-tryptophan). In these cases, no separation can be obtained on the RAC column. This seemed to be the mode of chiral recognition for 15 of the 49 compounds used in this study. As to be expected for this mode of enantioselectivity, it was found that the α values for these solutes were comparable on the R and HS columns, which have almost the same degree of CD substitution (Table I). This mode of chiral recognition would also be expected to lead to reversal of the elution order on the R column relative to the S and HS columns. This seems to be the case for the solutes in which the configuration is known (*e.g.* DL-threonine methyl ester, DL-norvaline, DL-norleucine, 1,2,3,4-tetrahydro-1-naphthol). In the case of the native CD phases, the enantiomeric elution order is usually difficult to predict and often not readily reversible. The incorporation of additional stereogenic centers on the CDs now surmounts this limitation in many instances.

In some cases, it is also possible for the base CD moiety to totally dominate chiral recognition, with little or no enantioselective contribution from the stereogenic substituents. The presence of the aromatic substituents on the CD may lead to retention but not necessarily enantioselectivity. In this case, elution order and α would be unaffected by the configuration of the substituents and would be similar on the R, HS and RAC columns because they have similar degrees of substitution. Five solutes are reported for which the configurations were known and elution order was found to be independent of the configuration of the CD substituents (1-cyclohexylethylamine, DL-phenylalanine, DL-homophenylalanine, DL-valine and DL-isoleucine).

Another possible chiral recognition mechanism is for both the CD and the CD substituents to contribute (in varying degrees) to chiral recognition. The chiral selectivity of the CD and one configuration of its substituent may be complementary (synergistic), leading to enhanced chiral recognition on one column relative to the RAC column or the column having the opposite configuration. In contrast, the oppositely configured substituent may have little effect or the opposite effect (antagonistic) of the CD moiety thereby diminishing the overall stereoselectivity of the phase and resulting in reduced stereoselectivity relative to the RAC column or the column having the opposite configuration. This mismatch of selectivity between stationary phases having roughly the same degree of substitution seemed to be the most common effect observed on these phases. Note that for some of these compounds (*e.g.* 1,3-dimethylbutylamine, 4-methyl-2-pentanol, DL-valine, 2-pentanol), the selectivity obtained on the RAC column was approximately the average of the selectivity obtained on the synergistic phase and the antagonistic phase. In some cases (*e.g.*, 1,5-dimethylhexylamine, DL-alanine, DL-O-methyltyrosine, 3-hexanol), the mutually antagonistic contributions to chiral selectivity from the chiral naphthylethyl carbamate substituent and the CD resulted in no separation on the "enantiomerically-pure" substituted CD phase. In general, elution order is less predictable for this mixed-mode of chiral recognition because it is dependent upon the relative contribution to enantioselectivity of the CD and the CD substituent.

Amines

In general, it is apparent that the best overall selectivities are obtained when the carbons α to the stereogenic center are 2° or 3° (*e.g.* 1,3-dimethylbutylamine *vs.* 2-amino-3,3-dimethylbutane; 2-aminoheptane *vs.* 3-aminoheptane; 1-cyclohexylethylamine). It is interesting to note, as well, that for the benzyl and naphthyl amines there is a reversal of elution order between the R and the S columns while the elution order remains the same for 1-cyclohexylethylamine on all four columns. This implies a difference in the retention and chiral recognition mechanism. The chirality of the R-CD substituents is complemented by the chirality of the CD, leading to enhanced enantioresolution for 1-cyclohexylethylamine on the R column. In contrast, for the aromatic amines, the enantioselectivity of the S-CD substituent is complemented by the chirality of the CD, leading to enhanced chiral recognition on both of the S columns. In the case of the aromatic amines, the RAC column exhibits the lowest stereoselectivity; therefore, the carbamate substituent is largely responsible for the chiral recognition of these compounds. This conclusion is supported by the reversal of elution order for these solutes obtained on the S columns relative to the R column.

Amino esters

In contrast to the homologous alcohols, increasing chain length on the amino esters clearly results in increasing selectivity on all four columns, as can be seen in Fig. 4 for the tryptophan esters. The same holds true for alanine methyl and ethyl ester. In all cases where known, the L enantiomer elutes first on the R column but the D enantiomer elutes first on all the other columns. For the aromatic esters, both the CD and the naphthylethyl group contribute to selectivity but the chiral co-recognition of the CD and the naphthylethyl moiety is less effective on the R column than on the S or RAC columns. For the aliphatic amino esters, however, the configuration of the CD substituent seems to be much more determinant for chiral recognition. The presence of the hydroxyl group on tyrosine methyl ester leads to increased selectivity relative to phenylalanine methyl ester but reduced stereoselectivity for serine methyl ester relative to alanine methyl ester.

Amino acids

For the aliphatic amino acids, enantioselectivity was only observed when using acetic acid–acetonitrile–ethanol mobile phases. This is no doubt due, in part, to the reduced mobile phase competition, relative to the acetic acid–methanol mobile phase,

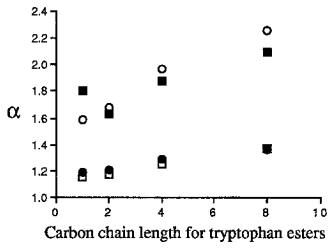


Fig. 4. Plot showing the effect of tryptophan ester chain length on selectivity (α) for the 3,5-dinitrobenzamide derivatives. □ = R column; ○ = S column; ■ = HS column; ● = RAC column.

for the CSP hydrogen bonding sites. This becomes important for the aliphatic amino acids because the absence of an additional aromatic ring reduces the potential for π–π interactions and steric or repulsive interactions, thereby making chiral discrimination more difficult than for the aromatic amino acids. As for the amines, the more substituents off the α carbon on the aliphatic amino acid, the higher the selectivity (*e.g.* leucine *vs.* isoleucine). Note however, that for leucine, the elution order is reversed on the R column relative to other columns and the D enantiomer elutes first. In contrast, for isoleucine, L enantiomer elutes first on all columns. It is also interesting to note that the elution order for DL-phenylalanine is the opposite of DL-homophenylalanine on all four columns. In addition, neither compound exhibits inversion of elution order between the R and the S columns.

Alcohols

It is apparent that, for the alcohols, the more similar the substituents on the stereogenic center are, the less predominant is the enantioselectivity (*e.g.* 2-hexanol *vs.* 3-hexanol). Increasing the chain length of one substituent enhances selectivity only up to about C5 and thereafter diminishes selectivity on all but the racemic column (Fig. 5). In all cases, the highest selectivities for the alcohols were obtained on either the S or the HS column. For the aliphatic alcohols and 2-methoxy-2-phenylethanol, the lowest selectivities were obtained on the R column. In contrast, the lowest selectivities for the aromatic alcohols, *sec*-phenethyl alcohol and 1,2,3,4-tetrahydro-1-naphthol, were obtained on the RAC column. It is interesting to note that the apparent relative elution order for 2-methoxy-2-phenylethanol on each of the columns is the opposite of that obtained for *sec*-phenethyl alcohol and 1,2,3,4-tetrahydro-1-naphthol and that the selectivities for 2-methoxy-2-phenylethanol on each of the columns more closely parallels that obtained for the aliphatic alcohols than that obtained for the aromatic alcohols. The configuration of the most retained enantiomers of 2-methoxy-2-phenylethanol and *sec*-phenethyl alcohols have the same configuration with respect to steric bulk but the opposite configuration with respect to the Cahn–Ingold–Prelog convention.

Carboxylic acids

Although only a limited number of carboxylic acids were analyzed on these phases, some of the trends observed for the other classes of compounds, such as reduced retention with increasing chain length and better selectivity for aromatic compounds, seems to hold for this class of compounds as well.

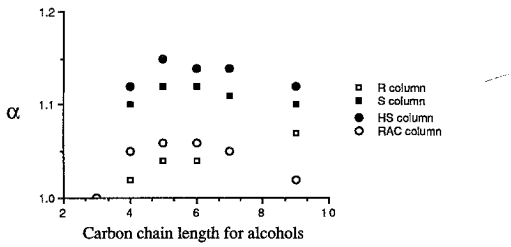


Fig. 5. Plot showing the effect of alcohol chain length on selectivity (α) for the 3,5-dinitrophenyl carbamate derivatives. □ = R column; ■ = S column; ● = HS column; ○ = RAC column.

CONCLUSIONS

In summary, carbamoylation of the CD moiety not only introduces new π–π interaction sites into the CSP but also additional hydrogen bonding sites and the potential for dipole stacking interactions as well. Consequently, it should not be too surprising that the resultant phases exhibit selectivities not unlike that obtained with the naphthylvaline-type CSP. The presence of stereogenic substituents on the derivatized CD moiety, in many cases, allows greater flexibility in control of the enantiomeric elution order than obtained on the native CD-CSP. In addition, the incorporation of the stereogenic substituents provides some valuable insight into the mechanisms responsible for chiral recognition on recently introduced derivatized CD CSP bonded phases which, unlike the native CD phases, exhibit enantioselectivity in the normal phase mode.

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