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REVERSED-PHASE CHROMATOGRAPHIC RESOLUTION OF N-(3,5-DINITROBENZOYL)-α-AMINO ACIDS ON CHIRAL STATIONARY PHASES

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SUMMARY

The enantiomers of N-(3,5-dinitrobenzoyl)-α-amino acids are readily separated by reversed-phase chromatography on chiral stationary phases. Lipophilic interactions are quite important and largely determine the relative contributions of two competing "opposite sense" chiral recognition processes. Selectivities are fairly high and, on chiral phase 2a, derived from (*R*)-N-(11-triethoxysilylundecanoyl)cyclohexyl(6,7-dimethyl-1-naphthyl)methylamine, are adequate for preparative separations as well as analytical separations. The (*S*)-enantiomers consistently elute last from the (*R*)-2a column. Chiral recognition models are proposed to accommodate the observed separability factors and elution orders.

INTRODUCTION

Much progress has been made in recent years in the development of chiral stationary phases for the direct chromatographic separation of enantiomers[1–3]. While many of these are biopolymers or synthetic polymers, a number are small chiral molecules anchored to a solid support. In this category are the silica-bonded α-arylalkylamine-derived chiral stationary phases reported recently by Ôi and co-workers[4,5] and by Pirkle and co-workers[6–8]. From the preliminary reports, it is evident that these chiral stationary phases are quite effective for the separation of the enantiomers of N-3,5-dinitrobenzoyl derivatives of α-amino acid esters and amines, amino alcohols, α-aminophosphonates, and amines.

Most separations to date have been performed using organic mobile phases (*e.g.* hexane–2-propanol). These chiral stationary phases can be used in the reversed-phase mode although the magnitude of α, the separability factor, usually diminishes in the more polar medium. One class of compounds which resolve nicely on some of these chiral stationary phases are N-(3,5-dinitrobenzoyl)-α-amino acids. The 3,5-dinitrobenzoyl group, important to chiral recognition, is easily appended and provides a strongly adsorbing chromophore to facilitate detection.

The α-arylalkylamine-derived chiral stationary phases are especially interesting from the mechanistic point of view, for they utilize multiple and competing chiral recognition mechanisms[7,8]. As a consequence, a change in mobile phase composition can alter the balance-point between competing mechanisms and possibly alter the

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elution order of the enantiomers. In this paper, we consider the reversed-phase resolution of N-(3,5-dinitrobenzoyl)-α-amino acids on the α-arylalkylamine-derived chiral stationary phases and consider explicitly the role played by lipophilic interactions in the observed chiral recognition.

EXPERIMENTAL

General

Chromatography was performed using an Altex 100A pump, an Altex 210 injector, and Altex model 165 variable-wavelength UV detector and a Kipp & Zonen BD 41 recorder. The preparation of chiral stationary phases 1b, 2a and 2b has been previously reported[6,7]. In this section, the preparation of chiral stationary phases 1a and 1c will be described. Chiral stationary phases were usually packed into 250 × 4.6 mm I.D. stainless-steel columns as a methanol slurry using conventional methods. Solutes used in this study were prepared by the procedure reported in an earlier paper[6].

Preparation of chiral stationary phase 1a

α-(6,7-Dimethyl-1-naphthyl)ethylamine. This amine was prepared by the reductive amination[9] of 6,7-dimethyl-1-naphthyl methyl ketone. Yield, 57%; ^{1}H NMR (C^2HCl_3) δ 1.52 (d, 3H), 1.67 (broad s, 2H), 2.43 (s, 3H), 2.47 (s, 3H), 4.85 (q, 1H), 7.13–7.60 (m, 4H), 7.80 (s, 1H). When 2 drops of ^2H_2O were added, the broad singlet at 1.67 ppm disappeared. IR (neat) cm^{-1} 3370, 3300, 3058, 3020, 2970, 2920, 2860, 1600, 1500. High-resolution mass spectrum calculated, for $C_{14}H_{17}N$: 199.1361; found: 199.1361.

N-(10-Undecenoyl)-α-(6,7-dimethyl-1-naphthyl)ethylamine. This amine was prepared from α-(6,7-dimethyl-1-naphthyl)ethylamine by the action of 10-undecenoyl chloride and triethylamine in methylene chloride in 99% yield. m.p. 97.0–98.5°C; ^{1}H NMR ($C^2HCl)_3$) δ 1.10–1.40 (m, 12H), 1.60 (d, 3H), 1.83–2.20 (m, 4H), 2.40 (broad s, 6H), 4.73–5.07 (m, 2H), 5.47–6.00 (m, 3H), 7.20–7.40 (m, 2H), 7.50–7.70 (m, 2H), 7.80 (s, 1H) IR (KBr) cm^{-1} 3300, 3080, 2980, 2920, 2850, 1630, 1540. Analysis: calculated for $C_{25}H_{35}NO$: C, 82.14; H, 9.65; N, 3.83; found: C, 82.25; H, 9.53; N, 4.13. This amide was resolved on a previously described preparation column.[10] High-R_F (*R*)-enantiomer: m.p. 107–108°C; $[\alpha]_D$ + 11.89 (c 0.19, CH_2Cl_2). Low-R_F (*S*)-enantiomer: m.p. 107–108°C; $[\alpha]_D$ − 10.85 (c 0.89, CH_2Cl_2).

(R)-N-(11-Triethoxysilylundecanoyl)-α-(6,7-dimethyl-1-naphthyl)ethylamine. This compound was prepared by hydrosilylation of the terminal double bond of the corresponding (*R*)-amide by the procedure described previously[6]. Yield, 53%; ^{1}H NMR (C^2HCl_3) δ 0.53–0.80 (m, 2H), 1.10–1.47 (m, 25H), 1.67 (d, 3H), 2.13 (t, 2H), 2.45 (broad s, 6H), 3.82 (q, 6H), 5.60–6.03 (m, 2H), 7.07–7.47 (m, 2H), 7.53–7.72 (m, 2H), 7.87 (s, 1H). IR (KBr) cm^{-1} 3293, 2975, 2925, 2860, 1630, 1540. High-resolution mass spectrum calculated for $C_{31}H_{51}NO_4Si$: 529.3555; found: 529.3571.

Chiral stationary phase 1a. This chiral stationary phase was prepared from (*R*)-N-(11-triethoxysilylundecanoyl)-α-(6,7-dimethyl-1-naphthyl(ethylamine by the procedure described previously[6]. Analysis: found: C, 7.988; H, 1.22; N, 0.20; Si, 42.01. Calculated: 0.14 mmoles of (*R*)-amide/g of stationary phase (based on N) and 0.25 mmoles of (*R*)-amide/g of stationary phase (based on C).

Preparation of chiral stationary phase 1c

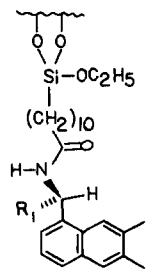
N-(10-Undecenoyl)cyclohexyl(6,7-dimethyl-1-naphthyl)methylamine. This amide was prepared by the action of 10-undecenoyl chloride and triethylamine on cyclohexyl(6,7-dimethyl-1-naphthyl)methylamine, the preparation of which was reported elsewhere[9], in methylene chloride in 92% yield. m.p. 118–121°C; ^{1}H NMR (C^2HCl_3) δ 1.00–2.30 (m, 27H), 2.42 (s, 3H), 2.48 (s, 3H), 4.80–5.10 (m, 2H), 5.60–5.90 (m, 3H), 7.23–7.42 (m, 2H), 7.53–7.70 (m, 2H), 7.95 (s, 1H). IR (KBr) cm^{-1} 3300, 2925, 2855, 1638, 1545. Analysis: calculated, for $C_{30}H_{43}NO$: C, 83.09; H, 9.99; N, 3.13; found: C, 83.11; H, 10.05; N, 3.33. This amide was resolved on a preparative chiral column as previously described[6]. High-R_F (*R*)-enantiomer: m.p. 11–114°C; $[\alpha]_D$ −15.67 (c 0.49, CH_2Cl_2). Low R_F (*S*)-enantiomer: m.p. 110–112°C; $[\alpha]_D$ + 15.61 (c 0.66, CH_2Cl_2).

(R)-N-(11-Triethoxysilylundecanoyl)cyclohexyl(6,7-dimethyl-1-naphthyl)methylamine. This compound was prepared by hydrosilylation of the preceding (*R*)-amide by the procedure described previously[6]. Dense liquid: Yield, 52%; ^{1}H NMR (C^2HCl_3) δ 0.50–0.80 (m, 2H), 1.00–2,20 (m, 38H), 2.40 (s, 3H), 2.43 (s, 3H), 3.80 (q, 6H), 5.50–5.77 (m, 1H), 5.90–6.07 (d, 1H) 7.20–7.34 (m, 2H), 7.50–7.67 (m, 2H), 7.95 (s, 1H). IR (Nujol) cm^{-1} 2860, 1670, 1580. High-resolution mass spectrum, calculated, for $C_{36}H_{59}NO_4Si$: 597.4213, found: 597.4210. $[\alpha]_D$ −10.84 (c 2.62, CH_2Cl_2). The (*S*)-enantiomer shows $[\alpha]_D$ + 10.47 (c 1.06, CH_2Cl_2).

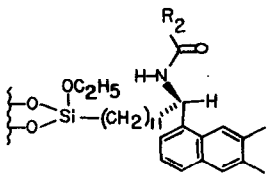
Chiral stationary phase 1c. chiral stationary phase 1c was prepared from (*R*)-N-(11-triethoxysilylundecanoyl)cyclohexyl(6,7-dimethyl-1-naphthyl)methylamine by the procedure described previously[6]. Analysis: found: C, 8.70; H, 1.15; N, 0.34; Si, 41.18. Calculated: 0.24 mmoles of (*R*)-amide/g of stationary phase (based on N) and 0.23 mmoles of (*R*)-amide/g of stationary phase (based on C).

RESULTS AND DISCUSSION

In our initial description of chiral stationary phase 1b[6], we noted that the elution order of the enantiomers of esters of N-(3,5-dinitrobenzoyl)-α-amino acids during direct-phase chromatography differs from that of the corresponding non-esterified acids during reversed-phase chromatography. For example, (*R*)-chiral stationary phase 1b selectively retains the (*R*)-enantiomers of the esters whereas it selectively retains the (*S*)-enantiomers of the acids. We have prepared several other



1 a R_1 = CH_3
b R_1 = Isopropyl
c R_1 = Cyclohexyl



2 a R_2 = CH_3
b R_2 = *tert.*-Butyl

TABLE I

REVERSED-PHASE RESOLUTION OF N-(3,5-DINITROBENZOYL)AMINO ACIDS UPON VARIOUS α-ARYLALKYLAMINE BASED CHIRAL STATIONARY PHASES

α = Separability factor; k' = capacity factor. Mobile phase: methanol–water (20:80) plus 0.2% $NaHCO_3$; flow-rate: 1 ml/min.

*α-Amino acids**	*Chiral stationary phase*					
	1a			*1b*		
	α	k'	*Configuration***	α	k'	*Configuration***
Alanine	1.00	1.5		1.28	1.0	*S*
Valine	1.08	2.7	*R*	1.31	1.2	*S*
Leucine	1.07	5.5	*S*	1.82	2.8	*S*
Isoleucine	1.05	3.9	*S*	1.51	2.3	*S*
Norleucine	1.00	6.3		1.51	5.0	*S*
Phenylglycine	1.59	7.1	*S*	1.90	3.3	*S*
4-OH-phenylglycine	1.76	4.5	*S*	1.77	2.6	*S*
Methionine	1.00			1.27	3.8	*S*
Phenylalanine	1.00	10.6		1.05	8.1	*S*
Tyrosine	1.00	4.2		1.00	4.3	
Tryptophan	1.00	18.9		1.00	12.3	

* N-3,5-Dinitrobenzoyl derivatives.

** Absolute configuration of the second eluted enantiomer.

chiral stationary phases in the α-arylalkylamine series and have investigated (see Table I) the reversed-phase chromatographic behavior of a number of N-(3,5-dinitrobenzoyl)-α-amino acids on these chiral stationary phases. Procedures for the preparation of chiral stationary phases 1b, 2a and 2b have been reported elsewhere[6,7]; phases 1a and 1c are prepared in analogous fashion. From Table I, one quickly notes that α, the separability factor, is enhanced as the R_1 group of the type 1 chiral stationary phases becomes larger (more lipophilic). Secondly, the type 2 chiral stationary phases, structurally similar to the type 1 phases, except that they are "turned" with respect to the silica, are superior in this application to the type 1 phases. Finally, of the two "turned" chiral stationary phases 2a and 2b, the one bearing the smaller acyl substituent, R_2, generally affords the larger α values. Overall, chiral stationary phase 2a affords, with but two exceptions, greater α values than any of the other phases.

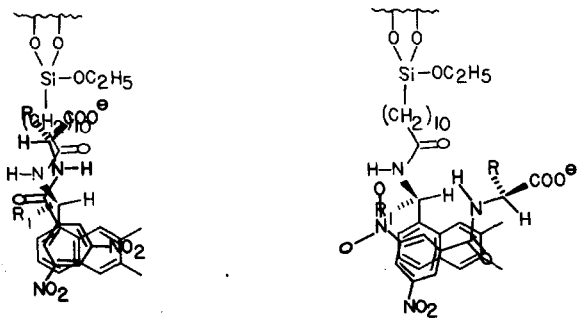
The rather systematic behavior encountered in this study can be explained in a manner somewhat analogous to that of the direct-phase behavior of the corresponding esters[6,7]. We believe that two competing chiral recognition processes are operative, that they have opposite enantioselectivities, and the extent to which each is operative is a function of the structure of the analyte, the chiral stationary phase, and to the composition of the mobile phase. The operative chiral recognition processes are though to be similar to those chiral recognition processes encountered during the direct-phase resolution but modified by additional solute–chiral stationary phase interactions, interactions which are encountered in aqueous mobile phases but are absent in non-polar organic mobile phases. For example, lipophilic interactions between the chiral stationary phase and the analytes provide strong bonding interactions that are essentially non-occurrent in non-polar mobile phases. These

1c			*2a*			*2b*		
α	*k'*	*Configuration***	α	*k'*	*Configuration***	α	*k'*	*Configuration***
1.62	0.8	*S*	1.62	1.1	*S*	1.71	0.8	*S*
1.36	2.2	*S*	1.80	1.6	*S*	1.54	1.7	*S*
1.85	5.2	*S*	2.79	3.0	*S*	2.46	3.7	*S*
1.64	3.3	*S*	2.22	2.2	*S*	1.96	2.5	*S*
1.97	5.2	*S*	2.59	3.1	*S*	2.13	3.7	*S*
2.19	4.4	*S*	2.92	3.4	*S*	3.18	3.5	*S*
2.28	1.9	*S*	3.25	2.0	*S*	3.60	2.0	*S*
1.64	2.6	*S*	2.26	1.7	*S*	1.98	2.0	*S*
1.25	8.8	*S*	1.84	4.7	*S*	1.30	5.9	*S*
1.21	2.5	*S*	1.69	1.8	*S*	1.26	2.3	*S*
1.13	12.7	*S*	1.57	7.9	*S*	1.27	10.8	*S*

lipophilic interactions provide a mechanism for the retention of both solute enantiomers and may also contribute to chiral recognition.

Fig. 1 illustrates the two competing chiral recognition processes that we suggest to be operative. The analyte enantiomer most strongly retained by each process is shown. The so-called "dipole-stacking" process[7,8] utilizes as associative interactions π–π interaction, electrostatic interaction between the stacked amide dipoles, and lipophilic interaction between the amino acid R group and the chiral stationary phase's connecting "arm" of methylene groups. The pH of the mobile phase is such that the amino acid carboxyl group is ionized, and this anionic group is directed away from the interactive strand of bonded phase and into the aqueous mobile phase, where it may be more effectively solvated. Imagine, in Fig. 1a, that the positions of the R group and the carboxylate group are interchanged (*i.e.* one is dealing with the adsorbate derived from the other analyte enantiomer). This results in loss of the lipophilic interaction and less effective solvation of the carboxylate group. Hence, this diasteromeric adsorbate is of higher energy than the one depicted and the analyte enantiomer incorporated into the high energy adsorbate will be less strongly retained than the enantiomer incorporated into the lower energy adsorbate.

Fig. 1 also depicts the competing "hydrogen-bonded" process. Here, the associate interactions are π–π bonding, hydrogen-bonding of the amino acid N*H* to the chiral stationary phase carbonyl oxygen, and lipophilic interaction between the amino acid R substituent and the R_1 substituent of the chiral stationary phase. Again, the polar carboxylate group is directed away from the chiral stationary phase and toward the polar mobile phase. Interchange of the carboxylate and R substituent, for the same reasons as just cited, should result in a higher energy adsorbate. Thus, the



Dipole-stacking process
(*R*)-enantiomer selectively retained

Hydrogen-bonded proces
(*S*)-enantiomer selectively retained

Fig. 1. Two competing chiral recognition processes for the reversed-phase resolution of N-(3,5-dinitrobenzoyl)-α-amino acids on type 1 chiral stationary phases.

dipole-stacking process selectively retains the (*R*)-enantiomers and the hydrogen bonding process selectively retains the (*S*)-enantiomers. What determines which of the two processes is dominant? In the dipole stacking process, the polar carboxylate group is intercalated between adjacent strands of bonded phase and presumably cannot be solvated as well as when it is more exposed to the mobile phase, as it is in the hydrogen-bonded process. Nevertheless, whatever contribution this solvation process may make toward dominance of the hydrogen-bonded process, it clearly is not the only factor. Consider the data in Table I for resolutions on chiral stationary phase 1a. Six of eleven amino acid derivatives are not resolved, those which resolve show small separability factors, and the (*R*)-enantiomer of N-3,5-dinitrobenzoylvaline is most retained whereas the (*S*)-enantiomers of the other resolvable analytes are most retained. This suggests that the two competing and opposing chiral recognition processes essentially cancel one another. As one increases the lipophilicity of the chiral stationary phase's R_1 group, the hydrogen-bonded process assumes greater importance. Chiral stationary phase 1b resolves nine of eleven amino acid derivatives [the (*S*)-enantiomers always being most retained] and phase 1c resolves all the amino acid derivatives [(*S*)-enantiomers retained] with improved separability factors. Although one can adduce additional arguments to disfavor the dipole-stacking process (*e.g.* long R groups on the analyte will have trouble intercalacting between adjacent strands of bonded phase), it would seem that lipophilic interactions need be invoked to rationalize the observed result. Thus, an increase in the lipophilicity of the chiral stationary phase's R_1 substituent causes increase dominance* of the hydrogen-bonded process and an increase in the magnitude of α, the separability factor. In type 2 chiral stationary phases, this R_1 group has been transformed into an even more lipophilic long chain of methylene groups. Hence, the superior performance of the

* The conformational rigidity of the chiral stationary phase achieved through use of a large R_1 group may also help the chiral recognition performance although this effect cannot yet be documented. However it must be noted that in direct-phase usage, the cyclohexyl group of chiral stationary phase 1c tends to suppress the hydrogen-bonding process because the size of this group leads to steric interaction with the alkoxy tails of the amino acid ester derivatives. For detailed descriptions of direct-phase resolution on chiral stationary phase 1c see ref. 11.

type 2 phases (compared to the type 1 phases) is attributable to enhanced lipophilic interaction and consequent enhanced contribution by the already dominant chiral recognition process.

In comparing the performance of chiral stationary phases 2a and 2b, we note that for the amino acid esters in organic mobile phases, the large *tert.*-butyl substituent of phase 2b tends to lessen the contribution of the dipole-stacking process (presumably for steric reasons) relative to the methyl substituent of phase 2a[7]. However, in an aqueous mobile phase, the greater bulk of the *tert.*-butyl group might be offset by its greater lipophilicity, and the strengthened lipophilic interaction with the R substituent of the analyte would tend to favor the minor dipole-stacking process. Hence, the reversed-phase performance of phases 2a and 2b tend to be more nearly comparable than is their direct-phase performance.

In conclusion, chiral stationary phase 2a has been shown to be quite effective for the reversed-phase separation of the enantiomers of N-(3,5-dinitrobenzoyl)-α-amino acids. Selectivities are such that both analytical- and preparative-scale separations might be conducted on this chiral stationary phase. Elution orders are consistent and are explained by a chiral recognition mechanism that takes into account specific chiral stationary phase-analyte interactions. These include not only π–π interactions, steric interactions and hydrogen-bonding interactions (which may occur in most mobile phases), but also the strong solvation of ionic groups and lipophilic interactions between alkyl substituents. These latter interactions are characteristic of aqueous mobile phases but not readily observed in organic mobile phases. Such mechanistic insight affords a basis for further optimization of this type of chiral stationary phase and is helpful, in general sense, in designing other chiral stationary phases intended for reversed-phase use.

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