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Use of homologous series of analytes as mechanistic probes to investigate the origins of enantioselectivity on two chiral stationary phases

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ABSTRACT

The normal-phase liquid chromatographic behavior of two homologous series of racemic carbamate derivatives of leucine anilides was examined on two chiral stationary phases, one of which has not been described heretofore. These chiral stationary phases are related structurally to the N-(3,5-dinitrobenzoyl)leucine chiral stationary phases originally developed in these laboratories, but differ from each other principally in the manner in which the chiral selector is attached to the silica gel support. While these selectors utilize the same interaction sites for bonding, the length of the analyte's linear alkoxyl substituent strongly influences enantioselectivity on only one of the chiral stationary phases. Similar but substantially less severe trends are noted as the length of a *p*-alkyl substituent on the second series of anilides is increased. Inferences concerning possible chiral recognition mechanisms are drawn from these observations.

INTRODUCTION

The use of chiral stationary phases (CSPs) to effect separation of the enantiomers of organic compounds has increased dramatically in the last decade (see, for example, ref. 1). For analytical applications, one desires a chiral selector which has a broad range of applicability but not necessarily a high degree of enantioselectivity. This will become increasingly the case as high-efficiency separation techniques (*e.g.* capillary electrophoresis) become more widespread. However, chiral selectors for preparative applications should show appreciable levels of enantioselectivity as well as broad applicability. The development of such chiral selectors is greatly facilitated by an understanding of the interactions involved in chiral recognition processes. Several of the broad-spectrum CSPs now available commercially have resulted from detailed investigations into the mechanistic basis for enantiodiscrimination. For example, the mechanism of chiral recognition between N-(3,5-dinitrobenzoyl)leucine derivatives and the esters and amides of N-(2-naphthyl)alanine (and related analytes) is believed to be fairly well understood, the proposed mechanism being supported by ^{1}H nuclear Overhauser effect studies [2] as well as by X-ray crystallographic data [3]. Guided by the mechanistic hypothesis, N-(aryl)amino acid-derived CSPs have been developed which show extremely high levels of enantioselectivity. In fact, separation factors exceeding one hundred have been observed in some cases.

To achieve high degrees of enantioselectivity, the selector must be tailored to have a high affinity for the more strongly complexed analyte enantiomer. In addition, the affinity of the selector for the less strongly complexed enan-

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tiomer should be minimal. This can be done, in part, by appropriate design of the selector. However, the enantioselectivity demonstrated by a particular selector is often influenced markedly by subtle factors. For example, we have previously demonstrated that the manner in which a chiral selector is linked to the underlying silica gel support can effect both retention and enantioselectivity [4,5].

Within a homologous series of analytes, enantioselectivity can be strongly dependent upon the length of a simple alkyl group in the analyte molecule. This is a clear demonstration that the alkyl group differentially influences the adsorption of the enantiomers. While relatively few such studies have been reported, we encounter this type of behavior fairly frequently. Mechanistic rationales accounting for this behavior can in many cases be tested by utilizing a modified version of the chiral stationary phase in which the selector presents the same interaction sites to the analyte enantiomers, but is "reoriented" so as to either exacerbate or relieve the interaction which causes enantioselectivity to be influenced by the length of the substituent in the analyte. Herein, we describe an instance of this type of behavior, offer a mechanistic rationale, and design a modified CSP to test the rationale.

EXPERIMENTAL

Chromatography

Chromatography was performed with an Anspec-Bischoff Model 2200 isocratic HPLC pump, a Rheodyne Model 7125 injector equipped with a 20-μl sample loop and two Milton Roy-LDC UV Monitor D fixed-wavelength detectors (254 and 280 nm) connected in series. The data was acquired using both a Kipp & Zonen BD 41 dual-pen chart recorder and a Hewlett-Packard 3394A recording integrator. Void volumes were determined using 1,3,5-tri-*tert.*-butylbenzene [6].

Chiral stationary phases

CSP I was prepared by a slight modification of a described procedure [7]. CSP II was prepared according to the scheme outlined in Fig. 1.

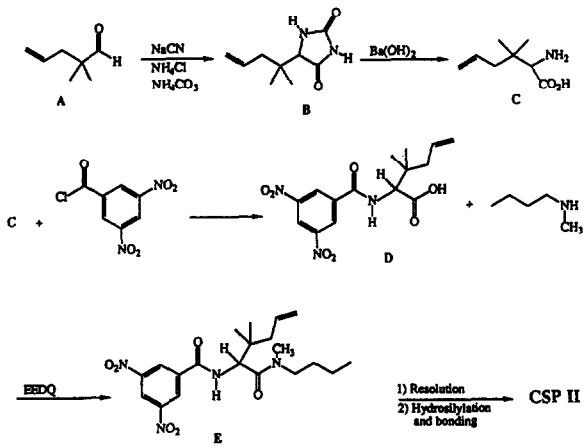


Fig. 1. Preparation of chiral stationary phase II.

2,2-Dimethyl-4-pentenylhydantoin (B)

To a solution of 1.15 g (23.4 mmol) of NaCN (poison!) and 1.26 g (23.5 mmol) of NH_4Cl in 10 ml of water, a solution of 2.50 g (22 mmol) of aldehyde **A** in 10 ml of methanol was added dropwise over 10 min. The addition funnel was rinsed with two 5-ml portions of methanol and the rinses were added to the reaction mixture. After 20 min, the reaction mixture was poured into a pressure bottle containing 10 g of NH_4CO_3 and sealed. The solution was heated on a steam bath and the progress of the reaction monitored by TLC using dichloromethane–diethyl ether (4:1). The R_F for the cyanohydrin is 0.80 and for the hydantoin is 0.10. After heating the reaction mixture for 6 h, it was cooled, and then concentrated under reduced pressure, diluted with 20 ml of water, and extracted with three 50-ml portions of dichloromethane. The combined organic layers were washed with two 20-ml portions of water, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was recrystallized from ethyl acetate:hexane to give 3.65 g of a while solid, yield 85%, m.p. 157–158°C. ^1H NMR (C^2HCl_3–[$^2H_6$]dimethyl sulfoxide, 6:1) 360 MHz δ 1.00 (m, 6H); 2.18 (m, 2H); 3.75 (s, 1H); 5.05–5.20 (m, 2H); 5.70–5.90 (m, 1H); 7.40 (br s, 1H); 10.20 (br s, 1 H). ^{13}C NMR (C^2HCl_3–[$^2H_6$]dimethyl sulfoxide, 6:1) 75 MHz δ 21.85, 22.08, 42.07, 64.65, 117.72, 132.20, 157.60, 174.10. Mass spectrum 70 eV *m/z* (relative intensity) 182(0.7), 100(100), 83(75), 55(96), 41(47). Analysis for $C_9H_{14}N_2O_2$:

calculated: C 59.32; H 7.74; N 15.37; found C 59.31; H 7.78; N 15.38.

2-Amino-3,3-dimethyl-5-hexenoic acid (C)

A stainless-steel tube was charged with 4.0 g (22 mmol) of **B**, 16 g (51 mmol) of $Ba(OH)_2$ octahydrate and 60 ml of water and sealed. The tube was placed in a tube furnace and heated to 145°C for 16 h. The tube was allowed to cool thoroughly, opened, and the contents were poured into a 500-ml beaker. The tube was rinsed with 100 ml of water, and the water rinses were added to the 500-ml beaker. Solid NH_4CO_3 was added until the precipitation of $BaCO_3$ was complete. The mixture was heated on a steam bath until gas evolution ceased, about 30 min. Celite was added to the warm reaction mixture and the solution was filtered. The filter cake was washed with two 20-ml portions of water and the combined filtrates were concentrated under reduced pressure. The residue was taken up in 100 ml of absolute ethanol and concentrated to dryness. Recrystallization from water–ethanol gave 2.80 g of a white solid, yield 80%. m.p. > 250°C. ^{1}H NMR ([$^2H_6$]dimethyl sulfoxide) 360 MHz δ 0.96 (s, 3H); 0.98 (s, 3H); 2.14 (m, 2H); 3.64 (d, 1H); 5.05–5.20 (m, 2H); 5.70–5.90 (m, 1H); 8.2 (br s, 2H); 14.00 (br s, 1H). Mass spectrum 70 eV *m/z* (relative intensity) 112(16.7), 83(25), 75(100), 55(61), 41(36). Analysis for $C_8H_{15}NO_2$: calculated: C 61.62; H 9.62; N 8.91; found C 60.94; H 9.68; N 9.08.

N-(3,5-Dinitrobenzoyl)-2-amino-3,3-dimethyl-5-hexenoic acid (D)

A 300-ml flask equipped with stir bar and nitrogen inlet was charged with 4.68 g (20 mmol) of 3,5-dinitrobenzoyl chloride, 3.10 g (19.5 mmol) of amino acid **C**, and 100 ml of dry tetrahydrofuran (THF). A solution of 1.6 ml (23 mmol) of propylene oxide in 40 ml of dry THF was added dropwise over a 30 min period to the cooled (0°C) heterogeneous reaction mixture. After an additional 30 min, the homogeneous reaction mixture was concentrated under reduced pressure and triturated with 50 ml of dichloromethane at 0°C to give 5.63 g of a white solid. A second crop of 0.53 g was obtained from the filtrate, yield 89%, m.p. 181–183°C. ^{1}H NMR (C^2HCl_3–[$^2H_6$]dimethyl sulfoxide, 20:1) 360 MHz δ 1.09 (s, 6H); 2.20 (m, 2H); 4.76 (d, 1H); 5.05–5.20 (m, 2H); 5.80–6.00 (m, 1H); 7.88 (m, 1H); 9.10 (m, 2H); 9.13 (m, 1H). Mass spectrum 70 eV *m/z* (relative intensity) 351(0.6), 251(10), 195(20), 149(15), 83(100), 55(93). Analysis for $C_{15}H_{17}N_3O_7$: calculated: C 51.28; H 4.88; N 11.96; found C 51.27; H 4.98; N 11.71.

N-(3,5-Dinitrobenzoyl)-2-amino-3,3-dimethyl-5-hexenoic acid methyl butyl amide (E)

A flask equipped with stir bar and nitrogen inlet was charged with 50 ml of dry dichloromethane, 1.44 g (5.8 mmol) of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ), and 1.50 g (4.3 mmol) of **D**. After stirring for 20 min at ambient temperature, 6 ml (58 mmol) of N-methyl butyl amine was added in one portion. A dark purple color developed, but faded to a pale yellow after 20 min. After this time, the reaction mixture was poured into a separatory funnel containing 100 ml of dichloromethane. The organic layer was washed sequentially with four 30-ml portions of 1 *M* HCl, 40 ml of water, and 40 ml of water–saturated NH_4Cl (3:1). The organic layer was dried with $MgSO_4$, filtered, concentrated under reduced pressure and dried *in vacuo* to give 1.65 g of colorless oil, 92% yield. Two amide rotamers are observed in the ^{1}H NMR in approximately a 60:40 ratio. ^{1}H NMR (C^2HCl_3) 200 MHz δ 0.90–1.70 (m, 14 H); 2.00–2.10 (m, 1H); 2.20–2.40 (m, 1H); 2.94 (s, 1.3H); 3.10–3.40 (2.7H); 3.40–3.80 (m, 1H); 5.10–5.30 (m, 3H); 5.80–6.10 (m, 1H); 7.40 (m, 1H); 9.00 (m, 2H); 9.20 (m, 1H). Mass spectrum 70 eV *m/z* (relative intensity) 420(4.9), 306(56), 195(59), 114(67), 95(100), 57(84), 44(68). Analysis for $C_{20}H_{28}N_4O_6$: calculated: C 57.13; H 6.71; 13.32; found C 57.09; H 6.70; N 13.09.

Resolution of the enantiomers of E

The enantiomers of **E** were separated by preparative chromatography on a 75 × 2.5 cm column containing the (*S*)-N-(1-naphthyl)leucine CSP bonded to 40 μm silica gel. A 2-propanol–hexane (30:70) mixture was used as the mobile phase. From mechanistic considerations, the first eluted enantiomer is assigned as (*R*)-**E**, and the second as (*S*)-**E**. Each enantiomer was obtained

as a colorless oil, and shown to be of greater than 99% enantiomeric purity by analytical HPLC. Each has a ^{1}H NMR spectrum identical to that of the racemate.

Preparation of CSP 3

An oven-dried 100-ml flask equipped with a stir bar, condenser and nitrogen inlet was charged with 10 ml of $(CH_3)_2SiHCl$, 10 ml of dry dichloromethane, 0.70 g of (*S*)-**E** and 30 mg of H_2PtCl_6, and the mixture was refluxed for 4 h. After this time, the mixture was concentrated to dryness and any residual $(CH_3)_2SiHCl$ was chased with two additional 30-ml portions of dry dichloromethane. The dark oil was diluted with 50 ml of dry ethyl ether and treated with 4 ml of triethylamine–absolute ethanol (1:1). The triethylamine hydrochloride which precipitated was removed by filtration using Celite. The filter cake was washed with two 10-ml portions of dry ethyl ether, the filtrate was concentrated and chromatogaphed on silica using dichloromethane–diethyl ether (10:1). The purified silane in 20 ml of dichloromethane was poured into a 100-ml flask containing 4.30 g of 5 μm silica which had been azeotropically dried with benzene. The solvents were removed under reduced pressure, and the silica and silane were heated at 110°C for 12 h with rocking on a Kugelrohr apparatus. After cooling, the modified silica was washed sequentially with 50 ml of methanol, 30 ml of ethyl acetate, 30 ml of dichloromethane and 30 ml of diethyl ether. The washed silica was slurry packed using methanol into a 250 × 4.6 mm I.D. stainless-steel HPLC column. After washing with 100 ml of dichloromethane, the CSP was endcapped using 2 ml of hexamethyldisilazane in 20 ml of dichloromethane. Elemental analysis of the residual CSP from the packing procedure showed a loading of 0.20 mmol/g based on C and 0.18 mmol/g based on N.

Synthesis of racemic analytes

All N-carbamoyl protected leucine derivatives were prepared by reaction of the racemic amino acid with the appropriate chloroformate using standard conditions [8]. In cases where the chloroformates were not commercially available, they were prepared by allowing phosgene to react with the appropriate alcohol in toluene [9].

All anilides were prepared from the corresponding N-carbamoyl protected amino acids by allowing the corresponding aniline to react with the amino acid in the presence of dicyclohexylcarbodiimide (DCC) in dry dichloromethane. All products were characterized at the N-protected amino acid stage by ^{1}H NMR and IR, and at the anilide stage by ^{1}H NMR and combustion analysis. All spectroscopic and elemental analysis data are in accord with the structures of the expected products.

RESULTS AND DISCUSSION

Linear series of racemic N-(*n*-alkoxycarbonyl)leucine-3,5-dimethylanilides and racemic N-(ethoxycarbonyl)leucine-*p*-alkylanilides were prepared (shown in Fig. 2), and their chromatographic behavior investigated on CSP I, an N-(3,5-dinitrobenzoyl)leucine-derived phase, and CSP II, an N-(3,5-dinitrobenzoyl)-"*tert.*-leucine"-like CSP, both shown in Fig. 3. Notice that while CSP II contains the same functional groups (interaction sites) as CSP I, CSP II is linked to the silica support via the amino acid alkyl sidechain and not through the C-terminal carboxamide, as is CSP I.

Plots of the chromatographic separation factor (α) *versus n* (the number of methylene units in the alkoxyl or alkyl substituent) are shown in Fig. 4 for the normal-phase separation of enantiomers of the N-(*n*-alkoxycarbonyl)leucine-3,5-dimethylanilides (Type 1 analytes) and the N-(ethoxycarbonyl)leucine-*p*-alkylanilides (Type 2 analytes) on CSP I.

On CSP I, the degree of enantioselectivity (*i.e.* α) afforded the enantiomers of the N-(*n*-alkoxycarbonyl)leucine-3,5-dimethylanilide analytes is highly dependent upon the length of the carba-

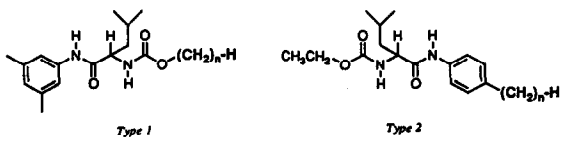


Fig. 2. Analytes prepared for use in the study with CSPs I and II.

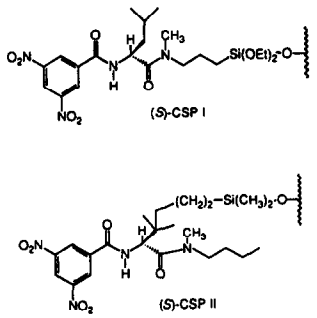


Fig. 3. Chiral stationary phases used in the investigation.

mate alkoxyl group, and is greatest when this group is long. Retention of both enantiomers decreases as the alkoxyl substituent is lengthened, but does so more quickly for the least retained enantiomer. Plots of the natural logarithm of the capacity factors, k', *versus* n (Fig. 5) show the hastened elution of the least retained enantiomer relative to the more retained enantiomer as n increases. In the nonpolar mobile phase used, this behavior suggests that unfavorable steric interactions are occurring to a greater extent for the least retained enantiomer, presumably as a consequence of the carbamate alkoxyl group having to intercalate between adjacent strands of bonded phase.

The use of Corey Pauling Koltung (CPK) space filling molecular models (Harvard Apparatus, South Natick, MA, USA) to explore possible structures of the more stable diastereomeric complex(es) between the CSP and the analyte [the (*S*)–(*S*) or (*R*)–(*R*) complex] leads one to conclude that interactions making the dominant contribution to the retention of the more retained enantiomer are similar to those postulated earlier for a different analyte–selector combination [2]. The essential bonding interactions are believed to be a face-to-face π–π interaction between the 3,5-dimethylanilide and the 3,5-dinitrobenzoyl systems, a hydrogen bond between the 3,5-dimethylanilide N–H and the C-terminal amide carbonyl oxygen of the CSP, and a hydrogen bond between the acidic 3,5-dinitrobenzamide N–H and the carbamate carbonyl oxygen. Cartoon-like representations of the selector and the analyte, intended to simplify aspects of the chiral recognition mechanism to be developed later, are introduced in Fig. 6.

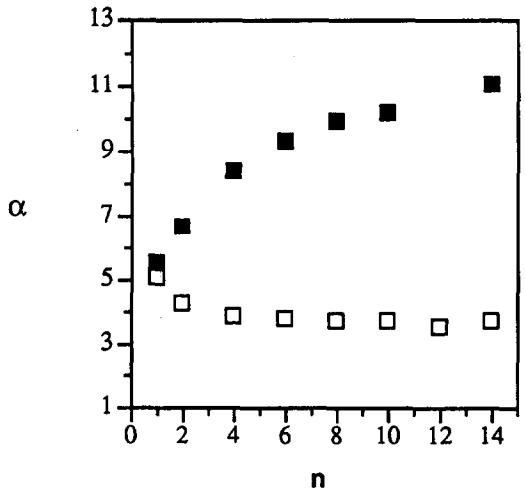


Fig. 4. Enantioselectivity (α) *versus* alkyl chain length (n) on CSP I; mobile phase consisting of 2-propanol–hexane (5:95, v/v). ■ = n-Alkoxy carbamates; □ = p-alkyl anilides.

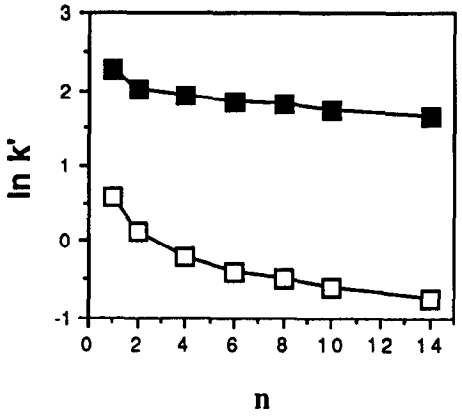
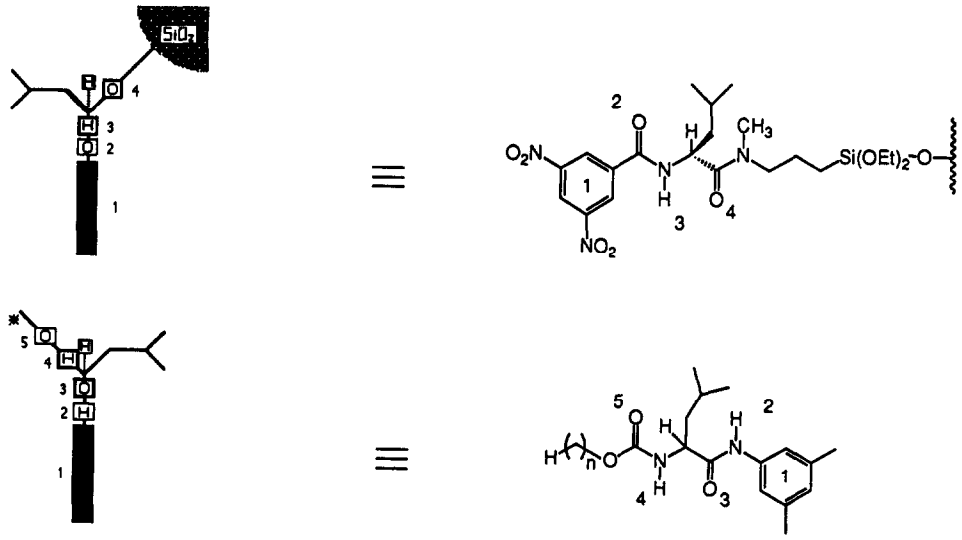


Fig. 5. Natural logarithm of k' (capacity factor) *versus* the number of methylene units in the alkoxyl substituent (n) for the Type 1 analytes on CSP I; mobile phase consisting of 2-propanol–hexane (5:95, v/v). □ = ln k_1'; ■ = ln k_2'.

From this model, depicted in Fig. 7, it may be seen that for the more retained enantiomer neither the alkoxyl groups of the Type 1 analytes nor the p-alkyl substituents of the Type 2 analytes are intercalated between the adjacent strands of the bonded phase.

In considering chiral recognition mechanisms, we have seldom made attempts to describe the structures of adsorbates which contribute to the retention of the least retained enantiomer. Often, these adsorbates are populated but a



Group is oriented toward the viewer

Group is oriented away from the viewer

* Location of lengthening alkoxyl group

Fig. 6. Legend to accompany Figs. 7 and 8.

small fraction of the time and are difficult to describe with confidence. While it has been suggested that the least retained enantiomer may utilize the same simultaneous bonding interactions as does its antipode [10], this is deemed unlikely when substantial enantioselectivity is encountered. If a retention mechanism (or combination of mechanisms) requires that the alkoxyl group of the least retained enantiomer be directed between adjacent strands of bonded phase and toward the silica support, this intercalation is expected, for steric reasons, to become increasingly unfavorable as the alkoxyl group is lengthened. This intercalation would be expected to hasten elution of the least retained enantiomer relative to its antipode, and likewise to do so to an increasing extent as the alkoxyl group becomes longer. The bonding interactions just invoked to explain the principle sources of retention for the more retained enantiomer do not, from study of the CPK models, seem to require any intercalation. Moreover, these bonding interactions do not appear to be simultaneously available to the least retained enantiomer. However, several alternate combinations of bonding interactions between the CSP and the least retained enantiomer can be envisioned, and some of these seemingly require intercalation of the alkoxyl group. Two conceivable structures for these least stable diastereomeric adsorbates which may arise from interaction of the least

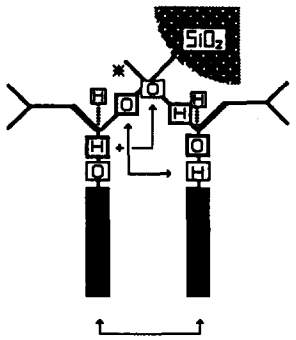


Fig. 7. Proposed chiral recognition mechanism for the more stable (*S*,*S*) diastereomeric complex between CSP I and the Type 1 and Type 2 analytes.

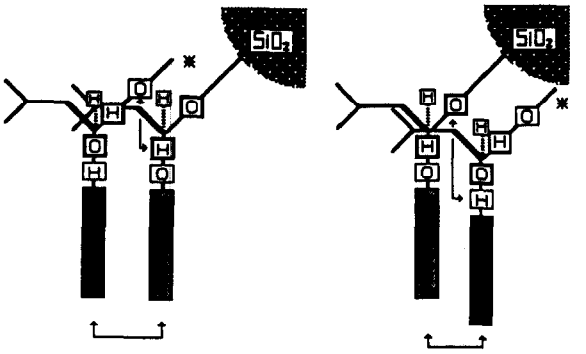


Fig. 8. Two hypothetical structures (chiral recognition mechanisms) for the less stable diastereomeric complex between CSP I and Type 1 and Type 2 analytes.

retained enantiomer with the CSP are depicted in Fig. 8. None of these combinations seem to entail intercalation of the *p*-alkyl substituents of the Type 2 analytes, consistent with the comparatively small effect the length of this substituent exerts on enantioselectivity. In the cases under discussion, the least stable diastereomeric adsorbates are heterochiral. That is, the analyte and the CSP bear dissimilar stereochemical descriptors [*e.g.* (*R*) and (*S*)].

In the first of the two bonding schemes depicted in Fig. 8, the carbamate alkoxyl group is directed alongside the tether and toward the silica support, an intercalative situation. In this representation, the least retained enantiomer is shown to approach the hindered face of the CSP, and in doing so undergoes two simultaneous bonding interactions (a face-to-face $\pi-\pi$ interaction, and a hydrogen bond involving the dinitrobenzamide N–H and the carbamate carbonyl oxygen). In the second representation, a face-to-face $\pi-\pi$ interaction and a hydrogen bond involving the anilide N–H and the carbonyl oxygen in the tether of the phase are invoked as bonding interactions. The strength of these interactions will be reduced since, owing to the difficulty of intercalation, the two components cannot approach one another as closely as in the case of the more stable complex. We hasten to point out that there is no experimental evidence that complexes like those depicted in Fig. 8 actually exist, and that these hypothetical bonding schemes are postulated to rationalize the experimental results. One must remember that the observed retention stems from a composite of many processes, some no doubt quite subtle. The *relative* importance of chiral recognition schemes like those represented in Fig. 8, and their respective contributions to the overall observed retention, is difficult to assess.

Retention for the Type 2 analytes falls off at more or less the same rate for both enantiomers throughout the series and α remains relatively constant as a consequence. The *p*-alkyl group in either enantiomer is directed away from the silica support and, consistent with the mechanistic picture developed, its length does not dramatically influence enantioselectivity.

To the extent that the proposed models represent reality, a chiral stationary phase which presents the same interaction sites to these analytes without requiring intercalation of any substituent should demonstrate enantioselectivity which is independent of the length of the substituent. CSP II was expected to be such a phase. Using the prior interaction schemes, neither enantiomer of the Type 1 analytes would be expected to intercalate its alkoxyl substituent between the strands of bonded phase and enantioselectivity should thus be rather constant throughout the homologous series. Considerations of the effect of the length of the *p*-alkyl substituent of the Type 2 analytes on enantioselectivity lead to similar expectations.

Plots of enantioselectivity (α) *versus* substituent length (n) for both homologous series of analytes on CSP II are shown in Fig. 9.

The chromatographic separation factor, α, for the enantiomers of the Type 1 analytes is always smaller on CSP II than on CSP I, and remains relatively constant throughout the series. Retention diminishes at more or less equal rates for both enantiomers as the carbamate alkoxyl substituent is lengthened. Elution order of the enantiomers of both types of analytes is the same on both CSP I and CSP II. These are precisely the observations expected on the basis of the preceding mechanistic rationale. CSP II affords less enantioselectivity for the Type 1 analytes than does CSP I because, in the absence of intercalation difficulties, the least retained enantiomers are retained longer and enantioselectivi-

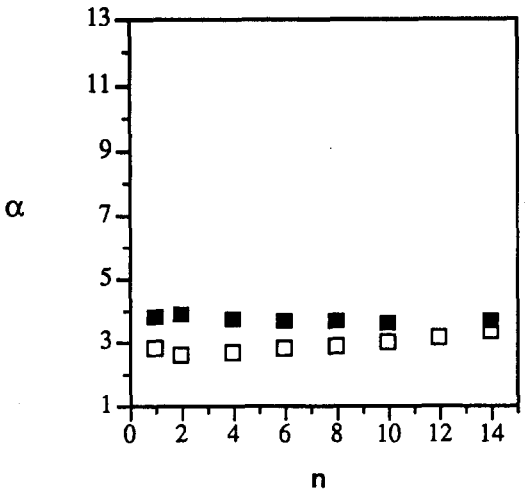


Fig. 9. Enantioselectivity (α) *versus* alkyl chain length (n) on CSP II; mobile phase consisting of 2-propanol–hexane (5:95, v/v). ■ = *n*-Alkoxy carbamates; □ = *p*-alkyl anilides.

ty is thus diminished. As predicted, enantioselectivity does not change appreciably throughout the homologous series of Type 2 analytes, since the *p*-alkyl substituents, according to the rationale, are intercalated on neither CSP I nor CSP II.

Note that the enantiomers of the Type 1 analyte having $n = 2$ show a greater separation factor on CSP 2 than do the enantiomers of the Type 2 analytes having $n = 1$ or 2. This is ascribed to the greater π-basicity of a 3,5-dimethylanilide relative to a *p*-toluamide or a *p*-ethylanilide. One expects, and typically finds, that similar amides derived from more π-basic arylamines (*e.g.* naphthylamines) show greater retention and enantioselectivity on CSPs I and II.

CONCLUSIONS

The enantiomers of homologous series of N-(*n*-alkoxycarbonyl)leucine-3,5-dimethylanilides and N-(ethoxycarbonyl)leucine-*p*-alkylanilides were separated on similar chiral stationary phases which differ principally in their respective modes of attachment to the silica gel support.

While the retention of each enantiomer results from the contributions of many different analyte-adsorbant interactions, the overall chromatographic behavior is rationalized in terms of one or two of the more important contributing mechanisms of retention. Different retention processes are proposed for each enantiomer. On CSP I, the most retained enantiomers of the N-(alkoxycarbonyl)leucine-3,5-dimethylanilides do not appear to intercalate the alkoxyl substituents between neighboring strands of bonded phase to as great an extent as do the less retained enantiomers. The longer the alkoxyl substituent, the more difficult the intercalation becomes. Consequently, the least retained enantiomer has its retention reduced relative to its antipode. This causes enantioselectivity to increase as the alkoxyl group becomes longer. On CSP II, neither enantiomer is thought to intercalate its alkoxyl group to any significant extent and enantioselectivity is essentially independent of the length of this substituent.

For the *p*-alkylanilide series, the length of the *p*-alkyl group has a modest effect on enantioselectivity on CSP I (long substituents diminish enantioselectivity slightly) and little effect on CSP II. In the former instance, one might assume that the *p*-alkyl substituents of the most retained enantiomers undergo some modest steric difficulty through encounter with neighboring strands of bonded phase. However, nothing more definite can be said about these interactions at present.

In principle, it should be possible to engineer a chiral stationary phase which, by virtue of its design, can use intercalative processes to advantage in enhancing enantioselectivity. Such investigations are currently underway.

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