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Contribution of specifiable hydrophobic interactions to chiral recognition

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ABSTRACT

The reversed-phase chromatographic behavior of several homologous series of racemic analytes was examined using chiral stationary phases (CSPs) derived from (*R*)-N-(2-naphthyl)alanine. For those analytes bearing an alkyl substituent on the stereogenic center, the degree of enantioselectivity is observed to increase as the length of this alkyl substituent is increased. This effect is attributed to a reduction in "wetted surface area" when the methylene chain "connecting arm" of the CSP contacts the alkyl substituent of the most retained analyte enantiomer owing to intercalation of this alkyl group between the strands of bonded phase. In these series, the alkyl substituents of the less retained enantiomers are differently oriented during interaction with the CPS and less effectively contact the bonded phase. Although hydrophobic interactions contribute the retention, they need not always contribute to chiral recognition as shown by other series of analytes. Cases may eventually be found where lengthening an alkyl substituent decreases enantioselectivity owing to greater hydrophobic interaction between the CSP and the initially eluted enantiomer.

INTRODUCTION

The retention of an analyte on a non-polar stationary phase during reversed-phase chromatography stems principally from the analyte being driven from the mobile phase into (or onto) the non-polar stationary phase so as to reduce the total "wetted surface area" in the system. In the case of enantiomeric analytes, hydrophobic effects provide equal motivation for each enantiomer to partition into (or onto) the stationary phase. However, it is necessary that there be contact between non-polar regions of both the analyte and stationary phase for such partitioning to occur, this contact being responsible for the reduction in "wetted surface area". On chiral stationary phases (CSPs), such contact is, in principle, dependent upon the stereochemistry of the analyte. Hence, enantioselective "hydrophobic interactions" might lead to differential retention of enantiomers. In most instances, such enantioselective hydrophobic interactions will be superimposed upon the more usual "polar effects" invoked to account for chiral recognition. In other words, hydrophobic interactions may be considered as just another type of intermolecular interaction which contributes to retention under reversed-phase conditions and *may* contribute to chiral recognition. Except for the pioneering efforts of Davankov [1] who has documented the contributions of hydrophobic interactions to chiral recognition during ligand-exchange chromatography, no mechanistic studies have been reported in which the role of these interactions in chiral recognition has been clearly defined. Davankov stands alone in the deliberate incorporation into the CSP of structural features intended to participate in enantioselective hydrophobic interactions. It is true that hydrophobic interactions are invoked as occurring during enantiomer separation on cyclodextrin [2] or protein-derived [3–5] CSPs. However, such effects are usually held to be the source of the major portion of the retention rather than the source of enantioselectivity. It seems fair to say that while hydrophobic effects promote analyte inclusion, differential contributions by hydrophobic interactions to the retention of enantiomers are usually not well understood

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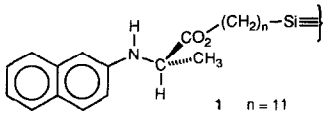
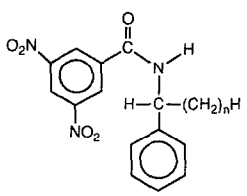
and, except for Davankov's work [1], cannot be said to be intentionally included into the design of the CSP.

RESULTS AND DISCUSSION

Because of our interest in chiral recognition mechanisms, we frequently examine homologous series of enantiomeric analytes by reversed-phase chromatography on the CSPs developed in our laboratories. It is found that retention increases as one proceeds through the homologous series but that enantioselectivity generally remains relatively constant. One prior instance of hydrophobic effects contributing to enantioselectivity was noted for an earlier π-basic CSP [6]. However, that system was but briefly studied.



1 n = 11
6 n = 3
7 n = 5



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The mechanistic basis by which CSP **1**, derived from N-(2-naphthyl)alanine, distinguished between the enantiomers of N-aroyl amino acid derivatives in normal-phase eluents is relatively well understood [7,8]. This CSP suffices to separate the enantiomers of many other compounds as well. For example, Fig. 1 shows the relation between n, the number of methylene units in the linear alkyl substituent of N-(3,5-dinitrobenzoyl)α-amino-α-phenyl alkanes, **2**, and the chromatographic separation factors of the enantiomers, α, when this homologous series of analytes is chromatographed on CSP **1** using either hexane–2-propanol (90:10) or methanol–water (90:10). In the normal mobile phase, α increases until n reaches 5, thereafter decreasing slowly. The retention of both enantiomers decreases as n increases (Fig. 2). In the reversed mobile phase, retention increases steadily (Fig. 2) as n increases as does the magnitude of α (Fig. 1). However, α is only 1.06 when $n = 3$ and increases to but 1.13 when $n = 17$, so the hydrophobic contributions to chiral recognition are quite small. Chromatography of similar series of analytes in which the aryl substituents are of greater hydrophobicity (*p*-anisyl, α-naphthyl, β-naphthyl) generates very similar α *vs.* n curves, retention increasing as expected. This type of observation has been encountered so frequently as to be surprising in the face of Davankov's persuasive reports of the contributions of hydrophobic interactions to chiral recognition [1]. If one truly understood how to utilize hydrophobic interactions to achieve chiral recognition, one might design CSPs capable of separating the enantiomers of relatively

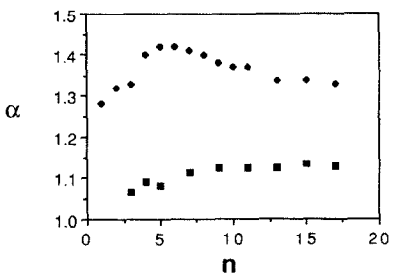


Fig. 1. Dependence of the separation factor, α, for the enantiomers of N-(3,5-dinitrobenzoyl)-α-amino-α-phenyl alkanes on the number of carbons, n, in the linear alkyl substituent. Column, CSP **1** (250 × 4.6 mm I.D.); mobile phases: ■ = methanol–water (90:10), flow-rate 1.0 ml/min; ◆ = hexane–propanol (90:10), flow-rate, 2.0 ml/min.

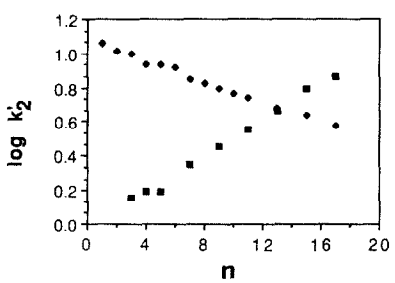
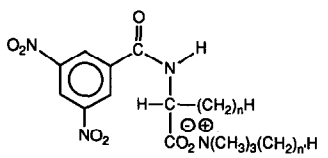


Fig. 2. Dependence of the logarithm of the capacity factor, k'_2, of the more retained enantiomer of N-(3,5-dinitrobenzoyl)-α-amino-α-phenyl alkanes on the number of carbons, n, in the linear alkyl substituent. Experimental conditions are the same as in Fig. 1. ■ = log k'_2 methanol–water; ◆ = log k'_2 hexane–2-propanol.

unfunctionalized compounds. Being purposely designed to operate under reversed-phase conditions, such CSPs might be useful to those wishing to monitor the enantiomeric composition of drugs or their metabolites in body fluids, possibly complimenting present-day cyclodextrin or protein-derived CSPs.

Recently, several homologous series of analytes were encountered which, when chromatographed on CSP **1** using a methanol–water mobile phase, do shown significant hydrophobic contributions to chiral recognition. These analytes, depicted in generalized form as **3**, **4** and **5**, were available from prior



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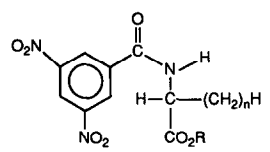

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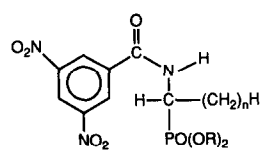

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studies. Some aspects of the reversed-phase chromatographic behavior of the type **3** ion pairs have been reported recently [9] as has the direct-phase chromatographic behavior of the ester analytes, **4** [7] and **5** [10]. It is important to note that, on CSP **1** using hexane–2-propanol (80:20), the separation factors for enantiomers of the type **4** and **5** analytes are fairly uniform within each series. Moreover, the enantioselectivity is relatively uninfluenced by the alcohol used to prepare the esters. This indicates that, under normal-phase conditions, intercalative effects, as noted for these analytes on other CSPs [11,12], occur to a minor extent on CSP **1**.

Under reversed-phase conditions, the number of methylene units in the alkyl substituent on the ana-

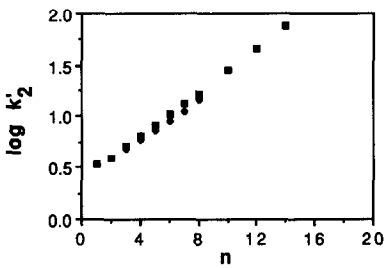

Fig. 3. Variation of the log k'_2 with the number of carbons in the linear alkyl substituent of (■) N-(3,5-dinitrobenzoyl)-α-amino acid ethyl esters and (◆) N-(3,5-dinitrobenzoyl-2-amino phosphonic acid dimethyl esters. Column, CSP **1**; mobile phase, methanol–water (80:20); flow-rate, 1.0 ml/min.

lyte's stereogenic center affects both retention (Fig. 3) and enantioselectivity (Fig. 4) of the ethyl esters of the type **4** α-amino acid derivatives and of the dimethyl esters of the type **5** 2-aminophosphonic acid derivatives. Similar observations are made for the enantioselectivity of the type **3** amino acid ion-pair derivatives (Fig. 5 and 6). Significantly, esters derived from these acids and higher alcohols (*n*-butanol, *n*-octanol) show the expected increase in retention under reversed-phase conditions but show no significant change in enantioselectivity relative to the corresponding ethyl esters. A similar observation was made recently when the hydrophobicity of the trimethylalkylammonium ion-pairing reagent was increased [9]. It is evident that, for these analytes, the hydrophobicity of the alkyl substituent on the stereogenic center influences enantioselectivity whereas the hydrophobicity of the alkoxyl or ammonium ion portions of these analytes has essen-

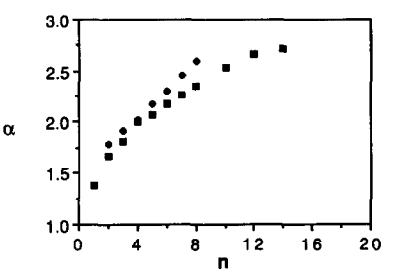

Fig. 4. Effect of the length of the alkyl substituent on the separation factor, α, for the enantiomers of (■) N-(3,5-dinitrobenzoyl)-α-amino acid ethyl esters and (◆) N-(3,5-dinitrobenzoyl)-2-amino phosphonic acid dimethyl esters. Column: CSP **1**; mobile phase, methanol–water (80:20); flow-rate 1.0 ml/min.

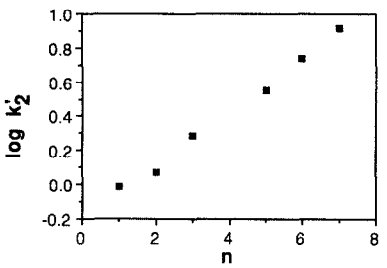

Fig. 5. Dependence of the logarithm of the capacity factor, k'_2, for the more retained enantiomer on the length of the linear alkyl substituent of the N-3,5-dinitrobenzoyl)-α-amino acids. Column: CSP **1**; mobile phase, methanol–water (70:30) with 5 m*M* octyltrimethylammonium phosphate with 0.01 *M* phosphate buffer, pH 6.86; flow-rate, 1.0 ml/min.

tially no such effect, influencing retention only. How may this be rationalized?

It is generally accepted that retention during reversed-phase chromatography stems principally from expulsion of a hydrophobic analyte from the aqueous mobile phase into the non-polar stationary phase. Enantiomers must undergo identical hydrophobic expulsion forces from the achiral mobile phase but, because they may differ in their "fit" to the CSP, may undergo differential hydrophobic interaction. "Fit' is a vague and unsatisfactory term, usually used in the absence of deeper understanding. In the present instance, "fit" is used to mean contact of hydrophobic portions of the analyte with hydrophobic portions of the stationary phase while (presumably) maintaining the stronger interactions normally used to attain chiral recognition. This contact reduces the surface area of these hydrophobic portions which must be wetted by the reversed mobile phase. This lower the energy of the system, contributing to the retention of the analyte. Some contacts occur equally well for either enantiomer, thus contributing equally to the retention of each. Whether or not a group contributes to chiral recognition through hydrophobic interactions bears upon the nature of the chiral recognition process and is of mechanistic relevance.

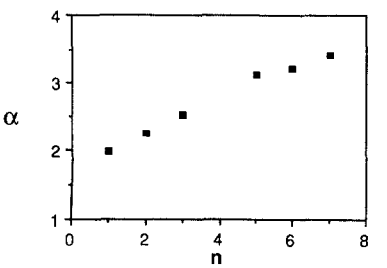

Fig. 6. Relationship between the separation factor, α, for the enantiomers of N-(3,5-dinitrobenzoyl)-α-amino acids on the length of the linear alkyl substituent. Experimental conditions are the same as in Fig. 5.

The elution orders of the enantiomers presently under discussion are the same using either direct or reversed-phase conditions. A chiral recognition mechanism has been advanced which is consistent with the observed elution orders [4], spectroscopic data [8], and the X-ray crystallographic structure [13] of a 1:1 complex of compounds similar in structure to the selector used in CSP **1** and to a type **4** analyte. The origin of the antioselectivity shown by CSP **1** toward the type **4** analytes is relatively well understood in non-polar solvents. It is reasonable to suppose that mechanistically similar processes operate under reversed-phase conditions *although they may be modified to some extent* by hydrophobic interactions, more extensive solvation of polar sites, and a weakening of the electrostatic components of some interactions owing to the higher dielectric constant of the medium. Although not now addressed, changes in the conformational preferences of either the analytes or the CSP or deep-seated changes in the structure of the bonded phase which occur with changes in the mobile phase composition might also influence chromatographic behavior.

The principal interactions occurring in the homochiral [*i.e.*, the (*R,R*) or the (*S,S*)] adsorbate formed from CSP **1** and a type **4** analyte are π–π interaction between the dinitrobenzoyl and naphthyl systems, hydrogen bonding of the dinitrobenzamide NH to the carbonyl oxygen of the CSP's C-terminal carboxyl group, and hydrogen bonding of the CSP's aniline-like NH to the analytes' C-terminal carbonyl oxygen. These interactions occur simultaneously and efficiently while the homochiral components are in low energy conformations. For these interactions to occur similarly in the heterochiral adsorbate, at least one of the components would have to assume a higher energy conformation. In hexane–2-propanol, the heterochiral adsorbates are formed to comparatively small extents. Consequently, their principal structures are uncertain. Similar mechanistic considerations are pre-

sumed to apply to the type **5** analytes where, owing to the Cahn–Ingold–Prelog [14] priority sequence, it is the heterochiral adsorbates which are most stable.

In the structures expected of the more stable diastereomeric adsorbates, study of space-filling models suggests that the alkyl group on the stereogenic center of the analyte parallels the methylene chain "connecting arm" linking the selector to silica. Although the structure of the adsorbate(s) formed from the less retained enantiomer is uncertain, we conclude that the analytes alkyl group is oriented differently. This inference is drawn from the chiral recognition mechanism and from the present observations. These suggest that the hydrophobic contribution to the chiral recognition of the type **3**, **4** and **5** analytes stems from contact of the methylene chains of the connecting arm of **1** with the alkyl substituent on the stereogenic center of the most retained analyte enantiomer. The alkyl group of the other enantiomer presumably is oriented differently and, when this group is short, does not efficiently contact non-polar regions of the CSP. As the alkyl groups of the less retained enantiomers become longer, they are better able to establish contact with a non-polar region of the stationary phase (presumably in the neighboring strands) and thus dimish the difference between the hydrophobic interactions undergone by each enantiomer. This leads to the "leveling-off" of enantioselectivity as shown in the α *vs. n* plots.

Why is the connecting arm of CSP **1** chosen as the site of the hydrophobic interaction which contributes to chiral recognition? This follows from the behavior of the ethyl esters of the type **4** analytes when these are chromatographed on CSPs **1**, **6** and **7**. These CSPs all use the same chiral selector but have, respectively, eleven, three, and five methylene groups in the connecting arms linking the selector to the silica. Fig. 7 presents plots of α, the separation factor for the enantiomers, *versus n*, the number of carbons in the linear alkyl substituents of the analytes as determined on CSPs **1**, **6** and **7**. The surface coverages of the three CSPs are similar although not identical. Hence, the vertical displacements of the curves may be influenced by the small differences in surface coverages [15]. However, it is the curve shapes which convey the information pertinent to the argument. Note that α initially increases on all three CSPs as the length of the alkyl substituent intercalated between (and presumably more or less parallel to) the strands is of a length comparable to that of the connecting arms. It appears that any further increase in the length of this group requires its reorientation owing to interaction with the underlying silica. The α *vs. n* curve from CSP **7** shows a pronounced slope change at $n = 5$, whereas the curve from CSP **1** shows no abrupt change in slope. Indeed, α is still increasing at $n = 14$. These curve shapes are clear indication of not only the intercalation of the alkyl substituent of the more retained enantiomer between the strands of bonded phase, but also demonstrate a lesser extent of intercalation by the less retained enantiomer.

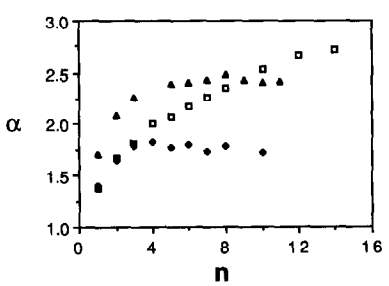

Fig. 7. The dependence of the separation factor, α, for the enantiomers of the ethyl esters of N-(3,5-dinitrobenzoyl)-α-amino acid on the length of the linear alkyl subsituent on three CSPs: □ = CSP **1**, ◆ = CSP **6**, △ = CSP **7**. Mobile phase, methanol–water (80:20); flow-rate 1.0 ml/min.

The data in this paper make it clear that the effect of hydrophobic interactions upon the enantioselectivity of a CSP is dependent upon the relative orientations and proximities of the nonpolar moieties involved. The hydrophobic interactions reported herein either enhance both retention and enantioselectivity or simply increase retention without altering enantioselectivity. Clearly, it should also be possible for hydrophobic interactions to *decrease* enantioselectivity. All that is required is that these interactions occur to a greater extent in the least retained enantiomer rather than the most retained enantiomer.

One additional comment on mechanistic differences between non-polar and aqueous mobile phases will be made. Many of the analytes and all of the CSPs we utilize have bulky groups projecting from one "face" so as to control, through steric interactions, the preferred mode of "face-to-face" ap-

proach. These bulky groups are typically hydrophobic. One must wonder whether in reversed-phase solvents, hydrophobic interactions between these groups and non-polar groups in the analytes might lessen the ability of the bulky groups to bias the mode of "face-to-face" approach, thus contributing to the reduction in enantioselectivity which generally accompanies a change from a non-polar to a reversed-phase eluent.

ACKNOWLEDGEMENTS

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