

CHROM. 25 011

High-performance chiral displacement chromatographic separations in the normal-phase mode

I. Retention and adsorption studies of potential displacers developed for the Pirkle-type naphthylalanine silica stationary phase

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(First received December 29th, 1992; revised manuscript received February 23rd, 1993)

ABSTRACT

Three families of potential displacers have been designed and synthesized for use with the Pirkle-type naphthylalanine silica stationary phases operated in the normal-phase mode. The displacers contain a π -acid head group (3,5-dinitrobenzoate, 3,5-dinitrobenzamide or 3,5-dinitrophenylcarbamate groups), an H-bonding mid-section (C=O and/or NH groups) and a solubility adjusting tail section (*n*-alkyl groups). By varying the length of the alkyl chain between methyl and tetracosanyl, a large number of homologous compounds were synthesized in each family. The capacity factors of the homologues were measured in *n*-hexane–tetrahydrofuran eluents; the k' values vary regularly with both the tetrahydrofuran concentration of the eluent and the length of the alkyl chain. The individual excess adsorption isotherms of the homologues were also determined and the isotherm parameters were correlated with the length of the alkyl chain. Because the capacity factors and the adsorption strengths of these compounds cover a broad range, they can serve as displacers in a variety of normal-phase chiral displacement chromatographic separations.

INTRODUCTION

The N-(2)-naphthyl-alaninate silica, a π -electron donor stationary phase developed by Pirkle and co-workers [1–5] is commonly used for

chiral separations, which are accomplished in the normal-phase mode with *n*-hexane–tetrahydrofuran eluents. In accordance with Dalglish's three-points-of-interaction rule [6], this stationary phase can be used to separate the enantiomers of solutes which contain a π -acid functional group (most often a 3,5-dinitrobenzoyl or 3,5-dinitrophenylcarbamoyl group) and an H-donor or H-acceptor group. Earlier, we published an HPLC method [7] for the analytical-scale separation of the enantiomers of platelet-aggregating

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factor analogues and 1,2-*O*-dihexadecyl-*rac*-glycerol ether derivatives which used the N-(2)-naphthyl-alaninate silica Pirkle-type phase. The success of this analytical separation prompted us to try to develop an analogous preparative-scale displacement chromatographic separation method to produce the individual glycerol ether enantiomers in quantities sufficient for the determination of their respective physico-chemical and pharmaco-chemical properties.

Because the principles of chiral displacement chromatographic separations have been recently discussed [8–10], they are not repeated here and the reader is referred to them for an in-depth review. The greatest hindrance to the development of any displacement chromatographic separation is the lack of readily available displacers and the paucity of the relevant adsorption isotherm data. Therefore, we have synthesized a large number of potential displacers to be used for chiral separations in the normal-phase mode and characterized their retention and adsorption behavior on the naphthylalanine silica stationary phase. The results of these studies are summarized in the present paper. The displacers were then used to develop a displacement chromatographic method for the separation of the enantiomers of the glycerol ether derivatives, the results of which will be discussed in Part II [11].

EXPERIMENTAL

Materials

A 250 mm × 4.6 mm I.D. Rexchrom column, packed with a 5- μ m D-naphthylalanine silica (DNAS) was obtained from Regis (Morton Grove, IL, USA) and used for the retention studies. Another, 100 mm × 2.6 mm I.D. column was slurry-packed in our laboratory [12] using another batch of the 5- μ m DNAS stationary phase (a gift by Dr. J. Perry of Regis). This small column was used for the adsorption isotherm determinations using the frontal chromatographic technique [13]. Both columns were equipped with water jackets and thermostatted by a UF-3 type recirculating water bath (Science and Electronics, Dayton, OH, USA) at 30°C, unless indicated otherwise.

HPLC-grade *n*-hexane (Baxter, Muskegon,

MI, USA) and tetrahydrofuran (Fisher Scientific, Fair Lawn, NJ, USA) were used for eluent preparation. Reagent-grade 3,5-dinitrobenzoyl chloride (DNB) was obtained from Aldrich (Milwaukee, WI, USA) and used as received. The mixtures of *n*-alcohols (ALFOL C₁₆–C₂₈) were obtained from Vista Chemical Co. (Houston, TX, USA); the other chemicals used were obtained from Aldrich, Eastman-Kodak (Rochester, NY, USA), Fisher Scientific or Wiley Organic (Cochocton, OH, USA), and used without further purification.

Apparatus

An isocratic liquid chromatograph consisting of a Type 2020 pump, a Type 2050 variable-wavelength UV detector (all from Varian, Walnut Creek, CA, USA), a pneumatically activated, computer-controlled Type 7000 injection valve (Rheodyne, Cotati, CA, USA) equipped with 10- and 50- μ l sample loops, and a Maxima 820 chromatographic work station (Millipore, Bedford, MA, USA) was used for the retention studies. A custom-built displacement chromatograph consisting of two Type 2020 pumps, a Type 2050 variable-wavelength UV detector and a Type RI-3 differential refractive index detector (all from Varian), as well as a pneumatically activated, computer-controlled Type 7000 injection valve (Rheodyne) equipped with 100- μ l to 5-ml sample loops, and a Type 7010 switching valve (Rheodyne) as described in ref. 12, was used for the adsorption isotherm determinations. A Type 4270 integrator (Varian), connected to a NEC Powermate I AT-compatible computer (Computer Access, College Station, TX, USA) and running the Chromplot-1 program developed in our laboratory [12], was used for system control, data collection and analysis.

Synthetic procedures

The details of displacer synthesis are described elsewhere [10]. Briefly, ester and amide-type displacers were synthesized according to the general Schotten–Baumann reaction schemes, while the carbamate-type displacers were obtained by reacting alcohols with 3,5-dinitrophenylisocyanate, generated *in situ* by thermal decomposition of the corresponding azide [5].

Adsorption isotherm measurements

The adsorption isotherms of the potential displacers were determined by frontal analysis using the small packed column. All isotherms were measured up to the solubility limit of the particular compound. In each successive step, the amount of displacer adsorbed on the stationary phase was calculated by the following equation:

$$q_i = [C_{mi}(V_m - V_0)] + q_{i-1} \quad (1)$$

where q_{i-1} is the amount of the compound adsorbed through the $(i-1)$ th step ($\mu\text{mole}/\text{column}$), C_{mi} is the mobile phase concentration (mM) of the compound in the i th step, V_m is the breakthrough volume (ml) of the concentration front in the i th step, and V_0 is the column hold-up volume (ml). The hold-up volume of the column was determined at 30°C by injecting an *n*-heptane solution into the eluent and recording the signal of the differential refractive index detector: it was 0.4 ml. The geometrical volume and the void volume were then used to calculate the void volume fraction and the nominal stationary phase volume [14] and resulted in a void volume fraction of $\varepsilon = 0.75$, a calculated phase ratio of $F = 0.333$ ($F = (1 - \varepsilon)/\varepsilon$), and a nominal stationary phase volume of $V_s = 0.133$ ml. With these values, both the mobile phase concentrations and the stationary phase concentrations could be expressed in mmol/l units.

RESULTS AND DISCUSSION

Retention studies of the potential displacers

The single most important factor that precludes the more widespread use of displacement chromatography is the lack of universally applicable displacers. The displacers must meet several contradictory criteria: they must have convex adsorption isotherms, be adsorbed more strongly on the stationary phase than the compounds to be separated, and be reasonably soluble in the carrier solvent to allow the preparation of concentrated displacer solutions. Furthermore, their chromatographic characteristics should, preferably, be similar to those of a large number of

different solutes over a wide range of experimental conditions.

Unfortunately, no single compound possesses all these characteristics. However, in the case of a Pirkle-type stationary phase, one can identify the intermolecular interactions that lead to the desired adsorption characteristics: carefully balanced π - π interactions, hydrogen bonding interactions and Van der Waals-type interactions. Thus, the generic structure of a displacer to be used on a Pirkle-type phase can be deduced: it should contain a π -acid (e.g. a 3,5-dinitrophenyl-) or π -base (e.g. a naphthylamine-) anchor group, a hydrogen-donor/acceptor group (e.g. an amide group), and a hydrophobic, solubility adjusting group (e.g. a long-chain alkyl group). Preferably, these groups should be readily attachable to a common core structure using inexpensive reagents and simple preparative methods.

In order to find broadly applicable displacers for the Pirkle-type phase, three series of homologous compounds were synthesized and studied in detail: the 3,5-dinitrobenzoyl esters of alcohols (DNB esters), the 3,5-dinitrophenylcarbamates of alcohols (DNP carbamates), and the *N*-3,5-dinitroamidoethyl-1-alkanoates (DNB amides).

The DNB esters with carbon numbers ranging from 1 to 28 were synthesized and their capacity factors were determined in tetrahydrofuran (THF)-*n*-hexane eluents at 30°C . The k' values are shown in Fig. 1 as a function of the alkyl

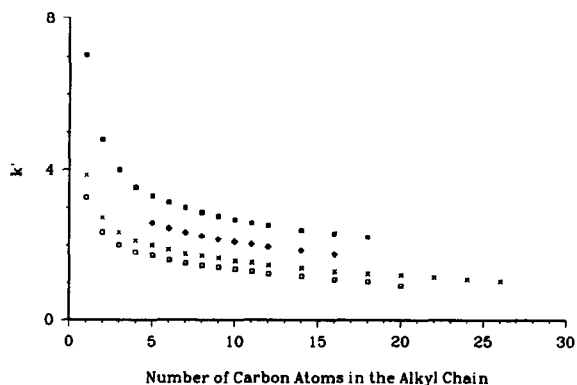


Fig. 1. The k' values of the DNB esters of *n*-alcohols as a function of the number of carbon atoms in their alkyl chain in different THF-*n*-hexane eluents at 30°C . Symbols: ■ = 2.5% THF; ◆ = 5% THF; × = 7.5% THF; □ = 10% THF.

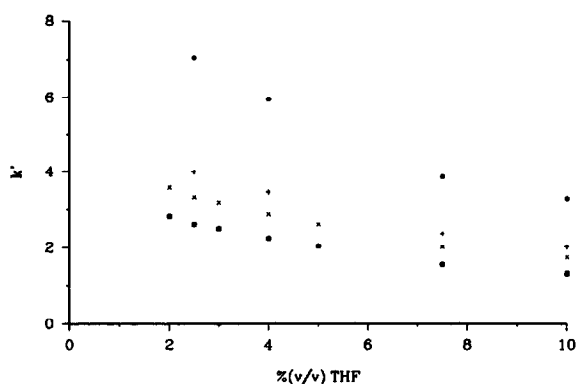


Fig. 2. The k' values of the DNB esters of *n*-alcohols with odd carbon numbers as a function of the % (v/v) THF concentration in different THF-*n*-hexane eluents at 30°C. Symbols: ● = C₁; + = C₃; × = C₅; ■ = C₁₁.

chain length. Initially, k' decreases very rapidly with the chain length. However, once the alkyl group becomes larger than hexyl, the change in k' becomes very small. This indicates that the first ten or so members of the DNB ester homologous series cover almost the entire of k' range that one can expect to see for these compounds. Therefore, the derivatives which have a very long alkyl chain will be of a lesser practical significance as displacers, and the disproportionately greater difficulties which are encountered when one tries to synthesize them in sufficiently high purity can be avoided.

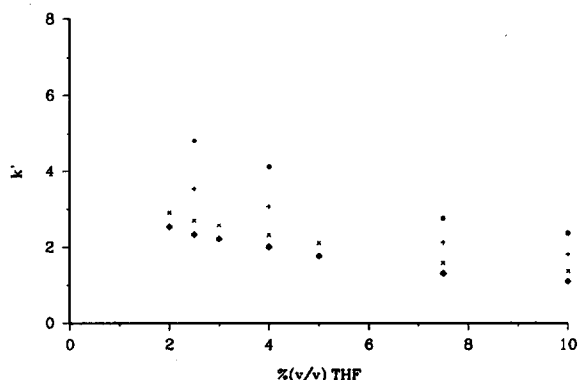


Fig. 3. The k' values of the DNB esters of *n*-alcohols with even carbon numbers as a function of the % (v/v) THF concentration in different THF-*n*-hexane eluents at 30°C. Symbols: ● = C₂; + = C₄; × = C₁₀; ◆ = C₁₆.

The k' values of the DNB esters are shown as a function of the THF concentration in Figs. 2 and 3 for the odd carbon number esters and the even carbon number esters, respectively. The k' values do not decrease with the THF concentration very rapidly, and the largest extrapolated capacity factor is less than about 20 (for the methanol derivative in pure *n*-hexane) indicating that the DNB esters do not form strong hydrogen bonds with the stationary phase.

DNP carbamate derivatives of *n*-alcohols were synthesized to produce more strongly retained displacers by increasing the hydrogen bonding opportunities with the additional NH group. The k' values of the DNP carbamates are shown in Fig. 4 as a function of the number of carbon atoms in the alkyl chain. A comparison of Figs. 1 and 4 shows that the DNP derivatives are indeed more strongly retained than the corresponding DNB esters, especially at low THF concentrations. Just as with the DNB esters, the capacity factor decreases very rapidly as the chain length increases from methyl to amyl, but from then on the decrease is slow, indicating that the promising displacer candidates are the methyl to decyl derivatives which can be synthesized in sufficiently high purity relatively easily. The k' values of the DNP carbamates are shown as a function of the THF concentration in Figs. 5 (odd number of carbon atoms) and 6 (even number of carbon atoms). A comparison of Figs. 2 and 5, and 3

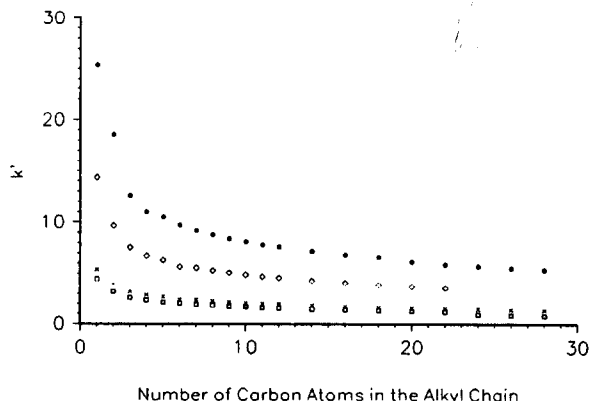


Fig. 4. The k' values of the DNP carbamates of *n*-alcohols as a function of the number of carbon atoms in their alkyl chain in different THF-*n*-hexane eluents at 30°C. Symbols: ● = 2.5% THF; ◇ = 4% THF; × = 7.5% THF; □ = 10% THF.

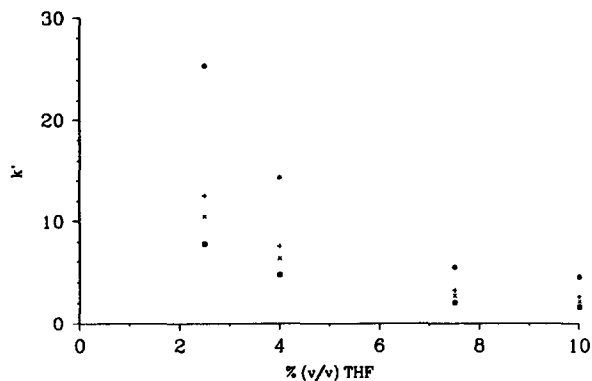


Fig. 5. The k' values of the DNP carbamates of *n*-alcohols with odd carbon numbers as a function of the % (v/v) THF concentration in different THF–*n*-hexane eluents at 30°C. Symbols: ● = C_1 ; + = C_3 ; × = C_5 ; ■ = C_{11} .

and 6 shows that the retention of the DNP carbamates decreases with the THF concentration much more rapidly than that of the DNB esters. The largest extrapolated capacity factor is about 50 (for the methanol derivative in pure *n*-hexane) indicating that the DNP carbamates do form strong hydrogen bonds with the stationary phase. The rapid decrease of the k' values brought about by the THF is quite understandable, considering that both the stationary phase and the THF cover the additional polar bonding site which is present in the DNP carbamates.

A third family of even more polar compounds was synthesized based on the bifunctional start-

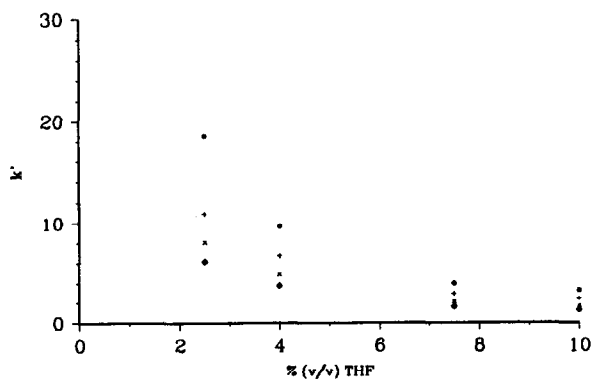


Fig. 6. The k' values of the DNP carbamates of *n*-alcohols with even carbon numbers as a function of the % (v/v) THF concentration in different THF–*n*-hexane eluents at 30°C. Symbols: ● = C_2 ; + = C_4 ; × = C_{10} ; ◆ = C_{16} .

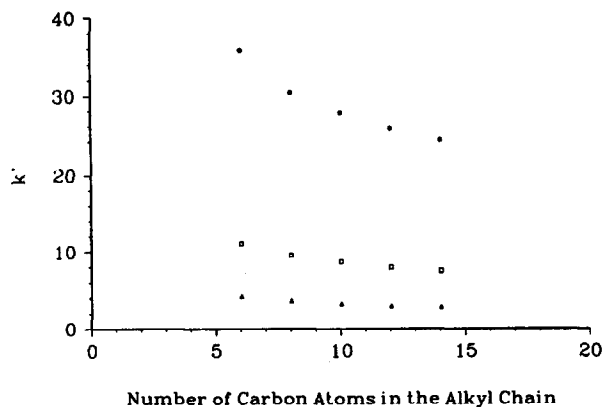


Fig. 7. The k' values of the N-3,5-dinitrobenzamidoethyl alkanoates as a function of the number of carbon atoms in their alkyl chain in different THF–*n*-hexane eluents at 30°C. Symbols: ● = 5% THF; □ = 10% THF; ▲ = 15% THF.

ing material, ethanolamine. The amino group was reacted with 3,5-dinitrobenzoyl chloride at subambient temperatures (0–4°C), followed by esterification of the hydroxyl group with *n*-alkanoyl chlorides (6–14 carbon atoms). The capacity factors of these compounds, measured in 5, 10 and 15% (v/v) THF in *n*-hexane eluents, are plotted in Fig. 7 as a function of the alkyl chain length, and in Fig. 8 as a function of the % (v/v) THF concentration. Much more than with the other homologous series, the k' values decrease rapidly with both the chain length and the THF concentration. These compounds are much more retained than either the DNB esters or the

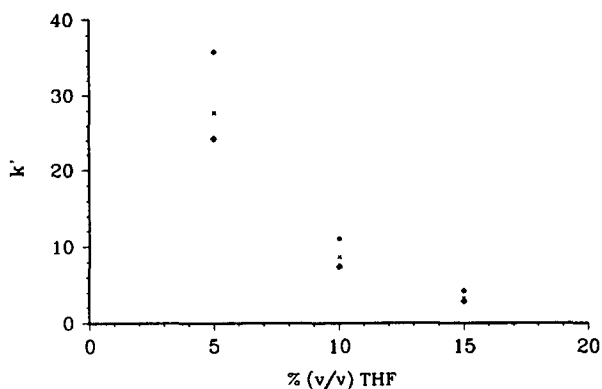


Fig. 8. The k' values of the N-3,5-dinitrobenzamidoethyl alkanoates as a function of the % (v/v) THF concentration in the THF–*n*-hexane eluents at 30°C. Symbols: ● = C_6 ; × = C_{10} ; ◆ = C_{14} .

DNP carbamates: the largest extrapolated capacity factor is around 100 (for the methanol derivative in pure *n*-hexane).

Since all members of these three homologous series display regular retention behavior and their k' values cover a sufficiently broad range, their adsorption isotherms were investigated to determine their utility as potential displacers for normal-phase chiral separations.

Determination of the adsorption isotherms

The individual excess adsorption isotherms of the DNB esters were determined by the breakthrough method using a carrier solution of 7.5% (v/v) THF in *n*-hexane at 30°C. Because the DNB esters are very soluble in this carrier solvent, quite high mobile phase concentrations could be tested. Some of the measured individual excess adsorption isotherms are shown in Fig. 9. The single-component, simple Langmuir isotherm equation was fitted to the measured data and the a and b isotherm parameters were evaluated using the least-squares method. The isotherm parameters were used to recalculate the individual excess adsorption isotherms, which are shown as solid lines in Fig. 9. There is a reasonably good fit between the measured isotherms (symbols) and the calculated isotherms (lines).

The logarithms of the isotherm parameters are linearly related to the number of carbon atoms in

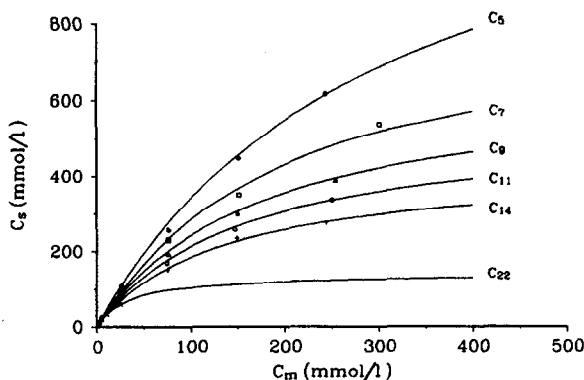


Fig. 9. Individual excess adsorption isotherms of the 3,5-dinitrobenzoyl esters of *n*-alcohols. Carrier: 7.5% (v/v) THF in *n*-hexane at 30°C. Symbols: ● = C_5 ; □ = C_7 ; △ = C_9 ; ○ = C_{11} ; + = C_{14} ; × = C_{22} .

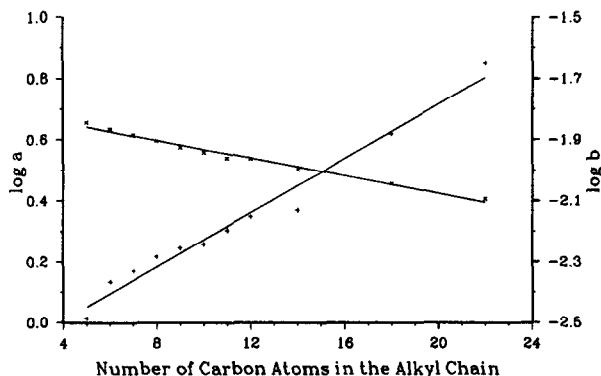


Fig. 10. The logarithm of the isotherm parameters a and b as a function of the length of the alkyl chain for the 3,5-dinitrobenzoyl esters of *n*-alcohols. Carrier: 7.5% (v/v) THF in *n*-hexane at 30°C. Symbols: × = $\log a$; + = $\log b$.

the alkyl chain, as shown in Fig. 10, and to each other, as shown in Fig. 11. This suggests that the adsorption strength in the family of DNB esters varies regularly with the structure of the compounds, and that quantitative structure–adsorption relationships, similar to the familiar quantitative structure–retention relationships [15] can be derived from these data.

The individual excess adsorption isotherms of the most polar displacer candidates, the DNB amides, are shown in Fig. 12. The measurements were limited to the 0 to 15 mM concentration range, because the solubilities of the DNB amides are low at 30°C in the 10% (v/v) THF in

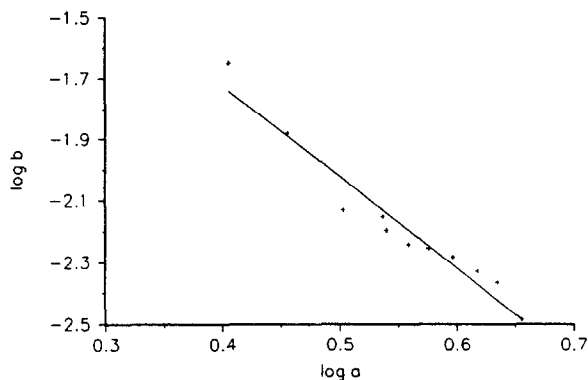


Fig. 11. $\log a$ as a function of $\log b$ for the 3,5-dinitrobenzoyl esters of *n*-alcohols. Carrier: 7.5% (v/v) THF in *n*-hexane at 30°C.

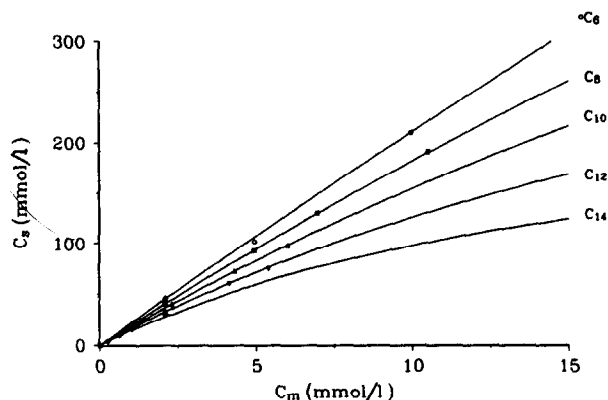


Fig. 12. Individual excess adsorption isotherms of the N-3,5-dinitrobenzamidoethyl alkanooates. Carrier: 10% (v/v) THF in *n*-hexane at 30°C. Symbols: $\circ = C_6$; $\square = C_8$; $\triangle = C_{10}$; $\nabla = C_{12}$; $\diamond = C_{14}$.

n-hexane solvent. The adsorption isotherm parameters, a and b , were determined as before and are shown in Figs. 13 and 14. It can be seen from Fig. 12 that the fit between the measured isotherms (symbols) and the calculated isotherms (solid line) is again reasonably good. The isotherm parameters, $\log a$ and $\log b$, are linearly related to the number of carbon atoms in the alkyl chain, as shown in Fig. 13, and to each other, as shown in Fig. 14. It can be seen by comparing the a parameters in Figs. 10 and 13 that the adsorption strength of the DNB amides is about five times higher than that of the DNB esters, and that the adsorption strength of the

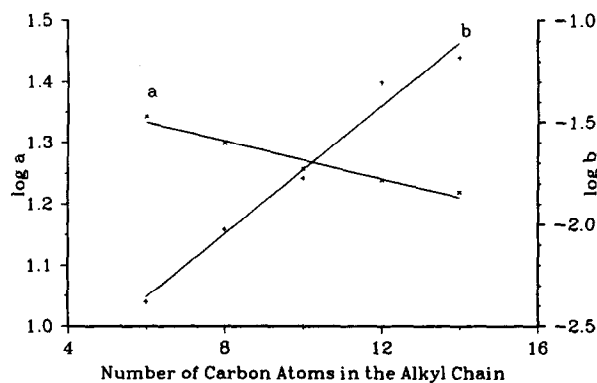


Fig. 13. The logarithm of the isotherm parameters a and b as a function of the length of the alkyl chain for the N-3,5-dinitrobenzamidoethyl alkanooates. Carrier: 10% (v/v) THF in *n*-hexane at 30°C. Symbols: $\times = \log a$; $+$ = $\log b$.

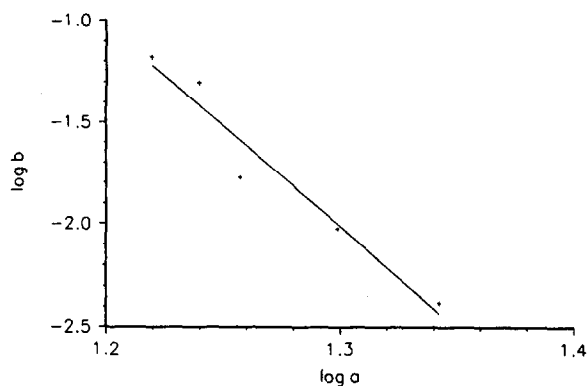


Fig. 14. $\log a$ as a function of $\log b$ for the N-3,5-dinitrobenzamidoethyl alkanooates. Carrier: 10% (v/v) THF in *n*-hexane at 30°C.

DNB amides decreases with the increasing carbon number more rapidly than the adsorption strength of the DNB esters.

Some of these displacers were successfully used for the development of chiral displacement chromatographic separations on Pirkle-type stationary phases, cyclodextrin-based stationary phases, and cellulose-based stationary phases [16]. These results will be discussed in Part II of this series.

CONCLUSIONS

Several displacer families covering a broad range of polarities (DNB esters, DNP carbamates and DNB amides) were designed and synthesized according to the concept of generic displacers. These displacers contain at least one anchoring group, one hydrogen bonding group and a solubility adjusting group. The retention and the adsorption properties of these displacers were determined and found to follow very regular patterns as a function of the length of their alkyl chain: longer chains result in smaller capacity factors and weaker adsorption. This regular adsorption behavior permits straightforward selection of a displacer for a Pirkle-type chiral stationary phase and eliminates most of the trial-and-error effort traditionally involved in the development of a displacement chromatographic separation.

ACKNOWLEDGEMENT

Partial financial support by the National Science Foundation (CH-8919151), the Texas Coordinating Board of Higher Education TATR Program (Grant No. 3376) and the Dow Chemical Company, Midland, MI to Gy.V., and by the Department of Energy, Office of Basic Energy Sciences (DE-FG 06-88 ER13963) to D.H.T. is gratefully acknowledged.

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