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Note

Non-ionic mobile phase dopants

I. Chiral charge-transfer acceptors and helicene resolution

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Reversed-phase liquid chromatography is the most widely used of the bonded-phase liquid chromatography modes. The wide range of applicability of this method has led to substantial commercial development efforts towards reproducible, stable column packing materials. It is therefore reasonable that much interest has developed concerning the design and use of mobile phase dopants with characteristics which adapt the reversed-phase high-performance liquid chromatographic method to otherwise difficult separation problems. Ion-pair reagents have become a popular tool for separating certain ionizable species and studies have been done to determine the separation mechanism[1]. Enantiomeric separation of amino acids in modified reversed-phase systems has been reported by several groups. Karger and co-workers[2–4] used chiral, metal-ion complexes with an alkyl side chain to provide affinity for the stationary phase. Using these dopants, they were able to separate both Dns and free amino acids by controlling the pH, mobile phase ionic strength, the nature of the metal ion in the complex, and the temperature. Gilon *et al.*[5] were able to resolve free amino acids using a chiral aspartylcyclohexylamide–Cu(II) ion complex, and recently Davankov *et al.*[6] reported the use of a chiral hydroxyproline–copper ion complex with an alkyl tail for this separation. Several complexes with varying tail lengths were used but the effect of length was not examined.

We report here the use of a non-ionic mobile phase modifier designed for a specific separation in the reversed-phase mode. A chiral dopant, N-(2,4-dinitrophenyl)-L-alanine-*n*-dodecyl ester (DNP-L-ALA-C_{12}) has been synthesized and used for the enantiomeric separation of 1-aza-hexahelicene (N^ 6). This dopant is analogous to the bonded phase previously described by Lochmüller and Ryall[7,8]. Other workers[9] have also developed techniques for helicene resolution using other bonded chiral charge-transfer agents. This new dopant takes advantage of the known ability of the DNP moiety to form charge-transfer complexes with polycyclic aromatic hydrocarbons and, through the addition of a chiral "site" near the DNP, resolves helicenes on the basis of a steric interaction[7–9]. The efficacy of this dopant is a function of both its concentration in the mobile phase and the mobile phase polarity.

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EXPERIMENTAL

The chiral dopant was synthesized as follows: L-alanine was reacted with *n*-dodecanol via a Fisher esterification using dry HCl gas. The resulting L-alanine dodecyl ester hydrochloride salt was neutralized with aqueous base, dried with anhydrous sodium sulfate, and reacted overnight at room temperature with an equivalent amount of 2,4-dinitrofluorobenzene in a mixture of tetrahydrofuran (THF) and water containing sodium bicarbonate to neutralize the resulting hydrofluoric acid. The THF was removed by rotary evaporation, and diethyl ether was added to the mixture to dissolve the product. The ether layer was separated and the product was recrystallized twice from ethanol–water to yield the final product. The non-chiral dopant 2,4-dinitro-N-dodecyl aniline (DNDA) was synthesized by reacting 2,4-dinitrofluorobenzene with an equimolar amount of *n*-dodecyl amine in an ethanol/water solution containing sodium bicarbonate. The solution was stirred at room temperature overnight. The resulting yellow oil was extracted into ether, dried with anhydrous sodium sulfate, and the product was recrystallized twice from ethanol–water. Purity was confirmed by thin-layer chromatography and proton magnetic resonance.

All chromatographic studies were carried out on a Varian 5000 liquid chromatograph equipped with a variable-wavelength UV–VIS detector and CDS-111 data collection system. A sampling valve with a 10-μl loop was used for all injections. The column (250 × 4.6 mm) was packed with 5-μm LiChrosorb RP-18 packing and thermostatted in a constant-temperature water bath at 26.40 ± 0.03°C. All mobile phase solvents used were glass distilled reagents and were used as received. The detector was operated at 254 nm for the undoped and at 290 nm for the doped experiments since the dopant has a minimal absorbance at that wavelength. System dead volumes at each set of conditions were determined by injection of ^2H_2O as recommended by McCormick and Karger[10]. Flow-rates were checked by timing the collection of eluent in a 10-ml buret.

RESULTS AND DISCUSSION

Fig. 1 is a plot of ln k' (k' = capacity ratio) *versus* percent water in methanol in the mobile phase for the chiral dopant DNP-L-ALA-C_{12}. It is seen that in the range of 15–20% water, k' for the dopant is quite large, and therefore, the hydrocarbonaceous stationary phase contains a significant amount of the charge-transfer agent in dynamic equilibrium. Conveniently, the N^6 has reasonable k' values in this composition range in both the doped and undoped experiments.

The retention data for N^6 in both the doped and undoped systems are listed in Table I, and the data has been plotted in Fig. 2. One effect which can immediately be noted is that for a given mobile phase composition, the retention volume for N^6 increased as the concentration of the dopant was increased. This increase in retention is proposed to be due to a charge-transfer interaction between the N^6 and the dopant in the stationary phase. The effect of increased retention with increased dopant concentration also appears to be a function of the mobile phase polarity, as the retention volume for N^6 increased more rapidly at 80% methanol than at 85% methanol. In addition to the increase in retention volume, enantiomeric separation of N^6 was observed at several dopant concentrations. The α values for these separa-

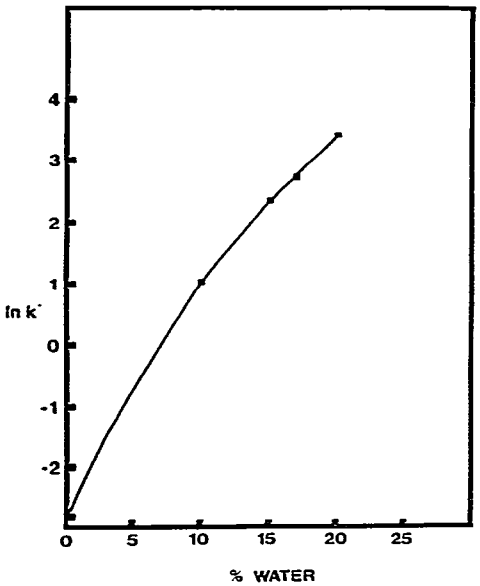

Fig. 1. Plot of ln k' of DNP-L-ALA-C_{12} ester *versus* percent water in the methanol–water mobile phase.

tions have been noted in parentheses in Table I along with the k' data. It can be seen that the α value increased as well when the mobile phase became more polar. However, at the maximum dopant concentration used in 80% methanol, all resolution was lost, and k' for the solute actually decreased. This phenomenon is not yet fully understood, but it is suspected that it may involve dopant–dopant interaction in or on the stationary phase.

TABLE I

k' FOR N Λ 6 AT VARIOUS DOPANT CONCENTRATIONS

Dopant concentration (mM)	*Methanol (%)*		
	85	*83*	*80*
0.0	1.91	2.38	3.77
0.50	2.74	3.66	6.36, 6.59 (1.04)
0.75	3.01	3.94, 4.07 (1.03)	7.19, 7.80 (1.08)
1.0	3.32, 3.41 (1.03)	4.82	5.25

The ability of this system to separate the enantiomers of N^6 is demonstrated in Fig. 3. Shown are three chromatograms of N^6 on a 5-μm ODS-C_{18} column. using three different mobile phase compositions containing the chiral dopant. Numerical data for these chromatograms are included in Table II. These data demonstrate

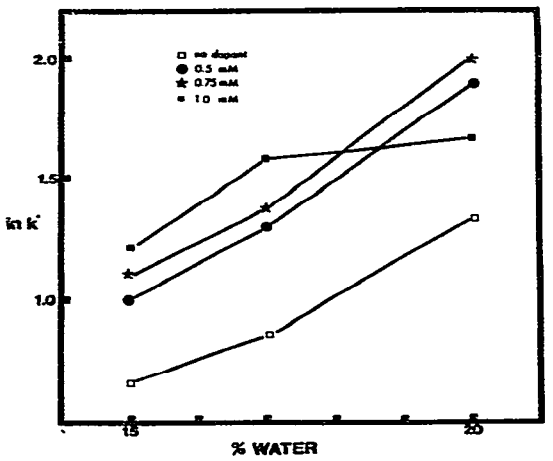

Fig. 2. Plots of ln k' of N^6 *versus* percent water in methanol–water mobile phase.

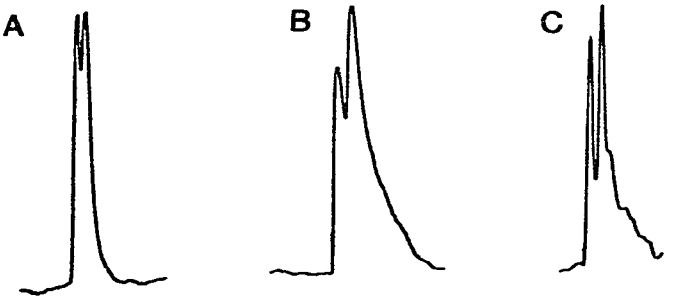

Fig. 3. Chromatograms of N^6 on Ultrasphere ODS-5 μm column with methanol–water mobile phase containing DNP-L-ALA-C_{12} ester. A, 85% Methanol containing 0.80 m*M* dopant; flow-rate 1.0 ml/min. B, 83% Methanol containing 0.70 m*M* dopant; flow-rate 1.0 ml/min. C, 82% Methanol containing 0.60 m*M* dopant; flow-rate 1.3 ml/min.

TABLE II

RETENTION DATA FOR N^6 ON ULTRASPHERE ODS-5 μm

V'_R = Retention volume (ml).

Mobile phase	V'_{R_1}	V'_{R_2}	α
85% Methanol, 0.80 m*M* dopant	12.15	12.54	1.039
83% Methanol, 0.70 m*M* dopant	15.16	15.94	1.060
82% Methanol, 0.60 m*M* dopant	19.29	20.87	1.091

the importance of determining the k' value of the dopant and the effect of changing the water concentration in the mobile phase. Attempts to improve the separation observed at 85% methanol by increasing the dopant concentration were only minimally successful. However, the α value for the separation increased noticably as the percent water in the mobile phase was increased, even though the concentration of dopant was concurrently decreased. The resolution also improved with each increment in water content, though some tailing occured as well, as can be seen in the last two chromatograms. No special attempts were made to optimize the system.

In order to demonstrate that the observed separations were indeed enantiomeric separations, the non-chiral dopant DNDA was used in the reversed-phase system under identical conditions as the chiral dopant. Only one peak for N^6 was observed at several sets of conditions where separation had been observed with the chiral reagent.

The improvement in enantiomeric resolution with the change in water content of the mobile phase suggests that the actual separation may be the result of increasing stationary phase mass, *i.e.*, as a result of increasing k' of the dopant. This dependence on mobile phase composition may provide the ability to "fine-tune" the system to produce an optimal result while avoiding an extremely high concentration of dopant. Studies are now underway to further investigate this effect.

The goal of this project is the development of a sound basis for the design of non-ionic mobile phase dopants. The choice of a chiral, charge-transfer dopant provides a very selective probe of chiral recognition, and studies currently in progress utilize this property to elucidate further the effect of concentration, length of dopant "tail", mobile phase composition, and stationary phase type (brush *vs.* polymeric). The practical value of such dopants in the reversed-phase high-performance liquid chromatographic separation of aromatic hydrocarbons is being actively investigated.

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