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3-(Phenyl-4-oxy)-5-phenyl-4,5-dihydro-(1*H*)-pyrazole: A fascinating molecular framework to study the enantioseparation ability of the amylose (3,5-dimethylphenylcarbamate) chiral stationary phase. Part I. Structure-enantioselectivity relationships

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ABSTRACT

Chiral stationary phases (CSPs) based on amylose (3,5-dimethylphenylcarbamate) (ADMPC) exhibit a wide-range of enantioselectivity in high-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC). Although this class of CSPs has been extensively used, chiral discriminations at receptorial level, which are useful to develop predictive molecular models, have been rarely reported in the literature.

Herein, we describe the results obtained in the enantioselective HPLC of a set of six C5-chiral 4,5-dihydro-(1*H*)-pyrazole derivatives on the ADMPC-based Chiralpak AD-3 CSP (CSP) under normal-phase and polar organic conditions. Using pure methanol as a mobile phase the exceptional enantioseparation factor value of 50 at 25 °C was found for one of the investigated analytes. To the best of our knowledge, the enantiomeric bias represents the most outstanding enantioseparation ever recorded on ADMPC-based CSPs.

Systematic variations in chemical groups in specific positions of the 3-(phenyl-4-oxy)-5-phenyl-4,5-dihydro-(1*H*)-pyrazole molecular framework resulted in peculiar changes in retention and enantioselectivity. A careful analysis of the chromatographic data permitted to advance some hypotheses concerning the role played by the individual chemical groups in determining the exceptional enantioseparation.

In particular, under methanol-rich mode, the prenyl moiety of the second eluted enantiomer of the better resolved analyte was recognized as a critical structural element to establish direct and favorable solvophobic interactions with apolar portions of selector.

1. Introduction

HPLC on chiral stationary phase (CSP) is the most widely used technique for separation of enantiomers of chiral compounds. Method development for a chiral separation involves the selection of an appropriate CSP. Despite the tremendous advances over the past few years in many aspect of chiral column technology, the strategy adopted to achieve the desired enantioseparation is remained essentially the same, being it again based on empirical approaches. This is mainly due to a limited knowledge of chiral recognition mechanism of the most CSPs.

Consequently, in order to minimize time-consuming and unproductive studies, it is convenient to screen a limited number of CSPs with recognized high chiral discrimination ability.

Although a number of enantioselective stationary phases have been described in the literature or are commercially available, only a few of them have demonstrated broad chiral selectivity and effectiveness in a wide range of eluent conditions. These include polysaccharide CSPs prepared by conversion of hydroxyl groups of monosaccharide units of cellulose or amylose to arylcarboxylate or arylcarbamate moieties and their fixing (by physical coating or chemical immobilization) onto macroporous silica particles [1–4].

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A survey by Okamoto et al. on the HPLC determination of the enantiomeric excess (ee) published in Angewandte Chemie International Edition in 2012 highlights that in 97% of cases the enantioselective analyses were carried out on polysaccharide-based CSPs [5]. Moreover, the 40% of enantioseparations were performed using the Chiralpak IA and Chiralpak AD CSPs which both contain amylose (3,5-dimethylphenylcarbamate) (ADMPC) as a chiral selector.

The field of application of the polysaccharide-based CSPs is not only restricted to non-racemic compounds obtained by asymmetric synthesis but extends to other important classes of chiral analytes. Huybrechts et al. have reported a survey on the HPLC resolution of a set of 150 chiral analytes of pharmaceutical interest variously functionalized (117 basic, 27 neutral and 6 acid compounds) [6]. In 85% of the investigated chiral drugs the HPLC enantioseparation was achieved using only four coated-type polysaccharide-based CSPs (Chiralcel OJ-H, Chiralcel OD-H, Chiralpak AD-H and Chiralpak AS-H CSPs). Once again, the ADMPC-based Chiralpak AD-H CSP produced the best enantioselectivity.

So, according to literature, ADMPC may be included among the chiral selectors with the broadest spectrum of enantioselectivity.

Despite the experimental works on ADMPC-based CSPs, not much is known about the nature of the selectand-selector interactions at molecular level, thus making their chromatographic behavior difficult to predict.

As demonstrated in our previous works, investigations on chiral discrimination mechanism of polysaccharide-based CSPs can be facilitated by designing and analyzing chiral probes whose enantiomers show large differences in the free energy of interaction with the CSP [7–9]. Selectand-selector systems that display receptor-like enantioselectivity [10,11] (i.e. $\alpha > 15$ corresponding to difference in free energy of interaction larger than 1.5 kcal mol^{-1} [12]) can be promising references for the *in silico* construction of molecular models of polysaccharide selectors able to account the observed discrimination and to elucidate at molecular level the interactions operating in the enantioseparation process [7,9].

Besides providing a very high enantioselectivity, an ideal molecular framework for developing reliable predictive models should meet the following requirements:

- be easily synthesizable and functionalizable;
- its functionalization should lead to a large fluctuation in enantioselectivity;
- the introduction of elements of molecular diversity should produce in one or more terms of the series of chiral compounds an inversion of the chiral discrimination and, therefore, of the enantiomer elution order.

In this article, the chromatographic behavior of a series of chiral compounds incorporating the 3-(phenyl-4-oxy)-5-phenyl-4,5-dihydro-(1*H*)-pyrazole scaffold on the coated-type ADMPC-based Chiralpak AD-3 CSP is shown. The functionalization of the molecular framework in the insertion points O, C5, and N1 is quite easy to achieve and offers the possibility of placing different functional groups in key positions for chiral recognition. The analytes were chromatographed under normal-phase (NP) and polar organic (PO) conditions and their enantiomer elution order was determined. Based on the experiential retention and enantioselectivity data, some hypothesis concerning the molecular fragments responsible for enantioselectivity and the nature of interactions occurring in the enantiodiscrimination process have been formulated.

2. Experimental

2.1. Enantioselective HPLC

Analytical HPLC analysis of **1–6** was performed using the commercially available 100 mm x 4.6 mm I.D. Chiralpak AD-3 column (Chiral Technologies Europe, Illkirch, France). HPLC solvents were purchased from Aldrich (St. Louis, MO, USA). HPLC apparatus consisted of a Perkin-Elmer (Norwalk, CT, USA) 200 Lc pump equipped with a Rheodyne (Cotati, CA, USA) injector, a 1000-μL sample loop, a HPLC Perkin-Elmer oven and a Perkin-Elmer detector. The signal was acquired and processed by Clarity software (DataApex, Prague, Czech Republic).

The standard solutions were prepared by dissolving about 1.0 mg of sample into 25 mL of ethanol. The injection volume was 20–50 μL.

2.2. Synthesis of *6*

Racemates **1–5** were synthesized by a chemical pathway reported elsewhere [8,13]. The synthetic procedure and ^{1}H and ^{13}C NMR characterization data for the novel pyrazoline **6** are reported in Fig. S1 of Supporting information (SI).

2.3. Absolute configuration and enantiomeric elution order

Semipreparative HPLC separations of the enantiomers of **1–4** and **6** were carried out on the commercially available 250 mm x 10 mm I.D. Chiralpak AD column using pure methanol (in the case of **2**, **3**, **4** and **6**) and ethanol (in the case of **1**) elution modes. The temperature was set at 25 °C. The flow rates were 2.5 mL min^{-1} with ethanol and 4.5 min^{-1} with methanol. The amounts of racemic samples resolved for single chromatographic runs ranged from 2 to 10 mg.

The absolute configuration of the enantiomers isolated on a semipreparative scale was empirically established by CD correlation method using as references the enantiomers of **1** [7,9,14] and a structurally related pyrazoline of known stereochemistry previously published by our group [15]. The CD spectra (Fig. S2 of SI) were recorded in ethanol at 25 °C by using a Jasco Model J-700 spectropolarimeter. The optical path was 0.1 mm. The spectra are average computed over three instrumental scans and the intensities are presented in terms of ellipticity values (mdeg).

The enantiomeric elution order on the Chiralpak AD-3 CSP was established by analyzing non-racemic samples enriched by the (*S*)-enantiomer and modulating the elution of second eluted enantiomer using the stopped-flow based procedure as previously described [14].

3. Results and discussion

3.1. High enantioselectivity of *1* under methanol mode

The preliminary step of our study was to analyze a series of chiral compounds with 3-(phenyl-4-oxy)-5-phenyl-4,5-dihydro-(1*H*)-pyrazole framework using a 100 mm × 4.6 mm ID column packed with 3-μm particles of Chiralpak AD-3 CSP and pure methanol as a mobile phase.

The highest value of enantioselectivity was achieved for the compound **1** (Fig. 1) which showed the exceptional enantioseparation factor of 50.3 at 25 °C (corresponding to a difference in free energy of interaction between the two enantiomers of 2.3 kcal mol^{-1}). Such a high value is uncommon in enantioselective HPLC on ADMPC-based CSPs which exhibits very broad spectrum of enantioselectivity but rarely α-values at receptorial level.