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High-performance liquid chromatographic enantioseparation of β-3-homo-amino acid stereoisomers on a (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid-based chiral stationary phase

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Abstract

High-performance liquid chromatographic methods were developed for the separation of the enantiomers of thirteen unusual β-3-homo-amino acids and three of its ethyl esters on a chiral stationary phase containing (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid as chiral selector. The effects of the mobile phase composition and the acidic modifiers on the separation were investigated. The structures of the substituents in β-position substantially influenced the retention and enantioseparation. The influence of ionic strength on the enantioseparation was established experimentally. The elution sequence was determined in all cases.

Keywords: Column liquid chromatography; β-3-Homo-amino acids; (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic acid-based chiral stationary phase

1. Introduction

During the past decade, there has been growing interest in β-amino acids with regard to their unique biological, neurological activity and are known to be receptor antagonist, enzyme inhibitors and also components of naturally occurring compounds with antitumor properties [1–3]. They are important constituents of natural products such as alkaloids, peptides, β-lactam antibiotics and intermediates for the synthesis of pharmaceuticals [4–6]. As a consequence of the wide-ranging utility of these compounds, considerable attention has been devoted to the preparation of β-amino acids in enantiopure forms via enantioselective synthesis or resolution of the racemates [7–9].

For control of the enantiopurity of the final products mainly high-performance liquid chromatographic (HPLC) separation methods are widely used. The application of chiral crown ethers as chiral stationary phases (CSPs) was initiated by Cram and co-workers [10] and chiral crown ethers like (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid are nowadays known to be very effective in the resolution of racemic compounds containing a primary amino group [11–13]. HPLC enantioseparations of β-amino acids have been performed by both indirect and direct methods. In the past few years, new types of chiral derivatizing agents and CSPs were applied for the enantioseparation of β-amino acids by D'Acquarica et al., Hyun et al. and Péter et al. [14–21].

The present paper describes direct HPLC methods for the enantioseparation of racemic β-substituted-β-amino acids (β-3-homo-amino acids) by applying the (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid-based CSP (for structure see [11,19]). The effects of different parameters on the selectivity, such as the nature of the organic modifier, the mobile phase composition, the ionic strength of the mobile phase and the structure

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of the analyte, were examined and are discussed. The elution sequence was determined in all cases.

2. Experimental

2.1. Chemicals and reagents

The amino acids evaluated in this study can be arranged into two classes (Table 1): β-alanine analogs which contain hydrocarbon aromatic substituents in the β position (compounds **1**–**6**), and β-substituted β-amino acids containing =N–, –O– and –S– heteroatoms (compounds **7**–**13**). Racemic 3-amino-3-phenylpropanoic acid (**1**), 3-amino-3-(3-pyridyl)propanoic acid (**9**), 3-amino-3-(2-furyl)propanoic acid (**10**), 3-amino-3-(3-furyl)propanoic acid (**11**), 3-amino-3-(2-thienyl)propanoic acid (**12**) and 3-amino-3-(3-thienyl)propanoic acid (**13**) were synthetized in a modified Rodionov synthesis [22–25]. The corresponding ester of **9**, **10** and **12** were prepared by esterification with ethanol in the presence of thionyl chloride according to a known procedure. Enantiomerically pure hydrochlorides of (*R*)-**14**–**16** were prepared by enzymatic kinetic resolution of the amino esters catalyzed by *Candida antarctica* lipase A in ethyl butanoate at ambient temperature [25]. The hydrochlorides of (*S*)-3-amino-3-phenylpropanoic acid (**1**) was prepared by acidic hydrolysis of the corresponding ethyl (*S*)-3-amino-3-phenylpropanoate [26]. The hydrochlorides of (*S*)-3-amino-3-heteroarylpropanoic acids (**10**–**13**) were prepared by acidic hydrolysis of the butyramides of the corresponding ethyl (*S*)-3-amino-3-heteroarylpropanoates.

Enantiomerically pure (*R*)- and (*S*)-3-amino-5-phenylpentanoic acid (**2**), (*R*)- and (*S*)-3-amino-4-(3-methylphenyl)-butanoic acid (**3**), (*R*)- and (*S*)-3-amino-4-(4-methylphenyl)-butanoic acid (**4**), (*R*)- and (*S*)-3-amino-4-(4-chlorophenyl)-butanoic acid (**5**), (*R*)- and (*S*)-3-amino-4-(2-naphthyl)-butanoic acid (**6**), (*R*)- and (*S*)-3-amino-4-(4-pyridyl)-butanoic acid (**7**) and (*R*)- and (*S*)-3-amino-4-(3-pyridyl)-butanoic acid (**8**) were from Solvay-Peptisyntha (Brussels, Belgium).

Acetonitrile (MeCN) and methanol (MeOH) of HPLC grade were from Merck (Darmstadt, Germany). Glacial acetic acid (AcOH), trifluoroacetic acid (TFA) and other reagents of analytical reagent grade were also from Merck. Milli-Q water was further purified by filtration it on a 0.45 μm filter, type HV, Millipore (Molsheim, France). The eluents were degassed in an ultrasonic bath, and helium gas was purged through them during the analyses.

2.2. Apparatus

The HPLC measurements were carried out on a Waters Breeze system consisting of a 1525 binary pump, a 487 dual-channel absorbance detector, a 717 plus autosampler and Breeze data manager software (Waters Chromatography, Milford, MA, USA) and equipped with Rheodyne Model 7125 injector (Cotati, CA, USA) with 20-μl loop.

Separations were carried out on a (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid-based CSP with 5-μm particle size, 150 × 4.0 mm i.d. [19].

3. Results and discussion

Amino acids **1**, **2**, **4** and **6** have already been resolved and published [27], but at different conditions and for comparison purposes their separations are included in the present paper too. Chromatographic results on the separation obtained at 20% (v/v), 50% (v/v), and 80% (v/v) MeOH contents, at a constant AcOH concentration of 10.0 mM are listed in Table 1. Table 1 reveals that an increase in the MeOH content led to an increase in the retention factor. This behavior is unusual in reversed-phase chromatography. As the content of MeOH in the aqueous mobile phase is increased, the polarity of the mobile phase is expected to decrease, and consequently the polar interactions between the mobile phase and polar β-amino acids should decrease leading to increased retention factors. This trend was observed for all of the investigated analytes. Similar behavior observed on a macrocyclic glycopeptide teicoplanin-based stationary phase was explained by the decreased solubility of amino acids in a MeOH-rich mobile phase [28,29].

The separation factor (α) and resolution (R_S) did not change significantly as the MeOH content of the mobile phase was increased (an exception was analyte **2**; Table 1). At high MeOH content the interactions of the first- and second-eluting enantiomers with the CSP were similar to those at low MeOH content. At high MeOH content owing to the favored non-chiral interactions the overall retention increased, while the role played by the chiral recognition and consequently the selectivity did not change significantly. The exception in the case of analyte **2** may be due to a different mechanism of interaction of 3-amino-5-phenylpentanoic acid with the CSP. The aromatic ring is one or two atoms farther from the chiral center (as compared with analytes **1** and **3**–**6**) and this may hinder the interaction with the CSP (smaller k'_1 values), but in this case increase of the MeOH content promoted the chiral recognition.

The structures of the substituents in β-position had substantial effects on the retention, but the selectivity (α) changed to a much lower extent than the k' value (Table 1). At three constant organic modifier and acid contents the chromatographic behavior revealed smaller retention factors for analytes **2**–**6** than for **1** (exception was analyte **6** for which smaller k' was observed at high MeOH content; Table 1). For analytes **2**–**6**, the greater distance of the aromatic ring from the stereogenic center may weaken the polar interaction with the selector and therefore the retention decreased. The slight increase in retention factor for analytes **5** and **6** as compared with **2**, **3** and **4** may be due to the increased H-bonding (through the chloro atom; **5**) or the polar interaction ability of analyte **6** with the protonated carboxy group or the –CO–NH– group of the selector. This increase in the retention factor was not accompanied by an increase in the separation factor, but the resolution slightly improved.

At a constant mobile phase composition, amino acids with substituents in the β position containing a =N–, –O– or –S– heteroatom (compounds **7**–**13**) demonstrated a significant change in retention factor as compared with compound **1**. For these analytes, higher retention factors were observed, especially in the mobile phases with higher MeOH contents. This behavior could be attributed to the possibility of H-bonding interac-