Journal of Chromatography A, 1218 (2011) 5653–5657

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

A chromatographic study on the exceptional enantioselectivity of cellulose tris(4-methylbenzoate) towards C5-chiral 4,5-dihydro-(1*H*)-pyrazole derivatives

Roberto Cirilli[a,*], Stefano Alcaro[b], Rossella Fioravanti[c], Rosella Ferretti[a], Adriana Bolasco[c], Bruno Gallinella[a], Cristina Faggi[d]

[a] *Istituto Superiore di Sanità, Dipartimento del Farmaco, Viale Regina Elena 299, I-00161 Rome, Italy*
[b] *Dipartimento di Scienze Farmacobiologiche, Laboratorio di Chimica Farmaceutica, Università degli Studi "Magna Græcia", Campus Universitario "Salvatore Venuta", Viale Europa, I-88100 Catanzaro, Italy*
[c] *Università "La Sapienza", Dipartimento di Chimica e Tecnologie del Farmaco, Piazzale A. Moro 5, I-00185 Rome, Italy*
[d] *Università degli studi di Firenze, Dipartimento di Chimica, Via della Lastruccia 13, 50019 Sesto Fiorentino, Firenze, Italy*

ARTICLE INFO

Article history:
Received 19 April 2011
Received in revised form 31 May 2011
Accepted 22 June 2011
Available online 30 June 2011

Keywords:
Enantiomer separation
Enantioselectivity
Chiral stationary phase
Chiralcel OJ-H
Temperature effects

ABSTRACT

A set of ten C5-chiral 4,5-dihydro-(1*H*)-pyrazole derivatives was synthesized and analyzed by high-performance liquid chromatography (HPLC) on the polysaccharide-based Chiralcel OJ-H chiral stationary phase (CSP). The enantioseparations were carried out using pure ethanol as eluent. Different structural elements of the investigated compounds were recognized for obtaining a very high enantioselectivity. In order to clarify some aspects of the chiral discrimination process, the thermodynamic parameters associated to the enantiorecognition and the enantiomer elution order were established.

1. Introduction

Enantioselective analysis covers a significant area of analytical chemistry and it has been gaining a growing interest within a wide variety of fields dealing with drugs, flavours, fragrances, natural products and so on [1]. The importance of this topic in view of both basic research and industrial application has given a strong impulse to the design of new and efficient chiral stationary phases (CSPs) to be used in HPLC. Currently more than 100 CSPs for HPLC are available on the market [2,3]. Among them the benzoates and arylcarbamates of cellulose and amylose have been successfully employed to resolve a broad range of chiral compounds on analytical and preparative scale [4–9].

An interesting and fashioning aspect of the enantioseparation on the polysaccharide-based CSPs is the understanding of the nature of the intermolecular interactions involved in the retention and discrimination processes. Despite the spectroscopic and chromatographic researches devoted on this topic, only a few aspects of the enantiorecognition mechanism have been clarified. This is basically due to: (i) a lacking of adequate crystallographic models to be employed in docking studies, (ii) the complexity of the three-dimensional structure of polysaccharide-based selectors and (iii) the presence of multiple active sites involved in the enantiorecognition process.

In this paper, we report the results of a chromatographic study on the chiral discrimination mechanism of cellulose tris(4-methylbenzoate) using as test molecules a series of C5-chiral 4,5-dihydro-(1*H*)-pyrazole derivatives. The commercially available CSPs based on the 4-methylbenzoate of cellulose are named Chiralcel OJ and are physically adsorbed on 3 (Chiralcel OJ-3), 5 (Chiralcel OJ-H), or 10 μm (Chiralcel OJ) particles of macroporous silica gel. Here, the Chiralcel OJ-H CSP was selected and its performances were studied using pure ethanol as eluent. It has been suggested that the main sites of the Chiralcel OJ-H CSP involved in the chiral adsorption and separation are probably the polar carbonyl groups of the ester function [10–12]. These groups, located inside the polymer chain, are able to act as an acceptor of hydrogen bonds and establish dipole–dipole interactions with the analyte.

The function of the aromatic fragments, located outside the polymer chain, lies in the introducing of site capable of π–π interactions with complementary portions of analyte and/or in the creation of steric impediment for the insertion of the enantiomers into the chiral helical grooves of CSP.

Recently we have reported on an interesting case of exceptionally high enantioselectivity of the Chiralcel OJ-H CSP towards N-thiocarbamoyl-3-(4-prenyloxy)-phenyl-5-phenyl-4,5-

* Corresponding author. Tel.: +39 06 49902407.
E-mail address: rcirilli@iss.it (R. Cirilli).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.06.081

dihydro-(1*H*) pyrazole (compound **1**) [13]. In order to elucidate some aspects of that unusual enantiorecognition phenomena, a set of molecules with structure analogue to **1** were synthesized and analyzed in the same chromatographic conditions. A systematic evaluation of the structure–enantioselectivity relationship data was carried out and some structural elements of both selector and selectands determining for enantioselectivity were recognized. In addition, the enantiomer elution order and the thermodynamic parameters associated to the discrimination process were determined.

2. Experimental

2.1. Enantioselective HPLC

Analytical HPLC analysis of **2**–**11** were performed using the commercially available 150 mm × 4.6 mm I.D. Chiralcel OJ-H column (Chiral Technologies Europe, Illkirch, France). HPLC grade ethanol was 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 50-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 0.5–2 mg of sample into 5 mL of ethanol. The injection volume was 20–50 μL.

The enantiomeric elution order on the Chiralcel OJ-H CSP was established by analyzing non racemic samples enriched by the (*R*)-enantiomer and modulating the second eluted enantiomer using the stopped-flow based procedure described in our recent paper [13].

2.2. Crystallographic data for (R)-**6** and (S)-**7**

2.2.1. Compound (R)-**6**

$C_{21}H_{23}N_3O_2S$, $M = 381.48$, Monoclinic, space group P 21, $a = 12.168(1)$, $b = 10.682(1)$, $c = 15.359(1)$ Å, $\beta = 90.909(2)$, $V = 1996.1(3)$ Å^3, $Z = 2$, $D_c = 1.269$, $\mu = 1.603$ mm^{-1}, $F(0\,0\,0) = 808$.

9296 reflections were collected with a $4.60 < \theta < 64.62$ range with a completeness to theta 96.4%; 5133 were independent, the parameters were 513 and the final R index was 0.0385 for reflections having $I > 2\sigma I$, and 0.0621 for all data.

Flack parameter = 0.045(17): due to the presence of a sulphur atom we can determine the absolute configuration by anomalous-dispersion effects in diffraction measurement on the crystal.

Two molecules are contained in the asymmetric unit, as we can notice from volume and density.

Colourless cubic shaped crystals suitable for collection were obtained by crystallization from acetone/ethyl acetate.

2.2.2. Compound (S)-**7**

$C_{22}H_{19}N_3S$, $M = 357.46$, Orthorhombic, space group P 21 21 21, $a = 5.796(1)$, $b = 9.016(1)$, $c = 34.125(1)$Å, $V = 1783.3(3)$ Å^3, $Z = 4$, $D_c = 1.331$, $\mu = 1.678$ mm^{-1}, $F(0\,0\,0) = 752$.

4328 reflections were collected with a $5.07 < \theta < 70.60$ range with a completeness to theta 96.3%; 2924 were independent, the parameters were 247 and the final R index was 0.0669 for reflections having $I > 2\sigma I$.

Flack parameter = 0.07(5): due to the presence of a sulphur atom we can determine the absolute configuration by anomalous-dispersion effects in diffraction measurement on the crystal.

In both cases RX-analysis were carried out with a Goniometer Oxford Diffraction KM4 Xcalibur2 at room temperature.

Cu/Kα radiation (40 mA/−40 KV), monochromated by an Oxford Diffraction Enhance ULTRA assembly, and an Oxford Diffraction Excalibur PX Ultra CCD were used for cell parameters determination and data collection.

The integrated intensities, measured using the ω scan mode, were corrected for Lorentz and polarization effects [14].

Direct methods of SIR2004 [15] were used in solving the structure and it was refined using the full-matrix least squares on F^2 provided by SHELXL97 [16].

Multi-scan symmetry-related measurement was used as experimental absorption correction type.

The non-hydrogen atoms were refined anisotropically whereas hydrogen atoms were refined as isotropic.

The X-ray CIF file for (*R*)-**6** was deposited at the Cambridge Crystallographic Data Center and allocated with the deposition numbers CCDC 712215, for compound (*S*)-**7** the deposition number is CCDC 816494.

Copies of the data can be obtained, free of charge, from CCDC, 12 Union Road, Cambridge, CB2 1EZ UK (e-mail: deposit@ccdc.cam.ac.uk; internet: www.ccdc.cam.ac.uk).

For compound (*R*)-**6** the packing shows that there were neither π-stacking interactions, in spite of the presence of two aromatic rings, nor T-shaped interactions, but there was a strong intramolecular hydrogen bond between hydrogen on OH group and atom N2; it forms a six members ring (O1, HO1, N2, C3, C11 and C12), the distance N2—HO1 being 1.945 Å and angle O1–HO1—N2 147.50°.

Compound (*S*)-**7** did not form significant hydrogen bonds (distances N–H–S too long or angles too narrow).

2.3. Synthesis of **2**–**11**

Racemates **2, 3**, **5** and **6** were synthesized by a chemical pathway reported elsewhere [17–19]. The ^{1}H NMR data of the novel pyrazolines **4** and **7**–**11** are reported in Supporting information.

3. Results and discussion

3.1. Effect of the structural modifications of **1** on the enantiorecognition process

Our first step was to analyze a small set of five compounds (**2**–**6**) with a structure similar to that of the target compound **1** on the Chiralcel OJ-H CSP using ethanol as mobile phase. A comparison between the enantioseparation factors of the compounds **1**–**6** is shown in Fig. 1. Although some of the separations obtained were extremely large, such as the one obtained for **4** and **6**, the α-values of **2**–**6** were dramatically lower than the compound **1**. Furthermore, several clear structure–enantioselectivity relationships could be identified. The most significant are: (i) the presence of hydroxyl group in ortho position of phenyl moiety led to compounds less efficiently resolved. As an example, the elimination of 2′-OH of **2** to give **3** yielded a consistent increase in the enantioselectivity from 2.6 to 6.7. (ii) The substitution of the thiocarbamoyl group on the N1-position with the acetyl group (compounds **4** and **6** vs. compounds **3** and **5**) produced a consistent lowering in enantioselectivity. (iii) The introduction of the prenyl fragment on the 4′-OH position had a marked positive effect on the chiral recognition. On the basis of this preliminary study, we may identify at least two key fragments involved in the exceptionally high chiral recognition of **1** (the prenyl and thiocarbamoyl groups) and two structural fragments (the acetyl and 2′-OH groups) reducing enantioselectivity of the pyrazoline analogues.

The next step was to increase our knowledge on the main interactions responsible for the enantioseparation of the compound **1** on the Chiralcel OJ-H CSP. Starting from the basic structure formed from the 4,5-dihydro-(1*H*)-pyrazole core we designed a set of compounds structurally related to **1** keeping at least one of two

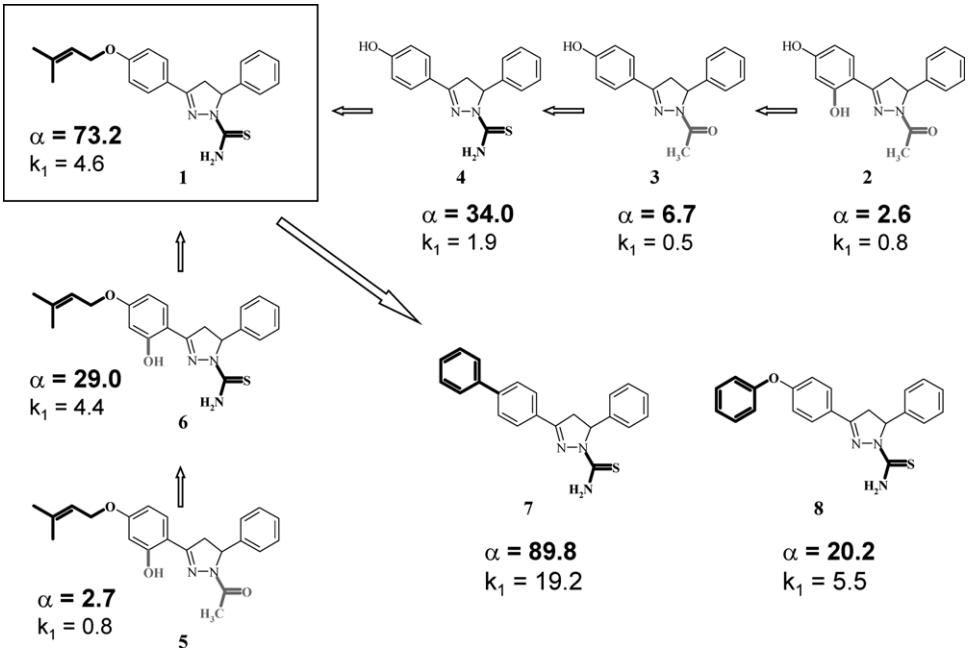

Fig. 1. Structures, enantioseparation and retention (for the first eluted enantiomer) factors of 1–8. Structural fragments necessary for a high enantioselectivity are showed in bold. The colour grey is used to visualize fragments reducing enantioselectivity. Chromatographic conditions: column, Chiralcel OJ-H 150 mm × 4.6 mm I.D.; eluent, ethanol; flow-rate, 1 mL min^{-1}; temperature, 25 °C; detection, UV at 340 nm.

most convenient moieties (prenyl or thiocarbamoyl groups) and analysing their chromatographic behaviour.

The prenyl moiety of the compound **1** may theoretically establish non-polar contacts with the aromatic side-chain of the OJ-H CSP or steric interactions. The potential "sandwich-like" π–π stacking was studied by replacing the prenyloxy group with fragments bearing a phenyl nucleus (phenyl and phenoxy groups). The chromatographic results, shown in Fig. 1, were discordant. In the case of the compound **7** the introduction of the phenyl group largely increased the chiral recognition degree: the α-value passed from 73.2 (compound **1**) to 89.8, which was the maximum value observed in this study. Conversely, even if the compound **8** was resolved very well, the replacement of the prenyl moiety with phenyl fragment reduced the enantioselelctivity to 20.2.

Probably, in the case of the compound **8** the π–π stacking was not centred onto the aromatic ring of the CSP with a reduced stabilizing component with respect to the compound **7**.

The initial chromatographic studies previously described, provided the evidence of the importance of the thiocarbamoyl moiety on the chiral discrimination degree. In fact, the replacement of the thiocarbamoyl of **6** with the acetyl group (compound **5**) gave a significant diminution of the chiral discrimination. Thus, we hypothesized that the amino group of thiocarbamoyl fragment of **1** could establish one or two hydrogen bonds with oxygen atoms of selector, probably belonging to the ester group. Starting from this observation the work design was carried out focusing the attention on compounds with the methyl substituent at N1-position (compounds **9** and **10**). Chromatographic results and analyte structures of **9** and **10** are both summarized in Fig. 2. A principal finding was that the high enantioselectivity ($\alpha > 29$) observed for the thiocarbamoyl-substituted compounds **1** and **6** dropped to the 1.3–1.4 range in the cases of methyl-substituted pyrazolines.

Furthermore, the first eluted enantiomer of the compounds **1** and **6** showed only slightly higher retention factor value compared to that of **9** and **10**. This suggests that in absence of productive donor hydrogen sites the retention of N1-methyl pyrazoline derivatives was again high.

The last structural modification of **1** was carried out with the aim to interpret the role of the phenyl group linked to the C5 stereogenic center. Thus, the compound **11** containing a methyl group in place of the phenyl moiety was prepared and analyzed on the Chiralcel OJ-H CSP. A total loss in enantioselectivity for the compound **11** was found. This data clearly highlights the basic function of the aromatic group for the high enantioselectivity of the parent compound **1**. Probably, this behaviour is correlated to its capability of establishing π–π interaction with the aromatic portion of selector.

3.2. Absolute configuration and enantiomeric elution order

In order to determine the enantiomer elution order, the chiral compounds investigated in this work were resolved on the mg-scale and the collected enantiomers submitted to chiroptical and structural analysis. The specific rotations and the chromatographic details used for the semipreparative enantioseparations are reported in Supporting information. The absolute configuration of **6** and **7** was directly secured by X-ray crystallography. A perspective view of the molecular structure of the (*R*)-(+)-**6** and (*S*)-(−)-**7** enantiomers is shown in Fig. 3. It is interesting to note the formation in **6** of an intramolecular H bond between the 2′-OH phenolic group and the nitrogen of the heterocyclic portion. Thus, both donor and acceptor sites are not available to establish intermolecular H-bond interactions with alcoholic solvents. This explains the very low solubility (<1 mg mL^{-1}) of the compound **6** and its analogue **2** in ethanol [20]. The absolute configuration assignment of the other enantiomers was empirically established by CD correlation using as references the enantiomers of the compounds **1**, **6**, **7** and **12** of known stereochemistry [13,20] (the structure of **12** and the CD analysis are reported in Supporting information). On the basis of the results arising from CD and X-ray studies, we could establish that, in all cases, the less retained enantiomer had (*R*) configuration.

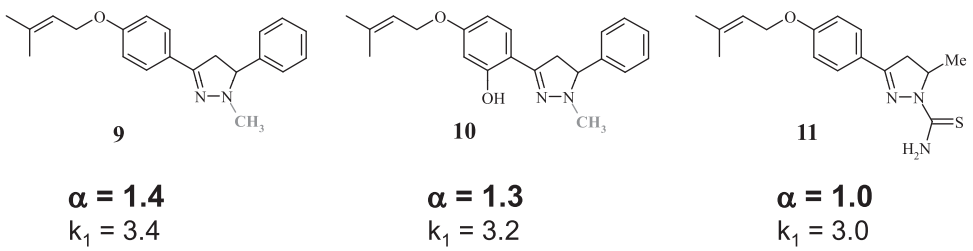

Fig. 2. Structures, enantioseparation and retention (for the first eluted enantiomer) factors of 9–11. Chromatographic conditions: column, Chiralcel OJ-H 150 mm × 4.6 mm I.D.; eluent, ethanol; flow-rate, 1 mL min^{-1}; temperature, 25 °C; detection, UV at 340 nm.

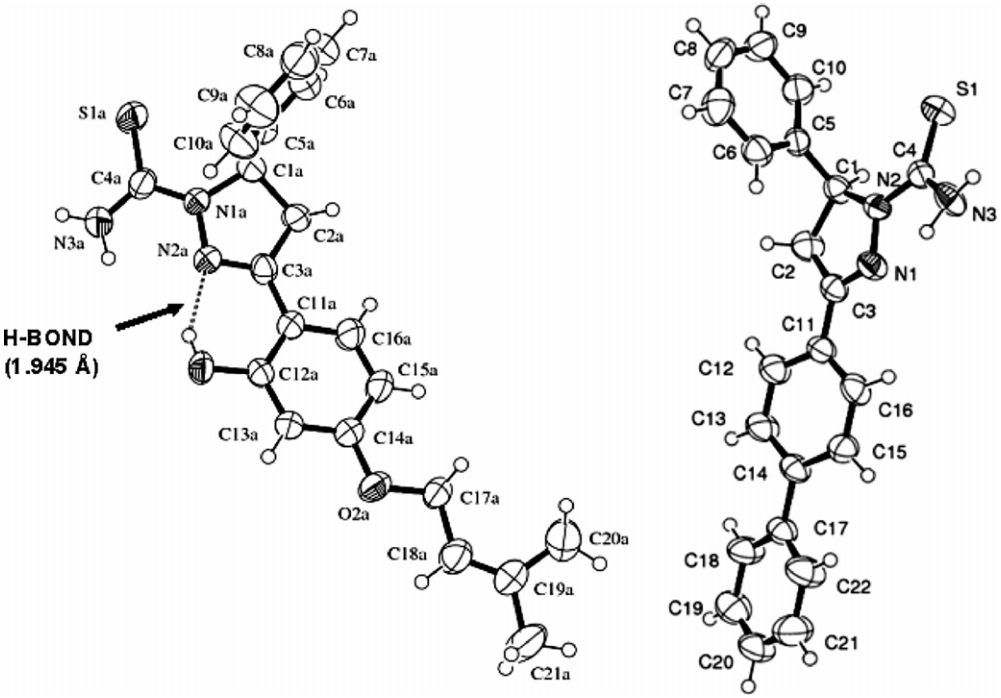

Fig. 3. An ORTEP view of the molecular structure of (*R*)-(+)-**6** (left) and (*S*)-(−)-**7** (right).

3.3. Thermodynamic aspect of the enantioseparation

The last series of experiments was devoted to the determination of the thermodynamic parameters associated to the enantioseparation of the compounds **2**–**11** on the Chiralcel OJ-H CSP. Each compound was submitted to a temperature-dependent study between 25 and 40 °C. The enantioseparation factors were calculated in 5 °C increments and correlated to the column temperature by the following equation:

$$\ln \alpha = \frac{\Delta\Delta H^\circ}{RT} + \frac{\Delta\Delta S^\circ}{R} \quad (1)$$

where $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ are the differences between two enantiomers in enthalpy and entropy of adsorption, respectively, onto stationary phase, R is the gas constant and T the absolute temperature. Typical variable temperature chromatograms of **7** are showed in Fig. 4. In all cases highly linear plots of lnα vs. $1/T$ were obtained ($r^2 > 0.9980$). The enthalpic and entropic terms, calculated from the slope and intercept, respectively, of van't Hoff plots are resumed in Table 1. For all pyrazolines the enantioselective factors decreased as the column temperature increased, as usually observed in enantioselective HPLC [21]. In most cases the $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ terms had the same negative sign. This indicated that the temporary formation of the more tightly structured enantiomer-OJ-H CSP diastereomeric complex caused a reduction in the molecular disorder. Only for the compounds **7** and **8**, the $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ terms had opposite sign. In the case of the compound **7**, a reduced favorable enthalpic contribution to enantioseparation with respect to the compound **1** ($\Delta\Delta H^\circ$ changed from −3.84 [13] to −2.26 kcal mol^{-1}) was observed. At the same time, the substitution of the prenyloxy moiety (compound **1**) with the phenyl group (compound **7**) had an opposite effect on the $\Delta\Delta S^\circ$ term which passed from the pejorative value of −4.36 cal mol^{-1} K^{-1} to a productive factor ($\Delta\Delta S^\circ$ = 1.37 cal mol^{-1} K^{-1}) for enantioselectivity. As a result, a substantial increase in the level of chiral discrimination was observed.

Table 1
Thermodynamic data.

Compound	$\Delta\Delta H^\circ$[a] (kcal mol^{-1})	$\Delta\Delta S^\circ$[b] (cal mol^{-1} K^{-1})	r^2
2	−1.07	−1.64	0.9993
3	−3.28	−7.21	0.9996
4	−4.15	−6.88	0.9983
5	−1.02	−1.48	0.9998
6	−3.45	−4.88	0.9999
7	−2.26	1.37	0.9989
8	−1.64	0.52	0.9994
9	−0.38	−0.65	0.9986
10	−0.33	−0.57	0.9981

Column, Chiralcel OJ-H 150 mm × 4.6 mm I.D.; flow-rate, 1 ml min^{-1}; detection, UV at 340 nm.

[a] Values are accurate at ±0.05 kcal mol^{-1}.

[b] Values are accurate at ±0.07 cal mol^{-1} K^{-1}.

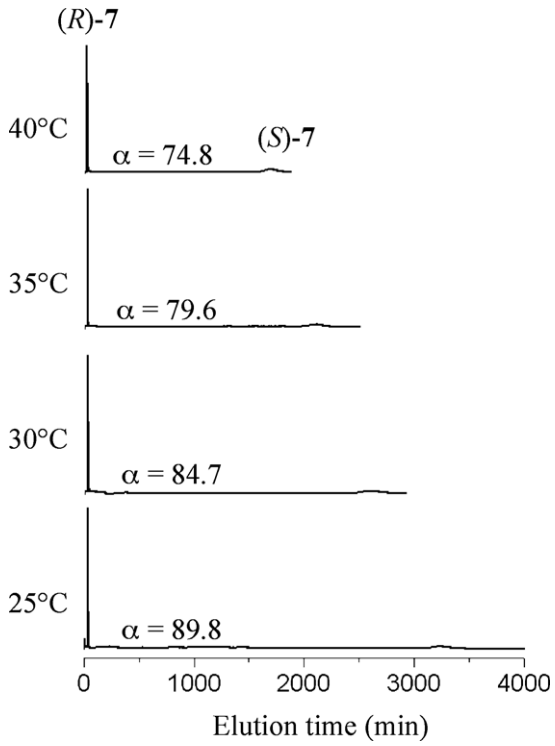

Fig. 4. Variable-temperature HPLC of **7**. Column, Chiralcel OJ-H 150 mm × 4.6 mm I.D.; eluent, pure ethanol; flow-rate, 1 mL min^{-1}; detection, UV at 340 nm.

4. Conclusions

Ten analytes were selected with the objective of identifying the factors that contribute to the exceptionally high enantioselectivity of compounds with 4,5-dihydro-(1*H*)-pyrazole skeleton on the Chiralcel OJ-H CSP. A detailed analysis of the chromatographic data pointed out the importance of the following fragments to obtain a high degree of chiral recognition: (i) an isoprenyloxy or phenyl group at the para position of the 3-aryl moiety; (ii) a thiocarbamoyl group at N1; (iii) a phenyl group linked to C5 stereogenic center. The more retained enantiomer of the best resolved thiocarbamoyl-substituted pyrazolines had (*S*)-configuration. In spite of the lower enthalpic contribute to enantiorecognition with respect to the analogues **1** and **8**, the compound **7** showed a higher value of enantioselectivity due to more favorable entropic conditions.

The chromatographic findings presented in this work constitute a significant support for further spectroscopic and *in silico* investigations on the enantiorecognition mechanism of this intriguing class of selectands.

Acknowledgments

We are grateful to Mrs. A. Mosca and Mr. L. Zanitti for their helpful technical collaboration.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.06.081.

References

[1] N.M. Maier, P. Franco, W. Lindner, J. Chromatogr. A 906 (2001) 3.
[2] M. Lämmerhofer, J. Chromatogr. A 1217 (2010) 814.
[3] T.J. Ward, K.D. Ward, Anal. Chem. 82 (2010) 4712.
[4] T. Ikai, Y. Okamoto, Chem. Rev. 109 (2009) 6077.
[5] X. Chen, C. Yamamoto, Y. Okamoto, Pure Appl. Chem. 79 (2007) 1561.
[6] I. Ali, H.Y. Aboul-Enein, Curr. Pharm. Anal. 3 (2007) 71.
[7] P. Franco, T. Zhang, J. Chromatogr. B 875 (2008) 48.
[8] T. Zhang, C. Kientzy, P. Franco, A. Ohnishi, Y. Kagamihara, H. Kurosawa, J. Chromatogr. A 1075 (2005) 65.
[9] T. Zhang, D. Nguyen, P. Franco, J. Chromatogr. A 1191 (2008) 214.
[10] Y. Okamoto, E. Yashima, Angew. Chem. Int. Ed. 37 (1998) 1020.
[11] K. Oguni, A. Matsumoto, A. Isokawa, Polym. J. 26 (1994) 1257.
[12] T. O'Brien, L. Crocker, R. Thompson, P.H. Toma, D.A. Conlon, B. Feibush, C. Moeder, G. Bicker, N. Grinberg, Anal. Chem. 69 (1997) 1999.
[13] R. Cirilli, S. Alcaro, R. Fioravanti, D. Secci, S. Fiore, F. La Torre, F. Ortuso, J. Chromatogr. A 1216 (2009) 4673.
[14] N. Walker, D. Stuart, Acta Crystallogr. Sect. A 39 (1983) 158.
[15] M.C. Burla, R. Caliandro, M. Camalli, B. Carrozzini, G.L. Cascarano, L. De Caro, C. Giacovazzo, G. Polidori, R. Spagna, J. Appl. Cryst. 38 (2005) 381.
[16] G.M. Sheldrick, SHELXL97: Program for Crystal Structure Refinement; Institut für Anorganische Chemie de Universitat Göttingen. Göttingen, Germany.
[17] F. Chimenti, E. Maccioni, D. Secci, A. Bolasco, P. Chimenti, A. Granese, O. Befani, P. Turini, S. Alcaro, F. Ortuso, R. Cirilli, F. La Torre, M.C. Cardia, S. Distinto, J. Med. Chem. 48 (2005) 7113.
[18] F. Manna, F. Chimenti, R. Fioravanti, A. Bolasco, D. Secci, P. Chimenti, C. Ferlini, G. Scambia, Bioorg. Med. Chem. Lett. 15 (2005) 4632.
[19] R. Fioravanti, A. Bolasco, F. Manna, F. Rossi, F. Orallo, M. Yáñez, A. Vitali, F. Ortuso, S. Alcaro, Bioorg. Med. Chem. Lett. 20 (2010) 6479.
[20] R. Cirilli, R. Ferretti, B. Gallinella, L. Turchetto, A. Bolasco, D. Secci, P. Chimenti, M. Pierini, V. Fares, O. Befani, F. La Torre, Chirality 16 (2004) 625.
[21] R. Cirilli, R. Ferretti, B. Gallinella, L. Zanitti, F. La, Torre, J. Chromatogr. A 1061 (2004) 27.