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Stereoselective binding of 2-(4-biphenylyl)-3-substituted-3-hydroxy-propionic acids on an immobilised human serum albumin chiral stationary phase

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Abstract

A series of 2-(4-biphenylyl)-3,3′-hydroxy-substituted phenyl propionic acid, with anti-inflammatory properties, bearing two chiral centres, were studied by HPLC upon HSA-CSP (human serum albumin-based chiral stationary phase). The compounds were analysed in their stereoisomeric erythro and threo forms. The study involved the enantioselective analysis on HSA-CSP, the determination of the racemate lipophilicity (log k'_w), a QSRR (quantitative structure–retention relationship) analysis and CD study for the assessment of the absolute configuration of the most retained enantiomer. Lipophilicity was found to be an important factor affecting the affinity of the compounds for the HSA stationary phase, but electronic properties seemed to play a role. The position of the substituent of the phenyl group on carbon 3 was found important to modulate stereoselective interaction, the highest value of enantioselectivities being found for the erythro ortho-substituted phenyl derivatives. The previously proposed two steps mechanism of enantiodiscrimination for cyclohexylphenyl substituted derivatives was confirmed for this series of derivatives bearing the biphenylyl moiety.

Keywords: Enantiomer separation; Chiral stationary phases, LC; Quantitative structure–retention relationship; Human serum albumin; 2-(4-Biphenylyl)-3,3′-hydroxyphenolpropionic acid

1. Introduction

Human serum albumin (HSA) immobilised on a stationary phase (HSA–CSP) has been extensively used in pharmaceutical analysis as a chiral stationary phase for determining the enantiomeric composition of chiral drugs [1–9]. Furthermore, the anchored protein, which maintains unchanged binding properties when compared to the native one, can be used to characterise the binding of drugs to the serum carrier, to elucidate the structure–retention relationship [10–15] and to reveal the drug–drug interaction in the binding to the main sites of the protein [16,17].

As the strength of drug binding to HSA can directly affect drug pharmacokinetics and pharmacodynamics, the mechanism of chiral discrimination on the main binding sites of albumin and the structural requirements essential for modulating the affinity are still the object of study to predict enantioseparation and to rationalise drug–HSA binding.

In a previous paper [18] we analysed a series of anti-inflammatory aryl propionic acids 2-(4-cyclohexyl phenyl)-3,3′-hydroxy-substituted phenyl propi-

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onic acid, with the aim to further define the mechanism and the structural requirements essential for enantiodiscrimination on the HSA–CSP.

The enantiomeric separation of each erythro and threo racemate was performed on the HSA column and the obtained retention parameters were used for the QSRR analysis. The lipophilic properties of the compounds (log k'_w), determined by RP-HPLC, proved to be very well correlated with the variation of the HSA retention indices within the series, the threo racemate showing higher lipophilicity than the corresponding erythro one.

Concerning the enantioselectivity, alpha values were found associated to the conformational behaviour of the molecules. This parameter was calculated for all the molecules, by means of molecular modelling study, with resulting prevailing linear and folded conformations. Racemates with linear conformation were found to show the highest enantioselectivity, and the absolute configuration of the carbon-2 adjacent to the carboxyl moiety, of the most retained enantiomer in the erythro and threo compounds was established to be always (S) by CD studies.

Therefore, as a consequence of these results a tentative proposal for chiral discrimination mechanism involving a two step interaction was suggested.

First the solute interacts with Site II, where arylpropionic acids have been shown to bind [4,19,20], through an electrostatic interaction between the anionic carboxylate moiety on the solute and the cationic group on the edge of the cleft. But the source of the enantiomeric discrimination is the process through which the solute–site II complex is completed and stabilised. This process involves conformational adjustments of the solute and protein to allow for insertion of the hydrophobic portion of the solute into the hydrophobic cavity at site II.

It can be assumed that the molecules with folded conformation will expend more energy than the linear one to interact with the hydrophobic cavity. While the conformation adjustments appear to affect the magnitude of the enantioselectivity, the actual source of the enantiodifferentiation appeared to be steric interaction between the solute and the chirality of site II. The conformational adjustment brings the carbon-2 into the position to interact with the chirality of the hydrophobic cleft and it is this interaction which drives the overall enantioselectivity.

It is here reported the analysis of another series of arylpropionic acids in which the cyclohexylphenyl moiety was replaced by a biphenylyl group (Fig. 1) and the position of the phenyl substituent at carbon 3 was ortho and para. The study involved the enantioselective analysis on HSA–CSP, the determination of the racemate lipophilicity (log k'_w), a QSRR analysis and CD study of some enantiomeric enriched samples.

The aim of this paper was to study how the enantioselective phenomenon could be modified by the insertion in the molecules of a more rigid and less hydrophobic part (biphenylyl moiety) and by the position of the substituent (ortho and para) on the phenyl ring at carbon 3.

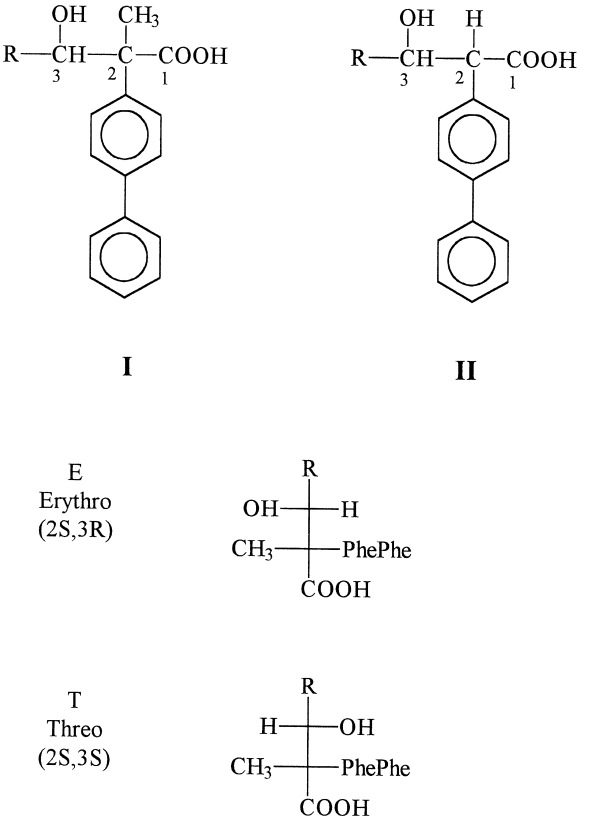


Fig. 1. General molecular structure of the compounds used in the study (R = Table 1) and Fisher projections of selected erythro and threo diastereoisomers.

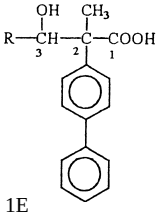
2. Materials and methods

2.1. Materials

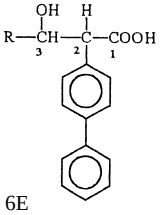
The studied arylpropionic acids (Table 1) were synthesised as previously reported [21–23].

HPLC grade methanol, 1-propanol, 2-propanol, hexane (Promochem, Germany) were used to prepare the mobile phases. Water was doubly distilled. To prepare the pH 7.0 phosphate buffer solution, potassium dihydrogenphosphate and dipotassium hydrogen phosphate trihydrate of analysis quality (Carlo Erba, Italy) were used. Octanoic acid (99%) was purchased from Aldrich Italia (Milan, Italy).

Table 1
Structures, capacity factors (k') and enantioselectivity (α) of racemates obtained on HSA column, mobile phase: 1-propanol:(pH 7.0, 0.1 *M*) potassium phosphate buffer (10:90) (v/v) containing 5 m*M* octanoic acid, flow-rate 1 ml/min. E = erythro, T = threo



	R	k'	α
1E	Ph	13.3–19.1	1.43
1T	Ph	19.6–27.1	1.38
2E	$PhCH_3(o)$	21.6–46.2	2.17
2T	$PhCH_3(o)$	23.3–38.0	1.63
3E	$PhCH_3(p)$	19.5–25.9	1.33
3T	$PhCH_3(p)$	25.6–41.3	1.61
4E	$PhOCH_3(o)$	16.3–31.9	1.96
4T	$PhOCH_3(o)$	17.2–17.2	1.00
5E	$PhOCH_3(p)$	10.9–12.3	1.11
5T	$PhOCH_3(p)$	15.5–18.6	1.20



	R	k'	α
6E	PhBr(*p*)	22.0–23.5	1.06
6T	PhBr(*p*)	39.4–48.1	1.22
7E	PhCl(*p*)	15.8–17.2	1.08
7T	PhCl(*p*)	33.4–44.6	1.33

The buffer solutions were filtered through a 0.45 μm membrane filter and degassed before their use for HPLC.

2.2. Apparatus

The solvent delivery system was a Jasco PU-980 Intelligent HPLC pump equipped with a Reodyne Model 7125 injector with a 20 μl sample loop. The eluents were monitored by a Jasco MD 910 Multi-wavelength Detector (DAD) connected to a computer station. For routine analyses the detector wavelength was set at 220 nm and 275 nm.

Circular dichroism (CD) measurements were carried out by a Jasco J-810 Spectropolarimeter (Jasco, Tokyo, Japan), using either methanol or phosphate buffer (0.1 *M*, pH 7.4) solutions (0.5–3 m*M*), with 1 cm pathlength, at room temperature. The analysed compounds showed the following values of $\Delta\epsilon$, as determined for a 100% e.e.: **(–)-1T** ($\Delta\epsilon$ −8.9, 253 nm), **(–)-2T** ($\Delta\epsilon$ −5.4, 249 nm), **(–)-3T** ($\Delta\epsilon$ −10.8, 248 nm), **(–)-4T** ($\Delta\epsilon$ +2.4, 278 nm; −6.1, 250 nm).

2.3. Determination of the lipophilic properties (log k'_w) by RP-HPLC

The stationary phase was an Alltima C_{18} 5 μm (150×4.6 mm I.D.) column. The flow-rate was 1 ml/min.

The mobile phase composition ranged from 40 to 80% (v/v) methanol with 0.02 *M* phosphate buffer at pH 7.0.

The dead volume of the system was measured as the first distortion of the baseline after injection of pure water. The stock solutions of tested compounds (1 mg/ml in methanol) were diluted with water to the final injected concentration (50 μg/ml). A 20 μl injection was made in duplicate. According to their chromatographic behaviour, the retention times of the solutes were determined at four different methanol–phosphate buffer mixtures ranging from 40 to 80% (v/v) methanol. At each mobile phase composition, the capacity factor k' was calculated according to $k' = (t_r - t_0)/t_0$ where t_r and t_0 were the retention times of analyte and the non-retained compound, respectively. The log k'_w values (log k' at 100% aqueous mobile phase) were obtained from the *y*-intercepts of plots log k' versus percent of metha-

Table 2
Dependence of the C_{18} capacity factors on the mobile phase composition: $\log k' = \log k'_w + a\ c$

No.	*Y* interc. Log k'_w	Slope	Corr. coeff.
1E	4.720±0.054	−0.0640±0.0008	0.99942
1T	4.831±0.052	−0.0651±0.0008	0.99969
2E	5.250±0.016	−0.0699±0.0002	0.99998
2T	5.369±0.026	−0.0715±0.0004	0.99996
3E	5.267±0.050	−0.0699±0.0008	0.99986
3T	5.563±0.031	−0.0736±0.0005	0.99995
4E	4.956±0.199	−0.0669±0.0033	0.99757
4T	4.893±0.163	−0.0665±0.0027	0.99983
5E	4.645±0.082	−0.0650±0.0014	0.99898
5T	4.802±0.118	−0.0663±0.0019	0.99913
6E	4.692±0.001	−0.0648±0.0000	1.00000
6T	5.218±0.070	−0.0688±0.0011	0.99971
7E	4.501±0.029	−0.0629±0.0004	0.99993
7T	5.049±0.062	−0.0674±0.0010	0.99976

a = slope; c = % methanol; $\log k'_w = y$ intercept.

nol in the mobile phase (Table 2). Correlation studies were performed using a statistical program (Graph Pad Prism).

2.4. Enantioselective chromatography

The chromatographic conditions for enantioseparation of the erythro and threo forms were studied on 7 μm HSA Shandon stationary phase (150×4.6 mm I.D.) at ambient temperature (Table 1).

The mobile phase composition was 1-propanol:(pH 7.0, 0.1 *M*) potassium phosphate buffer (10:90) (v/v) containing 5 m*M* octanoic acid. The flow-rate was 1 ml/min. The stock solutions of tested compounds (1 mg/ml in 1-propanol) were diluted either with buffer or with 2-propanol to the final injected concentration (50 $\mu g\ ml^{-1}$).

The values of enantiomeric excess (e.e% = $A_2 - A_1/A_2 + A_1$) were calculated from the chromatographic areas of the enantiomeric peaks. For the enantiomers of compounds **1T, 2T, 3T** a HSA column was used following the above reported conditions. The enantioselective separation of some compounds was also performed on Chiracel OJ (250×4.6 mm I.D.), Daicel Chemical Industries, using hexane:2-propanol:glacial acetic acid (90:10:0.2) (v/v) as eluent at 0.8 ml/min for **1E, 1T, 2T, 3E, 4T**, (95:5:0.2) (v/v) for **3T,** (70:30:0.2) (v/v) for **5E, 5T** with UV detection at 220–275 nm. The following values of e.e. were obtained: **(–)-1T** (e.e. 99.5%), **(–)-2T** (e.e. 45.0%), **(–)-3T** (e.e. 30.3%), **(–)-4T** (e.e. 83.0%). The elution order of the single enantiomer on HSA was performed by injecting enantiomer enriched mixtures.

2.5. QSRR analysis

Classical hydrophobic (log *P*), electronic (*s*, *F*) and steric (*MR*, *L*) parameters were either calculated (log *P*, *MR*) or retrieved (*s*, *F*, *L*) by the C-QSAR program Ver. 1.87 [24]. Regression analysis was also performed with the C-QSAR program.

3. Results

3.1. Determination of the lipophilic properties (log k'_w) by RP-HPLC

The capacity factors (log k') were measured at different concentrations (40–80%) of methanol in the mobile phase. The value corresponding to 0% of methanol (log k'_w) was then obtained by extrapolation of the linear portion of the curve log k' versus volume fraction of methanol (Table 2). This parameter (log k'_w) was retained as a valuable index of lipophilicity [25] and was used in the subsequent QSRR study. In fact by means of HPLC, hydrophobicity measures can be determined, which better describe the hydrophobic interaction of solutes with hydrophobic binding sites on proteins [26].

All the threo diastereoisomers showed higher log k'_w than the corresponding erythro ones (Table 2), demonstrating the threo higher chromatographic lipophilicity with respect to the other isomer. Compound **4E** was an exception, having higher log k'_w than **4T**. However, the range of lipophilicity of this class of compounds was found lower than that of the previous series bearing the cyclohexyl phenyl substituent, as well as the difference between threo and erythro log k'_w. This might be due to the higher lipophilicity of the cyclohexylphenyl moiety when compared to the biphenylyl one.

3.2. Retention on HSA

The enantiomeric separation of each erythro and threo racemate was performed by using 5 m*M* octanoic acid as additive in a mobile phase consisting of 1-propanol- (pH 7.0, 0.1 *M*) potassium phosphate buffer 10:90 (v/v), on the HSA column. The respective capacity (k') and enantioselectivity (a) factors are reported in Table 1.

The addition of the modifier (octanoic acid) was required to decrease the retention times on HSA, as reported for the highly bound non steroidal anti-inflammatory drugs [4,19,27].

All the threo diastereoisomers with a phenyl or para-substituted phenyl at carbon 3, showed higher k' than the corresponding erythro ones (Table 1), and the same behaviour was also displayed on a reversed-phase system (C_{18}), demonstrating the higher chromatographic lipophilicity of threo compounds. However on the reversed-phase system, the separation of erythro and threo stereoisomers was less neat than in the derivatives with the cyclohexylphenyl group [18], indicating a smaller difference in lipophilicity. Moreover, on the HSA column, the k'_2 of the ortho-substituted derivatives (2 and 4) for threo enantiomers were lower than the k'_2 of erythro ones: this behaviour was reflected in the **4E** lipophilicity value ($\log k'_w$) higher than that for **4T**.

The quantitative relationships between the chromatographic retention indices determined on the HSA column ($\log k'_1$, $\log k'_2$, Table 3) and the physico-chemical descriptors (QSRR) were studied by means of the multiple regression analysis method. The following equations were obtained:

$$\log k'_1 = 0.301(\pm 0.235) \log k'_w - 0.203(\pm 1.174)$$
$$n = 14\ r^2 = 0.393\ s = 0.121\ q^2 = 0.205$$
$$F_{1,12} = 7.84 \quad (1)$$

$$\log k'_2 = 0.480(\pm 0.238) \log k'_w - 0.963(\pm 1.188)$$
$$n = 14\ r^2 = 0.617\ s = 0.123\ q^2 = 0.492$$
$$F_{1,12} = 19.29 \quad (2)$$

$$\log k'_1 = 0.391(\pm 0.125) \log k'_w + 0.571(\pm 0.210)\sigma - 0.645(\pm 0.622)$$
$$n = 14\ r^2 = 0.857\ s = 0.061\ q^2 = 0.760$$
$$F_{1,11} = 36.22 \quad (3)$$

$$\log k'_2 = 0.558(\pm 0.175) \log k'_w + 0.490(\pm 0.294)\sigma - 1.342(\pm 0.871)$$
$$n = 14\ r^2 = 0.828\ s = 0.086\ q^2 = 0.755$$
$$F_{1,11} = 13.58 \quad (4)$$

Table 3
Enantioretention indices and physico-chemical parameters of the compounds studied

No.	Log k'_1	Log k'_2	Log k'_w	σ
1E	1.239	1.402	4.720	0.000
1T	1.415	1.531	4.831	0.000
2E	1.412	1.792	5.250	−0.130
2T	1.458	1.946	5.369	−0.130
3E	1.406	1.560	5.267	−0.170
3T	1.499	1.741	5.563	−0.170
4E	1.316	1.655	4.956	0.000
4T	1.347	1.374	4.893	0.000
5E	1.124	1.185	4.645	−0.270
5T	1.247	1.339	4.802	−0.270
6E	1.210	1.300	4.692	0.230
6T	1.560	1.790	5.218	0.230
7E	1.320	1.390	4.501	0.230
7T	1.630	1.810	5.049	0.230

k'_1, k'_2 = capacity factors of the first and second eluting enantiomer on HSA column; k'_w = capacity factor at 100% of aqueous mobile phase on RP-18 column.

All the equations (1)–(4) are statistically significant in terms of the partial F test, even if Eqs. (1) and (2) show poor correlation coefficients. One reason for that might be the narrow range of variation of the dependent variables $\log k_1'$ and $\log k_2'$.

Eqs. (1) and (2) are of no value as QSRR. However, the presence of the $\log k_w'$ term in Eqs. (3) and (4) confirms that lipophilicity is one important factor determining the differences in protein affinity throughout the series for both the first and the second eluted enantiomers. Eqs. (3) and (4) containing the σ term indicate that also the electronic properties of the molecules significantly affect the retention. It must be underlined that σ refers to the substituents located on the phenyl ring in position 3 of the 2-(4-biphenylyl)-3-hydroxy-2-methylpropionic acid derivatives (R in compounds **6–12**). The positive sign of the coefficients associated with σ in Eqs. (3) and (4) indicates that electron-attracting groups increase the retention times for both the first and the second eluted enantiomer.

The remarkable result coming from the above QSRR analysis is that the hydrophobic interaction of the analytes with the albumin binding site is reinforced by a further interaction involving the molecular skeleton of the solutes and some electron-rich secondary site on the protein. The retention mechanism might be interpreted as a non-specific inclusion in hydrophobic cavities in the HSA. This binding interaction takes place in the non specific binding sites, and while it relates to retention and surely to the extent of protein binding in the body, it probably plays no role in enantioselectivity. This view is supported by the fact that the enantioselectivity factor (α) does not correlate with $k'(1)$ for any of the series of compounds.

3.3. Stereochemical characterisation

The elution order for some of the compounds was determined by injection on the HSA based column of their enriched enantiomer mixtures. In particular, compounds (–)-**1T**, (–)-**2T** and (–)-**3-T** resulted in more retention with respect to the corresponding enantiomers. The stereochemistry of these compounds was characterised by enantioselective chromatography for determining their enantiomeric excess (see Experimental) and by circular dichroism (CD) for assigning their absolute configuration. The relative configuration of compounds **1–7** was previously determined by 1H-NMR studies [21–23]. The circular dichroism of compounds (–)-**1T**, (–)-**2T**, and (–)-**3T** gave a negative result, in methanol solution, at about 250 nm (Experimental section). In the case of structural analogues bearing a *p*-cyclohexyl substituted phenyl group at carbon 2 [18], the positive contribution to the CD, in methanol solution, was related to a (2*S*, 3*S*) or to a (2*S*, 3*R*) absolute configuration for the erythro or threo diastereomers, respectively. The assessment of the absolute configuration was done by applying the benzene sector rule proposed by Smith [28]. In the case of the threo diastereomers, the absolute configuration was assigned on the basis of the hypothesised higher contribution to the CD arising from the substituted carbon 3 group, and the inversion of the sign of the lowest energy CD band passing from buffer to methanol solution [28,29]. Some structural restrictions were necessary to reliably apply the sector rule, in particular the benzylic hydrogen should eclipse the edge of the benzene ring. This should be the conformational situation in the case of compounds **6** and **7**, but it is less obvious in the case of compounds **1–5**, where a methyl group replaces the benzylic hydrogen at carbon 2. Furthermore carbon 2 bears a biphenyl group instead of a cyclohexyl substituted benzene group as is the case of the above discussed structural analogues [18]. For these last the observed CD band between 280 and 250 nm can be safely assigned to the ^1L_b electronic transition of benzene. In the case of compounds **1–7** instead, the absorption spectrum around 250 nm should be dominated by the ^1L_a electronic transition of the biphenyl chromophore, as the contribution allied to the lowest energy ^1L_b electronic transition must probably completely submerged. Thus the observed CD band at about 250 nm for compounds (–)-**1T**, (–)-**2T**, and (–)-**3T**, and the bisegnate CD spectrum measured for (–)-**4T** (positive at 278 nm and negative at 250 nm), should be related to both the electronic transitions, ^1L_b and ^1L_a, of the biphenyl chromophore, as well as to the ^1L_b transition of the phenyl moiety at carbon 3. In the case of compound (–)-**4T**, the positive contribution to the CD in the lowest energy region could be related to the presence of the methoxy substituent at carbon 3. The presence of this substituent actually

gives a peculiar behaviour to compound (–)-**4T**, which does not show significant enantioselectivity in the binding to HSA, as shown by the absence of enantioresolution on the HSA based column.

These results do not allow a reliable assignment of the absolute configuration to these compounds. The CD investigation should be extended to a much larger series of compounds and information on their stereochemistry should be obtained by independent techniques.

The observed elution order of the (–) enantiomer as the more retained on the HSA based column should be in agreement with the enantioselective mechanism operating with the structural analogues previously discussed [18].

3.4. Enantioselectivity

From the above quantitative analysis of the physico-chemical factors influencing the retention on albumin, it is not evident which one (if any) determines the differences in enantioretention expressed by the parameter α reported in Table 1. In fact, in Eqs. (1)–(4), the parameters associated with the capacity factors of both the first and the second eluted enantiomers are the same (lipophilic and electronic). In an attempt to obtain some explanation of the variations of α, we examined the conformational aspects of compounds **1–7**. Unfortunately, we did not obtain any significant result, because the erythro and threo isomers of the 2-biphenylyl,2-methyl-substituted compounds (Fig. 1 general structure **I, II**), behave in a non homogeneous way when compared to the 2-(4′-cyclohexyl)-phenyl-substituted derivatives [18] in which the most populated (lowest energy) conformations were clearly characterized (fully extended for the erythro isomers and partially folded for the threo isomers).

Thus, in the present series the highest enantioselectivity within a pair of erythro–threo diastereoisomers could not be attributed to the same conformation.

What resulted was that, in this series, the erythro ortho-substituted derivatives (**2E–4E**) showed higher enantioselectivity than the corresponding threo isomers, the highest one among the two sets of derivatives, whereas considering the para-substituted compounds, the threo stereoisomers prevailed over the erythro one in the enantioresolution.

Considering the variations of α along the series, the properties of R appear to play a role. In the case of ortho-substituents on R, (2 and 4), one observes an increase in α and a big difference between erythro and threo stereoselectivity which might be related to sterically driven ligand–protein interactions critical for only one of the erythro enantiomers (Fig. 2a). The effect of the *o*-OCH3 and the *o*-CH3 might be a reflection of the steric restrictions of the cavity. These very favourable interactions for only one of the four diastereoisomers probably lead to an increase in the energy difference of the two diastereomeric complexes of **2E** and **4E** and increase the enantioselectivity.

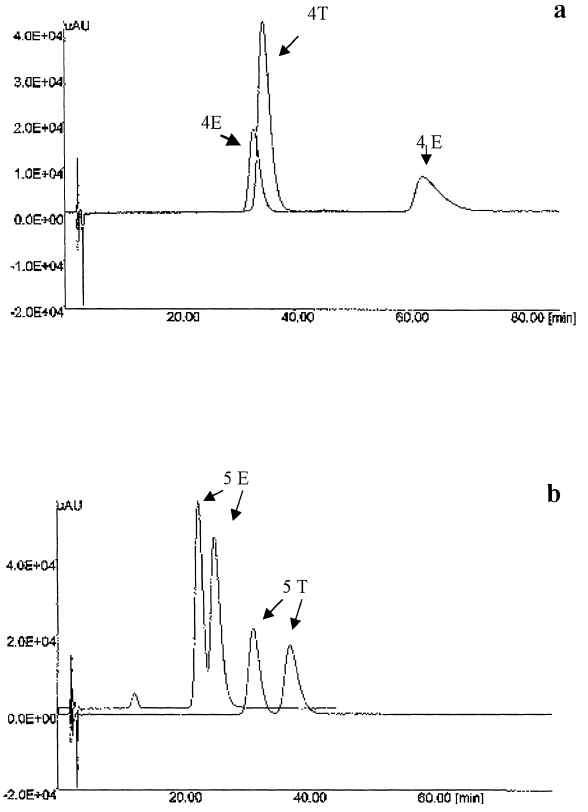


Fig. 2. Overlaid chromatograms on HSA–CSP of a) *rac*-4E and *rac*-4T, b) *rac*-5E and *rac*-5T. Chromatographic conditions: mobile phase: 1-propanol:(pH 7.0, 0.1 *M*) potassium phosphate buffer (10:90) (v/v) containing 5 m*M* octanoic acid, flow-rate 1 ml/min, UV detection at 220 nm.

On the other hand, the para-substituents might affect the interaction with the stereoselective site on the protein to an extent where the enantiomers have similar affinity, leading to a lower enantiodiscrimination and to a corresponding lower difference in enantioselectivities (Fig. 2b).

Also in this class of compounds in the erythro and threo series, the absolute configuration at carbon 2, the carbon adjacent to carboxyl moiety, should determine the elution order. It must be considered that the derivative not chiral at carbon 2, but only at carbon 3 bearing the hydroxy and biphenylyl substituent on this carbon, did not show enantioselective retention.

The key is again (see [18]) that the hydrophobicity does not play a role in the stabilization of the complex until it is formed. Thus, it must be the general steric bulk of the molecule and the energy required to assume an interactive conformation that result in the observed enantioselectivity.

4. Conclusion

The stereoselective binding of a series of arylpropionic acids was studied on HSA. The enantioseparation of the racemates was obtained by using a HSA column with the addition of octanoic acid in the mobile phase.

The retention properties of the compounds (k_1' and k_2') obtained with an HSA column were studied in terms of their physico-chemical properties, and statistically significant QSRR were calculated. Lipophilicity is an important factor affecting the affinity of the compounds for the HSA stationary phase, but also electronic properties played a role. Actually in this series, the biphenylyl instead of the cyclohexylphenyl moiety conferred lower lipophilicity to the molecules. As a consequence, the non-specific hydrophobic interactions were less important, such that electronic effects could be evidenced in the QSRR Eqs. (3) and (4).

On the other hand, higher values of enantioselectivities were found in this class of compounds. Also this phenomenon might be explained by the decreased hydrophobic effects and the consequent unmasking of other enantioselective interactions.

The previously proposed [18] two steps mechanism of enantiodiscrimination for cyclohexylphenyl substituted derivatives was confirmed for this series of derivatives bearing the biphenylyl moiety: first an electrostatic interaction establishes between the anionic carboxylate moiety and the cationic group on the edge of the binding area (carbon-2 in (S) gives rise to the most stable complex in the cyclohexylphenyl series), and then the hydrophobic portion of the molecules enters into the hydrophobic cleft of Site II forming stabilised diastereomeric solute–protein complexes. In the present series, additional electrostatic interactions contribute to the stability of such complexes.

Moreover, the position of the substituent of the phenyl group on carbon 3 was found important to modulate stereoselective interaction, the highest value of enantioselectivities being found for the ortho substituted phenyl erythro derivatives.

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References

[1] E. Domenici, C. Bertucci, P. Salvadori, S. Motellier, I.W. Wainer, Chirality 2 (1990) 263.
[2] E. Domenici, C. Bertucci, P. Salvadori, S. Motellier, I.W. Wainer, J. Pharm. Sci. 80 (1991) 164.
[3] T.A.G. Noctor, M.J. Diaz-Perez, I.W. Wainer, J. Pharm. Sci. 82 (1993) 675.
[4] T.A.G. Noctor, G. Felix, I.W. Wainer, Chromatographia 31 (1991) 55.
[5] E. Chosson, S. Uzan, F. Gimenez, I.W. Wainer, R. Farinotti, Chirality 5 (1993) 71.
[6] B. Loun, D.S. Hage, Anal. Chem. 66 (1994) 3814.
[7] P.J. Hayball, J.W. Holman, R.L. Nation, J. Chromatogr. B Biomed. Appl. 662 (1994) 128.
[8] J. Oravcová, B. Böhs, W. Lindner, J. Chromatogr. B. Biomed. Appl. 677 (1996) 1.
[9] J. Yang, D.S. Hage, J. Chromatogr. B 645 (1993) 241.
[10] R. Kaliszan, T.A.G. Noctor, I.W. Wainer, Chromatographia 33 (1992) 546.
[11] R. Kaliszan, T.A.G. Noctor, I.W. Wainer, Mol. Pharmacol. 42 (1992) 512.
[12] R. Kaliszan, A. Nasal, M. Turowski, J. Chromatogr. A 722 (1996) 25.
[13] T.D. Booth, I.W. Wainer, J. Chromatogr. A 737 (1996) 157.

[14] T.D. Booth, I.W. Wainer, J. Chromatogr. A 741 (1996) 205.
[15] V. Andrisano, T.D. Booth, V. Cavrini, I.W. Wainer, Chirality 9 (1997) 178.
[16] C. Bertucci, V. Andrisano, R. Gotti, V. Cavrini, Chromatographia 52 (2000) 319.
[17] C. Bertucci, A. Viegi, G. Ascoli, P. Salvadori, Chirality 7 (1995) 57.
[18] V. Andrisano, C. Bertucci, V. Cavrini, M. Recanatini, A. Cavalli, L. Varoli, G. Felix, I.W. Wainer, J. Chromatogr. A 876 (2000) 75.
[19] T.A.G. Noctor, D.S. Hage, I.W. Wainer, J. Chromatogr. A 577 (1992) 305.
[20] S. Wanwimolruk, D.J. Birkett, P.M. Brooks, Mol. Pharmacol. 24 (1983) 458.
[21] A. Guarnieri, S. Burnelli, L. Varoli, G. Fabbri, G. Scapini, Il Farmaco 38 (9) (1983) 686.
[22] A. Guarnieri, S. Burnelli, L. Varoli, N. Ghedini, G. Scapini, S. Ferri, E. Cavicchini, Pharmazie 40 (1985) 529.
[23] L. Varoli, A. Guarnieri, S. Burnelli, N. Ghedini, G. Scapini, S. Ferri, E. Cavicchini, Pharmazie 43 (1988) 15.
[24] C-QSAR, Ver. 1.87, BioByte, Claremont, CA, 1994.
[25] R.M. Smith, C.M. Burr, J. Chromatogr. A 475 (1989) 75.
[26] R. Kaliszan, A. Kaliszan, I.W. Wainer, J. Pharm. Biomed. Anal. 11 (1993) 505.
[27] T.A.G. Noctor, I.W. Wainer, J. Liq. Chromatogr. 16 (4) (1993) 783.
[28] H.E. Smith, Chem. Rev. 98 (1998) 1709.
[29] H.E. Smith, L.P. Fontana, J. Org. Chem. 56 (1991) 432.