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HPLC Separation of the Atropisomers of Some Substituted *N*-Arylthiazoline-2-Thiones with γ-Cyclodextrin as a Chiral Mobile Phase Additive: Size and Lipophilicity Effects of Substituents

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Abstract. The chromatographic separations of some substituted *N*-arylthiazoline-2-thione atropisomers are described, using reversed-phase HPLC with γ-cyclodextrin as a chiral mobile phase additive. The effects of the size and lipophilicity of various substituents are discussed and emphasize the close relationship between inclusion inside the cyclodextrin cavity and the chromatographic selectivity.

Key words: Chiral additive, cyclodextrin, RP-HPLC, atropisomers.

1. Introduction

In the past few years great attention has been paid to the use of inclusion phenomena in modern analytical separation methods [1–3]. In this field, extensive studies have been made on the use of cyclodextrins (CDs) in HPLC [4, 5]. In a previous paper [6], we described the chromatographic separation of eight *N*-arylthiazoline-2-thione and *N*-arylthiazoline-2-one atropisomers (Figure 1) in reversed phase HPLC using γ-cyclodextrin as a chiral mobile phase additive. A quantitative approach using experimental design has been developed, which allows us to determine the effect of three selected structural parameters on the separation and so on the inclusion process inside the CD cavities. In this paper, these first results, which proposed an inclusion model involving the aryl part, are applied to the prediction of chromatographic behaviour of other substituted *N*-arylthiazolinethione compounds.

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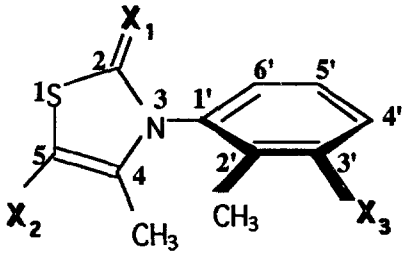
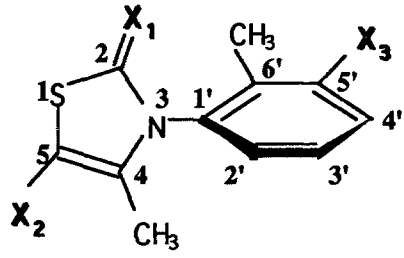


atropisomer S(+)



atropisomer R(-)

Fig. 1. Compound **1**. X_1 = oxygen or sulphur, X_2 = hydrogen or methyl, X_3 = hydrogen or methyl.

2. Material and Methods

2.1. Chemicals and Reagents

The synthesis of *N*-arylthiazolinethiones has been already described, as well as their barriers to rotation around the pivot bond [7–8]. The structure of compounds **2″d–f** and **3e** has been confirmed by ^{1}H and ^{13}C NMR analysis, mass spectra and chemical analysis.

Compound **2″d**. ^{1}H NMR 200MHz $CDCl_3$ δ ppm: 1.91 (3H, d, J = 1.2Hz); 3.81 (3H, s); 6.32 (1H, q, J = 1.2Hz); 7.15 (3H, m); 7.48 (1H, m). ^{13}C NMR 50MHz $CDCl_3$ δ ppm: 15.27 (Me-C4); 55.92 (OMe); 105.68 (C5); 112.67 (C′H); 121.21 (C′H); 126.02, 129.70 (C′H); 131.21 (C′H); 140.53 (C4); 154.59 (C = S).

Compound **2″e**. ^{1}H NMR 200 MHz $CDCl_3$ δ ppm: 1.93 (3H, d, J = 1.2Hz); 6.37 (1H, q, J = 1.2Hz); 7.32 (1H, m); 7.48 (2H, m); 7.60 (1H, m). ^{13}C NMR 50MHz $CDCl_3$ δ ppm: 15.35 (Me-C4); 106.37 (C5); 128.26 (C′H); 130.21 (C′H); 130.75, 131.12 (C′H); 132.53 (C′H); 135.25 (C′H); 139.49 (C4); 149.50 (C = S).

Compound **2″f**. ^{1}H NMR 200 MHz $CDCl_3$ δ ppm: 1.08 (3H, d, J = 5Hz); 1.19 (3H, d, J = 5Hz); 2.35 (1H, m, J = 6Hz); 6.35 (1H, d, J = 0.8Hz); 7.37 (1H, m); 7.49 (2H, m); 7.60 (1H, m). ^{13}C NMR 50MHz $CDCl_3$ δ ppm: 21.32, 22.47 (Me from iPr); 28.60 (CH of iPr); 104.75 (C5); 128.02 (C′H); 130.81 (C′H); 130.85, 131.11 (C′H); 132.97 (C′H); 136.00 (C′H); 150.67 (C = S).

Compound **3e**. ^{1}H NMR 200MHz $CDCl_3$ δ ppm: 1.80 (3H, d, J = 1.2Hz); 2.13 (3H, s); 6.37 (1H, q, J = 1.2Hz); 7.08 (1H, d × d, 6.6Hz × 1.2Hz); 7.33 (1H, t, 8Hz); 7.53 (1H, d × d, 8Hz × 1.2Hz).

Acetic acid and triethylamine (TEA) were purchased from Carlo Erba and Janssen, respectively. EtOH/H_2O (95 : 5) was purified by distillation.

CDs were obtained from Roquette Frères (Lestrem, France).

2.2. CHROMATOGRAPHY

HPLC was performed with a Merck Hitachi LichroGRAPH model L-6000 HPLC pump, a Merck Hitachi LichroGRAPH model L-4000 UV dectector and a Merck D2000 recorder. Separations were carried out with a Merck LichroCART 250-4 Lichrospher 100 RP18 endcapped (5 μm) (cat n°50838) column.

The mobile phase was prepared as follows: acetate buffer pH 4.1 (0.8% by volume of TEA + acetic acid to adjust pH), and ethanol were mixed in a ratio of 80 : 20 (v/v). 0.025 Mol of γ-CD were added to 1 L of the resulting solution. The mobile phase thus obtained was filtered through a membrane filter Millipore type HV (0.45 μm) and was degassed prior to use by a vacuum-ultrasonic method. Sample solutions were prepared so as to give a concentration of 4mg/L for each solute in ethanol. The amount of the sample injected was 20μL. All chromatograms were obtained at 22°C. The flow-rate was 0.8 mL/min, and UV detection was performed at 320 nm. The dead volume was determined by injection of sodium nitrate.

2.3. DETERMINATION OF THE ABSOLUTE CONFIGURATION AND OF THE ELUTION ORDER

As it is interesting to correlate the elution order of the two enantiomers with their spatial arrangement, the elution orders have been determined by injection of partially resolved mixtures obtained by preparative chromatography on a microcrystalline cellulose triacetate column [9] and on *p*-methylbenzoyl cellulose beads [10]. The barriers to rotation of compounds **2a–2d** and **2′a–2′d** (Figure 2) have been determined in previous work by the kinetics of racemization [8] and the absolute configuration of the (+) and (−) atropisomers determined by X-ray diffraction after derivatization with optically pure menthyl bromoacetate [11]. The absolute configuration related with the sign of rotation is shown in Figure 1.

For compounds **2″d–f** and **3** (Figures 2 and 4), the absolute configuration is unknown, and chromatographic data are reported according to the sign of the optical rotation.

2.4. CALCULATION OF THE STABILITY CONSTANT

Several methods have been reported for the determination of the association constants K_S of CD/complexes by means of solubility [12], potentiometry [13–14], spectroscopic methods [15–18] and fluorescence measurements [19–20]. These methods are not always suitable since it takes a long time to reach equilibrium.

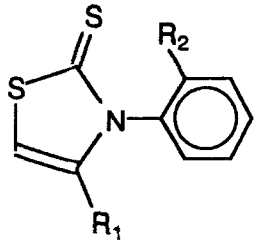


Fig. 2. Compounds **2**, **2'** and **2''**. **2**: $R_2 = CH_3$ and $R_1 = CH_3$(**2a**), C_2H_5 (**2b**), $CH(CH_3)_2$ (**2c**), $C(CH_3)_3$ (**2d**). **2'**: $R_1 = CH_3$ and $R_2 = C_2H_5$ (**2'a**), $CH(CH_3)_2$ (**2'b**), $C(CH_3)_3$ (**2'c**). **2''**: $R_1 = CH_3$ and $R_2 = OCH_3$ (**2''d**), Cl (**2''e**); $R_1 = CH(CH_3)_2$ and $R_2 = Cl$ (**2''f**).

The determination of K_s using the HPLC method with addition of cyclodextrin into an aqueous mobile phase presents many advantages in comparison with other methods [21].

For a neutral solute, some studies [22–25] have theoretically established the equation which expresses the stability constants K_s of a complex from chromatographic data. When a sample solute, S, is introduced into the column in the presence of γ-CD in the mobile phase, the following equilibria will be established

$$\begin{array}{ccc} & \overset{K_D}{\rightarrow} & \\ (S)_m + n(CD)_m & \leftarrow & (n\text{CD-S})_m \\ \downarrow\uparrow\ K_0 & & \downarrow\uparrow\ K_1 \\ (S)_s & & (n\text{CD-S})_s \end{array}$$

In these equilibria, subscripts m and s denote the mobile and stationary phases, respectively. The equilibrium constants are given as follows:

dissociation constant of CD-S

$$K_D = \frac{[(CD)_m]^n\,[(S)_m]}{[(CD\text{-}S)_m]} \tag{1}$$

distribution constant of S

$$K_0 = \frac{[(S_s]}{[(S)_m]} \tag{2}$$

distribution constant of CD-S

$$K_1 = \frac{[(n\text{CD-S})_s]}{[(n\text{CD-S})_m]}\,. \tag{3}$$

In the above schemes, the dissociation and/or the protonation of sample solutes is not taken into account, since the solutes examined are non-electrolytes. Furthermore, the distribution equilibrium of CD itself between the hydrophobic stationary phase and the hydrophilic mobile phase is assumed to be negligible because of the hydrophilic nature of the external faces of CD; this view is supported by the fact that the retention time of γ-CD was nearly the same as that of potassium nitrate used as a marker for measuring the column dead volume. For the same reason, the distribution equilibrium of the included species, (CD-S), onto the stationary phase expressed by Equation 3 may also be neglected.

The capacity factor, k', of the sample solute can therefore be written as

$$k' = \varphi \frac{[(\mathrm{S})_s]}{[(\mathrm{S})_m] + [(n\mathrm{CD\text{-}S})_m]} \tag{4}$$

where φ denotes the phase ratio of the column.

As the total concentration of CD, $[\mathrm{CD}]_T$, added in the mobile phase is given by $[\mathrm{CD}]_T = [(\mathrm{CD})_m] + [(n\mathrm{CD\text{-}S})_m] + \Sigma n_i[(n_i\mathrm{CD\text{-}M}_i)_m]$, where M_i represents the organic mobile phase modifiers (e.g. ethanol in our case), Equation 4 is expressed as

$$k' = \varphi \frac{K_0 K_\mathrm{D}}{K_\mathrm{D} + ([\mathrm{CD}]_T - n[(n\mathrm{CD\text{-}S})_m] - \Sigma n_i[(n_i\mathrm{CD\text{-}M}_i)_m])^n} . \tag{5}$$

For ethanol, the average literature value of the formation constant for CD-M_i is 0.9 M^{-1} with β-CD [26] and we assumed a value of 0 M^{-1} for γ-CD. So, $[(n_i\mathrm{CD\text{-}M}_i)_m]$ is negligible compared to $[\mathrm{CD}]_T$. Since the added concentration of the sample solute is very low in front of $[\mathrm{CD}]_T$ in the mobile phase, it can be assumed that $[\mathrm{CD}]_T - [(\mathrm{CD\text{-}S})_m] \sim [\mathrm{CD}]_T$. Furthermore, $K_0\varphi$ is equal to the capacity factor, k'_0, obtained in the absence of CD; therefore, Equation 5 reduces to

$$\frac{1}{k'} = \frac{1}{k'_0} + \frac{[\mathrm{CD}]_T^n}{K_\mathrm{D}\, k'_0} . \tag{6}$$

It is clear from Equation 6 that k' shows a hyperbolic dependence on $[\mathrm{CD}]_T$ and a plot of $1/k'$ vs $[\mathrm{CD}]_T^n$ gives a straight line whose slope is equal to $1/K_\mathrm{D}\, k'_0 = K\mathrm{s}/k'_0$. For the determination of the stoichiometries of CD complexes [25], several chromatographic analyses with different CD concentrations are needed. When the stoichiometry of the complexes is known or assumed, only two chromatographic analyses are useful: one to determine k'_0 and one to determine k' at a known CD concentration.

3. Results and Discussion

Chromatographic separations of the atropisomers of *N*-arylthiazoline(thi)ones **1** (Figure 1) have previously been described [6]. No separations have been obtained in the presence of β-CD. It emerges from the study investigated in the presence of γ-CD that the substituents X_1 and X_3 greatly influence the selectivity. Separations of the atropisomers have been obtained only for compounds with a thiocarbonyl function ($X_1 = S$): the substitution of oxygen by sulphur considerably enhanced the complex stability, as it has also been reported in the complexation of barbiturates with hydroxypropyl β-cyclodextrin [27]. The enantioselectivity was also dramatically affected by the presence of a methyl group in position 3′ of the aryl part (X_3). From these results, it was assumed that the polar part of the heterocycle was confined to the rim of the cavity whereas the benzene ring of the solute inserted into the lipophilic CD cavity, as in the case of the enantiomeric separation of diniconazole [28]. The substituent(s) played an important role from a steric point of view, a tight fit being important. The presence or absence of a methyl group in position 5 of the heterocycle (X_2) played only a minor role in the selectivity, but nevertheless contributed to increase the complex stability.

In agreement with these first investigations, the study has been extended here two series of substituted *N*-arylthiazolinethiones, compounds **2–2′–2″** and **3**. Compounds **2**, **2′** and **2″** (Figure 2) result from the replacement in **1** of a methyl by another group of different size and polarity, either in position 4 of the heterocycle (R_1 group in 2) or in position 2′ of the aryl part (R_2 group in **2′–2″**). So compounds **2** present an increased steric hindrance in the proximity of the previously studied X_2 substituent, whereas compounds **2′** present a similar increase of steric hindrance in a position close to X_3. The chromatographic data are given in Table I: k'_0 are the capacity factors without addition of γ-CD, $k'(+)$ and $k'(-)$ are the capacity factors respectively for the dextrorotatory and laevorotatory atropisomers in the presence of γ-CD, calculated from two racemate injections. α is the selectivity ($\alpha = k'(2)/k'(1)$, where $k'(1)$ is the capacity factor of the first eluted enantiomer).

Compounds **2** and **2′** behave similarly without the addition of γ-CD: on going through the series methyl, ethyl, isopropyl and tert-butyl either in position 4 of the heterocycle or in position 2′ of the aryl part, the hydrophobicity of the molecule increases as shown in both cases by an increase of k'_0. In reversed-phase liquid chromatography, it is known that the hydrophobic interaction, i.e., dispersion forces operating between the bonded alkyl moiety of the stationary phase and the nonpolar part of the sample molecule, plays an important role in determining the retention value of the sample solute. Belsner *et al.* [29] have demonstrated that $\log k'$ extrapolated to zero organic modifier content in the mobile phase for similar organic compounds, such as alkylbenzenes or alkylanilines, usually correlates fairly well with the logarithm of the octanol-water partition coefficient which is considered as a good measure of lipophilicity. There is a correlation between $\log k'$ values obtained for alkylbenzenes [30] and $\log k'$ values obtained for compounds

TABLE I. Chromatographic data for compounds **2**, **2′** and **2″**.[a]

cpd	R_1	R_2	k'_0	$k'(-)$	$k'(+)$	α	R_s	$K_s(-)$ (M^{-1})	$K_s(+)$ (M^{-1})
2a	$-CH_3$	$-CH_3$	30.36	4.95	5.29	1.068	0.54	205	189
2b	$-C_2H_5$	$-CH_3$	77.04	8.66	9.40	1.086	0.95	316	288
2c	$-CH(CH_3)_2$	$-CH_3$	157.19	16.91	18.80	1.112	1.56	331	294
2d	$-C(CH_3)_3$	$-CH_3$	356.56	24.68	22.85	1.080	1.14	538	584
2′a	$-CH_3$	$-C_2H_5$	77.27	8.23	8.23	1.000	0.00	335	335
2′b	$-CH_3$	$-CH(CH_3)_2$	194.73	20.13	20.13	1.000	0.00	346	346
2′c	$-CH_3$	$-C(CH_3)_3$	---[b]	24.71	24.71	1.000	0.00	---[b]	---[b]
2″d	$-CH_3$	$-OCH_3$	15.77	6.58	6.58	1.000	0.00	55	55
2″e	$-CH_3$	$-Cl$	36.56	3.45	3.81	1.108	0.00	384	343
2″f	$-CH(CH_3)_2$	$-Cl$	76.95	11.12	12.06	1.085	0.00	236	215

[a] Chromatographic conditions: column Merck LiChroCART 250-4 Lichrospher 100 RP18 endcapped (5 μm, 250 × 4 mm) (Cat. No. 50838); EtOH/acetate buffer pH 4.1 20 : 80 (v/v) + [γ-CD] = 25 mM, 0.8 ml/min, $T = 22.5°C$.

2 ($r = 0.978$) and **2′** ($r = 0.999$). The contribution of the group R_1 to lipophilicity is less pronounced than the one of the group R_2 (k'(**2c**) < k'(**2′c**)), since it is linked to the very polar heterocyclic ring: the slope of plots k'(**2′**) vs. k'(**2**) is larger than 1, with a good correlation ($s = 1.31$, $r = 0.992$).

Since the hydrophobic interaction is affected by various factors such as the chain length and the amount of bonded alkyl moieties in the stationary phase, as well as the type and the content of the organic solvent and the additives in the mobile phase, the addition of CD in the mobile phase is expected to cause a change of the retention value of the sample solute owing to the formation of an inclusion complex. With addition of γ-CD, the capacity factors, $k'(-)$ and $k'(+)$, of all solutes examined decreased to a great extent, as has been shown in the case of strong complexation [30–32]. The decrease in k' values caused by the addition of CDs in the mobile phase is based on the formation of a complex which results in a weakening of the hydrophobic interaction between solutes and the stationary phase. With addition of γ-CD to the mobile phase, retention decreases with the same magnitude for compounds **2** and **2′**, indicating that the complexation hinders both the participation of the R_1 and R_2 groups in the interaction with the support. This suggests that CD-complexes do not adsorb on RP18 silica, as it is always assumed for the calculation of the stability constant of complexes [22–25]. As noted in the absence of γ-CD (k'_0), the retention increases continuously with the series methyl to tert-butyl both for the R_1 and R_2 groups, pointing out the total contribution of the uncomplexed molecules to retention.

If the addition of γ-CD retains the similarity of the behaviour of compounds **2** and **2′** concerning retention, the chiral discrimination is affected differently: com-

pounds **2'** are not separated, whereas compounds **2** are nicely resolved. These α values (Table I) confirm the conclusions drawn from the factorial design applied to compounds **1** [6]: the role of the association CD cavity/aryl part, and the influence of the steric hindrance on the chiral resolution. For compounds **2**, the elution order remains constant, i.e. the dextrorotatory atropisomer is first eluted, when R_1 varies from methyl to isopropyl. On the other hand, when R_1 is a tert-butyl group (compound **2d**), the elution order of the two atropisomers is reversed. Since these four solutes have the same absolute configuration for a given sign (Figure 1), we assume another mechanism of chiral recognition for **2d**. Several examples of enantiomer separations through inclusion of a tert-butyl group are reported in the literature [33–35]. Nevertheless, when there is a possibility of inclusion of an aryl part or a tert-butyl group, for example in the case of the separation of the enantiomers of diniconazole using a β-cyclodextrin-bonded column [28], the insertion of the benzene ring is considered as responsible for the chiral discrimination. However, for **2d**, the inversion in the elution order compared to other compounds **2** might indicate the dominance of the tert-butyl inclusion. Whereas the selectivity α increases with the size of R_1 from methyl ($\alpha = 1.068$) to isopropyl ($\alpha = 1.112$), the possibility to include either the R_1 group or the aryl part inside the CD cavity for compound **2d** leads to decrease of α ($\alpha = 1.080$). Similar effects have already been observed in the resolution of thromboxane antagonists using β-cyclodextrin [36], where the replacement of a 2-chlorophenyl by a tert-butyl group resulted in a decrease of the separation factor from 1.19 to 1.06, all features of the molecules, in particular the presence of an aryl part, being elsewhere conserved.

The relation between the separation factor α and the stability constant of the complexes (as defined in Section 2.4) has been studied by Fujimura *et al.* [23], and is given by Equation 7:

$$\alpha = \frac{k'_2}{k'_1} = \frac{k'_{02}}{k'_{01}} \frac{K_{D2}}{K_{D1}} \left(1 + \frac{K_{D1} - K_{D2}}{K_{D2} + [CD]_T}\right). \tag{7}$$

In the case of enantiomers this equation reduces to:

$$\alpha = \frac{K_{D2}}{K_{D1}} \left(1 + \frac{K_{D1} - K_{D2}}{K_{D2} + [CD]_T}\right). \tag{8}$$

Thus, it is worth noting that the separation factor between isomers depends on the K_D values of the inclusion complexes and on the difference of magnitude between K_{D1} and K_{D2}. The apparent stability constants, K_s, of inclusion complexes for compounds **2** and **2'**, calculated from k'_0 and k at [γ-CD] 25 mM according to Equation 6, are listed in Table I (last columns). The assumptions used to derive Equation 6 (non-adsorption of CD and of CD complexes on the support, non-inclusion of the organic modifier) and the 1 : 1 stoichiometry have been controlled for compounds **1** [6], although it should always be borne in mind that the lack of a generally accepted substance and/or method for precise measurement of t_0 values

(retention times of a non-retained compound) in RPLC [37] may cause some errors in the determination of k' values. Furthermore, the binding constant of the inclusion complex, obtained by this reversed-phase liquid chromatographic method using an aqueous-organic mobile phase, is not independent of the mobile phase compositions being used, because the retention value of the solute is influenced not only by the concentration of CD but also by the type and the content of organic solvent in the mobile phase. All these assumptions lead to an error of ± 10% in the determination of the stability constants [6]. Nevertheless, the K_s values thus obtained, which vary from $55M^{-1}$ to $540M^{-1}$, are considered reasonable since they are similar to those reported in the literature for anthracene [38] ($340M^{-1}$ in γ-CD at 15°C), or drugs in β-CD [39] (propranolol: $220M^{-1}$, warfarin: $520M^{-1}$, indomethacin: $620M^{-1}$).

It is known that the inclusion process of cyclodextrin is quite selective because the degree of complex formation between the host and guest molecules is closely related to the compatibility of the cavity size and steric arrangement of the guest molecule. Besides, other factors such as hydrogen bonding and van der Waals forces may also play a role in determining the ease of complex formation [40–45]. The K_s values of compounds **2** and **2′** must be examined considering all these points. Since the host-guest interaction depends on the fit of the structural features of the guest molecule to the cavity of CD, two parts in our molecules may be included inside γ-CD: the heterocyclic ring and the aryl part. Nevertheless, according to the polarity of the cavity [46], the insertion of the benzene ring is more likely. For compounds **2**, the increase in size of the R_1 group leads to an increase in the complex stability and also to an improvement of the selectivity. The aryl part is included inside the γ-CD, whereas R_1 increases the basic and dipolar character of the thiocarbonyl function [47], and so by reinforcing the X_1/CD rim interactions the stability constant increases and leads to a better chiral discrimination. Although compounds **2′** are not separated, they present stability constants of the same magnitudes as those for their analogs **2**. It is assumed that the steric hindrance caused by R_2 precludes the discriminative insertion of the aryl part, with a loss of selectivity and probably a decrease in stability constant, which may be then well compensated by the inclusion or association of the heterocyclic part with the γ-CD.

Compounds **2″** differ from compounds **2** by the nature of the R_2 group. The replacement of the methyl group in **2a** by a methoxy group (**2′d**) or a chloro group (**2″e**) leads to a drastic change both in selectivity and complex stability, with comparable retention. Some examples have been reported on the marked effect of the methoxy group on the enantioselectivity [36, 48]: in the separation of thromboxane antagonists by β-CD [36], the presence of −OMe leads to small or non-existent selectivity, which may be due, according to the authors, to the bulk of the methoxyl substituent hindering inclusion by steric hindrance. The same considerations have been taken into account in the separation of derivatives of mandelic acid [48] in the presence of β-CD: the *o*-methoxy derivative was not resolved, whereas the *o*-chloro derivative was nicely resolved, as in the case of compounds **2″**. The poor stability of the complex formed with **2″d** shows that

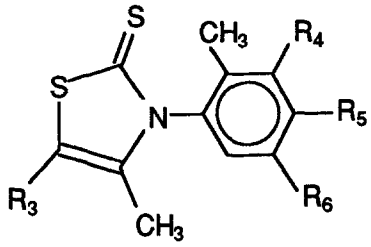


Fig. 3. Compounds **3**. $R_4 = R_5 = R_6 = H$ and $R_3 = H$ (**3a**) or CH_3 (**3f**). $R_4 = CH_3$, $R_5 = R_6 = H$ and $R_3 = H$ (**3b**) or CH_3 (**3g**). $R_4 = Cl$, $R_3 = R_5 = R_6 = H$ (**3e**). $R_4 = R_6 = H$ and $R_5 = CH_3$ and $R_3 = H$ (**3c**) or CH_3 (**3h**). $R_4 = R_5 = H$, $R_6 = CH_3$ and $R_3 = H$ (**3d**) or CH_3 (**3i**).

the absence of chiral discrimination is a consequence of a lack of inclusion. The results obtained for compounds **2a** and **2″e** show the influence of a chloro group: the introduction in the molecule of a chloro group (**2″e**), whose size is approximately that of a methyl group, but with a higher lipophilicity according to the k'_0 values, has a great positive effect on the selectivity ($\alpha = 1.108$ instead of 1.068) and on the complex stability ($\Delta K_s = +180\ M^{-1}$). The same considerations reflect the results obtained for compounds **3b** and **3e**, which differ by the group in position 3′ of the aryl part: the replacement of a methyl by a chloro group, which in terms of lipophilicity represent a great variation of retention ($\Delta k'_0$ 80 units), leads also to a drastic improvement of the enantioselectivity (1.000 to 1.073) and of the complex stability ($\Delta K_s = +100\ M^{-1}$). On the other hand, when R_1 is a group of larger size, such as isopropyl, the change from methyl (**2c**) to chloro (**2″f**) has an opposite influence, i.e. a decrease in lipophilicity ($\Delta k'_0 = -80$ units), in selectivity (from 1.112 to 1.085) and in the complex stability ($\Delta K_s = -100\ M^{-1}$). In the literature the effect of a chloro group has often been contradictory: the introduction of a chloro group in chiral benzo-1,4-diazepine [49, 50] leads to a loss of enentioselectivity, probably by steric hindrance, whereas the enantiomers of chloro diniconazole substituted in position 2 of the phenyl ring are well separated [28]. In the case of compound **2″f**, the isopropyl group may cancel the effect of the chloro group. Considering the spatial arrangement of the heterocyclic ring and the aryl part, the methyl groups of isopropyl are in a spatial proximity to the chloro group giving rise to a possible shielding of the effect of the chlorine.

Compounds **1** and **3** (Figure 3) differ in regard to the 3′, 4′ and 5′ substituents. Both have methyl at C-4 (heterocyclic ring) and C-2′ (aromatic ring). The chromatographic data are reported in Table II. They give information about the influence of the steric hindrance of the aryl part on the inclusion inside the CD cavity. Without the addition of γ-CD, the elution order given by the k'_0 values is **3b**, **3c**, **3d** respectively and **3g**, **3h**, **3f**, which corresponds to the elution order *o*-, *m*-, *p*- for the positional isomers of xylene or ethyltoluene [51] on a Lichrosorb RP-8 column. With addition of γ-CD, this elution order does not hold any more and is no

TABLE II. Chromatographic data for compounds **3**.[a]

cpd	R_3	R_4	R_5	R_6	k'_0	$k'(-)$	$k'(+)$	α	R_s	$K_s(-)$ (M^{-1})	$K_s(+)$ (M^{-1})
3a	−H	−H	−H	−H	30.36	4.95	5.29	1.068	0.54	205	189
3b	−H	$-CH_3$	−H	−H	64.70	11.10	11.10	1.000	0.00	193	193
3c	−H	−H	$-CH_3$	−H	88.34	9.53	10.10	1.060	0.63	330	309
3d	−H	−H	−H	$-CH_3$	161.25	17.23	18.49	1.074	0.98	334	308
3e	−H	−Cl	−H	−H	143.44	17.38	18.65	1.073	0.98	290	267
3f	$-CH_3$	−H	−H	−H	60.04	9.91	9.02	1.099	1.21	197	220
3g	$-CH_3$	$-CH_3$	−H	−H	127.30	21.37	21.37	1.000	0.00	188	188
3h	$-CH_3$	−H	$-CH_3$	−H	153.16	17.72	19.12	1.088	0.90	305	280
3i	$-CH_3$	−H	−H	$-CH_3$	248.36	29.96	34.27	1.144	1.84	291	249

[a] Same conditions as in Table I.

longer correlated with that observed for alkylbenzenes in the presence of β-CD. The contribution of the heterocyclic ring to the complexation is thus demonstrated. Considering the fact that the greater the complex stability the faster is the elution of the solute, the stability constants must follow an inverse relation to the elution order, i.e. $K_s(\mathbf{3b}) < K_s(\mathbf{3d}) < K_s(\mathbf{3c})$. The disagreement of the order of the stability constant ($K_s(\mathbf{3b}) < K_s(\mathbf{3d}) = K_s(\mathbf{3c})$) may be explained by the great difference of lipophilicity between compounds **3b** and **3d**. Figure 4 describes a simple model of inclusion which accounts for the stability constants as well as the selectivity. For compounds **3c**, **3d** or **3h**, **3i** the selective insertion of the aromatic ring may be considered towards the methyl group in position 4′ (R_5) or 5′ (R_6). The thiocarbonyl function is then in the proximity of the hydroxyl groups on the rim of the CD cavity. The dipole-dipole interactions carried out thus contribute to increase the separation factor. The α value for compounds **3c** ($\alpha = 1.060$) and **3d** ($\alpha = 1.074$) are similar. Even so, the model of insertion is different. For compound **3b**, the methyl group in position R_4 gives a great steric hindrance to the insertion of the aryl part toward the N-R_5 axis. The inclusion may occur only toward the methyl group in position 2′. Since the two rings of the molecule are in two perpendicular planes, the thiocarbonyl function is far from the CD rim and may not interact in a selective manner. Nevertheless, the group in position 3′ may play a role in the complex stability and in the enantioselectivity: when position 3′ is substituted by a methyl group (compounds **3b** and **3g**), its influence is only a steric hindrance, which causes a decrease of the complex stability ($193M^{-1}$ and $188M^{-1}$, respectively) and the absence of separation ($\alpha = 1.000$). On the other hand, the presence in 3′ of a chloro group (compound **3e**) which is similar in size to a methyl group but may interact with the hydroxyl groups of γ-CD, leads to a stabilisation of the complex ($K_s = 290M^{-1}$) with a good selectivity ($\alpha = 1.073$). As a consequence, the non-separation when the 3′ position is substituted by groups

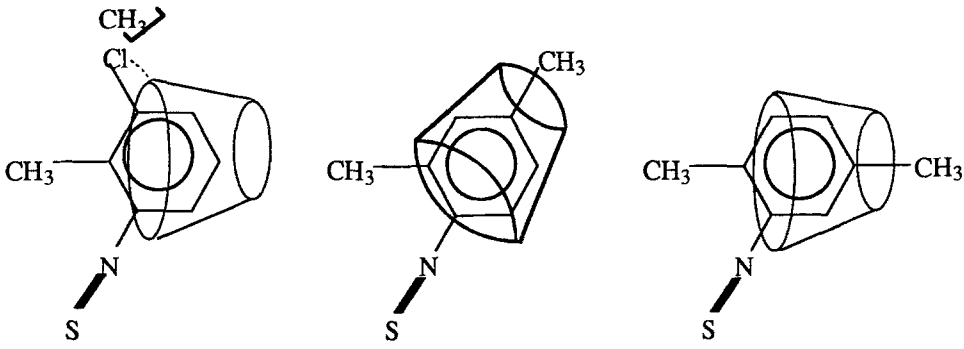


Fig. 4. Proposed inclusion models of compounds **3** inside the γ-CD cavity. (N-S is an edge view representation of the heterocycle, which is perpendicular to the aryl ring.)

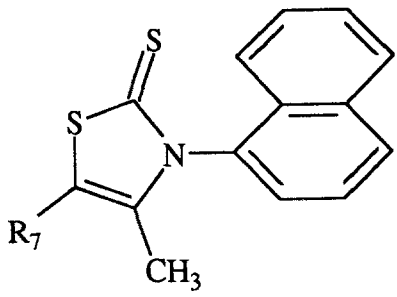


Fig. 5. Compounds **4**. R_7 = H (**4a**); R_7 = CH_3 (**4b**).

of similar size results from a steric hindrance toward the inclusion and from an eventual lack of stabilisation by interaction with the hydroxyl groups of the CD rim.

Results obtained for *N*-naphthyl-thiazoline-2 thione (compounds **4**, Figure 5) are reported in Table III: the benzoannulation mimics the substitution observed in **3b** and **3g**. However, the shape of a naphthyl group is adequate for the inclusion in the γ-CD cavity [52, 53]. It results in good separations and large K_s values.

TABLE III. Chromatographic data for compounds **4**.[a]

cpd	R_7	k'_0	$k'(-)$	$k'(+)$	α	R_s	$K_s(-)$ (M^{-1})	$K_s(+)$ (M^{-1})
4a	−H	85.89	15.35	14.44	1.063	0.90	183.8	197.9
4b	$-CH_3$	174.02	30.02	28.29	1.061	0.94	191.9	206.1

[a] Same conditions as in Table I.

4. Conclusion

Results obtained for compounds **2** and **3** show that the chromatographic resolution of the atropisomers of *N*-arylthiazoline-2 thiones in the presence of γ-CD is influenced not only by the inclusion of the aryl part, but also by interactions which may occur between the heterocyclic part or the substituents on the aryl part and the hydroxyl groups on the rim of the CD. The importance of the nature of a substituent has been demonstrated by the substitution of the same position by two groups which have a similar size but a different ability to interact, i.e. methyl and chloro groups. The substitution by an alkyl group either in position 4 of the heterocycle (**2**) or in position 2′ of the aryl part (**2′**) results in similar effects on retention, but the chiral discrimination is affected differently: compounds **2′** are not resolved whereas **2** are nicely resolved under similar complexation constants.

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