Structure and substituent effects on retention and chiral resolution of ketones and alcohols on microcrystalline cellulose triacetate plates

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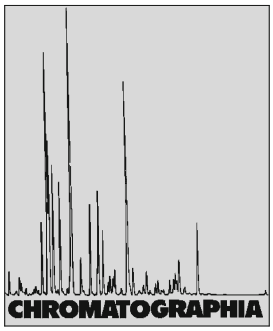

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Abstract

This paper reports a number of original thin layer chromatography enantioseparations of closely related ketones and alcohols such as tetralones, indanones, and benzhydrols carried out by elution with aqueous-alcoholic mixtures at different ratios. In order to investigate the structural and substituent effects on chiral recognition of microcrystalline cellulose triacetate, the results were compared with those obtained in previous papers for analogous compounds in similar experimental conditions. Even though the inclusion model of retention of analytes on this chiral stationary phase is confirmed, different and unexpected results were obtained for compounds having very favourable characteristics for resolution.

Keywords

Thin layer chromatography
Chiral separations
Mechanism of chiral recognition
Microcrystalline cellulose triacetate
Racemic ketones and alcohols

Introduction

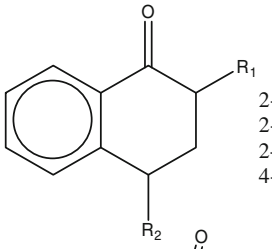
An excellent book [1], recently published, reports numerous applications of TLC for chiral separations and highlights the great potential of this technique for obtaining good resolution of the enantiomers and accurate quantitative determination of a large number of optical isomers. Among the various TLC applications of chiral stationary phases (CSPs), the most important were obtained on microcrystalline cellulose triacetate (MCTA), using both ready-to-use plates [2, 3] and non-commercial plates [4–11].

MCTA is an inexpensive material that does not adsorb UV light, enabling analyte detection and quantification by UV-densitometry; it was used for the resolution of more than 65 pairs of enantiomers eluting with aqueous-alcoholic mixtures containing methanol, ethanol or 2-propanol [2–11]. More specifically, a remarkable selectivity ($\alpha = 2.64$) was observed for Troger's base, which contains an asymmetric nitrogen atom (a tertiary amine rigidly locked in the ring) and is the usual standard for testing the resolving power of different chiral selectors [12].

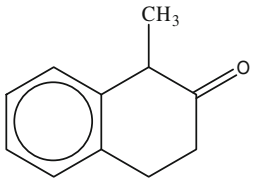
Some studies [8, 13, 14] have pinpointed several factors that favour and others that hinder the chiral discrimination of MCTA. In particular, the following factors play an important and positive role in the resolution:

1. inclusion of molecules in the asymmetric cavities of the supramolecular helical structures of MCTA which is mainly governed by the size of solutes and was proposed as a pre-requisite for the chiral recognition by Francotte et al. [14];
2. dipole–dipole interactions between the ester carbonyl groups of MCTA and the carbonyl group of solutes;
3. presence of a stereogenic centre on a rigid structure.

However, the general criteria reported above do not allow to predict with any certainty whether two enantiomers will be separated on MCTA



2-Acetyl-1-tetralone $R_1 = COCH_3$ $R_2 = H$
2-Methyl-1-tetralone $R_1 = CH_3$ $R_2 = H$
2-Bromo-1-tetralone $R_1 = Br$ $R_2 = H$
4-Methyl-1-tetralone $R_1 = H$ $R_2 = CH_3$



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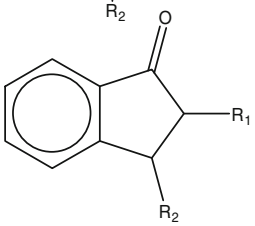
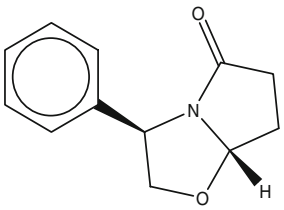


2-Ethyl-1-indanone $R_1 = CH_2CH_3$ $R_2 = H$
2-Butyl-1-indanone $R_1 = CH_2CH_2CH_2CH_3$ $R_2 = H$
2-Bromo-1-indanone $R_1 = Br$ $R_2 = H$

3-Phenyl-1-indanone $R_1 = H$ $R_2 =$ 



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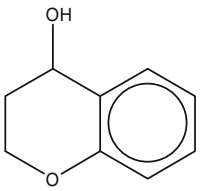

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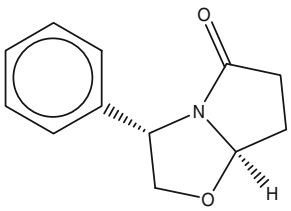
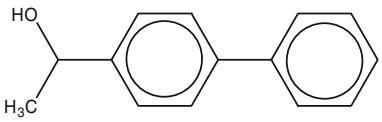
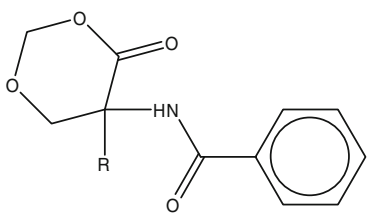


(*3S-cis*)- (+)-3-Phenyltetrahydropyrrolo-[2,1-*b*]oxazol-5(6*H*)-one



1-(4-Biphenyl)ethanol



DL-Benzoylamino-5-benzyl-4-oxo-1,3-dioxane R = 
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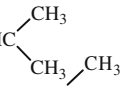
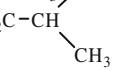
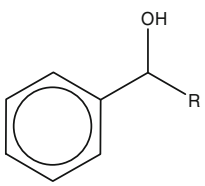
DL-Benzoylamino-5-isopropyl-4-oxo-1,3-dioxane R = 
DL-Benzoylamino-5-isobutyl-4-oxo-1,3-dioxane R = 



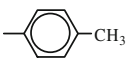
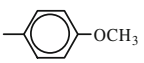
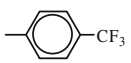
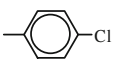
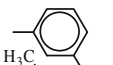
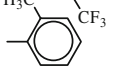
4-Methylbenzhydrol R = 
4-Methoxybenzhydrol R = 
4-(Trifluoromethyl)benzhydrol R = 
4-Chlorobenzhydrol R = 
3-(Trifluoromethyl)benzhydrol R = 
2-Methylbenzhydrol R = 

Fig. 1. The structures of the test solutes

Table 1. Retention (hR_{f1}, hR_{f2}) and resolution (α,R_S) data for racemic tetralones and indanones on non commercial MCTA/silica gel 60GF_{254} plates

Racemate	hR_{f1}*	hR_{f2}*	α**	R_s***	MCTA/silica gel ratio *(w/w)*	Reference
2-Acetyl-1-tetralone[a]	–	–	–	–	–	This study
2-Methyl-1-tetralone	53	57	1.19	1.4	4/1	This study
2-Bromo-1-tetralone	31	44	1.76	4.2	4/1	This study
4-Methyl-1-tetralone[a]	–	–	–	–	–	This study
1-Methyl-2-tetralone[a]	–	–	–	–	–	This study
2-Methyl-1-indanone	50	57	1.33	1.8	3/1	[7]
2-Ethyl-1-indanone	58	66	1.38	2.0	4/1	This study
2-Butyl-1-indanone	64	68	1.19	1.4	4/1	This study
2-Bromo-1-indanone	36	46	1.52	2.9	4/1	This study
3-Methyl-1-indanone	52	58	1.28	1.6	3/1	[7]
3-Phenyl-1-indanone[a]	–	–	–	–	–	This study

Eluent: ethanol/water 80/20 (*v*/*v*)
Migration distance ≅16 cm
* $hR_f = R_f \times 100$
** $\alpha = [(1/R_{f1})-1]/[(1/R_{f2})-1]$
*** $R_S = 2 \times$ (distance between the centres of two adjacent spots)/(sum of the widths of the two spots in the direction of development)
[a] Racemates unresolved under all the experimental conditions used in this work

plates; in fact, small variations of the chemical structure of solutes might have an adverse effect on resolution.

For this reason, the aim of this paper was to investigate the separation on MCTA plates of new structurally related enantiomeric pairs and to compare the results of this research with those previously obtained, thus increasing the knowledge of the mechanisms governing the selectivity of this CSP.

These studies are now possible because numerous racemates or enantiomeric pairs, such as new chiral ketones and alcohols, are commercially available, also at low cost.

Experimental

Chemicals

Racemates and high purity (≥95%) optical isomers were purchased from Sigma-Aldrich (St. Louis, MO, USA), Alfa Aesar (Ward Hill, MA, USA) and Acros Organics (Geel, Belgium).

TLC Analyses

Water, methanol, ethanol, and 2-propanol used for the preparation of analyte solutions and the elution of MCTA layers were all LC grade and were obtained from Merck (Darmstadt, Germany).

MCTA for LC (particle size <10 μm) was purchased from Fluka (Buchs, Switzerland). MCTA layers were prepared as reported in a previous paper [5]; in particular, layers with an MCTA/silica gel ratio 3/1 (*w*/*w*) were obtained by adding 3 g of silica gel 60 GF_{254} (particle size 15 μm, Merck) to 15 mL of water; the suspension obtained was mixed briefly with magnetic stirring, after which, 9 g of MCTA and 35 mL of ethanol were added; the suspension was stirred for 5 min and finally transferred into a Camag (Muttenz, Switzerland) automatic TLC-plate coater. The layers (10 × 20 cm or 20 × 20 cm, thickness 250 μm) were dried at room temperature and used within 2–5 h.

Layers with an MCTA/silica gel ratio 4/1 (*w*/*w*) were also prepared in this study as described above, by using 2.25 g of silica gel, 11.5 mL of water, 9 g of MCTA and 35 mL of ethanol.

Solutions (4–8 mg mL^{-1}) of racemates and pure optical isomers were prepared in either methanol or aqueous ethanol (80%). These solutions (0.5–1 μL) were applied 1 cm from the bottom and at least 1.5 cm from the sides of the plates, using a Microliter syringe (Hamilton, Reno, NV, USA). The plates were developed via the ascending technique in a Desaga (Wieslock, Germany) thermostatic chamber (22 × 22 × 6 cm), at 23 °C, after saturation for 1 h with the elution mixture.

Detection was carried out with UV lights at 254 nm, using a Camag UV Cabinet 3. Densitometric measurements were performed in the reflection mode at $\lambda = 254$ nm with a Shimadzu (Kyoto, Japan) CS-9001 PC densitometer coupled to a Pentium 1 IBM-compatible PC. Plates were scanned in a zigzag manner over the sample zones. All functions of the scanner were controlled and data were processed with TLC-specific software manufactured by Shimadzu. Real time background correction was automatically performed in the zigzag scanning mode.

Volume and Partition Coefficient Calculations

The molecular volume (V_m) and logarithm of octanol–water partition coefficient (log K_{ow}) of the target compounds were calculated after optimization (MM2 force field) of their three-dimensional structures using the software package CHEM3D Ultra 8.0 (Cambridge Soft Corporation, Cambridge, MA, USA).

Results and Discussion

The structures of the test solutes are illustrated in Fig. 1.

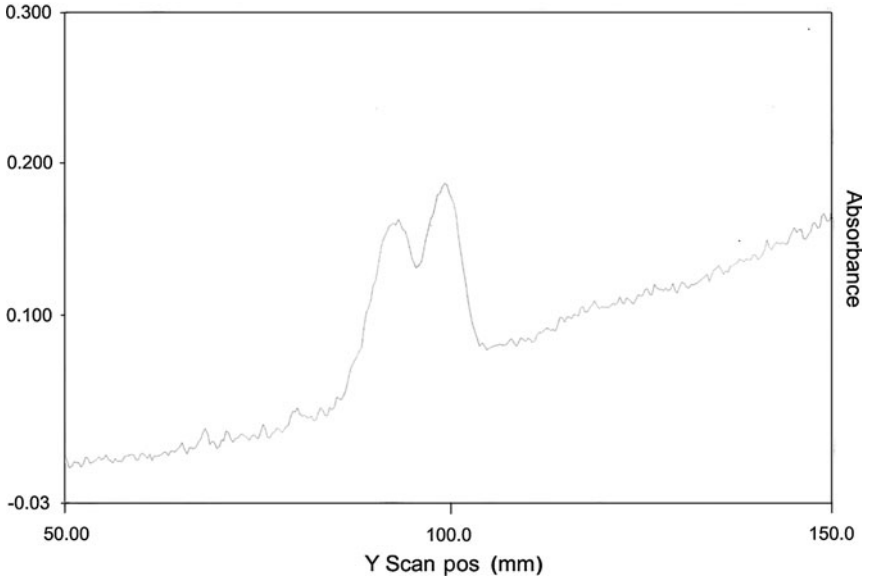


Fig. 2. Densitogram ($\lambda = 277$ nm) of racemic 2-butyl-1-indanone on MCTA/silica gel 4/1 layers eluted with ethanol/water 80/20 (*v*/*v*). The migration distance was 14 cm. 0.4 μL of a 5.0 mg mL^{-1} solution were applied to the plate

Table 2. Retention (hR_{f1}, hR_{f2}) and resolution (α, R_S) data of DL-5-benzoylamino-4-oxo-1,3-dioxane derivatives, substituted in the 5-position, on non commercial MCTA-silica gel $60GF_{254}$ plates. Migration distance ≅ 16 cm

Substituent	hR_{f1}*	hR_{f2}*	α**	R_S	Eluent mixture (*v*/*v*)	MCTA/silica gel ratio (*w*/*w*)
Methyl	55	61	1.28	1.4	2-Propanol/water 70/30	3/1
	65	70	1.26	1.4	Ethanol/water 70/30	3/1
	62	66	1.17	1.4	Ethanol/water 80/20	4/1
Isopropyl[a]	–	–	–	–	–	–
Isobutyl[a]	–	–	–	–	–	–
Benzyl[a]	–	–	–	–	–	–

* $hR_f = R_f \times 100$
** $\alpha = [(1/R_{f1})-1]/[(1/R_{f2})-1]$
*** $R_S = 2 \times$ (distance between the centres of two adjacent spots)/(sum of the widths of the two spots in the direction of development)
[a] Racemates unresolved under all the experimental conditions used in this work

Tetralones and Indanones

Several racemates with a carbonyl group in α or β-position with respect to the stereogenic centre were resolved on MCTA layers with aqueous-alcoholic mixtures as eluents [5–8, 11].

More specifically, this CSP shows a strong chiral recognition towards the optical antipodes with both the carbonyl group and the stereogenic centre on a rigid ring structure, such as flavanones [7, 8] and 2-oxazolidinones [6, 7, 11]. The enantiomers of 2-methyl and 3-methyl-1-indanone were also separated on MCTA layers [7] and Table 1 shows a comparison of their retention and resolution data with those obtained in this study for numerous structurally related tetralones and indanones. The results reported in Table 1 show two different types of behaviour among the various racemates; several were not resolved while the majority were baseline separated with R_s ranging from 1.4 to 4.2.

The use of layers with an MCTA/silica gel ratio of 4/1 allowed us to obtain R_s values higher than those observed with a 3/1 ratio owing to increased roundness and compactness of the spots; however, the selectivity coefficients for 3/1 and 4/1 ratios were the same.

The comparison between the selectivity coefficients of the different racemates gave the following information.

For tetralones the presence of the carbonyl group in the 1-position was essential for chiral discrimination, as shown by the resolution of 2-methyl-1-tetralone and the unsuccessful separation of the optical antipodes of 1-methyl-2-tetralone. The type of substituent in the ring played a key role in the chiral separation ($\alpha_{2\text{-bromo}} \gg \alpha_{2\text{-methyl}}$); unexpectedly, in the experimental conditions adopted, the resolution of racemic 2-acetyl-1-tetralone was not achieved. The presence of the stereogenic centre in the γ position with respect to the carbonyl group hindered the enantiomer resolution, as shown by the unsuccessful separation of the optical antipodes of 4-methyl-1-tetralone.

As in the case of tetralones, also for indanones the presence of the carbonyl group in the 1-position favours the resolution of various racemates. The sequence of selectivity coefficients for 2-substituted-1-indanones was the following: 2-bromo ≫ 2-ethyl ≥ 2-methyl > 2-butyl; thus, the presence of bromine in the 2-position determined the highest value of selectivity, as occurred in 2-substituted-1-tetralones. No great differences were observed between methyl and ethyl groups, also taking into account that the value for 2-methyl-1-indanone was related to layers with an MCTA/silica gel ratio of 3/1. As shown by comparison of the results obtained for 2-methyl-1-indanone and 3-methyl-1-indanone, the presence of the stereogenic centre in the 3-position with respect to the 2-position determined a lower selectivity; in addition, the substitution in the 2-position of a methyl group with a phenyl group precluded the resolution. These results highlighted yet again how steric factors play a crucial role in the chiral recognition of MCTA. As regards the results obtained for racemic 3-methyl-1-indanone, it should be noted that this racemate was studied and resolved on the MCTA column with water–alcohol mixtures as eluents [14], obtaining a significantly lower selectivity ($\alpha = 1.11$) than the one obtained in this work on MCTA plates ($\alpha = 1.28$).

The sequence of the selectivity coefficients for 1-tetralones (1T), 2-tetralones (2T), and 1-indanones (1I) was the following:

2-bromo-1T (1.76) > 2-bromo-1I (1.52) > 2-ethyl-1I (1.38) > 2-methyl-1I (1.33) > 3-methyl-1I (1.28) > 2-methyl-1T (1.19), 2-butyl-1I (1.19) > unresolved racemates (2-acetyl-1T, 4-methyl-1T, 1-methyl-2T, 3-phenyl-1I).

As previously observed, bromo derivatives of tetralone and indanone showed the highest α-values. Conversely, 2-methyl-1T had a selectivity coefficient much lower than 2-methyl-1I. Consequently, the effect of the same 2-substituent group on the chiral separation of 1-tetralones and 1-indanones was different.

By way of example, Fig. 2 contains a densitogram which is representative for separation of the enantiomers of 2-butyl-1-indanone, obtained at its maximum absorption wavelength (277 nm).

DL-5-Benzoylamino-4-oxo-1,3-dioxanes

The structure of 5-substituted-5-benzoylamino-4-oxo-1,3-dioxanes appears particularly suitable for the resolution of their enantiomers since these analytes contain a stereogenic centre located in a rigid structure and in α-position with respect to a carbonyl group. Moreover, the stereogenic centre is situated in β position with respect to a second carbonyl group present in the molecule. The racemates having methyl, isopropyl, isobutyl, and benzyl groups in 5-position were studied in order to determine the effect of the type of substituent on chiral separation.

The data in Table 2 show that only the enantiomers of 5-methyl-5-benzoylamino-4-oxo-1,3-dioxane were separated on MCTA/silica gel plates, both in the 3/1 and 4/1 ratios, eluting with mixtures of water and ethanol or 2-propanol. In all these cases, a high resolution factor ($R_s = 1.4$) was obtained for the 5-methyl derivative, although different selectivity coefficients (range of α values 1.17–1.28) were found. Figure 3 contains a densitogram obtained at the maximum absorption wavelength (236 nm) using MCTA/silica gel plates with a 4/1 ratio and ethanol/water 80/20 (*v*/*v*) as eluent.

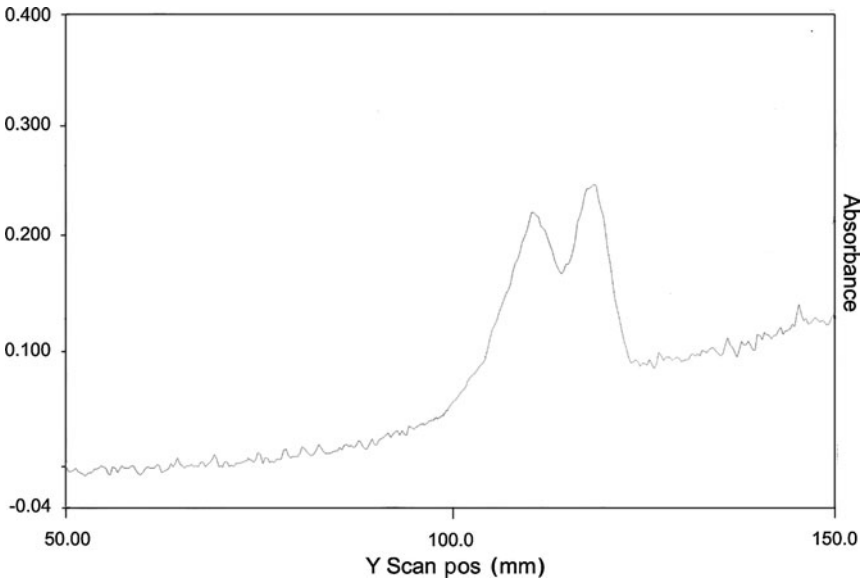


Fig. 3. Densitogram ($\lambda = 236$ nm) of DL-5-benzoylamino-5-methyl-4-oxo-1,3-dioxane on MCTA/silica gel 4/1 layers eluted with ethanol/water 80/20 (*v*/*v*). The migration distance was 14 cm. 0.2 μL of a 24.3 mg mL^{-1} solution were applied to the plate

Table 3. Retention (hR_{f1}, hR_{f2}) and resolution (α, R_S) data of the enantiomers of *cis*-3-phenyltetrahydropyrrolo-[2,1-*b*]oxazol-5(6*H*)-one, on non commercial MCTA-silica gel $60GF_{254}$ plates

MCTA/Silica Gel ratio *(w/w)*	Eluent mixture *(v/v)*	hR_{f1}*	hR_{f2}*	α**	R_S***
4/1	Ethanol/water 80/20	*3S*(+) 48	*3R*(−) 58	1.50	2.1
3/1	Ethanol/water 70/30	*3S*(+) 55	*3R*(−) 65	1.52	2.0

Migration distance ≅16 cm
* $hR_f = R_f \times 100$
** $\alpha = [(1/R_{f1})-1]/[(1/R_{f2})-1]$
*** $R_S = 2 \times$ (distance between the centres of two adjacent spots)/(sum of the widths of the two spots in the direction of development)

The absence of chiral discrimination for isopropyl, isobutyl, and benzyl derivatives can be related to the dimension of the substituent group which hinders the stereospecific interactions of analytes with MCTA in the experimental conditions used.

Enantiomers of *cis*-3-Phenyltetrahydropyrrolo-[2,1-*b*]oxazol-5(6*H*)-one

The structure of this compound favours the enantioseparation on MCTA because two stereogenic centres on a rigid structure are present in the β-position with respect to the carbonyl group located in one of the two rings.

As shown in Table 3, the two enantiomers 3*S* (+) and 3*R* (−) were markedly separated both on MCTA/silica gel 3/1 and 4/1 ratios, eluting with ethanol–water mixtures at different ratios and the enantiomer *cis*-3*S* (+) was retained the most. In both cases, similar and high α, R_s and ΔR_f values (0.10) were obtained.

Figure 4 contains a representative densitogram obtained for the mixture of the two enantiomers at the maximum absorption wavelength (200 nm), using MCTA/silica gel layers with a 4/1 ratio and ethanol/water 80/20 (*v*/*v*) as eluent.

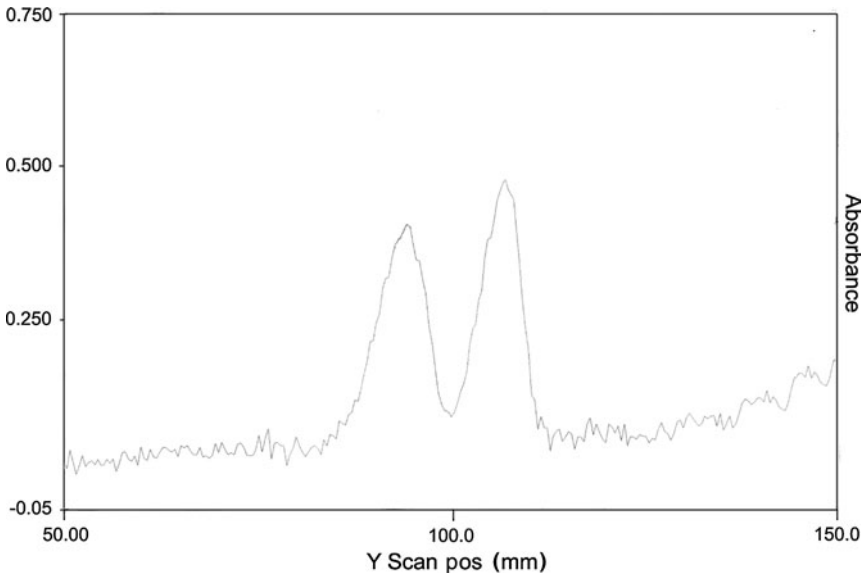


Fig. 4. Densitogram ($\lambda = 200$ nm) of the enantiomers of *cis*-3-phenyltetrahydropyrrolo-[2,1-*b*]-oxazol-5(6*H*)-one on MCTA/silica gel 4/1 layers eluted with ethanol/water 80/20 (*v*/*v*). The migration distance was 14 cm. 0.5 µL of a solution containing 25.8 mg mL-1 of the *R*-enantiomer and 22.7 mg mL-1 of the *S*-enantiomer were applied to the plate

Enantiomeric and Racemic Alcohols

Although the most suitable chiral stationary phase for the resolution of racemic aromatic alcohols is cellulose tribenzoate [15, 16], some chiral alcohols were baseline separated on MCTA plates [5, 6, 10], showing that the hydroxyl group is able to interact with the carbonyl groups of cellulose triacetate via hydrogen bonding.

α-Substituted Benzyl Alcohols

Table 4 contains a comparison of the results obtained in this study with those reported in previous work [5, 6] for nine α-substituted benzyl alcohols. The presence of a substituent such as the benzoyl group favours the resolving power of MCTA since the resulting compound (benzoin) contains a carbonyl group in α-position with respect to the stereogenic centre. On the contrary, the presence of a second hydroxyl group instead of the carbonyl group (hydrobenzoin) prevents the resolution. However, the chiral separation is possible if three phenyl groups (see 1,1,2-triphenyl-1,2-ethandiol) are present in the molecule as they increase the hydrophobicity of the racemate.

The six benzhydrol derivatives do not contain carbonyl groups or additional hydroxyl groups; however, they differ due to the presence of substituents such as $-CH_3$, $-OCH_3$, $-CF_3$ and -Cl in the phenyl moieties. Among these, 4-chlorobenzhydrol shows the highest selectivity coefficient value on layers with an MCTA/silica gel ratio of both 4/1 ($\alpha = 1.38$) and 3/1 ($\alpha = 1.34$), eluting with mixtures of water and ethanol or 2-propanol. The two enantiomers were baseline separated on both layers as shown by the values of the resolution factor ($R_s = 1.7$ and 1.5, respectively). A densitogram which is representative for separation of the two enantiomers of 4-chlorobenzhydrol, obtained at the maximum absorption wavelength (225 nm), using 2-propanol/water 70/30 (*v*/*v*) as eluent and an MCTA/silica gel 4/1 ratio, is shown in Fig. 5.

The 4-methoxy-benzhydrol showed a lower selectivity ($\alpha = 1.23$), giving rise to a spot with a shape similar to the figure "eight".

Partial separations were obtained for 2-methyl and 4-methyl-benzhydrol while the trifluoromethyl derivatives were not resolved; the behaviour of the trifluoromethyl benzhydrols can be attributed to the presence of the substituent group $-CF_3$ that substantially modifies the polarity of the molecule and/or the stereogenic centre, leading to different chiral discriminations.

Ethanol Derivatives and Alcohols with the Stereogenic Centre on a Rigid Ring Structure

Table 5 illustrates the results obtained for five ethanol derivatives and three alcohols with the stereogenic centre located on a rigid structure. Among these, only 1-(4-biphenyl) ethanol and 4-chromanol were studied in this work.

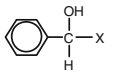
With regard to the ethanol derivatives, 1-(9-fluorenyl)ethanol and 2,2,2-trifluoro-1-(9-anthryl)ethanol showed high selectivity coefficients (2.24 and 2.02, respectively), whereas, among the other three compounds, only 1-(2-naphthyl)ethanol was resolved ($\alpha = 1.41$). Therefore, the positive role played by three aromatic ring fused together on the chiral recognition of MCTA, as well as by the 2-position of naphthyl group is evident, since the racemic 1-(1-naphthyl)ethanol was not resolved.

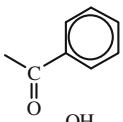
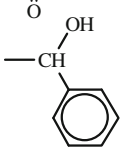
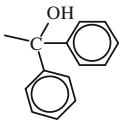
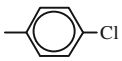
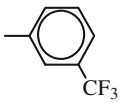
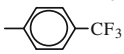
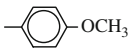
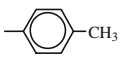
The fundamental importance of the lipophilicity of a solute is marked by the high selectivity coefficients of 7,8,9,10-tetrahydro-benzo[a]pyrene-7-ol ($\alpha = 2.04$) and 1-acenaphthenol ($\alpha = 1.28$). The racemic 4-chromanol, having only one aromatic ring, was not resolved, confirming the importance of hydrophobic interactions on chiral discrimination of MCTA, even when the stereogenic centre containing the hydroxyl group is locked in a rigid structure.

Mechanism of Chiral Recognition on MCTA

In order to relate the chiral resolving power of MCTA with selected characteristics of closely related solutes and with their capacity to permeate into the cavities of the polymer (an essential condition for chiral resolution), it is useful to evaluate the molecular volumes of the investigated compounds.

For example, aromatic hydrocarbons such as benzene and mesytilene behave differently on MCTA columns eluting with ethanol/water 95/5 *v*/*v*, with the former retained more than the latter.

Table 4. Retention (hR_{f1}, hR_{f2}) and resolution (α, R_S) data for enantiomeric and racemic benzyl alcohols  on non commercial MCTA-Silica Gel $60GF_{254}$ plates

	hR_{f1}*	hR_{f2}*	α**	R_s***	Eluent mixture *(v/v)*	MCTA/silica gel ratio *(w/w)*	Reference
	39 (S)	45 (R)	1.27	1.8	2-Propanol/water 80/20	3/1	[5]
	–	–	–	–	–	–	[6]
	34 (R)	42 (S)	1.40	1.8	Ethanol/water 80/20	3/1	[6]
	41	49	1.38	1.7	2-Propanol/water 70/30	4/1	This study
	37	44	1.34	1.5	Ethanol/water 70/30	3/1	This study
	–	–	–	–	–	–	This study
	–	–	–	–	–	–	This study
	41	46	1.23	1.0	2-Propanol/water 70/30	4/1	This study
	38	42	1.18	0.9	Ethanol/water 70/30	4/1	This study
	46	49	1.13	0.8	Ethanol/water 70/30	4/1	This study

Migration distance ≅16 cm
* $hR_f = R_f \times 100$
** $\alpha = [(1/R_{f1})-1]/[(1/R_{f2})-1]$
*** $R_S = 2 \times$ (distance between the centres of two adjacent spots)/(sum of the widths of the two spots in the direction of development)
[a] Racemates unresolved under all the experimental conditions studied

This behaviour is related to a better permeation of the MCTA cavities by benzene ($V_m = 72.6$ Å^3) than mesytilene ($V_m = 124.3$ Å^3). Conversely, 1,3,5-tri-tert-butylbenzene ($V_m = 288.7$ Å^3) is totally excluded from the cavities and is not retained by the stationary phase [17]. Therefore, the molecular volume of 1,3,5-tri-tert-butylbenzene can be considered as the exclusion volume from the MCTA cavities.

The R_f sequences for the enantiomers of tetralones (T) and indanones (I) eluted with ethanol–water 80:20 (*v*/*v*) for a MCTA/silica gel ratio of 4/1 (*w*/*w*), were the following (note that for resolved racemates, the R_f values of both enantiomers were reported):

1. 4-methyl-1T (0.57) ≥ 2-methyl-1T (0.57-0.53) > 1-methyl-2T (0.49) > 2-bromo-1T (0.44–0.31); 2-acetyl-1T (0.37)
2. 2-butyl-1I (0.68-0.64) ≥ 2-ethyl-1I (0.66-0.58) ≥ 3-methyl-1I (0.58–0.52); 2-methyl-1I (0.57–0.50) > 2-bromo-1I (0.46-0.36) ≥ 3-phenyl-1I (0.36)

The molecular volumes of tetralones and indanones (included between 132.9 and 186.7 Å^3) were much lower than the exclusion volume of MCTA; therefore, it can be drawn that the inclusion mechanism played an important role in the retention of such analytes. The strong retention of 2-acetyl-1T was due to additional interactions between the C=O of acetyl group and the ester carbonyl groups of MCTA. These kinds of interactions are achiral and make resolution problematic. Moreover, the high retention of 2-bromo-1T can also be

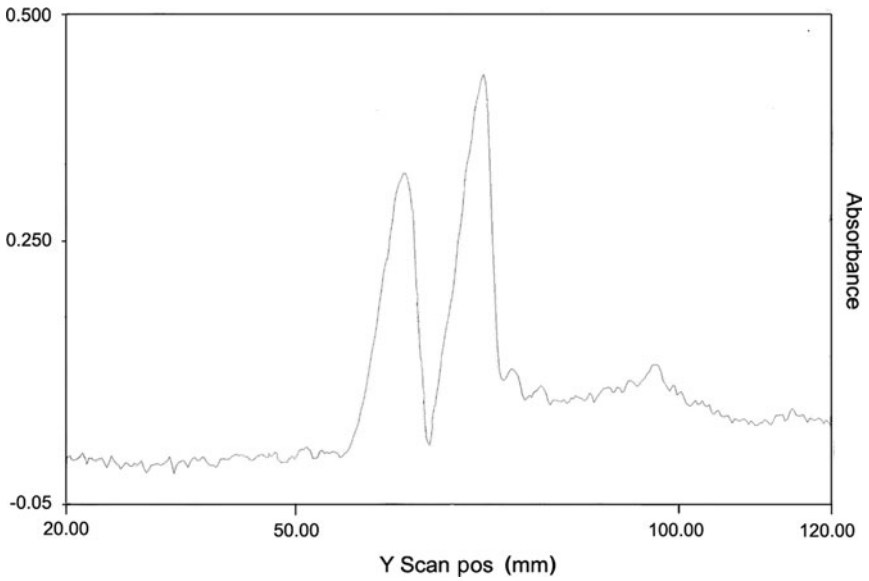


Fig. 5. Densitogram ($\lambda = 225$ nm) of racemic 4-chlorobenzhydrol on MCTA/silica gel 4/1 layers eluted with 2-propanol/water 70/30 (v/v). The migration distance was 10 cm. 0.4 μL of a 15.4 mg mL^{-1} solution were applied to the plate

attributed to dipole–dipole interactions of bromine with the functional groups of MCTA.

The unsuccessful resolution of 4-methyl-1T and 1-methyl-2T, which have the same or lower R_f values with respect to 2-methyl-1T (baseline separated), could be related to the lack of stereospecific interactions inside the cavity.

An absence of correlation between retention and hydrophobicity (log K_{ow}) was observed for indanones, the volumes of which were included between 132 $Å^3$ and 186 $Å^3$; when the homogeneous group of alkyl indanones was considered, a linear trend was obtained plotting V_m values as a function of the corresponding $\frac{1}{R_f} - 1$ data. R^2 values were 0.78 and 0.91 for the less and the more retained enantiomers, respectively, the latter being statistically significant at the 95% probability level ($P = 0.045$).

2-butyl-1I showed a lower selectivity coefficient with respect to the other three alkyl derivatives, probably owing to a poorer permeation of the cavities.

2-bromo- and 2-phenyl-1I were retained far more than alkyl derivatives owing to additional interactions between their substituent groups and the functional groups of MCTA. These supplementary interactions promoted the chiral resolution of 2-bromo-1I and were detrimental for 2-phenyl-1I, probably owing to steric effects of the phenyl group.

Further indications emerged from the chromatographic behaviour of 5-methyl ($V_m = 192.5$ $Å^3$), 5-isopropyl ($V_m = 233.9$ $Å^3$), 5-isobutyl ($V_m = 250.3$ $Å^3$) and 5-benzyl ($V_m = 269.4$ $Å^3$) DL-5-benzoylamino-4-oxo-1,3-dioxane; among these compounds only the 5-methyl derivative was resolved.

These analytes have the same structure and only differ in the dimension of the hydrophobic 5-substituent group. No relationship was observed when V_m values were plotted as a function of $\frac{1}{R_f} - 1$ data; consequently, a less important role of the inclusion mechanism can be hypothesized for the retention of 1,3-dioxane derivatives with respect to tetralones and indanones. For these solutes the retention seems to be governed to a greater extent by the reversed-phase mechanism.

The small dimensions of compounds belonging to the benzyl alcohol series (V_m included between 178.2 and 193.3 $Å^3$) suggested that their permeation is strongly facilitated in the molecular cavities of MCTA. Therefore, the unsuccessful resolution of some of these racemates (e.g. hydrobenzoin and the two trifluoromethyl derivatives) can be related to the presence of additional groups in the molecules with respect to those required for chiral recognition. These superfluous sites can give rise to some degree of competition and, therefore, to some decrease in enantioselectivity.

In line with this hypothesis, the addition of an extra phenyl group to the hydrobenzoin (see 1,1,2-triphenyl-1,2-ethandiol), increases its hydrophobicity while significantly reducing the achiral interactions due to the second hydroxyl group which increases the enantioselectivity.

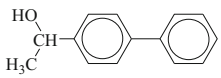
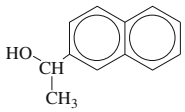
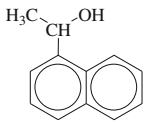
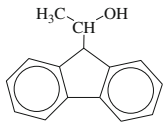
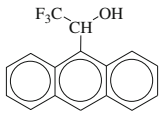
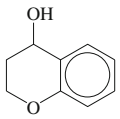
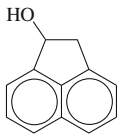
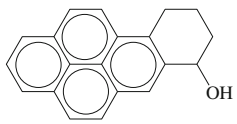
The possible cavity permeation of MCTA by chiral alcohols with larger dimensions is demonstrated by the behaviour of 7,8,9,10-tetrahydrobenzo [a]pyrene-7-ol ($V_m = 230$ A^3), which is baseline separated despite the presence of five rings fused together in the molecule. The lack of resolution of the 1-(4-biphenyl) ethanol with respect to the resolution of 1-(2-naphthyl)) ethanol and 1-(9-fluorenyl) ethanol highlights the key role of the aromatic moiety in chiral discrimination of MCTA.

Conclusions

MCTA plates can be successfully used for resolving a wide range of racemic compounds, providing useful information for analytical column applications. In fact, many enantiomeric pairs resolved in this work have not yet been studied on MCTA column and, for several of them, no chiral LC methods are reported in recent literature [18].

The results obtained in this study evidenced that the resolution of the investigated racemates on MCTA layers cannot be predicted with any certainty on the basis of their structure and substituent effects. In fact, the enantiomers of 2-methyl-1-tetralone and 1-(2-naphthyl)-ethanol were baseline separated while 1-methyl-2-tetralone and 1-(1-naphthyl)-ethanol were not resolved in their corresponding enantiomers. In this regard, it should be underlined that the molecules mentioned above had V_m values much lower than the MCTA exclusion volume and very similar log K_{ow}; therefore an easy permeation of MCTA cavities and similar interactions with the stationary phase could be hypothesized.

Table 5. Retention (hR_{f1}, hR_{f2}) and resolution (α, R_S) data for enantiomeric and racemic alcohols on non commercial plates of MCTA/Silica Gel $60GF_{254}$ 3/1 (*w*/*w*)

Alcohol	hR_{f1}*	hR_{f2}*	α**	R_s***	Eluent mixture *(v/v)*	Reference
	–	–	–	–	–	This study
	15 (S)	20 (R)	1.41	3.2	Ethanol/water 50/50	[5]
	–	–	–	–	–	[5]
	26	44	2.24	3.0	2-Propanol/water 80/20	[7]
	34 (R)	51 (S)	2.02	–	Ethanol/water 80/20	[3]
	–	–	–	–	–	This study
	57	63	1.28	1.4	2-Propanol/water 80/20	[10]
	14	25	2.04	2.8	Ethanol/water 80/20	[5]

Migration distance ≅16 cm
Migration distance ≅10 cm
* $hR_f = R_f \times 100$
** $\alpha = [(1/R_{f1})-1]/[(1/R_{f2})-1]$
*** $R_S = 2 \times$ (distance between the centres of two adjacent spots)/(sum of the widths of the two spots in the direction of development)
[a] Racemates unresolved under all the experimental conditions studied
[b] OPTI-T.A.C. F254 plates (Antec, Switzerland)

However, by comparing the various series of closely related ketones and alcohols, interesting data were obtained regarding the role of polar and hydrophobic groups on chiral recognition and the retention mechanism. In particular, for most compounds, the validity of the inclusion model of retention is confirmed, even though, for molecules with the highest V_m values such as 5-benzoylamino-4-oxo-1,3-dioxane derivatives, a significant role of reversed-phase mechanism was revealed.

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