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Direct separation of the stereoisomers of methoxytetrahydronaphthalene derivatives, new agonist and antagonist ligands for melatonin receptors, by liquid chromatography on cellulose chiral stationary phases

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Abstract

Analytical HPLC methods using derivatized cellulose chiral stationary phases were developed for the direct separation of the stereoisomers of disubstituted tetralin derivatives with two chiral centers, new agonist and antagonist ligands for melatonin receptors. The separations were made using normal-phase methodology with a mobile phase consisting of *n*-hexane–alcohol (methanol, ethanol, 1-propanol or 2-propanol) in various proportions, and a silica-based cellulose tris-3,5-dimethylphenylcarbamate (Chiralcel OD-H), or tris-methylbenzoate (Chiralcel OJ). The effects of concentration of various aliphatic alcohols in the mobile phase were studied. A better separation was achieved on cellulose carbamate phase compared with the cellulose ester phase. The effects of structural features of the solutes on the discrimination between the stereoisomers were examined. Baseline separation (R_s>1.5) was easily obtained in many cases. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Chiral stationary phases, LC; Methoxytetrahydronaphthalenes; Tetralins

1. Introduction

The interest in developing ligands for the melatonin receptors stems from the role of the neurohormone melatonin (*N*-acetyl-5-methoxytryptamin) (Fig. 1) principally synthesized by the pineal gland, in the control of various circadian and seasonal rhythms in vertebrates and from its influence on several neuroendocrine processes [1]. Therapeutic applications for melatonin ligands could be possible. Fourmaintraux et al. [2] recently described the synthesis, the pharmacological and biochemical studies of tetrahydronaphthalenic derivatives **I** and **II** (Fig. 1) as potent and specific new agonist and antagonist melatoninergic ligands. To continue this work we have further developed new compounds **1**–**5** in order to gain improved structure–

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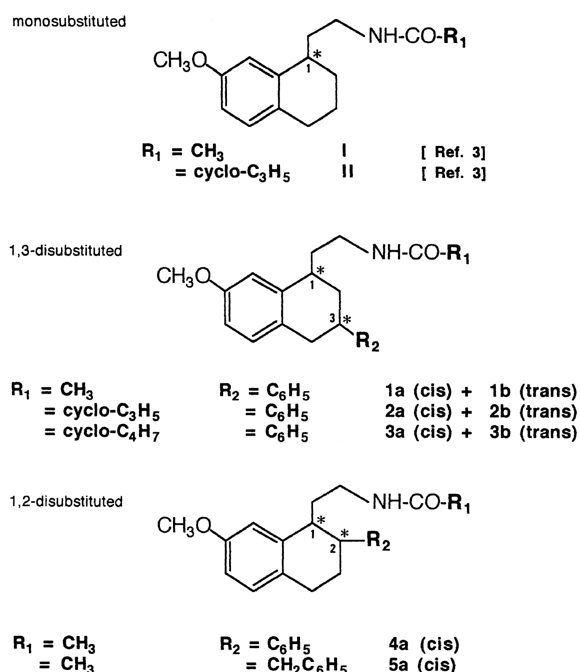

Fig. 1. Chemical structures of substrates **1**–**5**.

activity relationships and better characterize the melatonin receptors [3].

Compounds **1**–**5** have two chiral centers and as pharmacological studies have shown that enantiomers of many drugs differ in activity or in metabolism or in toxicity, the resolution of the racemic mixture seems advisable. To investigate the pharmacodynamics properties of each enantiomer, the search for a system to obtain a few milligram amounts of the relevant compounds are generally required and chiral high-performance liquid chromatography (HPLC) has been recognized as a useful methodology for this purpose [4], offering the advantage of furnishing both enantiomers. Cellulose and amylose esters and carbamates derivatives coated onto a large-pore silica gel backbone have proved to be extremely useful stationary phases for chiral resolution essentially used in the normal-phase mode [6–15]. Preliminary work has to be developed before scale-up to the preparative mode. In a previous paper we recently described [5] the analytical enantiospecific chromatographic separation of tetralins monosubstituted at the 1-position on those chiral stationary phases (CSPs).

In the continuity of our work [5] on the enantioseparation of racemates with potent biological activity, we examined in this study the direct separation of the four diastereoisomers (*cis*+*trans* forms) of **1**–**3**, disubstituted at the 1,3-positions, and the two enantiomers (*cis* form) of **4** and **5**, disubstituted at the 1,2-positions, on different CSPs of polysaccharide-derived types and particularly on (tris-3,5-dimethylphenylcarbamate) cellulose (Chiralcel OD-H) which shows a particularly high optical resolving ability among the other phenyl carbamate derivatives of cellulose developed so far [13].

2. Experimental

2.1. Reagents and materials

Compounds **1**–**3** were prepared according to the same general synthetic pathway described by Fourmaintraux et al. [2] leading to a mixture of the four diastereomers. Detailed synthesis will be published elsewhere. Attempts to resolve this diastereomeric mixture by fractional crystallization, in ethanol or in diisopropylether, only furnished enriched *cis*-form (**a**) and *trans*-form (**b**) at various percentage, the *trans*-racemate being more soluble than the *cis*-racemate. But the fractions obtained were not pure and the yields obtained were often poor after several crystallizations. Preliminary HPLC analytical works on an SiO_2 column (LiChrospher Si 100, 5 μm, 250×4.0 mm from Merck with hexane–ethanol or hexane–ethyl acetate as mobile phases at various percentages) were undertaken and were unsuccessful in separating *cis*/*trans* forms. The *cis* and *trans* absolute configurations were established by ^{1}H and ^{13}C homonuclear and heteronuclear nuclear magnetic resonance (NMR) spectroscopy [correlation spectroscopy, double quantum filtered (COSY, DQF) and rotating frame homonuclear Overhauser spectroscopy (ROESY)] on a Bruker AM 300 spectrometer operating, respectively, at 300.133 MHz and 75.469 MHz, using a 5 mm dual $^{1}H/^{13}C$ probehead at 303 K in $C^{2}HCl_3$: the complete assignment will be published with the synthesis of the products. The relative *cis*/*trans* proportions of the different fractions obtained after fractional crystallization were calculated by ^{1}H-NMR spectroscopy and by ana-

Table 1
Chromatographic parameters: retention factors (k'), enantioselectivity factor (α) and resolution (R_s) of **1a**, **1b**, **2a**, **2b**, **3a**, **3b** and **4a**, **5a** on Chiralcel OD-H

Compound[a]	Eluent[b]	k'_1	k'_2	α	R_s	First eluted enantiomer[c]
I	A	1.13	1.38	1.22	1.04 [5]	
1a *cis*	A	1.90	2.50	1.31	2.30	[+]
	B	4.75	6.18	1.80	7.10	[+]
	C	2.83	4.63	1.64	3.88	
	D	5.36	8.69	1.62	3.57	
	E	15.03	24.77	1.64	5.00	
1b *trans*	A	1.82	3.33	1.83[a]	4.87	[−]
	B	4.15	7.49	1.80	7.10	[−]
	C	2.54	5.29	2.08	5.25	
	D	4.23	–	1	nr	
	E	11.57	26.96	2.33	18.00	
II	A	1.52	–	1	nr [5]	
2a *cis*	A	2.88	3.63	1.26	2.85	[+]
	B	7.95	10.86	1.36	4.43	[+]
	C	4.68	7.21	1.54	3.65	
	D	9.82	15.58	1.59	5.39	
	E	25.85	41.65	1.61	6.53	
2b *trans*	A	2.40	5.35	2.22	10.76	[−]
	B	6.68	16.62	2.50	13.45	[−]
	C	4.05	10.53	2.60	9.84	
	D	7.49	21.56	2.88	13.26	
	E	19.23	57.32	2.98	15.64	
3a *cis*	B	5.00	8.57	1.71	7.90	[+]
	C	3.35	7.43	2.22	8.28	
	D	7.14	18.53	2.60	9.30	
	E	17.86	47.82	2.69	>20	
15°C	A	2.39	3.83	1.60	4.76	
20°C	A	2.33	3.79	1.62	5.05	
25°C	A	2.18	3.61	1.65	5.77	
30°C	A	2.17	3.62	1.67	6.20	[+]
35°C	A	2.06	3.48	1.69	6.23	
40°C	A	1.92	3.26	1.70	6.65	
0.7 ml/min	A	2.03	3.38	1.67	7.11	
3b *trans*	B	4.48	12.47	2.78	14.80	[−]
	C	2.74	8.00	2.92	12.08	
	D	5.24	17.32	3.31	14.00	
	E	12.96	44.26	3.42	21.33	
15°C	A	1.88	5.49	2.92	10.05	
20°C	A	1.86	5.18	2.76	9.14	
25°C	A	1.78	4.67	2.67	8.11	
30°C	A	1.76	4.42	2.48	8.05	[−]
35°C	A	1.70	4.10	2.41	7.10	
40°C	A	1.62	3.67	2.27	6.83	
0.7 ml/min	A	1.68	3.95	2.35	9.28	
4a *cis*	A	1.45	2.31	1.59	3.27	[+]
	B	3.55	5.59	1.58	5.22	
	C	2.03	2.33	1.15	1.21	
	D	3.45	5.69	1.65	4.00	
5a *cis*	A	1.61	1.89	1.17	1.18	[+]
	B	3.98	4.60	1.16	1.27	
	C	2.53	–	1	nr	
	D	3.93	4.77	1.21	1.47	

See legend of Table 2.

Table 2
Chromatographic parameters: retention factors (k'), enantioselectivity factor (α) and resolution (R_s) of **1a**, **1b**, **2a**, **2b**, **3a**, **3b** and **4a**, **5a** on Chiralcel OJ

Compound[a]	Eluent[b]	k'_1	k'_2	α	R_s
I	A	1.08	1.29	1.19	1.03 [5]
1a *cis*	A	3.68	5.01	1.36	2.49
	B	9.35	12.53	1.34	2.82
	C	4.17	5.16	1.24	1.45
	D	6.71	8.06	1.20	1.50
	E	17.07	20.18	1.18	1.68
	F	18.74	32.13	1.71	7.34
1b *trans*	A	3.68	5.01	1.36	2.49
	B	8.97	9.36	1.04	<0.5
	C	4.17	5.16	1.24	1.45
	D	5.52	10.20	1.85	4.41
	E	14.67	17.07	1.16	1.80
	F	20.49	24.62	1.20	2.51
II	A	1.15	1.92	1.67	3.97 [5]
2a *cis*	A	3.57	6.78	1.90	5.63
	B	9.09	16.53	1.81	6.12
	C	4.19	5.77	1.38	1.78
	D	6.40	7.51	1.17	1.41
	E	21.74	25.40	1.17	1.49
	F	18.53	20.92	1.13	1.53
2b *trans*	A	3.98	–	1	nr
	B	10.20	–	1	nr
	C	4.19	–	1	nr
	D	6.80	9.19	1.35	2.40
	E	18.98	25.40	1.34	2.60
	F	17.46	18.53	1.06	<0.5
3a *cis*	B	8.08	12.63	1.56	4.54
	C	3.61	4.78	1.32	1.86
	D	6.40	7.51	1.17	1.41
	E	21.74	25.40	1.17	1.49
	F	14.29	24.01	1.68	5.73
15°C	A	4.78	8.98	1.88	2.93
20°C	A	4.33	7.75	1.79	3.41
25°C	A	3.89	6.62	1.70	3.51
30°C	A	3.45	5.53	1.60	3.83
35°C	A	3.20	4.96	1.55	3.82
40°C	A	2.70	4.00	1.48	3.58
0.7 ml/min	A	2.93	4.55	1.55	4.17
3b *trans*	B	8.24	8.67	1.05	nr
	C	3.57	–	1	nr
	D	6.80	9.19	1.35	2.40
	E	18.98	25.40	1.34	2.60
	F	17.73	24.01	1.35	3.76
15°C	A	4.78	8.98	1.88	2.93
20°C	A	4.33	7.75	1.79	3.41
25°C	A	3.89	6.62	1.70	3.51

Table 2. Continued

Compound[a]	Eluent[b]	k'_1	k'_2	α	R_s
30°C	A	3.45	5.53	1.60	3.83
35°C	A	3.20	4.96	1.55	3.82
40°C	A	2.70	4.00	1.48	3.58
0.7 ml/min	A	2.93	4.53	1.55	4.17
4a *cis*	A	1.70	1.97	1.16	0.70
	B	4.45	5.09	1.15	0.90
	C	1.32	1.98	1.50	1.61
	D	2.83	5.35	1.89	3.29
	F	6.72	–	1	nr
5a *cis*	A	1.88	2.16	1.15	0.81
	B	4.95	5.82	1.18	1.12
	C	1.96	2.31	1.18	0.75
	D	3.39	4.27	1.26	1.14
	F	8.46	9.13	1.08	0.60

[a] Concentration ca. 0.75 m*M*.
[b] Eluents A: hexane–ethanol (90:10); B: hexane–ethanol (95:5); C: hexane–1-propanol (90:10); D: hexane–2-propanol (90:10); E: hexane–2-propanol (95:5); F: hexane–methanol (98:2). The flow-rate was 1 ml/min. The temperature was 30°C unless noted otherwise.
[c] Polarimetric detection.
nr: Not resolved.

lytical chiral HPLC and were in perfect accordance. Compounds **4**, **5** were prepared according to the same synthetic pathway and were obtained as pure *cis* forms. Methanol, ethanol, 1-propanol, 2-propanol and *n*-hexane were HPLC grade obtained from Merck (Nogent sur Marne, France) or Baker (Noisy le Sec, France). All the mobile phases were filtered through a membrane (0.45 μm) and degassed with a Waters in-line degasser apparatus. The mobile phases used were A: hexane–ethanol (90:10); B: hexane–ethanol (95:5); C: hexane–1-propanol (90:10); D: hexane–2-propanol (90:10); E: hexane–2-propanol (95:5); F: hexane–methanol (98:2). Compounds were chromatographed by dissolving them in the alcohol of the corresponding mobile phase to a concentration of about 0.75 m*M* (which corresponds to 15 nmol injected) and passed through a 0.45-μm membrane filter prior to loading the column.

2.2. Chromatography

Chiral chromatography was carried out on Chiralcel OD-H cellulose (tris-3,5-dimethylphenylcarbamate; 250×4.6 mm I.D., 5 μm), and on Chiralcel OJ cellulose (tris-methylbenzoate; 250×4.6

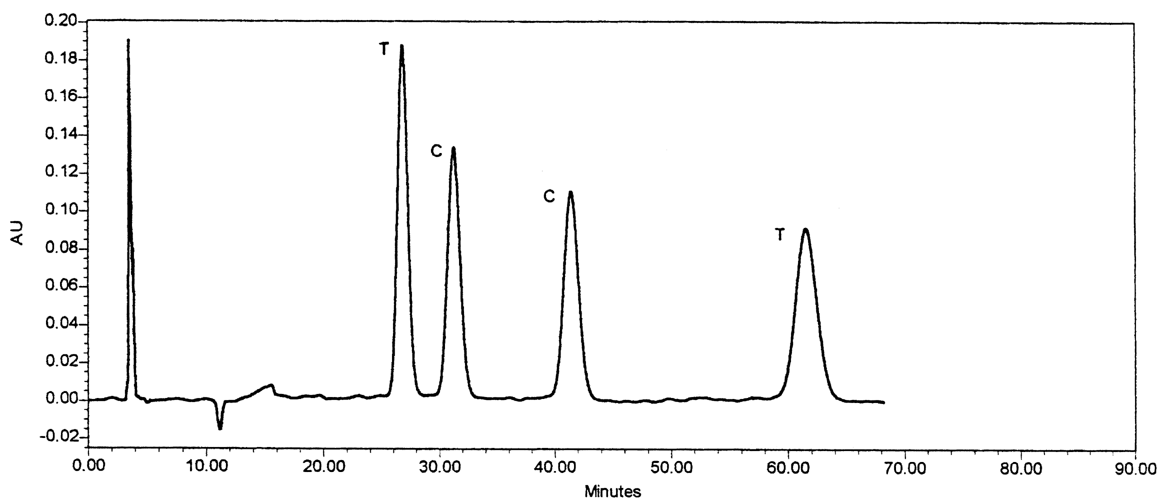

Fig. 2. Chromatogram (λ=200 nm) obtained for **2a** and **2b** (eluent B; 1 ml/min; Chiralcel OD-H).

mm I.D., 10 μm) (Daicel Chemical Industries, Baker, France) using a gradient Waters 600E metering pump model equipped with a Waters 996 photodiode array spectrophotometer. Chromatographic data were collected and processed on a Digital computer running Millennium 2010. The column eluate was monitored at 200; 210; 230; 275 nm. A polarimetric HPLC detector (Jasco OR 990) which utilizes a Xe–Hg lamp as a light source at full 350–900 nm range with the strong mercury line emission at 365 nm, a cell pathlength of 0.25 dm and volume 40 μl was used in series. An integrator (Merck 7500) was used to record the output from the polarimetric detector. The sample loop was 20 μl (Rheodyne 7125 injector). Mobile phase elution was made isocratically using *n*-hexane and a modifier (ethanol, 1-propanol or 2-propanol) at various percentages. The flow was 1 ml/min. The peak of the solvent front was considered to be equal to the dead time (t_0) and was taken from each particular run. It was about 3.20 min (1 ml/min) for Chiralcel OD-H and 3.30 min (1 ml/min) for Chiralcel OJ. Retention

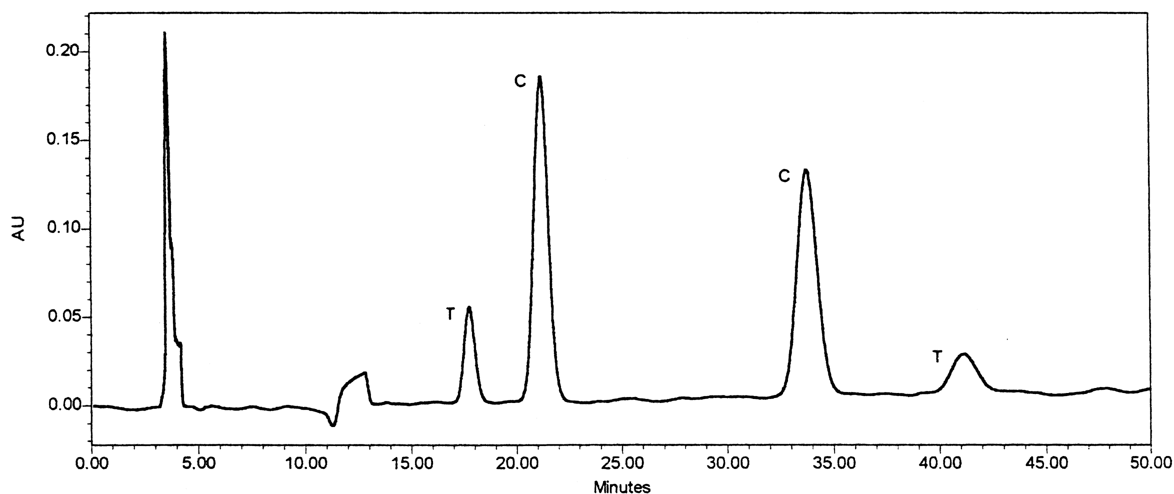

Fig. 3. Chromatogram (λ=200 nm) obtained for **3a** and **3b** (eluent B; 1 ml/min; Chiralcel OD-H).

times were mean values of two replicate determinations. All separations were carried out at 30°C unless noted otherwise to determine the temperature dependence on the optical resolution.

3. Results and discussion

The results of the chiral separation of the **1**–**5** racemates chromatographed are summarized in Table 1 for Chiralcel OD-H and Table 2 for Chiralcel OJ. The complete assignment, *cis* or *trans* forms, was made with the help of ^{1}H-NMR spectroscopy. Fig. 2 corresponding to compounds **2a**+**2b** and Fig. 3 corresponding to compounds **3a**+**3b** are examples typical of the separations achieved on Chiralcel OD-H (eluent B). Fig. 4 illustrates the results obtained for the separation of compounds **1a**+**1b** (eluent D) on Chiralcel OJ. The UV spectra of the isomers were identical and are, of course, very similar for compounds **1**–**5**. The maximum wavelengths are, respectively, 201.8, 279.8 nm for **1**; 200.6, 279.8 nm for **2**, **3** and 204.1, 279.8 nm for **4**, **5**.

Several kinds of mobile phase compositions were investigated, by changing the nature and the percentage of the alcohol, and baseline separations were generally obtained. The retention times and retention factors, k', of all compounds decrease by changing the modifier from 2-propanol (eluents D and E) to 1-propanol (eluent C) and to ethanol (eluents A and B) as expected from the higher polarity of ethanol [11–13]. This is illustrated in Fig. 5a and b (**3a**, **3b**; Chiralcel OD-H) which also show a reversal in elution order of the third peak (second eluted *cis*-enantiomer) and the fourth peak (second eluted *trans*-enantiomer) with a change in organic modifier from ethanol or 1-propanol to 2-propanol (Chiralcel OD-H). The chiral resolution of solutes **3a** and **3b** corresponds to an increase of 17% and 13%, respectively of the resolution factor R_s. The solvent polarity values for 1-propanol and 2-propanol are virtually the same. So the observed increases in retention and stereoselectivity are more likely due to the steric difference between the two molecules of solvent which can be expressed at both achiral and chiral binding sites. The increases in k' and α are consistent with a decreasing ability of 2-propanol to displace the solutes from the sites and this effect is greater with the *cis*-form (**3a**) than with the *trans*-form (**3b**) [20,21]. This result suggests that the use of 2-propanol may be exploited to affect changes in selectivity with this chiral column [19]. On Chiralcel OD-H for example, for **3a** the parameters k', α, R_s were 2.14, 1.67, 6.20; 3.55, 2.22, 8.28 and 7.14, 2.60, 9.30 with eluents A, C and D, respectively. The α and R_s parameters remain slightly constant or weakly increase for all *cis* and *trans* racemates on Chiralcel OD-H for A to C and D eluents. On

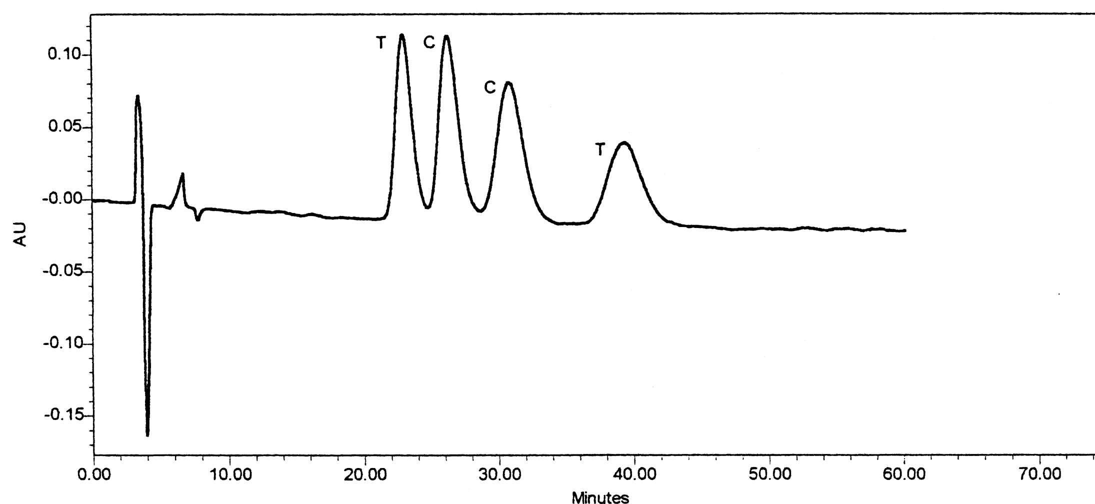

Fig. 4. Chromatogram (λ=200 nm) obtained for **1a** and **1b** (eluent D; 1 ml/min; Chiralcel OJ).

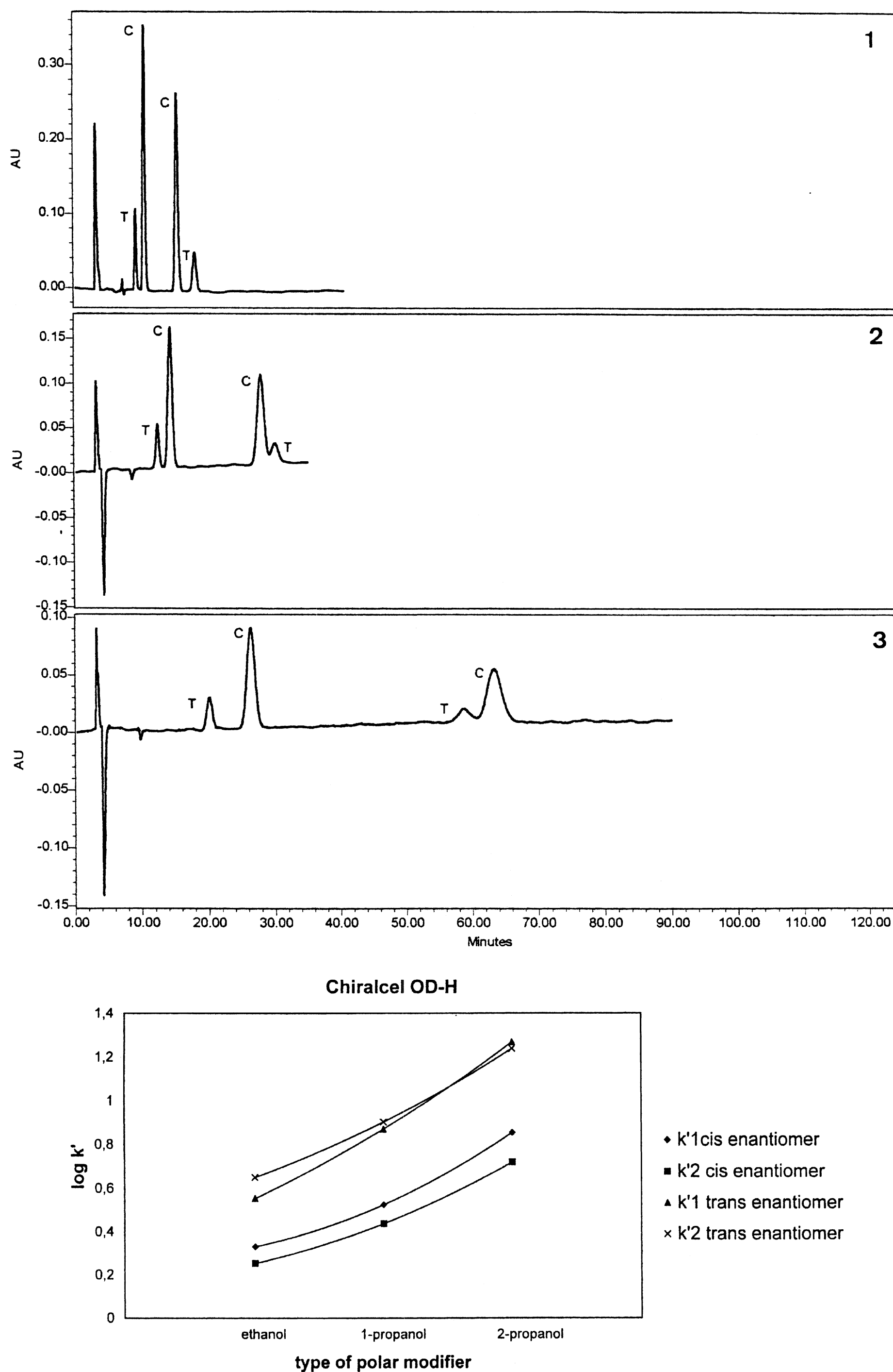

Fig. 5. (a) Chromatograms (λ=200 nm) for **3a** and **3b**; influence of the nature of polar modifier [eluents A (1), C (2), D (3); 1 ml/min; Chiralcel OD-H]. (b) Plot of log k' of the *cis*- and the *trans*-enantiomers versus the type of polar modifier (eluents A, C, D; 1 ml/min; Chiralcel OD-H).

Chiralcel OJ all parameters have the same behavior except for **1a**, **2a** and **3a** where the resolution parameter R_s decreases on changing polar modifier from ethanol (eluent A) to 2-propanol (eluent D). For example, for **2a** the parameter (R_s) was 5.63, 1.78, 1.41 with eluents A, C and D, respectively. So the resolution goes in an opposite way between the two columns when changing the polar modifier: this could be correlated to the recognition mechanism of the enantioseparation. This involves hydrogen bonding, dipole–dipole interaction, π–π interaction and inclusion into the chiral groove [8]. It has been reported that most of the cellulose tris(phenylcarbamate) derivatives form a lyotropic liquid crystalline phase in highly concentrated solution and when coated on silica gel from solution present an ordered structure in which the phenylcarbamate groups are regularly arranged [18]. In OD-H CSP the most important docking sites for the mechanism of chiral discrimination are considered to be the polar carba-

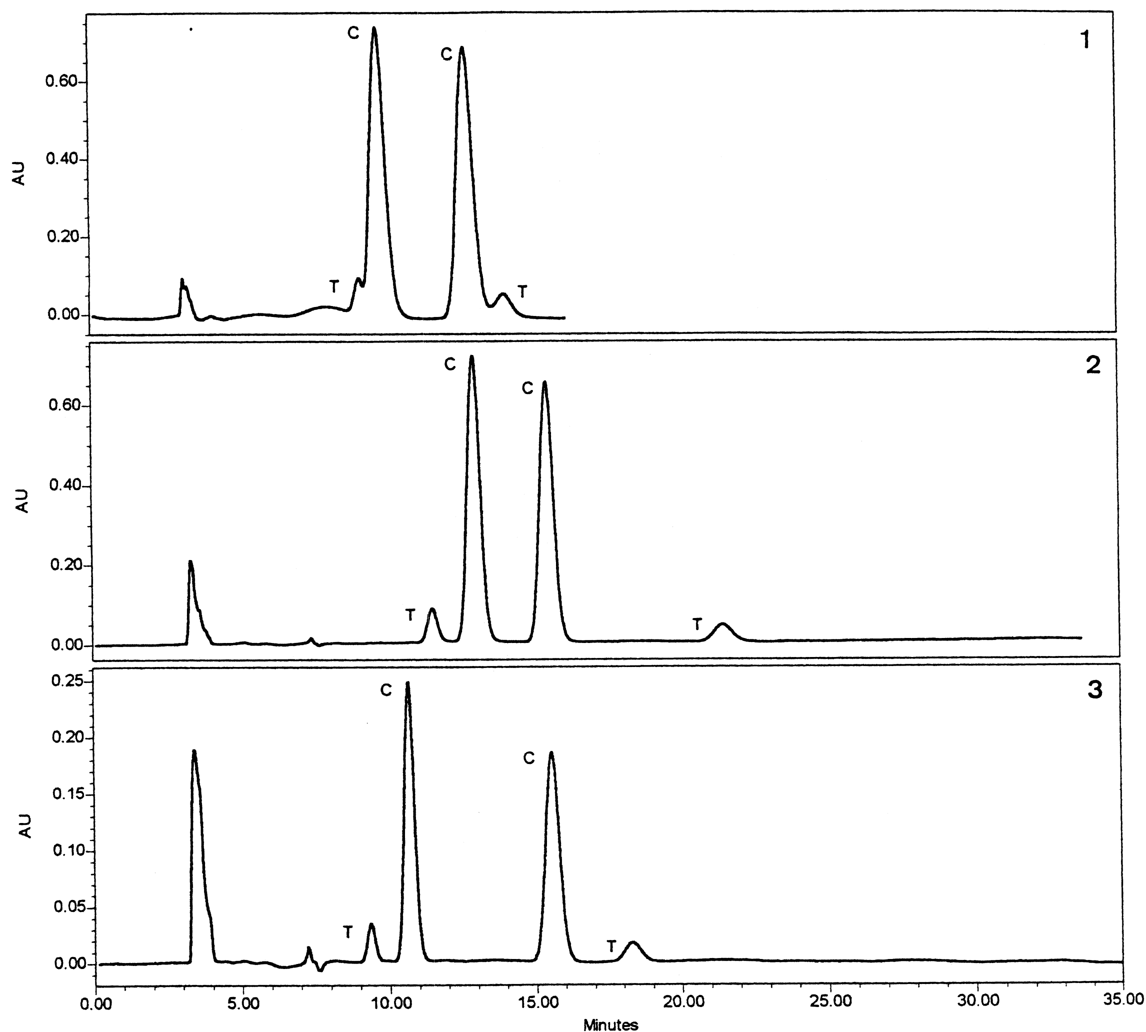

Fig. 6. (a) Chromatograms (λ=200 nm) for **1a** and **1b** (1); **2a** and **2b** (2); **3a** and **3b** (3); influence of the steric hindrance (eluent A; 1 ml/min; Chiralcel OD-H). (b) Plot of the selectivity and the resolution factors of the *cis*- and *trans*-enantiomers versus the number of carbon atoms nC; influence of the steric hindrance (eluent A; 1 ml/min; Chiralcel OD-H). (c) Chromatograms (λ=200 nm) for **1a** (1); **4a** (2); **5a** (3); influence of the position of substitution (eluent A; 1 ml/min; Chiralcel OD-H).

mate residues which can interact through H bondings with the corresponding groups on the analyte molecule [16,17]. Stronger solvents, such as alcohols, compete more effectively for the chiral sites than the analyte. The enantiomeric resolution increased as the size of the alcohol increased with isopropanol giving the maximum resolution. This might be due to the decrease in the capacity of larger alcohols to compete for hydrogen bonding sites because of steric hindrance [20,21]. In OJ CSP the best results are obtained with lower alcohols (methanol and ethanol added to the mobile phase). Partial resolution or total loss of resolution is observed when using 1-propanol or 2-propanol. This suggests that hydrogen bonding interactions are probably not predominant but separations might involve more π–π interactions between the aromatic moiety of the analyte and the phenyl groups of the stationary phase. This suggests also that the chiral cavity might change of geometry and/or change of size, which is caused by the kind of alcoholic modifier [20,22,23].

It can be seen that polar modifier concentration decrease (ethanol: eluents A and B or 2-propanol: eluents D and E) in the mobile phase, leads to an

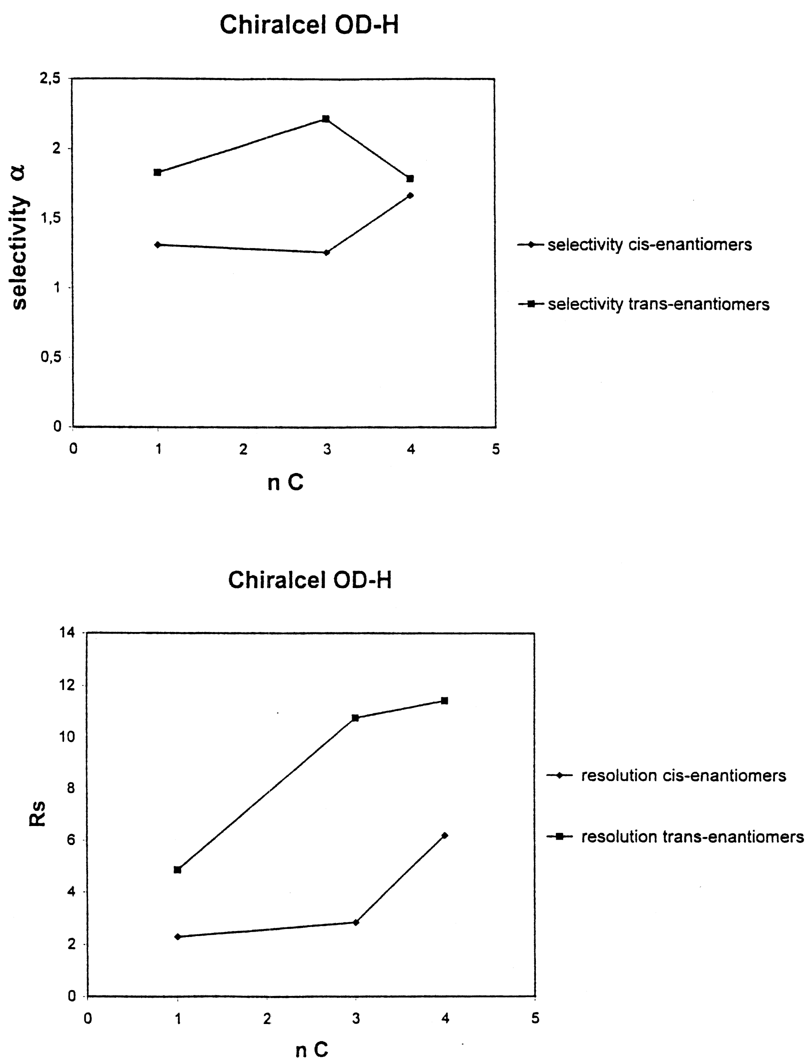

Fig. 6. (*continued*)

increase of both parameters (k', and R_s), for **1**, **2** and **3** racemates on Chiralcel OD-H and OJ columns, as shown by Refs. [9,11,12], except for **1b** where a decrease of resolution is observed. It must be noted that the enantioselectivity factor (α) remains slightly constant on the Chiralcel OD-H and Chiralcel OJ columns.

Increase of the bulkiness of the substituent group R (i.e., increase of the number of carbon atoms) leads to a significant increase in the resolution on Chiralcel OD-H CSP. When changing from **1a** to **2a** to **3a** (*cis*-form), the parameter (R_{sC1-C2}) was: 2.30, 2.85 and 6.20 (Chiralcel OD-H; eluent A). The resolution factor of the *trans*-enantiomers varies in the same way, when changing from **1b** to **2b** to **3b** R_{sT1-T2}: 4.87, 10.76 and 11.43 (Chiralcel OD-H; eluent A) (Fig. 6b). The same observation can be made with eluents C and D. The k' factors do not follow the same rule: the order of retention is **1**<**3**< **2**.

The variations of the resolution parameter R_{sC2-T2} between the second eluted *cis*-enantiomer and the

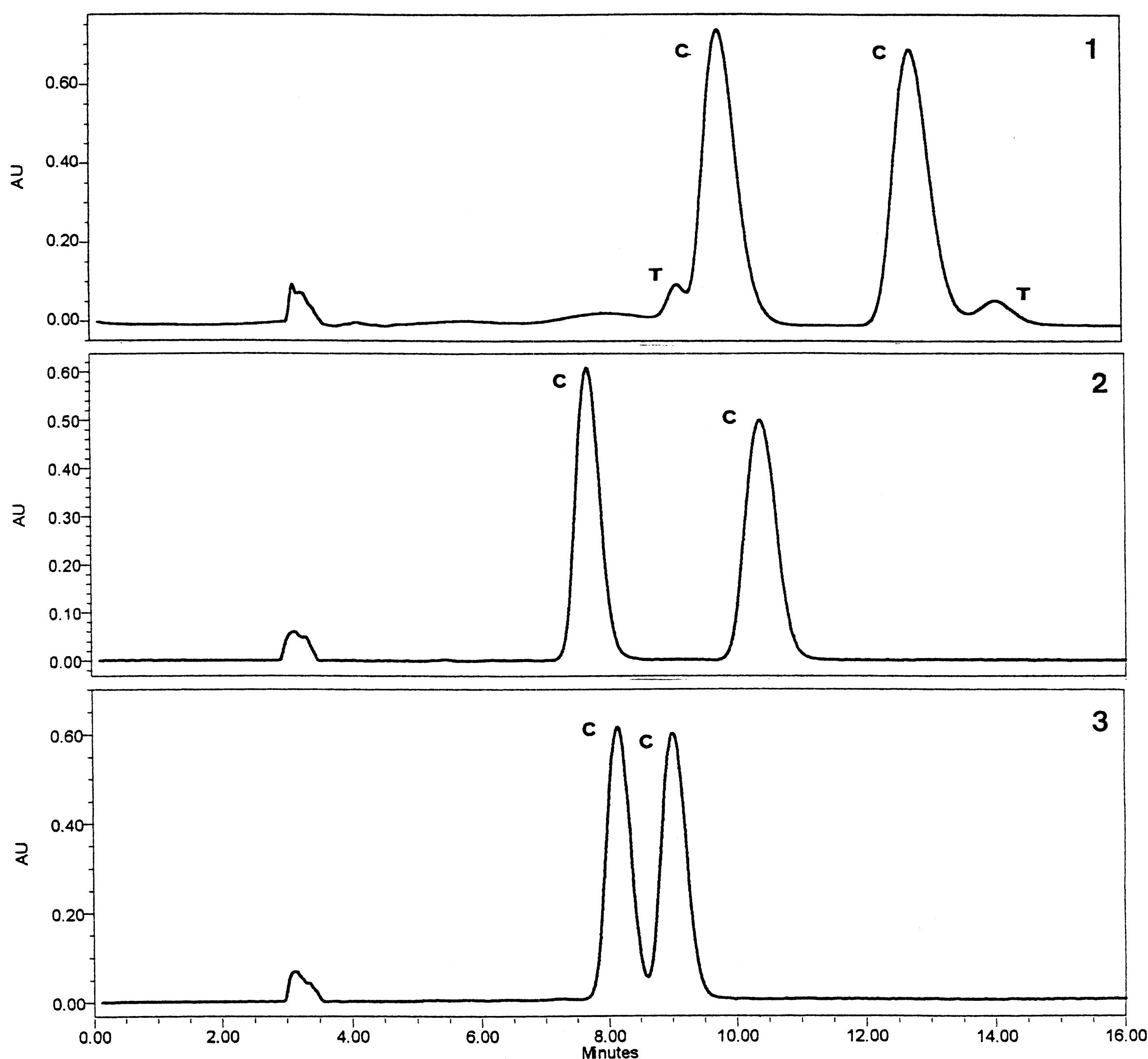

Fig. 6. (*continued*)

second eluted *trans*-enantiomer strikingly increase from **1** to **2** and decrease from **2** to **3**. This is illustrated in the chromatograms (Fig. 6a) [16,17]. This suggests that enantioselectivity is not mostly influenced by hydrogen bonds, but steric and hydrophobic interactions caused by the substituent groups R of the solute and the CSP have a more prominent effect in stabilizing the solute–stationary phase complex.

If we compare the disubstituted tetralins **1**, **2** to

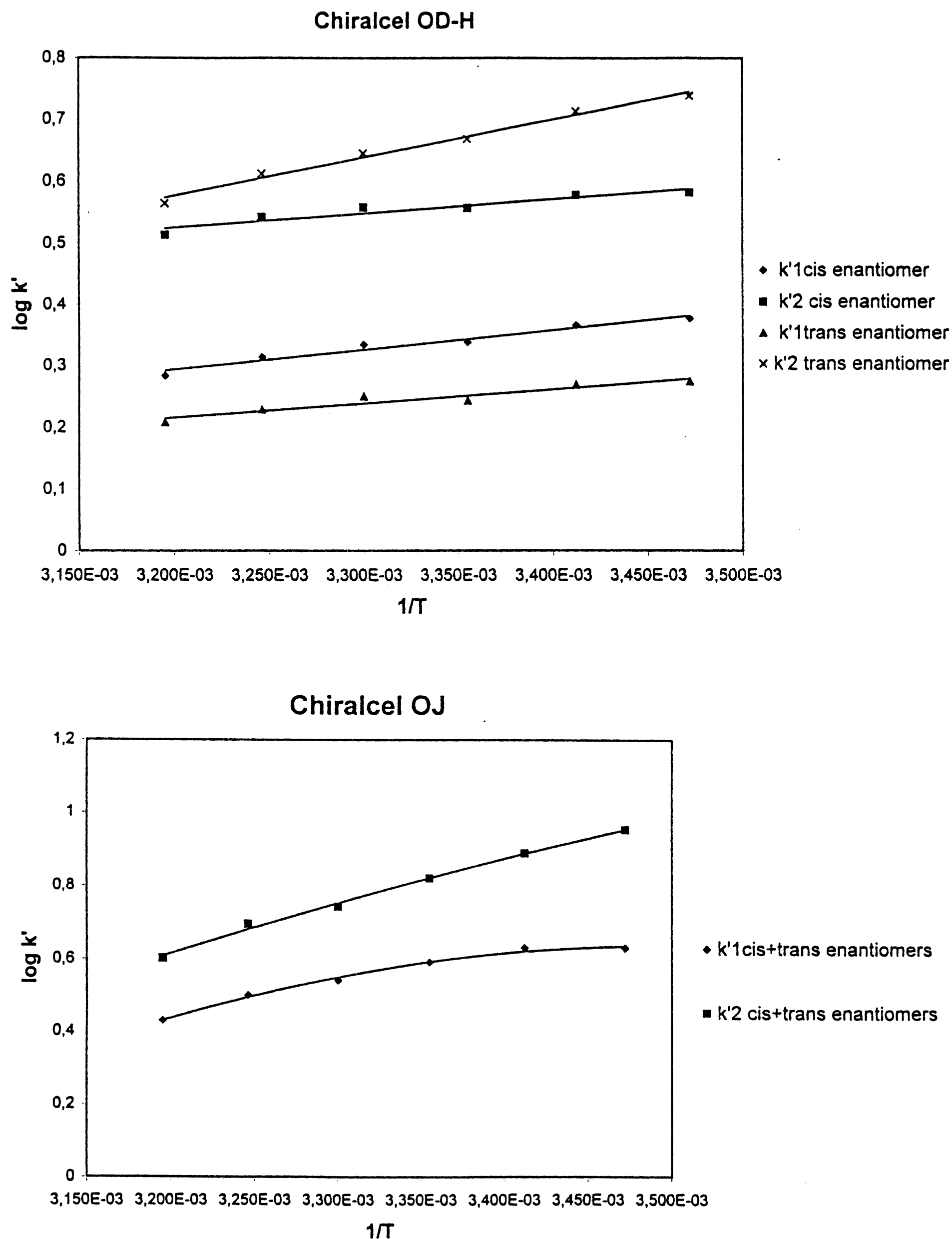

Fig. 7. Plot of log k' of the *cis*- and the *trans*-enantiomers versus the inverse of the temperature $1/T$ (K^{-1}) (eluent A; 1 ml/min; compounds **3a** and **3b**; Chiralcel OD-H and Chiralcel OJ).

their homologues **I**, **II**, respectively (Fig. 1) without a phenyl ring in the 3-position, we can see that this supplementary substitution induces in every case (kind of CSP and eluting phase) an increase of the retention factors (Tables 1 and 2) [5] and in a general manner an increase of enantioselectivity and resolution factors. Retention factors increase might correspond to a supplementary π–π interaction (solute–CSP). Resolution factors increase could be explained by both supplementary π–π interaction and steric hindrance. The comparison between **I**, **1**, **4** shows the effect of the phenyl substituent at the 3- and 2-positions: **4** is always less retained than **1** (on Chiralcel OD-H and OJ) but with higher separation (α, R_s) on Chiralcel OD-H and lower separation on Chiralcel OJ (Tables 1 and 2).The effect of substituent type at the 2-position is shown with compounds **4** and **5**: in a general manner **4** is always less retained than **5** but with higher separation (α, R_s) on Chiralcel OD-H and OJ (Tables 1 and 2). The phenyl group induces a better enantioseparation than the benzyl group (Fig. 6c).

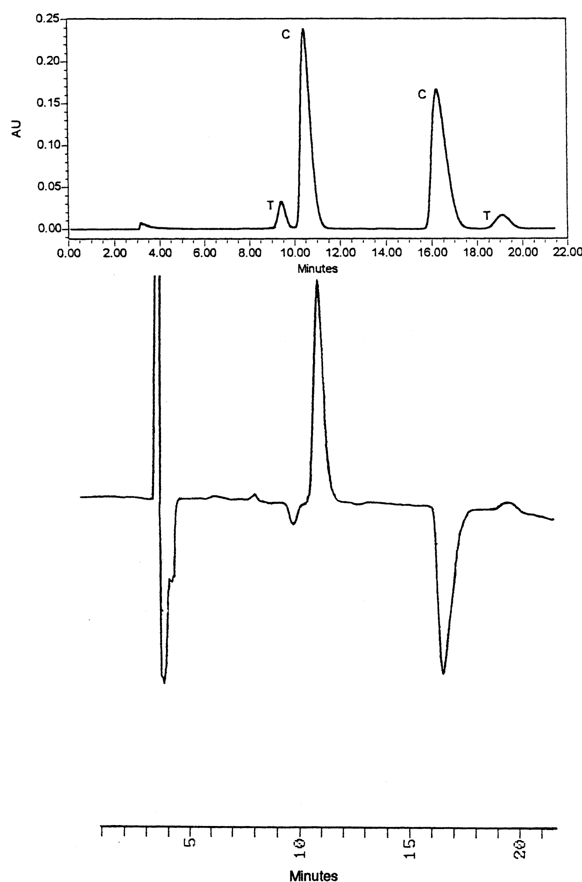

Fig. 8. Polarimetric detection and UV detection chromatograms (λ=254 nm) for **3a** and **3b** (eluent A; 1 ml/min; Chiralcel OD-H).

Moreover, the temperature of the surrounding of the chiral column has been partly investigated with compound **3** as a potential factor affecting the enantioselectivity as shown by Ref. [15]. Chromatograms for these compounds were run at 5°C intervals from 15 to 40°C, and before each injection the column was conditioned for 30 min. It was found that higher temperature improved the separation (Fig. 7) both on Chiralcel OD-H and OJ, and decreased the retention factors. α and R_s increase for **3a** but decrease for **3b.** On Chiralcel OJ, α decreases for **3a** and **3b** at higher temperature while R_s shows a maximum near 30°C.

Fig. 8 shows the polarimetric detection of the mixture of **3a**+**3b** obtained on a polarimetric detector Jasco OR 990. Under the same eluting conditions, on cellulose (tris-3,5-dimethylphenylcarbamate) with eluents A and B, for compounds **1**–**5**, first eluted *trans*-enantiomer is always (−) and first eluted *cis*-enantiomer is always (+). No reversal of elution is observed.

The cellulose (tris-3,5-dimethylphenylcarbamate) column gives better results compared to cellulose (tris-4-methylbenzoate) for the eight compounds. On Chiralcel OJ compounds **2b** and **3b** are not well resolved and under the same eluting conditions all compounds are usually more retained on OJ CSP than on OD-H CSP. On the Chiralcel OD-H column, the resolution of the four stereoisomers (**1**–**3**) was easily achieved using a different mobile phase for each compound. This chromatographic method could be suitable to determine optical purity. The preparative HPLC separation of each of the four diastereoisomers of the compounds **1**–**3** and the two enantiomers of compounds **4** and **5** are now under investigation.

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