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Cyclodextrin derivatives in the gas chromatographic separation of racemic mixtures of volatile compounds X. 2,3-Di-O-ethyl-6-O-*tert.*-butyldimethylsilyl-β- and -γ-cyclodextrins

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Abstract

2,3-Di-O-ethyl-6-O-*tert.*-butyldimethylsilyl-β- and -γ-cyclodextrins (ETTBS-β- and -γ-CDs) are proposed as stationary phases for GC separation of enantiomers. The influence of different polarity polysiloxanes (PS-347.5, PS-086, OV-1701) as diluting phase on the separation capacity of these CD derivatives was also investigated. The results of ETTBS-β- and -γ-CDs are compared to those of 2,3-di-O-methyl-6-O-*tert.*-butyldimethylsilyl-β- and -γ-cyclodextrins (METBS-β- and -γ-CDs). The advantages of ETTBS-β- and -γ-CDs in terms of minimum operative temperatures and separation capacity of the column are discussed. Each column coated with each of the new synthesized CD derivatives was evaluated by analysing a number of different racemates with different structures.

Keywords: Enantiomer separation; Chiral stationary phases, GC; Cyclodextrin-based stationary phases; Polysiloxanes

1. Introduction

Since they were introduced in 1983, cyclodextrin (CD) derivatives, pure or diluted in polysiloxanes, have been very successful in the gas chromatographic (GC) separation of underivatized enantiomers. CDs are now generally used diluted in moderately polar polysiloxane, as first introduced by Schurig and co-workers [1,2].

Blum and Aichholz [3] and Mosandl and coworkers [4–7] showed that 6-O-*tert.*-butyldimethylsilyl (TBS)-substituted CDs are successful chiral stationary phases (CSPs) because of their lipophilicity and miscibility with different polysiloxanes. In particular 2,3-di-O-methyl-6-TBS-β- and -γ-CDs (METBS-β- and -γ-CDs) and 2,3-di-O-acetyl-6-TBS-β- and -γ-CDs (ACTBS-β- and -γ-CDs) have been shown by several authors to be effective [8–14].

Previous studies investigated the influence of CD diluting phase on enantiomer separation [15,16]. This subject has also been investigated by other authors [10,17–21]. Buda et al. [22] showed that retention was not only due to chiral selector and diluting phase, as proposed by Jung et al. [23], but also to the competitive complexation of the polysiloxane with the CD.

In general, lower polarity CD diluting phases

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afford lower elution temperatures and as a consequence better enantiomer separation. We arbitrarily assumed that a CD/polysiloxane combination be considered effective when the resulting column has a minimum operative temperature (MIOT) equal to or below 40°C. When diluted in apolar to moderately polar polysiloxane (i.e. PS-086, and OV-1701), ACTBS-β- and -γ-CDs gave columns with MIOT within this range; METBS-β- and -γ-CDs diluted in PS-347.5, had a much higher MIOT (90°C), as was also shown by Dietrich et al. for diluting phases of similar (SE-30) and higher polarity (OV-215, and XE-60) [18]. With the aim of developing CDs of as general as possible a use, and since METBS-β- and -γ-CDs showed a very interesting enantioseparation capacity, 2,3-di-O-ethyl-6-O-TBS-β- and -γ-CDs (ETTBS-β- and -γ-CDs) and 2,3-di-O-pentyl-6-O-TBS-β- and -γ-CDs (PETBS-β- and -γ-CDs) were synthesized and tested as GC stationary phases. This article reports the results of our investigations on these four new CDs.

2. Experimental

2.1. Synthesis of 2,3-di-O-alkyl-6-O-tert.-butyldimethylsilyl-β- and -γ-CDs

Dialkyl-TBS-β- and -γ-CDs were synthesized in a two-step reaction following the method described by Takeo et al. [24,25] and Dietrich et al. [7]. The β- and -γ-CDs and all the reagents used in the synthesis were from Fluka (Buchs, Switzerland). Most racemate standards were from Roth (Karlsruhe, Germany), Fluka and Aldrich-Sigma (Stenheim, Germany). The 6-TBS-β- and -γ-CDs were obtained by reaction of TBS chloride dissolved in dry pyridine with β- and -γ-CDs and imidazole in dry pyridine following the procedure described by Dietrich et al. [6]. All the CDs were checked by NMR. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AC-200 spectrometer or a Jeol EX-400.

2.2. ETTBS-β-CD

Sodium hydride (0.3 g, 12.5 mmol) was stirred at 50°C with dry dimethyl sulphoxide (DMSO, 10 ml) and dry tetrahydrofuran (THF, 20 ml), under nitrogen, until evolution of hydrogen ceased. A solution of 0.25 mmol 6-TBS-β-CD (0.5 g) in dry DMSO and THF (50+20 ml respectively) was added to the previous solution, cooled to room temperature. After stirring for 2 h, 13 mmol ethyl iodide (1 ml) was added dropwise to the mixture cooled to 10°C over a period of 10 min. After stirring overnight, the sodium hydride excess was decomposed with methanol. The resulting solution was poured into ice-water, and then extracted three times with chloroform. The combined chloroform extracts were washed with water, dried over sodium sulfate and then concentrated. The impure product was submitted to column chromatography, using silica gel 60 (Merck, Darmstadt, Germany) as stationary phase and light petroleum (b.p. 40–60°C)–ethyl acetate (9:1) as mobile phase. White crystals of ETTBS-β-CD (210 mg, η=38%, m.p. 68°C) were obtained.

TLC was carried out on silica plate (Merck) using light petroleum (b.p. 40–60°C) as mobile phase. Analytes were detected with a 5% sulfuric acid–ethanol solution, after heating to 105°C for 5 min. ETTBS-β-CD R_F was 0.64.

2.2.1. ^{1}H NMR ($C^{2}HCl_3$) δ (ppm)

5.20 (d, 1H; H-1); 4.04 (m, 1H, H-5); 3.96 (m, 8H, H-3, H-4, H-6a, H-6b, 2×OCH_2 at C-2, C-3); 3.16 (dd, 1H, H-2), 1.22 (2t, 6H, CH_3-ethyl [CH_3–CH_2–O–]); 0.87 (s, 9H, $(CH_3)_3$–C), 0.02 (2×s, 6H, $(CH_3)_2$–Si).

2.2.2. ^{13}C NMR ($C^{2}HCl_3$) δ (ppm)

97.78 (C-1); 80.13 (C-2); 80.06 (C-3); 77.47 (C-4); 72.19 (C-5); 68.81 (O–CH_2); 66.35 (O–CH_2); 62.22 (C-6); 25.82 (CH_3)$_3$–C); 18.17 (CH_3)$_3$–*C*); 15.65 (CH_3–CH_2–O–), −4.88, −5.28 (2×(CH_3)$_2$–Si).

2.3. ETTBS-γ-CD

ETTBS-γ-CD was prepared by the same procedures and using the same molar amounts as for ETTBS-β-CD. The raw product was purified by column chromatography using silica gel 60 as stationary phase and light petroleum (b.p. 40–60°C)–ethyl acetate (95:5) as mobile phase. White crystals of ETTBS-γ-CD (210 mg, η=33%, m.p. 60°C) were obtained.

TLC and analyte detection were carried out under the conditions reported above. ETTBS-γ-CD R_F was 0.76.

2.3.1. ^1H NMR (C^2HCl_3) δ (ppm)

5.24 (d, 1H; H-1); 4.08 (m, 1H, H-5); 3.73–3.51 (m, 8H, H-3, H-4, H-6a, H-6b, 2×OCH_2 at C-2, C-3); 3.12 (dd, 1H, H-2), 1.21 (2t, 6H, CH_3–ethyl [CH_3–CH_2–O–]); 0.85 (s, 9H, $(CH_3)_3$–C), −0.01 (2×s, 6H, $(CH_3)_2$–Si).

2.3.2. ^{13}C NMR (C^2HCl_3) δ (ppm)

98.14 (C-1); 80.31 (C-2); 79.84 (C-3); 77.72 (C-4); 72.30 (C-5); 68.85 (O–CH_2); 66.57 (O–CH_2); 63.31 (C-6); 25.77 (CH_3)$_3$–C); 18.13 (CH_3)$_3$–*C*); 15.60 (CH_3–CH_2–O–); −4.96, −5.40 (2×(CH_3)$_2$–Si).

2.4. PETBS-β-CD

PETBS-β-CD was prepared with the same procedures and the same molar amounts as for ETTBS-β-CD. The raw product was purified by column chromatography using silica gel 60 as stationary phase and toluene–pentane (1:1) as mobile phase. Amorphous PETBS-β-CD (83 mg, η=12%) was obtained.

TLC and analyte detection were carried out under the conditions reported above using toluene–pentane (1:1) as eluent. PETBS-β-CD R_F was 0.73.

2.4.1. ^1H NMR (C^2HCl_3) δ (ppm)

5.20 (d, 1H; H-1); 3.90–3.62 (m, H-5, H-4, H-3 pentyl, H-6a, H-6b); 3.10 (dd, H-2); 1.61 (m, pentyl H-4); 1.30–1.27 (m, pentyl, 8 H); 0.99–0.75 (m, pentyl+(CH_3)$_3$–C), 0.02 (2×s, 6H, (CH_3)$_2$–Si).

2.4.2. ^{13}C NMR (C^2HCl_3) δ (ppm)

97.6 (C-1); 80.7 (C-2); 80.2 (C-3); 77.2 (C-4); 71.05 (C-5); 68.15 (O–CH_2); 29.90, 29.60 (pentyl), 28.31 (pentyl); 26.56 (CH_3)$_3$–C); 22.54, 22.40 pentyl, 18.21 (CH_3)$_3$–*C*); 13.96 (pentyl); −4.8 (2×(CH_3)$_2$–Si).

2.5. PETBS-γ-CD

PETBS-γ-CD was prepared with the same procedures and the same molar amounts as for ETTBS-β-CD. The raw product was purified by column chromatography using silica gel 60 as stationary phase and toluene–pentane (1:1) as mobile phase. Amorphous PETBS-γ-CD (53 mg, η=6.8%) was obtained.

TLC and analyte detection were carried out under the conditions reported above using toluene–pentane (1:1) as mobile phase. PETBS-γ-CD R_F was 0.77.

2.5.1. ^1H NMR (C^2HCl_3) δ (ppm)

5.19 (d, 1H; H-1); 3.91–3.53 (m, H-5, H-4, H-3 pentyl, H-6a, H-6b); 3.10 (dd, H-2); 1.60 (m, pentyl H-4); 1.31–1.27 (m, pentyl 8 H); 0.99–0.75 (m, pentyl+(CH_3)$_3$–C), 0.04,0.03 (2×s, 6H, (CH_3)$_2$–Si).

2.5.2. ^{13}C NMR (C^2HCl_3) δ (ppm)

96.94 (C-1); 80.7 (C-2); 80.3 (C-3); 77.6 (C-4); 71.0 (C-5); 66.3 (O–CH_2); 29.60 (pentyl), 25.8 (pentyl); 26.56 (CH_3)$_3$–C); 22.42 (pentyl), 18.3 (CH_3)$_3$–*C*); 13.94 (2×pentyl); −5.2 (2×(CH_3)$_2$–Si).

2.6. Column preparation and testing

Fused-silica columns (25 m×0.25 mm I.D., d_f 0.15 μm) were prepared by static coating. Columns coated with METBS-β-CD (30%) and METBS-γ-CD (50%), ETTBS-β-CD (30%) and ETTBS-γ-CD (50%) and PETBS-β-CD (50%) and PETBS-γ-CD (50%) in PS-347.5 (polymethyl siloxane) (Petrarch System, USA), PS-086 (polymethylphenylsiloxane, 12% phenyl) (Petrarch System), OV-1701 (polymethylphenylcyanopolysiloxane, 7% cyanopropyl, 7% phenyl) (Ohio Valley Speciality Chemical, Marietta, OH, USA), were prepared. Deactivation was with Carbowax 20M [26,27]. The procedures have been described in detail in a previous paper [28].

Column performances were first evaluated by means of a chiral test comprising ten compounds with highly different structural characteristics: its composition has been reported elsewhere [28]. Moreover, each column was tested with about 180 different racemates with different structural characteristics.

Only those racemates whose separation on ETTBS-CDs either differed significantly from their separation with METBS-CDs, and/or differed as a

function of the diluting phase were analyzed isothermally and the results included in this article.

2.7. Capillary GC conditions

Capillary GC was performed with a Carlo Erba 4160 and a 5160 gas chromatograph equipped with flame ionization detection (Fisons, Milan, Italy). Conditions were as follows: injection system, split; split ratio, 1:30; injector temperature, 230°C; detector temperature, 250°C; carrier gas, hydrogen; $\bar{u}$=40 cm/s, in agreement with Grob.

Except where specified otherwise, 1 μl of 300 μg/ml solutions of the racemates (prepared by dilution of 3 mg/ml standard solutions) were injected.

3. Results and discussion

The success of the four new synthesized CDs as GC chiral selectors differed considerably. PETBS-β-CD and PETBS-γ-CD were unsuccessful: columns prepared with these two CDs showed good efficiency (evaluated with the Grob test) but very low (PETBS-β-CD) or no (PETBS-γ-CD) enantioselectivity when submitted to the chiral test.

Our efforts therefore concentrated on ETTBS-β- and -γ-CDs. Columns prepared with either ETTBS-β-CD or ETTBS-γ-CD diluted in each polysiloxane (PS-347.5, PS-086 and OV-1701) were first submitted to the Grob test. ETTBS-β-columns operated successfully under the test conditions. In this case too, with the same CD, the elution order of some Grob test components differed with the diluting phase: this phenomenon has already been discussed in detail in a previous article [16]. In any case, columns prepared with ETTBS-β-CD diluted with any of the three polysiloxanes showed MIOTs determined with β-pinene (1) equal to or below the assumed value (i.e. 40°C). On the other hand, columns prepared with METBS-β-CD had a MIOT of 40°C only when PS-086 or OV-1701 were used as diluting phases.

Columns prepared with ETTBS-γ-CD were successful only when PS-086 was used as diluting phase, as the Grob tests reported in Fig. 1 clearly show. An explanation of why only the intermediate

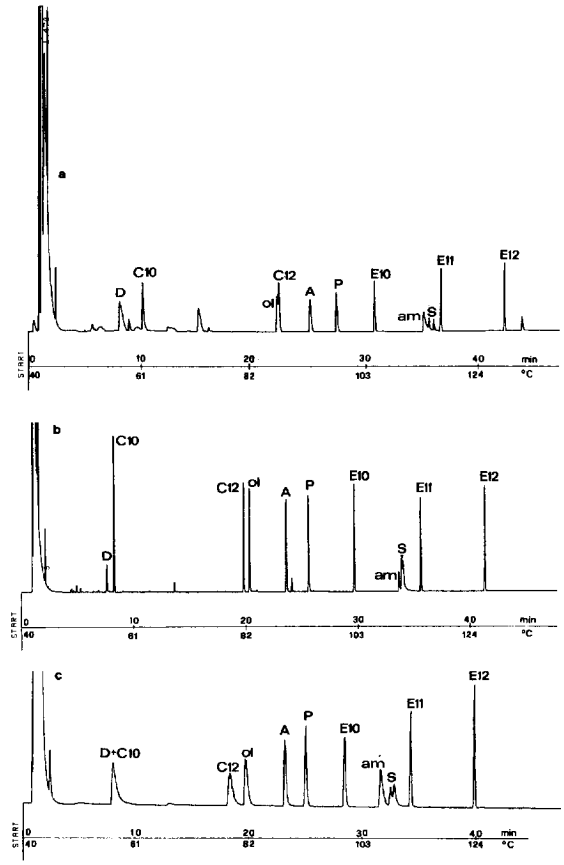


Fig. 1. Grob tests of columns prepared with ETTBS-γ-CD diluted in PS-347.5 (a), PS-086 (b) and OV-1701 (c). Temperature conditions: from 40–200°C at 2°C/min. D: 2,3-butanediol, C10: decane, ol: 1-octanol, C12: dodecane, A: 2,6-dimethylaniline, P: 2,6-dimethylphenol, am: dicyclohexylamine, S: 2-ethylhexanoic acid, E10, E11 and E12: methyl esters of fatty acid C_{10}, C_{11} and C_{12}.

polarity diluting phase worked at the assumed MIOT has not yet been found. The columns prepared with METBS-γ-CD had a MIOT of 40°C only with PS-086 or OV-1701 as diluting phases. The MIOTs determined with β-pinene (1) for both the 50% ETTBS-γ-CD/PS-347.5 and the 50% ETTBS-γ-CD/OV-1701 combinations were 60°C. In any case, the separation capacity of the ETTBS-γ-CD/PS-347.5 combination is lower than that of the ETTBS-γ-CD/PS-086 combination, up to about 100°C. On the other hand, those racemates which are separated by ETTBS-γ-CD at temperatures above 95°C [massoja lactones C_{10} and C_{12} (13 and 14), γ- and δ-lactones

C_9 (6 and 10), δ-lactone C_{10} (11) and compound (15)] show the highest resolution (R) and separation factor (α) with PS-347.5 as diluting phase: this may indicate that for this combination, and above this temperature, the racemate/CD interaction predominates over the CD/diluting phase interaction, which agrees with Buda et al. [22].

Table 1 reports R (calculated from peak widths at half-heights) and α (calculated from adjusted retention times) of those racemates whose separations with ETTBS-β-CD significantly differed from those obtainable with METBS-β-CD. Table 2 reports R and α of those racemates whose separations with ETTBS-γ-CD significantly differed from those obtainable with METBS-γ-CD. To make the two sets of results comparable, GC conditions suitable to obtain retention factor k values between 10 and 30 were applied. R values <1 are only reported for comparison.

The separation power of columns prepared with ETTBS-β-CD is generally higher than with the corresponding METBS-β-CD: for many of the investigated racemates, R is twice or three times higher. Only massoja lactones C_{10} and C_{12} (13 and 14), *cis*-rose oxide (29), carvone (33) and compound 35 were much better separated with METBS-β-CD. Fig. 2a reports the separation of linalol (18), linalyl acetate (19) and propanoate (20) with a 30% ETTBS-β-CD/PS-086 column, and Fig. 3 reports the separation of *cis*- and *trans*-nerolidol (21 and 22) racemates with a 30% ETTBS-β-CD/PS-347.5 column.

ETTBS-γ-CD columns behave differently. This CD derivative was competitive with METBS-γ-CD only when PS-086 was used as diluting phase. In this case too, the ETTBS-γ-CD/PS-086 combination very often gave R twice or three times higher than the corresponding METBS-γ-CD/PS-086 combination. Fig. 2b reports the separation of linalol (18), linalyl acetate (19) and propanoate (20) with a 50% ETTBS-γ-CD/PS-086 column, and Fig. 4 reports the separations of massoja lactones C_{10} and C_{12} (13 and 14) with a 50% ETTBS-γ-CD/PS-347.5 column.

The results reported in Tables 1 and 2 show that, in general, lower polarity diluting phases reduce the optimal separation temperature by between 5 and 15°C. With ETTBS-CDs, lower polarity diluting phases often give higher α values [15,16]; with ETTBS-β-CD, the only exceptions are *cis*-rose oxide (29) (see below) and compound (35), which, with PS-086 as diluting phase, unexpectedly show values significantly lower than they do with either PS-347.5 or OV-1701. Moreover, the analysis temperatures which produce the best separations with METBS-CDs and ETTBS-CDs with the same diluting phase are equal or very close to each other. The better ETTBS-CD racemate separations might therefore be due to this CD's better enantioselectivity.

Although a 'general' CD has not yet been found, ETTBS-CDs have shown themselves to be very effective as GC chiral selectors, affording the separation of several racemates with different skeletons and functionality. In particular, these derivatives have been successful in separating several racemates simultaneously which, hitherto, had never been separated together on a single column in a single run, e.g. ETTBS-β-CD for borneol (24), bornyl acetate (25), isoborneol (26), isobornyl isobutanoate (27) and camphor (28).

The behaviour of some of the investigated racemates with the CDs in question should be noted. *cis*-Rose oxide (29) racemate was well separated with METBS- and ETTBS-β- and -γ-CDs diluted in the different polysiloxanes adopted here, with the exception of the ETTBS-β-CD/PS-086 combination, where the separation unexpectedly fell. This might be explained by a predominant CD/diluting phase interaction vs. the racemate/CD interaction, in agreement with Buda et al. [22].

The separation of the racemates of δ-lactones C_{10} and C_{12} (11 and 12) and massoja lactones C_{10} and C_{12} (13 and 14) with the four CDs investigated is of interest in order to throw light on how their separation can vary because of the additional double bond in position 3–4 of the lactone ring. ETTBS-β-CD did not separate massoja lactone racemates, while the corresponding δ-lactones were almost base-line separated with PS-347.5 as diluting phase. METBS-β-CD separated both the couples of lactone racemates almost at the base-line with comparable R and α. ETTBS-γ-CD gave the highest R and α for massoja lactones, while the corresponding δ-lactones were baseline separated. METBS-γ-CD separated the massoja lactones almost at the baseline; the δ-lactones separation was very poor.

Interesting behaviour was also shown by linalol

Table 1
Resolutions (R) and separation factors (α) of some racemates obtained with the five columns prepared with β-CDs

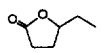
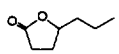
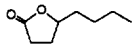
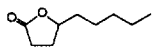
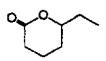
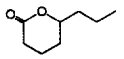
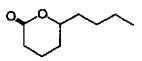
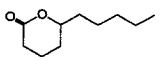
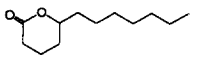
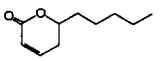
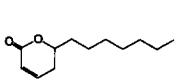
No.	Compound	Formula	Temp. (°C)	Column									
				METBS-β-CD/PS-086		METBS-β-CD/OV-1701		ETTBS-β-CD/PS-347.5		ETTBS-β-CD/PS-086		ETTBS-β-CD/OV-1701	
				R	α	R	α	R	α	R	α	R	α
1	β-Pinene		40	3.74	1.091	**4.00**	**1.112**	**4.94**	**1.158**	3.68	1.096	3.94	1.118
2	γ-Pentalactone		60	**8.02**	**1.135**	8.30	1.128	**12.72**	**1.552**				
			65							15.05	1.510	13.59	1.362
3	γ-Hexalactone		70	**6.55**	**1.103**	4.31	1.088	**10.55**	**1.299**				
			75							10.33	1.271		
			80									8.86	1.183
4	γ-Heptalactone		80	9.28	1.134	**8.79**	**1.140**	**13.23**	**1.308**				
			85							13.73	1.255		
			90									8.77	1.172
5	γ-Octalactone		100	**6.15**	**1.094**	5.81	1.086	**7.10**	**1.182**	9.92	1.166	6.08	1.092
6	γ-Nonalactone		100					**8.78**	**1.200**	10.17	1.184		
			105	**5.28**	**1.091**							5.32	1.100
			110			5.01	1.079						
7	δ-Hexalactone		75	NR	1.000	NR	1.000						
			80					1.47	1.029	**1.82**	**1.032**	1.12	1.022
8	δ-Heptalactone		80					**4.40**	**1.091**				
			85	**2.80**	**1.046**					3.90	1.068		
			90									2.96	1.056
			95			3.85	1.042						
9	δ-Octalactone		100	2.05	1.035	**1.72**	**1.038**	**2.54**	**1.076**	3.01	1.065	2.14	1.041
10	δ-Nonalactone		100					**1.42**	**1.025**	1.31	1.019		
			105	**1.83**	**1.028**							NR	1.000
			110			2.57	1.023						
11	δ-Decalactone		110					**1.18**	**1.024**	1.03	1.016		
			125	**1.09**	**1.048**	0.98	1.015					NR	1.000
12	δ-Dodecalactone		125					**1.33**	**1.022**				
			135			**1.36**	**1.017**			0.91	1.012		
			140	1.11	1.016							NR	1.000
13	Massoja decalactone		105					NR	1.000				
			110							NR	1.000	NR	1.000
			125	1.27	1.015	**1.22**	**1.016**						
14	Massoja dodecalactone		125					NR	1.000				
			130							NR	1.000		
			145									NR	1.000
			150	1.25	1.014	**0.95**	**1.016**						

Table 1 (Continued)

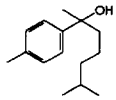
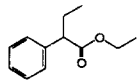
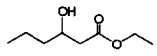
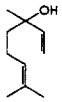
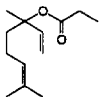
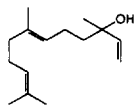
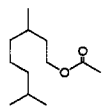
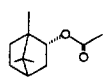
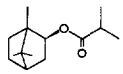
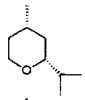
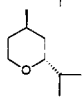
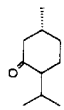
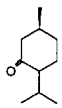
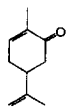
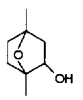
No.	Compound	Formula	Temp. (°C)	Column									
				METBS-β-CD/PS-086		METBS-β-CD/OV-1701		ETTBS-β-CD/PS-347.5		ETTBS-β-CD/PS-086		ETTBS-β-CD/OV-1701	
				R	α	*R*	α	*R*	α	*R*	α	*R*	α
15	2-(4-Methyl phenyl)-6-methyl heptan-2-ol		110	NR	1.000	NR	1.000	**1.13**	**1.018**				
			120							NR	1.000		
												NR	1.000
16	Ethyl 2-phenylbutanoate		80					**3.62**	**1.060**	3.38	1.052		
			85									2.39	1.043
			90			**1.09**	**1.018**						
			100	0.23	1.009								
17	Ethyl β-hydroxy hexanoate		70					**2.10**	**1.065**	1.59	1.034	0.39	1.021
			75										
			80	**2.75**	**1.045**	2.42	1.045						
18	Linalol		65	3.89	1.062	**3.97**	**1.071**	**8.41**	**1.178**				
			70							9.47	1.152		
			75									6.41	1.132
19	Linalyl acetate		70	NR	1.000	**1.38**	**1.018**	**4.86**	**1.088**	5.02	1.082		
			75									4.10	1.065
20	Linalyl propanoate		80					**1.39**	**1.022**	1.51	1.021	0.33	1.011
			90	NR	1.000	NR	1.000						
21	*cis*-Nerolidol		110					**5.18**	**1.064**	4.63	1.064	2.58	1.040
			115										
			120	2.31	1.033	**2.89**	**1.037**						
22	*trans*-Nerolidol		110					4.16	1.075	**5.30**	**1.075**	3.25	1.046
			115										
			120	3.42	1.041	**3.95**	**1.051**						
23	Citronellyl acetate		80					**2.11**	**1.041**				
			85							2.25	1.036	1.95	1.033
			90	NR	1.000	NR	1.000						
24	Borneol		65					**6.16**	**1.107**				
			70							4.42	1.070		
			75									3.69	1.065
			90	5.16	1.081	**6.54**	**1.105**						
25	Bornyl acetate		70					**2.13**	**1.026**	1.38	1.022		
			75									1.20	1.019
			80	NR	1.000	NR	1.000						

Table 1 (Continued)

No.	Compound	Formula	Temp. (°C)	Column									
				METBS-β-CD/PS-086		METBS-β-CD/OV-1701		ETTBS-β-CD/PS-347.5		ETTBS-β-CD/PS-086		ETTBS-β-CD/OV-1701	
				R	α	*R*	α	*R*	α	*R*	α	*R*	α
26	Isoborneol		65					**6.52**	**1.101**				
			70							5.95	1.070		
			75									4.36	1.067
			90	2.70	1.043	**3.46**	**1.066**						
27	Isobornyl Isobutanoate		85							1.43	1.021		
			90	1.17	1.015	**1.67**	**1.023**	1.19	1.018			**0.83**	**1.023**
28	Camphor		70	**3.72**	**1.052**	2.22	1.034	**4.33**	**1.075**	4.31	1.064	2.30	1.032
29	*cis*-Rose oxide		60					**1.64**	**1.042**	NR	1.000	1.37	1.037
			70	4.25	1.073	**5.50**	**1.082**						
30	*trans*-Rose oxide		60					1.10	1.019	**1.97**	**1.033**	1.36	1.023
			70	NR	1.000	**0.82**	**1.013**						
31	Menthone		65					**3.27**	**1.057**	2.43	1.039	3.01	1.050
			80	1.76	1.023	**1.58**	**1.024**						
32	Isomenthone		65					15.95	1.252	**17.27**	**1.257**	11.43	1.231
			80	10.19	1.140	**11.35**	**1.157**						
33	Carvone		80	0.95	1.015	**1.61**	**1.029**	NR	1.000	NR	1.000	NR	1.000
34	5,5,9-Trimethyl -2-oxa [4,5] spirodeca-5-ene		80					**4.51**	**1.065**				
			85							2.99	1.045		
			90	2.02	1.030	**3.30**	**1.048**					1.68	1.029
35	1,4-Dimethyl-7-oxabicyclo [2,2,1] heptan-2-ol		70					**3.32**	**1.049**	1.15	1.017		
			75									2.65	1.044
			80	3.51	1.058	**4.41**	**1.062**						

For each CD derivative, the best set of *R* and α values for each racemate, giving priority to α, is given in bold type.
NR=not resolved.

Table 2
Resolutions (R) and separation factors (α) of some racemates obtained with the five columns prepared with γ-CDs

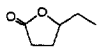
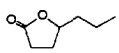
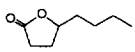
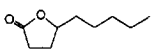
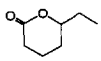
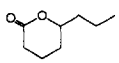
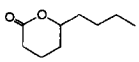
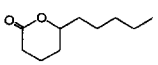
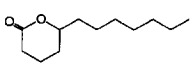
No.	Compound	Formula	Temp. (°C)	Column									
				METBS-γ-CD/PS-086		METBS-γ-CD/OV-1701		ETTBS-γ-CD/PS-347.5		ETTBS-γ-CD/PS-086		ETTBS-γ-CD/OV-1701	
				R	α	R	α	R	α	R	α	R	α
1	β-Pinene		40	**0.83**	**1.027**	0.88	1.021	NR	1.000	**0.96**	**1.028**		
			60									NR	1.000
2	γ-Pentalactone		50	NR	1.000	NR	1.000	NR	1.000				
			60							NR	1.000	NR	1.000
			70										
3	γ-Hexalactone		55							4.99	1.091		
			60	**0.1**	**1.012**	NR	1.000						
			65					**1.68**	**1.123**				
			70									1.30	1.045
4	γ-Heptalactone		70					**5.43**	**1.286**	12.26	1.212		
			80	**1.86**	**1.035**	2.20	1.027					3.27	1.105
5	γ-Octalactone		90					**4.74**	**1.165**	6.17	1.106		
			100	**1.11**	**1.023**	1.00	1.016					2.87	1.053
6	γ-Nonalactone		95					**6.20**	**1.119**	5.38	1.078		
			100									1.91	1.042
			105	**1.35**	**1.025**								
			110			0.86	1.015						
7	δ-Hexalactone		60							1.92	1.030		
			70	**0.10**	**1.009**	NR	1.000	**0.71**	**1.038**				
			80									NR	1.000
8	δ-Heptalactone		75							2.39	1.041	NR	1.000
			80					**0.80**	**1.045**				
			85	NR	1.000	NR	1.000						
9	δ-Octalactone		90					**1.64**	**1.071**	2.35	1.051		
			100	**2.05**	**1.035**	0.43	1.011					1.36	1.027
10	δ-Nonalactone		95					**3.73**	**1.069**				
			100							3.23	1.044		
			105	**1.28**	**1.019**	0.98	1.014					0.93	1.022
11	δ-Decalactone		110							**1.80**	**1.030**		
			125	**0.40**	**1.012**	0.32	1.009	1.96	1.026			0.31	1.013
12	δ-Dodecalactone		135	**0.29**	**1.010**					**1.06**	**1.015**	0.20	1.011
			150			NR	1.000	0.92	1.012				
13	Massoja decalactone		105					**6.11**	**1.104**	5.15	1.070		
			110									1.88	1.034
			125	**1.22**	**1.021**	1.17	1.015						

Table 2 (Continued)

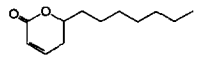
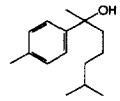
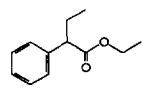
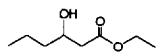
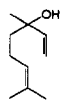
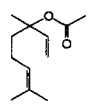
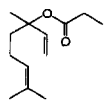
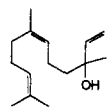
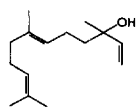
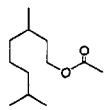
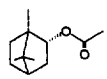
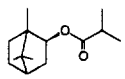
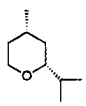
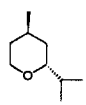
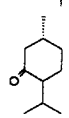
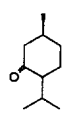
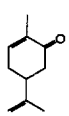
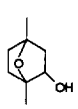
No.	Compound	Formula	Temp. (°C)	Column									
				METBS-γ-CD/PS-086		METBS-γ-CD/OV-1701		ETBS-γ-CD/PS-347.5		ETTBS-γ-CD/PS-086		ETTBS-γ-CD/OV-1701	
				R	α	*R*	α	*R*	α	*R*	α	*R*	α
14	Massoja dodecalactone		125					3.63	1.054	2.67	1.036		
			130									**1.15**	**1.091**
			135	**1.21**	**1.015**								
			150			0.10	1.007						
15	2-(4-Methyl phenyl)-6-methyl heptan-2-ol		110							1.57	1.023		
			120	NR	1.000	NR	1.000	**1.59**	**1.025**			NR	1.000
16	Ethyl 2-phenylbutanoate		80							2.02	1.027		
			85					**1.11**	**1.035**			NR	1.000
			90			**0.47**	**1.012**						
			95	0.1	1.007								
17	Ethyl β-hydroxy hexanoate		60							2.86	1.057		
			75					**1.01**	**1.062**			0.86	1.036
			80	**2.05**	**1.031**	1.67	1.026						
18	Linalol		60							**2.65**	**1.042**		
			70	**0.50**	**1.012**	0.42	1.010	0.20	1.030				
			75									NR	1.000
19	Linalyl acetate		70	0.85	1.013	**0.96**	**1.014**			2.36	1.034		
			75					**0.84**	**1.040**				
			85									NR	1.000
20	Linalyl propanoate		75							2.44	1.038		
			80					**1.00**	**1.051**			NR	1.000
			90	1.08	1.015	**1.17**	**1.015**						
21	*cis*-Nerolidol		110					**0.12**	**1.001**	NR	1.000		
			120	NR	1.000	NR	1.000					NR	1.000
22	*trans*-Nerolidol		110					NR	1.000	NR	1.000		
			120	NR	1.000	NR	1.000					NR	1.000
23	Citronellyl acetate		85							NR	1.000		
			90			NR	1.000	NR	1.000			NR	1.000
			100	NR	1.000								
24	Borneol		70							**1.54**	**1.029**		
			80									NR	1.000
			85					NR	1.000				
			90	NR	1.000	NR	1.000						

Table 2 (Continued)

No.	Compound	Formula	Temp. (°C)	Column									
				METBS-γ-CD/PS-086		METBS-γ-CD/OV-1701		ETTBS-γ-CD/PS-347.5		ETTBS-γ-CD/PS-086		ETTBS-γ-CD/OV-1701	
				R	α	*R*	α	*R*	α	*R*	α	*R*	α
25	Bornyl acetate		70 75 80	0.94	1.016	**1.29**	**1.017**	NR	1.000	**2.34**	**1.034**	NR	1.000
26	Isoborneol		60 70 90	0.10	1.009	**0.44**	**1.013**	NR	1.000	**1.13**	**1.006**	NR	1.000
27	Isobornyl isobutanoate		90	NR	1.000	NR	1.000	NR	1.000	NR	1.000	NR	1.000
28	Camphor		70	NR	1.000	NR	1.000	NR	1.000	NR	1.000	NR	1.000
29	*cis*-Rose oxide		55 65 70	6.25	1.080	**7.58**	**1.093**	2.39	1.167	**8.28**	**1.180**	NR	1.000
30	*trans*-Rose oxide		55 65 70	**2.43**	**1.038**	2.46	1.032	3.98	1.331	**19.59**	**1.361**	NR	1.000
31	Menthone		60 70 80	**3.38**	**1.080**	2.32	1.046	**3.43**	**1.223**	11.28	1.206	NR	1.000
32	Isomenthone		60 70 80	**5.60**	**1.083**	3.68	1.060	**3.52**	**1.216**	10.92	1.194	NR	1.000
33	Carvone		70 80	**1.26**	**1.021**	1.31	1.018	NR	1.000	**3.40**	**1.058**	0.82	1.035
34	5,5,9-Trimethyl-2-oxa [4,5] spirodeca-5-ene		85 90 105	0.10	1.009	0.10	1.009	**0.38**	**1.054**	2.97	1.044	NR	1.000
35	1,4-Dimethyl-7--oxa-bicyclo [2,2,1] heptan-2-ol		65 75 80	1.03	1.015	**1.24**	**1.017**	**1.18**	**1.049**	2.32	1.040	NR	1.000

For each CD derivative, the best set of *R* and α values for each racemate, giving priority to α, is given in bold type.
NR=not resolved.

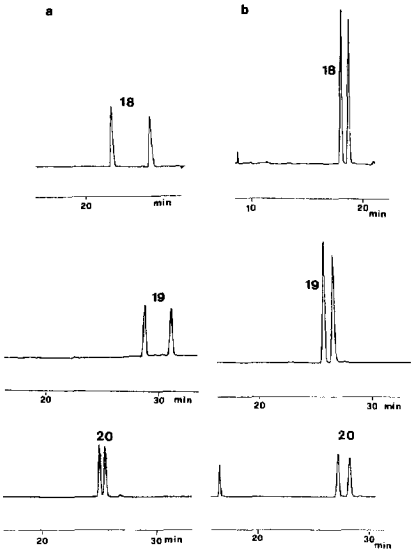


Fig. 2. (a) Separation of linalol (18) (70°C), linalyl acetate (19) (70°C) and propanoate (20) (80°C) racemates; column 30% ETTBS-β-CD/PS-086, 25 m×0.25 mm I.D., d_f: 0.15 μm; (b) Separation of linalol (18) (60°C), linalyl acetate (19) (70°C) and propanoate (20) (75°C) racemates; column 50% ETTBS-γ-CD/PS-086, 25 m×0.25 μm I.D., d_f: 0.15 μm.

(18), linalyl acetate (19) and propanoate racemates (20) (Fig. 2a and b). With β-CDs, the three racemates were much better separated with ETTBS-β-CD than with METBS-β-CD; linalol enantiomers

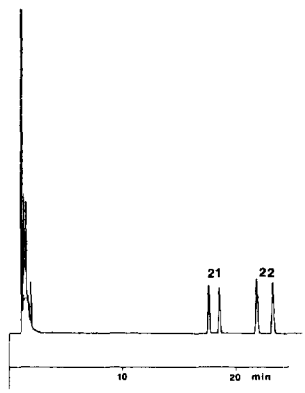


Fig. 3. Simultaneous separation of *cis*- and *trans*-nerolidol (21 and 22) racemates; column 30% ETTBS-β-CD/PS-347.5, 25 m×0.25 mm I.D., d_f: 0.15 μm; temperature: 110°C (isothermal).

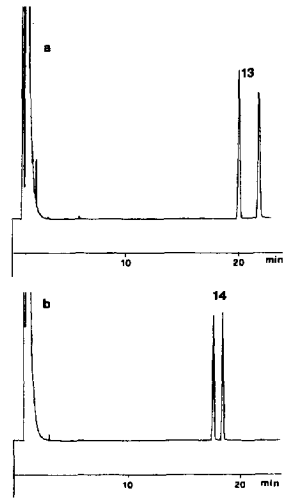


Fig. 4. Separations of massoja lactones C_{10} (13) (a) (105°C isothermal) and C_{12} (14) (b) (125°C isothermal); column 50% ETTBS-γ-CD/PS-347.5, 25 m×0.25 mm I.D., d_f: 0.15 μm.

had the highest R and α values in all cases while linalyl propanoate had the lowest, not being separated at all with METBS-β-CD. When γ-CDs were used, the separation of the three racemates in question became comparable: again, ETTBS-γ-CD gave better R and α than METBS-γ-CD. The different behaviour of the three racemates in question with METBS- and ETTBS-β-CDs, on one hand, and with METBS- and ETTBS-γ-CDs, on the other, may be due to the difference in the dimensions of β- and γ-CD mouth: the dimension of one of the groups bonded to the chiral centre in the three racemates may influence the interaction with the small-mouth β-CDs and not that with the big-mouth γ-CDs. This observation is in agreement with the separation of *cis*- and *trans*-nerolidols (21 and 22) which have the same structure as that of linalol at the chiral centre, but a further C_5 unit bonded to the carbon atom in position 9. *cis*- and *trans*-nerolidols are very well separated with both METBS- and ETTBS-β-CDs, but not at all with either METBS- or ETTBS-γ-CDs: the lack of enantioselectivity of the γ-CDs is probably due to the bigger mouth of γ-CDs, which makes it unable to discriminate between the enantiomers of either *cis*- or *trans*-nerolidols. Moreover, the similarity in the separation parameters of *cis*- and *trans*-

isomers with all four CDs shows that the stereoisomery of the C-9 side chain is not involved in the enantiomer discrimination.

4. Conclusions

The results reported here show that ETTBS-CDs can successfully replace METBS-CDs, and that, in general, they show higher GC separation capacity with the racemates investigated. ETTBS-β-CD can also be successfully used at low operating temperatures with diluting phases covering a wider range of polarity than can METBS-β-CD. In contrast, ETTBS-γ-CD can be successfully employed at a MIOT of 40°C only when combined with a polymethylphenylsiloxane diluting phase (PS-086).

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References

[1] V. Schurig and H.P. Nowotny, J. Chromatogr., 441 (1988) 155.
[2] H.P. Nowotny, D. Schmalzing, D. Wistuba and V. Schurig, J. High Resolut. Chromatogr., 12 (1989) 383.
[3] W. Blum and R. Aichholz, J. High Resolut. Chromatogr., 13 (1990) 515.
[4] H.-G. Schmarr, A. Kaunzinger and A. Mosandl, in P. Sandra (Editor), Proc. 13th Int. Symp. on Capillary Chromatography, Huethig, Heidelberg, 1991, p. 188.
[5] H.-G. Schmarr, A. Mosandl and A. Kaunzinger, J. Microcol. Sep., 3 (1991) 395.
[6] A. Dietrich, B. Maas, V. Karl, P. Kreis, D. Lehmann, B. Weber and A. Mosandl, J. High Resolut. Chromatogr., 15 (1992) 176.
[7] A. Dietrich, B. Maas, W. Messer, G. Bruche, V. Karl, A. Kauzinger and A. Mosandl, J. High Resolut. Chromatogr., 15 (1992) 590.
[8] B. Maas, A. Dietrich, D. Bartschat and A. Mosandl, J. Chromatogr. Sci., 33 (1995) 223 and related references.
[9] B. Maas, A. Dietrich, V. Karl, A. Kaunzinger, D. Lehmann, T. Koepke and A. Mosandl, J. Microcol. Sep., 5 (1993) 421.
[10] F. Kobor and G. Schomburg, J. High Resolut. Chromatogr., 16 (1993) 693.
[11] W.A. Koenig, A. Rieck, I.H. Hardt, B. Gehrcke, K.-H. Kubeczka and H. Muhle, J. High Resolut. Chromatogr., 17 (1994) 315.
[12] I.H. Hardt, C. Wolf, B. Gehrcke, D.H. Hochmuth, B. Pfaffenberger, H. Huehnerfuss and W.A. Koenig, J. High Resolut. Chromatogr., 17 (1994) 859.
[13] K. Jaques, W.M. Buda, A. Venema and P. Sandra, J. Chromatogr. A, 666 (1994) 131.
[14] J. Krupcik, E. Benicka, P. Majek, I. Skacani and P. Sandra, J. Chromatogr. A, 665 (1994) 175.
[15] C. Bicchi, G. Artuffo, A. D'Amato, V. Manzin, A. Galli and M. Galli, J. High Resolut. Chromatogr., 16 (1993) 209.
[16] C. Bicchi, A. D'Amato, V. Manzin, A. Galli and M. Galli, J. Microcol. Sep., 7 (1995), 326.
[17] A. Dietrich, B. Maas, G. Brand, V. Karl, A. Kauzinger and A. Mosandl, J. High Resolut. Chromatogr., 15 (1992) 769.
[18] A. Dietrich, B. Maas and A. Mosandl, J. Microcol. Sep., 6 (1994) 33.
[19] A. Dietrich, B. Maas and A. Mosandl, J. High Resolut. Chromatogr., 18 (1995) 152.
[20] B. Maas, A. Dietrich, T. Beck, S. Boerner and A. Mosandl, J. Microcol. Sep., 7 (1995) 65.
[21] I.H. Hardt and W.A. Koenig, J. Microcol. Sep., 5 (1993) 35.
[22] W.M. Buda, K. Jaques, A. Venema and P. Sandra, Fresenius J. Anal. Chem., 352 (1995) 679.
[23] M. Jung, D. Schmalzing and V. Schurig, J. Chromatogr., 552 (1991) 43.
[24] K. Takeo, K. Uemura and A. Mitoh, J. Carbohyd. Chem., 7 (1988) 293.
[25] K. Takeo, A. Mitoh and K. Uemura, J. Carbohyd. Chem., 187 (1989) 203.
[26] V. Schurig, D. Schmalzing, U. Muehleck, M. Jung, M. Schleimer, P. Mussche, C. Duvekot and J.C. Buyten, J. High Resolut. Chromatogr., 13 (1990) 713.
[27] L. Blomberg and T. Wannmann, J. Chromatogr., 148 (1978) 179.
[28] C. Bicchi, A. D'Amato, G. Artuffo, G.M. Nano, A. Galli and M. Galli, J. High Resolut. Chromatogr., 14 (1991) 301.