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Mixed chiral stationary phase containing modified resorcinarene and β-cyclodextrin selectors bonded to a polysiloxane for enantioselective gas chromatography

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Abstract

Both a resorcinarene with pendant L-valine diamide groups (used as hydrogen-bonding selector) and a permethylated β-cyclodextrin (used as inclusion-type selector) were chemically bonded to poly(hydromethyl)dimethylsiloxane in a one-pot reaction via Pt-catalyzed alkene hydrosilylation. This novel mixed chiral stationary phase (mixCSP) named Chirasil-Calixval-Dex resembles a combination of the known chiral stationary phases (CSPs) Chirasil-Calixval and Chirasil-Dex and it was used successfully in enantioselective gas chromatography toward a unified enantioselective GC separation system. It is demonstrated that Chirasil-Calixval-Dex retains the individual enantioselectivites of the single components. Thus the enantiomers of apolar hydrocarbons as well as polar amino acid derivatives can be separated with the mixed CSP.

Keywords: Enantiomer separation; Chiral stationary phases, GC; Cyclodextrins; Resorcinarene; Amino acids

1. Introduction

Cyclodextrins [1] and resorcinarenes [2–4] represent macrocyclic molecules with a defined cavity, but the individual polarity of these cavities in both types of supramolecular structures is different. Cyclodextrin derivatives are well established as chiral stationary phases (CSPs) in enantioselective gas chromatography (GC) for years [5,6]. Recently we reported on the use of an enantiomerically pure resorcinarene derivative, containing pendant L-valine diamide groups, linked to a poly(hydromethyl)dimethylsiloxane (Chirasil-Calixval, cf. Fig. 1, bottom) as CSP in enantioselective capillary gas chromatography [7,8]. Previously, resorcinarenes have been used as stationary phase only in achiral capillary gas chromatography for, e.g., the separation of positional isomers of disubstituted benzenes [9,10]. Xiao et al. were the first to use mixed non-polymer-bonded GC stationary phases comprising of a modified resorcinarene and β-cyclodextrin derivative for achiral separations observing synergistic effects [11]. Previous attempts to coat capillary columns with a mixture of octakis(3-*O*-butanoyl-2,6-*O*-di-*n*-pentyl)-γ-cyclodextrin (Lipodex E) [12] and polymeric Chirasil-Val [13] (both selectors represent-

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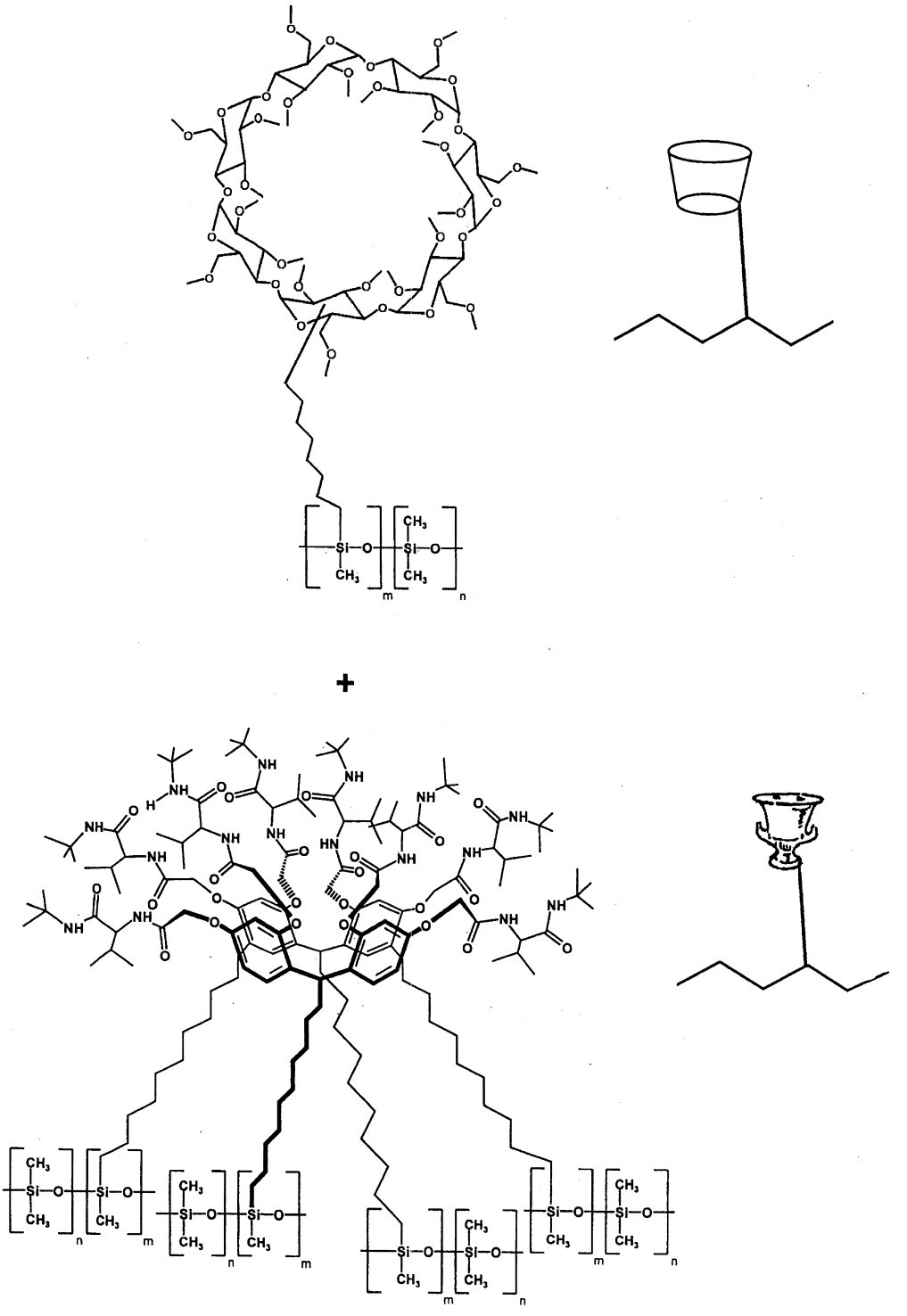

Fig. 1. Structures of the two CSPs Chirasil-Dex (top) and Chirasil-Calixval (bottom) which were bonded on the same polymeric backbone in the new mixed CSP Chirasil-Calixval-Dex. The molar ratio of the two selectors was 1:1. Depending on the reaction conditions, the octamethylene linker arises from the O6- and/or O2-position of cyclodextrin.

ing important CSPs for amino acid analysis) failed [14]. A report on a mixed CSP combining amino acid and cyclodextrin derived selectors bonded to a polysiloxane is revealed only in the secondary literature [15]. Efforts to prepare a mixed CSP from Chirasil-Val [13] and Chirasil-γ-Dex (Lipodex E bonded to poly(hydromethyl)dimethylsiloxane) [16] were not successful due to the different polarity of the polymers leading to droplet formation in coated fused-silica columns [17]. However, replacing Chirasil-γ-Dex by commercially available Chirasil-β-Dex (permethylated β-cyclodextrin bonded to poly(hydromethyl)dimethylsiloxane) (cf. Fig. 1, top) and Chirasil-Val by Chirasil-Calixval (cf. Fig. 1, bottom), respectively, resulted in a mixed CSP, prepared by linking the selectors in a one-pot hydrosilylation reaction to polydimethylsiloxane, which proved suitable for gas chromatographic separation of enantiomers retaining the individual enantioselectivities of the single components.

2. Experimental

2.1. Preparation of Chirasil-Calixval-Dex

The olefinic starting materials octakis-*O*-[(L-valine-*tert*.-butylamide)-*N*-acetyl]-C-decenyl-resorcinarene [8] and monokis-*O*-octenyl-permethyl-β-cyclodextrin [18] were prepared according to literature procedures.

The mixed CSP Chirasil-Calixval-Dex was synthesized as follows: 50 mg (0.0182 mmol) of the resorcinarene tetraalkene selector, 30 mg (0.0197 mmol) of the cyclodextrin monoalkene selector and 250 mg of poly(9.3% hydromethyl–90.7% dimethyl)siloxane (M_r~3000) were dissolved in a mixture of 4 ml of dry tetrahydrofuran and 8 ml of dry toluene in an inert atmosphere of nitrogen. A solution of 1 mg hexachloroplatinic acid (Wacker, Burghausen, Germany) in 1 ml of dry tetrahydrofuran was prepared separately and 125 μl thereof was added to the reaction mixture. After refluxing for 48 h at 90 °C the solution was allowed to cool down to room temperature. The solvent was removed under reduced pressure and the residue was dissolved in 5 ml of dry dichloromethane. After adding 20 ml of diethyl ether the mixture was kept at 5 °C for 1 h. The residual nonreacted particles were separated by filtration (pore size 0.45 μm). After evaporation of the solvent 230 mg (70%) of Chirasil-Calixval-Dex were obtained. Of the average 4.14 reactive hydromethyl moieties per polysiloxane, only 1.1 positions are statistically reacted with the selector alkenes. The resulting mixed CSP itself represents a mixture of various individual polymers containing statistical amounts of both selectors. The presence of the four olefinic arms of the resorcinarene tetraalkene selector should give rise to crosslinking of Chirasil-Calixval-Dex. To avoid further cross-linking of the polymer due to the residual Si–H moieties (needed for thermal immobilization on the fused-silica surface after coating) the product was stored as a solution in dry dichloromethane with exclusion of light in a refrigerator.

$[\alpha]_D^{20}$ +8.9 (c 1, $CHCl_3$). ^{1}H-Nuclear magnetic resonance (NMR) (C^2HCl_3, 250 MHz, ppm): 7.72, 6.69, 5.13, 4.61, 4.31, 4.02, 3.80, 3.63, 3.48, 3.36, 3.18, 3.15, 1.77, 1.28, 0.90, 0.05.

2.2. Gas chromatographic measurements

GC was carried out on a Carlo Erba 5300 Mega series instrument or a Hewlett-Packard HP 5890 system equipped with a flame ionization detection (FID) system and hydrogen as the carrier gas. Fused-silica capillaries (0.25 mm I.D.) were obtained on stock from Microquartz, USA. The capillaries were pretreated by heating the columns at 260 °C with a slow stream of hydrogen for 2 days and were coated subsequently with Chirasil-Calixval-Dex by the static method to yield a film thickness of 0.25 μm. The columns were conditioned by increasing the temperature gradually up to 190 °C and were kept at this temperature for 16 h before use. The Schurig test mixture (see Ref. [19]) was purchased from Varian-Chrompack, Middelburg, The Netherlands. Thermogravimetric analysis (TGA) and was performed on a Netzsch-Gerätebau STA 409C thermobalance whereby the sample (52 mg) was heated under nitrogen. The heating rate was 5 °C/min starting from room temperature to 450 °C.

3. Results and discussion

In preliminary experiments, a mixture of Chirasil-γ-Dex polymer [containing 40% (w/w) of monokis-

O-octenyl-octakis[3-*O*-butanoyl-2,6-*O*-*n*-pentyl]-γ-cyclodextrin] [16] and of Chirasil-Calixval polymer [containing 25% (w/w) of the resorcinarene derivative] [8] at a 7:4 molar ratio (as applied to the selectors) was coated and tested. However, the contribution of the γ-cyclodextrin selector to enantioselectivity was so dominant that no difference of the mixed phase compared to the pure Chirasil-γ-Dex was discerned. Therefore the permethylated β-cyclodextrin CSP Chirasil-Dex [18] was employed for further investigations. Thus both selectors were simultaneously linked to poly(hydromethyl)-dimethylsiloxane via the concomitant Pt-catalyzed hydrosilylation of the alkenes in a one-pot reaction. In order to implement this kind of polymer-bonded CSP, a combination of the alkene precursors octakis-*O*-[(L-valine-*tert*.-butylamide)-*N*-acetyl]-C-decenyl-resorcinarene and monokis-octenyl-permethyl-β-cyclodextrin were used to prepare the novel mixed CSP at a molar selector ratio of 1:1 (cf. Fig. 1).

Chirasil-Dex alone is incapable of separating all proteinogenic amino acid derivatives (*N*-trifluoroacetyl-ethyl esters) into enantiomers (cf. Fig. 2). Only alanine, serine, cysteine, phenylalanine and proline can be separated with acceptable separation factors. For threonine, methionine, isoleucine and leucine, poor separation has been noticed. In case of valine, aspartic acid and glutamic acid no enantiomeric bias was observed at all and lysine, ornithine and tryptophan were not eluted within 30 min. However, Chirasil-Dex is a versatile commercial selector for other classes of compounds prone to molecular inclusion [18]. By contrast, Chirasil-Calixval is capable of separating most proteinogenic amino acid derivatives but fails to separate compounds devoid of hydrogen-bonding interactions [7].

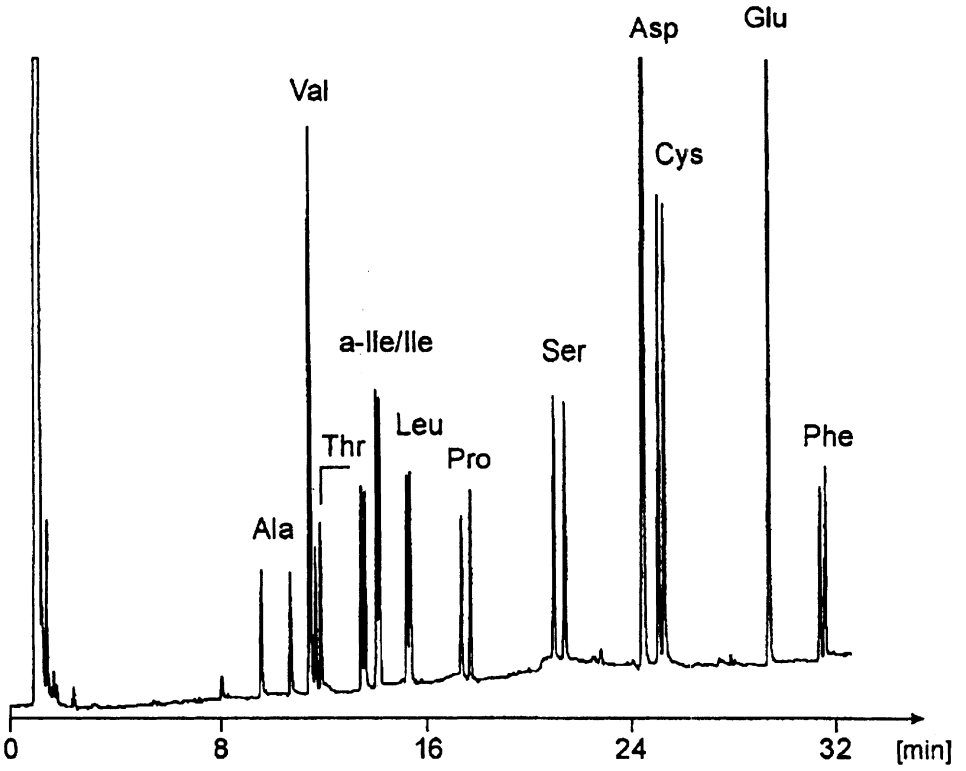

Fig. 2. Gas chromatographic enantiomeric separation of some *N*(*O*)-trifluoroacetyl-DL-amino acid ethyl esters on Chirasil-Dex (30%, w/w). 20 m×0.25 mm fused-silica capillary with a film thickness of 250 nm; temperature program: 70 °C for 3 min, 3 °C/min, 170 °C; carrier gas: 0.5 bar hydrogen; split: 1:100.

Thus, in the mixed CSP Chirasil-Calixval-Dex the contribution to enantioselectivity of the individual CSPs can be probed. Chiral recognition in chromatography is the result of various kinds of enantioselective interactions. In case of Chirasil-Calixval-Dex three different modes of molecular recognition may be invoked, i.e., hydrogen bonding with diamide moieties as well as molecular inclusion into the two cavities of different polarities.

Fig. 3 shows the separation of some racemic *N*(*O*)-trifluoroacetyl-amino acid ethyl esters on the mixed CSP Chirasil-Calixval-Dex. In comparison to Chirasil-Val [13] a better separation for proline, but inferior separation of the amino acids aspartic acid, phenylalanine and tryptophan were observed. This observation clearly resembles the properties of the single CSP Chirasil-Calixval [7]. Although the separation factors of alanine and proline are larger on Chirasil-Dex as compared to Chirasil-Calixval, the separations of these amino acids on the mixed CSP were nearly the same as on Chirasil-Dex. Despite the reduced amount of the cyclodextrin selector in the mixed CSP it may therefore be concluded that an additive effect is operating in determining the overall enantioselectivity for the two amino acid derivatives. Yet the separation factors are still low in all cases. As valine, glutamic acid, lysine and ornithine are not separated on Chirasil-Dex, the nearly same retention times of these amino acids on Chirasil-Calix and Chirasil-Calixval-Dex implies that the observed enantioselectivity in the mixed CSP is entirely governed by the diamide selector. This notion can directly be proved for threonine. Threonine exhibits a change of the elution order between Chirasil-Calixval and Chirasil-Dex. The elution order observed with the mixed CSP Chirasil-Calixval-Dex resembles that of the single CSP Chirasil-Calixval rather than that of Chirasil-Dex. Obviously, the diamide selectors overrides the influence of the cyclodextrin selector due a weak or peripheric interaction of threonine with the latter. Since the resorcinarene selector may compete with the cyclo-

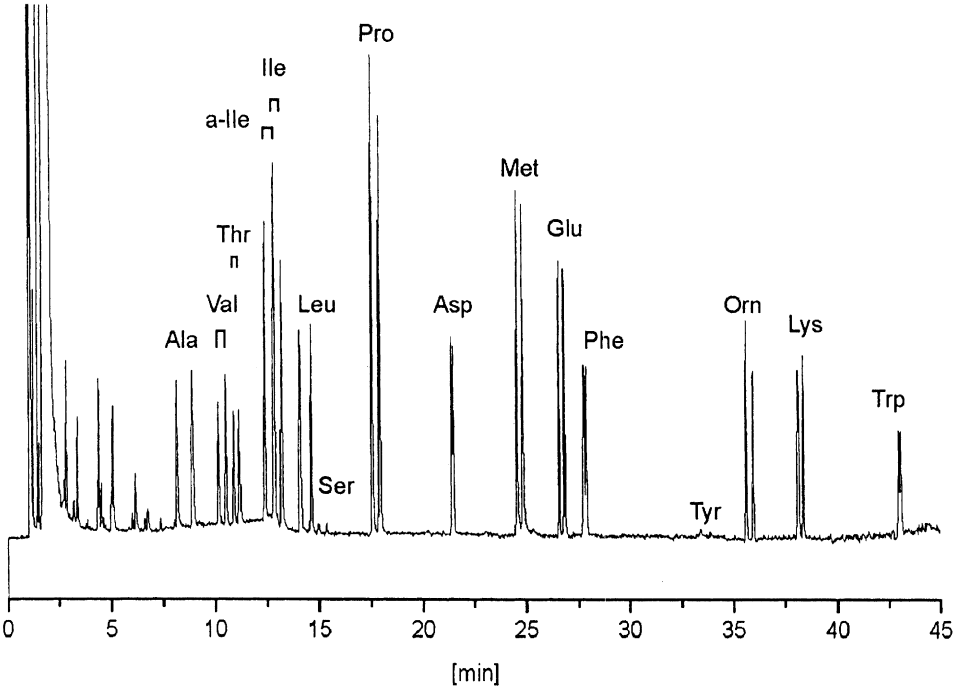

Fig. 3. Gas chromatographic enantiomeric separation of some *N*(*O*)-trifluoroacetyl-DL-amino acid ethyl esters on Chirasil-Calixval-Dex (15/10%, w/w). 20 m×0.25 mm fused-silica capillary with a film thickness of 250 nm; temperature program: 70 °C for 3 min, 3 °C/min, 170 °C; carrier gas: 0.5 bar hydrogen; split: 1:100.

dextrin selector, an optimization may in the future be carried out by varying the ratio of the selectors in the mixed CSP, although preliminary investigations along this line were not particularly successful. Despite an increase of the amount of the cyclodextrin selector from 10 to 15% (w/w) in the mixed CSP, the separation factor for phenylalanine and aspartic acid (as *N*-trifluoroacetyl-ethyl ester derivatives) could not be increased.

An unusual elution order of the critical enantiomeric pairs of the two diastereomers isoleucine (Ile) and *allo*-isoleucine (a-Ile), respectively, were observed when the single selectors and the mixed selectors are compared (cf. Fig. 4). Whereas the elution order of the enantiomers is always D<L for both diastereomers the total elution orders of all stereoisomers are different.

Chirasil-Calixval: D-a-Ile<D-Ile<L-a-Ile<L-Ile.
Chirasil-Dex: D-a-Ile<L-a-Ile<D-Ile<L-Ile.
Chirasil-Calixval-Dex: D-a-Ile<<L-a-Ile<D-Ile<<L-Ile.

As compared to Chirasil-Dex the separation factors for the enantiomers of Ile and a-Ile are clearly improved due to an additive effect caused by the presence of Chirasil-Calixval in the mixed CSP. Yet the inverted elution order D-Ile<L-a-Ile observed for the diastereomers on Chirasil-Calixval is not transmitted into the mixed CSP although a shift of the retention times toward a change of the elution order is already evident leading to partial peak overlap of L-a-Ile and D-Ile.

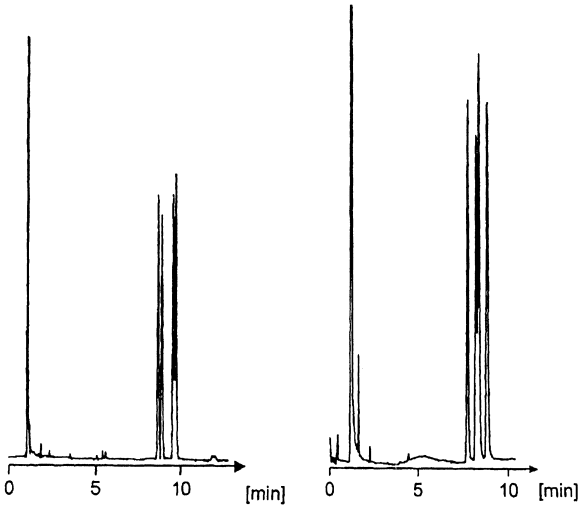

Fig. 4. Gas chromatographic enantiomeric separation of the stereoisomers of DL-isoleucine (Ile) and DL-*allo*-isoleucine (a-Ile) (as *N*-trifluoroacetyl- and *O*-ethyl ester derivatives) on Chirasil-Dex (30%) (left) and Chirasil-Calixval-Dex (25%, 1:1) (right). Both columns: 20 m×0.25 mm fused-silica; oven temperature: 90 °C; carrier gas: 0.4 bar hydrogen; split: 1:100.

Chirasil-Val is a versatile commercial selector for hydrogen-bonding enantiomeric mixtures such as derivatized amino acids [13]. However, the CSP fails to separate analytes devoid of strong hydrogen-bonding interactions. The same holds true for Chirasil-Calixval. Therefore it was considered a challenge to provide a mixed CSP which is capable of simultaneously separating the enantiomers of polar amino acid derivatives and of apolar enantiomers such as unfunctionalized hydrocarbons. In Fig. 5 the enantiomeric separation of compounds which (except for the hydroxy-γ-lactone) cannot be separated on diamide selectors is demonstrated. The separability rests entirely on the presence of the cyclodextrin selector in the mixed CSPs. The separation factors α for these compounds were generally lower on Chirasil-Calixval-Dex compared to single Chirasil-Dex. This is due to the reduced concentration of the cyclodextrin selector in the mixed CSP (10%, w/w). For selectors diluted in polysiloxane matrices a concentration dependence of the separation factor has been predicted by theory and was verified by the experiment [20]. The selector concentration of cyclodextrin in Chirasil-Dex varies between 30 and 40% (w/w). There is no evidence of an adverse effect on enantioselectivity imparted by the Chirasil-Dex component due to the presence of the Chirasil-Calixval component in the mixed CSP. Obviously, the sterically constrained resorcinarene cavity imparts no striking influence on enantioselectivity. Thus, the presence of totally different selectors combined in one CSP represents an interesting novel approach in enantioselective gas chromatography.

The Schurig test mixture is a tool for testing the quality of commercial Chirasil-Dex columns [19]. The chromatogram in Fig. 6 demonstrates the possibility to separate enantiomers of this test mixture with the new mixed CSP Chirasil-Calixval-Dex, although the components of the mixture are not amenable to enantiomeric separation on diamide-based CSPs. Only the elution of strong basic and acidic racemates such as 1-phenylethylamine and 2,3-butanediol (*rac* and *meso*), respectively, in the

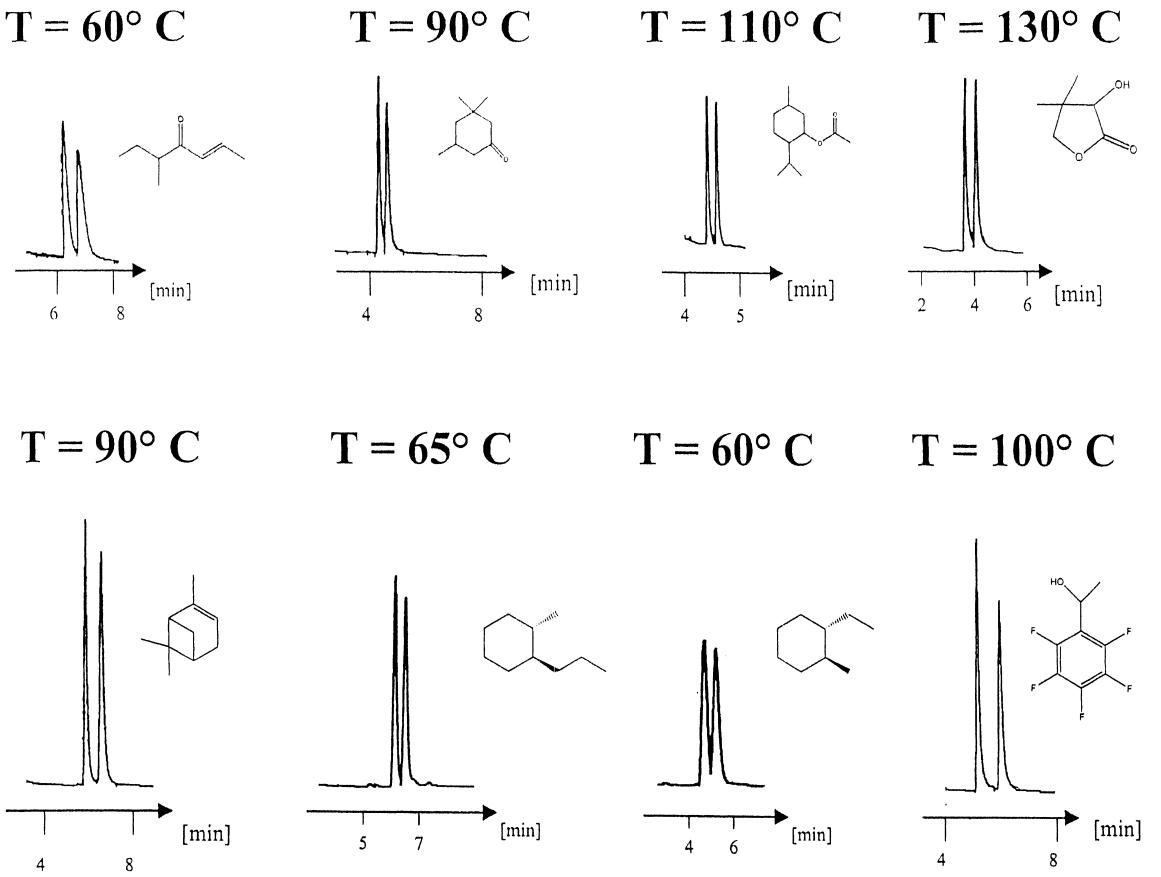

Fig. 5. Gas chromatographic enantiomeric separation of different classes of compounds on Chirasil-Calixval-Dex not amenable to resolution on conventional diamide based CSPs (except of the hydroxy-γ-lactone). 20 m×0.25 mm fused-silica capillary; film thickness: 250 nm; carrier gas: 0.5 bar hydrogen; split: 1:100.

test mixture caused problems. This fact may be due to the not yet solved problem of an effective immobilization and deactivation of the mixed CSP. Due to the limited thermal stability of the resorcinarene diamide selector [7], as compared to the cyclodextrin selector, the thermal immobilization employed for Chirasil-Dex (240 °C/36–40 h) [18] could not be performed. To avoid bleeding and decomposition of the resorcinarene selector only a thermal immobilization at 200 °C for 48 h was practiced. A modified immobilization procedure via a copolymer under more gentle thermal conditions will be a challenge for further investigations. Although the surface of the fused-silica column was not deactivated prior to the coating procedure, no adverse effect was observed for most separations (cf. Figs. 3–6). Only if peak tailing or incomplete recovery of analytes was observed, established deactivation procedures should be employed as ancillary options for the approach described here.

The thermal stability of the mixCSP Chirasil-Calixval-Dex polymer prior to coating was evaluated by thermal analysis (TGA). The results are comparable to the thermal behavior of single Chirasil-Calixval [7,8]. The polymer began to smoothly decompose at about 220 °C. The onset point was determined at 340 °C. These results corroborate the observed gas chromatographic behavior of Chirasil-Calixval-Dex as a mixed CSP. Thus, the new mixed stationary phase is as thermally stable as Chirasil-Calix but less stable as Chirasil-Dex. The mixCSP Chirasil-Calixval-Dex can be used up to 200 °C without decompo-

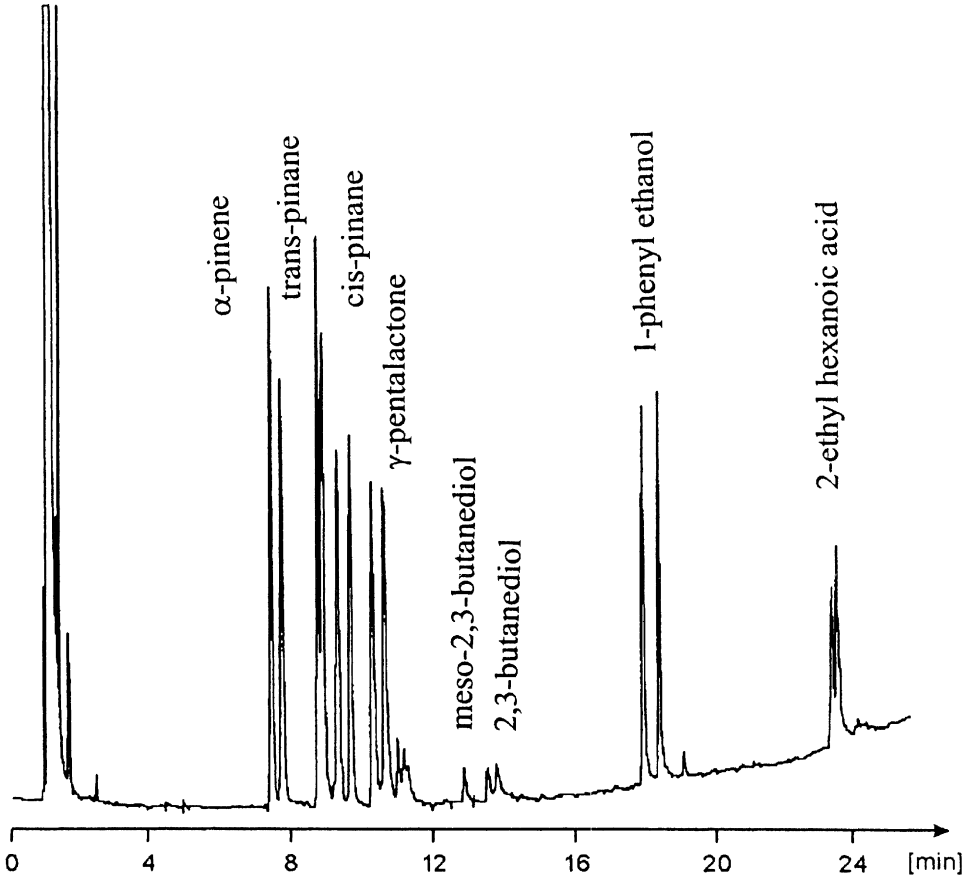

Fig. 6. Gas chromatographic enantiomeric separation of racemates of the Schurig test mixture [19] with Chirasil-Calixval-Dex. 20 m×0.25 mm fused-silica capillary; film thickness: 250 nm; temperature program: 60 °C for 3 min, rate: 4 °C/min, 140 °C; carrier gas: 0.45 bar hydrogen; split: 1:100.

sition and can be used even up to 230 °C for short periods of time in enantioselective GC. In this respect it should be mentioned that high temperatures are always detrimental to enantioselective separation when the enthalpy term, determining the Gibbs energy change, is responsible for chiral recognition [21]. Therefore the use of short columns has been advocated recently in enantioselective gas chromatography [22,23]. It can be predicted that the use of short columns coated with Chirasil-Calixval-Dex will lead to a considerable reduction of the elution temperature increasing selectivity which compensates the loss of efficiency.

4. Conclusion

The feasibility of a simultaneous reaction of two olefinic macrocyclic chiral selector precursors with a poly(hydromethyl)dimethylsiloxane via Pt-catalysed hydrosilylation in a one-pot reaction is demonstrated. The resulting mixCSP combines the enantioselectivities of the single selectors Chirasil-Dex and Chirasil-Calixval. Neither cooperative nor inhibitory but additive effects of the two selectors on enantioselectivity have been observed. The slight decrease of the separation factor for racemates which are only separated by one selector component is due to the

reduced concentration of the active selector in the mixed CSP. The demonstration of the concept of mixed CSPs combining the enantioselectivities of individual CSPs may be regarded as a first step toward the goal of a unified CSP in enantioselective gas chromatography. By changing the chirality of the diamide selectors from L to D (inverted) or to DL (racemic), further interesting insights of the action of the two individual selectors in the mixed CSP can be obtained in.

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