ELSEVIER

Journal of Chromatography A, 761 (1997) 181–193

JOURNAL OF CHROMATOGRAPHY A

Enantioselective capillary gas chromatography and capillary supercritical fluid chromatography on an immobilized γ-cyclodextrin derivative

Heiko Grosenick[1], Volker Schurig*

Institut für Organische Chemie, Universität Tübingen, Auf der Morgenstelle 18, D-72076 Tübingen, Germany

Received 28 June 1996; revised 19 September 1996; accepted 24 September 1996

Abstract

Octakis(3-O-butanoyl-2,6-di-O-*n*-pentyl)-γ-cyclodextrin (3-bu-2,6-pe-γ-CD) was chemically linked to a polysiloxane via an octamethylene spacer, yielding a chiral stationary phase called Chirasil-γ-Dex. Its immobilization onto the surface of capillary columns is possible by thermal treatment. The influence of the percentage of 3-bu-2,6-pe-γ-CD in the stationary phase was systematically investigated. With an increasing percentage of cyclodextrin, chiral separation factors, α, and retention factors, k', increased, whereas the degree of immobilization decreased. A value of 40% (w/w) 3-bu-2,6-pe-γ-CD was found to be optimal, since high separation factors are combined with a good degree of immobilization. Besides applications in enantioselective gas chromatography, some enantiomer separations by capillary supercritical fluid chromatography are presented.

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

1. Introduction

Cyclodextrin-based stationary phases play an important role in enantioselective gas chromatography (GC), supercritical fluid chromatography and high-performance liquid chromatography (HPLC). For GC, alkylated/acylated cyclodextrin (CD) derivatives (e.g., permethylated β-CD), diluted in an appropriate polysiloxane, have been employed for coating capillary columns [1,2]. Cyclodextrin derivatives, which are viscous liquids at room temperature [e.g., octakis(3-O-butanoyl-2,6-di-O-*n*-pentyl)-γ-cyclodextrin (3-bu-2,6-pe-γ-CD) [3]], may directly be used for coating columns [4]. The approach of polysiloxane-diluted CD derivatives was extended to polysiloxane-bonded derivatives (Chirasil-Dex) [5,6]. For Chirasil-Dex, one oct-7-enyl moiety is introduced at the primary hydroxy group C(6)OH of one glucose unit. The residual hydroxy groups of all glucose units are methylated. In a platinum-catalyzed hydrosilylation reaction, a Si–H bond of a poly(90% dimethyl–10% hydromethylsiloxane) is added to the C=C double bond of the octenyl group. As a result, the cyclodextrin derivative is covalently bonded to the polysiloxane via one octamethylene spacer. The most important advantage of a Chirasil-Dex station-

*Corresponding author.

[1] Present address: BASF AG, ZAX-Analytical Dept., D-67056 Ludwigshafen, Germany

PII S0021-9673(96)00805-9

ary phase is that it can be thermally immobilized onto fused-silica surfaces [7]. Immobilized stationary phases offer the following advantages:

- In case of contamination with non-volatile compounds, the column can be rinsed with solvents.
- Injection techniques such as large-volume injection, on-column injection and splitless injection and the application of low-boiling solvents are feasible without deterioration of the stationary phase film.
- The stationary phase can be employed for chromatographic modes involving condensed mobile phases such as capillary (open-tubular) supercritical fluid chromatography (cSFC) [7–10], open-tubular liquid chromatography (OTLC) [11] and open-tubular capillary electrochromatography (OTCEC) [11].

3-Bu-2,6-pe-γ-CD was introduced by König et al. [3] into GC and numerous chiral solutes belonging to different classes of compounds could be resolved into their respective enantiomers [4]. Whereas in the first reports the viscous 3-bu-2,6-pe-γ-CD was directly coated onto the surface of capillary columns [3], dilution in OV-1701 which was performed subsequently proved to be advantageous [12]. Besides analytical applications, preparative enantiomer separations on 3-bu-2,6-pe-γ-CD were also described [13]. Recently, the first use of immobilized polysiloxane-bonded 3-bu-2,6-pe-γ-CD, called Chirasil-γ-Dex, was reported [14] and the advantages of immobilized stationary phases mentioned above could be utilised also for this CD derivative.

For permethylated β-CD (perme-β-CD) dissolved in the polysiloxane OV-1701, it was shown previously that the chiral separation factor, α, increases with increasing percentage of CD, but that this increase is reduced at higher CD concentrations [15]. Graphs of α vs. CD concentration level off at higher concentrations. These results were explained using the concept of the retention increment, R'. Similar observations were reported for polysiloxane-bonded perme-β-CD (Chirasil-Dex) [16] and for 3-bu-2,6-pe-γ-CD dissolved in OV-1701 [12]. There is an optimum CD concentration. A percentage of CD above the optimum value would induce loss of characteristic advantages inherent to polysiloxanes, such as high chromatographic efficiency (due to high diffusion coefficients), wide temperature range, good film quality and favourable film stability. A percentage of CD that is below the optimum value causes reduced separation factors.

In this work, the synthesis and application of Chirasil-γ-Dex is described in detail. The influence of the percentage of 3-bu-2,6-pe-γ-CD on the immobilization properties, the chiral separation factor, α, and the retention factor, k', in GC was studied. The first applications of Chirasil-γ-Dex in enantioselective cSFC are shown.

2. Experimental

2.1. Synthesis

All IR spectra were in agreement with the expected chemical structures. Unless otherwise indicated, ^{1}H and ^{13}C NMR spectra were recorded at 250 and 62.9 MHz, respectively. The assignment of ^{13}C NMR signals was supported by spinecho experiments. All chemical shifts are referenced to Me_4Si. Carbon and hydrogen atoms of the alk(en)yl and butanoyl moieties are designated a, b, c, etc., e.g. butanoyl: $-OCOCH_2(a)CH_2(b)CH_3(c)$. The indices 2, 3, 6 and oc (e.g., a_{oc}) are used to indicate 2-O-pentyl, 3-O-butanoyl, 6-O-pentyl and 6-O-octenyl, respectively. Diastereotopic protons are distinguished by the symbol "′" (e.g., a_3 and a_3') or by A and B (H-6A and H-6B) [17].

2.2. Monooctenylation

6-O-oct-7-enyl-γ-CD was synthesized analogously to 6-O-oct-7-enyl-β-CD [7,16], purified by repeated silica gel chromatography [methanol–toluene (1:1, v/v)] and obtained as a white solid in a yield of 24%. The complete separation of 6-O-oct-7-enyl-γ-CD from non- and dioctenylated γ-CD was confirmed by electrospray mass spectrometry (ESI–MS). The melting point was 250°C (decomposition); R_F=0.17 [methanol–toluene (1:1, v/v)]; $[\alpha]_D^{25}$+ 133±5 (c=1, H_2O).

^{1}H and ^{13}C NMR spectra corresponded to the spectra of native γ-CD, but with broadened signals

due to the absence of C_8 symmetry; additionally, the signals of $-O(CH_2)_6CH_{gem}=CH_{cis}H_{trans}$ appeared:

^1H NMR ([$^2H_6$]dimethyl sulfoxide): δ 5.76 [m, $=CH_{gem}$, overlapping with OH(2) and OH(3)], 4.99 (d, $J_{cis,trans}$; 17.6 Hz, $=CH_{cis}$), 4.80 (d, $J_{cis,trans}$ 17.6 Hz, $=CH_{trans}$, overlapping with H-1), 2.01 (m, f_{oc}).

^{13}C NMR ([$^2H_6$]dimethyl sulfoxide): δ 138.8 (=CH–), 114.7 ($=CH_2$), 69.8 (C-6, substituted), 59.9 (C-6, non-substituted), 39.2–25.0 ($a_{oc}-f_{oc}$). The downfield shift of 9.9 ppm for C-6(substituted) compared to C-6(non-substituted) is in agreement with the empirical ^{13}C NMR shift rules described for the alkylation of primary alcohols [18] (see also [19]).

ESI–MS: m/z 1407.6 ($[M+H]^+$, calculated 1407.3).

2.3. n-Pentylation

6-O-Oct-7-enyl-2-O-*n*-pentyl-heptakis(2,6-di-O-*n*-pentyl)-γ-CD was synthesized analogously to octakis(2,6-di-O-*n*-pentyl)-γ-CD [3,17]. Repeated silica gel chromatographic purification [stepwise gradient from 40 to 100% (v/v) diethyl ether in *n*-hexane] of the crude product afforded a viscous, liquid mixture of 6-O-oct-7-enyl-2-O-*n*-pentyl-heptakis(2,6-di-O-*n*-pentyl)-γ-CD and higher pentylated derivatives. A complete separation, however, is not necessary at this stage, as it occurs readily after subsequent butanoylation. Depending on the required purity, about 16 to 53% of the theoretical amount was obtained. R_F of the mixture: 0.46–0.48 [ethyl acetate–toluene (15:85, v/v); $[\alpha]_D^{25}+81\pm5$ (c=1, *n*-hexane).

^1H and ^{13}C NMR spectra corresponded to the spectra of heptakis(2,6-di-O-*n*-pentyl)-β-CD [17,20] and octakis(2,6-di-O-*n*-butyl)-γ-CD [17]; additionally, the signals of $-O(CH_2)_6CH_{gem}=CH_{cis}H_{trans}$ appeared:

^1H NMR (C^2HCl_3): δ 5.74 (m, $=CH_{gem}$), 4.92 (d, $=CH_{cis}$, overlapping with H-1), 4.87 (d, $=CH_{trans}$, overlapping with H-1), 1.95 (dt, $J_{f(oc),gem}=J_{f(oc),e(oc)}=6.8$ Hz, f_{oc}).

^{13}C NMR (C^2HCl_3): δ 138.6 (=CH–), 114.1 ($=CH_2$), 29.0–22.0 ($a_{oc}-f_{oc}$).

ESI–MS: m/z 1229.3, 1264.9, 1299.4, 1335.5, decreasing signal intensity in this order ($[M+2H]^{2+}$ for CD derivatives with fifteen, sixteen, seventeen and eighteen pentyl groups, respectively; calculated for fifteen pentyl groups: 1229.7).

2.4. Butanoylation

3-O-Butanoyl-6-O-oct-7-enyl-2-O-*n*-pentyl-heptakis(3-O-butanoyl-2,6-di-O-*n*-pentyl)-γ-CD was synthesized analogously to octakis(3-O-butanoyl-2,6-di-O-*n*-pentyl)-γ-CD [3], except that triethylamine was used as the solvent instead of dichloromethane. A mixture of CD derivatives was obtained, which showed no OH absorption in IR spectra. The composition of the mixture depended on the purity of the starting material. Silica gel chromatography with a stepwise gradient from 10 to 66% (v/v) ethyl acetate in *n*-hexane afforded the desired derivative as a slightly yellowish, viscous oil in an overall yield of 4%. The purity was confirmed by ESI–MS and SFC.

R_F=0.22 [ethyl acetate–*n*-hexane (1:4, v/v)]. The use of Polygram-Sil G/UV_{254} plates (plastic) instead of Sil-G25/UV_{254} plates (glass), both obtained from Macherey-Nagel (Düren, Germany), caused changes to the elution order. $[\alpha]_D^{25}+67\pm5$ (c=0.9, chloroform).

^1H NMR (assignment by ^1H–^1H-COSY 600 MHz spectrum, [$^2H_{12}$]-cyclohexane): δ 5.77 (m, $=CH_{gem}$), 5.32 (dd, H-3, $J_{3,2}=J_{3,4}=9.1$ Hz), 5.11 (d, H-1, $J_{1,2}$ 2.9 Hz), 4.96 (ddt, $=CH_{cis}$, $J_{cis,trans}$ 17.1 Hz, $J_{cis,gem}$ 2.1 Hz, $J_{cis,f(oc)}$ 1.6 Hz), 4.87 (ddt, $=CH_{trans}$, $J_{trans,cis}$ 10.3 Hz, $J_{trans,gem}$ 2.2 Hz, $J_{trans,f(oc)}$ 1.1 Hz), 3.99 (d, H-6A, $J_{6A,6B}$ 9.8 Hz), 3.88 (d, H-5, $J_{5,4}$ 9.3 Hz), 3.79 (dd, H-4, $J_{4,3}=J_{4,5}=9.1$ Hz), 3.57 (m, a_2), 3.49 (m, a_6) 3.46 (d, H-6B, $J_{6B,6A}$ 9.8 Hz), 3.39 (m, a_6') 3.35 (m, a_2'), 3.22 (dd, H-2, $J_{2,3}$ 9.5 Hz, $J_{2,1}$ 2.9 Hz), 2.40 (dt, a_3, $J_{a3,a3'}$ 15.8 Hz, $J_{a3,b3}$ 7.2 Hz), 2.13 (dt, a_3', $J_{a3',a3}$, 16.0 Hz, $J_{a3',b3}$ 7.5 Hz), 2.06 (dt, f_{oc}, $J_{f(oc),gem}=J_{f(oc),e(oc)}=7.2$ Hz), 1.67 (m, b_3), 1.58 to 1.50 (m, b_2 and b_6), 1.40 to 1.34 (m; d_2, d_6, e_{oc} and solvent), 0.95 bis 0.92 (m; e_2, e_6 and c_3). The signals of b_{oc}, c_{oc} and d_{oc} are located within the non-resolved signal groups 1.58–1.50 or 1.40–1.34 ppm.

^{13}C NMR (C^2HCl_3): δ 171.6 (O-CO), 139.1 (=CH–), 114.1 ($=CH_2$), 97.7 (C-1), 78.4 (C-4), 75.9 (C-2), 72.0 (C-3), 71.5 (a_6), 71.1 (C-5), 70.8 (a_2), 69.2 (C-6), 36.0 (a_3), 33.8 to 18.1 (b_2–d_2, b_6–d_6, b_{oc}–f_{oc}, b_3), 14.1 to 13.7 (e_2, e_6, c_3).

ESI–MS: m/z 1510.6 ($[M+2H]^{2+}$, calculated 1510.4).

SFC: Retention time of 3-O-butanoyl-6-O-oct-7-enyl-2-O-*n*-pentyl-heptakis(3-O-butanoyl-2,6-di-O-*n*-pentyl)-γ-CD, 16.2 min. Purity, 98.7 area% (flame ionization detector). Fused-silica capillary column (5 m×0.05 mm I.D.), coated with immobilized Chirasil-Dex [8]. Supercritical CO_2, asymptotically density-programmed from 0.42 (5 min) to 0.54 g/ml, 150°C.

2.5. Hydrosilylation

Chemical bonding of 3-O-butanoyl-6-O-oct-7-enyl-2-O-*n*-pentyl-heptakis(3-O-butanoyl-2,6-di-O-*n*-pentyl)-γ-CD to poly(90% dimethyl–10% hydromethylsiloxane) via a H_2PtCl_6-catalyzed hydrosilylation was carried out as described for Chirasil-Dex [6] and gave a colorless, viscous oil. No further clean-up was performed after drying in vacuo.

$[\alpha]_D^{20}$ +12.8±3, +28.1±3, +41.1±3 (for 20, 40 and 60%, w/w, respectively) (c=0.9, chloroform).

^1H NMR (C^2HCl_3): corresponded to the spectrum of 3-O-butanoyl-6-O-oct-7-enyl-2-O-*n*-pentyl-heptakis(3-O-butanoyl-2,6-di-O-*n*-pentyl)-γ-CD, however, there were no olefinic protons and in addition, polysiloxane signals appeared: δ 4.61 (SiH), 0.00 (SiMe).

2.6. Column preparation

Fused-silica tubing (for GC: 0.25 mm I.D., Microquartz, Munich, Germany; for SFC: 0.05 mm I.D., Chrompack, Middelburg, Netherlands) was heated at 260°C for 2 h in a slow stream of hydrogen and was statically coated without further deactivation with a filtered solution of the stationary phase (4 and 20 mg/ml, respectively) in *n*-pentane, yielding a film thickness of 0.25 μm. Immobilization was accomplished thermally at 190°C for 24 h with a very low flow of hydrogen, as described previously [7,16]. The 0.25 mm I.D. columns were subsequently rinsed with 20 ml of pure filtered dichloromethane in order to remove the non-immobilized portion of the stationary phase. In order to avoid plugging, the 0.05 mm I.D. columns were rinsed with a stepwise gradient from methanol, methanol–dichloromethane to dichloromethane. The degree of immobilization was estimated by calculating k_b'/k_a' for the test solutes *n*-tridecane, *n*-tetradecane and 1-phenylethanol. The terms k_a' and k_b' are the retention factors determined before and after the rinsing procedure, respectively.

2.7. Instrumentation

GC was performed with a Carlo-Erba MEGA gas chromatograph (Fisons, Milan, Italy) equipped with a split injector and a flame ionization detector. Hydrogen (99.999%, average linear velocity 45–70 cm/s) or helium (99.96%, 30–45 cm/s) served as the carrier gas.

SFC was performed with a Carlo-Erba SFC 3000 system (Fisons) equipped with a SFC 300 syringe pump, a pneumatic Valco valve injector (0.2-μl sample loop) and a flame ionization detector. The split ratio was approximately 1:50. Carbon dioxide (99.9995%, Messer-Griesheim, Düsseldorf, Germany) was used as the mobile phase. The flow was controlled with a deactivated frit restrictor (Dionex/Lee Scientific, Salt Lake City, UT, USA).

2.8. Determination of the chiral separation factor, α, and the retention factor k′

Correct net retention times were used for the determination of correct and comparable α and k' values. Net retention times were measured relative to the methane peak. The methane retention time was determined for each column temperature separately, thus taking into account the changed carrier gas velocity due to its changed viscosity. Also after readjusting the split ratio, the methane retention time had to be redetermined. Temperatures below 35°C were maintained by placing the columns in a water bath, the temperature of which could be easily and reproducibly controlled.

2.9. Solutes

The *cis*- and *trans*-isomers of 1,1-difluoro-2-ethyl-3-methylcyclopropane were kindly provided by Professor W.R. Dolbier, University of Florida (Gainsville, FL, USA) and Professor W. Borden, University of Washington (Seattle, WA, USA). Bromochlorofluoromethane was obtained from Professor A. Collet, ENS Lyon, France. The other solutes were

commercial samples. Trifluoroacetylation of nornicotine and propranolol was performed with trifluoroacetic acid anhydride at 80°C according to standard procedures.

3. Results and discussion

3.1. Synthesis

Chirasil-γ-Dex batches containing 20, 40 and 60% (w/w) 3-bu-2,6-pe-γ-CD were prepared by varying the ratio of monooctenylated 3-bu-2,6-pe-γ-CD and poly(dimethyl–hydromethylsiloxane) for the hydrosilylation reaction. A polysiloxane with 10% OSiHMe units was employed (for 60% 3-bu-2,6-pe-γ-CD, a polysiloxane with 15% OSiHMe units was also used). The absence of olefinic proton signals in the ^{1}H NMR spectra revealed complete linking of the CD derivative.

3.2. Immobilization

Chirasil-γ-Dex containing 20, 40 and 60% (w/w) 3-bu-2,6-pe-γ-CD was coated and subsequently thermally immobilized onto fused-silica capillary columns [7,16]. The degree of immobilization was estimated using the test solutes *n*-tridecane, *n*-tetradecane and 1-phenylethanol. As expected, increasing CD content caused decreasing immobilization, due to the reduced concentration of reactive Si-H groups. Whereas the degree of immobilization with 20 and 40% 3-bu-2,6-pe-γ-CD was satisfactory (78 and 71%, respectively), that of the stationary phase with 60% 3-bu-2,6-pe-γ-CD was not acceptable (25%). A stationary phase with 60% 3-bu-2,6-pe-γ-CD prepared from a polysiloxane with 15% OSiHMe showed a degree of immobilization of 41%. A further column was made by adding a small amount of a carboxy-functionalized polysiloxane [21] to this stationary phase and the degree of immobilization was found to be 51%. This column (60% 3-bu-2,6-pe-γ-CD) and two of columns mentioned above (20 and 40% 3-bu-2,6-pe-γ-CD) were used to study the influence of the percentage of CD on the separation factor and on the retention factor.

The chiral separation factor of the test solute 1-phenylethanol determined before and after the immobilization procedure was nearly constant. Hence, the CD derivative obviously experienced neither epimerization nor decomposition during the immobilization reaction. However, the plate number determined with the test solutes decreased by 5 to 15%.

3.3. Influence of the 3-bu-2,6-pe-γ-CD percentage on the chiral separation factor and the retention factor in GC

In order to determine the optimum concentration of CD, the chiral separation factor, α, and the retention factor k' were determined for sixteen chiral solutes on immobilized Chirasil-γ-Dex with 20, 40 and 60% (w/w) CD (Table 1). Some typical chromatograms are shown in Fig. 1. The corresponding plots show that, for most solutes, no significant increase in α can be observed with a percentage of CD above 40% (Fig. 2, solid lines). For enflurane ($CHFClCF_2OCHF_2$), methyl lactate and 1,4-pentandiol, the final α values were achieved using only 20% CD. Only for the hydrocarbons α-pinene and *trans*-pinane does α significantly increase at concentrations above 40% (Fig. 2, solid lines). Thus, both hydrocarbons show a lower retention increment R' [15] at this stationary phase than the other solutes, and the apolar polysiloxane contributes relatively strongly to their retention compared to the CD. For 2-hexanol and 1,4-pentandiol, the α values at 60% are smaller than those at 40% (Fig. 2, solid lines). This surprising result, however, is probably due to deviations from the Gaussian peak shape, so that peak maxima obtained from the chromatograms do not correspond to the correct retention times.

In contrast to the separation factor, the retention factor, k', of each solute increases approximately linearly with the concentration of CD (Fig. 2, dotted line). As 60% CD gave rise to prolonged analysis times, but only slightly improved separation factors, compared to 40% CD, we consider 40% 3-bu-2,6-pe-γ-CD to be the optimum concentration.

For comparison, the α values of the test solutes on 3-bu-2,6-pe-γ-CD dissolved in OV-1701 (30%, w/w) were also determined (see Fig. 2, circles). For most of the solutes investigated, these values were slightly lower than those obtained on Chirasil-γ-Dex, after interpolation to 30%. Obviously, the solutes interact

Table 1
Influence of the weight percentage of 3-bu-2,6-pe-γ-CD on the chiral separation factor, α, and on the retention factor k_2' of different solutes

Solute	Temperature (°C)	Percentage (w/w) of 3-bu-2,6-pe-γ-CD						First eluted enantiomer
		20%		40%		60%		
		$k_2'^a$	α	$k_2'^a$	α	$k_2'^a$	α	
α-Pinene	26	22.07	1.032	33.27	1.058	48.51	1.068	(1*R*)-(+)
trans-Pinane	26	28.28	1.018	44.85	1.036	63.51	1.044	(1*R*)-(+)
Isoflurane $CF_3CHClOCHF_2$	27	1.34	1.302	3.35	1.366	5.55	1.381	(*S*)-(+)
Desflurane $CF_3CHFOCHF_2$	27	0.38	1.717	0.97	1.906	1.59	1.894	(*S*)-(+)
Enflurane $CHFClCF_2OCHF_2$	27	4.10	1.970	11.71	2.093	20.16	2.113	(*R*)-(−)
2-Hexanol	36	13.21	1.124	24.72	1.117	45.18	1.148	
1-Phenylethanol	90	6.30	1.041	13.25	1.048	19.15	1.050	
Menthol	75	22.47	1.036	41.21	1.047	71.16	1.044	
2-Methylcyclohexanone	70	7.13	1.119	14.53	1.162	25.06	1.173	
Filbertone[b]	75	4.92	1.107	9.45	1.166	15.43	1.173	
Carvone	65	59.35	1.035	109.21	1.057	183.04	1.052	
Menthone	80	10.49	1.088	20.79	1.160	32.25	1.164	(2*R*,5*S*)-(+)
Fenchone	70	13.04	1.083	24.06	1.131	38.90	1.130	(1*R*)-(−)
1,4-Pentandiol	90	13.99	1.117	30.99	1.134	64.35	1.114	
Methyllactate	80	1.54	1.198	3.80	1.236	6.22	1.220	(*S*)-(−)
Serinemethylester (TFA)	110	6.00	1.172	13.58	1.229	22.45	1.228	

For chromatograms, see Fig. 1; for plots, see Fig. 2. For some solutes, the elution order was determined by means of enantiomerically enriched samples.

[a] As the degrees of immobilization ranged from 78 to 51%, the k_2' values listed here were obtained by extrapolation of the measured k_2' values to 100% (corresponding to a film thickness of 0.25 μm).

[b] (*E*)-5-methylhept-2-en-4-one.

more strongly with the moderately polar OV-1701 than with the apolar polysiloxane of Chirasil-γ-Dex, thus causing lower retention increments (R'), and the final α value is only reached at higher concentrations of CD. Only for the hydrocarbons, α-pinene and *trans*-pinane, did the α values obtained on the dissolved CD fit well with the curves of the bonded CD.

Separation factors and retention factors at varying concentrations of 3-bu-2,6-pe-γ-CD were also determined for a series of γ-lactones (Fig. 3). As for the other solutes, there was only a small increase in α when the percentage of CD was increased from 40 to 60% (see the distances between the three plots in Fig. 3, left). Within the homologous series, the pronounced α_{max} for γ-heptalactone is remarkable. Again, the retention factor k_2' of each lactone increases almost proportionally with the concentration of CD (this cannot be deduced directly from the logarithmic plot in Fig. 3, right). From C_9 to C_{12}, a linear relationship between carbon number and ln k_2' is observed, which is characteristic of homologous series in GC and which represents the additive contribution of each methylene group to the vaporization enthalpy [22]. As for all homologous series (with the exception of *n*-alkanes), there is a deviation from linearity in the lower range (C_5 to C_9).

3.4. Application of Chirasil-γ-Dex in GC

As expected, the immobilized stationary phase shows a similarly broad applicability for enantiomer separations as pure and diluted 3-bu-2,6-pe-γ-CD [3,4,12]. In addition to the test solutes in Table 1, Table 2 lists further chiral solutes that could be baseline-separated (some chromatograms are shown in Fig. 1). Columns with 40% CD were used throughout. Many separations succeeded within short analysis times. This is supported by the use of short columns (10 m), which permit lower elution temperatures and, therefore, provide higher separation factors [23]. The enantioselectivity towards multiply halogenated *n*-alkanes, cycloalkanes and ethers is remarkable (often $\alpha > 1.3$). Especially notable are the high separation factors for multiply fluorinated compounds, i.e., $\alpha = 2.1$ for enflurane, 1.9 for desflurane

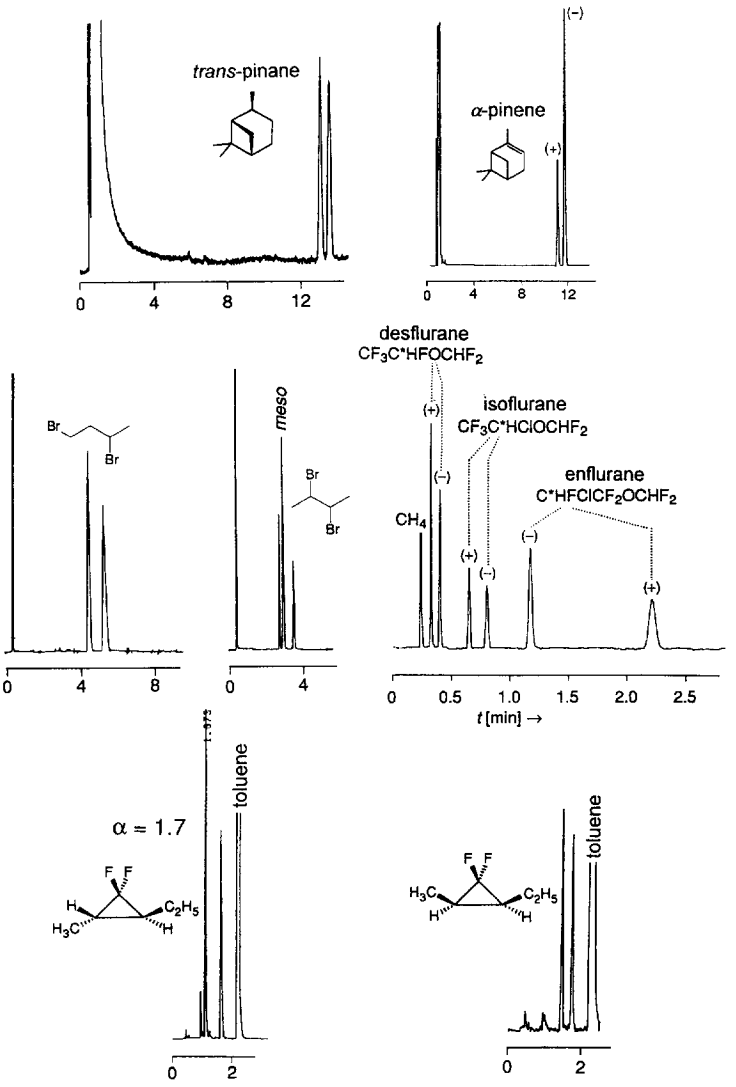

Fig. 1. GC enantiomeric separation of different chiral solutes. The fused-silica capillary column (10 m×0.25 mm I.D.) was coated with immobilized Chirasil-γ-Dex (40%, w/w). After removing the non-immobilized portion of the stationary phase by rinsing, the film thickness was about 0.18 μm (degree of immobilization=71%). For values of column temperature, α and k'_2, see Tables 1 and 2, respectively. Helium was used as the carrier gas for α-pinene, *trans*-pinane, pulegone and carvone and hydrogen was used as the carrier gas for the remaining solutes.

($CF_3CHFOCHF_2$) and 1.7 for *trans*-1,1-difluoro-2-ethyl-3-methylcyclopropane. The high volatility of these solutes enables low elution temperatures to be used and, hence, contributes to some extent to the surprisingly high enantioselectivity. The high α value for enflurane even enables rapid complete enantiomer separation in only 7 s (column 50 cm× 0.05 mm I.D.) representing the fastest enantiomer separation to date [14].

NMR studies suggest that the enantioselective interaction between 3-bu-2,6-pe-γ-CD and (*R*)- and (*S*)-enflurane is attributed to hydrogen bonding between the acidic protons of enflurane and oxygen atoms of 3-bu-2,6-pe-γ-CD [24]. These forces effect

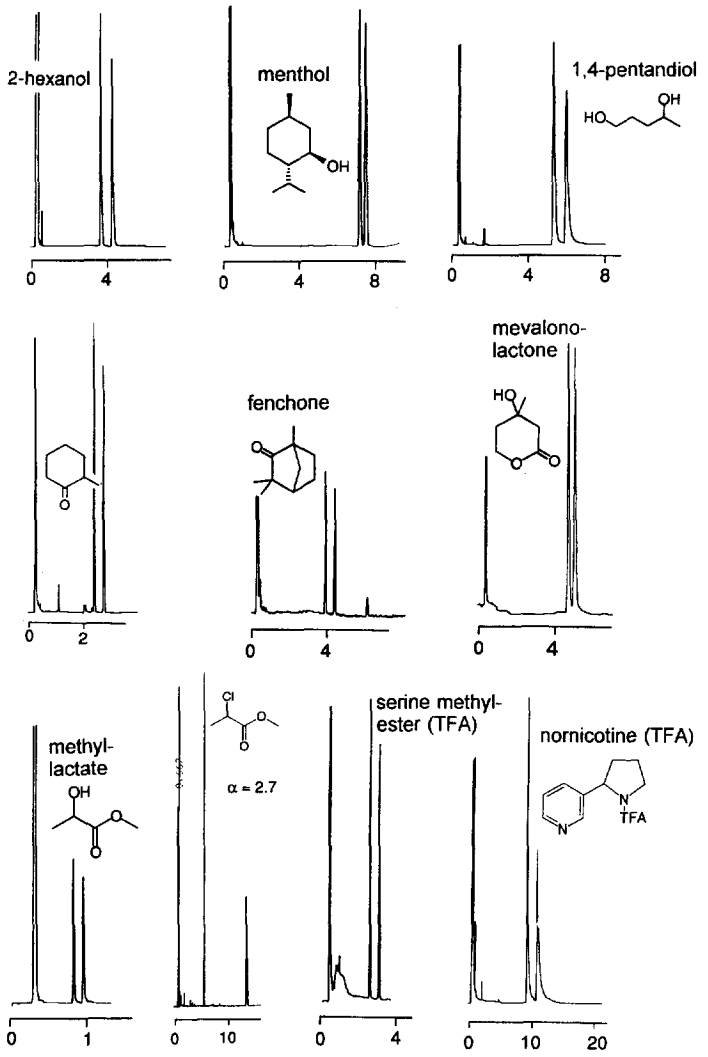

Fig. 1. (*continued*)

the formation of inclusion complexes, the existence of which was recently proved by intermolecular ^{1}H NOE experiments [24].

The δ-lactone series can also be separated into enantiomers. Here, a surprising order of elution is observed, since δ-heptalactone is eluted together with δ-nonalactone after δ-octalactone (Fig. 4, dotted line; for temperature-programmed chromatogram, see Fig. 5). For comparison, a chromatogram with an achiral stationary phase (polysiloxane SE-52) was recorded, which exhibited the expected elution order. Hence, δ-heptalactone preferentially interacts with the CD. δ-Heptalactone also shows the maximum α value within the homologous series (isothermally at 120°C, Fig. 4, solid line; no chromatogram is shown). It should be recalled that interaction with the chiral selector is a prerequisite for enantiomer separations, but a strong interaction does not definitely provide high enantioselectivity [2]. Systematic studies of the temperature-programmed enantiomer separation of the γ- and δ-lactones on modified CDs that were diluted in polysiloxanes of different polarity have also been reported by Bicchi et al. [25,26].

As on other CD phases [27,28], the enantiomers of

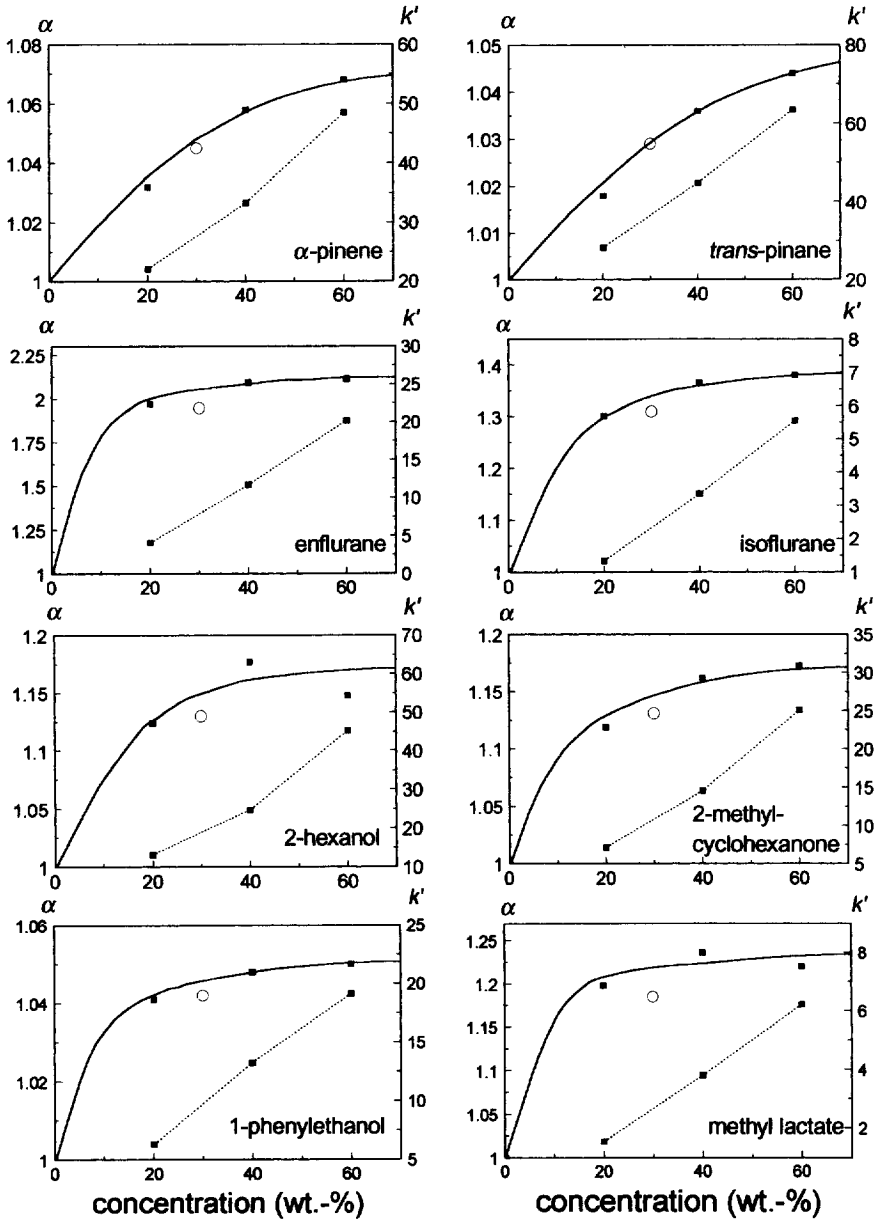

Fig. 2. Influence of the percentage of 3-bu-2,6-pe-γ-CD in Chirasil-γ-Dex on the chiral separation factor α (solid line) and on the retention factor k_2' (dotted line). See Table 1 for data and Fig. 1 for chromatograms. All k_2' values are referenced to a film thickness of 0.25 μm (see footnote to Table 1). For comparison, the α values determined on 3-bu-2,6-pe-γ-CD dissolved in OV-1701 (30% w/w) are also shown (○).

α-substituted propionic acid esters can be resolved with high separation factors. The extreme separation factor of $\alpha=2.7$ was observed for 2-chloromethyl propionate (55°C, Fig. 1).

Unusual findings were reported previously for the enantiomeric separation of methyl lactate (2-hydroxymethyl propionate) on pure 3-bu-2,6-pe-γ-CD at between 50 and 100°C [29]. A non-linear ln α vs. $1/T$ plot, with a minimum at 80°C and inversion of the elution order at 60°C, was obtained, and an

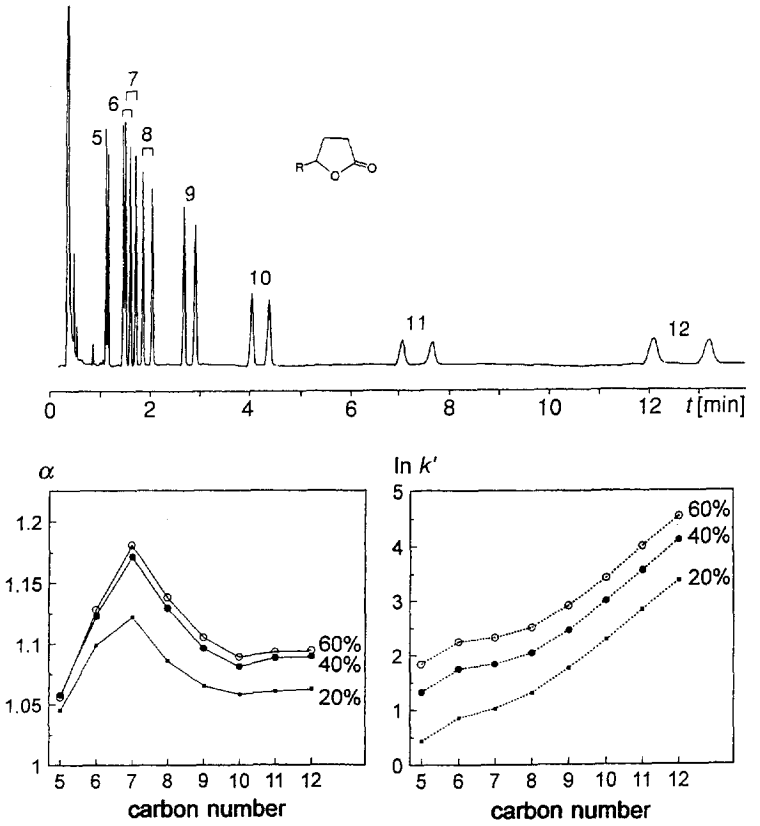

Fig. 3. GC enantiomeric separation of γ-lactones (γ-valerolactone to γ-dodecalactone) at 135°C. Fused-silica capillary columns (10 m×0.25 mm I.D.) were coated with immobilized Chirasil-γ-Dex. Top: gas chromatogram, 40% (w/w) 3-bu-2,6-pe-γ-CD, the carrier gas was 55 cm/s hydrogen. Left: plot of α vs. γ-lactone carbon number for different percentages of 3-bu-2,6-pe-γ-CD. Right: plot of ln k_2' vs. γ-lactone carbon number for different percentages of 3-bu-2,6-pe-γ-CD. All k_2' values are referenced to a film thickness of 0.25 μm (see footnote to Table 1).

unusual broadening of the (*S*)-methyl lactate peak, even as the first-eluted enantiomer, was consistently observed. In our laboratory, it was not possible to reproduce these results either on immobilized 3-bu-2,6-pe-γ-CD or on dissolved 3-bu-2,6-pt-γ-CD (30% in OV-1701). On the dissolved CD, the ln α vs. $1/T$ plot is linear between 45 and 110°C and (*S*)- is consistently eluted before (*R*)-methyl lactate. The intercept with the $1/T$ axis was extrapolated and found to be 0.00254 K^{-1}, corresponding to 121°C.

From the unfunctionalized hydrocarbons tested on Chirasil-γ-Dex, only the complete enantiomeric separation of *trans*-pinane and α-pinene and the partial separation of *cis*-pinane and 1,2,2-trimethylcyclohexane succeeded.

Frequently, small changes in the solute molecule cause large differences in enantioselectivity. For instance, the enantiomers of 2-methylcyclohexanone can be baseline-separated at 70°C, whereas no partial or even complete separation could be achieved for 3-methylcyclohexanone at between 70 and 40°C.

For many solutes, good column efficiencies were observed. Hence, with a column length of 10 m, the separation factor $\alpha=1.03$ is sufficient for the baseline separation of *trans*-pinane (hydrogen 55 cm/s, Fig. 1). On the other hand, the efficiency for halogenated substrates is often unsatisfactory (broadened, but symmetric peaks for enflurane; asymmetric peaks for 1,3-dibromobutane). This behaviour of halogenated compounds might be attributed to a strong interaction with 3-bu-2,6-pe-γ-CD. Not surprisingly for non-deactivated fused-silica

Table 2
Column temperature, retention factor, k'_2, and chiral separation factor, α, for GC baseline enantiomeric separations on immobilized Chirasil-γ-Dex (40%, w/w)

Solute	Temperature (°C)	k'_2	α
2-Octanol	35	82.84	1.033
1,3-Butandiol	65	52.64	1.071
3,3,5-Trimethylcyclohexanone	120	1. 21	1.111
Pulegone	85	17.87	1.054
5-Methylnorborn-5-en-2-one	70	13.18	1.108
Mevalonolactone	160	13.58	1. 078
2,5-Dimethoxytetrahydrofuran	35	9. 71	1.230
2-Chloromethyl propionate	55	15.38	2.698
Nornicotine (TFA-derivatized)	125	30.13	1.182
Propranolol (TFA-derivatized)	140	293.48	1.040
2-Bromobutane	26	3.24	1.092
2-Bromopentane	26	11.55	1.311
2-Iodobutane	26	7.99	1.063
1,3-Dibromobutane	60	12.33	1.200
2,3-Dibromobutane	60	7.95	1.337
1,4-Dibromopentane	70	27.13	1.320
Bromochlorofluoromethane	−22	27.50	1.042
trans-1,1-Difluoro-2-ethyl-3-methylcyclopropane	23	3.83	1.715
cis-1,1-Difluoro-2-ethyl-3-methylcyclopropane	23	4.17	1.258

For column dimensions and some chromatograms, see Fig. 1.

columns, the peak shape for underivatized diols and amines is rather poor. Nevertheless, the enantiomers of 1,4-pentandiol (Fig. 1) and 1,3-butandiol were baseline-separated without derivatization. The enantiomer separation of nornicotine (Fig. 1) and propranolol succeeded after trifluoroacetylation.

Using hydrogen as the carrier gas, elution profiles indicating solute conversion during the chromato-

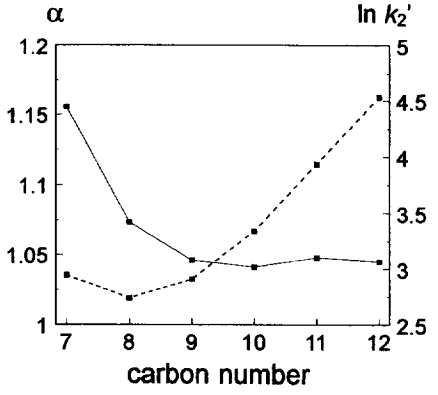

Fig. 4. Plot of α (solid line) and ln k'_2 (dotted line) vs. carbon number for GC enantiomeric separation of δ-lactones (isothermally at 120°C). The fused-silica capillary column (10 m×0.25 mm I.D.) was coated with immobilized Chirasil-γ-Dex (40%, w/w). After removing the non-immobilized portion of the stationary phase by rinsing, the film thickness was about 0.18 μm (degree of immobilization=71%). For temperature-programmed chromatogram, see Fig. 5.

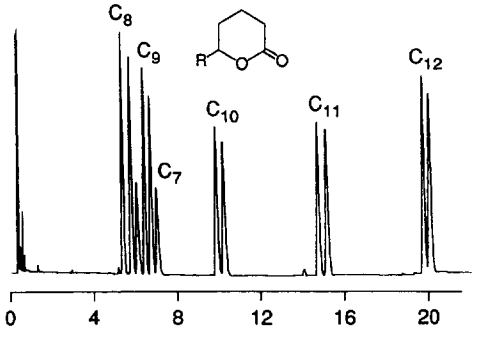

Fig. 5. GC enantiomeric separation of δ-lactones (δ-heptalactone to δ-dodecalactone; temperature programme 119°C (8 min), 2°C/min to 145°C). The fused-silica capillary column (10 m×0.25 mm I.D.) was coated with immobilized Chirasil-γ-Dex (40%, w/w). After removing the non-immobilized portion of the stationary phase by rinsing, the film thickness was about 0.18 μm (degree of immobilization=71%). The carrier gas was 56 cm/s hydrogen. For α and ln k'_2, determined using isothermal conditions, see Fig. 4.

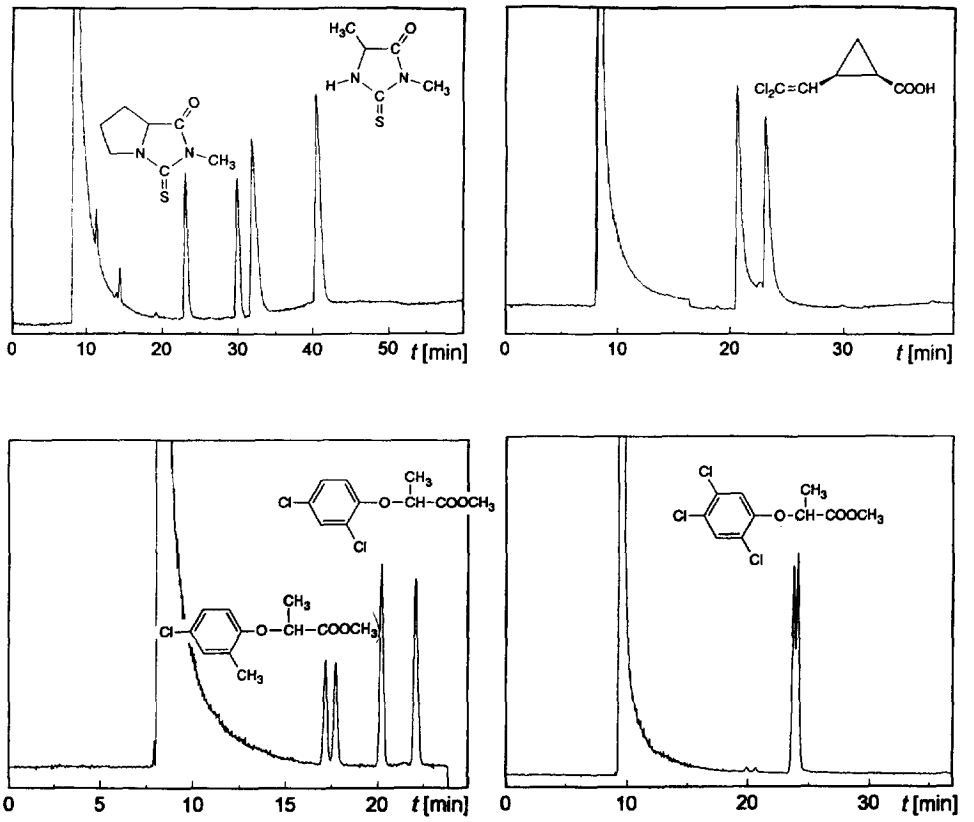

Fig. 6. SFC enantiomeric separation of MTH-proline and MTH-alanine (top left), *cis*-permethrinic acid (top right), mecopropmethylester and dichlorpropmethylester (bottom left) and fenopropmethylester (bottom right). The fused-silica capillary column (5 m×0.05 mm I.D.) was coated with immobilized Chirasil-γ-Dex (40%, w/w). For the CO_2 density programme and the column temperature, see Table 3.

graphic process have been observed for α,β-unsaturated ketones (pulegone, carvone, filbertone) on some columns. Obviously, hydrogenation of the solutes takes place, which is caused by traces of platinum (hydrosilylation catalyst) that are present in the stationary phase. However, chromatograms with

Table 3
Column temperature, CO_2 density programme and retention time for SFC enantiomeric separations on immobilized 3-bu-2,6-pe-γ-CD (40%, w/w)

Solute	Temperature (°C)	Density programme		Retention time of second eluted peak (min)
		Start ($g\ ml^{-1}$)	Gradient ($g\ ml^{-1}\ min^{-1}$)	
MTH-proline	55	0.25 (5 min)	0.005	30.1
MTH-alanine	55	0.25 (5 min)	0.005	40.8
cis-Permethrinic acid	50	0.25 (5 min)	0.005	23.3
Mecopropmethylester	50	0.25 (10 min)	0.010	17.8
Dichlorpropmethylester	50	0.25 (10 min)	0.010	22.1
Fenopropmethylester	45	0.25 (10 min)	0.005	24.2
Benzoin	40	0.20 (10 min)	0.003	20.9

For column dimensions and chromatograms, see Fig. 6.

excellent peak shapes are obtained if helium is used as the carrier gas.

3.5. Application of Chirasil-γ-Dex in capillary SFC

For use in SFC, Chirasil-γ-Dex containing 40% 3-bu-2,6-pe-γ-CD was coated and immobilized onto fused-silica capillary columns with an inner diameter of 0.05 mm. Fig. 6 shows the enantiomeric separation of the methylhydantoin derivatives of the amino acids proline and alanine, *cis*-permethrinic acid (precursor of the insecticide permethrin) and the methyl esters of the phenoxypropionic acids mecoprop, dichlorprop and fenoprop, which are used as herbicides (see Table 3). MTH-proline, MTH-alanine and dichlorpropmethylester are resolved into enantiomers with remarkably high peak resolutions of 6, 5 and 3.5, respectively.

References

[1] V. Schurig and H.-P. Nowotny, J. Chromatogr., 441 (1988) 155.
[2] V. Schurig and H.-P. Nowotny, Angew. Chem., Int. Ed. Engl., 29 (1990) 939.
[3] W.A. König, R. Krebber and P. Mischnick, J. High Resolut. Chromatogr., 12 (1989) 732.
[4] W.A. König, Gas Chromatographic Enantiomer Separation with Modified Cyclodextrins, Hüthig, Heidelberg, 1992.
[5] P. Fischer, R. Aichholz, U. Bölz, M. Juza and S. Krimmer, Angew. Chem., Int. Ed. Engl., 29 (1990) 427.
[6] V. Schurig, D. Schmalzing, U. Mühleck, M. Jung, M. Schleimer, P. Mussche, C. Duvekot and J.C. Buyten, J. High Resolut. Chromatogr., 13 (1990) 713.
[7] V. Schurig, Z. Juvancz, G.J. Nicholson and D. Schmalzing, J. High Resolut. Chromatogr., 14 (1991) 58.
[8] M. Jung and V. Schurig, J. High Resolut. Chromatogr., 16 (1993) 215.
[9] J. Dönnecke, W.A. König, O. Gyllenhaal, J. Vessman and Ch. Schulze, J. High Resolut. Chromatogr., 17 (1994) 779.
[10] G. Yi, J.S. Bradshaw, B.E. Rossiter, A. Malik, W. Li and M.L. Lee, J. Org. Chem., 58 (1993) 4844.
[11] V. Schurig, M. Jung, S. Mayer, S. Negura, M. Fluck and H. Jakubetz, Angew. Chem., Int. Ed. Engl., 33 (1994) 2222.
[12] I. Hardt and W.A. König, J. Microcol. Sep., 5 (1993) 35.
[13] V. Schurig, H. Grosenick and B.S. Green, Angew. Chem., Int. Ed. Engl., 32 (1993) 1662.
[14] V. Schurig, H. Grosenick and M. Juza, Recl. Trav. Chim. Pays-Bas, 114 (1995) 211.
[15] M. Jung, D. Schmalzing and V. Schurig, J. Chromatogr., 552 (1991) 43.
[16] M. Jung and V. Schurig, J. Microcol. Sep., 5 (1993) 11.
[17] G. Wenz, Carbohydr. Res., 214 (1991) 257.
[18] E. Breitmaier and W. Voelter, Carbon-13 NMR Spectroscopy, VCH, Weinheim, 1987, pp. 213 and 379.
[19] A. Uena and R. Breslow, Tetrahedron Lett., 23 (1982) 3451.
[20] W. Meier-Augenstein, B.V. Burger and H.S.C. Spies, Magn. Reson. Chem., 29 (1991) 681.
[21] G. Lai, G. Nicholson, U. Mühleck and E. Bayer, J. Chromatogr., 540 (1991) 217.
[22] G. Schomburg, Gaschromatographie, VCH, Weinheim, 1987, p. 54.
[23] M. Lindström, J. High Resolut. Chromatogr., 14 (1991) 765.
[24] H. Grosenick, M. Juza, J. Klein and V. Schurig, Enantiomer, in press.
[25] C. Bicchi, G. Artuffo, A. D'Amato, V. Manzin, A. Galli and M. Galli, J. High Resolut. Chromatogr., 16 (1993) 209.
[26] C. Bicchi, G. Artuffo, A. D'Amato, G. Pellegrino, A. Galli and M. Galli, J. High Resolut. Chromatogr., 14 (1991) 701.
[27] W.-Y. Li, H.L. Jin and D.W. Armstrong, J. Chromatogr., 509 (1990) 303.
[28] A. Berthod, W. Li and D.W. Armstrong, Anal. Chem., 64 (1992) 873.
[29] W.A. König, D. Icheln and I. Hardt, J. High Resolut. Chromatogr., 14 (1991) 694.