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Chiral π-donor stationary phases with (*R*)-N-pivaloylnaphthylethylamide groups for direct enantiomer separation by gas, liquid and supercritical fluid chromatography

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ABSTRACT

Several monomeric and polymeric stationary phases, all with (*R*)-N-pivaloylnaphthylethylamide as the chiral selector group, were synthesized and tested for direct enantiomer separation by high-performance liquid, capillary gas, packed column supercritical and capillary supercritical chromatography.

INTRODUCTION

In the last 10 years there have been rapid developments in chromatographic methods for the direct separation of enantiomers. High-performance liquid chromatography (HPLC) is now very well established in this domain for both analytical [1,2] and preparative [3] separations and there is a wide choice of commercially available stationary phases [4]. Capillary gas chromatography (cGC) is developing very fast [5–7] and supercritical fluid chromatography (SFC) [8], either in packed (pSFC) or in capillary columns (cSFC) has profited from the theoretical and experimental knowledge in HPLC and GC.

The aim of this work was to synthesize different stationary phases for HPLC, cGC and SFC with packed and capillary columns, all with the same chiral group, and to investigate their chromatographic suitability. We chose for this purpose a derivative of (*R*)-N-pivaloylnaphthylethylamide ((*R*)-2,2-dimethyl-N-{1-[1-(4-amino) naphthylenyl]ethyl}propanamide, NH_2-PNEA; Fig. 1), the PNEA group having a

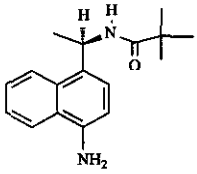

Fig. 1. Chiral group of all stationary phases, a derivative of (*R*)-N-pivaloylnaphthylethylamide: (*R*)-2,2-dimethyl-N-{1-[1-(4-amino)naphthalenyl]ethyl}propanamide, NH_2-PNEA.

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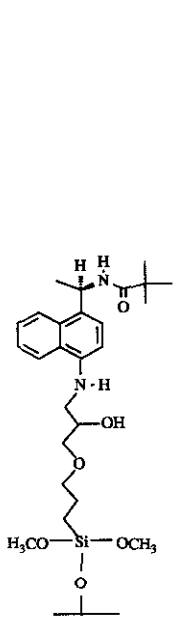
broad range of applications in HPLC [9]. It was outside the scope of this work to evaluate chiral separation mechanisms of the PNEA group with different kinds of samples (see, *e.g.* refs. 10 and 11) and for this reason elution orders were not determined.

LIQUID CHROMATOGRAPHY

Experimental

Three chiral stationary phases (CSPs) were synthesized: a monomeric bonded phase, CSP I (M) (Fig. 2), and two polymer-coated phases, CSP II (PG) and CSP III (G-Co) (Fig. 3). CSP I (M) contains 3-glycidoxypropyltrimethoxysilane as a spacer between the NH_2-PNEA groups and the silica matrix. In CSP II (PG), polyglycidoxypropylmethylsiloxane serves as a backbone carrying the chiral groups and the bonds to the aminopropylated silica matrix. In CSP III (G-Co), glycidoxypropylmethyldimethylsiloxane copolymer is used as a backbone.

Materials. LiChrospher Si 100 (Merck, Darmstadt, Germany) with a particle size of 5 μm and a specific surface area of S_{BET} = 264.4 ± 5.4 m^2/g was used. Polyglycidoxypropylmethylsiloxane and glycidoxypropylmethyl(45–55%)dimethylsiloxane copolymer were purchased from Petrarch Systems (Bristol, PA, USA) and all other chemicals either from Fluka (Buchs, Switzerland) or Merck (Zürich, Switzerland). For liquid chromatography, HPLC-grade solvents from Romil Chemicals (Shepsheed, UK) were used. The chiral selector NH_2-PNEA was available through a



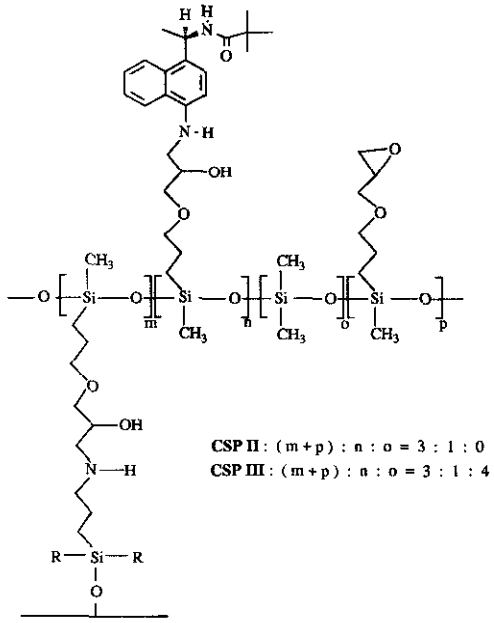


Fig. 2. Chiral stationary phase CSP I (M).

Fig. 3. Chiral stationary phases CSP II (PG) and CSP III (G-Co). For HPLC and pSFC, R = OC_2H_5; for cGC and cSFC, R = CH_3. m,n,o and p denote coefficients of indicated groups according to manufacturer and elemental analysis.

three-step reaction starting from (*R*)-(+)-1-(1-naphthyl)ethylamine. The general synthetic procedure has been reported previously [9,12].

CSP I (M). A 0.23 g (0.86 mmol) amount of NH_2-PNEA and 0.20 g (0.85 mmol) of 3-glycidoxypropyltrimethoxysilane were stirred in 20 ml of dry toluene (dried over Pb–Na alloy) during 1h. A 2.01 g amount of LiChrospher Si 100 [dried at 150°C and 1.3 Pa for 6 h] was added. The mixture was stirred slowly at 110°C for 6 h. After cooling, the derivatized silica was washed with toluene and methanol and dried at 90°C and 1.3 Pa for for 12 h. Weight increase: 0.18 g (8.99%). Analysis: found, C 6.99, H 1.51, N 0.69%; calculated, 0.23 mmol of (*R*)-amide per gram of stationary phase or about 0.60 groups/nm^2 (based on C), or 0.25 mmol of (*R*)-amide per gram of stationary phase or 0.63 groups/nm^2 (based on N).

Aminopropylated silica. A 6.97 g amount of LiChrospher Si 100 (dried at 150°C and 1.3 Pa for 8 h) was mixed with a solution of 4.42 g (20 mmol) of 3-aminopropyltriethoxysilane in 50 ml of dry toluene. The mixture was slowly stirred and refluxed under dry conditions for 24 h. After cooling, the derivatized silica was washed with toluene, methanol, diethyl ether and *n*-pentane and dried at 80°C and 10^{-2} Pa for 6 h. Weight increase: 0.88 g (12.7%). Analysis: found, C 4.78, H 1.43, N 1.61%; calculated, 0.57 mmol amine per gram of stationary phase or 1.5 groups/nm^2 (based on C).

CSP II (PG). A 0.18 g (0.68 mmol) amount of NH_2-PNEA and 0.37 g of polyglycidoxypropylmethylsiloxane (2.14 mmol of glycidoxy units) were dissolved in 25 ml of dry toluene and heated under nitrogen for 1 h at 70°C. Thereafter 1.97 g of aminopropylated silica were added and the suspension was slowly stirred at 110°C for 6 h. After cooling, the derivatized silica was washed with toluene, diethyl ether, ethanol and methanol and dried at 80°C and 1.3 Pa for 6 h. Weight increase: 0.21 g (10.6%). Analysis: found, C 10.67, H 2.28, N 1.43%.

CSP III (G-Co). A 2.02 g amount of aminopropylated silica was treated with 0.82 g of glycidoxypropyl(45–55%)dimethylsiloxane copolymer (2.36 mmol of glycidoxy units) and 0.22 g (0.82 mmol) of NH_2-PNEA in 25 ml of dry toluene under the same experimental conditions as with CSP II (PG). Weight increase after drying: 0.19 g (9.64%). Analysis: found, C 11.04, H 2.37, N 1.40%.

Procedures. The elemental analyses were done with a routine analyser in the Microanalytical Department of Ciba-Geigy (Basle, Switzerland).

To eliminate fines the CSPs were sedimented five times in methanol. Stainless-steel tubes (25 cm × 3.2 mm I.D.) were used as columns. A slurry prepared from about 1.9 g of the phase material and 30 ml of methanol–triethylene glycol (1:9) for CSP I and dibromomethane–*n*-hexane (8:2) for the other stationary phase was filled into the column with a Haskel air-driven fluid pump (Model 27486-4; Haskel Engineering and Supply, Burbank, CA, USA) at a pressure of 68 MPa. The columns were conditioned with methanol and *n*-hexane.

Chromatography was performed using an Altex (Berkeley, CA, USA) Model 110 solvent metering pump. A Hitachi Model 100-10 variable-wavelength UV detector (Kontron, Zürich, Switzerland) at 254 nm, a Rheodyne (Berkeley, CA, USA) Model 7125 syringe-loading sample injector with a 20-μl loop and as recording devices a Tarkan W & W Model 600 recorder (Kontron), a Servograph REA 310 pen drive (Radiometer, Copenhagen, Denmark) and an HP 3396 A integrator (Hewlett-Packard, Widen, Switzerland) were used.

The mobile phase used in the chromatographic experiments were *n*-hexane–2-propanol (78:22) and (90:10) at a flow-rate of 1 ml/min. The columns and the mobile phase container were maintained at 20°C (Assistant WTE var 3185 thermostat; R. C. Kuhn, Berne, Switzerland).

Toluene as a non-retained standard, dissolved in the appropriate mobile phase, was used to determine the dead time, t_0, and number of theoretical plates, N_0. The measured values were in the ranges $1.56 \leqslant t_0 \leqslant 1.66$ min and $6000 \leqslant N_0 \leqslant 8000$ for the three stationary phases tested.

The samples were synthesized according to common laboratory methods. Table I lists all samples used with the methods described below.

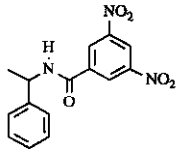
Results and discussion

Chromatography of the chiral α-phenylalkylamide derivatives DNB-PEA and DNB-PNA (Table II) shows different elution properties for the three stationary phas-

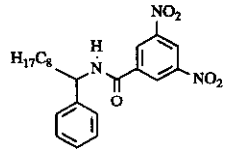
TABLE I

SAMPLES USED IN CHROMATOGRAPHIC EXPERIMENTS

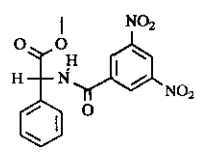
DNB = 3,5-dinitrobenzoyl; TFA = trifluoroacetyl; HFB = heptafluorobutyryl.



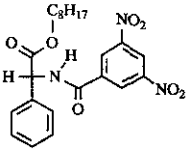
N-DNB-Phenylethylamine
(DNB-PEA)



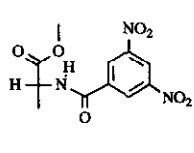
N-DNB-Phenylnonylamine
(DNB-PNA)



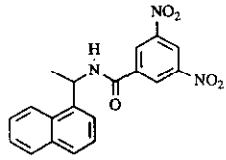
N-DNB-Phenylglycine methyl ester
(PG-ME)



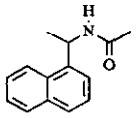
N-DNB-Phenylglycine octyl ester
(PG-OE)



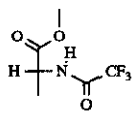
N-DNB-Alanine methyl ester
(DNB-Ala)



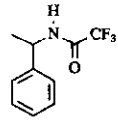
N-DNB-Naphthylethylamine
(DNB-NEA)



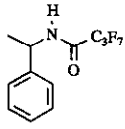
N-Acetylnaphthylethylamine
(Ac-NEA)



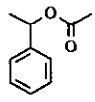
N-TFA-Alanine methyl ester
(TFA-Ala)



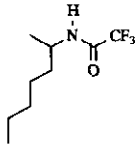
N-TFA-Phenylethylamine
(TFA-PEA)



N-HFB-Phenylethylamine
(HFB-PEA)



O-Acetylphenylethanol
(Ac-POH)



N-TFA-Heptylamine
(TFA-HAm)

TABLE II

RESOLUTION OF SOME 3,5-DINITROBENZOYLAMIDES BY LIQUID CHROMATOGRAPHY

HPLC conditions: mobile phase, (a) *n*-hexane–2-propanol (78:22) and (b) *n*-hexane–2-propanol (90:10); flow-rate, 1 ml/min; column, 25 cm × 3,2 mm I.D., 5 μm; detection, UV at 254 nm. k'_1 = Capacity factor of the first-eluted enantiomer; α = separation factor.

Compound	CSP I (M)[a]		CSP II (PG)[a]		CSP III (G-Co)[b]	
	k'_1	α	k'_1	α	k'_1	α
DNB-PEA	9.48	2.08	2.93	1.63	2.47	1.20
DNB-PNA	8.77	2.52	1.92	1.78	1.11	1.59
PG-ME	12.05	1.21	2.79	1.07		
PG-OE	5.21	1.23	1.22	1.09		

[a] Mobile phase a.
[b] Mobile phase b.

es. The glycidoxypropylmethyldimethylsiloxane copolymer, where about half of the epoxy-containing side-chains are replaced by methyl groups, together with a relatively low concentration of the chiral selector NH_2-PNEA, produces a non-polar separation quality of CSP III (G-Co). The DNB-PEA and DNB-PNA samples had to be separated with the less polar mobile phase hexane–2-propanol (90:10), because otherwise they would have been eluted in the dead time, t_0. CSP II (PG) contains more side-chains with epoxy groups and therefore a higher concentration of the chiral selector. The polarity of the phase is higher and the samples elute in a more polar mobile phase with higher capacity factors. For CSP I (M) these effects are even more pronounced. On all stationary phases the samples with longer alkyl chains elute before the corresponding homologues with shorter alkyl chains. CSP I (M) has the highest selectivity for the samples considered. This was also observed with other chiral aromatic samples. The separation factors decrease in the order CSP I (M) > CSP II (PG) > CSP III (G-Co). This behaviour is mainly due to the decrease in the surface concentration of the chiral selector. For the α-phenylalkylamide derivatives DNB-PEA and DNB-PNA, the separation factors increase on all the phases with increasing length of the alkyl chain. For the N-3,5-dinitrobenzoylphenylglycine derivatives PG-ME and PG-OE, the separation factors are scarcely influenced by the length of the alkyl chain.

CAPILLARY GAS CHROMATOGRAPHY

Experimental

Synthesis of stationary phase. The monomeric phase was prepared by heating NH_2-PNEA with an excess of butene oxide for 2 h at 60°C [CSP IV (M), Fig. 4]. The reaction of NH_2-PNEA with the glycidoxypolysiloxanes occurred after the coating in the capillaries during the conditioning process at 120°C.

Gas chromatography. Glass capillaries were drawn from Duran glass tubes with a modified Hupe & Busch capillary drawing machine. The capillaries (I.D. 0.3 mm)

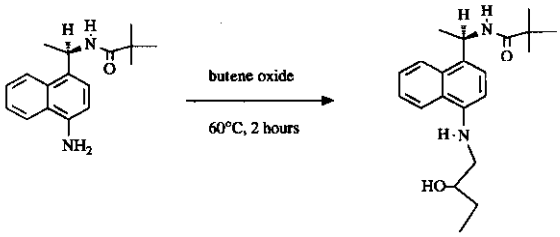


Fig. 4. Synthesis of chiral stationary phase CSP IV (M).

were leached according to Grob [13] at 170°C for 12 h. Deactivation was performed with aminopropyldimethylethoxysilane at 300 °C for 12 h. The deactivated capillaries were coated by the static method. The concentration of the monomeric phase CSP IV (M) was 1 mg/ml and the concentration of the polymeric phase CSP II (PG) was 1.3 mg of NH_2-PNEA and 3 mg of polyglycidoxypropylmethylsiloxane in 1 ml of dichloromethane. The concentration of the polymeric phase CSP III (G-Co) was 0.85 mg of NH_2-PNEA and 4.3 mg of glycidoxypropylmethyldimethylsiloxane copolymer in 1 ml of dichloromethane. The gas chromatograph (Hewlett-Packard, HP-5794A) used was equipped with a flame ionization detector and a split/splitless injector. Helium was used as the carrier gas at a velocity of 30 cm/s. The splitting ratio was set at 1:20.

Results and discussion

The stationary phases separate chiral amides and esters. The best separation factor (α) was obtained with the monomeric phase (Table III). However, the bleeding rate was high and the columns could be used only for short periods at temperatures higher than 100°C. The phase also formed droplets, even on non-deactivated surfaces. This caused a very low efficiency (maximum 650 plates/m).

The polymeric phases had some advantages over the monomeric phase. The wettability on the deactivated capillary surface was clearly better. This led to an improved efficiency (maximum 1500 plates/m). The thermal stability was also improved, but it was still lower than that with columns which were coated with polygly-

TABLE III

RESOLUTION OF SOME AMIDES AND ESTERS BY CAPILLARY GAS CHROMATOGRAPHY

GC conditions: carrier gas helium; injector temperature, 220°C; column, 10 m × 0.3 mm I.D.; flame ionization detection at 280°C. k'_1 = Capacity factor of the first-eluted enantiomer; α = separation factor.

Compound	Temperature (°C)	CSP IV (M)		CSP II (PG)		CSP III (G-Co)	
		k'_1	α	k'_1	α	k'_1	α
TFA-Ala	90	5.5	1.077	13.2	1.032	17.8	1.024
TFA-HAm	90	5.3	1.053	18.9	1.029	41.3	1.019
HFB-PEA	100			31.8	1.073	94.5	1.050
TFA-PEA	120	9.2	1.101	19.2	1.061	25.2	1.041
Ac-POH	90			14.9	1.025		

cidoxypropylmethylsiloxane and an achiral amine, *e.g.*, Jeffamine M-2070. The greatest advantage of the polymeric phases was the simple immobilization process during the conditioning. After rinsing with dichloromethane, more than 85% of the stationary phase remained in the column.

PACKED COLUMN SUPERCRITICAL FLUID CHROMATOGRAPHY

Experimental

The laboratory-constructed SFC apparatus [14] consisted of a System Gold 116 HPLC pump (Beckman Instruments, Basle, Switzerland) controlled by an NEC PC-8201A computer. The pump head was cooled by an ethanol cooling bath at −10°C. The cooling jacket was laboratory built. The sample was introduced by a Rheodyne Model 7125 HPLC injection valve with a 5-μl loop. Temperature control for the column was provided by an oven from a gas chromatograph (Sigma 2; Perkin-Elmer, Küsnacht, Switzerland). The outlet pressure was regulated at 40°C by a Tescom restrictor (Matkemi, Therwil, Switzerland). Inlet and outlet pressures were controlled by a laboratory-built pressure controller. A Uvikon 720 LC UV detector (Kontron) was used at 254 nm. The results were recorded with a Hewlett-Packard HP 3396A integrator. Carbon dioxide (40-grade with 5% of methanol) was obtained from Carbagas (Berne, Switzerland). Chromatography was performed with the CSPs used for HPLC.

Results and discussion

Some 3,5-dinitrobenzoylamides could be separated into the enantiomers with CSPs I–III by pSFC (Table IV).

The selectivities obtained with the SFC system are lower than those with the HPLC system, for different reasons. SFC separation was executed with another modifier (methanol) and at higher temperatures. On the other hand, the efficiency is much better with the SFC system because the solute diffusion coefficients are 5–10 times greater in SFC than in HPLC (Fig. 5).

TABLE IV

RESOLUTION OF SOME 3,5-DINITROBENZOYLAMIDES BY PACKED COLUMN SUPERCRITICAL FLUID CHROMATOGRAPHY

SFC conditions: mobile phase, carbon dioxide with 5% methanol; flow-rate, 1.8 ml/min; temperature, 40°C; column inlet pressure, 220 bar; column outlet pressure, 180 bar; column, 25 cm × 3.2 mm I.D., 5 μm; detection, UV at 254 nm. k'_1 = Capacity factor of the first-eluted enantiomer; α = separation factor.

Compound	CSP I (M)		CSP II (PG)		CSP III (G-Co)	
	k'_1	α	k'_1	α	k'_1	α
DNB-PEA	11.29	1.69	4.50	1.17	2.54	1.07
DNB-PNA	12.93	1.93	4.05	1.28	2.22	1.13
PG-ME	8.03	1.09	2.39	n.r.[a]	1.18	n.r.
DNB-NEA	17.97	3.21	7.30	1.51	4.41	1.23
DNB-Ala	4.86	1.45	2.17	1.09	1.26	n.r.

[a] Not resolved.

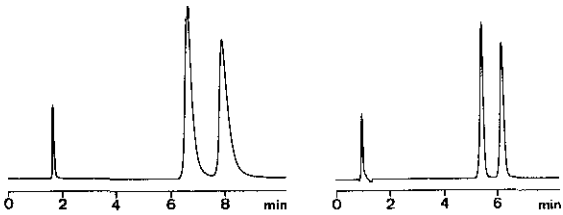


Fig. 5. Separation of N-DNB-phenylethylamine (DNB-PEA) on stationary phase CSP II (PG) in HPLC (left) and pSFC (right). Column, 25 cm × 3.2 mm I.D., 5 μm; UV detection at 254 nm. HPLC: mobile phase, hexane–2-propanol (80:20); flow-rate, 1 ml/min; temperature, 22°C. SFC: mobile phase, carbon dioxide with 5% methanol; flow-rate, 1.8 ml/min at −10°C; average column pressure, 200 bar; temperature, 40°C.

CAPILLARY SUPERCRITICAL FLUID CHROMATOGRAPHY

Experimental

The equipment was laboratory built [14]. The transport of the mobile phase (only carbon dioxide was used) was effected by a piston pump (ISCO SFC-500 Mikro flow pump with cooling jacket). Sampling was done with a four-way injection valve (Valco CI4W) with a 60-nl loop (Valco, Rotor 0.06). Temperature control for the column was provided by an oven from a gas chromatograph (Hewlett-Packard Model 5710A). The flame ionization detector (Hewlett-Packard Model 18710 A) was equipped with a capillary jet (Hewlett-Packard, part No. 18740-80230). A frit restrictor (Lee Scientific, batch No. 1028-1.4) was connected to the capillary column and situated in the capillary jet of the flame ionization detector. Chromatograms were recorded with a Model 3390A or 3396A integrator (Hewlett-Packard). The capillary column was made as described under capillary gas chromatography [15].

Results and discussion

Table V shows some of the results obtained. The efficiency of this column is very low, only 80 theoretical plates/m, and it seems that the stationary phase does not completely wet the capillary surface. In contrast to the separations in cGC, the capillary column was stable under the operating conditions.

TABLE V

RESOLUTION OF TWO SAMPLES BY CAPILLARY COLUMN SUPERCRITICAL FLUID CHROMATOGRAPHY

SFC conditions: mobile phase carbon dioxide (99.998%); temperature, 90°C; column, 5 m × 0.08 mm I.D.; flame ionization detection at 300°C; sample volume, 60 nl. k'_1 = Capacity factor of the first-eluted enantiomer; α = separation factor.

Compound	Pressure (bar)	Dead time, t_0 (min)	CSP II (PG)	
			k'_1	α
DNB-PEA	283	5.76	9.82	1.48
Ac-NEA	177	9.60	7.10	1.07

CONSLUSIONS

It is possible to use stationary phases with the same chiral selector group for direct enantiomer separations with different chromatographic methods. The synthesized phases are useful for the separation of enantiomeric mixtures of samples with π-acceptor groups. The monomeric and polymeric phases tested seem to be stable under the experimental conditions of HPLC and SFC in packed and capillary columns. Owing to wetting problems, the phases are not thermally stable in cGC and subject to bleeding; the stability of these immobilized columns towards organic solvents is good, which makes them suitable for cSFC.

ACKNOWLEDGEMENTS

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