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Development of chiral stationary phases for the enantiomeric resolution of dihydrodiols of polycyclic aromatic hydrocarbons by π-donor–acceptor interactions

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ABSTRACT

Chiral stationary phases (CSPs) derived from (*R*)-(−)-2-(2,4,5,7-tetranitrofluoren-9-ylideneaminooxy)propionic acid (TAPA) covalently bound to silica gel have been developed by altering the alkyl group at the chiral centre or the number of aromatic rings and their degree of nitration. The chromatographic properties of the CSPs were characterized by use of a racemic model solute. Depending on the solvent strength of the mobile phase, the CSPs exhibit the quality of a normal or a reversed phase. The chromatographic behaviour of 30 racemic hydroxylated derivatives of polycyclic aromatic hydrocarbons (PAHs) on (*R*)-(−)-TAPA CSP revealed the structural requirements for chiral recognition. The applicability of the CSPs for the enantiomeric separation of *trans*-dihydrodiols of PAHs on an analytical as well as preparative scale and for investigating the enantioselectivity of the biotransformation and genotoxicity of PAHs is demonstrated.

INTRODUCTION

The class of polycyclic aromatic hydrocarbons (PAHs) contains some of the most powerful chemical carcinogens [1]. PAHs have to be enzymatically transformed to biologically reactive metabolites in order to exert their carcinogenic properties [2]. The sequential attack of cytochrome P-450 dependent monooxygenase(s) and of microsomal epoxide hydrolase leads to the regio- and stereoselective formation of *trans*-dihydrodiols [3]. The positional isomers as well as the enantiomers of the *trans*-dihydrodiols of PAHs possess widely varying biological activities [4]. Studies concerned with this aspect of chemical carcinogenesis, therefore, require efficient separation methods for the isomers of *trans*-dihydrodiols. Reversed-phase high-performance liquid chromatography (HPLC) is unsurpassed for the separation of positional isomers of *trans*-dihydrodiols [5]. For their enantiomeric resolution *trans*-dihydrodiols are in most cases converted with a suitable chiral acid to diastereomeric esters, which can be separated by normal-phase HPLC; ester cleavage then yields the enantiomerically pure *trans*-dihydrodiols [6]. For a long time attempts have been made to replace this rather cumbersome technique by direct enantiomeric separation of *trans*-dihydrodiols via chiral stationary phases (CSPs) [7]. Recently CSPs on the basis of (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine or -leucine bonded to γ-aminopropylsilanized silica have become commercially available and were successfully used for the chromatographic resolution of racemic dihydrodiols of phenanthrene [8], chrysene [8], benz[*a*]anthracene [8–11] and benzo[*a*]pyrene [11].

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Since we have been interested in the stereoselective biotransformation of dibenz[*a*,*h*]anthracene [12] and picene [13], we tried to apply the direct chromatographic separation on CSPs to the enantiomeric resolution of *trans*-dihydrodiols of these PAHs. Unfortunately, a wide variety of commercial CSPs employing donor–acceptor interactions as well as CSPs based on chiral polymers, γ-cyclodextrin or cellulose triacetate were not suited to solve our separation problem; this failure was later independently confirmed [14]. Therefore we developed CSPs starting with 2-(2,4,5,7-tetranitrofluoren-9-ylideneaminooxy)carboxylic acids covalently bound to silica gel [15]. These CSPs could successfully be applied for the enantiomeric resolution of 18 *trans*-dihydrodiols of pyrene, chrysene, benz[*a*]anthracene, dibenz[*a*,*h*]anthracene, dibenz[*a*,*j*]anthracene, picene, benzo[*a*]pyrene and benzo[*e*]pyrene.

EXPERIMENTAL

Chemicals

The majority of the racemic *trans*-dihydrodiols used in this study (*cf.* Fig. 1) was prepared by reduction of suitable *o*-quinones with sodium borohydride in the presence of oxygen, *e.g.* **5**, **7**, **12**, **17**, **24** and **25** [16], **1–4**, **6**, **15**, **22** and **23** [17], **8** and **10** [18], **11**, **13** and **14** [19], **9**, **19** and **20** [20], **22c** and **22d** [21], **18** [22], **21** [23]. The syntheses of **16** [24] as well as of **7a**, **22a** and **22b** [25] were performed as described in the literature.

(−)-(1*R*,2*S*)-Ephedrine was purchased from Janssen Chimica (Brüggen, Germany). Fluoren-9-one, 2-nitro-, 2,7-dinitro-, 2,4,7-trinitro- and 2,4,5,7-tetranitrofluoren-9-one were obtained from Aldrich (Steinheim, Germany).

(*R*) - (−) - Isopropylideneaminooxypropionic acid (R_1 = methyl, Fig. 2) and its enantiomerical-

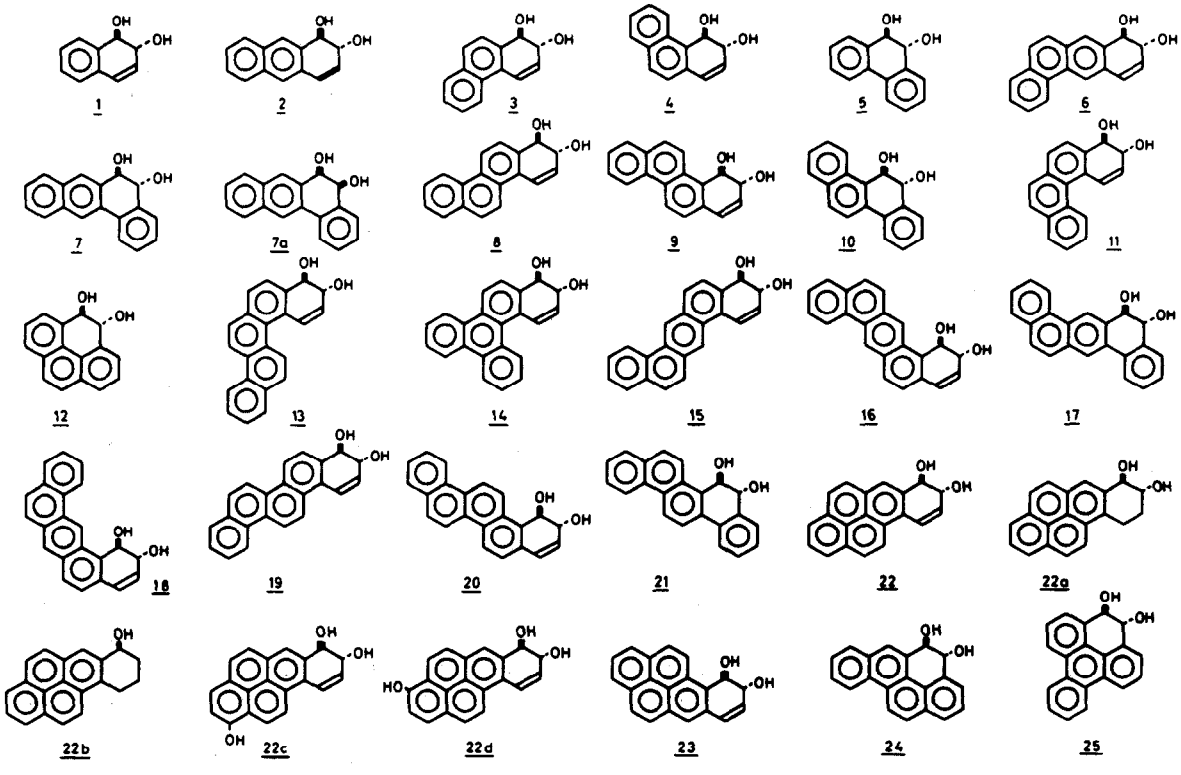


Fig. 1. Structure of dihydrodiols and related derivatives of polycyclic aromatic hydrocarbons (in the case of the *trans*-dihydrodiols only the enantiomer with *R*,*R* absolute configuration is shown in a position where the benzylic hydroxyl group points upward).

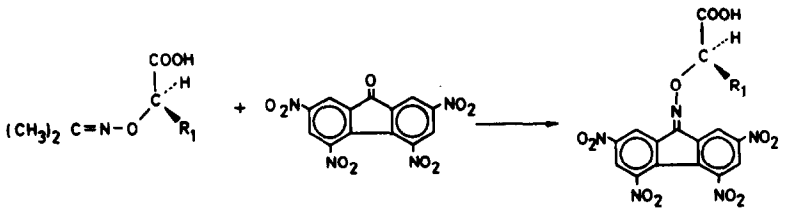


Fig. 2. Synthesis of chiral ligands by transoximation of (+)-2-isopropylideneaminooxy carboxylic acids with nitrated fluorenones.

ly pure homologues with R_1 = ethyl, propyl, isopropyl and butyl were synthesized analogously to Block and Newman [26] and characterized by elemental analysis, electron impact mass spectrometry (EI-MS), NMR, melting point (m.p.) determination and polarimetry (data not shown).

2,6,8 - Trinitro - 4*H* - cyclopenta[*def*]phenanthren - 4 - one (precursor for CSP 6) was prepared as described by Minabe *et al.* [27]. 1,3,5,8 - Tetranitro - 10*H* - benzo[*b*]fluoren - 10 - one (precursor for CSP 7) and 2,5,9,11 - tetranitro - 7*H*-benzo[*c*]fluoren - 7 - one (precursor for CSP 8) were obtained according to Ishikawa and Masubuchi [28] by nitration of 10*H*-benzo[*b*]-fluoren-10-one [29] and 7*H*-benzo[*c*]fluoren-7-one [30]. Purity and structural identity of the nitrofluorenones were confirmed by elemental analysis, EI-MS, NMR and m.p. determination (data not shown).

Methanol and dichloromethane for HPLC were supplied by Baker (Gross-Gerau, Germany), all other chemicals of analytical grade were from different commercial sources.

Preparation of CSPs

The synthesis of the chiral ligands was carried out according to Block and Newman [26]. A 14-mmol amount of the (+)-2-isopropylideneaminooxy carboxylic acid and 9 mmol of the corresponding fluorenone were dissolved in 30 ml glacial acetic acid and 0.12 ml concentrated sulphuric acid and heated under reflux for 2 h. Recrystallization from water–glacial acetic acid yielded pale yellow crystals, which were characterized by elemental analysis, EI-MS, NMR (data not shown), m.p. determination and polarimetry (Table I).

The coupling of the chiral ligands to LiChrosorb Si 100 (Merck, Darmstadt, Germany) was carried out by modifying the method described by Mikes *et al.* [15]. In a first step 8.34 mmol 3-aminopropyltriethoxysilane, 8.34 mmol of the chiral fluorenylideneaminooxy carboxylic acid and 9.16 mmol N,N′-dicyclohexylcarbodiimide were stirred at room temperature in 240 ml chloroform for 20 h. After filtration of the solution for separating the dicyclohexylurea and removing the solvent under reduced pressure the residue was dissolved in a suspension of 6.4 g LiChrosorb Si 100 (5 μm) in 100 ml *p*-xylene. The mixture was kept under an argon atmosphere and heated to 120°C for 8 h without stirring. After cooling to room temperature the modified silica was filtered through a glass diaphragm (porosity D5) and washed with 800 ml chloroform, acetone and methanol, respectively, with yields averaging 8 g of CSP. The CSPs were characterized by elemental analysis, which was used for calculating the amount of bound ligand (Table I).

Stainless-steel columns (250 × 4 mm) were filled with the CSPs by application of the balanced-density slurry packing method [31] by Knauer (Berlin, Germany). When the CSP columns were stored at room temperature with methanol and/or dichloromethane, their chromatographic performance deteriorated considerably within several months, probably due to partial cleavage of the covalent linkage of the chiral ligand to silica. Yet storage of the CSP columns at 4°C with *n*-hexane as eluent improved their stability considerably; these columns have retained their chromatographic properties for several years now.

Chromatographic conditions

For liquid chromatography a modular chromatographic system was used consisting of a

TABLE I

CHARACTERIZATION OF CHIRAL LIGANDS AND CSPs DERIVED THEREOF

All chiral ligands possess (*R*) absolute configuration.

Chiral ligand leading to CSP	Formula	Elemental analysis (%)			m/z[a] (M^+ – COOH)	Melting point (°C)[b]	Specific rotation[c,d] $[\alpha]_D^{20}$	Elemental analysis (%) of the CSPs			Bound chiral ligand (mmol/g)
		C calc./ found	H calc./ found	N calc./ found				C	H	N	
1	$C_{16}H_{13}NO_3$	71.90/72.06	4.90/4.89	5.24/5.27	222 (32)	165–166	−41.1	12.53	1.84	2.38	0.41
2	$C_{16}H_{12}N_2O_5$	61.54/61.55	3.87/3.76	8.97/8.90	267 (41)	210–211	−52.1	12.05	1.68	2.48	0.56
3	$C_{16}H_{11}N_3O_7$	53.79/53.34	3.10/2.97	11.76/11.62	312 (19)	234–235	−50.0	12.91	1.70	3.00	0.55
4	$C_{16}H_{10}N_4O_9$	47.77/47.73	2.51/2.47	13.92/13.83	357 (45)	200–202	−81.4	11.55	1.93	2.92	0.46
5	$C_{16}H_9N_5O_{11}$	42.97/42.17	2.03/2.18	15.66/15.20	402 (27)	189–191 (200–201)	−100.2 (−90)	9.80	1.48	3.13	0.40
5a	$C_{17}H_{11}N_5O_{11}$	44.26/44.18	2.40/2.59	15.18/14.84	416 (73)	172–173 (184–185)	−79.4 (−88)	9.36	1.49	2.90	0.37
5b	$C_{18}H_{13}N_5O_{11}$	45.48/45.49	2.76/2.95	14.73/14.52	430 (100)	168–169	−85.0	12.05	1.84	3.40	0.44
5c	$C_{18}H_{13}N_5O_{11}$	45.48/45.39	2.76/2.88	14.73/14.63	430 (100)	215–217 (200–202)	−129.7 (−103)	13.10	2.13	3.45	0.46
5d	$C_{19}H_{15}N_5O_{11}$	46.63/46.21	3.09/2.99	14.31/14.03	444 (100)	183–185 (207–209)	−69.9 (−22)	12.64	1.89	3.67	0.46
6	$C_{18}H_{10}N_4O_9$	50.72/49.90	2.36/2.74	13.14/12.58	380 (24)	207–209	−75.5	12.71	1.68	2.90	0.45
7	$C_{20}H_{11}N_5O_{11}$	48.30/47.48	2.23/2.45	14.08/13.72	451[e] (13)	215–216	−56.8	12.02	1.62	3.03	0.40
8	$C_{20}H_{11}N_5O_{11}$	48.30/48.11	2.23/2.54	14.08/13.50	451[e] (7)	230–232	−94.3	12.41	1.64	2.93	0.40

[a] Given is m/z of a diagnostic fragment ion and its relative intensity (in parentheses).
[b] Melting points from the literature [15] are given in parentheses.
[c] Specific rotation values [°]; Concentration 1.5 mg/ml in chloroform.
[d] Specific rotation values measured at 633 nm [15] are given in parentheses.
[e] Fragment ion resulting from $M^+ - NO_2$.

solvent-delivery system SP 8700 (Spectra-Physics, Darmstadt, Germany), a sample injection valve (Model C6U, Valco, Schenkon, Switzerland) with a 25-μl sample loop, an UV detector (254 nm; Model D, LDC Analytical, Gelnhausen, Germany) and an integrator–plotter (Model CI-10, LDC Analytical). The mobile phase consisted of mixtures of dichloromethane and methanol. The standard operating conditions were a flow-rate of 0.8 ml/min and room temperature. The dead time t_0 was determined as the retention time of tetrachloromethane. The solutes were dissolved in dichloromethane, methanol or acetone depending on their solubility; 1–3 μg per 25 μl were injected onto the column.

RESULTS AND DISCUSSION

(*R*) - (−) - 2 - (2,4,5,7 - Tetranitrofluoren - 9 - ylideneaminooxy)propionic acid (TAPA) coupled to 3-aminopropyltriethoxysilane and then bonded to silica gel has first been used for the enantiomeric separation of *trans*-dihydrodiols of benz[*a*]anthracene and benzo[*a*]pyrene by Kim *et al.* in 1981 [7]. In order to explore scope and limitations of this type of chiral stationary phases for the separation of enantiomers, especially of dihydrodiols of PAHs, and to determine the electronic and structural parameters which govern this separation process, we developed stationary phases based on (*R*)-(−)-TAPA by altering (i) the π-electron density in the fluorenylidene moiety by the degree of nitration, (ii) the spatial requirement at the center of chirality by modifying the substituent R_1 (*cf.* Fig. 2), and (iii) the dimensions of the aromatic ring system (*cf.* Fig. 3; CSPs 6–8).

Preparation of chiral stationary phases

The CSPs 1–5, 5a–d, 6–8 used in this study (*cf.* Fig. 3) were prepared by covalent coupling of suitable chiral ligands to silica via an aminopropyl spacer.

For constructing the chiral ligand the oxime derivative bearing the chiral center and the nitrofluorenone were synthesized separately and coupled by transoximation as described by Block and Newman [26] (Fig. 2). The characteristic data of elemental analysis, m.p. determinations, mass spectra and specific rotation values are shown in Table I. In some cases differences as compared to data published earlier [15] have been encountered (Table I).

The covalent binding of the chiral ligand to silica was carried out via an aminopropyl spacer by modifying the method of Mikes *et al.* [15]. First the spacer was bound to the chiral ligand catalyzed by N,N′-dicyclohexylcarbodiimide. Then the resulting triethoxysilane amide was tempered with the silica to achieve covalent binding. The binding of the organic material to the silica was verified by elemental analysis (Table I). The amount of bound ligand was calculated to be in the range of 0.37 to 0.56 mmol/g modified silica, which compares well to other CSPs [32].

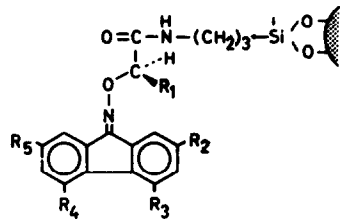
Reaction of residual silanol groups in CSP 5 with trimethylsilylimidazole yielded a CSP which did not exhibit a significant improvement of selectivity determined with the model solute **16** ($\alpha = 1.50$ in the case of CSP 5 *versus* $\alpha = 1.54$ in the case of "end-capped" CSP 5).

Influence of the structure of chiral stationary phases on the enantiomeric separation of a model solute

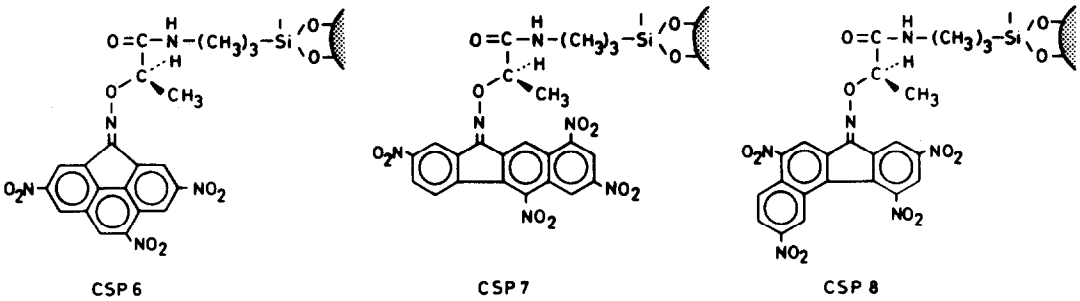
In order to characterize the interactions between solute and CSP leading to chiral recognition and thus to enantiomeric resolution, the capacity factor k' and the selectivity α of dibenz[*a*,*h*]anthracene (DBA) 1,2-diol **16** (*cf.* Fig. 1) on model CSPs based on TAPA (Fig. 2, R_1 = methyl) were determined (Table II). Dihydrodiol **16** was chosen as solute on the basis of its optimal enantiomeric separation by TAPA (CSP 5) thus being potentially a good indicator of alterations in the interaction between solute and CSP.

According to the chiral recognition model described by Pirkle and Pochapsky [33] one π-donor–acceptor interaction is required and additionally one stereochemically dependent interaction, in our case between the chiral center of the ligand and (at least) one of the hydroxyl groups of the dihydrodiol.

When the nitro groups in CSP 5 were stepwise



CSP	1	2	3	4	5	5a	5b	5c	5d
R_1	CH_3	CH_3	CH_3	CH_3	CH_3	C_2H_5	nC_3H_7	iC_3H_7	nC_4H_9
R_2	H	NO_2	NO_2	NO_2	NO_2	NO_2	NO_2	NO_2	NO_2
R_3	H	H	H	NO_2	NO_2	NO_2	NO_2	NO_2	NO_2
R_4	H	H	H	H	NO_2	NO_2	NO_2	NO_2	NO_2
R_5	H	H	NO_2	NO_2	NO_2	NO_2	NO_2	NO_2	NO_2



CSP 6 CSP 7 CSP 8

Fig. 3. Structures of chiral stationary phases (CSPs).

removed leading to CSP 4, 3, 2 and 1, the strength of the charge-transfer interaction weakened and consequently the capacity factors of **16** decreased; concomitantly the chiral discrimination deteriorated as indicated by the declining selectivity (Table II).

Variation of the alkyl group at the chiral center (R_1 in Fig. 3) demonstrates the influence of a weaker stereochemically dependent interaction, expressed as capacity factor, on the retention of **16**. CSP 5a is a good example of this effect. While the retention time has increased as compared to TAPA the selectivity is smaller; thus probably the less effective stereochemical interaction leads to an arrangement of the planar aromatic system which allows a stronger charge-transfer interaction.

The more bulky ligands in CSP 5b, 5c, 5d exhibit decreasing k' and α values indicating that both interactions are diminished.

The linearly annellated CSP 7 is the optimized system for the resolution of **16**. In addition to the strong chiral interaction the more effective charge-transfer interaction expressed by the large capacity factor, $k_1' = 12.00$, causes an increase of selectivity from $\alpha = 1.61$ (TAPA) to 2.01 (CSP 7). CSP 6 exhibits a weak π–π interaction ($k_1' = 4.82$) which is too small for an efficient chiral recognition. CSP 8 and **16** obviously form a strong charge-transfer complex as shown by the large capacity factor, $k_1' = 10.9$, but the lack of enantiomeric resolution indicates that this arrangement might not allow any chiral interaction.

TABLE II

CHROMATOGRAPHIC BEHAVIOUR OF *trans*-1,2-DIHYDROXY-1,2-DIHYDRODIBENZ[*a*,*h*]ANTHRACENE (**16**) ON CHIRAL STATIONARY PHASES (*cf.* FIG. 3)

k_1' is the capacity factor of the first-eluted enantiomer, $k_1' = (t_{R1}/t_0) - 1$, where t_{R1} is the retention time of the first-eluted enantiomer and t_0 the retention time of a non-retained solute. The selectivity, α, between two enantiomers is the ratio of their respective capacity factors (k_1'/k_2'). Operating conditions: flow-rate, F, 2 ml/min; room temperature; column dimensions, 250 × 4 mm; $V_0 = 2.0$–2.3 ml, where $V_0 = Ft_0$; mobile phase: methanol–dichloromethane 30:70, v/v); UV detection at 280 nm.

CSP	k_1'	α
1	0	NR[a]
2	0.09	NR
3	2.11	1.09
4	3.12	1.38
5	9.74	1.61
5a	16.80	1.34
5b	5.02	1.36
5c	2.05	NR
5d	3.27	1.15
6	4.82	1.10
7	12.00	2.01
8	10.90	NR

[a] NR = No resolution, $\alpha = 1$

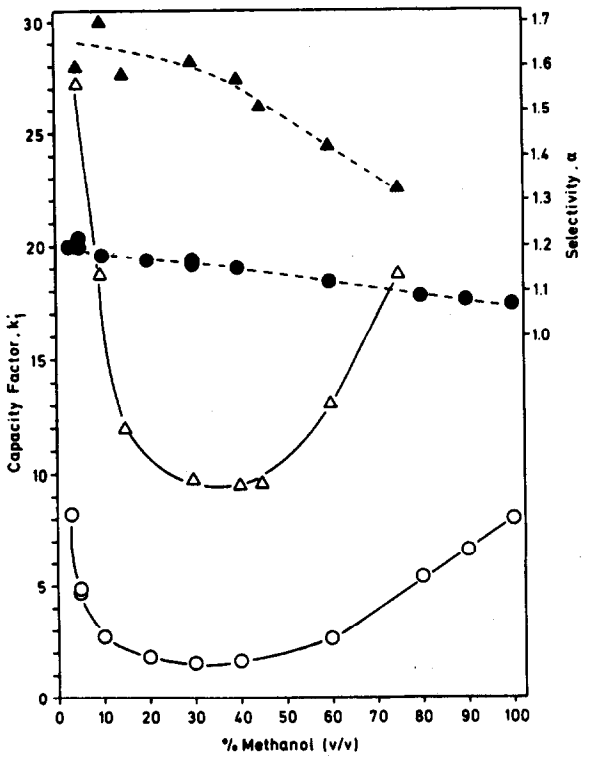


Fig. 4. Dependence of the capacity factor, k_1' (solid lines), of the first eluted enantiomer and of the selectivity, α (dotted lines), between the two enantiomers from the composition of the mobile phase consisting of methanol and dichloromethane; solutes: **16** = △, ▲(CSP 5); **22** = ○, ●(CSP 5a).

Influence of the composition of the mobile phase on the enantiomeric separation

Mixtures of methanol and dichloromethane proved to be suitable mobile phases for the enantiomeric resolution of *trans*-dihydrodiols on chiral stationary phases based on (*R*)-(−)-2-(2,4,5,7-tetranitrofluoren-9-ylideneaminooxy)-carboxylic acids. Variation of the composition of the binary mobile phase from pure dichloromethane to pure methanol resulted in a marked bimodal change of the capacity factor which reached its minimum at 30–35% (v/v) methanol in the binary mixture (Fig. 4). This behaviour is a general phenomenon as it is observed with different solutes as well as with different CSPs (Fig. 4) [32,34]. Similar results have also been obtained with diol and cyano phases applying mobile phases of different solvent strength [35]. This retention property indicates that the stationary phase exhibits an intermediate polarity, *i.e.* a polarity which is in between the polar unmodified silica (normal phase) and the non-polar, aliphatic hydrocarbon-modified silica (reversed phase). In pure dichloromethane, a rather non-polar solvent with a solvent strength parameter of $P' = 3.4$ [36], strong interaction with the CSP is shown by a high capacity factor k'; this interaction is of polar nature since it is weakened by an increase in the content of methanol, a polar solvent with a solvent strength parameter of $P' = 6.6$ [36]. In pure methanol, on the other hand, a strong non-polar interaction between solute and CSP is observed, which is weakened by increasing the dichloromethane content leading to a decrease in the value of the capacity factor k'. The lowest k' value is obtained at a methanol content in the binary mixture which represents a solvent strength of $P' = 4.36$–4.52 [37].

The hydrophobic interaction between solutes and CSP at high methanol content is furthermore confirmed by the observed differences in the capacity factors between solutes **16** and **22** (*cf.* Fig. 4). Thus the larger k' values and their stronger increase with increasing methanol content ($>40\%$) in the case of **16** as compared to **22** is a consequence of the different hydrophobic nature of the two *trans*-dihydrodiols. Using the partition coefficient P, which is a quantitative description of the lipophilicity of a compound, and estimating log P by summation of the hydrophobic fragmental constants according to Rekker [38] leads to the conclusion that **16** is more lipophilic with log $P \approx 4.56$ as compared to **22** with log $P \approx 3.84$. This results in stronger hydrophobic interactions of **16** with the CSP and consequently larger k' values in comparison to **22**.

Not only the retention of the solute but also the degree of chiral discrimination demonstrated by the selectivity α is influenced by the change in the composition of the mobile phase (Fig. 4) [32,34]. Polar interactions between the CSP and at least one of the chiral hydroxyl groups of the solute clearly play a more important role for chiral recognition than the non-polar interactions dominating at high methanol content. Additionally the *trans*-dihydrodiol and methanol could compete for hydrogen bonding sites in the CSP thereby impairing the chiral interactions. Furthermore, it should be emphasized that the chiral discrimination is only slightly influenced by the change in the composition of the mobile phase as compared to the marked alteration of the capacity factor.

Structural requirements for the enantiomeric separation of trans-dihydrodiols on chiral stationary phases based on (R)-(−)-2-(2,4,5,7-tetranitrofluoren-9-ylideneaminooxy)carboxylic acids

In order to account for the structural features of chiral compounds governing their chromatographic separation into enantiomers 30 hydroxylated derivatives (Fig. 1) of PAHs were applied to CSP 5; 27 of these solutes were metabolically relevant *trans*-dihydrodiols which exist as just two optical isomers, the *R,R*- and the *S,S*-enantiomer, in spite of their two centres of chirality.

Interaction between the dihydrodiols and the CSP leading to retention of the solute can take place between the aromatic ring system of the former and the nitrated fluorenylidene moiety of the latter (π-donor–acceptor interactions) as well as between the hydroxyl group(s) of the dihydrodiol and the amide carbonyl or the aminooxy moiety of the stationary phase (hydrogen bonds).

The $\pi-\pi$ interaction seems to play the most important role since an increase in the number of aromatic rings in the solute leads to stronger retention, expressed as capacity factor, k' (Table III): while compounds with one (**1**) or two isolated aromatic rings (**5**) are very weakly retained, the capacity factor increases with the number of annellated rings, *e.g.* **5**→**10**→**21**. The strongest retention is observed in the case of compounds with four annellated rings, *e.g.* **15**, **16**, **18–20** whereas the interaction of solutes with four unsubstituted condensed rings, *e.g.* **22**, **22a**, **22b**, **23**, is somewhat weaker. Increasing the basicity of the aromatic ring system by the +M effect of a phenolic hydroxyl group leads to stronger π-donor–acceptor interactions thus raising the retention considerably as in the case of **22c** and **22d** compared to **22**. Additionally, hydrogen bonds between the phenolic hydroxyl groups and the CSP could add to the retention of **22c** and **22d**. An olefinic double bond in the ring containing the diol moiety (**22**) leads to a higher capacity factor as in the case of the saturated ring system (**22a**) probably caused by the larger number of π-electrons or by the greater planarity of the ring system thus improving the interaction between solute and stationary phase.

For the enantiomeric recognition of a chiral compound by a CSP a three-dimensional interaction is necessary. π-Donor–acceptor interactions provide in principle two of the three interactions requiring just a single addition interaction for chiral discrimination [33]. For optimal chiral resolution the $\pi-\pi$ interaction between the nitrated fluorenylidene moiety and the aromatic ring system has to fix the chiral solute in a position allowing maximal interaction between one of the hydroxyl groups and the CSP. There-

TABLE III

CHROMATOGRAPHIC BEHAVIOUR OF DIHYDRODIOLS AND RELATED DERIVATIVES OF POLYCYCLIC AROMATIC HYDROCARBONS (*cf.* FIG. 1) ON CHIRAL STATIONARY PHASE CSP 5 (*cf.* FIG. 3)

For the meaning of k_1' and α see Table II. Operating conditions: flow-rate, 0.8–2.0 ml/min; room temperature; column dimensions, 250 × 4 mm; V_0 = 2.35 ml; mobile phase: the percentage (v/v) of methanol in dichloromethane is given; UV detection at 280 nm.

Dihydrodiol	Methanol (%)	k_1'	α
1	30	0.51	NR[a]
	0	0.09	NR
2	30	0.34	NR
	1	13.57[b]	1.05
3	30	0.25	NR
	1	5.46[b]	1.04
4	30	0.22	NR
	1	2.31	NR
5	30	0.23	NR
	5	0.44	NR
6	30	1.88	1.08
	5	5.91	1.10
7	30	0.92	1.07
	1	8.95	1.08
7a	30	0.53	NR
	1	3.58	NR
8	30	1.59	1.08
	1	10.09	1.20
9	30	0.77	NR
	1	5.61[b]	1.05
10	30	0.16	NR
	1	1.90	1.12
11	30	0.62	NR
	1	9.95	NR
12	30	0.74	NR
	1	5.48	1.11
13	30	3.50	NR
14	30	2.13	NR
	5	5.50	NR
15	30	14.03	1.09
	10	20.90	1.09
16	30	9.74	1.61
	5	27.25	1.65
17	30	4.23	1.06
	10	6.39	1.06
18	30	7.66	1.27
	10	13.48	1.27
19	30	10.98	1.07
	5	31.45	1.10
20	30	4.18	1.09
	5	15.05	1.08
21	30	0.75	1.20
	1	8.11	1.20
22	30	4.31	1.14
	10	7.42	1.16
22a	30	3.02	1.09
	10	4.57	1.11
	5	7.61	1.11
22b	30	2.85	1.12
	1	5.32	1.13
22c	30	16.10	1.09
	10	43.22	1.10
22d	30	17.39	1.12
23	30	1.79[b]	1.05
	5	6.48	1.06
24	30	3.66	1.20
	5	10.24	1.19
25	30	1.98	1.21
	5	6.02	1.24

[a] NR = No resolution, $\alpha = 1$.
[b] Eluted as shoulder.

fore a marked π-donor–acceptor interaction is a necessary but not a sufficient requirement for chiral separation. This is demonstrated by the behaviour of **11**, **13**, **14** which exhibit a high capacity factor but lack any enantiomeric resolution. It is interesting to note that not only the enantiomers of **11**, **13**, **14** but also those of other *trans*-dihydrodiols possessing the structural element of a fjord-region[a] could not be separated by the CSPs depicted in Fig. 3. Obviously the helicity of fjord-region PAHs prevents the formation of the diastereomeric complex required for chiral discrimination.

In general the increase in the number of aromatic rings leads to an improvement in stereoselectivity exemplified by **5**, **10** and **21** (Table III). Two or three aromatic rings in the dihydrodiol are the minimum for chiral recognition by CSP 5 as shown by the partial separation of **2**, **3** and **9** into the enantiomers. The separation could not further be improved by modification of the mobile phase as was expected from the results of Fig. 4. In the case of *trans*-dihydrodiols **6**, **7**, **10**, **12**, **15**, **17**, **19**, **20**, **22**, **22c**, **22d**, **23** a more favourable chiral interaction leads to an improvement of enantiomeric separation (α = 1.06–1.19) which is further enhanced (α = 1.20–1.27) in the case of **8**, **16**, **18**, **21**, **24**, **25**. It should be noted that neither an olefinic double bond, *e.g.* **22a** as compared to **22**, nor two hydroxyl groups, *e.g.* **22b** as compared to **22a**, are required for chiral resolution. In the case of vicinal hydroxyl groups in the solute the *trans*-orientation seems to allow better chiral interactions with the CSP than the *cis*-orientation as in **7** *versus* **7a**. A similar observation has already been reported [7]. Apparently the two hydroxyl groups mutually compete for hydrogen bonds with the chiral center of the CSP.

The successful enantiomeric separation of a large number of metabolically relevant and biologically active *trans*-dihydrodiols of PAHs can now be applied to studies concerned with the

[a] The term fjord-region denotes a sterically crowded molecular region in a PAH, *e.g.* the region between C-1 and C-12 in benzo[*c*]phenanthrene; thus the fjord-region is a special case of a bay-region, *e.g.* the region between C-4 and C-5 in phenanthrene.

stereoselective formation of these metabolites [13,39] or with the biological activity of the enantiomers [12] and their covalent binding to DNA [40].

Applicability of the chiral stationary phases for the enantiomeric separation of metabolites of PAHs and of drugs

Although many of the *trans*-dihydrodiols depicted in Fig. 1 could be resolved into their enantiomers by application of CSP 5, in some cases this separation was not fully satisfactory. Thus in the case of the two non-bay-region *trans*-dihydrodiols of DBA the enantiomers of the 1,2-isomer, **16**, are well separated ($k_1' = 9.74$, $\alpha = 1.61$; *cf.* Table II) while those of the biologically very important 3,4-isomer **15** [12,39,40] were only poorly resolved ($\alpha = 1.09$, $R_s = 0.35$; Fig. 5A) despite of the stronger π-donor–acceptor interactions as expressed by a capacity factor of $k_1' = 14.03$ (Table III).

Assuming that the aromatic ring system of both *trans*-dihydrodiols is fixed in a similar manner to the tetranitrofluorenylidene system of CSP 5 the 3,4-substitution pattern obviously does not allow a similar effective chiral interaction as in the case of the 1,2-isomer.

An attempt to improve the chiral recognition by replacing the methyl group (R_1 in Fig. 3) in the center of chirality of CSP 5 by more bulky alkyl groups leading to CSP 5a, 5b, 5c, 5d did not succeed (data not shown). Therefore another strategy was pursued by modifying the geometry of the π-acceptor moiety in the CSP by annellating the original tetranitrofluorenylidene system with additional nitroaromatic rings in different positions leading to CSP 6, 7, 8. Indeed this approach finally yielded an almost base-line separation of the enantiomers of **15** with CSP 8 ($\alpha = 1.20$, $R_s = 1.07$; Fig. 5B) although at the expense of a rather long separation time; this CSP could also be employed for the separation of (+)- and (−)-**15** on a preparative scale [12]. As an example of the application of CSP 8 in the investigation of the biotransformation of tumorigenic DBA Fig. 5C demonstrates the stereoselective formation of (−)-(3*R*,4*R*)-dihydrodiol by hepatic microsomes of Sprague–Dawley rats pretreated with 3,4,3′,4′-tetrachlorobiphenyl.

The applicability of the CSPs described in this study is not restricted to the enantiomeric separation of *trans*-dihydrodiol but can also be expanded to other metabolically relevant deriva-

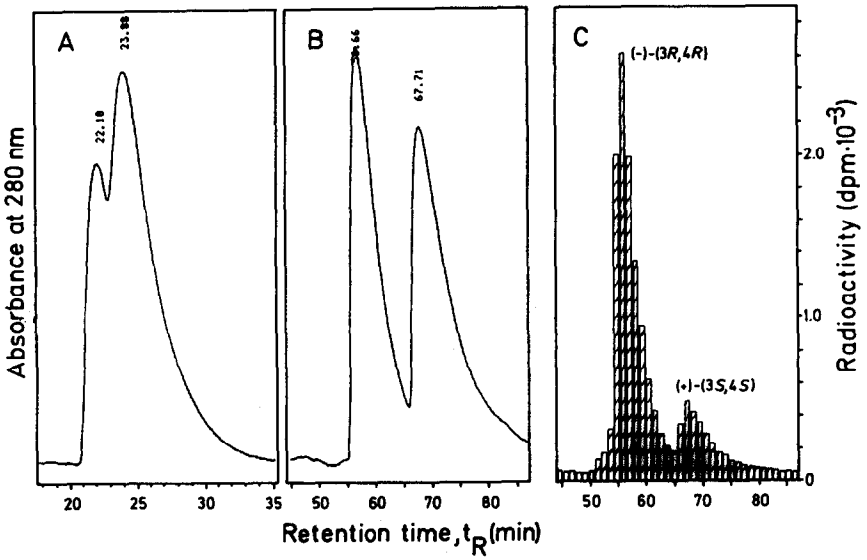


Fig. 5. Chromatographic separation of the enantiomers of synthetic (A, B) and metabolically formed (C) *trans*-3,4-dihydroxy-3,4-dihydrodibenz[*a*,*h*]anthracene on CSP 5 (A) and CSP 8 (B, C). Mobile phase: methanol–dichloromethane (30:70, v/v); flow-rate: 1.6 ml/min; $t_0 = 1.48$ min.

tives of PAHs like arene oxides [39], tetrahydrotetraols [39] phenoldihydrodiols (Fig. 1, Table III) and dihydrodiol oxides [7]. The enantiomeric separation of (±)-DBA 5,6-oxide on CSP 5b [39] indicates that not only hydroxyl groups but also an oxiranyl oxygen is able to form stereoselective interactions with the chiral center of the CSP. Finally these CSPs could successfully be applied to the preparation of enantiomeric pure intermediates for the synthesis of metabolically relevant derivatives of PAH [22].

However, attempts to employ these CSPs for the separation of racemic drugs, *e.g.* β-blockers, antiinflammatory agents, calcium-channel antagonists, anaesthetics and anticoagulants, failed in spite of marked charge-transfer interactions expressed by capacity factors in the range of $k' = 3–7$. Apparently the interactions between the substituents at the asymmetric carbon atom of the drug and the chiral center of the CSP or the distance between them did not permit any chiral discrimination.

CONCLUSIONS

Nitrated fluorenylideneaminooxy carboxylic acids covalently bound to silica gel via an aminopropyl spacer yielded CSPs suitable for the chromatographic separation of *trans*-dihydrodiols and related derivatives of PAHs thus avoiding the cumbersome separation of the enantiomers as their diastereomeric esters. Charge-transfer interactions between the π-acceptor of the CSP and the aromatic ring system of the solute in combination with chiral interactions via hydrogen bonds provide the basis for chiral discrimination. The retention of the solutes on the CSPs is strongly influenced by the composition of the mobile phase consisting of methanol–dichloromethane mixtures while the selectivity of the enantiomeric separation is only weakly affected. A minimum of two annellated aromatic rings in the solute were required for stereochemically discriminating interactions while an enlargement of the π-donor system to four aromatic rings resulted in an increase in stereoselectivity. The chiral interaction could furthermore be improved by modifications of the geometry of the π-acceptor part of the chiral ligand.

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