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Note

R-N-(Pentafluorobenzoyl)phenylglycine as a chiral stationary phase for the separation of enantiomers by high-performance liquid chromatography

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Much progress has recently been made in the separation of enantiomers by high-performance liquid chromatography (HPLC)[1,2]. The most attractive methods are those in which a chiral stationary phase is applied for the direct separation of the enantiomers. In this respect the work of Pirkle *et al.*[1,3–5] and of Oi *et al.*[6,7] significantly advanced the rational design of selective chiral stationary phases.

In this work we report the preparation of a novel chiral stationary phase for HPLC, *R*-N-(pentafluorobenzoyl)phenylglycine. This chiral phase was ionically bonded to the γ-aminopropyl silica via the acid–base reaction occurring between the amino group on the silica and the carboxylic acid group in the chiral phase. The selectivity of this chiral phase for various types of racemates, such as succinimides, hydantoins and mandelates, was investigated and compared with the selectivity of the 3,5-dinitrobenzoylphenylglycine phase developed by Pirkle.

EXPERIMENTAL

Apparatus

The liquid chromatograph consisted of a high-pressure reciprocating pump (Orlita, Giessen, F.R.G.), a flow-through manometer, acting as a pulse dampener, a high pressure sampling valve (Model 7125 Rheodyne, Berkeley, CA, U.S.A.) equipped with a 20-μl sample loop and an UV-LC spectrophotometer (Pye-Unicam, U.K.). The wavelength of the latter was usually set at 254 nm. The columns were made of 316 stainless steel (150 × 4.6 mm I.D.).

Materials

All solvents were of analytical grade (Merck, Darmstadt, F.R.G.). Tetrahydrofuran (THF) was freshly distilled over $LiAlH_4$. The solutes were obtained from Janssen Chimica (Beerse, Belgium) or synthesized by ourselves. The γ-aminopropyl silica was LiChrosorb NH_2, 5 μm (Merck). *R*-α-Phenylglycine and pentafluorobenzoyl chloride were purchased from Janssen Chimica. (*R*)-N-(3,5-Dinitrobenzoyl)phenylglycine was obtained from Sigma Chemicals (St. Louis, MO, U.S.A.).

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Procedures

Synthesis of R-N-(pentafluorobenzoyl)phenylglycine. To a slurry of 3.0 g (20 mmol) of *R*-α-phenylglycine in 50 ml of fresly distilled THF, a solution of 4.5 g (30 mmol) of pentafluorobenzoyl chloride in 100 ml freshly distilled THF was added. This mixture was stirred at room temperature, under a nitrogen atmosphere, for 7 days, then evaporated to dryness *in vacuo*. The residue was dissolved in 100 ml water, containing 10% (w/w) of sodium bicarbonate. This solution was extracted twice with 50 ml of diethyl ether and then adjusted to pH = 5.3 with citric acid. The aqueous solution was continuously extracted with diethyl ether for 24 h. The pH of the aqueous layer was tested during the extraction and kept at pH = 5.3 with citric acid. The organic layer was collected and the diethyl ether removed by distillation. The dry residue was recrystallized from dry diethyl ether–pentane (1:1, v/v). The white crystals were isolated by filtration, washed with pentane and dried *in vacuo* (25°C). The yield of *R*-N-(pentafluorobenzoyl)phenylglycine was 2.9 g (45%), m.p. 190–192°C ^{1}H NMR [C^2HCl_3–$(C^2H_3)_2SO$]: δ 5.00–6.40 (br; 1H), 5.44 (d, 1H), 7.09–7.34 (m, 5H), 8.52 (d, 1H). IR (KBr) (cm^{-1}): 3500–2500,3280, 3080, 1705, 1660, 1550, 1520, 1490, 1420, 1350, 1230, 1100, 1000. High resolution mass spectrum: calculated for $C_{15}H_8NO_3F_5$, 345.0424; found, 345.0430.

Column preparation. The coating of the chiral phases on the γ-aminopropyl silica was performed in pre-packed columns. These columns were packed with a pressurized slurry technique, using isopropanol as slurry liquid and methanol as displacer liquid up to 600 bar. The columns were washed successively with 50 ml of methanol and 50 ml of freshly distilled THF. The chiral phase was coated on the pre-packed column as described by Pirkle and Finn[8].

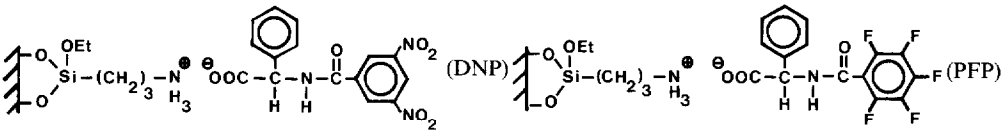
RESULTS AND DISCUSSION

The *R*-N-(pentafluorobenzoyl)phenylglycine phase (PFP) is a modification of the *R*-N-(3,5-dinitrobenzoyl)phenylglycine phase (DNP) developed by Pirkle and Finn[8]. The phases differ only in the type and number of functional groups in the benzoyl ring. The presence of five fluorine atoms makes the benzoyl ring very electron poor, which might favourably influence the resolution of certain enantiomers because π–π donor–acceptor interactions seem to play a rôle in chiral recognition.

In order to investigate the effect of the replacement of two NO_2 groups in the Pirkle phase (DNP) by five fluorines, the retention and selectivity of a number of racemates were measured, under identical conditions, on both chiral phases. The results of these measurements are given in Table I. Some significant differences between the two chiral phases were observed. The capacity factors for all solutes are remarkably smaller on the PFP phase. Because the amount of chiral phase loading was found to be the same for both phases, the difference in retention must be attributed to the change in the nature of the benzoyl ring. The greater retention on the DNP phase might indicate that the retention is also due to non-stereoselective interactions of the solutes with the rather polar NO_2 groups in the benzoyl ring. The enantioselectivity also differs on the two phases. Some racemates, like alcohols, show larger α values on the DNP phase, but solutes containing nitrogen, like succinimides, hydantoins and mandelates, show larger α values on the PFP phase. From Table I it is seen that the presence of a larger carbon substituent on the nitrogen in hydantoins

TABLE I

CAPACITY FACTORS, k', AND SELECTIVITY FACTORS, α, OF SOME ENANTIOMERS ON THE DNP AND PFP PHASES



Compound	*DNP*		*PFP*		*% Iso-propanol*
	k'	α	k'	α	
RR/SS-1-Phenylsulphinyl-2-methyl-3-buten-2-ol	22.8	1.08	14.4	1.05	2.5
RS/SR-1-Phenylsulphinyl-2-methyl-3-buten-2-ol	17.8	1.10	11.8	1.12	2.5
3-Phenylphthalide	6.6	1.04	2.3	1.00	5.0
1,1'-Bi-2-naphthol	17.1	1.37	3.6	1.10	10.0
2,2,2-Trifluoro-1-(9-anthryl)ethanol	2.8	1.37	0.8	1.13	10.0
3-Methyl-3-phenylsuccinimide	10.1	1.03	3.8	1.13	10.0
5-Cyclopentyl-5-phenylhydantoin	7.9	1.33	5.1	1.61	10.0
5-Methyl-5-phenylhydantoin	17.9	1.18	8.7	1.27	10.0
Methyl mandelate	8.3	1.11	5.3	1.08	2.5
Mandelamide	18.2	1.03	11.4	1.12	10.0
Ethyl N-(pentafluorobenzoyl)phenylglycinate	4.1	1.00	3.1	1.14	10.0
Ethyl N-(3,5-dinitrobenzoyl)phenylglycinate	12.6	1.11	4.9	1.00	10.0
2-Phenylsuccinimide	17.1	1.12	7.1	1.19	10.0

(cyclopentyl *vs.* CH_3) increases the α value, in agreement with earlier findings[9].

The esters of *R*-N-(pentafluorobenzoyl)phenylglycine and *R*-N-(3,5-dinitrobenzoyl)phenylglycine show a remarkable behaviour. The enantiomers of these esters can be well separated on the chiral phase based on the parent compound, but not on the other phase. This behaviour somewhat contradicts the assumed need of π–π donor–acceptor interactions to obtain enantioselectivity, as postulated in the three point interaction model. Rather it supports theories in which dipole–dipole stacking and Van der Waals interaction are also considered as important[10].

The results described so far indicate that the PFP phase shows a higher selectivity for nitrogen-containing racemates than the DNP phase and might be a welcome addition to the known chiral stationary phases. Fig. 1 shows a typical chromatogram of a hydantoin racemate obtained on the PFP phase.

Mixed chiral stationary phase

From Table I it is seen that significant differences in α values are found on the DNP and PFP phases. For certain types of solutes the DNP phase is superior, for others the PFP phase. It should be advantageous to have a chiral column which combines to a large extent the properties of both chiral stationary phases. This can be realized by connecting a DNP and a PFP column in series or by coating of the γ-aminopropyl silica with a mixture of DNP and PFP. The latter approach was investigated in more detail.

When the γ-aminopropyl silica column is loaded with a mixture of chiral phases, the amount of each of the individual chiral phases will be less than when the

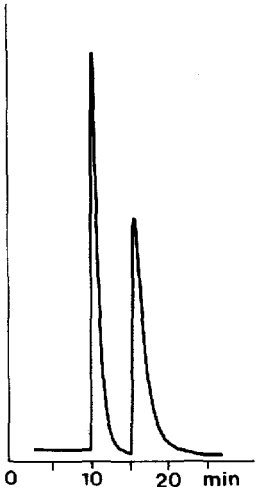


Fig. 1. Separation of the enantiomers of 5-phenyl-5-cyclopentylhydantoin on the PFP phase. Mobile phase: hexane–isopropanol (9:1, v/v).

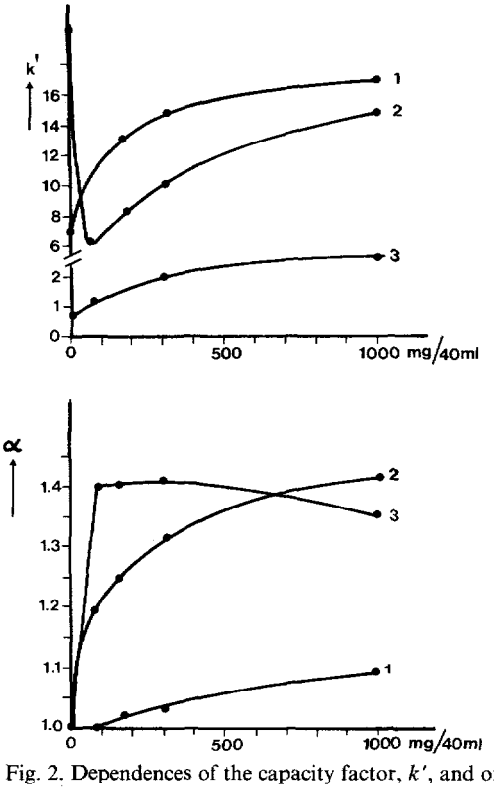


Fig. 2. Dependences of the capacity factor, k', and of the selectivity factor, α, on the degree of loading of the γ-aminopropyl silica column with the DNP phase. Solutes: 1 = 1-phenylsulphinyl-2-methyl-3-buten-2-ol; 2 = 1,1'-bi-2-naphthol; 3 = 2,2,2-trifluoro-1-(9-athryl)ethanol. Mobile phases as in Fig. 1.

TABLE II

CAPACITY FACTORS, k', AND SELECTIVITY FACTORS, α, OF SOME ENANTIOMERS ON THE DNP, PFP AND THE 1:1 MIXED DNP + PFP CHIRAL PHASES

Compound	*DNP*		*PFP*		*DNP + PFP*		*% Isopropanol*
	k'	α	k'	α	k'	α	
RR/SS-1-Phenylsulphinyl-2-methyl-3-buten-2-ol	22.8	1.08	14.4	1.05	17.2	1.07	2.5
RS/SR-1-Phenylsulphinyl-2-methyl-3-buten-2-ol	17.8	1.10	11.8	1.12	13.8	1.12	2.5
3-Phenylphthalide	6.6	1.04	2.3	1.00	4.3	1.03	5.0
1,1'-Bi-2-naphthol	17.1	1.37	3.6	1.10	6.3	1.34	10.0
2,2,2-Trifluoro-1-(9-anthryl)ethanol	2.8	1.37	0.8	1.13	1.5	1.30	10.0
3-Phenylsuccinimide	17.1	1.12	7.1	1.19	10.5	1.17	10.0
5-Cyclopentyl-5-phenylhydantoin	7.9	1.33	5.1	1.61	6.3	1.53	10.0
Methyl mandelate	8.3	1.11	5.3	1.08	6.3	1.11	2.5
Mandelamide	18.2	1.03	11.4	1.12	13.4	1.09	10.0

column is loaded with only one chiral phase. Because this might considerably affect the selectivity, the influence of the loading on the capacity factors and α values of some racemates was investigated with the DNP phase. The results of these measurements are shown in Fig. 2. As is seen, the capacity factors and α values increase steeply with increasing DNP loading of the column and then level off at higher loadings. Clearly the change in α values, when going from a medium to a high loading, is not dramatic. Therefore we expect that the α values will still be in an acceptable range when the γ-aminopropyl column is loaded with a 1:1 mixture of DNP and PFP. This can be simply obtained by dissolving equal amounts (mmol) of each phase in the recycling solvent used to load the column with the chiral phase, *e.g.*, both chiral phases have about the same acidity.

Table II shows the α values of some enantiomers on the 1:1 mixed DNP and PFP phase. As expected, the α values lie between those obtained on the separate phases. However, on the mixed phase all compounds show an acceptable α value, in contrast to the situation on the separate phases.

These results with the mixed chiral phase show that it must be possible to design such phases for a broader spectrum of racemates. In particular, chiral phases which show large α values at low loadings are attractive for mixed chiral phases.

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