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π-ACCEPTOR AMIDE GROUP FOR LIQUID CHROMATOGRAPHIC CHIRAL SEPARATIONS WITH SPECIAL EMPHASIS ON THE 3,5-DINITROBENZOYL AMIDE[a]

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SUMMARY

A series of achiral π-acceptor benzene derivatives and of achiral benzamide derivatives were chromatographed on a chiral and an achiral stationary phase to study the specific interactions of π-acceptor samples with a chiral π-donor stationary phase. The specific interactions with the chiral moiety of the stationary phase are expressed by the relative selectivity k'^* [$k'^* = k'$ (chiral phase)/k' (achiral phase)]. To study the chiral recognition, a series of chiral phenylethylamine derivatives were chromatographed on the same two phases to determine the influence of the π-acceptor amide group on the chiral separation. The separation factor α does not correlate with the capacity factor k_2' but it correlates with the relative selectivity $k_2'^*$. If the aromatic moiety of the π-acceptor is flat, there is a correlation of the AMPAC-calculated LUMO (lowest unoccupied molecular orbital) with the relative selectivity k'^*. The 3,5-dinitrobenzoyl group ideally combines flatness with a low LUMO.

INTRODUCTION

The 3,5-dinitrobenzoyl (3,5-DNB) group was first incorporated into many solutes to extend the scope of a π-donor (chiral 9-anthryl-1,1,1-trifluoroethanol grafted to silica) chiral stationary phase[1]. This led to the synthesis of the first chiral stationary phase (CSP) containing the 3,5-DNB group[2,3] (*e.g.*, 3,5-dinitrobenzoylphenylglycine phase). Many more chiral separations using such a π–π interaction have since been reported.

Pirkle and co-workers made substantial investigations of the chiral recognition mechanisms in which π–π interactions are involved. Based on the fact that the elution

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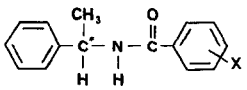


Fig. 1. General structure of the chiral phenylethylamine (PEA) derivatives investigated.

order can change within a series of homologous compounds, the concept of two competing chiral recognition processes was proposed[4]. Such processes ("dipole-stacking" and "hydrogen-bonded" mechanisms) have been found in many related separations[5–11]. These processes include the interaction of amide groups of the CSP and the analyte combined with a parallel arrangement of the aromatic systems and steric interactions. One of these systems was investigated also by spectroscopic methods[12,13], confirming the importance of π–π interactions. In this paper we introduce an alternative method for studying π–π interactions in chiral chromatography.

To investigate the chiral separation mechanism of 3,5-DNB compounds on π-donor phase, we synthesized a series of π-acceptor compounds. The general structure of the chiral phenylethylamine (PEA) derivatives is shown in Fig. 1. These PEA compounds were chromatographed on (*R*)-N-pivaloylnaphthylethylamide [(*R*)-PNEA], a π-donor stationary phase considered previously[14] (see Fig. 2).

Despite the structural similarity of the compounds, the capacity factors varied considerably. To study the chiral recognition, it was necessary to separate non-specific achiral effects due to the general polarity of the compounds from specific chiral effects due to complexation of the sample with the chiral moiety. We therefore use the relative selectivity k'^* [$k'^* = k'$(chiral phase)/k'(achiral phase)] to determine the tendency for each enantiomer to complex with the chiral stationary phase. An achiral phase is used as a reference phase to measure the polarity of a compound. Of course, the k'^* values depend on the choice of the reference phase. However, within a series of compounds, one can expect low k'^* values for compounds that are retained non-specifically and high k'^* values for compounds that are retained specifically on the chiral columns. On chiral π-donor columns, the latter should apply for compounds with a good π-acceptor group being retained by π–π interactions. For the other compounds, k'^* should be small and constant. If π–π interactions are important, there should be a correlation of the k'^* values with the electron affinity of the π-acceptor group[15]. As a measure of the electron affinity we use the LUMO (lowest unoccupied molecular orbital[16]) of the π-acceptor group. The concept of relative selectivity can also be used for a detailed discussion of the separation of a homologous series of 3,5-dinitrobenzoylamides on structurally related π-donor chiral stationary phases[17].

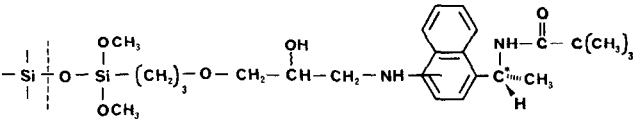


Fig. 2. Structure of the chiral π-donor phase (*R*)-N-pivaloylnaphthylethylamide [(*R*)-PNEA] used (mixture of positional isomers).

EXPERIMENTAL

The compounds were synthesized according to literature methods. Their identities were checked by NMR and mass spectrometry and elemental analysis.

Chromatography was performed on a Shimadzu LC-6A system at room temperature with *n*-hexane–isopropanol (4:1) as eluent. The dead volume was determined with toluene. The chiral phase (*R*)-PNEA was synthesized according to the literature[14] based on Daltosil (4 μm) (Serva, Heidelberg, F.R.G.). The achiral phase was aminopropyl-Si100 polyol (5 μm) (Serva). Columns of 250 × 4.6 mm I.D. were used at a flow-rate of 2 ml/min.

Structures were minimized by the semi-empirical quantum mechanical AMPAC program (QCPE Program No. 506) on a VAX-8650 and a FPS-164 computer using the AM1 and precise options. Conformational analysis was performed utilizing the molecular modelling package Chem-X (Molecular Modelling Systems, Chemical Design, Oxford, U.K.) and the local minima so found were reoptimized with AMPAC.

RESULTS AND DISCUSSION

Table I gives the chromatographic data for some achiral benzene derivatives on the chiral phase (*R*)-PNEA and on an aminopropyl phase, using the same eluent. The choice of the achiral reference column is arbitrary. An aminopropyl phase was chosen because there is a secondary amine present in the chiral phase. Without doing quantitative correlations, it can be seen that the k' values in Table I (on the chiral and achiral phases) do not correlate with the calculated molecular parameter LUMO. To do this, additional parameters for the general polarity of the compounds need to be considered, *e.g.*, the logarithm of the octanol–water partition coefficient[18,19]. This would include hydrophobicity effects.

With the exception of 1,4-dinitrobenzene, the relative selectivity k'^* generally increases with decreasing LUMO. This means that the chiral phase with the π-donor moiety shows a greater tendency than the achiral phase to retain the π-acceptor

TABLE I

π–π-EFFECTS FOR ACHIRAL BENZENE DERIVATIVES ON (*R*)-PNEA RELATIVE TO AN AMINOPROPYL PHASE

The capacity factors were measured with *n*-hexane –isopropanol (4:1). Each LUMO was calculated with AMPAC. The relative selectivity k'^* is the ratio k' (PNEA)/k'(aminopropyl).

Compound	*k'(PNEA)*	*k'(amino)*	*k'**	*LUMO*
Benzene	0	0	–	0
Nitrobenzene	0.49	0.20	2.5	−1.06
1,4-Dinitrobenzene	0.93	0.37	2.5	−2.21
1,2-Dinitrobenzene	4.15	1.44	2.9	−1.84
1,3-Dinitrobenzene	1.62	0.54	3.0	−2.08
1,3-Dinitro-5-cyanobenzene	2.59	0.83	3.1	−2.26
1,3,5-Trinitrobenzene	2.81	0.82	3.4	−2.53

samples in Table I. For a series of structurally related compounds the empirical k'^* value can therefore be used as an indicator of π–π interactions in retention processes.

Table II shows similar chromatographic data for some substituted achiral benzamide derivatives. There is no correlation between the relative selectivity k'^* and the LUMO. With the exception of 3-nitrobenzamide, k'^* correlates with the LUMO for those benzamides whose substituents are in the plane of the aromatic ring. As in Table I, k'^* generally increases with decreasing LUMO. 3,5-Diaminobenzoylamide, a π-donor compound, also has a low relative selectivity.

Of course, the relative selectivity of a compound does not depend exclusively on the π-acceptor group. The effects of the N-substituent is shown in Table III for 3,5-dinitrobenzamides. Relative to the unsubstituted 3,5-dinitrobenzamide, the substituted test compounds have smaller capacity factors on the aminopropyl phase, which reflects the general polarity of these compounds. However, on the chiral phase, not all capacity factors are smaller. This shows that the complexation of the samples with the chiral phase can be very tight owing to additional interactions. Therefore, in order to rationalize the retention mechanism of 3,5-dinitrobenzoyl amides on PNEA, attractive interactions between the N-substituents and the phase must be considered.

The data in Tables I–III show that the chiral phase is on average 2.5 times more polar than the aminopropyl phase used in our investigation. A relative selectivity k'^* between 2 and 3 corresponds to non-specific complexation of the sample due to some sort of hydrophobic effects on the chiral and the achiral phases. Compounds with a relative selectivity higher than 3 are retained on the chiral phase due to specific interactions. Only for such compounds can one expect a large chiral separation to occur. On the other hand, small α-values can be due to interactions that are less specific or due to various specific interactions that cancel the effect of each other. These aspects were studied with the chiral PEA derivatives in Fig. 1.

TABLE II

π–π EFFECTS FOR ACHIRAL BENZAMIDE DERIVATIVES ON (*R*)-PNEA RELATIVE TO AN AMINOPROPYL PHASE

Details as in Table I. In each instance the LUMO of the N-methyl compound was calculated.

Substituent	*k' (PNEA)*	*k' (amino)*	*k'**	*LUMO*
None	3.31	1.71	1.9	−0.19
3,5-Diamino	31.5	11.2	2.8	+0.18
3-Cyano	7.23	2.85	2.5	−0.74
4-Cyano	7.76	3.09	2.5	−0.89
2-Nitro[a]	21.3	6.06	3.5	−1.33
3-Nitro	9.04	2.69	3.4	−1.31
4-Nitro	7.70	2.48	3.1	−1.51
3-Nitro-5-methoxycarbonyl	7.80	2.57	3.0	−1.57
2,6-Dinitro[a]	25.4	12.1	2.1	−1.87
3,4-Dinitro[a]	22.1	7.12	3.1	−2.14
3,5-Dinitro-2-methyl[a]	7.22	2.79	2.6	−2.11
3.5-Dinitro	9.78	2.82	3.5	−2.23

[a] Flat arrangement of the molecule very unfavourable according to AMPAC.

TABLE III

RELATIVE SELECTIVITIES, k'^*, FOR SOME N-SUBSTITUTED 3,5-DINITROBENZOYL AMIDES ON (*R*)-PNEA RELATIVE TO AN AMINOPROPYL PHASE

Details as in Table I.

Substituent	*k' (PNEA)*	*k' (amino)*	*k'**
None	9.78	2.82	3.5
CH_3	7.51	2.13	3.5
C_5H_5[a]	7.58	2.22	3.4
cyclo-C_6H_{11}	4.08	1.01	4.0
CH_2–C_6H_5	7.92	1.60	5.0
CH_2–1-naphthyl[b]	15.7	2.06	7.6
CH_2–C_6H_5 and CH_3	2.16	0.82	2.6
CH_2–C_6H_4–*o*-F	7.42	1.60	4.6
CH_2–C_6H_4–*o*-OCH_3	8.94	1.87	4.8
CH_2–C_6H_3–*m,p*-Cl_2	9.22	2.53	3.6
CH_2–C_6H_3–*o,p*-Cl_2	6.98	1.63	4.3

[a] Significantly different LUMO owing to conjugation with the N-substituent.

[b] Highest *k'* (PNEA), although the compound is less polar than many of the other compounds within this series.

Table IV gives the chromatographic and calculated data for the chiral test compounds. The changes in the *k'* and α-values must be attributed to the benzamide moiety, the only part varied. It is obvious that the k_1' and k_2' do not correlate with the separation factor α. The capacity factors are very high if the amide group is far out of the plane of the aromatic ring (2-nitro- and 2,6-dinitro compounds). The corresponding retention process does not give a large chiral separation. The specific retention of the test compounds on (*R*)-PNEA, expressed as k'^*, is high if the amide group is almost coplanar with the aromatic group and if the compound has a low LUMO (3-nitro-5-methoxycarbonyl-, 3,5-dicyano-, 3-nitro-5-cyano- and 3,5-dinitro-compounds). For these compounds without any substituents *ortho* to the amide and with planar substituents, the chiral separation factor α increases with decreasing LUMO. Additionally, both $k_1'^*$ and $k_2'^*$ increase with decreasing LUMO. One can therefore conclude that the first and second eluted enantiomers of these compounds are retained by similar retention processes, both involving π–π interactions. For the other compounds in Table IV alternative retention processes are probable.

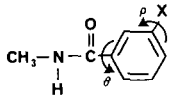
As is to be expected, the separation factor α correlates generally with the relative selectivity k'^*. Within the series, k_1^* is not constant and can reach significant values, *e.g.*, the relative selectivity $k_1'^*$ of the 3,5-DNB compound is higher than most of the $k_2'^*$ values in Table IV. Therefore, a proper discussion of retention mechanisms for a series of compounds should include the specific complexation of the first and second eluted enantiomers. In our series of compounds we did not find a derivative of PEA with high $k_1'^*$ and $k_2'^*$ (greater than 3) and a small α-value. No competing chiral processes are operating in our system of PEA–benzamides of (*R*)-PNEA.

The naphthoyl derivatives have a very low LUMO. Unfortunately, the nitro groups are not in the plane of the aromatic system. The chiral recognition is not better than that for the 3,5-DNB derivative. The naphthoyl compounds were not eluted

TABLE IV

CHROMATOGRAPHIC RESULTS OF THE CHIRAL PEA COMPOUNDS ON (*R*)-PNEA AND ON AN AMINOPROPYL PHASE, AND CALCULATED DATA

k_1' = capacity factor of the first eluted enantiomer; k_2' = capacity factor of the second eluted enantiomer; α = separation factor; k' (*a*) = k' (amino); k'* and LUMO, see Tables I and II. AC, absolute configuration of the better retained enantiomer. The naphthoyl derivatives were chromatographed with *n*-hexane–isopropanol (3:2); ne = not eluted. R according to Fig. 1. The net atomic charges on the amide hydrogens were virtually constant throughout the series. Definition of angle θ, ρ_1, ρ_2 and ρ_3 as illustrated.



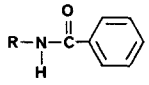
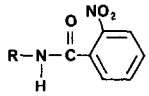
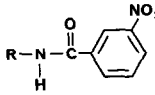
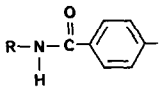
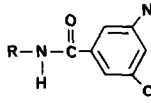
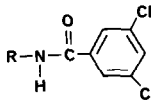
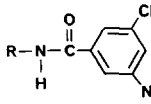
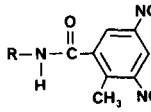
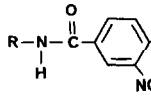
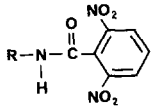
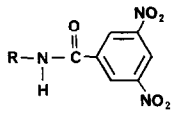
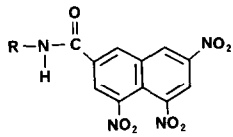
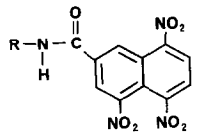
Structure	k_1'	k_2'	α	k' (*a*)	k_1'*	k_2'*	*AC*	*LUMO*	θ	ρ_1	ρ_2	ρ_3
	1.88	1.97	1.04	0.91	2.07	2.16	*R*	—0.11	37[a,b]			
	8.57	8.91	1.04	2.96	2.90	3.01		—1.27	70	33		
	3.75	4.48	1.14	1.52	2.47	2.82	*R*	—1.31	34	0		
	2.89	3.20	1.11	1.43	2.02	2.24		—1.46	40	0		
	4.36	5.98	1.37	1.22	3.57	4.90	*R*	—1.57	39	0	0	
	4.34	6.52	1.50	1.81	2.36	3.54		—1.20	37			
	5.58	9.15	1.70	1.71	3.15	5.35		—1.73	38	0		
	2.40	2.60	1.08	1.29	1.86	2.02	*R*	—2.11	74	20	0	
	5.53	6.78	1.23	3.12	1.77	2.17		—2.11	37	38	38	
	17.2	17.9	1.04	7.07	2.43	2.53		—1.93	80	35	15	

TABLE IV (*continued*)

Structure	k_1'	k_2'	α	*k' (a)*	k_1'*	k_2'*	*AC*	*LUMO*	θ	ρ_1	ρ_2	ρ_3
	7.09	13.3	1.88	1.52	4.66	8.75	*R*	—2.23	37	0	0	
	8.30	14.5	1.75	ne	–	–		—2.71	35	40	40	0
	6.4	11.5	1.80	ne	–	–		—2.85	35	40	40	35

[a] In good agreement with X-ray data (26.1° for benzamide[20]).
[b] Planar conformation less stable by 1.05 kcal/mol.

from the aminopropyl column with *n*-hexane–isopropanol. Therefore, the relative selectivity was not accessible.

The relative selectivities of the chiral compounds correlate well with the relative selectivities of the corresponding achiral compounds in Table II. This indicates that the π-acceptor amide group controls the retention in both instances. To study further the retention mechanism of 3,5-dinitrobenzoyl compounds with π-donors, we calculated the most favourable conformations of simplified PNEA and the chiral PEA–3,5-DNB as shown in Fig. 3. The chosen 3,5-DNB sample is a simple case with only two conformational minima. (*R*)-PNEA is more complicated, with three minima. The corresponding data are given in Table V. Fig. 4 shows stereo views of PEA–3,5-DNB and (*R*)-PNEA in their minimum energy conformations. Using these conformations, diastereomeric adsorption complexes according the "hydrogen-bonded" or the "dipole-stacking" process[4] cannot be formed without considerable conformational changes. This was studied using computer assisted molecular modeling; the results will be published elsewhere[21]. Although we do not propose any structure for the diastereomeric adsorption complexes in this paper, we would point out that the relative selectivity (and the capacity factor) for the achiral benzyl–3,5-DNB (see Ta-

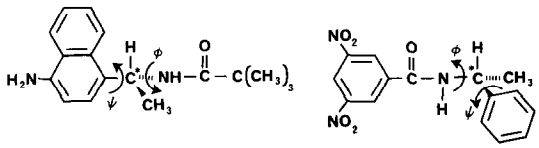


Fig. 3. Structure of the compound used to simulate (*R*)-PNEA with the computer and the chiral test compound (*R*)-PEA–3,5-DNB. For (*R*)-PNEA $\Phi = C_1$–C*–N–C and for $\Psi = C_2$–C_1–C*–N [analogously for (*R*)-PEA–3,5-DNB].

TABLE V

COMPUTED AMPAC ENERGETIC MINIMA, E (kcal/mol), OF (*R*)-PNEA AND THE 3,5-DINITROBENZOYLAMIDE OF (*R*)-PEA

The torsion angles Φ and Ψ are defined as shown in Fig. 3.

Parameter	*(R)-PNEA (min. 1)*	*(R)-PNEA (min. 2)*	*(R)-PNEA (min. 3)*	*(R)-PEA–3,5-DNB (min. 1)*	*(R)-PEA–3,5-DNB (min. 2)*
Φ	114.4°	120.8°	−82.1°	102.8°	−79.1°
Ψ	91.4°	−118.8°	68.9°	24.2°	68.7°
E	−17.00	−15.32	−14.09	24.17	26.17

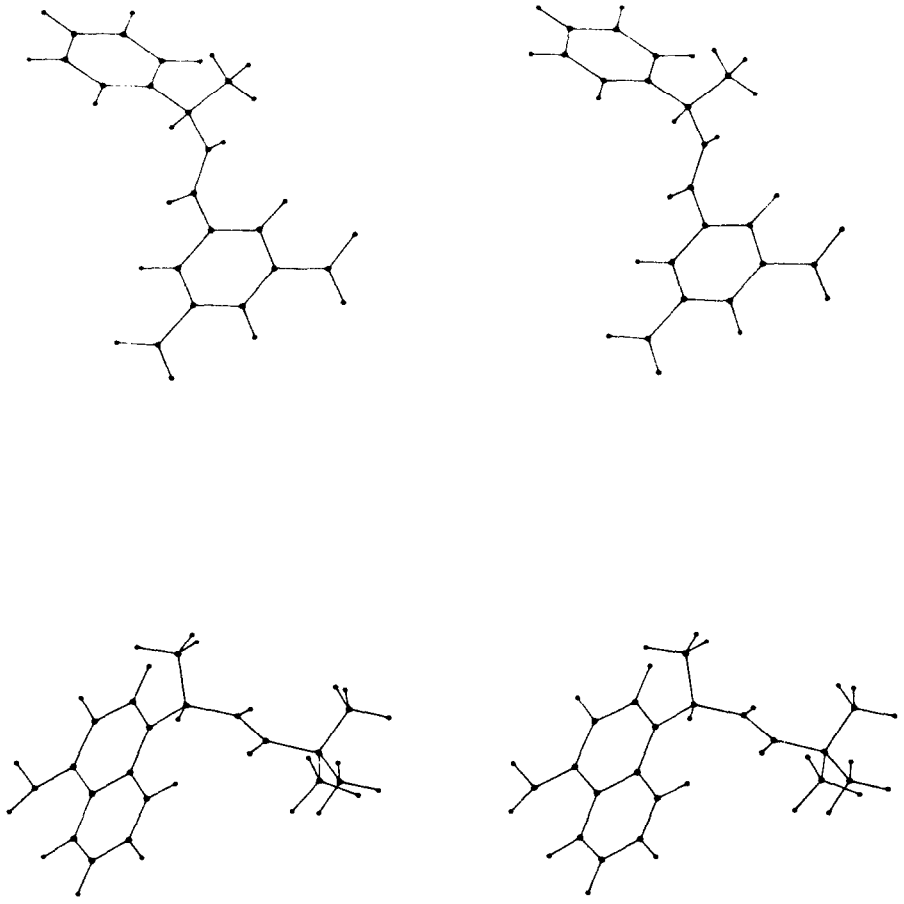


Fig. 4. Stereo view of the energetically most favourable conformations of (*R*)-PNEA and (*R*)-PEA–3,5-DNB. as calculated with AMPAC.

ble III) is closer to the relative selectivity of the first eluted (*S*)-PEA–3,5-DNB (see Table IV) than the last eluted (*R*)-PEA–3,5-DNB. This is not easily rationalized using the concept of attractive interactions of the π- and amide groups combined with steric repulsion of alkyl and aryl groups. To rationalize this elution order we therefore propose to consider several attractive interactions of (*R*)-PEA–3,5-DNB with (*R*)-PNEA (interaction of the benzamide group and interactions of the phenyl and the methyl group). For the (*S*)-PEA–DNB less attractive interactions (interaction of the benzamide group and interaction of the phenyl or methyl group) would be possible. The achiral benzyl–3,5-DNB also has less possibility of interacting with (*R*)-PNEA than the (*R*)PEA–3,5-DNB (interaction of the benzamide group and interaction of the phenyl group), resulting in a similar retention to that of the (*S*)-PEA–3,5-DNB.

CONCLUSIONS

For the series of phenylethylamine–π-acceptor amides on the π-donor stationary phase (*R*)-PNEA, the separation factor α generally increases with increasing relative selectivity $k_2'^*$. There was no case with high $k_2'^*$ and small α. However, the relative selectivities $k_1'^*$ are neither constant nor negligible. The 3,5-dinitrobenzoylamide group has a great tendency to complex with the chiral phase. This seems to be possible owing to lack of *ortho* substituents (favouring a flat arrangement of the benzamide moiety) and a low LUMO. The π–π interaction and the interaction of the amide groups bring the sample into very close proximity with the phase, so that the other groups at the chiral centre can interact with the phase. More investigations are necessary to decide whether the concept of steric repulsion or a concept based on Van der Waals attractions must be used to discuss the chiral recognition processes.

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