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Unusual examples of the liquid chromatographic resolution of racemates
Resolution of π-donor analytes on a π-donor chiral stationary phase

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Abstract

A π-basic chiral stationary phase (CSP) derived from (*S*)-1-(6,7-dimethyl-1-naphthyl)isobutylamine was used for the resolution of the enantiomers of π-basic 3,5-dimethylanilide derivatives of non-steroidal anti-inflammatory drugs related to α-arylpropionic acids. The separation factors are large enough for analytical purposes, baseline resolution being obtained. From the resolution of 3,5-dimethylanilide, N-methyl-3,5-dimethylanilide, N-alkylamide and N,N-dialkylamide derivatives of naproxen, it was concluded that the π-basic aryl derivatizing group plays a role as a hydrogen bond acceptor in the chiral recognition process and the amide NH hydrogen of analytes is essential for chiral recognition.

1. Introduction

Pirkle-type chiral stationary phases (CSPs) typically resolve the enantiomers of racemic analytes through π-donor–acceptor interactions between the CSP and the analyte [1–3]. To utilize π-donor–acceptor interactions, Pirkle-type CSPs incorporate π-acidic and/or π-basic aryl functional groups to interact with complementary aryl groups in the analytes. Racemic analytes which lack π-acidic or π-basic aryl functional groups are commonly derivatized with achiral π-acidic or π-basic reagents. CSPs containing π-acidic aryl groups have been used for the resolution of the enantiomers of π-basic analytes [4,5]. Similarly, CSPs containing π-basic aryl groups have been used for the resolution of the enantiomers of π-acidic analytes [6]. CSPs containing both of π-acidic and π-basic aryl groups have been utilized to resolve either π-basic or π-acidic analytes [7,8]. In each case, a π–π interaction between the CSP and the analyte is believed to play an important role in chiral recognition. In the absence of π–π interaction, the ability to separate enantiomers on Pirkle-type CSPs is expected to be greatly reduced. Accordingly, the resolution of π-acceptor racemates on π-acceptor CSPs or the resolution of π-donor racemates on π-donor CSPs is of some interest in that they are unexpected.

The resolution of π-acceptor racemates on π-acceptor CSPs has been reported. For exam-

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ple, Lienne et al. [9] reported that N-(3,5-dinitrobenzoyl) derivatives of α-aminoamides or α-amino esters (π-acceptor racemates) are resolved with reasonable separation factors on the π-acceptor CSP derived from N-(3,5-dinitrobenzoyl)-(*S*)-tyrosine. However, it should be noted that the π-basic aryl group present in Tambute's CSP may conceivably serve as a π-donor in the chiral recognition process. In contrast, the resolution of π-donor analytes on π-donor CSPs with more than marginal separation factors has not been reported except for the resolution of the enantiomers of arylhydantoins on N-aryl-α-amino acid CSPs described in a thesis [10]. The recent report by Oliveros et al. [11] of the resolution of some π-donor analytes on π-donor CSPs has been questioned by Pirkle et al. [12] and aroused our attention in this area.

In this paper, we report that CSP **1**, widely used for the resolution of a variety of π-acidic racemates [13–17], can be successfully used for the resolution of the enantiomers of the 3,5-dimethylanilide derivatives of non-steroidal anti-inflammatory drugs (NSAIDs) related to α-arylpropionic acids. This study may provide good examples for the unusual liquid chromatographic resolution of π-donor analytes on π-donor CSPs.

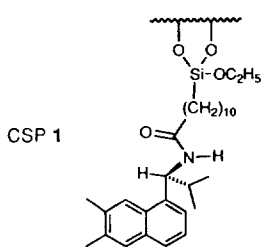
2. Experimental

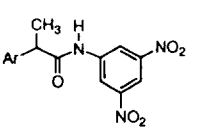
The HPLC system consisted of a Waters (Milford, MA, USA) Model 510 pump, a Rheodyne (Cotati, CA, USA) Model 7125 injector with a 20-μl sample loop, a Youngin (Seoul, Korea) Model 710 absorbance detector with a 254-nm UV filter and a Youngin D520B computing integrator. All chromatographic data were obtained using 2-propanol–hexane (90:10) as the mobile phase at a flow-rate of 2.0 ml/min. The column void volume was measured by injecting 1,3,5-tri-*tert.*-butylbenzene obtained from Regis (Morton Grove, IL, USA) [18].

A stainless-steel chiral HPLC column (250 mm × 4.6 mm I.D.) packed with CSP **1** (support, Spherisorb 5-μm silica gel; loading level, 0.21 mmol of chiral selector per gram of stationary phase based on either C or N) was available from previous studies [15] and was used unchanged. Analytes were also available from previous studies or were prepared by treating acid chlorides with arylamines or alkylamines as described previously [19,20].

3. Results and discussion

CSP **1** has been used for the resolution of the 3,5-dinitroanalide derivatives, **2**, of NSAIDs related to α-arylpropionic acids [21]. In these resolutions, a chiral recognition mechanism utilizing a face-to-face π-donor–acceptor interaction, a face-to-edge π–π interaction and a hydrogen-bonding interaction between the CSP and the analytes was proposed [20]. Based on the chiral recognition mechanism proposed, the 3,5-dimethylanilide derivatives, **3**, of NSAIDs are expected to show little or no resolution on CSP **1** because of the lack of a π-acidic group in the type **3** analytes. Surprisingly, we found that CSP **1** resolves the enantiomers of the type **3** analytes with reasonable separation factors.





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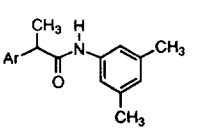

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The chromatographic data for the resolution of the enantiomers of 3,5-dimethylanilide and 3,5-dinitroanilide derivatives of α-phenylpropionic acid and NSAIDs on CSP **1** are compared in Table 1. Retention factors, *k'*, for the resolution of the 3,5-dimethylanilide derivatives, **3**, on CSP **1** are much smaller than those of the 3,5-dinitroanalide derivatives **2**, as shown in

Table 1
Resolution of 3,5-dinitroanilide derivatives **2** and 3,5-dimethylanilide derivatives **3** of α-phenylpropionic acid and NSAIDS on CSP **1**

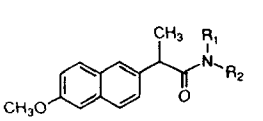
NSAID	2			3		
	k_1'	α	Conf.	k_1'	α	Conf.
α-Phenylpropionic acid	13.36	1.84	*R*	2.86	1.27	*R*
Ibuprofen	7.76	2.12	*R*	1.59	1.28	*R*
Naproxen	20.17	1.42	*R*	4.14	1.31	*R*
Fenoprofen	10.83	1.61		3.02	1.29	
Flurbiprofen	11.93	1.64		3.88	1.31	
Ketoprofen	10.10	2.00		3.95	1.23	
Alminoprofen	4.47	3.01		3.66	1.25	

Conditions: mobile phase, 2-propanol–hexane (10:90); flow-rate, 2.0 ml/min; temperature, ambient. k_1' = Retention factor for the first-eluted enantiomer; α = separation factor; Conf. = absolute configuration of the second-eluted enantiomer.

Table 1. The separation factors, α, are also lower when the derivatizing group is changed from the π-acidic 3,5-dinitroanalide to the π-basic 3,5-dimethylanilide. However, the separation factors for the 3,5-dimethylanilide derivatives, **3**, of NSAIDs on CSP **1** are large for CSP **1** to be used for analytical purposes. The elution orders shown in Table 1 were determined by comparing the chromatograms from racemic samples with those from optically active samples prepared from commercially available α-phenylpropionic acid, ibuprofen and naproxen. The elution orders for the 3,5-dimethylanilide derivatives, **3**, on CSP **1** determined in this way are in accord with those for the 3,5-dinitroanilide derivatives, **2**, as shown in Table 1.

To see the effect of the aryl derivatizing group on the resolution trends, various anilide derivatives of α-phenylpropionic acid, ibuprofen, naproxen and simple N-alkyl- and N,N-dialkylamide derivatives of naproxen were prepared and resolved on CSP **1**. The results from the chromatography of various anilide derivatives of α-phenylpropionic acid, ibuprofen and naproxen on CSP **1** are summarized in Table 2. One example is shown in Fig. 1. The results obtained by chromatographing simple N-alkyl- and N,N-dialkylamide derivatives, **4**, of naproxen on CSP **1** are given in Table 3. It is noted from Table 2 and Fig. 1 that the retention of both enantiomers decreases drastically as the derivatizing aryl group is changed from 3,5-dinitrophenyl to 3-nitrophenyl to phenyl itself. In contrast, the retention decreases only slightly as the aryl derivatizing group is changed from phenyl to 3-methylphenyl to 3,5-dimethylphenyl. In addition, the retention of a simple ethyl- or propylamide derivative of naproxen is comparable to that of a π-basic aryl derivative of naproxen, as noted from Tables 2 and 3. From these observations, it is presumed that the face-to-face π–π interaction between the π-basic aryl group of the CSP and the π-acidic aryl group such as 3,5-dinitrophenyl or 3-nitrophenyl of the analyte is the major component of the interactions leading to retention of the enantiomers. However, π-basic aryl derivatizing groups such as phenyl, 3-methylphenyl and 3,5-dimethylphenyl do not seem to contribute to a face-to-face π–π interaction between the CSP and the analyte. The slight decrease in retention as the aryl derivatizing group is changed from phenyl to 3-methylphenyl to 3,5-dimethylphenyl may come from the increased lipophilicity of the analyte.

The separation factors, α, also vary according to the aryl derivatizing group, as shown in Table 2. In general, the analytes containing a π-acidic aryl derivatizing group have larger separation factors than those containing a π-basic aryl derivatizing group. However, the analytes containing a π-basic aryl derivatizing group show baseline resolution with reasonably good separation factors, as shown in Fig. 1. It is interesting to note in Table 2 that changing the π-basic aryl derivatizing group does not have any notable effect on the separation factors of the enantiomers, indicating that the π-basicity or the bulkiness of the aryl derivatizing group plays no significant role in the chiral recognition.



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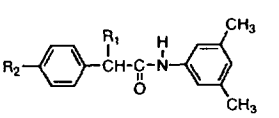

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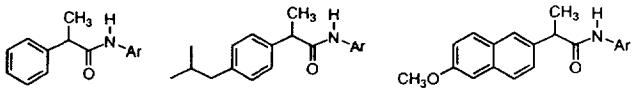
Simple N-alkylamide derivatives of naproxen are also resolved on CSP **1** even though the

Table 2
Resolution of various anilide derivatives of (A) α-phenylpropionic acid, (B) ibuprofen and (C) naproxen on CSP **1**



(A) (B) (C)

Parent	Ar	k'_1	k'_2	α	Conf.
A	3,5-Dimethylphenyl	2.86	3.62	1.27	*R*
	3-Methylphenyl	3.02	3.74	1.24	*R*
	Phenyl	3.46	4.22	1.22	*R*
	3-Nitrophenyl	5.58	8.00	1.43	*R*
	3,5-Dinitrophenyl	13.36	24.56	1.84	*R*
B	3,5-Dimethylphenyl	1.59	2.04	1.28	*R*
	3-Methylphenyl	1.64	2.11	1.29	*R*
	Phenyl	1.87	2.34	1.25	*R*
	3-Nitrophenyl	3.15	4.83	1.53	*R*
	3,5-Dinitrophenyl	7.76	16.45	2.12	*R*
C	3,5-Dimethylphenyl	4.14	5.43	1.31	*R*
	3-Methylphenyl	5.55	7.29	1.31	*R*
	Phenyl	6.43	8.41	1.31	*R*
	3-Nitrophenyl	10.53	15.21	1.44	*R*
	3,5-Dinitrophenyl	20.17	28.64	1.42	*R*

Conditions: mobile phase, 2-propanol–hexane (10:90); flow-rate, 2.0 ml/min; temperature, ambient. k'_1 = Retention factor for the first-eluted enantiomer; k'_2 = retention factor for the second-eluted enantiomer; α = separation factor; Conf. = absolute configuration of the second-eluted enantiomer.

separation factors, α, are small, as shown in Table 3. However, N,N-dialkylamide derivatives of naproxen are not resolved at all on CSP **1**. Similarly, the N-methyl-3,5-dimethylanilide of naproxen is not resolved on CSP **1**. It is also noted from Table 3 that variation in the length or the bulkiness of the N-alkyl substituent of an N-alkylamide derivative of naproxen does not produce a marked effect on the separation factors.

From these results, it is presumed that the π-basic aryl derivatizing group does play a role

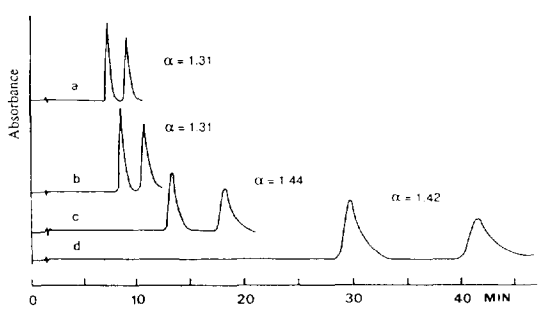


Fig. 1. Resolution of (a) 3,5-dimethylanilide, (b) anilide, (c) 3-nitroanilide and (d) 3,5-dinitroanilide derivatives of racemic naproxen on CSP **1**. For chromatographic conditions, see Experimental.

Table 3
Resolution of N-alkyl- and N,N-dialkylamide derivatives **4** and *N*-methyl-3,5-dimethylanilide of naproxen on CSP **1**

4		k'_1	k'_2	α
R_1	R_2			
H	CH_2CH_3	6.72	7.33	1.09
H	$CH_2CH_2CH_3$	4.59	5.08	1.11
H	$(CH_2)_9CH_3$	2.42	2.71	1.12
H	$C(CH_3)_3$	2.20	2.46	1.12
CH_2CH_3	CH_2CH_3	1.98	1.98	1.00
$CH(CH_3)_2$	$CH(CH_3)_2$	0.88	0.88	1.00
CH_3	3,5-Dimethylphenyl	0.88	0.88	1.00

Conditions: mobile phase, 2-propanol–hexane (10:90); flow-rate, 2.0 ml/min; temperature, ambient. k'_1 = Retention factor for the first-eluted enantiomer; k'_2 = retention factor for the second-eluted enantiomer; α = separation factor.

in the chiral recognition of the NSAID derivatives on π-basic CSP **1**. The role does not seem to be simply steric since π-basic aryl or N-alkyl groups of different size do not notably affect separation factors. However, hydrogen bonding interactions which utilize the π-basic aryl group as a hydrogen-bonding acceptor [22] could play a role in chiral recognition. It does seem that the amide NH is essential for chiral recognition of the analytes, as none of the tertiary amides of naproxen are resolved on CSP **1**, as shown in Table 3. However, a definitive statement concerning the mechanism by which these enantiomers are separated on π-basic CSP **1** must await further study.

In order to obtain more information about the chiral recognition mechanism, the 3,5-dimethylanilides, **5**, a homologous series of α-(*p*-alkylphenyl) alkanoic acids, were prepared and resolved on CSP **1**. In a previous study [20], we were able to provide a mechanistic rationale for the resolution of the 3,5-dinitroanilide derivatives of NSAIDs on CSP **1** by studying the chromatographic resolution of the enantiomers of 3,5-dinitroanilide derivatives of α-(*p*-alkylphenyl) alkanoic acids. Similarly, we thought that by variation of the length of the *p*-alkyl and α-alkyl substituents of the type **5** compounds, we might determine whether intercalation processes play a significant role in the chiral recognition of these analytes. However, variation of the lengths of these substituents has only a small effect on the separation factors for the enantiomers of the members of this series of analytes. These data are summarized in Table 4. Lengthening the α-alkyl group of **5** slightly diminishes the separation factors whereas lengthening the *p*-alkyl group of **5** slightly enhances the separation factors. Hence intercalation processes play no significant role in the chiral recognition of these analytes by CSP **1**.

In conclusion, we have shown that 3,5-dimethylanilide derivatives of NSAIDs can be resolved on CSP **1** with reasonably useful separation factors. These resolutions are unusual examples of the resolution of π-donor analytes on a π-basic chiral stationary phase. These resolutions are practically important in terms of saving analytical time and, in consequence, saving eluting solvent. For example, the resolution of the 3,5-dimethylanilide derivative of naproxen can be accomplished within 12 min whereas that of the 3,5--dinitroanilide derivative of naproxen requires almost 45 min for complete resolution, as shown in Fig. 1. The details of the chiral recognition mechanism employed by CSP **1** for the resolution of π-donor analytes is not yet clear and efforts to elucidate the chiral recognition mode are in progress.

Table 4
Resolution of 3,5-dimethylanilide derivatives **5** of α-(*p*-alkylphenyl)alkanoic acids on CSP **1**

5		k_1'	k_2'	α
α-Alkyl (R_1)	*p*-Alkyl (R_2)			
CH_3	H	2.86	3.62	1.27
CH_2CH_3	H	2.96	3.64	1.23
$(CH_2)_2CH_3$	H	2.84	3.62	1.27
$(CH_2)_4CH_3$	H	2.61	3.19	1.22
$(CH_2)_6CH_3$	H	2.38	2.90	1.22
$(CH_2)_9CH_3$	H	2.04	2.45	1.20
CH_3	CH_3	2.56	3.16	1.23
CH_3	$(CH_2)_2CH_3$	2.11	2.80	1.33
CH_3	$(CH_2)_5CH_3$	1.67	2.24	1.34
CH_3	$(CH_2)_7CH_3$	1.51	2.06	1.36
CH_3	$(CH_2)_9CH_3$	1.38	1.90	1.38

Conditions: mobile phase, 2-propanol–hexane (90:10); flow rate, 2.0 ml/min; temperature, ambient. k_1' = Retention factor for the first-eluted enantiomer; k_2' = retention factor for the second-eluted enantiomer; α = separation factor.

Acknowledgement

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