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IMPROVED CHIRAL STATIONARY PHASE FOR THE SEPARATION OF THE ENANTIOMERS OF CHIRAL ACIDS AS THEIR ANILIDE DERIVATIVES

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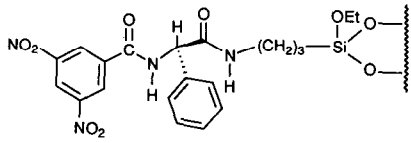
SUMMARY

Liquid chromatographic separation of the enantiomers of anilide derivatives of chiral carboxylic acids is facile on a chiral stationary phase derived from a conformationally restricted β-amino acid. This new chiral stationary phase, a variant of the (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine-derived chiral stationary phase, is markedly superior to its predecessor for separation of the enantiomers of a wide variety of anilides derived from carboxylic acids.

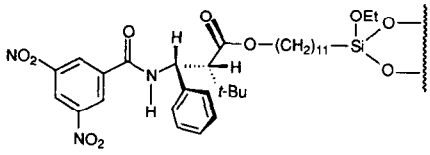
INTRODUCTION

A variant of the widely used and commercially available phenylglycine-derived chiral stationary phase (CSP), **1**, was recently described[1]. This stationary phase, CSP **2**, differs structurally from CSP **1** in two ways. First, a second center is, in effect, interposed between the single stereogenic center of CSP **1** and the carbonyl moiety which links it to the solid support. Secondly, CSP **2** is ester-linked to an eleven carbon connecting arm rather than amide-linked to a three carbon connecting arm. The enantiomers of many analytes show larger separation factors on CSP **2** than on CSP **1**. This is perhaps surprising since the distance between the primary interaction sites, the 3,5-dinitrobenzoyl group and the C-terminal carbonyl, is greater in CSP **2** than in CSP **1**. The second stereogenic center is not believed to be a "primary" source of enantiodifferentiation, but, in those cases where separation is greater on CSP **2**, is thought to complement the phenylglycine-like stereogenic center. Normally, increasing the distance between the interaction sites of either a CSP or an analyte will engender increased conformational freedom and a reduced level of chiral recognition. However, the bulky substituents on the vicinal stereogenic centers of CSP **2** confer considerable conformational rigidity, apparently holding the interaction sites in such spatial positions as to frequently enhance the chiral recognition properties of CSP **2**. Even so, the mode of enantiodifferentiation by CSP **2** is expected to be analogous to that of CSP **1**[2].

0021-9673/89/$03.50



CSP 1



CSP 2

As part of the on-going evaluation of CSP **2**, a number of anilides of chiral carboxylic acids were prepared and chromatographed, the data being presented herein. From an analysis of the relationships between analyte structure and chromatographic behavior, a chiral recognition model is advanced to rationalize the experimental observations.

EXPERIMENTAL

Apparatus

Chromatography was performed using a Bischoff Model 2200 isocratic high-performance liquid chromatography pump, a Rheodyne Model 7125 injector with a 20-μl sample loop, a 250 × 4.6 mm stainless-steel column packed with CSP **2** as described previously[1], two Milton Roy UV Monitor® D fixed-wavelength detectors (254 and 280 nm) connected in series, and a Kipp & Zonen Model BD 41 dual-pen chart recorder.

Reagents

Racemic ibuprofen was isolated from a Motrin® tablet. Ibuprofen was partially resolved according to the procedure of Nicoll-Griffth[3]. Fenoprofen was a gift of Eli Lilly and Company. The racemate and the (S)-(+) enantiomer of 2-phenylbuteric acid was obtained from Aldrich as were 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) and the various anilines and amines used herein. The remaining acids were available from prior studies.

Derivatization

The anilides were made either via the acid chloride or through the agency of EEDQ. Acids **1** and **5** were converted to the acid chlorides using thionyl chloride. The remaining acids were converted to the mixed anhydrides with EEDQ. The former derivatization sequence has been described[3].

General anilide synthesis using EEDQ

Equal quantities (*ca.* 10 mg) of the acid and EEDQ were added to a 5-ml screw-capped test tube followed by two drops of the aniline and 0.5 ml of

dichloromethane. After 30 min, 1.5 ml additional dichloromethane and 1 ml of 1 *M* sodium hydroxide was added, the mixture was shaken vigorously, and the upper layer was removed with a pipet. The lower layer was similarly washed several times with water, than 1 ml of 1 *M* hydrochloric acid was added. The mixture was shaken vigorously, centrifuged if necessary to separate layers (the higher-molecular-weight *p*-alkylanilines form emulsions when acidified; excesses of these reagents were avoided) and the upper layer was withdrawn. The lower layer was repeatedly washed with water, especially in the case of the higher-molecular-weight alkylanilines. The resulting solution was dried over anhydrous magnesium sulfate and analyzed directly. Early eluting impurities were noted in some instances but did not interfere with the analyses[3].

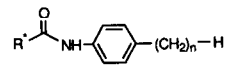
Conversion of otherwise non-functionalized acids to anilide derivatives is quite straightforward. To verify that the reported procedure does afford the intended derivatives, *p*-ethylanilides of acids **2–5** were prepared on a larger scale and characterized by NMR and IR spectroscopy and by elemental analysis. These characterization data are in accord with the assigned structures.

RESULTS AND DISCUSSION

The enantiomers of a variety of anilides of chiral carboxylic acids are separable on CSP **2**. Table I provides chromatographic data for the normal-phase separation of the enantiomers of a homologous series of *p*-alkyl anilide derivatives of 2-(α-naphthyl)propionic acid (**1**), ibuprofen (**2**), fenoprofen (**3**), α-isopropoxyphenylacetic acid (**4**) and 2-phenylbuteric acid (**5**) on CSP **2**. All analytes show considerably larger

TABLE I

NORMAL-PHASE SEPARATION ON (2*R*,3*R*)-CSP **2** OF CHIRAL ACIDS **1–5** AS THEIR *p*-ALKYLANILIDES



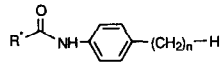
n	**1**		**2**		**3**		**4**		**5**	
	α^a	$k_1'^b$	α^a	$k_1'^b$	α^a	$k_1'^b$	α^a	$k_1'^b$	α^a	$k_1'^b$
0	1.94	8.37	1.54	3.71	1.49	5.87	1.71	2.76	1.64	4.20
1	1.91	11.4	1.57	5.01	1.47	8.08	1.71	3.60	1.68	6.00
2	1.91	9.40	1.56	4.13	1.47	6.73	1.70	3.07	1.69	4.80
4	1.98	8.00	1.55	3.57	1.47	5.73	1.74	2.51	1.69	4.20
6	1.96	6.93	1.51	3.33	1.46	5.13	1.71	2.33	1.68	3.73
8	1.96	6.27	1.53	2.96	1.46	4.67	1.73	2.09	1.66	3.44
10	1.96	5.71	1.51	2.77	1.44	4.40	1.71	1.87	1.65	3.13
12	1.99	5.20	1.52	2.56	1.44	3.97	1.73	1.73	1.67	2.80
14	1.98	4.83	1.52	1.37	1.43	3.73	1.75	1.60	1.65	2.67

[a] Chromatographic separation factor.

[b] Capacity factor for the first eluted enantiomer using 10% (v/v) 2-propanol in hexane as the mobile phase; flow-rate 2 ml/min.

TABLE II

REVERSED-PHASE SEPARATION ON (2*R*,3*R*)-CSP **2** OF CHIRAL ACIDS **1–5** AS THEIR *p*-ALKYLANILIDES

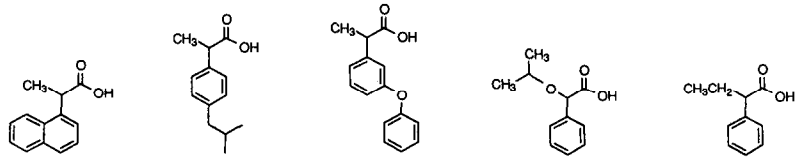


n	*1*		*2*		*3*		*4*		*5*	
	α^a	$k_1'^b$	α^a	$k_1'^b$	α^a	$k_1'^b$	α^a	$k_1'^b$	α^a	$k_1'^b$
0	1.68	0.87	1.32	0.49	1.21	0.66	1.31	0.42	1.34	0.22
1	1.68	1.22	1.32	0.75	1.25	0.90	1.30	0.64	1.38	0.39
2	1.64	1.39	1.35	0.77	1.25	0.97	1.27	0.71	1.38	0.42
4	1.65	1.74	1.34	1.08	1.25	1.26	1.27	0.92	1.33	0.58
6	1.62	2.32	1.34	1.42	1.23	1.73	1.28	1.26	1.31	0.84
8	1.61	3.16	1.32	1.92	1.23	2.26	1.27	1.71	1.32	1.16
10	1.61	4.24	1.32	2.58	1.36	2.74	1.24	2.39	1.28	1.58
12	1.60	5.68	1.30	3.45	1.22	4.13	1.24	3.11	1.30	2.10
14	1.61	7.61	1.30	4.64	1.21	5.54	1.23	4.21	1.27	2.93

[a] Chromatographic separation factor.

[b] Capacity factor for the first eluted enantiomer using methanol–water (9:1, v/v) as the mobile phase; flow-rate 1 ml/min.

chromatographic separation factors on CSP **2** than on CSP **1**[2]. Additionally, excellent reversed-phase separations are obtained for these analytes on CSP **2** (Table II).



1 **2** **3** **4** **5**

In order for enantiodifferentiation to occur, a minimum of three simultaneous interactions, at least one of which is enantiodependent, must occur between the analyte and the CSP[4]. From prior investigation of these anilide analytes on CSP **1**, there is reason to expect two competing chiral recognition mechanisms, having different modes of dipole stacking as important associative interactions[2,3,5,6]. These are shown in Figs. 1 and 2. The three interactions which lead to enantiodifferentiation in Fig. 1 are

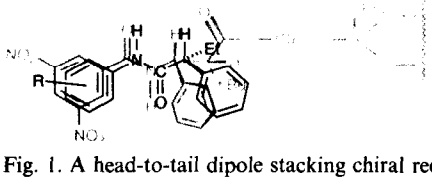


Fig. 1. A head-to-tail dipole stacking chiral recognition model.

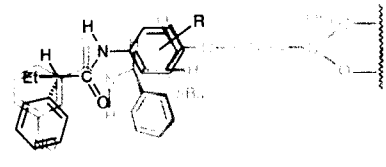


Fig. 2. A head-to-head dipole stacking chiral recognition model.

a "head-to-tail" dipole stack, a π–π association between the 3,5-dinitrobenzoyl moiety of the CSP and the anilide functionality of the analyte and a stereochemically dependent steric interaction between the analyte and the CSP. The alternate chiral recognition model (Fig. 2) is one in which the dipole stacking is of a "head-to-head" nature. In Fig. 2, a π–π interaction can occur between the aryl portion of the acid and the 3,5-dinitrobenzoyl group pf the CSP, the enantiodifferentiating interaction again being a steric interaction between the alkyl group on the stereogenic center of the analyte and the CSP.

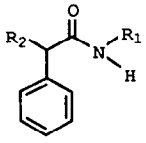
For analytes in which the π-basic aryl group is either the aniline system or is an aryl substituent on the stereogenic center of the acid portion of the molecule, the mechanism deemed most important for the retention of the more strongly retained enantiomer is clear; it is the dipole-stacking mode which permits simultaneous π–π bonding with the 3,5-dinitrobenzoyl group. For analytes having π-basic groups in both locations, the two processes are in competition. Since both stacking modes give rise to the same elution order, this datum is of no particular aid in distinguishing between the two competing stacking modes.

Note that in the head-to-tail mode, a substituent of the anilide in the *para*-position is directed away from the solid support and into the mobile phase, the converse being true for the head-to-head arrangement. Consequently, analysis of the chromatographic data for analytes derived from a homologous series of *p*-alkylanilides was expected to yield considerble mechanistic information. For example, if the head-to-tail arrangement is the dominant contributor to chiral recognition, the length of the *p*-alkyl substituent should have little effect on the chromatographic separation factor of the enantiomers. However, if the head-to-head arrangement is dominant, then the *p*-alkyl substituent should interact sterically with the neighboring strands of bonded phase and with the underlying solid support. This effect increases as the length of the *p*-substituent increases, causing a decrease in the stability of the diastereomeric adsorbate in which this is occurring. This results in reduced retention of this enantiomer relative to its antipode and hence a change in the magnitude of the chromatographic separation factor. This effect is expected to be the most pronounced for CSPs having short connecting arms (such as CSP **1**) and somewhat attenuated for CSPs having long connecting arms, such as CSP **2**. Examination of the data in Table I for the separation of the enantiomers of homologous series of the *p*-alkylanilides of chiral acids revels that no definite diminution of α is observed. This observation, though suggestive, does not necessarily rule out head-to-head stacking, owing to the aforementioned attenuation expected from the long connecting arm of CSP **2**.

If the head-to-tail stacking process is indeed operative, then increasing the π-basicity of the N-aryl substituent should increase the separation factors for the enantiomers. That this is the case is shown by the data for the N-1-naphthyl-, N-2-naphthyl- (Table III) and N-phenylamide (Table I) derivatives of **5**. Similarly, introduction of substituents on to the anilide ring, by altering π-basicity, should affect α. This is observed for anilides of **5**. The unsubstituted anilide has an α of 1.64 while the *p*-methylanilide gives an α of 1.68. The *p*-CF_3-, *p*-CN- and *p*-NO_2-anilides show α values of 1.52, 1.32 and 1.26, respectively. Similar trends are noted for 3,5-disubstituted anilides of **5**. The observation that α is directly affected by the π-basicity of the anilide moiety provides strong evidence for head-to-tail stacking being the dominant process involved in chiral recognition. Similar observations for ibuprofen

TABLE III

SEPARATION ON (2*R*,3*R*)-CSP 2 OF VARIOUS ANILIDES OF α-PHENYLBUTANOIC ACID AND 2-ISOPROPOXYPHENYLACETIC ACID



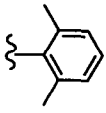
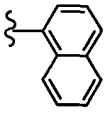
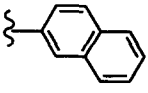
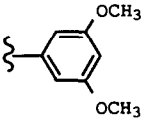
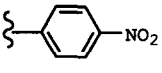
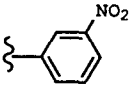
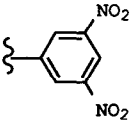
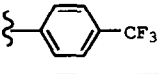
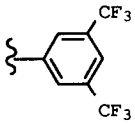
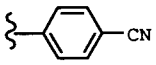
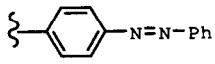
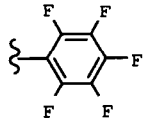
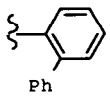
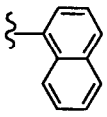
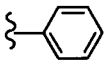
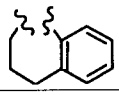
R_1	R_2	*Normal phase*		*Reversed-phase*	
		α^a	$k'_1{}^b$	α^a	$k'_1{}^c$
	C_2H_5	1.39	4.13	1.07	0.94
	C_2H_5	3.03	31.9	2.26	1.13[d]
	C_2H_5	2.28	27.7	1.81	2.00
	C_2H_5	2.01	10.5[d]	1.48	0.68[d]
	C_2H_5	1.26	3.24[d]	1.13	0.39[d]
	C_2H_5	1.29	3.04[d]	1.10	0.44
	C_2H_5	1.15	2.73[d]	1.06	0.52[d]
	C_2H_5	1.52	1.40[d]	1.20	0.26[d]

TABLE III *(continued)*

R_1	R_2	*Normal phase*		*Reversed-phase*	
		α^a	$k_1'^b$	α^a	$k_1'^c$
	C_2H_5	1.00	0.51	1.00	0.40
	C_2H_5	1.32	4.71	~1.00	0.32
	C_2H_5	1.58	4.37	1.20	129
	C_2H_5	1.43	1.27	1.00	≈1.00
	C_2H_5	1.31	2.13	1.15	0.56
	$(CH_3)_2CH$–O–	2.55	17.4	1.74	1.74
	$(CH_3)_2CH$–O–	1.76	2.87	1.28	0.45
		1.28	1.40[d]	1.10	0.68[d]

[a] Chromatographic separation factor.

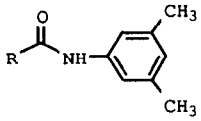
[b] Capacity factor for the first eluted enantiomer using 10% (v/v) 2-propanol in hexane as the mobile phase; flow-rate 2 ml/min.

[c] Capacity factor for the first eluted enantiomer using methanol–water (9:1, v/v) as the mobile phase; flow-rate 1 ml/min.

[d] The (*S*)-(+) enantiomer was most strongly retained.

TABLE IV

SEPARATION ON (2*R*,3*R*)-CSP **2** OF THE 3,5-DIMETHYLANILIDES OF VARIOUS CARBOXYLIC ACIDS



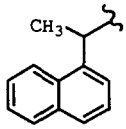
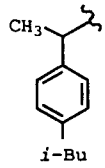
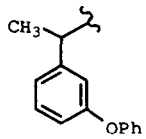
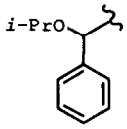
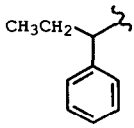
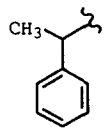
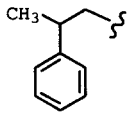
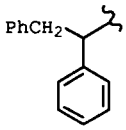
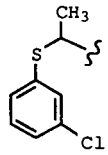
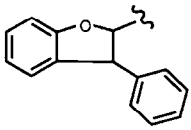
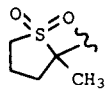
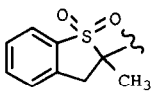
R	*Normal phase*		*Reversed-phase*	
	α^a	$k'_1{}^b$	α^a	$k'_1{}^c$
	2.90	16.3[d]	2.13	2.35[d]
	2.09	8.27[d]	1.70	1.48
	1.77	12.80	1.41	1.81
	2.21	4.16	2.02	0.76
	2.20	9.20[d]	1.77	0.77[d]
	2.25	9.40	1.65	0.77

TABLE IV

R	*Normal phase*		*Reversed-phase*	
	α^a	$k'_1{}^b$	α^a	$k'_1{}^c$
	1.33	11.3	1.22	0.84
	1.51	9.73	1.77	0.84
	1.74	8.71	1.48	1.14
	1.24	4.73	1.12	2.55
	1.46	9.20	1.22	0.57
	1.70	14.1	1.30	0.97

[a] Chromatographic separation factor.

[b] Capacity factor for the first eluted enantiomer using 10% (v/v) 2-propanol in hexane as the mobile phase; flow-rate 2 ml/min.

[c] Capacity factor for the first eluted enantiomer using methanol–water (9:1, v/v) as the mobile phase; flow-rate 1 ml/min.

[d] The (*S*)-(+) enantiomer was most strongly retained.

anilides have been reported by Nicoll-Griffith[3] and were used to support a similar mechanistic conclusion for the separation of these enantiomers on CSP **1**.

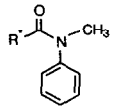
Table IV contains chromatographic data for the separation of a variety of chiral acids as their 3,5-dimethylanilides on CSP **2**. Note that the 3,5-dimethylanilide derivatives show enantioselectivity which, using both normal and reversed-phase conditions, surpasses that shown by the enantiomers of the *p*-alkylanilide derivatives of acids **1–5** (Tables I and II). This observation is also consistent with the dominance of a head-to-tail process.

Tertiary amide derivatives of acids **1–5** were prepared to determine the effects of hydrogen bonding interactions between the anilide proton of the analyte and a basic site on the CSP. If the anilide N–H participates in some essential hydrogen bonding interaction, its replacement by an alkyl substituent would be expected to seriously erode chiral recognition, consequently reducing α significantly. Chromatographic data for the separation of the N-methylanilides of acids **1–5** on CSP **2** are provided in Table V. The chromatographic separation factors for the enantiomers of the N-methyl anilides of acids **2**, **3** and **5** are slightly reduced, but still comparable in magnitude to those noted for the corresponding anilides (Table I). However, the capacity ratios of the former are significantly reduced relative to the latter. These results indicate that hydrogen bonding of the anilide N–H proton is not essential to chiral recognition of these analytes, although its presence may lead to achiral retention. Indeed, the N-methyl anilide of **1** exhibits a considerably larger α than does the anilide itself.

The chromatographic separation factor for separation of the enantiomers of the N-methylanilide of acid **4** is reduced and the capacity ratio for the first eluted enantiomer is much larger than that noted for the corresponding anilide. Apparently, hydrogen bonding of the anilide N–H proton of this analyte contributes significantly to the overall chiral recognition process. However, the hydrogen bond may be to the oxygen of the neighboring isopropoxyl group rather than to the CSP. This could explain the reduced retention and might entail a change in conformational preference. Since elution orders are not yet established for the derivatives of acid **4**, a chiral recognition model is not presently suggested for these analytes.

TABLE V

CHROMATOGRAPHIC BEHAVIOR ON (*R,R*)-CSP **2** OF CHIRAL ACIDS **1–5** AS THEIR *N*-METHYLANILIDES



	1	*2*	*3*	*4*	*5*
α^a	2.46	1.47	1.32	1.34	1.45
$k'_1{}^b$	2.44	1.48	2.69	4.94	1.27

[a] Chromatographic separation factor.

[b] Capacity factor for the first eluted enantiomer using 10% (v/v) 2-propanol in hexane as the mobile phase; flow-rate 2 ml/min.

CONCLUSION

Chiral carboxylic acids are readily resolved as their anilide derivatives by high-performance liquid chromatography (HPLC) on the β-amino acid-derived CSP **2**. The enantiomeric purity of chiral carboxylic acids can be determined and, in many cases, enantioselectivity is sufficient for facile preparative resolution.

ACKNOWLEDGEMENTS

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