DISCUSSION OF A CONTROVERSIAL CHIRAL RECOGNITION MODEL

WILLIAM H. PIRKLE* and JOHN E. McCUNE

School of Chemical Sciences, Box 44, Roger Adams Laboratory, University of Illinois, 1209 W. California Street, Urbana, IL 68108-3731 (U.S.A.)

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SUMMARY

Wainer and Doyle and McDaniel and Snider have proposed a "head-to-head" chiral recognition model to rationalize the separation of the enantiomers of amide and anilide derivatives of chiral acids such as ibuprofen, naproxen and fenoprofen on a phenylglycine-derived chiral stationary phase. Pirkle and Reno and Nicoll-Griffith have proposed an alternative "head-to-tail" model. Evidence is presented which suggests that for a series of amide and anilide derivatives, both mechanisms are possible, additional structural features determining the contribution made by each to the observed time-averaged chiral recognition. For anilides, the head-to-head mechanism is less prevalent, its operation again requiring the presence of certain structural features in the analyte.

INTRODUCTION

Several groups have reported the chromatographic separation of the enantiomers of amide derivatives of α-arylpropionic and α-substituted arylacetic acids, compounds of pharmaceutical interest, on the chiral stationary phase (CSP) derived from (R)-N-(3,5-dinitrobenzoyl)phenylglycine. Conflicting proposals have been tendered as to the nature of the dominant chiral recognition mechanism(s) employed by this CSP toward these analytes. We now present new results which bear upon the nature of the chiral recognition processes.
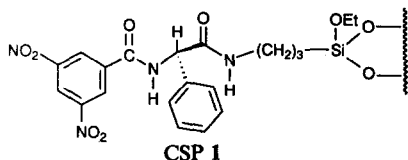
CSP 1

Initially, Wainer and Doyle[1] described the separation of amide derivatives of ibuprofen, naproxen, fenoprofen and benoxaprofen on CSP 1. They rationalized their observations by means of a "face-to-face" approach of analyte to CSP promoted by "dipole stacking" of amide dipoles. Fig. 1 shows the arrangement of the components
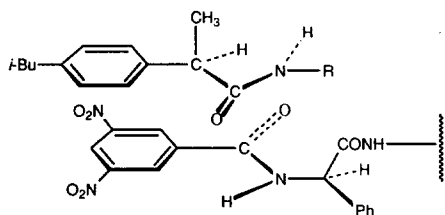
Fig. 1. The Wainer–Doyle model for the more stable diastereomeric adsorbate between ibuprofen derivatives and CSP 1 (representation of the chiral recognition mechanism published by the authors[1]).

of the more stable diastereomeric adsorbate as proposed by Wainer and Doyle. Additionally, a π–π interaction between the dinitrobenzoyl group of the CSP and the aryl substituent of the acid-derived portion of the analyte was proposed, as were steric interactions between the CSP and the analyte enantiomers. This model, adapted from one proposed for a different type of amide analyte[2], we term a "head-to-head" arrangement. It was earlier noted that dipole stacking can occur in either a head-to-head arrangement, where the carboxylic acid components are oriented in the same direction, or a "head-to-tail" mode[3]. It has also been noted that when two competing chiral recognition processes are possible, additional structural features present in the analyte can be expected to determine the relative contribution made by each process to the overall time-averaged behavior ultimately observed[4]. In the Wainer–Doyle proposal (Fig. 1), the proposed π–π interaction makes the head-to-head approach plausible for amides prepared from amines lacking π-basic substituents. However, some of the amides utilized by these authors were prepared from amines containing π-basic aryl groups. Might this not alter the preferred mode of dipole stacking? This point was considered and dismissed by Wainer and Doyle, who suggested that, "If π–π interactions were the primary driving force in the formation of the CSP–solute complex, a reversal in elution order of the ibuprofen enantiomers would be expected with the addition of the 1-naphthalenemethyl group. This reversal would reflect the preferred π–π bonding between the naphthyl ring and the 3,5-dinitrobenzoyl ring". However, the reader will note that for the conformation having a 180° dihedral angle between the methine hydrogen and the carbonyl oxygen, 180° rotation of the analyte about the axis parallel to the C–O bond of the carbonyl group and a slight lateral displacement affords a "head-to-tail" arrangement which, in the case of derivatives prepared from amines containing π-basic aryl groups, would allow π–π interaction *without an inversion of elution order*. After such a rotation, the sterically large groups (*i.e.*, the phenyls) are still "external" to the stack. Hence, the observations that the separation factor for the enantiomers of ibuprofen 1-naphthalenemethylamide is not reduced (indeed, it is greater) relative to the methylamide and elution order is unchanged *cannot* be taken as evidence of an absence of head-to-tail stacking brought about by π–π bonding between the naphthyl and 3,5-dinitrobenzoyl moieties.

A reviewer raised the issue discussed by Wainer and Alembik[5] concerning the "directionality" of amide dipoles. An instance was reported using (*R*)-CSP 1 in which "there is an inversion in the enantiomeric elution order for the amide derived from amines compared to those derived from carboxylic acids" ... "The major difference between these two compounds is the position of the chiral center relative to the amide moiety". The examples cited were N-benzoyl-α-methylbenzyl amine, α = 1.17,

R elutes before S, and the anilide of α-phenylpropionic acid, $\alpha = 1.10$, S elutes before R. We disagree with the notion that "directionality" of the amide dipole determines the elution order of the analytes in question, believing rather that it is simply a matter of which group is preferentially used as the π-base (benzoyl $<$ phenyl $<$ anilide) during dipole stacking that determines the predominant sense of dipole stacking (*i.e.*, head-to-head or head-to-tail) and, in these instances, elution order.

McDaniel and Snider[6] subsequently reported the separation of enantiomers of amide derivatives of ibuprofen, flurbiprofen and α-methoxyphenylacetic acid on the same CSP. They noted that amides derived from α-naphthylamine show greater enantioselectivity on CSP **1** than do the amides derived from any of the other amines used in the study and they proposed the chiral recognition model shown in Fig. 2, a model essentially that of Wainer and Doyle[1]. They did not consider the likelihood that the enhanced enantioselectivity noted in this instance arises from a dominant π–π interaction between the 3,5-dinitrobenzoyl group of the CSP and the α-naphthylamido portion of the analyte, possibly because of the prior assertation that such π–π interaction (and the resultant head-to-tail arrangement) would lead to an elution order different than that actually observed[1].

Not all workers have agreed that head-to-tail arrangements would lead to "inverted" elution orders. Such head-to-tail stacking between N-(3,5-dinitrobenzoyl)-leucine derivatives and N-acylated-α-amino acid amides derived from aniline, p-tolidine, α-naphthylamine and β-naphthylamine has been invoked to explain enantioselectivity noted in both chromatography and asymmetric synthesis[7,8]. Moreover, Nicoll-Griffith[9] has also examined the chromatographic behavior of various ibuprofen anilides and amides on CSP **1**. Based on the observation that electron-withdrawing *para* substituents on the anilide moiety diminish selectivity whereas electron-donating substituents enhance selectivity, Nicoll-Griffith proposed the head-to-tail dipole stacking model shown in Fig. 3 and suggested that this model should also be valid for other α-methylarylacetic acid anilides, specifically citing naproxen, benoxaprofen and fenoprofen. Nicoll-Griffith inferred that "alternate substituents on the drug aromatic ring should not affect the chiral recognition mechanism, aromatic amide derivatives of these drugs should exhibit enhanced enantiomer separations and the same elution order". Finally, the ibuprofen amides were proposed to resolve by the same head-to-tail dipole stacking model, with the π–π interaction between the anilide moiety and the 3,5-dinitrobenzoyl group of the CSP being replaced by a σ–π interaction.
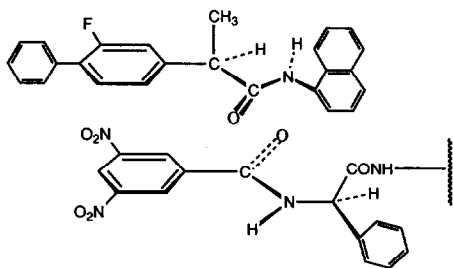

Fig. 2. The McDaniel–Snider model for the more stable diastereomeric adsorbate between ibuprofen derivatives and CSP **1** (representation of the chiral recognition mechanism published by the authors[6]).
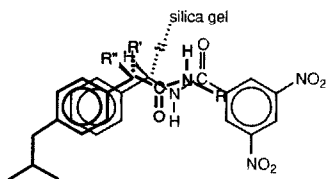
Fig. 3. The Nicoll-Griffith model for the more stable diastereomeric adsorbate between ibuprofen derivatives and CSP 1 (representation of the chiral recognition mechanism published by the author[9]).

EXPERIMENTAL

Apparatus

Chromatography was performed using a Bischoff isocratic pump, a Rheodyne injector, a Regis covalent Pirkle 1A column, two Milton Roy–LDC UV Monitor D® detectors (254 and 280 nm) in series and a Kipp-Zonen BD-41 dual-pen recorder.

Reagents

Racemic ibuprofen was isolated from a Motrin® tablet. Ibuprofen was partially resolved according to the procedure of Nicoll-Griffith[9]. Fenoprofen was a gift from Eli Lilly. Racemic and (S)-(+)-2-phenylbutyric acid, 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) and the various anilines and amines were obtained from Aldrich. 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[6]. The amides of acids **1** and **2** were prepared from the corresponding acid chlorides by addition of the appropriate amine and using an extractive work-up as described below for the anilides.

Anilide synthesis using EEDQ

Equal amounts (*ca.* 10 mg) of the acid and EEDQ were placed in 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 of dichloromethane and 1 ml of 1 *M* sodium hydroxide were added, the mixture shaken vigorously and the upper layer was removed with a pipet. The lower layer was similarly washed several times with water, then 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 withdrawn. The lower layer was repeatedly washed with water. The resulting solutions were dried over anhydrous magnesium sulfate and analyzed directly. Early eluting impurities were noted in some instances, but did not interfere with the analyses[10]

RESULTS AND DISCUSSION

We recently described a variation of CSP 1 which usually affords enhanced enantioselectivity[10]. To evaluate this new CSP, we prepared and chromatographed a number of anilides of chiral acids[11]. Some of these were prepared to distinguish head-to-head from head-to-tail alignments, alignments which cannot be distinguished by elution order as they are "same sense" mechanisms. These same analytes should also distinguish between the two stacking modes on CSP 1. The basis for this distinction is simple. Note from Figs. 1 and 2 that were the head-to-head mechanism operative, amides made from n-alkylamines or from anilines having para-alkyl substituents would direct these alkyl groups toward the underlying silica support, the alkyl group being intercalated between adjacent strands of bonded phase. There is considerable precedent that, when such intercalation occurs, increasing the length of the alkyl substituent decreases, through steric interaction of the alkyl substituent with the flanking strands of the CSP and the underlying silica support, the stability of the diastereomeric adsorbate containing the enantiomer intercalating this group. This results in a reduced retention of this enantiomer relative to its antipode. Hence a change in the magnitude of α results. The shape of the resultant α vs. n plot (n is the number of carbons in the linear alkyl group) thus conveys considerable mechanistic information[4,12]. We hasten to add that the mobile phase in these instances is normal, not reversed.

The chiral acids 2-(α-naphthyl)propionic acid (1) (a model for naproxen), ibuprofen (2), fenoprofen (3), 2-isopropoxyacetic acid (4) and 2-phenylbutyric acid (5) were used to prepare homologous series of amide derivatives using n-alkylamines and p-alkylanilines. These derivatives were chromatographed on CSP 1 and the effect of the length of the alkyl group on α, the separation factor for enantiomers, was noted.
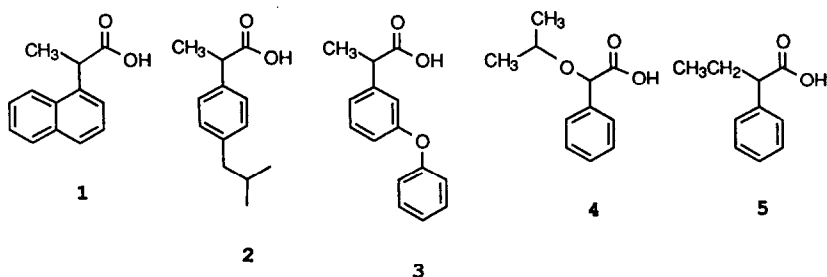
Amide derivatives

Data pertinent to the separation of a series of n-alkylamine-derived amides of 1 and 2 appear in Table I. Note that, for the ibuprofen derivatives, α decreases as n, the number of carbons in the linear alkyl group, increases and no separations were observed with $n > 6$. This is in accord with the expectations generated by the Wainer–Doyle model. For the corresponding amides of 1, the magnitude of α again decreases as n increases but enantiomer separation persists even when $n = 18$. Presumably, the greater π-basicity of the α-naphthyl substituent leads to a strong π–π interaction, one more difficult to disrupt in less favorable steric circumstances (i.e.,

TABLE I

CHROMATOGRAPHIC BEHAVIOR ON (R)-CSP 1 OF CHIRAL ACIDS 1 AND 2 AS THEIR n-ALKYLAMIDES

$$R \overset{*}{\underset{}{\diagup}} \overset{O}{\underset{\|}{C}} NH-(CH_2)_n-H$$
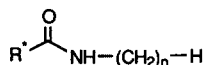
Acid (n)	1		2	
	α^a	$k_1'^b$	α^a	$k_1'^c$
18	1.12	2.80	1.00	1.73
14	1.16	3.14	1.00	1.87
10	1.19	3.42	1.00	2.13
8	1.20	3.57	1.00	2.37
6	1.21	4.05	1.06	2.40
4	1.28	4.85	1.08	2.83
3	1.33	5.44	1.11	3.07
2	1.31	6.60	1.13	4.37
1	1.27	8.57	1.14	6.33
0	1.14	7.96	1.00	7.13

[a] Chromatographic separation factor.

[b] Capacity factor for the first eluted enantiomer using 2-propanol–hexane (10:90, v/v) as the mobile phase; flow-rate, 2 ml/min.

[c] Capacity factor for the first eluted enantiomer using 2-propanol–hexane (5:95, v/v) as the mobile phase; flow-rate, 2 ml/min.

when a long alkyl group is involved). Note the significantly greater capacity ratios for derivatives of 1 relative to those of 2. It also seems likely that the greater size of the naphthyl group permits a "shift" in the relative positions of the two π–π components which allows relaxation of some of the steric effects generated by the longer alkyl groups. The R enantiomers are preferentially retained on (R)-CSP 1, as expected from all the mechanisms advanced and from the elution orders reported for amides of ibuprofen and naproxen[1].

Anilide derivatives

Homologous series of p-(n-alkyl)anilides were prepared from acids 1–5 and examined chromatographically on CSP 1. The results are given in Table II. If the enantiomers of these anilides separate owing to a head-to-tail stacking model as shown in Fig. 3, the p-alkyl groups would be directed away from the silica and their length would be expected to have little effect on enantioselectivity. However, the absolute retention would be expected to decrease as n increases owing to increasing analyte solubility in the mobile phase. For the homologous series of anilides derived from ibuprofen, fenoprofen and 2-phenylbutyric acid, the length of the p-alkyl substituent affects the retention but, to a good approximation, not enantioselectivity. We consider this to be compelling evidence that the chromatographic behavior of these analytes is not significantly influenced by processes similar to the Wainer–Doyle and McDaniel–Snider mechanisms. The observed lack of dependence of the selectivity on the length of the alkyl substituent is consistent with the mechanistic proposals offered by

TABLE II

CHROMATOGRAPHIC BEHAVIOUR ON (R)-CSP 1 OF CHIRAL ACIDS 1–5 AS THEIR p-ALKYLANILIDES
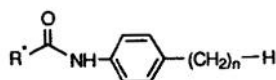
Acid (n)	1		2		3		4		5	
	α^a	$k_1'^b$	α^a	$k_1'^b$	α^a	$k_1'^b$	α^a	$k_1'^b$	α^a	$k_1'^b$
14	1.22	4.96	1.23	1.45	1.20	3.10	1.28	1.45	1.09	1.80
12	1.22	5.10	1.22	1.50	1.16	3.20	1.29	1.47	1.10	1.84
10	1.22	5.50	1.22	1.54	1.19	3.30	1.30	1.60	1.10	2.00
8	1.23	5.80	1.21	1.63	1.20	3.46	1.29	1.63	1.10	2.06
6	1.23	6.30	1.21	1.81	1.21	3.75	1.32	1.81	1.08	2.27
4	1.25	6.91	1.20	2.10	1.22	4.15	1.37	1.93	1.11	2.50
2	1.30	8.50	1.22	2.60	1.20	5.40	1.39	2.41	1.10	3.20
1	1.34	10.40	1.23	3.20	1.21	6.46	1.42	2.96	1.12	3.97
0	1.36	10.40	1.21	3.20	1.20	6.44	1.34	2.80	1.11	4.00

[a] Chromatographic separation factor.
[b] Capacity factor for the first eluted enantiomer using 2-propanol–hexane (5:95, v/v) as the mobile phase; flow-rate, 2 ml/min.

Nicoll-Griffith[9] and ourselves. The greater enantioselectivity noted for ibuprofen and fenoprofen anilides relative to those of 2-phenylbutyric acid can be explained by either stacking mode, as it is implicit in the models that differences in size between the alkyl and aryl groups on the stereogenic center are ultimately responsible for the observed enantioselectivity. Fig. 4 expresses this idea in a slightly different format than was used by Nicoll-Griffith, but the models are essentially the same.

The enantiomers of the anilides derived from 4 are separable on CSP 1. Interestingly, the selectivity decreases as the length of the p-alkyl substituent increases, but never disappears. This suggests that chiral recognition is occurring by both head-to-head and head-to-tail arrangements. As the alkyl group becomes longer, the contribution of the head-to-head process lessens and the remaining enantioselectivity presumably stems from the head-to-tail process. Dipole stacking seems probable for the anilides of 1 and seemingly occurs by both head-to-head and head-to-tail arrangements. In this instance, the head-to-head contribution is rationalized by the greater π-basicity of the α-naphthyl substituent relative to the aryl substituents present in acids 2, 3 and 5. The two stacking modes lead to the same sense of enantioselectivity,
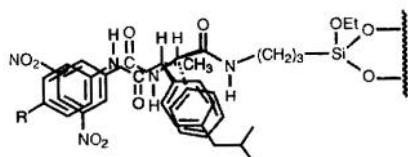

Fig. 4. Head-to-tail dipole stacking chiral recognition model.

the head-to-head contribution diminishing as the length of the *p*-alkyl substituent increases. With higher values of *n*, the remaining chiral recognition is considered to come principally from the head-to-tail contribution.

The elution orders for the enantiomers of the amides derived from acids **1** and **2** and of the anilides derived from acids **1–3** and **5** are known and conform to the chiral recognition mechanisms discussed. The elution order of the enantiomeric anilides derived from acid **4** is not yet established.

Dipole stacking

Similar to an approach used by Wainer and Doyle[1], 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 the CSP on α. If the anilide N–H participated in some essential hydrogen bonding interaction, its replacement by an alkyl substituent would seriously erode chiral recognition.

Chromatographic data for the separation of the N-methylanilide derivatives of acids **1–5** on CSP 1 are given in Table III. The chromatographic separation factors for the tertiary amide derivatives of acids **1–3** and **5** are comparable to those noted for the corresponding secondary amides in Table II. However, the capacity ratios are significantly reduced. These results indicate that hydrogen bonding of the anilide N–H proton is not essential to chiral recognition but, if present, may lead to achiral retention. These conclusions differ only slightly from those of Wainer and Doyle[1] who, noting chromatographic separation factors and capacity ratios of 1.11 and 15.5 for the enantiomers of ibuprofen benzylamide and 1.07 and 6.4 for those of the N-methyl-benzylamide, suggest that hydrogen bonding increases the stability of the benzyl amide–CSP complex relative to that of the N-methylbenzylamide complex. The reduction in k'_1 which accompanies N-methylation clearly indicates a reduction in the energy of adsorption. However, multiple "complexes" are involved during chromatography and the added retention may not stem from the complex(es) which afford chiral recognition. Therefore, we do not consider the small changes in

TABLE III

CHROMATOGRAPHIC BEHAVIOUR ON (*R*)-CSP 1 OF CHIRAL ACIDS 1–5 AS THEIR N-METHYLANILIDES
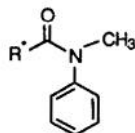
Parameter	Acid				
	1	*2*	*3*	*4*	*5*
α[a]	1.21	1.20	1.17	1.00	1.18
k'_1[b]	3.47	1.67	3.57	4.27	1.36

[a] Chromatographic separation factor.

[b] Capacity factor for the first eluted enantiomer using 2-propanol–hexane (10:90, v/v) as the mobile phase; flow-rate, 2 ml/min.

separation factors to be compelling evidence for any hydrogen bonding *during the chiral recognition process*; the modest difference in separation factors may simply result from differences in conformational preferences which accompany N-methylation. Primary amides show substantial preference for population of the *Z* rotamer (about the carbonyl carbon–nitrogen bond) which will be markedly reduced upon N-methylation. Changes in conformational preferences might well influence both selectivity and retention, especially when the amide's nitrogen substituents intercalate between the strands of bonded phase.

Interestingly, the enantiomers of the tertiary amide of acid **4** do not separate on CSP 1 and the capacity ratio is much larger than that noted for the corresponding secondary amide. Apparently, hydrogen bonding of the anilide N–H proton is essential to the chiral recognition process for this analyte and we make no claim that dipole stacking is involved.

CONCLUSION

When chromatographing amide analytes on amide stationary phases, one should be aware that a variety of transient bonding interactions are possible. Hence, ascribing a retention mechanism or a chiral recognition mechanism is not as straightforward as might initially be thought. We have presented data demonstrating the occurrence of a head-to-tail chiral recognition mechanism for anilide derivatives when chromatographed on CSP 1. Additionally, a head-to-head mechanism can contribute to chiral recognition for those analytes in which the acid component contains a substituent, suitably located and of adequate π-basicity, to compete with the aryl portion of the anilide for π–π interaction with the 3,5-dinitrobenzoyl group. Hence, alternate substituents on the drug aromatic ring *do* affect the partitioning between competing chiral recognition mechanisms but not, in these cases at least, the elution order. We have also presented evidence that is consistent with the operation of a head-to-head Wainer–Doyle type of mechanism in the separation of ibuprofen amides derived from *n*-alkylamines.

We emphasize that enantiodifferentiation is a time-weighted average of multiple processes and cannot be stringently ascribed to a single mechanism in all instances.

ACKNOWLEDGEMENTS

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