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STERIC AND ELECTRONIC EFFECTS IN THE RESOLUTION OF ENANTIOMERIC AMIDES ON A COMMERCIALLY AVAILABLE PIRKLE-TYPE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC CHIRAL STATIONARY PHASE

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SUMMARY

The steric and electronic effects in the resolution of enantiomeric amides on a commercially available (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine chiral stationary phase (CSP) have been investigated. Several homologous series of enantiomeric amides were synthesized from alkyl and aromatic amines and from alkyl and aromatic acids. The results of the study indicate that chiral recognition is based on the formation of diastereomeric solute–CSP complexes that are due to attractive interactions located on a single bond in both the solute and CSP and on steric interactions within the complexes. The magnitude of the chiral resolution appears to depend on the steric bulk at the chiral center. In addition, when the amides synthesized from chiral amines were chromatographed, the (*R*)-enantiomers eluted first, whereas the opposite elution order was found for the amides synthesized from enantiomeric carboxylic acids. Thus, the amide moiety not only provides the sites of attractive interaction between the solute and CSP, but also influences the spatial orientation of the two molecules, thereby affecting the relative stabilities of the two diastereomeric complexes and determinging the enantiomeric elution order.

INTRODUCTION

The rapid development of high-performance liquid chromatographic (HPLC) chiral stationary phases (CSPs) provides the analyst with a powerful array of analytical tools. The maximum use of these CSPs depends on an understanding of how they work, *i.e.*, the mechanism of chiral recognition.

The first liquid chromatographic chiral recognition model was proposed by Dalgliesh[1] as an explanation of the resolution of aromatic amino acids by cellulose paper chromatography. The proposed model consisted of a "three-point" attachment between the solute and CSP. The attachment was accomplished through attractive interactions between the solute and CSP that included hydrogen bonding and adsorption of the aromatic moiety.

The "three-point" model takes into account the fact that the determination of

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the configuration about a chiral carbon involves at least three of the four bonds attached to that center. This is the basis, for example, of the Cahn–Ingold–Prelog convention [the (*R*), (*S*) convention] for the assignment of configuration to a chiral molecule[2].

The "three-point" attractive interaction model has been widely used as the basis of chiral recognition models. This has been true especially since the introduction of the CSPs developed by Pirkle *et al.*[3,4], which are designed to permit attractive hydrogen bonding and π–π interactions with the solute.

However, the number of attractive interactions between the solute and CSP need not be limited to three; there can be more, or there can be less. Chiral recognition models based on two attractive interactions[5,6] and just one[7] have been proposed. Lochmüller and Souter[8] have also proposed that an environmental chirality where there are no attachments could distinguish between enantiomers.

The latter situation has been suggested as the chiral recognition mechanism for cyclodextrin-based CSPs[9]. In those CSPs chiral recognition involves the inclusion of the solute in the chiral cavity of the CSP.

The situation can also exist where there is more than one attractive interaction between the solute and CSP, all of which are located on a single bond of both molecules. In this instance, only one element of the chirality of both te solute and CSP is used in the formation of the solute–CSP complex. The relative stabilities of the two resulting diastereomeric complexes must, therefore, be due to the interaction of at least two other stereochemical elements of both the solute and CSP, *i.e.*, the relative steric fit of the two solute enantiomers with the single configuration of the CSP.

This situation exists in the resolution of enantiomeric amides on a commercially available (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine CSP. Pirkle and Welch[10] have reported the resolution of a number of amides on this CSP and have proposed a chiral recognition model based on an attractive dipole stacking interaction between the solute and the CSP.

We have investigated this general mechanism by using several homologous series of enantiomeric amides. Our results support this approach and indicate that the chiral recognition model is based on the following: (1) attractive interactions located on a single bond in both the solute and the CSP which are responsible for the formation of the diastereomeric solute–CSP complex and for the orientation of the molecules within the complex; and (2) steric interactions within the complexes based on the stereochemical configurations of the two molecules.

EXPERIMENTAL

Apparatus

The chromatography was performed with a Spectra-Physics (Santa Clara, CA, U.S.A.) modular liquid chromatograph equipped with a Spectra-Physics Model 4270 integrator, a Spectra-Physics Model 8440 UV–VIS detector set at 254 nm and a Spectra-Physics 8700 solvent delivery system. The column was a stainless-steel, J. T. Baker-packed, Pirkle covalent column (25 cm × 4.6 mm I.D.) with a packing of 5-μm spherical particles of aminopropyl silica modified with (*R*)-N-(3,5-dinitrobenzoyl)-phenylglycine (J. T. Baker, Phillipsburg, NJ, U.S.A.). The temperature of the column was controlled by a Lazar HPLC water jacket (Bodman Chemicals, Media, PA, U.S.A.).

Amines

The following amines were used as purchased: 1-methyl-3-phenylpropylamine, *d*-(+)-α-methylbenzylamine, *dl*-α-methylbenzylamine, 1-naphthylethylamine, 1-methylbutylamine, 2-methylbutylamine, 2-ethylhexylamine, 2-aminoheptane, *sec.*-butylamine, 1-methylheptylamine, 1,5-dimethylhexylamine, 1,3-dimethylbutylamine, 1,2-dimethylpropylamine, 1-ethylpropylamine, 2-amino-1-butanol, *d*-2-amino-1-butanol, 2-amino-1-hexanol, 2-amino-1-pentanol, *n*-butylamine, *exo*-2-aminonorbornane, *endo*-2-aminonorbornane hydrochloride and aniline (Aldrich, Milwaukee, WI, U.S.A.); (*S*)-(−)-2-amino-1-propanol and *dl*-2-amino-1-propanol (Pfaltz & Bauer, Stamford, CT, U.S.A.); *n*-propylamine, *n*-pentylamine, *n*-hexylamine and *n*-heptylamine (Fluka, Switzerland); isopropylamine (Eastman Laboratory and Specialty Chemicals, Rochester, NY, U.S.A.); (+)-2-aminoheptane, (−)-2-aminoheptane, (+)-2-aminopentane and (+)-2-aminobutane, (Chemical Dynamics, South Plainfield, NJ, U.S.A.); 2-amino-5-methylhexane, 3-aminononane, 3-aminoheptane, 3-aminohexane, *dl*-amphetamine sulfate and 2-aminohexane (ICN K & K Labs., Plainview, NY, U.S.A.).

Acid chlorides

The following acid chlorides were used as purchased: 2-naphthoyl chloride (Fluka); *p*-anisoyl chloride, 4-cyanobenzoyl chloride, *p*-chlorobenzoyl chloride and *p*-nitrobenzoyl chloride (Aldrich); *p*-toluoyl chloride and *p*-phenylbenzoyl chloride (Pfaltz & Bauer); benzoyl chloride (Allied Chemical, New York, NY, U.S.A.); thionyl chloride (Fisher Scientific, Pittsburgh, PA, U.S.A.).

Carboxylic acids

The following carboxylic acids were used as purchased: 2-methylhexanoic acid (Fluka); 2-methylbutyric acid, 2-phenylpropionic acid and 2-phenylbutyric acid (Aldrich). 2-Methylheptanoic acid was provided by Millard Maienthal (Food and Drug Administration, Washington, DC, U.S.A.). The 2-bromopentane used in synthesizing 2-methylpentanoic acid was purchased from Aldrich, and the 2-bromoheptane used in synthesizing 2-methylheptanoic acid was purchased from Fluka.

Other chemicals

The HPLC solvents, hexane, isopropanol and acetonitrile, were purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.) All other chemicals were reagent grade and were used as purchased.

General procedure for the synthesis of amides

The amides derived from amines were synthesized by using the appropriate acid chloride according to a procedure previously outlined[11]. The amides based on acids were synthesized by a previously reported procedure which involved converting the acids into acid chlorides and condensing them with the appropriate amines[12].

Synthesis of carboxylic acids from secondary aliphatic bromides

2-Methylpentanoic acid and 2-methylheptanoic acid were synthesized from the corresponding secondary aliphatic bromides. The bromides were first converted to secondary aliphatic nitriles[13] and then hydrolyzed in base[14] to yield the desired acids.

Resolution of 2-phenylpropionic acid

The partial resolution of 2-phenylpropionic acid was accomplished by fractional crystallization of the diastereomeric (*S*)-(−)-α-methylbenzylamine salts[15].

Resolution of aliphatic acids with quinine

The partial resolution of the 2-methylalkyl acids was accomplished by fractional crystallization of the diastereomeric quinine salts[16].

Elution order

When the resolved compounds were available, the elution order of the amides derived from the amines and from the carboxylic acids was determined by chromatographing enantiomeric mixtures containing unequal proportions of each isomer.

Chromatographic conditions

The mobile phases were mixtures of hexane–isopropanol–acetonitrile. A column temperature of 20°C and a flow-rate of 2 ml/min were maintained throughout the analyses.

RESULTS AND DISCUSSION

Formation of the diastereomeric solute–CSP complex

A general structure of the solutes used in this study is presented in Fig. 1. For these molecules all the major sites of attractive interaction with the CSP lie along the amide bond. These sites are the amide dipole, the amide carbonyl (hydrogen bond acceptor), the amide hydrogen (hydrogen bond donor) and the aromatic substituent of the amide. The corresponding sites on the CSP also apparently lie along the dinitrobenzoyl amide bond (Fig. 1). Thus, it appears that the attractive interactions responsible for the formation of the diastereomeric solute–CSP complexes are based on some combination of dipole stacking, hydrogen bonding and π–π interactions along these bonds. This hypothesis is consistent with previous work on the resolution of amides by this CSP[10,12], in which a primary attractive interaction was identified between the amide dipoles of the solute and the CSP.

Effect of the amide bond on the enantiomeric elution order

The enantiomeric elution orders were determined for the amides synthesized from resolved aliphatic and aromatic amines (Table I, 2b–f, 6a–c, 7; Table II,

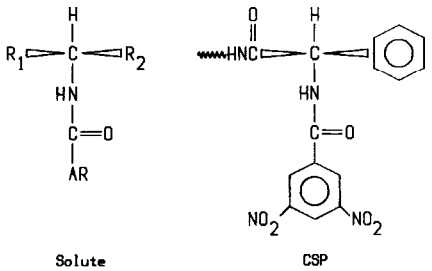
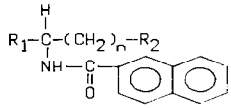


Fig. 1. General structure of the solutes and the CSP used in this study.

TABLE I

RESOLUTION OF ENANTIOMERIC AMIDES CONTAINING A NAPHTHYL SUBSTITUENT



*Compound**	*n*	k_1'**	α	*Elution order*
R_1 = H, R_2 = CH_3				
1a	1	13.20		
b	2	10.75		
c	3	9.47		
d	4	8.70		
e	5	8.23		
R_1 = CH_3, R_2 = CH_3				
2a	0	11.56	1.00	
b	1	9.53	1.03	*R,S*
c	2	7.88	1.06	*R,S*
d	3	7.08	1.09	*R,S*
e	4	6.44	1.10	*R,S*
f	5	6.00	1.11	*R,S*
R_1 = CH_2OH, R_2 = CH_3				
3a	0	32.35	1.04	*S,R*
b	1	20.46	1.09	ND***
c	2	15.87	1.11	ND
d	3	13.43	1.15	ND
R_1 = CH_3CH_2, R_2 = CH_2CH_3				
4a	0	8.16	1.00	
b	1	7.98	1.03	ND
c	2	5.93	1.07	ND
d	4	5.01	1.10	ND
R_1 = CH_3, R_2 = $CH(CH_3)_2$				
5a	0	8.16	1.06	ND
b	1	6.84	1.09	ND
c	2	6.43	1.11	ND
d	3	6.40	1.11	ND
R_1 = CH_3, R_2 = C_6H_5				
6a	0	15.22	1.24	*R,S*
b	1	11.41	1.07	*R,S*
c	2	13.79	1.05	*R,S*
Miscellaneous				
7, 1-(1-naphthyl)ethylamide		25.88	2.10	*R,S*
8, 2-methylbutylamide		9.41	1.00	
9, 2-ethylhexylamide		15.83	1.00	
10, 1-amido-2-propanol		30.20	1.00	

* The compounds in series 1 are not optically active.

** Capacity factor of the first eluted enantiomer.

*** ND = Not determined.

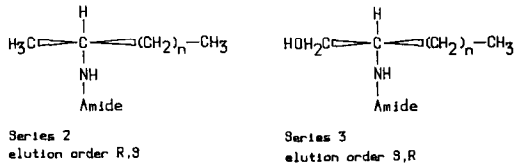


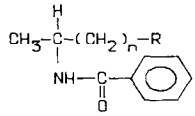
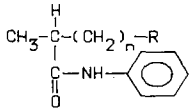
Fig. 2. Configurations of the best-retained enantiomers for series 2 and 3.

13a–e, 14). In each case, the (*R*)-enantiomer eluted first. There was an apparent inversion of this elution order for an aliphatic aminoalcohol (Table I, 3a) for which the (*S*)-enantiomer eluted first. The configurations of the best retained isomers for series 2 and 3 are presented in Fig. 2. From these configurations it is clear that the configuration with respect to the steric bulk of the substituents around the chiral center has not changed. What has changed is the configuration with respect to the Cahn–Ingold–Prelog nomenclature.

The enantiomeric elution order for the benzyl amides synthesized from resolved aliphatic and aromatic acids (Table II, 15a–e, 16) was also determined. For these compounds, the (*S*)-enantiomers eluted first.

TABLE II

RESOLUTION OF ENANTIOMERIC AMIDES CONTAINING A PHENYL SUBSTITUENT

Compound	*n*	*R*	k_1'*	α	*Elution order*
					
13a	1	CH_3	12.84	—**	*R,S*
b	2	CH_3	9.74	1.03	*R,S*
c	3	CH_3	8.33	1.06	*R,S*
d	4	CH_3	8.22	1.08	*R,S*
e	5	CH_3	8.10	1.08	*R,S*
14	0	C_6H_5	23.25	1.17	*R,S*
					
15a	1	CH_3	11.19	1.02	*S,R*
b	2	CH_3	8.64	1.04	*S,R*
c	3	CH_3	7.10	1.07	*S,R*
d	4	CH_3	7.06	1.06	*S,R*
e	5	CH_3	6.79	1.08	*S,R*
16	0	C_6H_5	12.30	1.10	*S,R*

* Capacity factor of the first eluted enantiomer.

** The (*R*)-isomer appears as a shoulder preceding the (*S*)-isomer.

There is an inversion in the enantiomeric elution order for the amides derived from amines compared to that for the amides derived from carboxylic acids (which is not the result of the Cahn–Ingold–Prelog nomenclature). The structures for two of these amides, the benzamides of α-methylbenzylamine (Table I, 14) and α-methylphenylacetic acid (Table I, 16), are given in Fig. 3. The major difference between these two compounds is the position of the chiral center relative to the amide moiety. For the amides derived from amines (*e.g.*, 14), the chiral center is attached to the amido portion of the amide, whereas for the amides derived from carboxylic acids (*e.g.*, 16), the chiral center is bonded to the carbonyl portion of the amide.

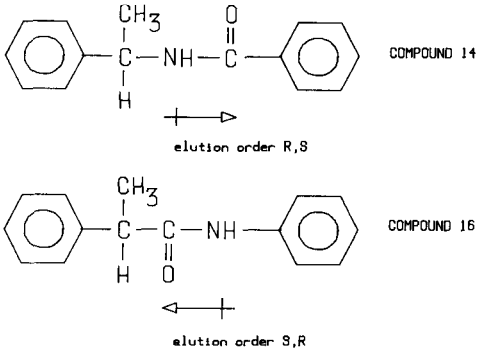


Fig. 3. Structure and the direction of the dipole moment for compounds 14 and 16.

Thus, from the results of this study, it appears that in these series of amides, the attractive interaction between the amide dipoles of the solute and CSP has a directional character. The interaction not only forms the diastereomeric solute–CSP complex but also orients the two molecules within the complex, thereby determining the enantiomeric elution order.

Effect of the position of the amide bond

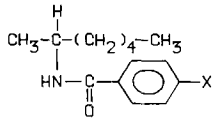
The results of this study also indicate that for the solutes resolved on this system the attractive sites must be directly attached to the chiral center of the solute. If the amide bond is removed from this center by even a single methylene group, there is no chiral resolution, as illustrated by the failure of this system to resolve compounds 8–10 (Table I).

Effect of π-basicity on stereoselectivity

The effect on chiral separation (α) of the electronegativity of the aromatic substituent of the amide moiety is presented in Table III. The results indicate that the electronegativity of the *para*-substituent, as defined by the Hammett constants[17], has very little effect on the stereoselectivity. The amides containing strong electron-donating substituents, *i.e.*, *para*-methyl and methoxy, are resolved with stereochemical resolution factors of 1.07 and 1.08, respectively, whereas for those amides with strong electron-withdrawing substituents, *i.e.*, *para*-chloro, cyano and nitro, $\alpha = 1.04$.

TABLE III

EFFECT OF ELECTRONEGATIVITY OF AROMATIC SUBSTITUENTS ON RESOLUTION



X	σ^*	$k_1'^{**}$	α
CH_3	−0.13	3.31	1.07
OCH_3	−0.11	7.02	1.08
H	0.00	3.52	1.06
C_6H_5	0.01	4.96	1.06
Cl	0.24	2.55	1.04
CN	0.67	9.36	1.04
NO_2	0.78	6.73	1.04

* Hammett constant[17].

** Capacity factor of the first eluted enantiomer.

These results suggest that the π–π interactions between an aromatic substituent on the amide moiety of the solute and the dinitrobenzoyl substituent of the CSP apparently have a limited stabilizing effect on the diastereomeric solute–CSP complexes and are not necessary for chiral recognition.

This approach is consistent with a previous report from our laboratory[12] in which enantiomeric alkyl amides of the α-methylarylacetic acid compound ibuprofen were resolved on the same CSP. In that study, the N-methyl- and cyclohexylamides were resolved with stereochemical resolution factors of α = 1.05 and 1.04, respectively, whereas the benzylamide was resolved with an α value of 1.11. In addition, the (*S*)-enantiomer of those amides eluted first, indicating that the directional effect of the amide bond was also observed for that series of amides.

This effect may explain the increased stereoselectivity when a naphthyl substituent is present in the molecule in contrast to a phenyl substituent, as illustrated by the lower separation factors found for compounds 13a–e and 14 (Table II) compared to those for compounds 2b–f and 6a (Table I). With the naphthyl moiety, there is a better overlap of the π-systems of the solute and CSP, which should also result in increased stabilization of the diastereomeric complexes.

Effect of steric bulk at the chiral center

The results of this study indicate that the stereoselectivity of this chromatographic system is a function of the steric bulk at the chiral center of the solute. As the steric bulk increases, α increases. For example, in the homologous series of β-naphthoylamides derived from straight chain alkanes, series 2–4, the addition of a methylene group increases the relative steric bulk at the chiral center and produces a corresponding increase in stereoselectivity.

The effect of the steric bulk at the chiral center is more pronounced in the β-naphthoylamides of the aromatic amides. An increase in the size of the aromatic group itself from a phenyl ring (6a) to a naphthyl ring (7) is reflected by a change in

α, which increases from 1.24 to 2.10. Conversely, if the phenyl ring is moved away from the chiral carbon by the insertion of methylene groups, the stereoselectivity decreases, as in series 6.

This effect may also be seen in series 5 when n is changed from 2 to 3; the steric bulk gained by the additional methylene group appears to be balanced by the added distance between the isopropyl moiety and the chiral carbon, resulting in no increase in α.

Chiral recognition model

The results of this study suggest a chiral recognition model that is based on differences in steric fit between the enantiomeric solutes and the CSP. This model has two interdependent aspects:

(1) formation of the diastereomeric solute–CSP complexes and the orientation of the molecules within these complexes through attractive interactions located on a single bond in both the solute and CSP;

(2) steric interactions within the complexes that are based on the stereochemical configurations of the two molecules.

The latter aspect of the model reflects the fact that in the solute molecule there is relatively free rotation around the C–N bond between the chiral carbon and the nitrogen atom of the secondary amide. The steric interaction between the solute and the CSP, therefore, must reflect the sum total of all the possible conformations of the solute.

CONCLUSION

For the amides investigated in this study, the solute–CSP complex is formed by a single attractive interaction between the molecules, followed by chiral discrimination based on steric fit. Similar results were found for a series of carbamate derivatives of aliphatic amines[18] and for a series of enantiomeric oxazolidines[19]. In the latter case, the key attractive interaction is a single π–π interaction between aromatic moieties on the solute and CSP, whereas the steric fit between the two molecules within the complex determines the enantiomeric resolution. These proposed chiral resolution models are consistent with the hypothesis that stereoselectivity is based on the chirality of the solute and the CSP and that it is within the solute–CSP complex that the stereochemical differences between the solute enantiomers are expressed.

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