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RESOLUTION OF ENANTIOMERIC AMIDES ON A CELLULOSE-BASED CHIRAL STATIONARY PHASE

STERIC AND ELECTRONIC EFFECTS

IRVING W. WAINER* and MARC C. ALEMBIK
Division of Drug Chemistry, Food and Drug Administration, Washington, DC 20204 (U.S.A.)
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SUMMARY

A series of enantiomeric amides were resolved on a commercially available chiral stationary phase (CSP) consisting of cellulose tribenzoate coated on macroporous silica. The amides were synthesized from several homologous series of aliphatic and aromatic chiral amines and a series of aliphatic chiral acids. The results of the study indicate that the formation of the solute–CSP diastereomeric complex is based on a combination of hydrogen bonding, π–π and amide dipole interactions. These interactions not only form the diastereomeric complex but also appear to position the solute and CSP within the complex. This is suggested by the fact that the enantiomeric elution order for a series of amides formed from aliphatic amines is *R*, *S*, whereas the enantiomeric elution order for the corresponding series of amides formed from aliphatic acids is *S*, *R*. The effects of steric bulk at the chiral center were also investigated; the results indicate that for the homologous series of aliphatic amides, an increase in the length of the alkyl chain attached to the chiral center results in an increase in the chiral resolution (α). This is not the case, however, for the homologous series of aromatic amides; the results suggest that chiral recognition is a function of the fit of the asymmetric portion of the solute in a chiral cavity (or channel) of the CSP and that this fit has rigid steric requirements.

INTRODUCTION

The naturally occurring chiral biopolymer cellulose was one of the earliest liquid chromatographic chiral stationary phases (CSPs). Cellulose paper chromatography, for example, was used by Dalgliesh[1] for the resolution of aromatic amino acids. Although natural cellulose lacks the mechanical strength necessary for use as a high-performance liquid chromatographic (HPLC) phase, a number of HPLC CSPs have been developed from cellulose derivatives.

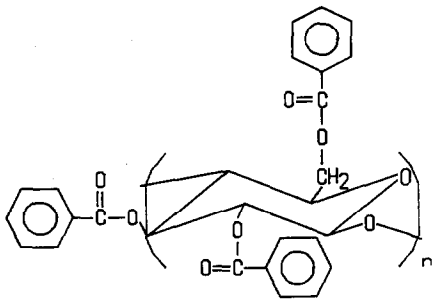
One of the initial cellulose-based CSPs was microcrystalline cellulose triacetyl (CTA-I), introduced by Hesse and Hagel[2], which has proved effective in the resolution of a number of chiral compounds. The CTA-I-CSP is obtained by heteroge-

neous acetylation. If the acetylation is carried out in solution (homogeneous conditions) and the cellulose derivative is then recrystallized, a different crystalline form of cellulose triacetate (CTA-II) is obtained[3]. CTA-II has been shown to be a less effective CSP than CTA-I[4].

Recently, Ichida *et al.*[5] reported the synthesis and application of a series of five HPLC CSPs that were obtained by using homogeneously generated cellulose derivatives adsorbed on macroporous silica gel. These CSPs have a wide and varied range of applications. However, it is not yet clear which of these CSPs and which conditions are best for a particular application. This situation will be resolved by a thorough understanding of the chiral recognition mechanisms operating in these phases.

A chiral recognition mechanism for phenyl-substituted solutes on the CTA-I-CSP has been proposed by Hesse and Hagel[6], Blaschke[7] and Francotte *et al.*[4]. This mechanism involves the penetration of the aromatic portion of the solute into the cavities that are formed between the *d*-α-glucose units of the CSP. The observed stereoselectivity is due to differences in fit or inclusion of the enantiomers of the solute in the cavities of the CSP. Armstrong[8] has suggested a similar inclusion mechanism for resolutions obtained on cyclodextrin-based CSPs.

In the work recently reported by Francotte *et al.*[4], the authors suggest that CTA-I- and CTA-II-based phases should be considered as entirely different sorbents. With this suggestion in mind, we investigated the applicability of an "inclusion" chiral recognition mechanism to resolutions obtained on the commercially available cellulose tribenzoate CSP (OB-CSP) (Fig. 1) described by Ichida *et al.*[5]. The solutes were several homologous series of enantiomeric amides synthesized from aliphatic and aromatic chiral amines and achiral acid chlorides and from aliphatic chiral carboxylic acids and achiral amines.



OB

Fig. 1. Structure of the CSP used in this study.

The results of our study suggest a chiral recognition mechanism which differs from the mechanism proposed for the CTA-I-CSP. This mechanism involves (i) the formation of diastereomeric solute–CSP complexes through attractive interactions between the amide moiety of the solute and an ester moiety of the CSP; (ii) the positioning of the solute and the CSP within the complex; and (iii) the steric fit of the aliphatic portion of the solute in the chiral cavity of the CSP.

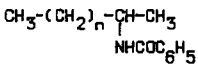
EXPERIMENTAL

Apparatus

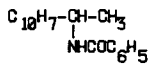
The chromatography was performed with a Perkin-Elmer (Norwalk, CT, U.S.A.) Series 400 liquid chromatograph equipped with a Perkin-Elmer LC auto-control, a Perkin-Elmer LC-85B variable-wavelength spectrophotometric detector and a Perkin-Elmer LCI-100 laboratory computing integrator. The column used with this system was stainless-steel (25 cm × 4.6 mm I.D.) packed with a tribenzoate cellulose adsorbed on microporous silica (OB column, Diacel Chemical Industries, New York, NY, U.S.A.).

Materials

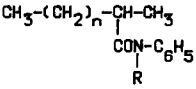
The amides used in this study (Fig. 2, compounds 1–8, 10, 11; and N-acetyl-2-aminoheptane) were prepared according to previously described procedures[9].



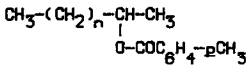
Compound 1



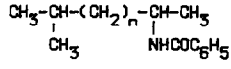
Compound 8



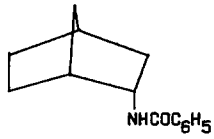
Compound	R
2	H
3	C_2H_5



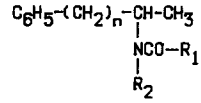
Compound 9



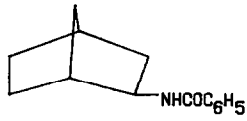
Compound 4



Compound 10



Compound	R_1	R_2
5	C_6H_5	H
6	C_6H_5	CH_3
7	CH_3	H



Compound 11

Fig. 2. Structures of aromatic compounds used in this study.

HPLC-grade hexane, 1-propanol and acetonitrile were purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.).

Sample preparation

The solutes (0.1 mg) were dissolved in 10 ml of methylene chloride.

Chromatographic conditions

A temperature of 20°C and a flow-rate of 1 ml/min were maintained throughout the study. The mobile phases were composed of various mixtures of hexane, 1-propanol and acetonitrile. The solutes containing aromatic substituents were monitored at 254 nm and those without aromatic moieties were monitored at 220 nm.

RESULTS AND DISCUSSION

The chiral recognition process reflects the total of all the interactions between the solute and the CSP. Among the most important are the interactions which lead to the formation of the diastereomeric solute–CSP complexes and those which determine the stability of these complexes. Although these two aspects are inseparable in the chiral recognition process as a whole, the results of this study, presented in Tables I and II, provide an assessment of their relative contributions and an insight into the mechanism of chiral resolution for the CSP used in this study.

Factors affecting the formation and stability of the solute–CSP complex

There are three possible modes of interaction between the amide portion of the solute and one or more of the benzoyl moieties of the CSP: (i) hydrogen bonding interaction between the amide hydrogen and the ester carbonyl; (ii) π–π interaction between the phenyl moieties; and (iii) dipole–dipole interaction between the amide and ester dipoles. In this study, we have not been able to determine whether these interactions take place between the solute and a single benzoyl ester or simultaneously with a number of these groups.

The relative importance of the hydrogen-bonding interaction appears to vary with the solute. The magnitude of chiral resolution (α) for the amides derived from aliphatic carboxylic acids is unchanged when the amide hydrogen is replaced by an ethyl group, as shown in Table I by the compounds in series 2 and 3, respectively. However, for the amide based on phenethylamine (5b), there is a total loss of stereoselectivity when the amide hydrogen is replaced by a methyl group (6); α = 1.59 and 1.00, respectively.

The effect of changing the π-basicity of the phenyl moiety on the amide group of the solute, and thus the contribution of π–π interactions, is presented in Table II. For this factor, the greater the relative π-basicity, as represented by the correspondingly smaller Hammett constant (σ)[10], the greater the chiral resolution. The presence of the strong electron-donating *p*-methyl substituent (σ = −0.13) leads to an α of 2.06, whereas the substitution of the strong electron-withdrawing *p*-nitro substituent (σ = 0.78) results in an α of 1.29. These results are consistent with the postulation of a π–π interaction involving a phenyl moiety of the CSP which acts as a π-acid (σ = 0.45) because of the presence of the carboxylic ester moiety.

The chiral resolution obtained with the *p*-nitro substituent also appears to

TABLE I

RESOLUTION OF AROMATIC COMPOUNDS USED IN THIS STUDY

*Compound**	*n*	k_1'**	α	*Mobile phase****	*Enantiomeric elution order*
1a	1	2.72	1.24	A	*R, S*
b	2	2.11	1.53	A	ND§
c	3	1.73	1.55	A	*R, S*
d	4	1.56	1.69	A	ND
e	5	1.21	1.92	A	*R, S*
2a	1	2.60	1.32	A	*S, R*
b	2	2.52	1.57	A	*S, R*
c	3	1.98	1.49	A	ND
d	4	1.56	1.56	A	*S, R*
e	5	1.24	1.77	A	ND
3a	1	2.88	1.38	A	ND
d	4	1.47	1.59	A	ND
e	5	1.19	1.81	A	ND
4a	0	2.12	1.19	A	ND
b	1	1.68	1.53	A	ND
c	2	1.42	1.66	A	ND
d	3	1.24	1.85	A	ND
5a	0	4.93	1.10	B	*R, S*
b	1	2.04	1.59	B	*R, S*
c	2	2.00	1.45	B	ND
6	1	1.50	1.00	B	
7	1	1.49	1.00	B	
8		6.04	1.00	B	
9a	1	2.97	1.00	C	
b	2	3.05	1.00	C	
c	3	3.07	1.00	C	
e	4	3.35	1.00	C	
10 *endo*-2-amino-norbornane		3.35	1.23	A	ND
11 *exo*-2-amino norbornane		4.11	1.00	A	ND

* See Fig. 2 for structure.

** Capacity factor of the first eluted enantiomer.

*** Mobile phase A = hexane–1-propanol (95:5); mobile phase B = hexane–1-propanol–acetonitrile (96:3:1); mobile phase C = hexane–1-propanol (97:3).

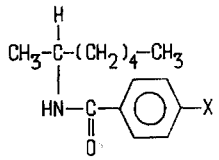
§ ND = not determined.

represent the stereoselectivity obtained without the contribution of π–π interaction. The N-acetyl derivative of 2-aminoheptane, for example, was also resolved in this chromatographic system with an α of 1.22. However, for some solutes, the lack of π–π interaction results in a loss of stereoselectivity. This is the case for the N-acetyl derivative of phenethylamine (7), which was not resolved under these chromatographic conditions.

Both the amide and ester moieties possess dipole moments, *i.e.* 3.5–3.8 D (ref. 11) and 1.7–2.0 D (ref. 12), respectively. Although the dipole–dipole interactions present in this system are less important than the amide–amide dipole interactions proposed for the chiral resolution of amides on the Pirkle-type CSP[9,13,14], they do

TABLE II

EFFECT OF *para* SUBSTITUENTS ON RESOLUTION



X	σ^*	α
CH_3	−0.13	2.06
OCH_3	−0.11	1.78
H	0.00	1.69
Cl	0.24	1.34
CN	0.67	1.45
NO_2	0.78	1.29

* Hammett constant[10].

appear to play a role in the chiral recognition process. This is suggested by the resolution of the solutes without hydrogen-bonding capabilities (3a, d, e) or without a phenyl substituent on the amide moiety (N-acetyl-2-aminoheptane).

The results from the chromatography of a series of enantiomeric esters (Table I, 9a, b, c, e) derived from optically active alcohols and *p*-toluoyl chloride indicate that at least two of the three possible interactions must be present for chiral recognition to occur. Although these esters contain a strong π-basic substituent, they lack both an available hydrogen and a strong dipole and were not resolved in this system.

The presence of two or more interactions along the amide and ester bonds is also indicated by the directional nature of the bonding. For the amides derived from amines, the *R*-enantiomer elutes first, whereas the opposite elution order is found for amides derived from enantiomeric acids. This difference indicates that the interactions along the amide and ester bonds not only form the solute–CSP complexes, but also position the two molecules within the complex, thereby determining the relative stability of the two diastereomeric complexes. For this to occur, there must be at least two points of attachment along these bonds.

The effect of the structure of the ester moiety of the CSP on stereoselectivity was also investigated by using the cellulose triacetyl CSP (OA-CSP) described by Ichida *et al.*[5]. Solutes 1d, 2d, 4c and the *p*-toluoyl derivative of 4d were not resolved on this CSP. The OA-CSP lacks the ability to interact with these solutes through π–π interactions, and this could explain the observed lack of stereoselectivity. The OA-CSP does, however, possess the ability to interact with the solutes through dipole–dipole interactions and by the formation of hydrogen bonds between the acetyl carbonyl of the CSP and the amide hydrogens of the solute. Since the dipole moments of an acetyl ester and a benzoyl ester are equivalent (1.88 D for ethyl acetate and 1.80 D for ethyl benzoate[15]), the difference between the two CSPs most probably lies in their abilities to form hydrogen bonds with the solutes. This difference could be due to the greater electronegativity of the benzoyl carbonyl oxygen compared to

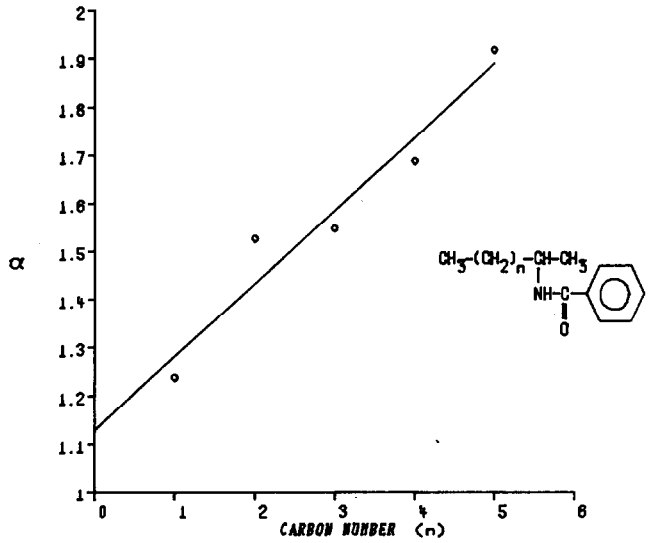


Fig. 3. Plot of stereoselectivity *versus* chain length for the compounds in series 1 (Table I).

that of the acetyl carbonyl oxygen because of the conjugative effects of the phenyl ring[15].

Factors affecting the magnitude of chiral recognition

The effect of the length of the aliphatic carbon chain on stereoselectivity for the compounds in series 1 and 2 (Table I) is presented graphically in Figs. 3 and 4, respectively. From these graphs, it is evident that an increase in steric bulk through the addition of a methylene group increases α. This is also the case for the compounds

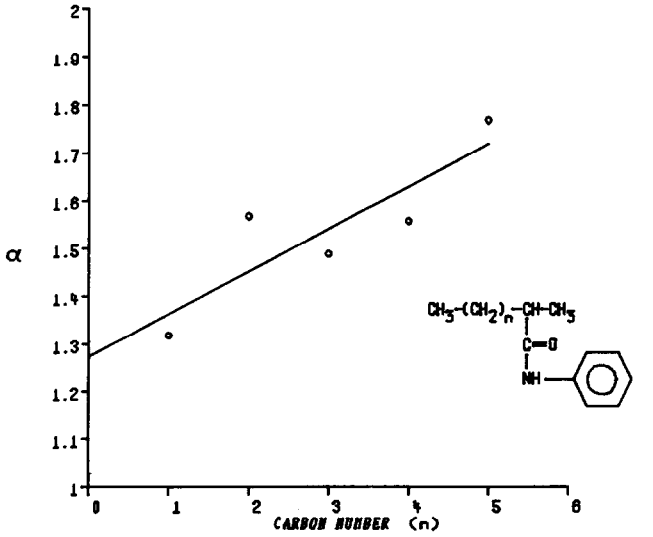


Fig. 4. Plot of stereoselectivity *versus* chain length for the compounds in series 2 (Table I).

in series 4 (Table I), which contains an isopropyl group in one of the side-chains. Thus, it appears that steric bulk at the chiral center plays a key role in determining the magnitude of α.

This, however, does not appear to be the case for the resolution of the aromatic amines in series 5 and compound 8. For these solutes, it appears that the presence of a bulky, rigid aromatic ring at the chiral center does not enhance stereoselectivity: with a phenyl group at the chiral center (5a), $\alpha = 1.10$; with a naphthyl ring (8), $\alpha = 1.00$. When a methylene group is inserted between the chiral center and the phenyl moiety (5b), α increases to 1.59. The addition of a second methylene group (5c) decreases α to 1.45.

These results suggest that the solute has to conform to a rigid spatial arrangement which is defined by the CSP. The solutes with the aromatic group attached directly to the chiral center do not have enough flexibility to attain the optimum fit. The addition of the methylene group gives the solute the needed flexibility, thus increasing the observed stereoselectivity. However, the addition of a second methylene group, which should, *a priori*, lead to an increase in chiral recognition, results instead in a decrease in α. The added steric bulk appears, in this case, to decrease the ability of both of the enantiomers of the solute to fit into the chiral cavity of the CSP. This decreased ability is most likely a result of steric hindrance between the rigid phenyl group of the solute and sections of the CSP which are not part of the chiral cavity.

An additional example of the rigid spatial requirements of the CSP are the results from the chromatography of the benzoylamides of *endo*- and *exo*-2-aminonorbornane (Table I, compounds 10 and 11, respectively). The *endo*-form was resolved with an α of 1.23, whereas the *exo*-form was not resolved under these chromatographic conditions.

CONCLUSION: A PROPOSED CHIRAL RECOGNITION MODEL

The results from this study are consistent with a chiral recognition model based on three interrelated aspects:

(i) The formation of diastereomeric solute–CSP complexes through attractive interactions between the solutes and the CSP. In this case, the attractive interactions involve hydrogen bonding, π–π and dipole interactions between the amide bond of the solute and the ester bond of the CSP. The stronger these interactions are, the tighter the binding of the solute to the CSP and the greater the expression of the stereochemical differences between the enantiomeric solutes.

(ii) The positioning of the solute and CSP within the diastereomeric complex, using at least two of the possible interactions.

(iii) The steric fit of the asymmetric portion of the solute in the chiral cavity of the CSP. This determines the relative stabilities of the diastereomeric complexes and thus the magnitude of the chiral resolution.

The proposed mechanism for the OB-CSP used in this study is an attractive binding-steric fit formulation which differs from the inclusion mechanism proposed for the CTA-I-CSP[4,6,7]. The difference in mechanisms for the two CSPs, which are based on two different forms of derivatized cellulose, is consistent with the observation by Francotte *et al.*[4] that reprecipitated cellulose triacetate (CTA-II) does not

have the same ability to form inclusion complexes that CTA-I has. Since the CSPs used in this study are based on reprecipitated cellulose derivatives coated on silica, it is likely that they would reflect the properties of CTA-II rather than those of CTA-I and that a chiral recognition process based on the formation of inclusion complexes would not predominate in this system.

ACKNOWLEDGEMENTS

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