CHIRAL RECOGNITION MODEL FOR THE RESOLUTION OF EPHEDRINE AND RELATED α,β-AMINOALCOHOLS AS ENANTIOMERIC OXAZOLIDINE DERIVATIVES

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SUMMARY

The mechanism of chiral recognition has been investigated for a series of enantiomeric *cis*-oxazolidines on a commercially available high-performance liquid chromatographic chiral stationary phase (HPLC-CSP). The oxazolidine molecules were synthesized through the condensation of ephedrine and ephedrine-related molecules with aromatic aldehydes. The resulting molecules are rigid five-membered rings whose configuration has been determined by proton magnetic resonance and single-crystal X-ray diffraction. The oxazolidines derived from the condensation of ephedrine and aldehydes containing a π-basic moiety such as naphthaldehyde were resolved on the HPLC-CSP as were those oxazolidines synthesized by using a π-acidic aldehyde such as *p*-nitrobenzaldehyde. However, there was a reversal in the elution order for the two types of oxazolidines. Oxazolidines resulting from the condensation of ephedrine and a π-neutral aldehyde such as benzaldehyde were not resolved. The results of this study suggest a chiral recognition model based on the formation of diastereomeric solute–CSP complexes through a single attractive interaction and chiral discrimination resulting from the difference in steric fit.

INTRODUCTION

We have previously reported[1] the direct enantiomeric resolution of *dl*-ephedrine (I) on a commercially available high-performance liquid chromatographic (HPLC) chiral stationary phase (CSP) developed by Pirkle *et al.*[2]. Racemic ephedrine was first converted by condensation with 2-naphthaldehyde to an enantiomeric oxazolidine. After recrystallization from ethanol, the oxazolidine was resolved on a CSP which consisted of (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine ionically bound to an aminosilica support.

This resolution is one of many direct separations of enantiomers that have been reported recently for this CSP in both its ionically and covalently bonded forms[3]

and for the closely related ionically and covalently bonded (*S*)-N-(3,5-dinitrobenzoyl)leucine CSPs[4]. These CSPs have been particularly successful in resolving molecules of pharmacological interest, such as amphetamine[5], propranolol[6], α-methylarylacetic acids[7], benzodiazepin-2-ones[4], hexobarbital[8] and others.

Concomitant with these rapid developments has been a growing interest in the underlying mechanism of chiral recognition. A fully developed interaction model should both explain and predict enantiomeric elution order. In addition, the model should enable the chromatographer to readily decide what molecules will be resolved without modification on a particular CSP or, if necessary, what modifications must be made to the molecule to facilitate resolution. Molecules of the latter type include primary amines, which are best resolved as their amide[5] or carbamate[9] derivatives.

Developing a clear picture of the mode of chiral recognition is an extremely difficult and often misleading process. Chiral resolution is the result of a number of competing interactions between the solute and the CSP and between the solute and the silica support[10]. If the solute has conformational mobility, the various conformations must also be considered. In addition, even when the CSP is considered to reside preferentially in one conformation, as suggested by Pirkle *et al.*[10], this conformation has two faces, both of which can, in principle, interact with the solute.

Most of the recent chiral recognition models developed for the CSP used in this study have been based on the "three-point" interaction model initially proposed by Dalgliesh[11]. The models involve attractive π–π, hydrogen bonding and dipole–dipole interactions as well as repulsive steric interactions[10]. In this approach, only one of the interactions needs to be stereochemically controlled.

However, different chiral recognition models have been proposed in other chiral chromatographic systems. These models are based on two interactions between the solute and the CSP[12,13] or just a single one[14]. In addition, a chiral recognition model based on a single dipole–dipole interaction has been proposed for the resolution of aliphatic and aromatic amides on the (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine CSP[15]. In this model the dipole–dipole interaction is crucial to the formation of the solute–CSP diastereomeric complex and also aligns the molecules within the complex. Thus chiral recognition is a function of the steric fit between the solute and CSP.

A similar single-interaction chiral recognition model can be postulated for molecules that do not possess an amide dipole but are resolved on Pirkle-type CSPs. For these molecules, the single attractive interaction may occur between the 3,5-dinitrobenzoyl moiety on the CSP (a strong π-acid) and π-bases in the solute. Such interactions have been proposed as an integral part of the chiral recognition model[10].

The *cis* form of the oxazolidines derived from ephedrine and related α,β-aminoalcohols is well suited to the study of an interaction model based on an initial π–π interaction which is integral to the formation of the diastereomeric solute–CSP complex and which orients the molecules within the complex. The oxazolidines used in this study are rigid five-membered rings with a precisely known spatial orientation. This eliminates the added problem of conformational mobility and allows a clear picture of the solute-CSP interactions.

In this paper we present our chromatographic studies of a series of *cis*-2-aryl-5-phenyl-1,3-oxazolidines on the ionically bonded form of the phenylglycine CSP. Our results again suggest that chiral recognition models do not necessarily have to

involve a number of attractive and repulsive interactions, but, rather, that they may be due in some cases to the formation of the diastereomeric CSP–solute complex by a single attractive interaction[15]. Thus chiral discrimination is based on the difference in steric fit between the enantiomeric solutes and the CSP, which is a function of the chirality of the molecules involved.

EXPERIMENTAL

Apparatus

Chromatography was performed with a Spectra-Physics (Santa Clara, CA, U.S.A.) Model 8000 liquid chromatograph equipped with an SP 8000 data system, a Spectra-Physics Model 8310 UV–VIS detector set at 254 nm and a temperature-controlled column compartment. The column packing was an ionically bonded form of the (R)-N-(3,5-dinitrobenzoyl)phenylglycine CSP; the column (25 cm × 4.6 mm I.D.) was prepacked and purchased from J. T. Baker (Phillipsburg, NJ, U.S.A.). Proton magnetic resonance (PMR) spectra were obtained on a 200-MHz Fourier transform nuclear magnetic resonance spectrometer (Varian XL-200, Varian Assoc., Instrument Group, Palo Alto, CA, U.S.A.). Mass spectra were obtained with a double-focusing, electron-impact mass spectrometer (Varian MAT 311A, Finnigan MAT, San Jose, CA, U.S.A.).

Materials

The d- and l-isomers of ephedrine were purchased from Aldrich (Milwaukee, WI, U.S.A.). The d- and l-isomers of pseudoephedrine were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.). Racemic halostachine and 4-methoxy-ephedrine were experimental samples from the stores of the U.S. Food and Drug Administration (Washington, DC, U.S.A.). The aldehydes used in this study (2-naphthaldehyde, 2-methoxy-1-naphthaldehyde, 4-methoxy-1-naphthaldehyde, benzaldehyde, 4-bromobenzaldehyde and 4-nitrobenzaldehyde) were purchased from Aldrich. The HPLC solvents were purchased from Burdick & Jackson (Muskegon, MI, U.S.A.). The remaining chemicals and solvents were reagent grade and were used as purchased.

Synthesis procedure

The oxazolidines were synthesized from the free bases according to our previously described procedure[1]. The free base of the aminoalcohol (0.1 mole) and the appropriate aldehyde (0.1 mole) were dissolved in 100 ml of benzene and refluxed for 2 h. The calculated amount of water formed in the reaction was removed by using a Dean-Stark trap. The excess benzene was removed by distillation under reduced pressure. A portion of the residue was analyzed by PMR and by HPLC. The remaining material was recrystallized from absolute ethanol and then submitted to the same tests.

Chromatographic conditions

The mobile phases were mixtures of hexane and isopropanol. A flow-rate of 1 ml/min and a column temperature of 20°C were maintained throughout the analysis.

Fig. 1. Structures of the aminoalcohols used in this study.
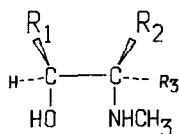
COMPOUND		R$_1$	R$_2$	R$_3$
I	EPHEDRINE	PHENYL	METHYL	H
II	4-METHOXYEPHEDRINE	4-MEO-PHENYL	METHYL	H
III	PSEUDOEPHEDRINE	PHENYL	H	METHYL
IV	HALOSTACHINE	PHENYL	H	H

Elution order

Where possible the elution order of the enantiomeric oxazolidines was established by using an enantiomerically pure aminoalcohol as the starting material and chromatographing mixtures of the enantiomeric derivatives in known but unequal proportions.

RESULTS

Synthesis and structure of the oxazolidines

The α,β-aminoalcohols used in this study (Fig. 1) were condensed with a variety of aromatic aldehydes according to a previously described procedure[1]. This reaction yielded, in every case, an oxazolidine of fully defined structure and configuration. Fig. 2 shows the general reaction using the dextrorotatory enantiomer of ephedrine [I, (1S,2R)-1-phenyl-2-methylaminopropanol] as an example.

Ephedrine contains two asymmetric carbons (C$_1$ and C$_2$), and condensation to the cyclic, five-membered oxazolidine proceeds with retention of configuration at these carbons while a third asymmetric center is introduced. Thus, two stereoisomeric oxazolidines, V *cis* and V *trans*, are possible.

In the case of the condensation of *l*-ephedrine with 2-naphthaldehyde, both stereoisomers are formed initially. However, recrystallization of the product mixture from ethanol converts the mixture to the energetically more favorable *cis* isomer (Vd, (2S,4S,5R)-2-β-naphthyl-3,4-dimethyl-5-phenyl-1,3-oxazolidine).
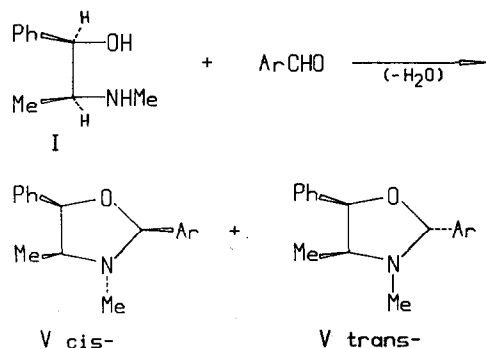

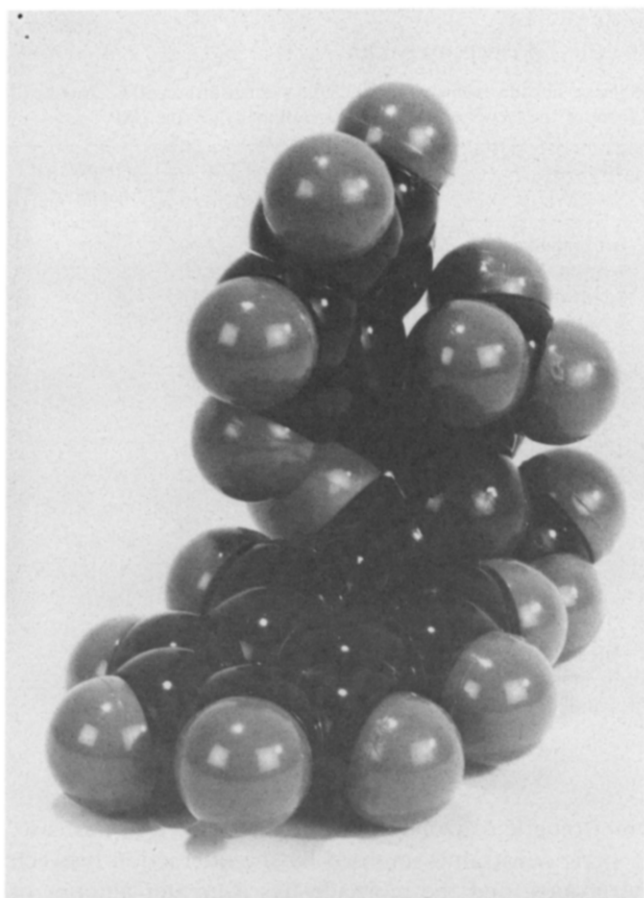Fig. 2. Stereochemistry of the oxazolidine ring formation.

Fig. 3. The structure of the *cis*-oxazolidine, Vd, formed from the condensation of ephedrine and 2-naphthaldehyde.

The structure of the *cis* stereoisomer is presented in Fig. 3. In this molecule the C_1-phenyl, C_2-methyl and naphthyl moieties are on the same side of the oxazolidine ring, whereas the N-methyl group is on the opposite side. In addition, the steric interaction between the C_1-phenyl and C_2-methyl groups holds the phenyl group at a 90° angle from the plane of the oxazolidine ring. This structure has been unambiguously determined by single-crystal X-ray diffraction[16] and PMR studies[17].

The structures of the oxazolidines derived from condensation of various aldehydes with ephedrine and 4-methoxyephedrine (Va–f and VIa–c, respectively) were determined by PMR by using Vd as a model. In all cases both the *cis* and *trans* stereoisomers were present in the initial product mixture, but only the *cis* isomer was present after recrystallization from ethanol.

Pseudoephedrine (III) is diastereomeric with respect to ephedrine (I). In this molecule, the C_1-phenyl and C_2-methyl groups are *trans* to each other, rather than *cis* as in the case of I. Condensation of III with 2-naphthaldehyde also initially yields

TABLE I

CHROMATOGRAPHIC RESULTS FOR *cis* STEREOISOMERS

Chromatographic conditions: mobile phase, hexane–isopropanol (99.5:0.5); temperature, 20°C; flow-rate, 1 ml/min; column, ionically bonded form of the (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine CSP.

Oxazolidine	Aminoalcohol	Aldehyde	k'^{*}	α	First eluted enantiomer**
Va	Ephedrine	Benzaldehyde	2.36	1.00	
b		4-Bromobenzaldehyde	2.44	1.00	
c		4-Nitrobenzaldehyde	7.70	1.13	*d*
d		2-Naphthaldehyde	3.71	1.10	*l*
e***		2-Methoxy-1-naphthaldehyde	3.82	1.36	*l*
f***		4-Methoxy-1-naphthaldehyde	8.76	1.09	*l*
VIa	4-Methoxyephedrine	4-Nitrobenzaldehyde	14.90	1.17	
b		2-Naphthaldehyde	11.06	1.16	
c***		2-Methoxy-1-naphthaldehyde	3.72	1.32	
VIIa	Pseudoephedrine	4-Nitrobenzaldehyde	11.28	1.00	
b		2-Naphthaldehyde	2.71	1.00	
VIII	Halostachine	2-Naphthaldehyde	4.72	1.00	

* Capacity factor of the first eluted enantiomer.
** Configuration of the ephedrine moiety in the oxazolidine.
*** Mobile phase, hexane–isopropanol (99:1).

a mixture of *cis* and *trans* stereoisomeric oxazolidines (VIIb). However, in this case, the *cis* stereoisomer lacks the steric constraints imposed by the interaction between the C_1-phenyl and C_2-methyl groups, and the molecule has a greater amount of conformational mobility. This is true for all of the oxazolidines based on pseudo-ephedrine and halostachine (VIIa–b and VIII, respectively).

Chromatographic results

The chromatographic results for the *cis* stereoisomers of the 12 oxazolidines studied are presented in Table I. In the case of ephedrine, the *cis* stereoisomers were resolved except for the oxazolidines resulting from the condensation with benzalde-hyde and 4-bromobenzaldehyde (compounds Va and Vb, respectively). For deriva-tives Vd, Ve andVf, the oxazolidine derived from the condensation with *l*-ephedrine eluted first. The elution order was reversed for the 4-nitrobenzaldehyde oxazolidine (Vc). In this case, the oxazolidine derived from condensation with *d*-ephedrine eluted first.

The best resolution was obtained with the 2-methoxy-1-naphthaldehyde de-rivative (Ve; $\alpha = 1.36$). Similar results were obtained when 4-methoxyephedrine was used as the aminoalcohol, although the elution order could not be determined be-cause the pure enantiomers were not available.

The *cis*-oxazolidines derived from pseudoephedrine and halostachine (VIIa–b and VIII, respectively) were not resolved under the chromatographic conditions used.

DISCUSSION

One of the most striking features of the chromatographic results for the *cis* stereoisomers of the ephedrine-based oxazolidines (Va–f) is the obvious importance of an attractive π–π interaction between the solute and the CSP. When a weak π-base is incorporated into the molecule, there is no observed resolution (Va–b). However, when a strong π-base is used, a rather good resolution is obtained (Vd–f). It is noteworthy that the addition of a π-acid also results in a resolution (Vc), but with a reversal in the observed elution order. Any proposed mechanism must satisfactorily account for these results.

A general mechanism consistent with all the results in Table I involves the interaction between a π-base on the oxazolidine molecule with the π-acid dinitrobenzoyl (DNB) moiety on the CSP. This interaction is the major driving force in the formation of the solute–CSP complex and orients the molecules within the complex. Stereoselectivity results from a difference in the stability of the two diastereomeric complexes that arises from differences in the steric fit of the enantiomeric solutes on the CSP.

It could be argued that there are additional attractive interactions involving the electron pairs of the oxazolidine nitrogen and oxygen atoms. Both can act as hydrogen bond acceptors and can interact with corresponding moieties on the CSP. However, the chromatographic results do not suggest any major contribution from these interactions either in the formation of the solute–CSP complexes or in influencing the stability of these complexes.

When a strong π-base such as a naphthyl moiety is incorporated into the oxazolidine at C_5, the attractive π–π interaction takes place between this moiety and the DNB moiety on the CSP. In this case, the chiral recognition appears to be due to the rigid conformations of the C_1-phenyl and C_2-methyl groups and differences in stability between the steric fit of the oxazolidines based on *l*- and *d*-ephedrine. For these molecules, the *l*-ephedrine-based oxazolidines form the most stable complexes with the CSP and are eluted last.

When 4-nitrobenzaldehyde (a π-acid) is used to form the oxazolidine, the situation is reversed. In this case, the only π-base available is the C_1-phenyl group of the ephedrine moiety, and it must interact with the DNB group of the CSP. The solute is, in essence, rotated 180° in its orientation to the CSP, thereby causing a reversal in elution order. In this case, the differences in steric fit appear to be due to the C_5-substituent and the C_2-methyl.

When a weak π-base is used in the condensation, there are two mechanisms working in opposite directions. Solute–CSP complexes based on the interaction between the added phenyl group and the DNB group on the CSP lead to one elution order, whereas the complexes based on the phenyl group of ephedrine and the DNB group give the opposite elution order. The net result is the observed lack of resolution (Va–b).

The lack of resolution of the oxazolidines derived from pseudoephedrine and halostachine, (VIIa–b and VIII, respectively) appears to be due to the loss of conformational rigidity afforded by the steric interaction between the C_1-phenyl and C_2-methyl groups. In this case, the added conformational mobility allows both of the enantiomers to minimize steric interactions with the CSP, and the diastereomeric solute–CSP complexes are of equal stability.

CONCLUSION

The chromatographic results for a series of relatively simple oxazolidines of known steric structure suggest that chiral recognition models do not need to be based on three interactions, but rather that they can involve a single attractive interaction. This interaction forms diastereomeric solute–CSP complexes with different stabilities that arise from differences in steric fit between the enantiomeric solutes and the CSP. This mechanism has already been suggested by chromatographic results for the amides and carbamates of simple secondary amines, in which all the sites of attractive interaction are situated on a single bond in both the solute and the CSP[9,15,18]. In this work, π–π interactions play a similar role in the formation of the solute–CSP complexes and in the orientation of the solute in these complexes.

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