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Some aspects of the enantiorecognition of derivatized primary amines on a Pirkle-type chiral stationary phase utilizing tocainide and mexiletine as model compounds

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ABSTRACT

The principles of chiral recognition responsible for the operation of the Pirkle (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine chiral stationary phase (CSP) were employed for generating complementary functionality by achiral derivatization of chiral amines. The model amines chosen were tocainide and mexiletine considering their common structural features. The chromatographic behaviour of four types of derivatives was studied on the covalent and ionic versions of the CSP. Chiral discrimination mechanisms are proposed to expain the results obtained and to account for the observed elution orders.

INTRODUCTION

Since the preparation and evaluation of the Pirkle chiral stationary phase (CSP) based on the immobilization of (*R*)-N-(3,5-dinitrobenzoyl)-phenylglycine (DNBPG) on aminopropyl functionalized silica [1], this unique CSP has received considerable attention in terms of its areas of applications and principles of operation [2].

The relative contributions to the chiral recognition mechanism(s) of donor-acceptor interactions, dipole stacking of amide dipoles, hydrogen bonding and steric repulsive forces arising between the CSP and the chiral solute have been discussed by many workers. Interesting research has been carried out on the role of the amide group in the formation of the diastereomeric complexes between the CSP and the solute's antipodes [3–12]. Dipole stacking and hydrogen bonding of amides together with the $\pi-\pi$ donor–acceptor interactions have been considered as being the major associative interactions responsible for the formation of the diastereomeric adsorbates [9]. The successful operation of the CSP is a result of the joint action of groups of factors, one of which in some specific instances could attain a predominant position over the others, whereas in other instances they would contribute equally to the overall performance of the CSP.

A good understanding of what kinds of interactions are necessary for chiral recognition on this Pirkle-type CSP is essential in the derivatization of a chiral molecule to introduce functionalities complementary to those which the CSP utilizes for its operation. Derivatization is carried out with chiral or achiral reagents. The latter may serve to block polar functional groups that cause excessive band broadening or to introduce groups that interact favourably with the CSP [3]. Chiral derivatizing agents (CDA) suffer several drawbacks, such as different reaction rates of the analyte's enantiomers with the CDA, the fact

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that the CDA could be contaminated with its antipode and racemization.

Generally, primary chiral amines require derivatization prior to chromatography on a DNBPG CSP because the highly polar amine functionality gives rise to superfluous achiral interactions with the CSP [13]. Amines can undergo reactions with various achiral acylating agents to produce amides (–NHCO– group). Ureas and thioureas are provided easily by isocyanates and thioisocyanates, respectively, creating a urea (–NHCONH–) linkage. Hence, by modifying a chiral amine first with an acylating agent and then with the corresponding isocyanate (bearing the same alkyl or aryl substituent), one could visualize the effec of the additional –NH– group of the urea on the chiral recognition mechanism.

The aim of this study was to elucidate some principles for the modification of chiral compounds bearing an amine group on their stereogenic centre and possessing a π-basic aromatic moiety not linked to the latter. The purpose of this modification is to enhance the selectivity of the DNBPG CSP towards the chiral analyte and to provide resolvable derivatives.

As model compounds we chose the antiarrhythmic agents tocainide (Toc) and mexiletine (Mex) (Fig. 1). They both possess an amine group on the stereogenic centre and have a xylyl moiety exhibiting π-basic properties. Considering the structural difference between Toc and Mex, we could assess the role of the amide group on the chiral recognition mechanism by reacting the model analytes with the same derivatizing agent. Gal *et al.* [14] have reported on the resulation of Toc and Mex ($R = 1.5$) via derivatization with homochiral derivatizing agents (thioisocyanates) on a reversed-phase column. Another RP-HPLC method of separating and determining Toc enantiomers employs an α-1

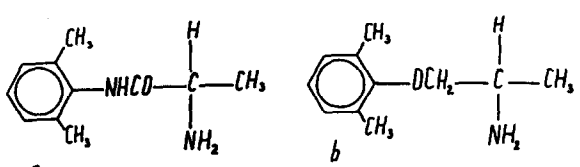

Fig. 1. Structures of the model compounds: (a) tocainide; (b) mexiletine.

acid glycoprotein CSP [15]. Derivatization of Mex with an achiral acylating agent, 2-naphthoyl chloride, and subsequent separation on ionic DNDPG CSP has been reported by McErlane *et al.* [16].

EXPERIMENTAL

Column preparation

Two laboratory-made columns (250 mm × 4.5 mm I.D.) were employed: (1) DNBPG covalently bound to aminopropyl silica, 5 μm (CSP I) and (2) DNBPG ionically bound to aminopropyl silica, 5 μm (CSP 2).

The silica, silanized with γ-aminopropyltriethoxysilane, and the chiral selector (DNBPG) were synthesized according to Pirkle *et al.* [1]. CSP 1 was prepared and filled in the column as described [1] and CPS 2 was generated *in situ* by the procedure of Pirkle *et al.* [17].

Derivatives

The 3,5-dinitrophenyl urea derivatives were prepared starting from 3,5-dinitrobenzoyl chloride according to Pirkle *et al.* [18]. The other ureas were synthesized by mixing 2 mg of the free base with a 5 molar excess of the corresponding isocyanate in 1 ml of dry acetonitrile. After shaking vigorously for 5 min, the reaction mixture was allowed to stand for 30 min at room temperature, then 10-μl aliquots of the sample were injected on to the column immediately after filtration and dilution with acetonitrile. The same procedure was carried out in pyridine. After the reaction had taken place, the mixture was evaporated to dryness and the residue was taken up in chloroform and again evaporated to dryness. After dissolving the derivative in 2 ml of acetonitrile, 10-μl aliquots were analysed after filtration.

The 3,5-dinitrobenzoyl derivatives of Toc and Mex were prepared by the action of a 2 molar excess of the reagent on the respective free base in dry acetonitrile. The reaction mixture was stirred for 1 h at room temperature. All derivatization reactions were controlled by thin-layer chromatography [mobile phase toluene–ethanol–acetonitrile (7:2:1), silica gel plate; Merck, Darmstadt, Germany]. Elution orders were es-

tablished by injecting an excess of one of the enantiomer derivatives.

All derivatives, unless specified otherwise, were analysed using a mobile phase consisting of 2-propanol–*n*-hexane (5:95). Fig. 2 shows the structures of the derivatives prepared and their abbreviations.

Equipment

A Perkin-Elmer (Norwalk, CT, USA) Series 2 liquid chromatograph with a Rheodyne Model 7105 injector and an LC-75 UV detector operating at 254 nm were used.

Reagents and materials

All solvents were of analytical-reagent or HPLC grade (Merck). The mobile phase was degassed and filtered through a 0.5 μm Millipore membrane filter. Triethylamine and pyridine of purities over 99% were supplied by Merck.

The derivatizing agents 3,5-dinitrobenzoyl chloride, 1-naphthyl isocyanate and methyl isocyanate (Fluka, Buchs, Switzerland) were of purities better than 99%. The silica gel Nucleosil 100-5 was obtained from Macherey–Nagel (Düren, Germany). 3–Aminopropyltriethoxysilane (99%) was purchased from Janssen Chimica (Beerse, Belgium).

(±)-, (−)- and (+)-Mex and (±)-, (−)- and (+)-Toc were kindly donated by Boehringer (Ingelheim, Germany) and Astra Hässle (Mölndal, Sweden), respectively.

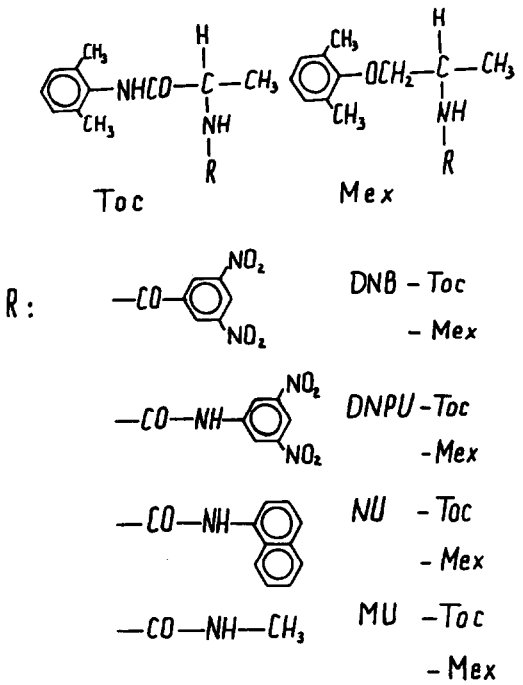

Fig. 2. Structures of the 3,5-dinitrobenzoyl (DNB), 3,5-dinitrophenylurea (DNPU), 1-naphthylurea (NU) and methylurea (MU) derivatives.

RESULTS AND DISCUSSION

The results of the initial testing of the two columns for efficiency (number of theoretical plates, N) and enantiocelectivity (α) are given in Table I. We used substance with 1 to compare the efficiencies of the two CSPs, because it was similar to the derivatives of Tox and Mex. Using substance 2 we wanted to see the influence of the silonol groups. The ionic column provides a resolution R twice as large as that given by the covalent column because the efficiency of the former is higher. Taking into account their application for enantioseparation, howerer, we could qualify them as comparable because the values of the selectivity are similar. Considering the reported resolution of the enantiomers of Mex as their 2-naphthoyl derivatives on an ionic DNBPG CSP [16], we decided to prepare a derivative possessing a urea (–NHCONH–) functionality (Table II). Thus, an additional amido group was introduced (as compared with the amide bond).

Whereas no resolution was observed for the 1-naphthylurea derivative of Toc, the corresponding Mex derivative was resolved. As indicated in Table II, the covalent CSP provides a greater enantiomeric separation than the ionic CSP of the 3,5-dinitrobenzoyl derivative of Toc. This is seen also on the chromatograms in Fig. 3.

It is of interest to elucidate the mechanism underlying this different operation of the two CSPs. Despite its higher efficiency, the ionic column affords $R = 1.15$ for DNB-Toc whereas on the covalent CSP $R = 3.42$, because of the greater selectivity. The chiral recognition mechanism that we suggest is shown in Fig. 4. Three simultaneous interactions take place: an interaction between the π-basic 2,6-dimethylphenyl

TABLE I

EFFICIENCY AND ENANTIOSELECTIVITY OF THE COVALENT AND IONIC DNBPG COLUMNS

Test compound	Type[a]	k'^{b}	α	R	N^{c}
(1) CH_3CO–D,L-alanine-β-naphthylamine $CH_3CHCONHC_{10}N_8$ | $HNCOCH_3$	C	8.6	1.52	4.32	5900
	I	5.3	1.60	6.23	10 800
(2) 2,2,2-Trifluoro-9-anthrylethanol $HOCHC_{14}H_9$ | CF_3	C	3.1	1.40	2.71	12 000
	I	5.0	1.44	5.35	19 000

[a] C = Covalent column; I = ionic column.
[b] Capacity factor of the more retained enantiomer.
[c] Number of theoretical plates.

moiety of the derivative and the CSP's π-acidic 3,5-dinitrobenzoyl group; a "head-to-tail" amide dipole stacking between the analyte's amide bonded to the 2,6-dimethylphenyl group and the amide dipole of the 3,5-dinitrobenzoyl moiety; and a "head-to-tail" alignment of the amide group introduced by the derivatization with the amide linkage of the chiral selector to the silica support. The latter interaction does not take place on the ionic CSP. The enantiodifferentiation is achieved by the spatial position of the methyl group on the stereogenic centre of the derivative. The *S*-configuration offers an orientation of the methyl group such that it remains in

TABLE II

CHROMATOGRAPHIC BEHAVIOUR OF THE STUDIED DERIVATIVES OF TOCAINIDE AND MEXILETINE

Derivative[a]	Type[b]	Tocainide					Mexiletine				
		$k_1'^{c}$	$k_2'^{d}$	α^{e}	R^{f}	Elution order	$k_1'^{c}$	$k_2'^{d}$	α^{e}	R^{f}	Elution order
DNB	C	8.4	11.0	1.31	3.42	*R*, *S*	6.6				
	I	7.8	8.5	1.06	1.15	*R*, *S*	8.2				
DNBU	C	7.4					7.2				
	I	7.0					6.3				
MU	C	9.4	11.6	1.23	2.88	*R*, *S*	7.0				
	I	8.2	11.5	1.02	1.11	*R*, *S*	8.2				
NU	C	9.5					11.0	12.2	1.15	1.05	*R*, *S*
	I	8.6					12.0	13.2	1.15	1.05	*R*, *S*

[a] For derivative abbreviations, see caption to Fig. 2.
[b] C = Covalent chiral stationary phase; I = ionic chiral stationary phase.
[c] k_1' = Capacity factor of the first-eluted enantiomer in case of chiral recognition. If no resolution occurs, $k_1' = k'$, where k' = capacity factor of racemic analyte; $k = (t_R - t_0)/t_0$, t_R = retention time of the solute, t_0 = retention time of a non-retained solute.
[d] k_2' = Capacity factor of the more retained enantiomer.
[e] α = Enantioseparation factor (selectivity).
[f] Resolution factor, $R = 2\Delta t/w_1 + w_2$, where w = peak width at peak half-height.

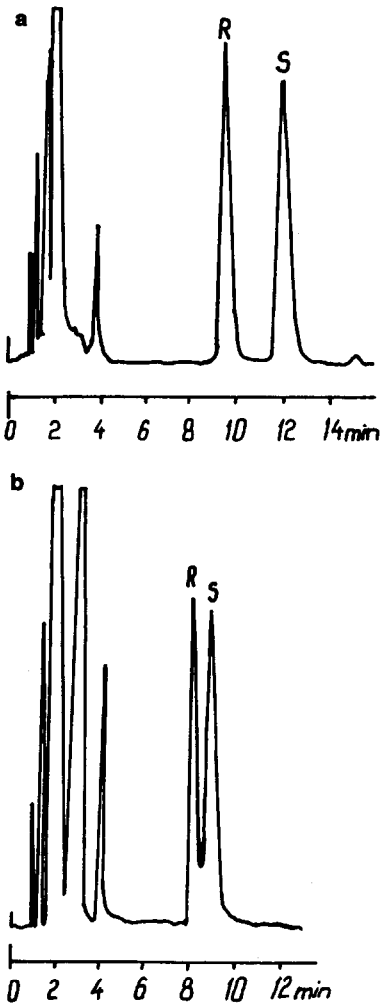

Fig. 3. Chromatograms of the 3,5-dinitrobenzoyl derivative of tocainide obtained on (a) the covalent column and (b) the ionic column. Mobile phase, 2-propanol–*n*-hexane (5:95); detection wavelength, 254 nm; temperature, 25°C; flow-rate, 2 ml/min.

the plane of the neighbouring carbonyl group, thus not causing any steric interference with the CSP's interaction face. The *R*-enantiomer has its methyl group positioned towards the "free" face of the CSP and the phenyl ring of the chiral selector. Hence the corresponding diastereomeric complex is not as stable as that formed by the *S*-antipode.

The contribution of the "head-to-tail" stacking of amide dipoles to the separation of the enantiomers of DNB-Toc is demonstrated by the lack of resolution of DNB-Mex. The only difference between the DNB derivatives of Toc and Mex is one amide (–NHCO–) functionality.

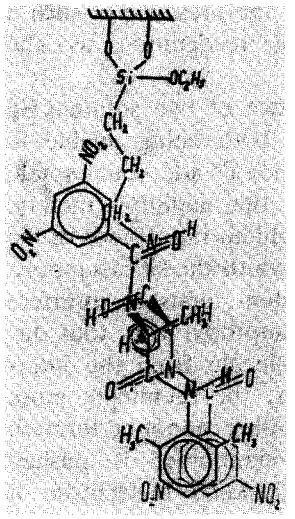

Fig. 4. Proposed chiral recognition mechanism of the 3,5-dinitrobenzoyl derivative of tocainide on the covalent DNBPG CSP.

The proposed interaction mechanism between the 1-naphthylurea (NU) derivative of Toc and the covalent CSP is presented in Fig. 5. No separation occurs with the 1-naphthylurea (NU)

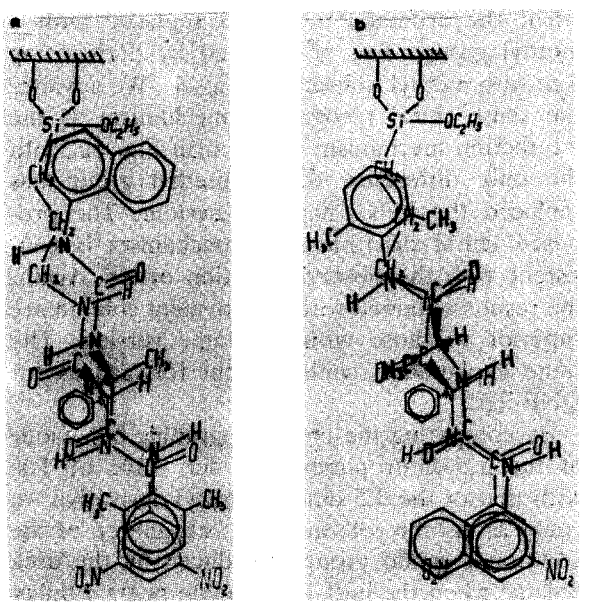

Fig. 5. Proposed solute–CSP interaction mechanism of tocainide 1-naphthylurea on the covalent DNBPG CSP.

derivative of Toc despite the presence of such a valuable site to chiral recognition as the naphthylurea moiety.

We suggest the existence of two solute–CSP interaction mechanisms, both being unable to resolve the analyte. The urea's additional amido group (Fig. 5a) moves the naphthyl moiety, intercalated between neighbouring strands of the bonded phase, further towards the silica support. This bulky aromatic system tends to interfere with the connecting aminopropyl arm, thus displacing the analyte's molecule from the interaction sites. As a result, the seemingly more stable diastereomeric complex cannot be formed, as the corresponding enantiomer is pushed "downwards'. The other possible mechanism of chiral recognition is shown in Fig. 5b. It is most unlikely, however, that this mechanism is responsible for any chiral recognition, as the additional amido group of the urea functionality displaces the chiral centres from their former position (Fig. 5a), and it turns out that the configuration about the stereogenic centre of the analyte has little or no effect on the stability of the represented associative interactions.

Support for the role of the naphthyl moiety in destroying the diastereomeric complex in Fig. 5a is provided by the observed chiral resolution when the aromatic moiety is replaced with a methyl group. This is achieved by derivatizing Toc with methyl isocyanate (Fig. 6). We advance the same chiral recognition mechanism as the interaction mechanism depicted in Fig. 5a, with the only difference that a methyl group has replaced the bulky naphthyl system. The proposed chiral discrimination mechanism is consistent with the observed elution order. Again, the results obtained with the covalent column are superior to those with the ionic version. The same explanation could be put forward as for DNB-Toc.

By comparing the chromatographic behaviour of the 3,5-dinitrophenylurea derivative of Toc with that of the 3,5-dinitrobenzoyl derivative we could assess the role in chiral recognition of the additional amido group introduced by the urea linkage when the initial isocyanate bears a bulky aromatic substituent. This role is simply mechanistic, because owing to the amido group the

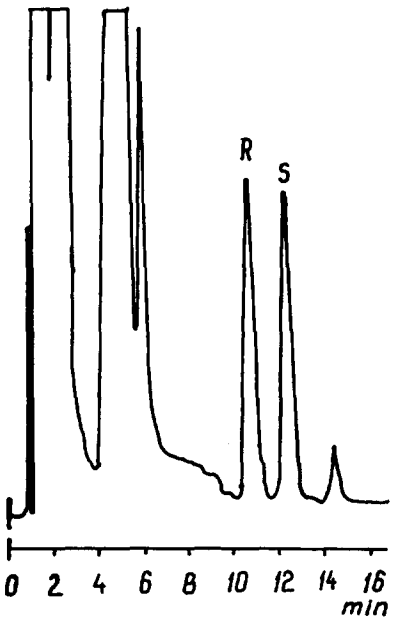

Fig. 6. Separation of the enantiomers of the methylurea derivative of tocainide on the covalent column. Experimental conditions as in Fig. 3.

aromatic moiety is moved "a step" further to the surface of the silica support.

The corresponding urea derivate of Mex (DNPU-Mex) affords a measure of the valuable contribution to chiral recognition of the "head-to-tail" amide dipole stacking between the chiral

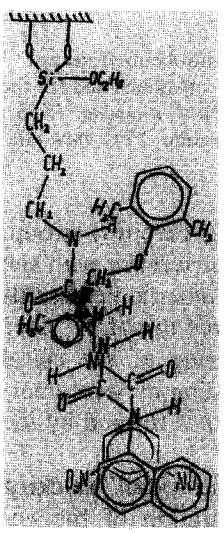

Fig. 7. Proposed chiral recognition mechanism of mexiletine 1-naphthylurea on the covalent DNBG CSP.

analyte and the CSP. The presence of an O-methylene linkage instead of an amide functionality in the molecule of DNPU-Mex is responsible for the lack of resolution. The 1-naphthylurea derivative of Mex is being resolved, however, with an enantiomeric separation of 1.15. Actually this is the only derivative of Mex to be resolved in this study. Obviously, this is due to the presence of a π-basic moiety introduced through the derivatization with 1-naphthyl isocyanate (Fig. 7). We assume that in order for the enantiomeric species to be differentiated by the CSP, their chiral centres should be in close proximity to each other. In this way the spatial arrangement about the analyte's stereogenic centre would give rise to a sterically dependent destabilization of one of the diastereomeric solute–CSP complexes. This enantiodifferentiating role is played by the methyl group on the asymmetric chiral carbon of the derivative. On carrying out the separation at 0°C, no change in the elution order of NU-Mex enantiomers was observed. This means that the $\pi-\pi$ interaction is a more important factor than the conformational mobility of the moieties with regard to the stabilization of the complex.

Finally, we present a rapid method for the separation of the enantiomers of Toc derivatized with 3,5-dinitrobenzoyl chloride. With a mobile phase consisting of 2-propanol–ethanol–*n*-hexane (10:3:87) we achieved a selectivity $\alpha = 1.21$ and a resolution $R = 1.00$ in less than 3 min on the covalent column. An eluent of 2-propanol–*n*-hexane (10:90) with no addition of ethanol afforded on the same column a better resolution ($R = 2.44$) and a greater selectivity ($\alpha = 1.30$) The analysis time was 6 min.

CONCLUSIONS

We propose that the additional amide functionality introduced in the molecule of tocainide by achiral derivatization is capable of becoming involved in a "head-to-tail" dipole-dipole stacking with the covalent amide linkage between the DNBPG chiral selector and the silica support. This interaction has proved to be of significant value in the chiral recognition process. The additional amide bond can be generated by the action of an acylating agent or an isocyanate. The achiral derivatizing agent employed for this purpose should not possess a bulky substituent, as it would otherwise sterically interfere with the underlying support, resulting in loss of resolution.

Chiral compounds resembling mexiletine in structure require the introduction of a π-base along with the necessary amide functionality. This aromatic moiety is expected to interact with the π-acidic 3,5-dinitrobenzoyl moiety of the CSP.

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