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Mechanistic investigation into the enantioselective separation of mexiletine and related compounds, chromatographed on an amylose tris(3,5-dimethylphenylcarbamate) chiral stationary phase

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Abstract

Mexiletine and a series of structurally related compounds have been chromatographed on an amylose tris(3,5-dimethylphenylcarbamate) chiral stationary phase. By application of quantitative structure–enantioselective retention relationship and thermodynamic approaches, two separate retention mechanisms were identified. These mechanisms are based on either the presence or absence of secondary hydrogen-bonding groups. Highly statistically significant regression equations have been derived which describe the retentions of the first and second eluting enantiomers in terms of non-empirical molecular descriptors.

Keywords: Chiral stationary phases, LC; Quantitative structure–retention relationships; Retention mechanisms; Enantiomer separation; Amylose tris(3,5-dimethylphenylcarbamate); Mexiletine

1. Introduction

Mexiletine [1-(2,6-dimethylphenoxy)-2-aminopropane] is an oral antiarrhythmic drug, similar to lidocaine in structure and physiological effects [1]. It has been shown to be effective in the treatment of neuropathic pain of different aetiologies.

In this paper we report the findings of a study aimed at identifying the relationships between mexiletine and its analogues (at the enantiomeric level), and their respective affinities for a given receptor site. This is most conveniently achieved through extrathermodynamic linear free-energy relationships (LFERs). An extrathermodynamic approach to isolating the governing factors of chromatographic retention and enantioselectivity entails the combination of detailed models of the process being studied, with specific concepts of thermodynamics [2]. The demonstration of LFERs in a system insinuates a genuine link between the quantities being correlated, and means that the form of the underlying connection is probably identifiable.

Quantitative structure–enantioselective retention relationships (QSERRs) require reliable input data to ensure validity of the resultant equations. Chromatographic data lends itself to studies of this kind due to ready reproducibility and high precision. However, intercolumn variability introduces the potential for

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less universally meaningful relationships. The Chiralpak AD chiral stationary phase (CSP) (Daicel Chemical Industries, Tokyo, Japan) column was chosen for this study as it is representative of a class of stationary phases which are uniform, reliable and robust. These CSPs are widely used for the separation of many diverse racemic compounds [3–14], but little is actually known about the mechanisms of enantioseparation, operating on any of the amylosic phases. However, recently our group identified evidence for a conformationally driven chiral recognition mechanism operating on the Chiralpak AD CSP [15]. It is believed that by attempting to study specific molecular interactions operating during the chromatographic process, the information obtained may be exploited to gain the ability to predict enantioseparations and also to further development of highly efficient and even customised CSPs.

2. Experimental

2.1. Chemicals

Compounds 1–12 were all gifts from Boehringer Ingelheim (Burlington, Canada). HPLC-grade ethanol and hexane were obtained from Anachemia Science (Montreal, Canada). Diethylamine was purchased from Aldrich (Milwaukee, WI, USA).

2.2. Chromatography

The chromatographic system was composed of a Spectra-Physics P1500 binary pump, a Spectra-Physics UV1000 variable-wavelength detector and a Spectra-Physics SP8875 autosampler equipped with a 20-μl loop (Thermo Separation Products, Toronto, Canada). Separation was performed on a Chiralpak AD chiral column, 250×4.6 mm I.D. (Chiral Technologies, Exton, PA, USA). A Spectra-Physics Datajet integrator acted as an interface for electronic data collection using Winner on Windows software run on a 386 personal computer.

Column temperature regulation was achieved by using a Haake D1-G refrigerated bath/circulator (Fischer Scientific, Montreal, Canada) and a column water jacket.

The mobile phase consisted of hexane–ethanol (95:5)+0.1% diethylamine, filtered and degassed. All samples were prepared in mobile phase.

2.3. Computational chemistry

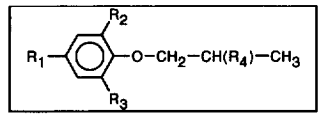
Molecular models were created using Insight II release 230 (Biosym, San Diego, CA, USA) run on an IBM RS6000 RISC workstation (IBM Corporation, Austin, TX, USA). All energy minimisations were performed using Discover ver. 2.9 (Biosym), running within Insight II

3. Results

A series of 12 mexiletine-related compounds were chromatographed on an amylose tris(3,5-dimethylphenylcarbamate) (AD) CSP, using a hexane–ethanol (95:5, v/v)+0.1% diethylamine mobile phase. Retention data were collected over the temperature range 0 to 30°C, and the results interpreted from both QSERR and thermodynamic aspects. The structures of the compounds studied are presented in Fig. 1.

The compounds were chosen in order that the small structural variations could be investigated in terms of their physico-chemical significance towards retention and enantioselectivity. Table 1 lists the relative retention (k') and enantioseparation (α) values of the twelve sample compounds, chromatographed at the highest and lowest limits of the temperature range.

The retention data were subjected to multiparame-



COMPOUND	R_1	R_2	R_3	R_4
1.	OH	CH_3	CH_3	OH
2.	H	CH_3	CH_2OH	OH
3.	H	CH_3	CH_2OH	NH_2
4.	CH_3	CH_3	CH_2OH	NH_2
5.	CH_3	CH_3	H	NH_2
6.	CH_3	CH_3	CH_3	NH_2
7.	NO_2	CH_3	CH_3	NH_2
8.	NH_2	CH_3	CH_3	NH_2
9.	OH	CH_3	CH_3	NH_2
10.	H	CH_3	CH_3	NH_2
11.	H	OCH_3	OCH_3	NH_2
12.	H	CH_2CHCH_2	CH_2CHCH_2	NH_2

Fig. 1. Structures of the compounds used in this study.

Table 1
Retention and selectivity data at 0°C and at 30°C, for compounds 1–12 chromatographed on a Chiralpak AD CSP

Compound	ln k'_1 at 0°C	ln k'_2 at 0°C	α at 0°C	ln k'_1 at 30°C	ln k'_2 at 30°C	α at 30°C
1	1.42	1.58	1.17	1.36	1.45	1.12
2	1.72	2.27	1.73	1.49	1.74	1.29
3	2.39	2.56	1.18	1.84	1.88	1.05
4	2.46	2.77	1.36	1.83	1.96	1.14
5	0.58	0.66	1.08	−0.01	−0.01	1.00
6	−0.48	−0.48	1.00	−0.80	−0.80	1.00
7	1.51	1.57	1.06	0.87	0.87	1.00
8	2.38	2.65	1.31	1.65	1.92	1.19
9	1.75	2.02	1.31	1.42	1.59	1.19
10	−0.16	−0.16	1.00	−0.67	−0.67	1.00
11	1.60	1.60	1.00	0.91	0.91	1.00
12	−1.24	−1.24	1.00	−1.43	−1.43	1.00

See Section 2.2 for chromatographic conditions.

ter regression analysis against various non-empirical molecular descriptors (Table 2), intuitively chosen for their suspected role in solute–stationary phase interactions (Table 1). The simplest possible relationships between the ln k' values at 0°C and these descriptors were established and are presented in Eq. (1) and Eq. (2) (see Table 3). Relationships were derived for retention at 0°C, as this temperature enabled the largest number of compounds to be separated.

In view of these two equations, it would appear that the major contribution to retention arises from the hydrophobicity of the substituents at R_1 and R_3.

Table 2
Structural descriptors used in the final QSERR

Compound	π_{R1}	π_{R3}	A_c	S_d
1	−0.67	0.56	−0.332	−2.742
2	0.00	−1.03	−0.505	0.187
3	0.00	−1.03	−0.498	0.134
4	0.56	−1.03	−0.436	−2.433
5	0.56	0.00	−0.470	−2.413
6	0.56	0.56	−0.406	−2.417
7	−0.28	0.56	−0.195	−2.840
8	−1.23	0.56	−0.389	−4.190
9	−0.67	0.56	−0.335	−3.003
10	0.00	0.56	−0.471	0.102
11	0.00	−0.02	−0.278	0.141
12	0.00	1.10	−0.458	0.118

π_{R1}=fragmental hydrophobicity of substituent R_1, π_{R3}= fragmental hydrophobicity of substituent R_3, A_c=total aromatic excess charge, S_d=substructure dipole. (Descriptors calculated according to Kaliszan et al. [20]).

With the electronic charge and dipole terms added, more differentiation between the first and second eluting enantiomers becomes possible. The hydrophobicity terms are common for both ln k'_1 and ln k'_2 and, being negative, indicate that retention increases with increasing hydrophilicity. This is to be expected for chromatographic systems operating under normal-phase conditions, due to solute solubility in the mobile phase. However, previous studies with aromatic acids [15] have shown that hydrogen-bonding ability can have a higher correlation to retention than hydrophobicity when a large series of compounds is investigated. Within this series, the term π not only relates to hydrophobicity, but also hydrogen-bond donor ability through an interrelation between the two descriptors of -0.8669 ($n=12$) at R_1 and -0.9234 ($n=12$) at R_3. Hence the model developed for the aromatic acids is comparable to the present series of mexiletine analogues. The incorporation of more specific descriptors is unfortunately not facilitated by the small sample set, therefore π is adopted with its inherent duality.

The inclusion of fragmental hydrophobicity, as compared to total molecular hydrophobicity, is believed to emphasise solute orientation during the partitioning process between mobile and stationary phases. Fragmental hydrophobicity is therefore more useful in describing aspects of specific retention, in contrast to general retention. The initial interaction is thus assumed to take place between the polar functional group at R_4 and a hydrogen-bond acceptor

Table 3
Equations (1) and (2)

$$\ln k'_1 = 3.1791 - 1.3383(\pm 0.5547)\pi_{R1} - 1.6451(\pm 0.4157)\pi_{R3} + 4.9370(\pm 3.2306)A_c$$

$t=5.31$, $p=0.0002$ $t=8.71$, $p<0.0001$ $t=3.36$, $p=0.0063$

$n=12$, $R=0.9580$, $F_{3,8}=29.74$, $F_{3,8\alpha=0.05}=4.07$, $p<0.0001$ (1)

$$\ln k'_2 = 0.9128 - 1.4554(\pm 0.5214)\pi_{R1} - 1.7345(\pm 0.3696)\pi_{R3} - 0.2834(\pm 0.1718)S_d$$

$t=6.14$, $p<0.0001$ $t=10.33$, $p<0.0001$ $t=3.63$, $p=0.0040$

$n=12$, $R=0.9702$, $F_{3,8}=42.71$, $F_{3,8\alpha=0.05}=4.07$, $p<0.00009$ (2)

π_{R1}: Fragmental hydrophobicity at position R_1;
π_{R3}: Fragmental hydrophobicity at position R_3;
A_c: Total aromatic excess electronic charge;
S_d: Substructure dipole.

The figures in parentheses are the standard deviations of the regression coefficients, n is the number of data points used to derive the regression, R is the correlation coefficient, F is the f-test value, t is the t-test value and p is the significance level of the individual variables and of the whole equation.

situated in the outside of the helical stationary phase cavity. Examination of the retention of compounds devoid of any further functional group suggests that this initial interaction is relatively weak, leading to low retention and practically no enantioselectivity. An increase in the stabilisation of the diastereomeric complex, sufficient to allow observable discrimination between enantiomers at high temperature, is only achieved via further hydrogen-bonding interactions. At low temperature, stabilisation, and consequently discrimination, is far more significant for those compounds containing secondary hydrogen-bonding interactions. Computer docking interactions employing the functional group at R_4 as the primary tethering interaction, followed by conformational adjustment and energy minimisation, enabled favourable stabilising interactions between the stationary phase and solute aromatic substituents to take place. Bulky substituents at both R_2 and R_3 seemed to sterically hinder the ability of the molecule to completely enter the stationary phase cavity, which is consistent with the chromatographic results.

A complimentary approach to investigating chromatographic retention mechanisms involves enthalpy–entropy compensation [16]. Analogously to QSERRs, enthalpy–entropy compensation manifests itself in a linear dependence of the overall free energy changes on the corresponding enthalpy change for intrinsically similar physico-chemical phenomena. By application of the Gibbs-Helmholtz equation ($\Delta G=\Delta H - T\Delta S$), it can be deduced that, in the vicinity of T, changes in ΔH are offset by changes in ΔS so that the free energy change is practically independent of temperature.

The capacity factor k', is the dimensionless unit employed to measure chromatographic retention, such that $k'=(t_R - t_0)/t_0=KV_s/V_m$, where t_R is the retention time of the solute, t_0 is the retention time of an unretained solute, K is the thermodynamic equilibrium constant for solute binding, V_s is the volume of stationary phase in the column and V_m is the volume of mobile phase in the column. V_s/V_m is also referred to as the column phase ratio, φ. The free energy change for the chromatographic process is expressed by $\Delta G=-RT \ln K=-RT \ln (k'/\varphi)$. Substitution into the Gibbs–Helmholtz equation for the capacity factor yields the final relationship $\ln k'=-\Delta H/RT+\Delta S/R+\ln \varphi$. If the mechanism of the chromatographic recognition process is invariant over the temperature range studied and the enthalpy is constant, then a linear dependence between $\ln k'$ and $1/T$ (Van't Hoff plot) is exhibited. Non-linearity within these plots is attributed to the presence of multiple retention mechanisms or distinct shape

Table 4
Van't Hoff plot data obtained for k'_1

Compound	Gradient	Intercept	Correlation
1	0.381	0.037	0.9090
2	0.722	−0.919	0.9780
3	1.590	−3.438	0.9966
4	1.866	−4.355	0.9966
5	1.511	−4.936	0.9872
6	0.991	−4.061	0.9516
7	1.845	−5.220	0.9936
8	2.171	−5.541	0.9957
9	0.936	−1.684	0.9980
10	1.399	−5.311	0.9939
11	1.865	−5.221	0.9984
12	0.743	−3.928	0.7120

Table 5
Van't Hoff plot data obtained for k'_2

Compound	Gradient	Intercept	Correlation
1	0.493	−0.216	0.9380
2	1.541	−3.375	0.9963
3	1.978	−4.689	0.9969
4	2.332	−5.757	0.9988
5	1.671	−5.469	0.9906
6	0.991	−4.061	0.9516
7	1.965	−5.620	0.9969
8	2.443	−6.263	0.9964
9	1.225	−2.471	0.9986
10	1.399	−5.311	0.9939
11	1.865	−5.221	0.9984
12	0.743	−3.928	0.7120

differences between molecules with similar retention [17].

In order to evaluate the effect of temperature on enantiomer retention, capacity factors for the twelve mexiletine analogues were measured over the temperature range 0–30°C. Table 4 and Table 5 list data obtained from Van't Hoff plots constructed for k'_1 and k'_2. The data for compounds 6, 10, 11 and 12 are the same for k'_1 and k'_2 as no separation was achieved at any temperature. Compound 12 clearly demonstrates non-linear behaviour suggesting multiple recognition mechanisms. This may be an artefact of this compound's very low retention in this chromatographic system and was subsequently removed from any further analysis. For the remaining eleven compounds, all plots are linear with positive slopes, thus the enthalpies of association are constant and negative over the temperature range studied.

Compensation temperatures may be used to determine whether or not all the solutes display similar retention mechanisms in a given system. A close correspondence of the compensation temperatures may be accepted as proof that the mechanisms are essentially identical [18]. Linearity in plots of ln k' vs. ΔH are indicative of compensation due to similar solute–stationary phase recognition mechanisms. Compensation plots for compounds 1–11 are shown in Fig. 2 and Fig. 3. Values for the capacity factors recorded near the harmonic mean of the temperature range were used in the plots to enhance accuracy

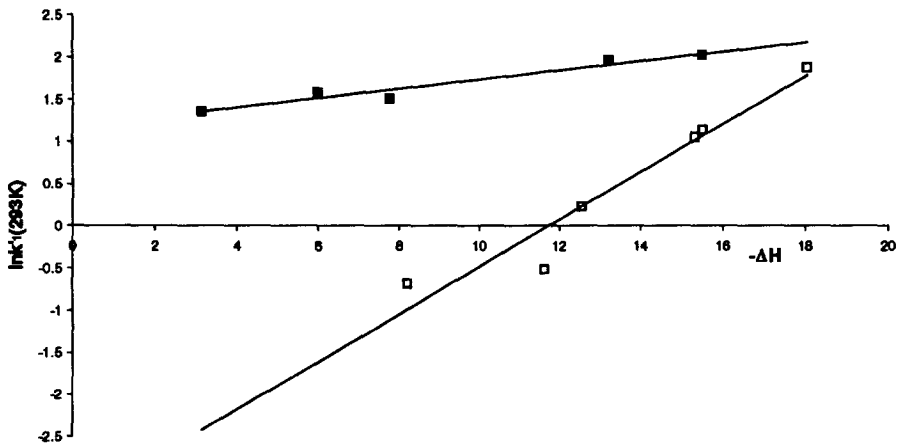


Fig. 2. Compensation plot for the first eluting enantiomers.

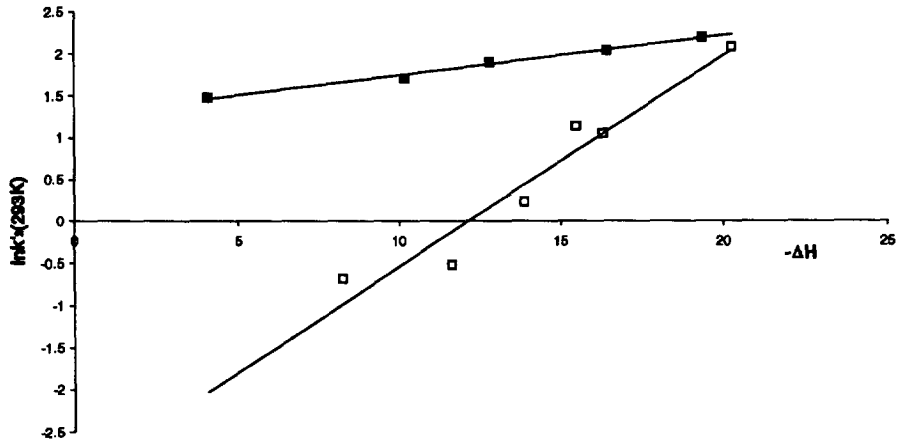


Fig. 3. Compensation plot for the second eluting enantiomers.

[19]. It is observed that in both plots the data fall into two distinct groups, with linearity being observed in both groups. All the compounds containing hydroxyl groups form the upper set, while the remaining compounds form the lower set. Although each set is linear, demonstrating enthalpy–entropy compensation within each set, the slopes and intercepts differ greatly. For ln k'_1 upper set, R=0.9736, slope=0.056 and intercept=1.1677. For ln k'_1 lower set, R=0.9651, slope=0.283 and intercept=−3.3238. For ln k'_2 upper set, R=0.9941, slope=0.0476 and intercept=1.2646. For ln k'_2 lower set, R=0.9665, slope=0.251 and intercept=−3.047. This indicates that the upper and lower sets have different compensation temperatures and therefore different retention mechanisms. The negligible differences between the two upper sets and also between the two lower sets indicates that, within each pair of separated enantiomers, the retention mechanisms are essentially the same, with only the free energy of the binding interactions differing slightly.

4. Discussion

Retention for this series of mexiletine analogues, chromatographed on a Chiralpak AD CSP, under the present conditions, has been shown to conform to two different mechanisms, primarily based upon substituent characteristics. The overwhelming ability of solute hydrogen-bond donor groups to influence retention and enantioselectivity has been demonstrated by the division in the compensation plots. The data for k'_1 and k'_2 are grouped into two sets, distinguished by their respective retention mechanisms. The first set contains solutes with secondary hydrogen-bonding sites in the form of hydroxyl groups and the second set contains the remainder of the compounds. Compound 8 is observed to be capable of being a member of both sets. It can be present in the first set due to the hydrogen-bonding capacity of the para amino group, but as this interaction is weaker than with a para hydroxyl group (as demonstrated by compound 1), it may also be grouped with the second set. These observations conform to the initial retention model proposed via computer docking simulations. Retention is believed to be a two-step process, the first interaction being a hydrogen bond, formed between the outer edge of the CSP helical cavity and the functional group at the solute stereogenic centre. This is followed by conformational adjustment and, steric interactions permitting, stabilisation of the diastereomeric complex via any remaining hydrogen-bonding groups.

Enantioselectivity arises mainly from increasing the stability of the diastereomeric complex via multiple hydrogen-bonding interactions. This forces a reduction in the separation between the solute stereogenic centre and the CSP, thus enhancing any discriminating steric interactions.

Further studies into the recognition mechanisms operating on amylosic CSPs have been conducted.

The results of these studies will be published elsewhere.

Acknowledgments

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