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Non-linear structure–enantioselective retention relationships in a homologous series of 1,4-disubstituted piperazine derivatives

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Abstract

A high-performance liquid chromatography method for the direct separation of enantiomers of a homologous series of 1,4-disubstituted piperazine derivatives, using a chiral cellulose tris(4-methylbenzoate) stationary phase and a hexane–propan-2-ol eluent, is described. An atypical relationship was found between enantioselectivity and the carbon number of the alkyl substituent with a maximum corresponding to four–five carbon atoms in the substituent. The optimum enantioselectivity observed suggests the existence of a limited size interaction site on the stationary phase and an input to retention due to the steric exclusion of individual solutes. © 1997 Elsevier Science B.V.

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1. Introduction

The limited availability of enantiospecific retention parameters for a longer series of congeneric solutes is the reason why only a few quantitative structure–enantioselective retention relationship (QSERR) studies have been reported [1–7]. Such studies might be of use in the investigation of the origins of enantioselectivity on a given chiral stationary phase and could help to optimize separation conditions.

Pirkle et al. [8,9], in their studies on the nature of enantioselectivity, used a homologous series of analytes as mechanistic probes. When there is a strong dependence of enantioselectivity upon the length of the alkyl group in the solute molecule, Pirkle et al. [9] propose the use of a modified chiral phase in which the interaction sites of the selector are "reoriented" so as to either exacerbate or relieve the interaction that causes enantioselectivity to be influenced by the length of the alkyl substituent. Another strategy could be to sufficiently lengthen to surpass maximum enantioselectivity.

In a search for new hypnotic and sedative agents, a homologous series of racemate 1,4-disubstituted piperazine derivatives (**1–9**, Fig. 1) was synthesized [10,11]. As stereoisomers often show different pharmacological activities, it seemed advisable to resolve

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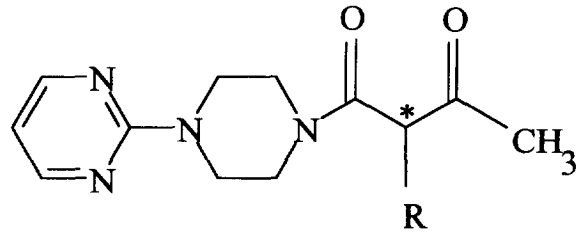

Comp. No	1	2	3	4	5	6	7	8	9
R	CH_3	C_2H_5	C_3H_7	C_4H_9	C_5H_{11}	C_6H_{13}	C_7H_{15}	C_8H_{17}	C_9H_{19}

Fig. 1. Chemical structures of the test analytes.

the racemic mixtures obtained. At the same time, the relatively long homologous series produced appeared to be uniquely appropriate for a study of the relationship between alkyl chain length and enantioselectivity.

2. Experimental

2.1. Chemicals

A series of nine homologous test solutes (Fig. 1) was synthesized and identified previously [10,11].

The solvents used were of high-performance liquid chromatography (HPLC) grade.

2.2. Apparatus

A Varian 9000 liquid chromatograph, equipped with a UV–Vis variable wavelength detector and a gradient pump (Varian Associates, Palo Alto, CA, USA) was used. A Valco valve injector (10 μl loop) was employed. The HPLC system was operated under a Star Workstation 4.0 program.

2.3. Chromatographic conditions

An achiral column, ODS80TM, TosoHaas (250× 4.6 mm I.D.) was purchased from Candela (Warsaw, Poland).

2.3.1. Chiral columns

The columns used were as follows: Chiralcel OJ, Daicel (250×4.6 mm I.D.), packed with cellulose tris(4-methylbenzoate) and immobilized on silica; Chiralcel CA-1, Daicel (250×4.6 mm I.D.), packed with microcrystalline cellulose triacetate; Chiralcel OD-R, Daicel (250×4.6 mm I.D.), packed with cellulose tris(3,5-dimethylphenylcarbamate) and immobilized on silica and AGP, Baker (100×4.6 mm I.D.), packed with acid α_1-glycoprotein and immobilized on silica.

All chiral columns were purchased from Witko-Eurocolor (Łódź, Poland).

2.3.2. Mobile phases

RP ODS80TM: methanol–0.01 *M* phosphate buffer, pH 7.0, (60:40, v/v); flow-rate, 1 ml/min.

Chiralcel OJ: hexane–propan-2-ol (80:20, v/v); flow-rate, 1 ml/min.

Chiralcel CA-1: ethanol 100%; methanol 100%; 30 min gradient from hexane–ethanol (70:30, v/v) to 100% ethanol; hexane–ethanol 70:30 (v/v); flow-rate, 0.5 ml/min.

Chiralcel OD-R: acetonitrile–water (40:60, v/v); flow-rate, 0.5 ml/min.

AGP: phosphate buffer (0.01 *M*; 100%) and propan-2-ol–0.01 *M* phosphate buffer, pH 7.0 (5:95, v/v); flow-rate, 0.9 ml/min.

The temperature was kept at 21°C and detection was at 242 nm.

3. Results and discussion

Compounds **1–9** exhibited typical behaviour when examined in an achiral chromatographic environment (C_{18} reversed-phase stationary phase). The capacity factors (Table 1) exhibited very good linear correlations with the calculated lipophilicities (Eq. (1)).

$$\log k' = 0.531(\pm 0.025) \cdot \log P - 0.074(\pm 0.058) \quad (1)$$

$$n = 9,\ r = 0.992,\ s = 0.0970,\ p \leq 0.001,\ F = 451.30$$

The retention of the homologous series of analytes **1–9** can, in principle, be described by the aid of methylene group increments according to the formula given in Eq. (2) [12–17]:

$$\log k'_s = \log k'_p + \sum_{n=1}^{n} \tau_n \quad (2)$$

where $\log k'_p$ is the retention of a parent compound; $\log k'_s$ is the retention of a derivative carrying n substituents; $\tau_X = \log k'_{R-X} - \log k'_{R-H}$.

The average methylene group contribution for the compounds was found to be $\tau_{CH2} = 0.26 \pm 0.11$ (Table 1). Three of the methylene increments went substantially outside of the average range: $\tau_{C2-C1} = 0.16$, $\tau_{C8-C7} = 0.52$ and $\tau_{C9-C8} = 0.14$. The remaining increments fell within the range, $\tau = 0.21–0.27$, with the average value being $\tau = 0.25 \pm 0.02$ (which is not very different from the former value, 0.26). The results were in good agreement with the results obtained by Smith et al. [14,16,17]. The separation of compounds **1–9** into enantiomers was tested on several columns under different conditions. Resolution of all of the compounds was achieved on cellulose tris(4-methylbenzoate).

Table 1
Capacity factors and methylene groups increments for compounds **1–9**

Analyte number	log k'	log P^{a}_{cal}	Methylene group increment	
			Group	τ
1	0.0048	−0.06	C_2–C_1	
2	0.1682	0.44	C_2–C_1	0.16
3	0.3909	0.94	C_3–C_2	0.22
4	0.6024	1.44	C_4–C_3	0.21
5	0.8593	1.94	C_5–C_4	0.26
6	1.1274	2.45	C_6–C_5	0.27
7	1.3959	2.95	C_7–C_6	0.27
8	1.9142	3.45	C_8–C_7	0.52
9	2.0528	3.95	C_9–C_8	0.14

Chromatographic parameters: Column C_{18} (250×4.6 mm I.D.); mobile phase, methanol–0.01 *M* phosphate buffer, pH 7.0, 60:40 (v/v).

[a]Log P_{cal} values were calculated with the aid of the ProLogP 4.2 program, CompuDrug, Hollán Ernö, Budapest, Hungary.

Chromatograms of the test solutes obtained on this stationary phase are presented in Fig. 2. The assignment of two chromatographic peaks as arising from enantiomers was verified according to Pirkle et al. [18]. The enantioselectivity of the chiral phase used for the analytes studied is very good, in contrast to that found with several of the other chiral phases tested, i.e., microcrystalline cellulose triacetate (Chiralcel CA-1), cellulose tris(3,5-dimethylphenylcarbamate) (Chiralcel OD-R) and acid α_1-glycoprotein (AGP). Whereas no chiral separation was obtained on Chiralcel CA-1 and OD-R phases, the heptyl, octyl and nonyl derivatives were separated on AGP (Table 2).

Capacity factors for the less retained enantiomer (k'_1) and for the more retained enantiomer (k'_2), determined on Chiralcel OJ, are shown in Table 2, along with the selectivity (α) and the resolution (R_S).

In Fig. 3, the logarithms of capacity factors for the first-eluting isomer and for the second-eluting one are plotted against the carbon number of the alkyl substituent, N_C. The plots are unusually smooth.

Fig. 3 shows that enantioselectivity increases initially, with elongation of the aliphatic substituent. A similar observation was reported previously [8,9]. However, in our case, maximum selectivity is evident for four–five carbon alkyl groups. For longer aliphatic substituents, the selectivity decreases and eventually attains a more or less constant level. That tendency is also evident in Table 1 for selectivity, α, and resolution, R_S. The resolution appears to reach a maximum level for three–five carbon atom long alkyl groups.

An optimum alkyl chain length for enantioseparation (Fig. 3, Table 2) is an unusual feature of our HPLC system and of the analytes tested. Pirkle et al. [9] had not observed a maximum on their enantioselectivity versus alkyl chain length relationship for a series of seven–eight *n*-alkoxy carbamates and *p*-alkyl anilides chromatographed on an *N*-(3,5-di-

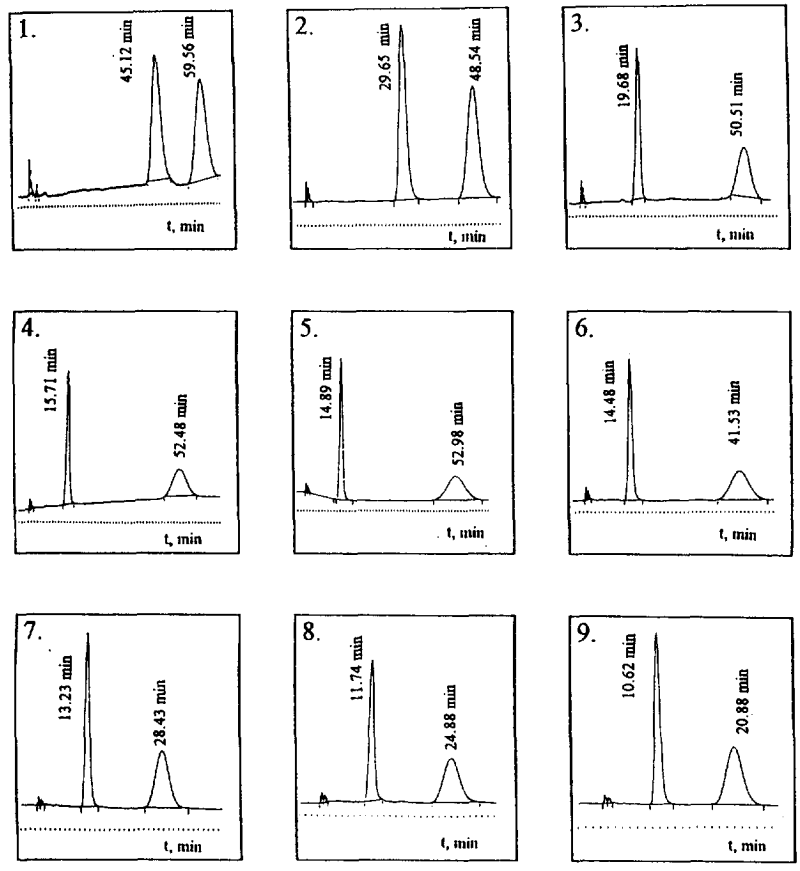

Fig. 2. Chromatograms of the test compounds. Numbers correspond to the structures presented in Fig. 1.

nitrobenzoyl)leucine-derived phase. The alkyl chain length of the analytes referred to in Ref. [9] varied from one to fourteen carbon atoms. Enantioselectivity increased steeply up to seven–eight carbons and then did not change up to fourteen carbons. However, no further tendency of enantioselectivity to decrease with further elongation of the alkyl chain length can be anticipated.

The maximum enantioselectivity observed for four–five carbon alkyl groups (for which there is also the strongest retention of the more retained enantiomer) would suggest that this is the size of the alkyl-bonding site on the stationary phase. Because of steric restrictions, only one–two carbons of the four–five atoms long substituent of the corresponding less retained enantiomers can reach the hydrocarbon bonding site, giving rise to maximum enantioselectivity. With longer alkyl groups, the enantioselectivity decreases again because an increase in the attractive interactions of both enantiomers with the hydrocarbon-binding site on the stationary phase is greater than the attraction with the hydrocarbonaceous eluent.

The structure–enantioselectivity relationships described provide some insight into the mechanism of enantioseparation at the molecular level. A single type of enantioselective recognition site on Chiracel OJ suffices to explain the observations. This is unlike the enantioseparation of acidic drugs on a human serum albumin chiral stationary phase in which two enantioselective interaction sites have been postulated [19].

Table 2
Capacity factors of the first-eluting enantiomer (k'_1) and the second-eluting enantiomer (k'_2), selectivity (α) and resolution (R_S) for the set of analytes given in Fig. 1 and chromatographed on chiral columns

Analyte number	Chiralcel OJ				AGP		
	k'_1	k'_2	α	R_S	k'_1	k'_2	α
1	11.10	14.99	1.35	2.42	0.61	0.61	1.00
2	7.04	12.17	1.75	4.09	0.99	0.99	1.00
3	4.33	12.69	2.93	6.51	1.26	1.26	1.00
4	3.24	13.17	4.12	6.89	1.96	1.96	1.00
5	3.04	13.36	4.39	6.05	3.21	3.21	1.00
6	2.91	10.22	3.51	5.31	6.09	6.09	1.00
7	2.60	6.74	2.59	4.33	12.24	15.51	1.27
8	2.18	5.74	2.63	3.84	29.85	40.25	1.35
9	1.87	4.65	2.49	3.43	71.92	111.5	1.55

The chiral columns used were a cellulose tris(4-methylbenzoate) stationary phase (Chiralcel OJ) with hexane–propan-2-ol (80:20, v/v) as the eluent and an acid α_1-glycoprotein stationary phase (AGP) with 0.01 *M* phosphate buffer, pH 7.0,–propan-2-ol (95:5, v/v) as the eluent.

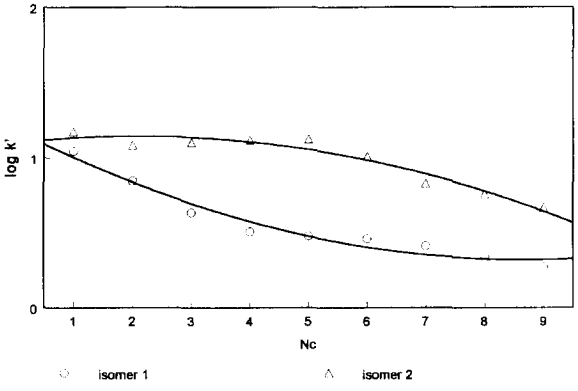

Fig. 3. Relationships between logarithms of capacity factors, log k' and the number of carbon atoms in the alkyl substituent of the test analytes, N_C. Circles denote the first-eluting enantiomer and triangles denote the second-eluting enantiomer.

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