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Short communication

Influence of temperature on the enantioseparation of rolipram and structurally related racemates on Chiracel-OD

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Abstract

The temperature dependence of the chiral separations of rolipram and structurally related racemates was investigated in liquid chromatography with Chiralcel-OD as stationary phase. The thermodynamic data reveal that the enantioseparation of rolipram and two other racemates belong to the unusual case of entropy-controlled separations whereas for the remaining racemates the expected enthalpy-controlled separations were observed. In particular, at 20°C not even a partial separation is obtained for rolipram whereas a complete baseline resolution is achieved at 65°C.

Keywords: Enantiomer separation; Temperature effects; Thermodynamic parameters; Rolipram

1. Introduction

During enantioseparation of racemates on chiral stationary phases, a decrease of enantioselectivity at higher column temperatures is very often observed since most separations are enthalpy controlled. Davankov was the first to report on an improved enantioselectivity at rising column temperature when separating racemic N-benzylproline on a ligand exchange column (L-proline-incorporated resin) [1]. It was found that the entropy contribution dominated the enthalpy contribution over the whole temperature range studied. Although enantioseparation on chiral stationary phases is nowadays a routine procedure, only a few studies have demonstrated increased enantioselectivity at higher column temperatures. Such a behaviour has been reported by Isaksson [2] for the separation of propranolol on immobilized cellulase (CBH I), by Cabrera [3] for the enantioseparation of Prominal on ChiraDex and in a previous work from our group [4] for the separation of 4-hydroxyphenylmethyl-sulphoxide on Chiralcel-OB.

The influence of temperature on the elution order has been well documented. A change in elution order was predicted by Koppenhöfer and Bayer [5] based on thermodynamic considerations and later observed experimentally by Schurig [6] and Watabe [7] in gas chromatography and by Pirkle [8] in high-pressure liquid chromatography.

It is the scope of this paper to demonstrate the feasibility of Chiralcel-OD for the enantioseparation of rolipram derivatives. In addition, the separation of rolipram is investigated at different temperatures and the thermodynamic parameters $\Delta_{(R,S)}\Delta H^0$ and $\Delta_{(R,S)}\Delta S^0$ have been determined.

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2. Experimental

2.1. Materials

The structures of rolipram (**1**) and its derivatives (**2–10**) investigated in this study are given in Fig. 1. All racemates were kindly donated by Dr. J. Demnitz (Sandoz Pharma, Basel, Switzerland). The solvents used for the preparation of the mobile phases were of LiChrosolv grade from Merck (Darmstadt, Germany). The mobile phase compositions and chromatographic parameters are given in Table 1 and Table 2.

2.2. Liquid chromatography

A Kontron HPLC pump (Model 420) was used in conjunction with a Kontron variable-wavelength UV detector (Model 430). The column temperature was maintained with a Henggeler (Riehen, Switzerland) column thermostat. The HPLC column was Chiralcel-OD (25 cm×4 mm I.D.) from Daicel Chemical

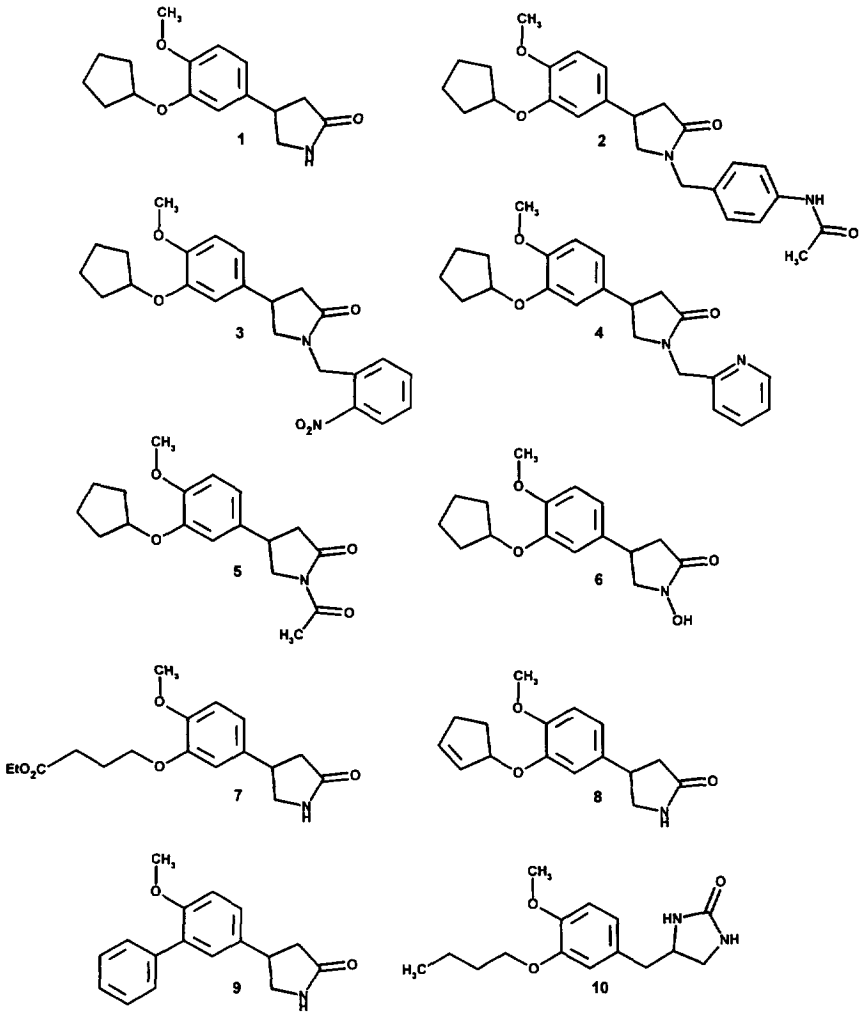

Fig. 1. Structures of investigated racemates (compound **1** refers to rolipram).

Table 1
Retention times and enantioselectivity of chiral rolipram derivatives on Chiralcel-OD

Racemate	40°C 40% 2-propanol			40°C 10% 2-propanol			60°C 40% 2-propanol			60°C 10% 2-propanol		
	t_R (min)[a]	k'_2	α	t_R (min)[a]	k'_2	α	t_R (min)[a]	k'_2	α	t_R (min)[a]	k'_2	α
1	10.26	1.23	1.00	33.04	6.18	1.03	8.61	0.87	1.00	26.72	4.80	1.07
2	11.72	1.54	1.35	150.26	31.66	1.29	10.01	1.17	1.41	128.82	27.00	1.34
3	13.18	1.86	1.10	43.15	8.38	1.10	9.53	1.07	1.00	29.09	5.32	1.06
4	9.93	1.15	1.06	30.93	5.72	1.07	8.05	0.75	1.00	22.46	3.88	1.00
5	9.44	1.05	1.00	19.15	3.16	1.04	7.72	0.67	1.00	13.83	2.00	1.00
6	10.94	1.37	1.00	39.95	7.68	1.00	8.94	0.94	1.00	32.18	5.99	1.00
7	12.03	1.61	1.00	64.52	13.02	1.08	9.48	1.06	1.00	47.14	9.24	1.08
8	11.00	1.39	1.00	42.15	8.16	1.00	9.16	0.99	1.00	33.82	6.35	1.00
9	11.77	1.55	1.00	43.05	8.35	1.00	9.76	1.12	1.00	34.99	6.60	1.03
10	10.62	1.30	1.37	45.22	8.83	1.35	8.71	0.89	1.28	36.6	6.95	1.24

Mobile phases: *n*-hexane–2-propanol (9:1) and *n*-hexane–2-propanol (6:4) with a flow-rate of 0.5 ml/min; temperatures: 40°C and 60°C, respectively; UV detection: 210 nm; injection volume: 20 μl (1–2 mg/ml).
[a]Retention time of second eluted enantiomer.

Industries (Tokyo, Japan). The chiral stationary phase consists of cellulose tris(3,5-dimethylphenylcarbamate) coated on macroporous silica gel with a particle size of 10 μm.

3. Results and discussion

To compare the retention times and enantioselectivities of all the racemates, all separations were done under identical conditions within each screening. The amount of organic modifier, usually 10% 2-propanol, was adjusted in the second series to 40%. In addition, all separations were carried out at 40°C and 60°C. The capacity factors $[k'=(t_R-t_0)/t_0]$ and separation factors ($\alpha=k'_2/k'_1$) obtained are given in Table 1.

As expected, the retention times increase with decreasing column temperature and/or amount of organic modifier. With the exceptions of **6** and **8**, chiral discrimination is observed for all described racemates. Nevertheless the most surprising result is the different behaviour of enantioselectivity α.

Table 2
Effect of temperature on enantioselectivity of rolipram **1** on Chiralcel-OD

Temperature		4% 2-propanol		2% methanol		7% 4-methyl-2-pentanol	
°C	K	t_R (min)[a]	α	t_R (min)[a]	α	t_R (min)[a]	α
10	283.17	113.71	1.00	n.p.		n.p.	
25	298.17	79.14	1.03	n.p.		n.p.	
40	313.17	66.71	1.08	70.83	1.04	78.30	1.12
60	333.17	63.42	1.11	64.28	1.07	70.59	1.14
65	338.17	64.41	1.12	n.p.		n.p.	
		$\Delta_{(R,S)}\Delta H^0 = 1.62$ kJ/mol					
		$\Delta_{(R,S)}\Delta S^0 = 5.72$ J/mol K					

[a]Retention time of second eluted enantiomer.
n.p = Experiment not performed. Mobile phases: *n*-hexane–2-propanol (96:4), *n*-hexane–methanol (98:2) and *n*-hexane–4-methyl-2-pentanol (93:7) with a flow-rate of 0.5 ml/min; UV detection: 210 nm; injection volume: 20 μl (1–2 mg/ml); temperature as indicated.

Whereas for racemates **3**, **4**, **5** and **10** enantioselectivity decreases with increasing temperature, enantioselectivity increases with increasing temperature for racemates **1**, **2** and **9**. No temperature dependance is observed for racemate **7**. Obviously, the change in temperature influences differently the interaction of the enantiomers with Chiralcel-OD. This may be better understood if one considers the Gibbs–Helmholtz parameters $\Delta_{(R,S)}\Delta H^0$ and $\Delta_{(R,S)}\Delta S^0$.

The difference between the free energies of association can be calculated from the difference in retention time via enantioselectivity α according to the equation:

$$\Delta_{(R,S)}\Delta G^0 = RT \ln K_R/K_S = RT \ln \alpha \quad (1)$$

where the subscript R refers arbitrarily to the later- and S to the earlier-eluting enantiomer. The temperature dependence of the enantioselectivity α can be employed to calculate the Gibbs–Helmholtz parameters $\Delta_{(R,S)}\Delta H^0$ and $\Delta_{(R,S)}\Delta S^0$ of chiral recognition according to the equation

$$\ln \alpha = -(\Delta_{(R,S)}\Delta H^0/RT) + (\Delta_{(R,S)}\Delta S^0/R) \quad (2)$$

According to Eq. 2, a plot of ln α against $1/T$ is linear, the slope being the difference between the enthalpy of association of the enantiomers with the stationary phase.

Table 1 indicates that the effect of entropy controlled separation is best expressed for rolipram (**1**). The Gibbs–Helmholtz parameters $\Delta_{(R,S)}\Delta H^0$ and $\Delta_{(R,S)}\Delta S^0$ have therefore been carefully determined in a second experiment. Because of a better resolution with 10% 2-propanol, the concentration of the organic modifier was further decreased to 4%. In addition, different alcohols were tested as modifiers to investigate whether the nature of the alcohol is responsible for the unusual thermodynamic behaviour. Therefore, the temperature dependence of the separation was additionally investigated with methanol and 4-methyl-2-pentanol. The results of this study are summarized in Table 2. The Gibbs–Helmholtz parameters $\Delta_{(R,S)}\Delta H^0$ and $\Delta_{(R,S)}\Delta S^0$ have been calculated according to Eq. 2 with 4% 2-propanol as organic modifier and the results are plotted in Fig. 2. A value of $\Delta_{(R,S)}\Delta H^0 = 1.62$ kJ/mol supports the assumption that chiral discrimination seems to be the result of an additional weak $\pi-\pi$ interaction or weak hydrogen bonding.

With 4% 2-propanol in n-hexane no separation occurs at 10°C and 20°C whereas an excellent separation is obtained at 60°C (Fig. 3). With methanol and 4-methyl-2-pentanol as modifier, similar results are obtained. Again, in both cases which have

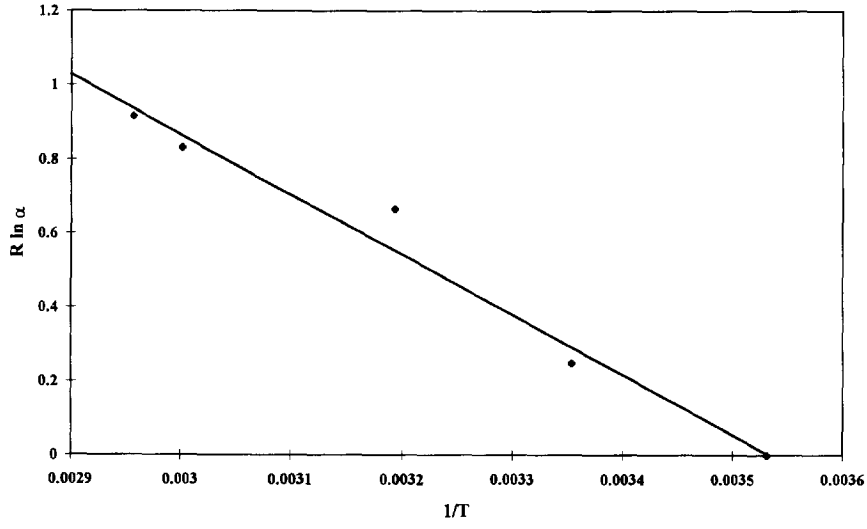

Fig. 2. Plot of R ln α against $1/T$ for rolipram **1** on Chiralcel-OD with 4% 2-propanol in n-hexane as mobile phase (chromatographic conditions as indicated in Table 2)

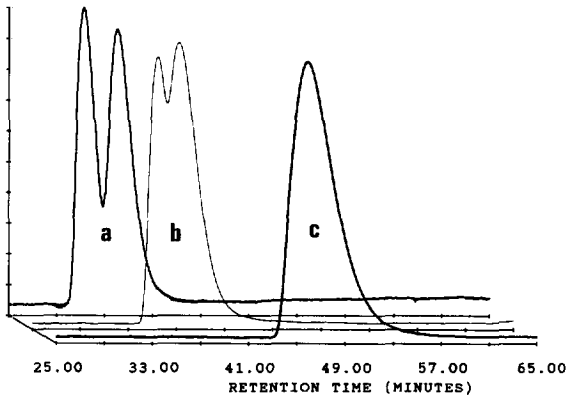

Fig. 3. Effect of temperature on enantiomeric separation of rolipram **1** on Chiralcel-OD. Mobile phase: *n*-hexane–2-propanol (96:4), 1.0 ml/min, UV detection: 210 nm; temperature: 60°C (a), 40°C (b) and 20°C (c).

only been investigated at 40°C and 60°C, enantioselectivity increases with increasing temperature. All separations are thus entropy controlled.

Since the separation factor is linked to changes in the free energy of association and the latter depends on the interplay between enthalpy change, entropy change and temperature, at lower temperature the separation factor may, in principle, increase again with simultaneous reversal of the order of elution. Unfortunately, it was not possible to confirm this prediction. With 4% 2-propanol the enantiomers of rolipram are not separated at 10°C. At this temperature, long retention times are observed and strong peak broadening prevents chiral separations because lower separation efficiencies due to peak broadening compensate enantioselectivity.

In conclusion, it has again been demonstrated that variation of temperature should be considered to improve a chiral separation in liquid chromatography, especially if enantiomerization at elevated temperatures can be ruled out and/or the separation itself is entropy controlled.

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