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Thermodynamic studies of complexation and enantiorecognition processes of monoterpenoids by α- and β-cyclodextrin in gas chromatography

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Dedicated to the memory of Professor Danuta Sybilska.

Abstract

Gas–liquid chromatography was applied in thermodynamic investigations of processes of complexation and enantioseparation by α- and β-cyclodextrins of chiral monoterpenoids. The distribution constants, stability constants and thermodynamic parameters enthalpy, entropy and free energy of the complexation processes were determined. It has been found that enantioseparation of monoterpenes by α- and β-cyclodextrins is the result of formation of 1:2 stoichiometric complexes. When 1:1 stoichiometric complexes are formed, enantioselectivity is not observed. All investigated processes of complexation are enthalpy-driven regardless of the stoichiometry of the formed complexes. $-\Delta H$, $-T\Delta S$ and $-\Delta G$ of complexation process have higher values for bicyclic than for monocyclic monoterpenoids as well as for α-CD than for β-CD. The first or second step of complexation may be responsible for enantioselectivity.

Keywords: Gas chromatography; Cyclodextrins; Enantiomer separation; Chiral monoterpenoids; Stability constants; Inclusion complexes; Thermodynamics

1. Introduction

Chiral gas chromatography today is dominated by capillary columns with various derivatives of cyclodextrins as stationary phases. Enantioseparation is obtained mainly owing to the process of complexation of a chiral guest-molecule by enantiomerically pure host-cyclodextrin. Such a system, characterized by great efficiency of the capillary column, enables the separation of enantiomers even when enantioselectivity of the system is low ($\alpha \leq 1.02$).

In spite of very diverse practical applications, the mechanism of complexation and structural relations are still unsolved or only partly solved problems and remain open for discussion. In this matter, thermodynamic studies could supply some valuable information facilitating identification of the physicochemical basis of complexation processes. Gas chromatography modified with cyclodextrins is one of a variety of experimental methods employed in the determination of thermodynamic quantities for the formation of cyclodextrin inclusion complexes [1–4]. The system of classical gas chromatographic columns with a solution of native cyclodextrins as the chiral stationary phase, developed in our laboratory in the early 1980s, lost its significance as an analytical tool mainly because of low efficiency [5]. However, it can be a useful tool for investigation of mechanisms of chiral recognition by cyclodextrins, as we have recently shown [6,7].

The aim of the current work was to look inside the mechanism of complexation and to establish some structural relations using α- and β-CD complexes with terpenoids as the model compounds. The stoichiometry, stability constants of the α- and β-CD complexes as well as the thermodynamic function were determined using gas–liquid chromatography.

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Monoterpenoids were chosen because of the diversity of their composition and isomeric forms which makes them an interesting object in the search for structural relations.

2. Theoretical considerations

Our experimental set-up consists of a GLC system with pure solvent without CDs (system I) and with CDs as a chiral agent (system II).

According to Eq. (1), the distribution constants were calculated from retention data and the measured density of the stationary phase.

$$K = \frac{jt'_R F_C \rho_S}{w_S} \tag{1}$$

where F_C is the volume flow-rate of gas phase; t'_R, adjusted retention time; ρ_S, density of stationary phase; w_S, weight of stationary phase; j, compressibility correction factor $j = 1$.

In system I, without a chiral agent, the chromatographic process is dependent only on the partitioning process between the mobile and stationary phase:

$$G_{(g)} \overset{K_0}{\rightleftarrows} G_{(l)}$$

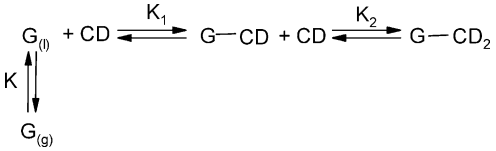
$$K_0 = \frac{[G_{(l)}]}{[G_{(g)}]} \tag{2}$$

where K_0 is the distribution constant of solute G between the gaseous (g) and liquid (l) phase. The van't Hoff expression for such a chromatographic system is:

$$\ln K_0 = -\frac{\Delta H_0}{R}\frac{1}{T} + \frac{\Delta S_0}{R} \tag{3}$$

where K_0 is the distribution constant in system I, ΔH_0 and ΔS_0 represent enthalpy and entropy changes of transfer of the solute G between the mobile and stationary phase, R is the gas constant and T is the absolute temperature.

In system II with CDs as a chiral agent, both processes of transfer and complexation occur. The process of complexation of solute G with n molecules depends on the stoichiometry. In our system, both 1:1 and 1:2 complexes can be formed. The equilibria are then as follows:



where K is the distribution constant in system II, K_1 and K_2 are the stability constants of complexes of 1:1 and 1:2 stoichiometry, respectively. The van't Hoff expression for system II is:

$$\ln K = -\frac{\Delta H}{R}\frac{1}{T} + \frac{\Delta S}{R} \tag{4}$$

where ΔH and ΔS represent enthalpy and entropy changes of transfer of the solute G between the mobile and stationary phase and complexation of the solute by CD.

The stability of the CD complexes was determined using the following simplified equation [6]:

$$K = K_0\left(1 + \sum_{n=1}^{N}\prod_{i=1}^{n} K_i[\mathrm{CD}]^n\right) \tag{5}$$

where K_0 and K are the distribution constants in system I and II, K_i is the stability constant of complexes G[CD]$_i$ of 1:i stoichiometry.

For 1:1 stoichiometry, the van't Hoff expression is as following:

$$\ln K_1 = -\frac{\Delta H - \Delta H_0}{R}\frac{1}{T} + \frac{\Delta S - \Delta S_0}{R} - \ln\left(\frac{1}{K_1} + [\mathrm{CD}]\right) \tag{6}$$

while for 1:2 stoichiometry the equation is:

$$\ln K_1K_2 = -\frac{\Delta H - \Delta H_0}{R}\frac{1}{T} + \frac{\Delta S - \Delta S_0}{R} - \ln\left(\frac{1}{K_1K_2} + \frac{[\mathrm{CD}]}{K_2} + [\mathrm{CD}]^2\right) \tag{7}$$

where K_1 and K_2 are the stability constants of CD complexes of 1:1 and 1:2 stoichiometry.

Enthalpy changes of complex formation (ΔH^*) in the stationary phase in system II can now be defined by Eq. (8).

$$\Delta H^* = \Delta H - \Delta H_0 \tag{8}$$

Differences in the change of Gibbs free energy of complexation for each enantiomer at temperature 333 K were determined according to the equation:

$$\Delta G^* = -RT\ \ln K_1 \tag{9}$$

for enantiomers of 1:1 stoichiometry and:

$$\Delta G^* = -RT \ln K_1K_2 \tag{10}$$

for enantiomers of 1:2 stoichiometry.

The entropy changes of complexation were determined from the relation:

$$\Delta G^* = \Delta H^* - T\Delta S^* \tag{11}$$

The enthalpy of complex formation was calculated from Eq. (8).

The enantioseparation factor α is given by the equation:

$$\alpha = \frac{K^{II}}{K^{I}} \tag{12}$$

where K^I and K^{II} are distribution constants of enantiomers eluted as a first and second ones.

For 1:2 stoichiometric complexes, we can write:

$$\alpha = \frac{1 + K_1^{II}[CD] + K_1^{II}K_2^{II}[CD]^2}{1 + K_1^{I}[CD] + K_1^{I}K_2^{I}[CD]^2} \quad (13)$$

Enantioselectivity factor α changes asymptotically with CD concentration from 1.0 for [CD] = 0 to the constant value $K_1^{II}K_2^{II}/K_1^{I}K_2^{I}$ giving absolute enantioselectivity E that should be independent from the CD concentration [8].

$$E = \frac{K_1^{II}K_2^{II}}{K_1^{I}K_2^{I}} \quad (14)$$

where I and II refer to enantiomers eluted from the column as the first and the second ones, K_1 and K_2 are the stability constants. E should be constant and independent of the CD concentration. A comparison of the absolute enantioselectivity separately for the first and second step of complexation (K_1^{II}/K_1^{I} and K_2^{II}/K_2^{I}) provides information about which step of complexation is responsible for enantioseparation.

The enthalpy–entropy compensation study was used by many authors [2,9–11] to investigate the retention mechanism of separated enantiomers. It can be expressed by the following equation:

$$\Delta H^\circ = \beta \Delta S^\circ + \Delta G^\circ{}_\beta \quad (15)$$

where $\Delta G^\circ{}_\beta$ is Gibbs free energy of physicochemical interaction at a compensation temperature β. ΔH° and ΔS° are the corresponding free enthalpy and entropy, respectively. It is expressed as a linear relationship between ΔH and ΔS. The slope of this relation is called the compensation temperature β.

When the enthalpy–entropy compensation is observed in GC or HPLC for a group of compounds, all of them have the same free energy change $\Delta G^\circ{}_\beta$ and net retention time at compensation temperature β. The similarity of the compensation temperature values for the group of compounds indicate similarity of the mechanism of their separation.

3. Experimental

3.1. Chemicals

α- and β-Cyclodextrin were supplied by Cyclolab (Budapest, Hungary). The Chromosorb W NAW (60–100 mesh) for GC was a product of Johns-Manville (Litho, USA). The model compounds (+)- and (−)-β-pinene, (+) and (−)-fenchone, (+)-menthone, (+)- and (−)-pulegone, were supplied by Fluka (Buchs, Switzerland). (+)- and (−)-α-Pinene and (−)-menthone were from Aldrich (Milwuakee, USA). (+)- and (−)-Limonene were from Merck-Schuchardt (Hohenbrunn, Germany). (+)- and (−)-Isomenthone were components of (−)- and (+)-menthone, respectively. The structures of the model compounds are presented in Table 1. All other substances were of analytical reagent grade and were used without further purification.

3.2. Columns

Glass columns with dimensions 2 m × 4 mm I.D. were packed with Chromosorb W NAW (60–100 mesh), which was coated with α-CD or β-CD dissolved in glycerol or with pure glycerol as matrix column. To improve solubility of α-CD, lithium nitrate (0.005 mol cm^{-3}) was added to the solution. The same amount of $LiNO_3$ was added to the solution of β-CD and pure glycerol (matrix column). Detailed information on cyclodextrin concentrations is shown in Table 2.

Table 1
The names and structural formulae of investigated compounds

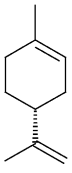
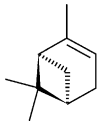
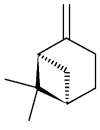
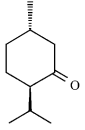
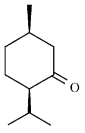
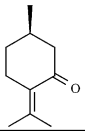
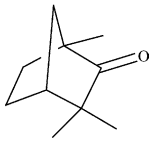
	Monocyclic	Bicyclic
Hydrocarbons	(+)-Limonene 	(+)-α-Pinene  (+)-β-Pinene 
Ketones	(+)-Menthone  (+)-Isomenthone  (+)-Pulegone 	(+)-Fenchone 

Table 2
Column characteristics

Column code	Cyclodextrin	Concentration of CD in stationary phase (m)[a]	Stationary phase
Matrix	–	0.000	Glycerol
A1	α-CD	0.054	Glycerol
A2	α-CD	0.081	Glycerol
A3	α-CD	0.108	Glycerol
A4	α-CD	0.136	Glycerol
A5	α-CD	0.162	Glycerol
B1	β-CD	0.054	Glycerol
B2	β-CD	0.108	Glycerol
B3	β-CD	0.136	Glycerol
B4	β-CD	0.162	Glycerol

[a] m, molality.

3.3. Apparatus and procedure

Chromatographic studies were performed using a Hewlett-Packard (Waldbronn, Germany) Model 5890 series II gas chromatograph equipped with dual flame ionisation detectors. The peak areas and retention time were measured by means of an HP 3396 series II integrator. Chromatographic measurements were performed at five temperatures: 333, 338, 343, 348 and 353 K. Each measurement was carried out two or three times. Methane was co-injected as a marker to determine the hold-up time (t_M). The flow-rate (40 ± 0.50 ml/min) was carefully maintained.

Density measurements were performed using an Anton Paar (Austria) densitometer. Measurements were performed at the same temperatures as in the chromatographic studies.

4. Results and discussion

4.1. Determination of distribution constants

The distribution constants K were calculated on the basis of Eq. (1) and measured densities of stationary phases and retention times of investigated compounds. Table 3 presents the distribution constants of monoterpenoids at temperature 333 K on the matrix, α- and β-CD columns.

It can be seen that for all compounds the distribution constants are higher on the α- and β-CD column than on the matrix column. This suggests that for those compounds inclusion complexes are formed with α- and β-CDs.

4.2. Determination of stability constants of inclusion complexes

The relation (linear or parabolic) of the distribution constant from CD concentration provides information about the stoichiometry (1:1 or 1:2) of the formed complexes. For α-CD, this relation is parabolic for most of the investigated compounds except (±)-pulegone, while for β-CD, it is linear except for (+)- and (−)-fenchone and (+)- and (−)-isomenthone. Moreover, in all cases where a parabolic relation is obtained, enantioseparation is observed. For the linear relation, enantiorecognition is not observable.

Sample plots of relation of the distribution constants versus cyclodextrin concentrations are presented in Fig. 1. The values of stability constants calculated from Eq. (5) are listed in Table 4. The example of separation of α-pinene enantiomers on α-CD column is presented in Fig. 2.

For all bicyclic compounds, stability constants of the first step of complexation with α-CD are much higher than those of the second step of complexation. For monocyclic compounds K_2 is higher than K_1 or has a comparable value, for instance for limonene.

An interesting observation was made for enantiomers of fenchone. For α-CD, K_1 is higher for the (+)-enantiomer and K_2 has equal value for both enantiomers while for β-CD, K_1 is higher for the (+)-enantiomer but K_2 is higher for the (−)-enantiomer (the second step of complexation has the opposite direction from the first one). As a result, the final enantioseparation is higher on the α-CD than on the β-CD column. Examples of chromatograms are presented in Fig. 3. It can be seen that while the separation of enantiomers is well

Table 3
Distribution constants of monoterpenoids in temperature 333 K on matrix, α- and β-CD columns

Compound	M	A1	B1	A3	B2	A5	B4
(+)-Menthone	560	750	760	1090	1000	1470	1280
(−)-Menthone	560	750	760	1020	1000	1370	1280
(+)-Isomenthone	820	1270	1430	2070	2150	3030	3857
(−)-Isomenthone	820	1350	1430	2430	2070	3660	3658
(+)-Fenchone	310	1100	1090	3010	2070	5000	4849
(−)-Fenchone	310	630	1090	1370	2190	2180	5128
(±)-Pulegone	1600	1880	2260	2230	3150	2770	3841
(+)-α-Pinene	10	100	30	352	60	660	130
(−)-α-Pinene	10	220	30	794	60	1400	130
(+)-β-Pinene	15	130	60	400	120	670	280
(−)-β-Pinene	15	220	60	780	120	1320	280
(+)-Limonene	25	70	30	190	50	310	60
(−)-Limonene	25	60	30	170	50	270	60

Relative errors less than 10%.

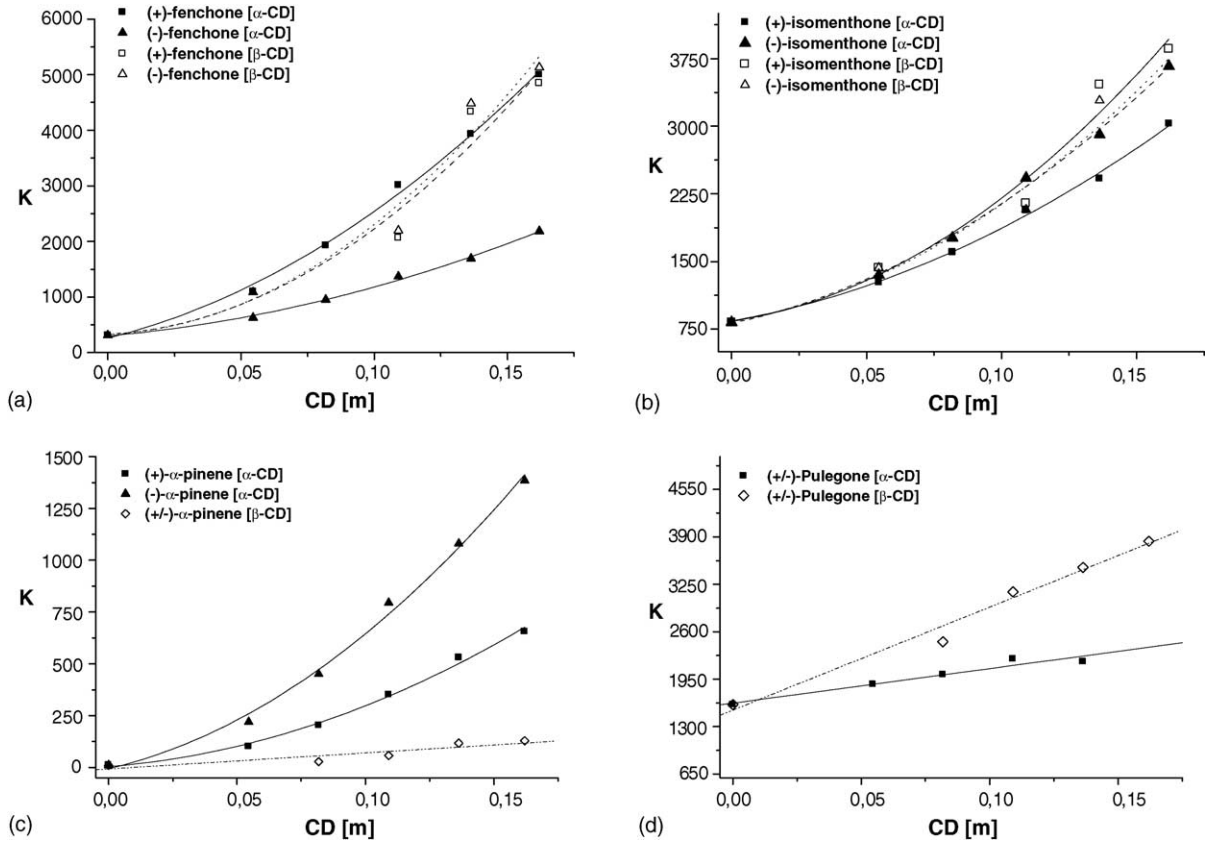


Fig. 1. Relation between adjusted partition coefficient K vs. CD concentration for enantiomers of fenchone (a), isomenthone (b), α-pinene (c) and pulegone (d).

visible on α-CD; on β-CD, only a widening of a single peak is observed.

4.3. Determination of thermodynamic parameters of complexation

The calculated distribution constants were used to determine ΔH of partition and complexation of monoterpenoids by α- and β-CD.

The enthalpy of partition was determined from the slope of linear variation $\ln K$ versus $1/T$. Examples of the obtained plots are presented in Fig. 4. A very good linear variation of $\ln K$ versus $1/T$ allowed ΔH to be calculated with good precision.

The enthalpy of complexation was calculated from Eq. (8).

On the basis of Eqs. (9)–(11), calculated stability constants and ΔH^* of complexation, it was possible to determine ΔG^*

Table 4
Stability constant of α- and β-cyclodextrin complexes with selected investigated monoterpenoids determined in glycerol at 60 °C

	α-CD				β-CD			
	K_1	K_2	K_1K_2	R^2	K_1	K_2	K_1K_2	R^2
(+)-Menthone	3.8	8.9	34	0.999	8.6	–	–	0.998
(−)-Menthone	4.4	6.1	27	0.999	8.6	–	–	0.998
(+)-Isomenthone	6.4	9.0	58	0.999	6.9	15	104	0.994
(−)-Isomenthone	7.4	11	81	0.999	7.4	13	96	0.995
(+)-Fenchone	34	10	340	0.996	12	44	528	0.986
(−)-Fenchone	13	11	143	0.999	9.9	57	564	0.988
(±)-Pulegone	3.4	–	–	0.943	9.0	–	–	0.999
(+)-α-Pinene	84	21	1764	0.998	65	–	–	0.961
(−)-α-Pinene	163	26	4238	0.999	65	–	–	0.961
(+)-β-Pinene	110	9	990	0.997	101	–	–	0.963
(−)-β-Pinene	210	10	2100	0.998	101	–	–	0.963
(+)-Limonene	20	16	320	0.993	8.8	–	–	0.992
(−)-Limonene	18	15	270	0.997	8.8	–	–	0.992

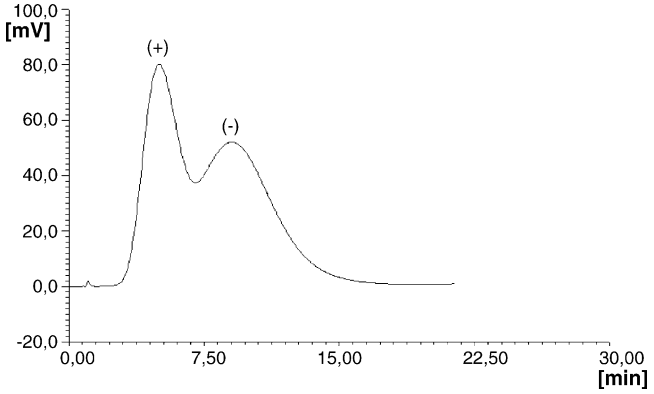


Fig. 2. Chromatogram of (±)-α-pinene obtained on column A5. Temperature, 80 °C.

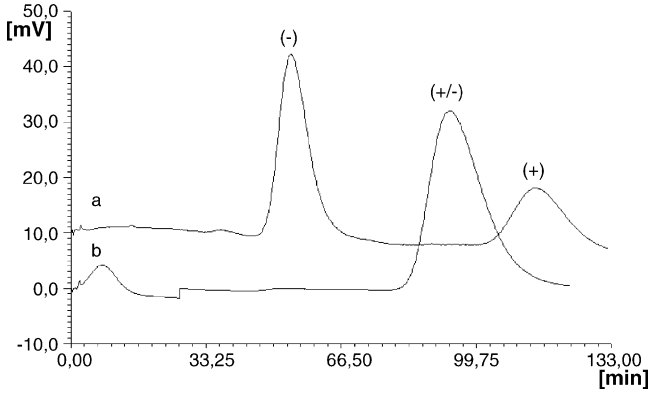


Fig. 3. Chromatograms of (±)-fenchone obtained on columns A5 (a) and B4 (b). Temperature, 65 °C.

and ΔS^* of complexation. The determined thermodynamic parameters of complexation of monoterpenoids by α- and β-CD are reported in Table 5. It is clear that complexation processes are enthalpy-driven. In all cases, ΔH^* is more negative than $T\Delta S^*$.

The higher values of $-\Delta H^*$, $-T\Delta S^*$ and $-\Delta G^*$ for bicyclic than for monocyclic monoterpenoids for α- and β-CD can be explained by deeper penetration and better fitting of the guest compound into the CD cavity. Comparing the thermodynamic parameters of monoterpenoid complexation by α- and β-cyclodextrins, it can be observed that in general they have higher values for α-CD complexes. This can signify that α-CD is a better host molecule for monoterpenoids guests.

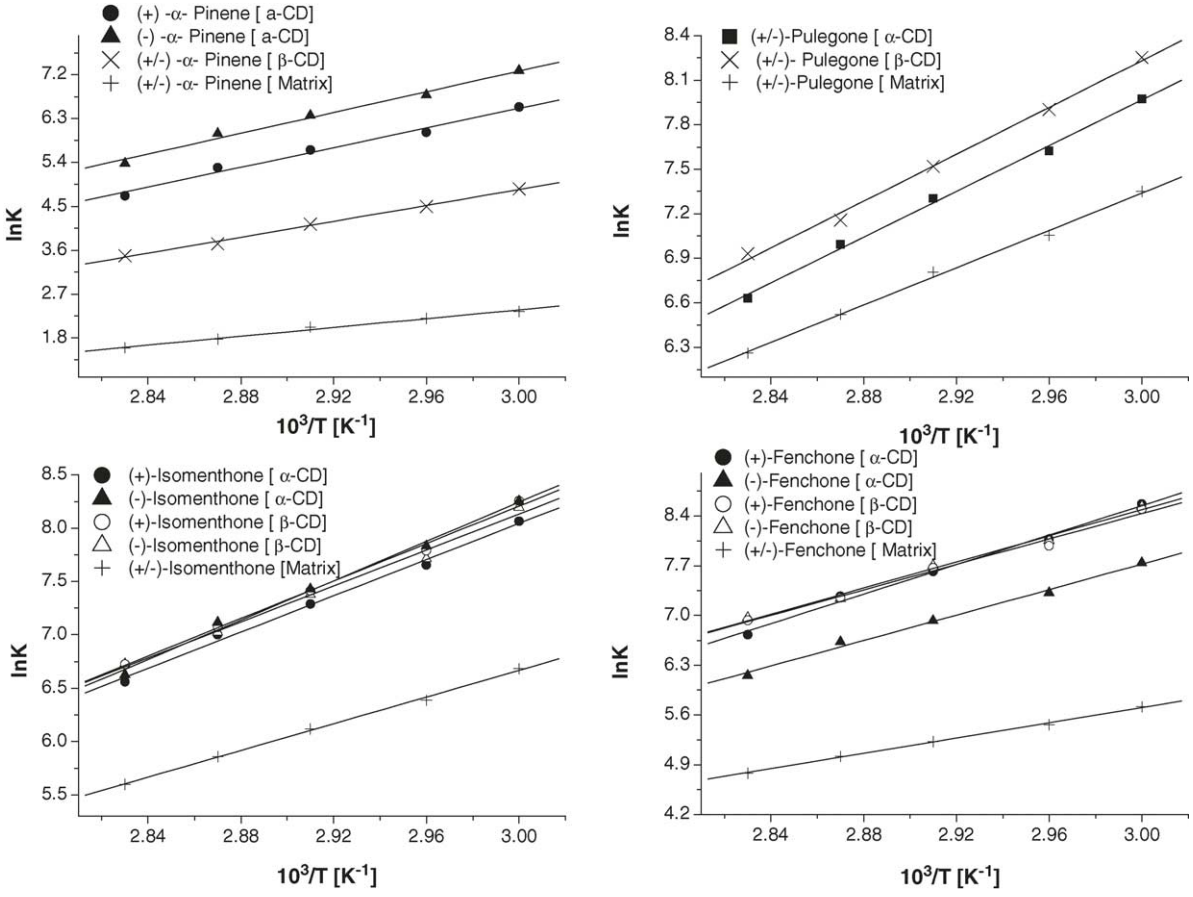


Fig. 4. van't Hoff plots of ln(K) vs. 1/T for enantiomers of fenchone (a), isomenthone (b), α-pinene (c) and pulegone (d) obtained on columns M, A5 and B4.

Table 5
Thermodynamic parameters of complexation of monoterpenoids by α- and β-CD

	$-\Delta H_0$ (kJ/mol)	α-CD			β-CD		
		$-\Delta H^*$ (kJ/mol)	$-T\Delta S^*$ (kJ/mol)	$-\Delta G^*$ (kJ/mol)[a]	$-\Delta H^*$ (kJ/mol)	$-T\Delta S^*$ (kJ/mol)	$-\Delta G^*$ (kJ/mol)[a]
(+)-Menthone	51.7 ± 0.3	22.0 ± 4.0	12	10	9.8 ± 0.5	4	6
(−)-Menthone	51.7 ± 0.3	19.6 ± 3.0	11	9	9.8 ± 0.5	4	6
(+)-Isomenthone	52.7 ± 0.3	18.8 ± 2.9	8	11	18.0 ± 1.0	5.1	12.9
(−)-Isomenthone	52.7 ± 0.3	24.9 ± 3.2	13	12	15.5 ± 2.0	2.9	12.6
(+)-Fenchone	45.1 ± 0.6	42.2 ± 0.3	26	16	32.4 ± 1.4	15.1	17.3
(−)-Fenchone	45.1 ± 0.6	30.3 ± 3.4	16	14	33.2 ± 1.0	15.7	17.5
(±)-Pulegone	53.0 ± 0.7	12.0 ± 2.0	8	4	12.6 ± 5.0	7	6
(+)-α-Pinene	37.8 ± 2.4	47.4 ± 7.4	26	21	37.0 ± 5.0	25	12
(−)-α-Pinene	37.8 ± 2.4	52.0 ± 7.0	29	23	37.0 ± 5.0	25	12
(+)-β-Pinene	38.4 ± 2.7	46.3 ± 5.7	27	19	37.0 ± 5.0	24	13
(−)-β-Pinene	38.4 ± 2.7	50.5 ± 5.4	30	21	37.0 ± 5.0	24	13
(+)-Limonene	42.3 ± 1.7	39.7 ± 4.1	24	16	9.0 ± 2.0	3	6
(−)-Limonene	42.3 ± 1.7	38.6 ± 5.8	24	15	9.0 ± 2.0	3	6

[a] ±2.0.

4.4. Absolute enantioselectivity

The absolute enantioselectivity E calculated from Eq. (14) for separated enantiomers is presented in Table 6. The data in Table 6 indicate that on the α-CD column, the second step of complexation is enantioselective for the monocyclic ketones: menthone and isomenthone, while for bicyclic fenchone, α-pinene and β-pinene the observed enantioselectivity is the

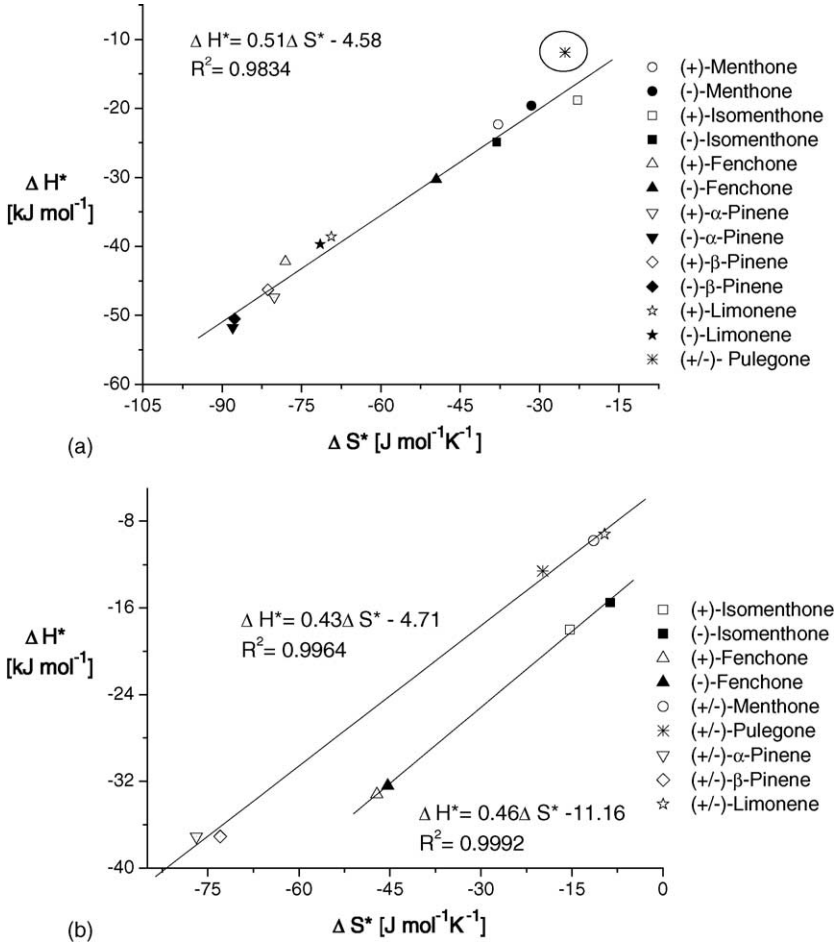


Fig. 5. Plots of enthalpy–entropy compensation for the investigated compounds on columns A5 (a) and B4 (b). (±)-Pulegone is excluded from the regression line on plot (a).

Table 6
The absolute enantioselectivity and the ratio of stability constants of the first and second step of complexation

Cyclodextrin	Compound	First eluted	E	K_1^{II}/K_1^{I}	K_2^{II}/K_2^{I}
α-CD	(+/−)-Menthone	(−)-Menthone	1.26	0.86	1.46
α-CD	(+/−)-Isomenthone	(+)-Isomenthone	1.43	1.16	1.23
α-CD	(+/−)-Fenchone	(−)-Fenchone	2.46	2.72	0.90
α-CD	(+/−)-α-Pinene	(+)-α-Pinene	2.34	1.93	1.21
α-CD	(+/−)-β-Pinene	(+)-β-Pinene	2.06	1.92	1.07
β-CD	(+/−)-Isomenthone	(−)-Isomenthone	1.14	0.93	1.22
β-CD	(+/−)-Fenchone	(+)-Fenchone	1.09	0.85	1.29

result of the first step of complexation. On the β-CD column, the second step of complexation is enantioselective for both compounds.

4.5. Enthalpy–entropy compensation

Fig. 5 shows enthalpy–entropy plots (ΔH versus ΔS according to Eq. (15)) for investigated compounds on A5 and B4 columns at 333 K. It can be seen from the plots that on β-CD column (Fig. 5b), the analytes can be divided into two groups: the first ((±)-α-pinene, (±)-β-pinene, (±)-limonene, (±)-menthone and (±)-pulegone) forming 1:1 stoichiometric complexes with β-CD, and the second ((+)- and (−)-fenchone, (+)- and (−)-isomenthone) forming complexes of 1:2 stoichiometry. It seems that enthalpy–entropy compensation enable recognition of two different mechanisms of separation with cyclodextrins: the first where complexes of 1:1 stoichiometry are formed (without enantioseparation) and the second with 1:2 stoichiometric complexes (with enantioseparation). Unfortunately, on α-CD column (Fig. 5a) such a correlation is not clearly visible because only one compound ((±)-pulegone) forms 1:1 stoichiometric complexes with α-CD. The regression lines are given in Fig. 5.

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