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Chiral recognition ability of α-cyclodextrin with regard to some monoterpenoids under gas–liquid chromatographic conditions

Monika Asztemborska[a,*], Danuta Sybilska[a], Robert Nowakowski[a], Giorgio Perez[b]

[a]*Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland*
[b]*Istituto per lo Studio dei Materiali Nanostrutturati del Consiglio Nazionale delle Ricerche; 00016 Monterotondo Scalo, Italy*

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Abstract

Gas–liquid chromatography was applied to investigate the mechanism of α-cyclodextrin (α-CD) complexation processes with some chiral monoterpenoids differing from each other in chemical properties and structure. They were chosen from hydrocarbons, alcohols, aldehydes and ketones of acyclic, monocyclic and bicyclic structure. The relationships between the retention factor, *k*, of a guest solute (G) and α-CD concentration were studied. The obtained data enabled the stoichiometry, the stability of individual complexes and the separation factor of enantiomers to be determined. It was found that almost all the investigated monoterpenoids, apart from the acyclic ones, form inclusion complexes with α-CD. Straight-line relations (*r* vs. [α-CD]) were observed for monocyclic alcohols and pulegone, without any trace of enantioselectivity. This behaviour indicates that the 1:1 stoichiometry of the G–CD complexes does not lead to chiral recognition. Parabolic relations arising from 1:2 stoichiometry were found for limonene, α-phellandrene, some monocycylic ketones and all the investigated bicyclic terpenoids. It appeared that only the second step of complexation displayed marked enantioselectivity. However, a loss of efficiency resulting from slower equilibration is then noticeable. Attempts are made to rationalize the chromatographic results with respect to the structure of the investigated compounds.

Keywords: Enantiomer separation; Stability studies; Cyclodextrins; Monoterpenoids; Terpenoids

1. Introduction

Chiral chromatography is one of the most important and fast-developing branches of chromatography. Many enantioselective chromatographic columns are commercially available nowadays both for gas and liquid chromatography. The modern analytical applications of chiral gas chromatography are dominated by capillary columns, mainly those modified with various derivatives of cyclodextrins [1–4]. The practical achievements of these methods are difficult to overrate. However, in spite of these analytical successes the mechanisms of chiral recognition are not fully known.

Since the early 1980s we have been developing gas–liquid chromatographic methods using solutions of cyclodextrins (in formamide or glycerol) as stationary phases in packed columns. Such systems

*Corresponding author.
E-mail address: monika@ichf.edu.pl (M. Asztemborska).

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are endowed with great selectivity with regard to isomers of various kinds: constitutional isomers, geometric isomers, diastereomers and enantiomers [5–9]. Unfortunately, the efficiency of packed columns is only moderate. These classical methods lose their analytical significance when compared with capillary columns characterised by high resolution.

On the other hand, as we have recently shown [10,11], systems comprised of classical columns packed with an inert support coated with a solution of native cyclodextrin, may serve as a potential tool for physicochemical studies of complexation processes between guest and cyclodextrin molecules. A similar physicochemical approach was developed by Schurig et al. [12] using the more precise technique employing capillary columns.

In this paper gas–liquid chromatography is applied to study the stability, stoichiometry and some structural relations of inclusion complexes formed between α-CD and monoterpenoids.

The main goal of this study was to find how the enantioselectivity arising from α-CD complexation is related to the stoichiometry of the G–CD association and the shape of the G molecule.

Monoterpenes were chosen as the model compounds because many chiral compounds of more or less similar structure occur within this group. Such variability was important in seeking the relationship between the structure of the guest molecule and the physicochemical properties of its α-cyclodextrin complexes. The commercial availability of both enantiomers was also taken into account.

2. Theoretical consideration

About 10 years ago Armstrong et al. [13] applied molten cyclodextrin derivatives with relatively volumetric bounded groups as stationary phase of GC system and suggested multiple mechanisms of chromatographic enantioseparation. These authors always used the same stationary phase and followed temperature relations of chromatographic parameters that enabled them to determine some thermodynamic data. Contrary to the earlier Armstrong work, we used various stationary phases differing from each other in α-CD concentration in glycerol. Retention factors and separation factors were determined as function of α-CD concentration in glycerol. This enabled the determination of stoichiometry and the stability of α-CD complexes.

From the point of view of theoretical considerations and from the examination of experimental results we accepted the idea that in a very polar solvent, hydrophobic interaction is the major contributor towards inclusion complex formation. In effect, the inclusion phenomenon is the main driving force in chromatographic chiral separations.

In the studied gas–liquid chromatographic system the stationary phases were comprised of dilute solutions of cyclodextrin (CD) in an achiral solvent (S). If a volatile substance (guest) G is eluted through the column, the process of partition in gas–liquid chromatography without the addition of cyclodextrin is characterised by the equilibrium:

$$G_{(g)} \overset{K^0}{\rightleftarrows} G_{(l)}$$

where K^0 is the coefficient of partition of solute G between the gaseous (g) and liquid (l) phase.

$$K^0 = \frac{[G_{(l)}]}{[G_{(g)}]} \tag{1}$$

After addition of cyclodextrin to the stationary phase we are dealing with an additional process of complexation of solute G with cyclodextrin CD.

Under the assumption that only G–CD complexes of 1:1 stoichiometry are formed, the equation for the partition coefficient, K, is reduced to the form [14]:

$$K = K^0(1 + K_1[CD]) \tag{2}$$

where K_1 is the stability constant of a G–CD complex of 1:1 stoichiometry.

In this case the relation of the net retention time (t'_R) versus concentration of cyclodextrin is linear:

$$t'_R = t'^0_R(1 + K_1[CD]) \tag{3}$$

Taking into consideration the possibility that complexes of 1:2 stoichiometry ($G–CD_2$) are also formed, the equation is as follows [10]:

$$K = K^0(1 + K_1[CD] + K_1K_2[CD]^2) \tag{4}$$

where K_1 and K_1K_2 are the stability constants of the

G–CD and G–CD_2 complexes, respectively. In this case a parabolic relation of net retention time versus concentration of cyclodextrin is expected:

$$t'_R = t'^0_R(1 + K_1[CD] + K_1K_2[CD]^2) \quad (5)$$

As it is experimentally impossible to prepare a series of columns of identical parameters differing only by cyclodextrin concentration, it was useful to apply the concept of relative retention according to Schurig et al. [12,15]. As we prefer not to use the relative retention but rather work on retention time scale in minutes we have supplemented Schurig's formulae t_R/t^*_R and t^0_R/t^{0*}_R with the factor t_{Rav}:

$$r = \frac{t_R}{t^*_R} \cdot t_{Rav} \text{ and } r^0 = \frac{t^0_R}{t^{0*}_R} \cdot t_{Rav} \quad (6)$$

where r is the relative net retention time of solute G with respect to an inert reference standard R, t^{0*}_R and t^*_R are the retention times of an inert reference standard R on the matrix and α-CD columns and t_{Rav} is the average retention time of an inert reference standard on the matrix and α-CD columns calculated as follows:

$$t_{Rav} = \frac{t^0_s + t^1_s + \ldots + t^n_s}{n+1} \quad (7)$$

where t^0_s, $t^1_s, \ldots t^n_s$ are retention times of an inert standard on matrix column and columns with various concentrations of α-CD columns and $n+1$ is the number of columns.

Finally the equation for 1:1 stoichiometry has the following form:

$$r = r_0(1 + K_1[CD]) \quad (8)$$

and for 1:2 stoichiometry:

$$r = r_0(1 + K_1[CD] + K_1K_2[CD]^2) \quad (9)$$

3. Experimental

3.1. Materials

The names and structural formulae of the investigated monoterpenoids are presented in Table 1. They were chosen from hydrocarbons, alcohols, aldehydes and ketones and were divided according to their structures into acyclic, monocyclic and bicyclic compounds.

α-Cyclodextrin (α-CD) was supplied by Chinoin (Budapest, Hungary); the (+) and (−)-α- and β-pinenes, citronellene, citronellal, menthone, pulegone, fenchone, linalool, menthol, neomenthol, α-terpineol and isopinocampheol were from Fluka (Buchs, Switzerland); (+) and (−)-limonene and (−)-α-phellandrene were from Merck-Schuchardt (Hohenbrunn, Germany); (+) and (−)-camphene, carvone and terpinen-4-ol were from Aldrich (Milwaukee, USA). Instead of (+)-α-phellandrene standard the dill oil was applied.

All other reagents and solvents were of analytical grade and were used as received.

3.2. Apparatus and procedures

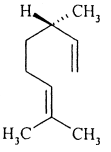
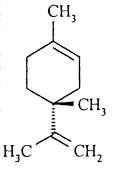
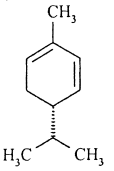
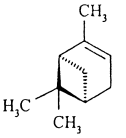
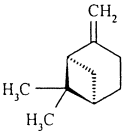
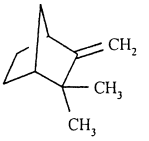
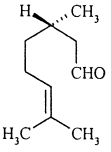
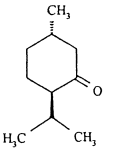
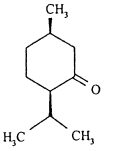
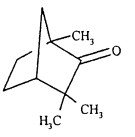
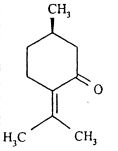
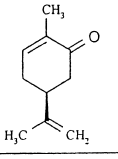
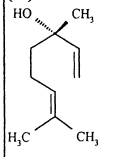
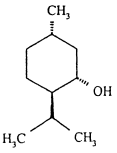
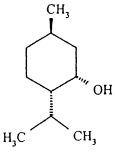
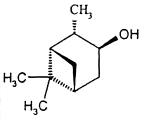
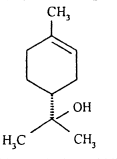
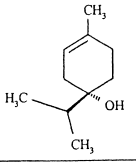
Gas chromatographic studies were performed using an Hewlett-Packard Model 5890 gas chromatograph equipped with a dual flame ionization detection system. The peak areas and retention times were measured by means of an Hewlett-Packard 3390 A integrator.

The glass columns (2 m×4 mm I.D.) were packed with Chromosorb W non-acid washed (60–80 mesh) coated with glycerol or glycerol solutions of α-CD of appropriate molality. To improve the solubility of α-CD, some lithium nitrate ($LiNO_3$) was added to achieve a final concentration 3.8 *M*. The same procedure was applied in the preparation of the reference matrix column. Thus salting effects were not taken into account.

To simplify GC calculations, α-CD concentrations are expressed as molal solutions as described in [15]. Detailed information on CD molalities and other specific conditions are given with the corresponding chromatograms and graphs. Hold up time t_M was measured from the methane peak.

The stability constants of α-CD complexes were calculated from Eq. (3) for methanol, ethanol, 1-propanol and 2-propanol, from Eq. (8) for pulegone, menthol, neomenthol, α-terpineol and terpinen-4-ol and from Eq. (9) for (+) and (−)-fenchone, (+) and (−)-isopinocampheol (+) and (−)-carvone and (+) and (−)-isomenthone.

Table 1
Names and structural formulae of investigated compounds

	Acycylic	Monocyclic		Bicyclic	
Hydrocarbons	(+)-citronellene 	(+)-limonene 	(+)-α-phellandrene 	(+)-α-pinene 	(+)-β-pinene 
				(+)-camphene 	
Aldehydes and ketones	(+)-citronellal 	(+)-menthone 	(+)-isomenthone 	(+)-fenchone 	
		(+)-pulegone 	(+)-carvone 		
Alcohols	(+)-linalool 	(+)-menthol 	(+)-neomenthol 	(+)-isopinocampheol 	
		(+)-α-terpineol 	(+)-terpinen-4-ol 		

4. Results and discussion

4.1. Inert standard selection

All calculations of stability constants and separation factors were performed applying the Schurig relative retention concept [15,12]. Thus an appropriate inert standard had first to be found.

Fig. 1 presents the dependence of the retention times of methanol, ethanol, 1-propanol and 2-propanol on α-CD concentration. Only the retention time of methanol is independent of α-CD concentration. This indicates that under the conditions of

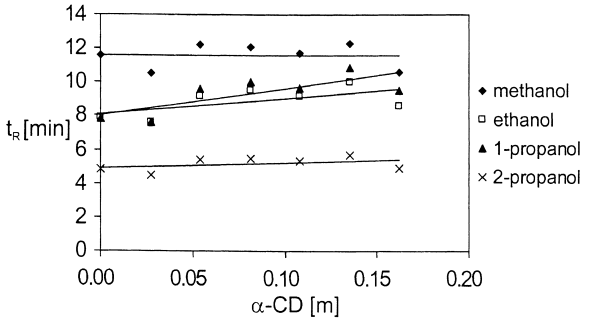


Fig. 1. Dependence of t'_R vs. α-CD molality for methanol, ethanol, 1-propanol and 2-propanol determined at 70 °C.

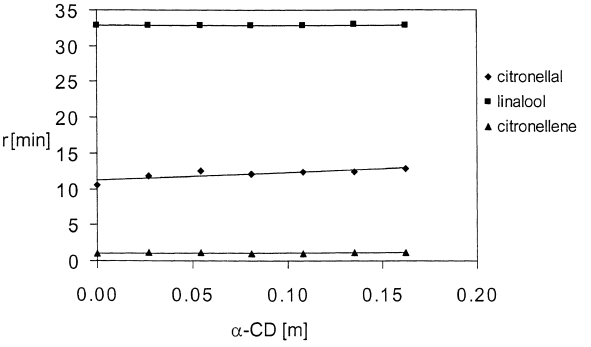


Fig. 2. Dependence of r vs. α-CD molality for the acyclic monoterpenoids, citronellene, citronellal and linalool, determined at 70 °C.

the experiment, methanol does not form inclusion complexes with α-CD making it suitable as an inert standard. The calculated stability constants of aliphatic alcohols are collected in Table 2.

The values of the stability constants of alcohols C_1–C_3 determined in glycerol at 70 °C differ significantly from those observed by Matsui et al. [16] in water. However, their order corresponds fairly well.

4.2. Chromatographic behaviour of monoterpenoids

4.2.1. Acyclic monoterpenoids

The dependences of retention times of acyclic monoterpenoids citronellene, citronellal and linalool on α-CD concentration are presented in Fig. 2.

Under the conditions of the experiment, regardless of the functional group (hydrocarbon, alcohol or aldehyde), none of the acyclic monoterpenoids form inclusion complexes with α-CD as the relations r vs. [α-CD] are straight lines with a slope close to zero. As a consequence no separation of enantiomers should be expected.

Table 2
Stability constants (K[a]) of α-cyclodextrin complexes with aliphatic alcohols C_1–C_3 determined in glycerol at 70 °C

Alcohol	K	K[b]
Methanol	0.0	0.93
Ethanol	1.1	5.62
1-Propanol	1.9	23.44
2-Propanol	0.7	4.90

[a] Stability constants K calculated from Eq. (3).
[b] Data taken from Ref. [16].

4.2.2. Monocyclic monoterpenoids

The plots of the retention times of monocyclic alcohols menthol, neomenthol, α-terpineol and terpinen-4-ol vs. α-CD concentration are presented in Fig. 3. All of these are straight lines. The slope is slightly steeper for menthol and terpinen-4-ol than for the other compounds. These results indicate that complexes of 1:1 stoichiometry are formed between α-CD and alcohols, and complexes with menthol and terpinen-4-ol are more stable. However, the α-CD complexation process of 1:1 stoichiometry does not differentiate enantiomers. The values of stability constants are listed in Table 3.

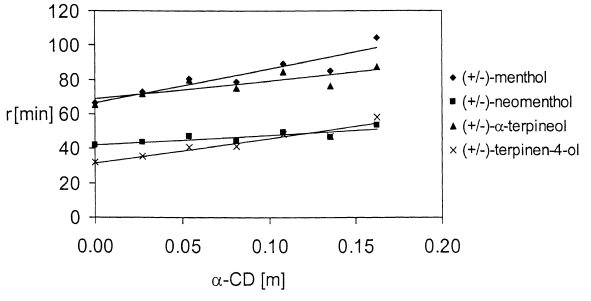


Fig. 3. Dependence of r vs. α-CD molality for monocyclic alcohols: (+/−)-menthol, (+/−)-neomenthol, (+/−)-α-terpineol and (+/−)-terpinen-4-ol determined at 70 °C.

Table 3
Stability constants of α-cyclodextrin complexes with selected investigated monoterpenoids determined in glycerol at 70 °C[a]

	K_1K_2	K_1	K_2
(+)-Fenchone	562	20	28
(−)-Fenchone	225	13	17
(−)-Isopinocampheol	57	7	8
(+)-Isopinocampheol	39	6	7
(+)-Carvone	41	9	5
(−)-Carvone	27	9	3
(+)-Isomenthone	112	7	16
(+)-Isomenthone	75	8	9
(+/−)-Pulegone		5	
(+/−)-Menthol		3	
(+/−)-Neomenthol		1	
(+/−)-α-Terpineol		2	
(+/−)-Terpinen-4-ol		5	

[a] Stability constants calculated from Eq. (8) for 1:1 stoichiometry complexes and from Eq. (9) for 1:2 stoichiometry.

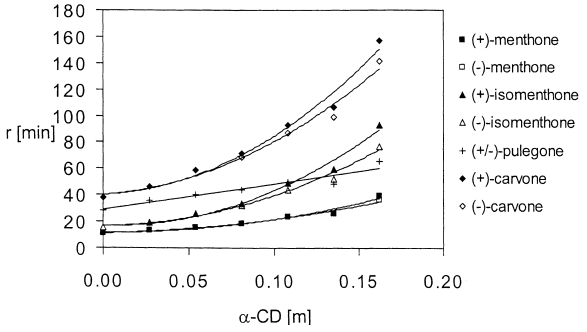


Fig. 4. Dependence of r vs. α-CD molality for monocyclic ketones: (+/−)-menthone, (+/−)-isomenthone, (+/−)-pulegone and (+/−)-carvone determined at 70 °C.

Interesting changes in the t_R vs. [α-CD] relation (Fig. 4) are observed for the monocyclic ketones menthone, isomenthone, pulegone and carvone. While for pulegone the dependence is a straight line, for menthone the straight line changes slightly into a parabolic curve, and for isomenthone a parabolic dependence is clearly seen. α-CD forms complexes of 1:1 stoichiometry with pulegone, while complexes of 1:2 stoichiometry with menthone are formed at higher concentrations of α-CD, and complexes of 1:2 stoichiometry with isomenthone originate at lower concentrations of α-CD. Interestingly the best enantioseparation was achieved for isomenthone, for menthone the separation of enantiomers is visible only at higher concentration of α-CD, while for pulegone no enantioseparation occurs.

The group of monocyclic ketones (pulegone, menthone and isomenthone) seems to be a boundary group between compounds which do not form complexes or form complexes of 1:1 stoichiometry, where enantioselectivity is not observed, and monoterpenoids which form complexes of 1:2 stoichiometry where enantioselectivity is clearly visible, as presented in Table 5. Since the shape of the guest molecule seems to be of crucial importance in the processes of CD complexation, molecular modelling of menthone, isomenthone and pulegone was performed.

One example is presented in Fig. 5 where the geometry of three monocyclic ketones (pulegone, menthone and isomenthone) is compared. Geometry optimization was simulated using the optimization

Table 4
Enantioseparation factor α of investigated monoterpenoids obtained on column with 0.162 M of α-CD in glycerol at 70 °C

	Acycylic	Monocyclic		Bicyclic	
Hydrocarbons	(+/−)-Citronellene 1.00	(+/−)-Limonene 1.11	(−/+)-α-Phellandrene 1.24	(−/+)-α-pinene 2.22 (−/+)-Camphene 2.31	(−/+)-β-pinene 1.86
Aldehydes and ketones	(+/−)-Citronellal 1.00	(+/−)-Menthone 1.10	(−/+)-Isomenthone 1.21 (+/−)-Pulegone 1.00	(−/+)-Fenchone 2.01 (+/−)-Carvone 1.11	
Alcohols	(+/−)-Linalool 1.00	(+/−)-Menthol 1.00 (+/−)-α-Terpineol 1.00	(+/−)-Neomenthol 1.00 (+/−)-Terpinen-4-ol 1.00	(−/+)-Isopinocampheol 1.15	

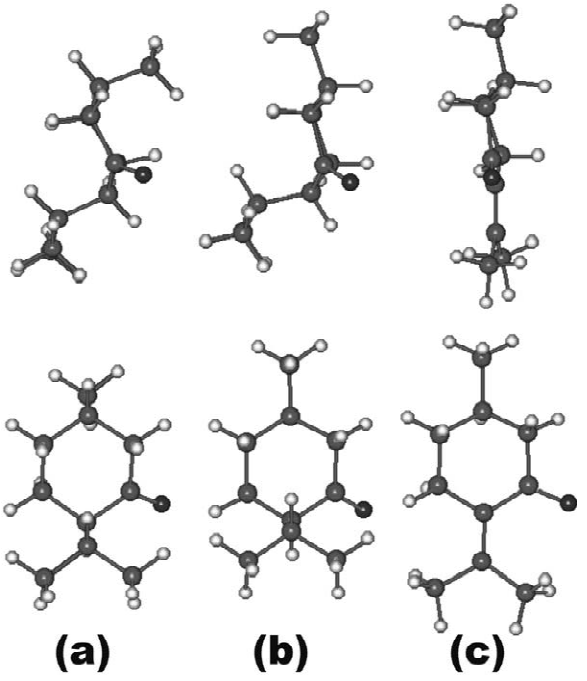


Fig. 5. Comparison of molecular dimensions and shape of isomenthone (a), menthone (b) and pulegone (c).

procedure from HYPER CHEM v. 6.0 software. Differences in the shape (length and width) are evident. Comparing the shapes of the three compounds one can see that isomenthone is the shortest and the most 'volumetric' molecule, which may explain the ease of its complexation with two molecules of α-CD. Pulegone is probably too flat and too long to be complexed by two α-CD molecules. The menthone molecule is a borderline case.

Confirmation of the above requires further simulation of molecule–CD complexes in a solvent. A comparison of the complexes energies will be of interest with respect to understanding the mechanism of α-CD complexation with the analysed molecules.

Fig. 6 presents the dependence of retention times vs. α-CD concentration for monocycylic hydrocarbons—limonene and α-phellandrene. In each case parabolic dependences indicate the formation of complexes of 1:2 stoichiometry endowed with a relatively poor enantioselectivity.

4.2.3. Bicyclic monoterpenoids

The retention times versus α-CD concentration for bicyclic monoterpenoids fenchone and isopinocampheol are shown in Fig. 7a. In each case the parabolic dependence of t_R vs. [CD] provides evidence for 1:2 stoichiometry of the formed complexes. The observed enantioselectivity is much higher for fenchone than for isopinocampheol.

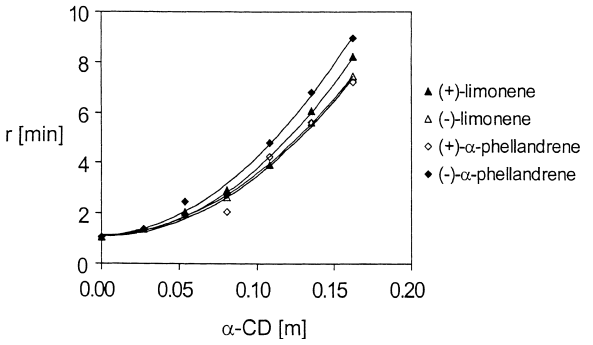


Fig. 6. Dependence of r vs. α-CD molality for monocyclic hydrocarbons: (+/−)-limonene and (+/−)-α-phellandrene determined at 70 °C.

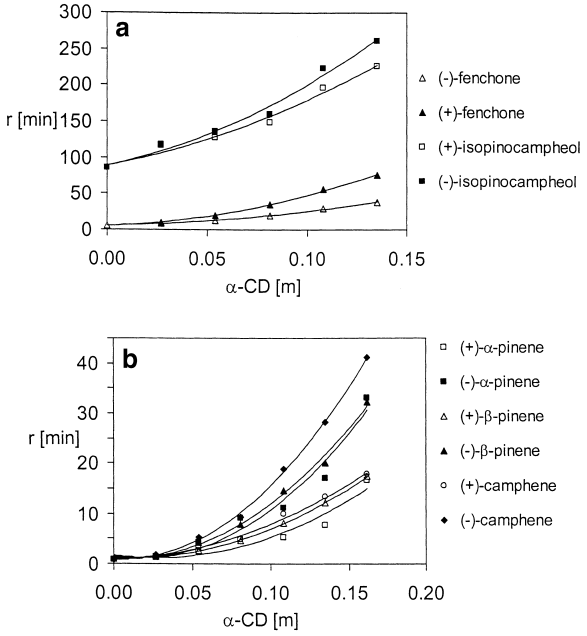


Fig. 7. Dependence of r vs. α-CD molality for bicyclic monoterpenoids: (+/−)-fenchone and (+/−)-isopinocampheol (a); (+/−)-α-pinene, (+/−)-β-pinene and (+/−)-camphene (b); determined at 70 °C.

Table 5
Stoichiometry and enantioselectivity

		Stoichiometry	Enantioseparation
Acyclic	Alcohols	Lack of complexation	No
	Aldehydes	Lack of complexation	No
	Hydrocarbons	Lack of complexation	No
Monocycylic	Alcohols	1:1	No
	Ketones	1:1/1:2	No/Yes
	Hydrocarbons	1:2	Yes
Bicyclic	Alcohols	1:2	Yes
	Ketones	1:2	Yes
	Hydrocarbons	1:2	Yes

The relation between the retention times of bicyclic hydrocarbons: α-pinene camphene and β-pinene vs. α-CD concentration is shown in Fig. 7b. For each compound the parabolic relation suggests the formation of complexes of 1:2 stoichiometry. High enantioseparation factors have also been achieved for all the compounds. Similar observations were made by Möder et al. [17] under HPLC conditions.

The enantioseparation factors of all the investigated compounds are collected in Table 4. However, due to the slower equilibration process accompanying the addition of two α-CD molecules to the guest molecule, the column's efficiency is reduced twice or thrice. In effect, the remarkable enantioselectivity is gained at the expense of slower kinetics.

5. Concluding remarks

All results presented in this study lead to the conclusion that the remarkable enantioselectivity of α-CD with respect to some monoterpenoids appears in the second step of complexation. Under the conditions of the experiment the process of 1:1 stoichiometry does not differentiate enantiomers. Examples of chromatograms of pulegone (without enantioseparation) and fenchone (with baseline separation of enantiomers) are presented in Fig. 8.

The fact that monocyclic alcohols do not attach a second molecule of α-CD while monocyclic hydrocarbons are endowed with this ability indicates the great positive role of hydrophobic interactions and some hindering effect produced by the hydroxyl group.

Bicyclic hydrocarbons, ketones as well as alcohols form associates of 1:2 stoichiometry with α-CD but higher enantioselectivity was observed for hydrocarbons and ketones than for alcohols. It seems that the presence of the hydroxyl group does not favour enantioselectivity.

Unfortunately the remarkable enantioselectivity arising from 1:2 stoichiometry is accompanied by a considerable loss of efficiency. Although α-CD is relatively small and forms complexes with aliphatic alcohols and hydrocarbons it does not include acyclic terpenoids regardless of their chemical nature. This phenomenon requires further investigation.

It appears that under the conditions of the experiment three terpenoids do not form complexes with α-CD while five of them form complexes of 1:1 stoichiometry and ten of them form complexes of 1:2 stoichiometry. However, more general statements require further study. At present the statement that the second step of complexation is endowed with remarkable enantioselectivity is limited only to the terpenoids and α-CD.

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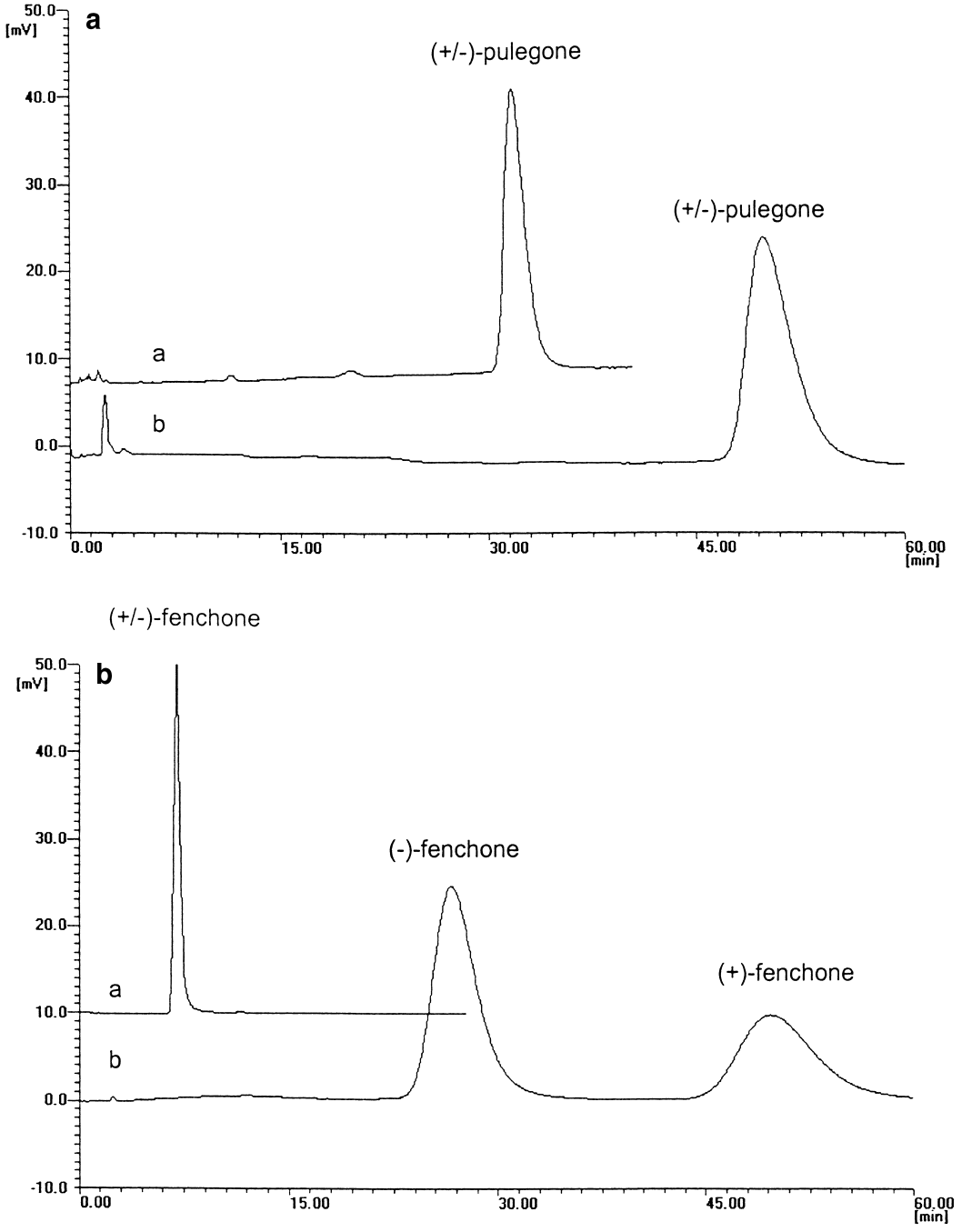

Fig. 8. Chromatograms of (+/−)-pulegone (a) and (+/−)-fenchone (b) obtained on matrix (a) and α-CD (0.108 *M*) (b) columns; temperature, 70 °C; flow-rate, 40 ml/min.

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