Carbohydrate Polymers 91 (2013) 110–114

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Interactions with β-cyclodextrin as a way for encapsulation and separation of camphene and fenchene

Magdalena Ceborska[a,*], Monika Asztemborska[a], Roman Luboradzki[a,b], Janusz Lipkowski[a]

[a] Institute of Physical Chemistry, Polish Academy of Sciences, 01-224 Warsaw, Poland
[b] Cardinal Stefan Wyszynski University, Dewajtis 5, 01-815 Warsaw, Poland

ARTICLE INFO

Article history:
Received 14 December 2011
Received in revised form 17 May 2012
Accepted 27 July 2012
Available online 9 August 2012

Keywords:
Cyclodextrin host–guest complex
Separation of regioisomers
Monoterpene
X-ray
Chromatography
^{1}H NMR

ABSTRACT

The separation of isomeric monoterpenes, camphene and fenchene by complexation with β-cyclodextrin is presented. Both of the monoterpenes form complexes with β-cyclodextrin (as shown by both gas chromatography and ^{1}H NMR) with similar stability constants nevertheless it is possible to separate them by re-crystallization. The crystal structure of β-cyclodextrin with fenchene was also studied by X-ray diffraction.

1. Introduction

Cyclodextrins (CDs) are macrocyclic carbohydrate, toroid-like shaped organic compounds, consisting of glucopyranose units linked by 1,4-glucosidic bonds. The most common cyclodextrins include α, β, and γ CDs containing 6, 7 and 8 glucopyranoside units respectively. The internal cavity exhibits hydrophobic properties, which enable complexation of a variety of guest molecules including both organic and inorganic species. The character of the guest compound can vary significantly, from polar compounds including alcohols (Caira, Griffith, & Nassimbeni, 1996), amines (Knoll, Bobek, Giester, & Brinker, 2001) and inorganic (Spencer, He, Wu, & Fetter, 1998) and organic (Hbaieb, Kalfat, Chevalier, Amdouni, & Parrot-Lopez, 2008) anions to apolar ones (for example many hydrocarbons; Neoh, Koecher, Reineccius, Furuta, & Yoshii, 2010). This particular property has been widely used in pharmaceutical (Arun, Ashok, & Sravanthi, 2008) and food (Astray, Gonzalez-Barreiro, Mejuto, Rial-Otero, & Simal-Gandara, 2009) applications. When added to the aqueous solution of the biologically active compound they often increase the solubility and bioavailability of the guest molecule (Wu, Liang, Yuan, Wang, & Yan, 2010). Cyclodextrins are used as chiral separators in chromatography (Lämmerhofer, 2010); they may also be applied for separation of enantiomers by synthetic chemists (Grandeury, Petit, Gouhier, Agasse, & Coquerel, 2003).

Camphene is one of the monoterpenes, a class of molecule that is commonly used in cosmetic, food and pharmaceutical industry as components of drugs, fragrances and flavours (Bledsoe, 1997; Whittaker, 1972). It can be found in essential oils (e.g. turpentine oil, cypress oil, and ginger oil). It is also an important reagent for the preparation of more complicated oxygenated compounds obtained by catalytic oxidation (da Silva & Gusevskaya, 2001) or carbonylation reactions (Tao, Yeh, Tu, & Chow, 1991). Other important processes include acetoxylation (Castanheiro, Fonseca, Ramos, & Vital, 2008) and acid catalysed hydration (da Silva, Kozhevnikov, & Gusevskaya, 2003). Camphene expresses antilithic and expectorant properties. Tiwari and Kakkar (2009) from research on rat alveolar macrophages showed better resistance to oxidative stress caused by *t*-BHP (*t*-butyl hydroperoxide) following treatment with camphene. Moreover treating with camphene decreased the amount of nitric oxide produced, harmful species that, by reacting with superoxide radicals, gives peroxynitrate radicals, which cause damage, e.g. nitration of DNA or breaking of the DNA strand. Because of its importance for fragrance and pharmaceutical industries, as well as its use as an intermediate in organic synthesis camphene is prepared on a large scale. The common reaction used for obtaining camphene is a catalytic rearrangement of pinene (Ebmeyer, 2002; Findik & Gündüz, 1997). Given its great importance for various industries it is really important to have this compound pure, but it usually is used as a mixture of camphene and fenchene.

* Corresponding author. Tel.: +48 22 3433414; fax: +48 22 3433333.
E-mail addresses: mceborska@ichf.edu.pl (M. Ceborska), monika@ichf.edu.pl (M. Asztemborska), rluboradzki@ichf.edu.pl (R. Luboradzki), klatrat@ichf.edu.pl (J. Lipkowski).

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2012.07.072

Fenchene is a bicyclic terpene, regioisomer of camphene, with a different position of two methyl substituents. It is present in some essential oils (e.g. eucalyptus, French turpentine). Usually it is prepared by dehydration of fenchyl alcohol (Yuasa et al., 1992); it is also obtained as a by-product of the synthesis of camphene via rearrangement of pinene. Commercially available camphene is available in the form of a mixture with fenchene.

The present paper reports preparation and characterization of the supramolecular complexes of β-cyclodextrin with isomeric monoterpenes: camphene and fenchene and the separation of camphene and fenchene by re-crystallization with β-cyclodextrin.

Here we present the separation of isomeric monoterpenes camphene and fenchene by crystallization with β-cyclodextrin. In addition the, crystal structure of the complex of β-cyclodextrin with fenchene is determined and analysed.

2. Materials and methods

2.1. Materials

β-Cyclodextrin (β-CD) was supplied by Cyclolab (Budapest, Hungary) and was recrystallized from water before use. (+)- and (−)-camphene/fenchene mixtures were purchased from Fluka (Buchs, Switzerland) and used without further purification.

2.2. Preparation of the complexes

1 ml of a commercially available mixture of camphene/fenchene (40.8 mg, 0.01 mM) in dioxane was added to 30 ml of 0.01 M β CD I water. The addition was carried out at room temperature with stirring. After 15 min a precipitate was formed. It was filtered off and dried in vacuum yielding the product (1:1 complex) in a form of white powder (171 mg), 45% yield,[1] which was characterized by NMR.

The white powder (150 mg) previously obtained was added to water (5 ml), stirred at 75 °C for 5 h, and left for slow cooling to RT (5–6 h) and kept at RT for additional 72 h, until a precipitate in the form of crystals was formed (12 mg, quantitatively – for 1:1 β CD/fenchene complex).[2]

2.3. Methods

2.3.1. Chromatography – apparatus and procedures

Gas chromatographic studies were performed using a Hewlett–Packard Model 5890 gas chromatograph equipped with a dual flame ionization detector. The peak areas and retention times were measured by means of a Hewlett–Packard 3390 A integrator. Four glass columns (2m × 4 mm ID) were packed with Chromosorb (60–80 mesh) coated with formamide solutions of 0; 0.03; 0.06 and 0.09 M of β-cyclodextrin. A holding time t_M was measured from the methane peak. Chromatographic conditions were as follows: column temp. 40 °C; flow rate 40 ml/min.

2.3.2. NMR studies

NMR spectra were recorded on a Bruker Avance II 300 spectrometer operating at 300.17 MHz for ^{1}H. Standard pulse sequences were used for recording proton spectra. The measurements were made in DMSO-solutions, chemical shifts were given in δ (ppm) relative to TMS as the internal standard. All experiments were performed at 298 K and the sample concentration was 0.02 M/dm^3.

2.3.3. X-ray crystallography

Colourless crystal of approximate dimensions 0.20 × 0.14 × 0.09 was used. Diffraction data were collected at 100 K using Bruker Kappa CCD diffractometer with graphite monochromated Mo Kα radiation. The structure was solved by direct methods (SHELXS-97) and refined on F^2 by full-matrix least-squares method (SHELXL-97) (Sheldrick, 2008). The structure contains one βCD molecule and one guest in asymmetric unit. Non-hydrogen atoms of β-cyclodextrin and water were refined with anisotropic thermal displacement parameters, while the disordered guest molecule was refined isotropically. All βCD's hydrogen atoms were placed in geometric positions and treated as riding with C—H = 0.95 Å and O—H = 0.84 Å.

3. Results and discussion

3.1. Chromatography

The propensity of cyclodextrins and their derivatives to form inclusion complexes with a variety of compounds of different chemical nature has been widely used in many chromatographic techniques, mainly for analytical purposes. Various kinds of binding forces are involved in cyclodextrin complexation. Among them, geometric fitting plays the most important role. In consequence, these processes are very frequently endowed with a great selectivity with regard to isomers. For that reason chromatographic systems modified with CDs seem to be the most universal tool for separation of isomers of different kinds: constitutional, diastereoisomers as well as enantiomers. The processes of complexation between cyclodextrin–host and guest molecules themselves may be traced by chromatographic studies. The results could be helpful in understanding the mechanisms of separation and in optimization of resolution processes in which CD complexation is involved. Moreover, the chromatographic studies may sometimes constitute a valuable tool indicating subjects of interest for further structural investigations. Gas chromatography using a column modified with cyclodextrins was used for elucidation of thermodynamic parameters of complexation (Jung, Schmalzing, & Schurig, 1991; Schurig & Juza, 1997) as well as for evaluation of the stability constants of cyclodextrin-guest complexes (Asztemborska, Bielejewska, Sybilska, & Duszczyk, 2000a; Asztemborska, Nowakowski, & Sybilska, 2000b; Asztemborska, Sybilska, Nowakowski, & Perez, 2003). In the studied system of gas–liquid chromatography the stationary phases were comprised of dilute solutions of β-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 characterized by the equilibrium:

$$G_{(g)} \overset{K^0}{\rightleftharpoons} G_{(1)}$$

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

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

After addition of cyclodextrin to the stationary phase an additional process of complexation of solute G with cyclodextrin CD is being performed. The process of complexation of solute G with n molecules of cyclodextrin depending on stoichiometry can be written as follows:

$$G_{(l)} + n \cdot CD \overset{K_1, K_2, \ldots, K_n}{\longleftrightarrow} G \cdot CD_n$$

[1] leaving the reaction for a longer time (1 h, 5 h and 24 h) did not improve the yield of obtained product.

[2] The whole procedure was repeated for larger amounts of substances (100 ml of 0.01 M solution of β CD and 136 mg of the camphene/fenchene mixture confirming previously obtained results).

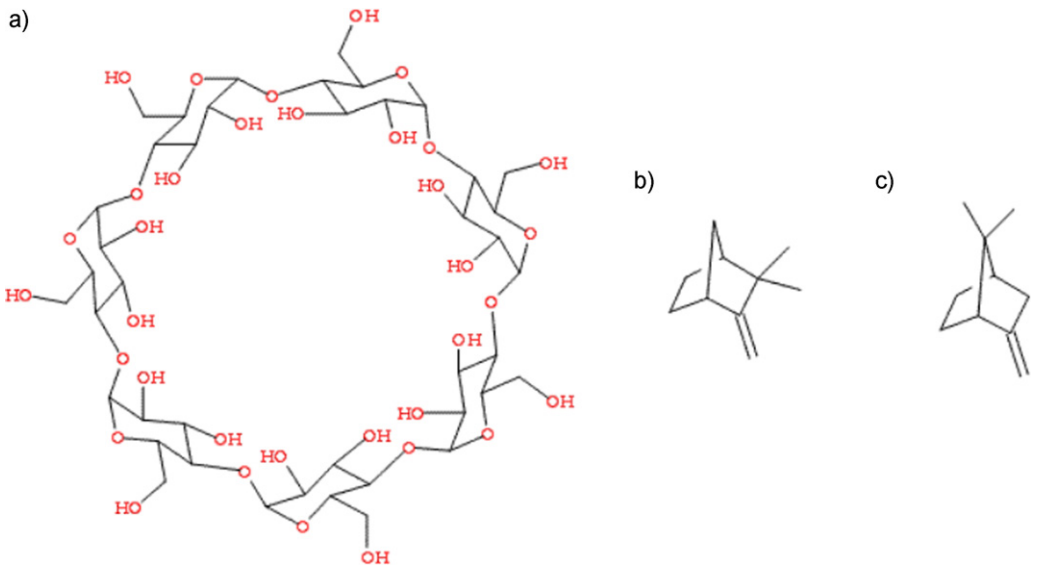

Fig. 1. Molecular structures of (a) β-cyclodextrin, (b) camphene and (c) fenchene.

where K_1, K_2, …, K_n means the stability constants of complex of solute G with 1, 2 or n molecules of cyclodextrin depending on stoichiometry.

$$K_1, K_2, \ldots, K_n = \prod_{i=1}^{n} K_i = \frac{[G \cdot \mathrm{CD}_n]}{[G][\mathrm{CD}]^n} \quad (2)$$

When the process of partition is accompanied by process of complexation the partition coefficient is equal to:

$$K = K^0 \left(1 + \sum_{n=1}^{N} \prod_{i=1}^{n} K_i [\mathrm{CD}]^n\right) \quad (3)$$

When only complexes G·CD of 1:1 stoichiometry are formed, the equation for partition coefficient K is reduced to the form (Purnell, 1966):

$$K = K^0(1 + K_1[\mathrm{CD}]) \quad (4)$$

where K_1 is the stability constant of a G·CD complex of 1:1 stoichiometry. In this case the relation of net retention time (t'_R) versus concentration of cyclodextrin is linear:

$$t'_R = t'^0_R(1 + K_1[\mathrm{CD}]) \quad (5)$$

The stability constants of complexes formed between β-cyclodextrin and (+/−)-camphene and β-cyclodextrin and (+/−)-fenchene were estimated using Eq. (5) as it was done previously (Asztemborska et al., 2000a, 2000b, 2003).

Linear relation (t'_R) vs. β-cyclodextrin concentration has been observed (Fig. 2). This behaviour suggests the formation of 1:1 stoichiometry complexes. Similar observations were made for complexes of β-cyclodextrin with other monoterpenoids – α- and β-pinenes (Asztemborska et al., 2000b).

The stability constants evaluated at 40 °C in formamide medium using Eq. (5) for complexes of β-cyclodextrin with (+/−)-camphene was 270 [M^{-1}] and with (+/−)-fenchene was 297 [M^{-1}].

3.2. Camphene/fenchene complexes with β-cyclodextrin obtained by fast precipitation and their separation via re-crystallization

Commercially available camphene consists of mixture of compounds, where the main compound is camphene with the other being fenchene, regioisomer of camphene, in which the only difference is the position of two methyl substituents (as it is presented in Fig. 1). Both regioisomers form complexes with β-cyclodextrin with a stoichiometry 1:1 and no separations of enantiomers. Since complexes of β-cyclodextrin with both enantiomers of camphene are equally strong, there is no selectivity towards any of them, only one enantiomer – (+) camphene/fenchene – was chosen for our studies. The stability constants evaluated for complex of camphene/β-cyclodextrin and fenchene/β-cyclodextrin suggest the possibility of separation of the isomers, nevertheless, due to only small difference (with fenchene/β-cyclodextrin complex being stronger) between them this task appeared to be challenging. The addition of dioxane solution of camphene/fenchene mixture to the solution of β-cyclodextrin in water results in fast precipitating product, which composition was analysed by ^{1}H NMR. ^{1}H NMR spectra were recorded for camphene/fenchene mixture (92:8) and for the complex obtained with β-cyclodextrin. Signals of the terminal alkene protons resonating at 4.50 and 4.69 for camphene and at 4.63 and 4.83 for fenchene appeared to be the most diagnostic. The camphene/fenchene ratio in the obtained complex was the same as in the starting material (Fig. 2). In Fig. 3b signals of one of terminal alkene protons in both camphene and fenchene in the complex with β-cyclodextrin are presented. The signals of the other protons are omitted for clarity, as they overlap with the signals for β-cyclodextrin.

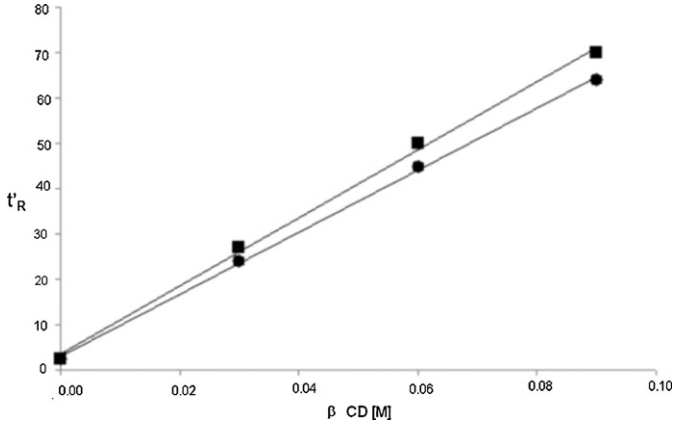

Fig. 2. Retention times of (+/−)-camphene and (+/−)-fenchene depending on β-CD concentration in formamide (■, fenchene; ●, camphene).

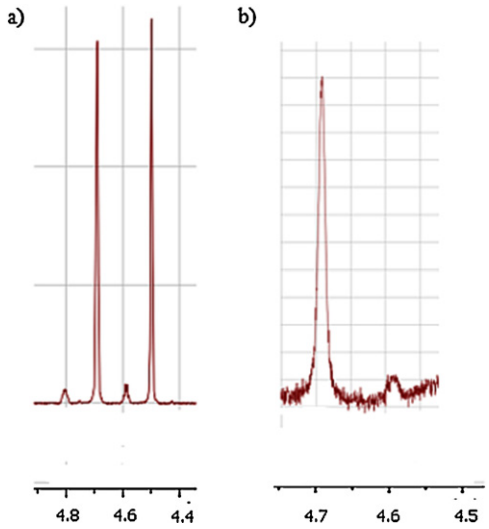

Fig. 3. Partial ^{1}H NMR spectra showing signals for (a) terminal alkene protons of camphene and fenchene, (b) one of terminal alkene protons of camphene and fenchene in the complex with β-cyclodextrin.

To conclude: fast precipitation of the product leads to the formation of the β-cyclodextrin complexes with both camphene and fenchene, but does not lead to the separation of the regioisomers. Time of formation of complexes is one of the key factors determining the characteristics of the obtained compound, with temperature being the second. Therefore, obtained mixture of complexes of β-cyclodextrin/camphene and β-cyclodextrin/fenchene in water was again added to water and dissolved in higher temperature (75 °C), stirred in that temperature for an additional 5 h and cooled down to room temperature. Contrary to the described earlier formation of the mixture of complexes in the form of precipitation, the precipitate did not appear until 72 h after being cooled down to room temperature suggesting different mechanisms of complex formation. The product precipitates in a form of monocrystals and it was subjected to single crystal X-ray analysis to establish the composition of the obtained complex.

3.3. Characterization of β-cyclodextrine/fenchene complex

Single crystal X-ray analysis was performed. It confirms the formation of β-cyclodextrin/fenchene complex, with no formation of β-cyclodextrin/camphene complex (Table 1).

The obtained β-CD–fenchene complex crystallizes in an orthorombic group $C222_1$. It shows the typical β-cyclodextrin form of head-to-head dimer, which is stabilised by hydrogen bonds.

All of the glucose units are in the $^4C_1$ conformation and each β-cyclodextrin has approximate 7-fold axis, which maintains the round shape of the macrocycle. Every head to head β-cyclodextrin dimer is stabilised by hydrogen bonds between secondary hydroxyl groups. All primary hydroxyl groups point outwards from the β-cyclodextrin cavity. Usually, β-cyclodextrins form 1:1 inclusion compounds with guest molecules. This kind of species are observed also with the guest compounds that are of similar size to camphene and fenchene, e.g. benzamide (Wang, Lian, & Cai, 2007) or benzoic acid (Aree & Chaichit, 2003). Although the obtained β-cyclodextrin–fenchene complex also exhibits the stoichiometry 1:1, instead of inclusion complex, there is exclusion complex present. The guest compound, surprisingly, does not enter the hydrophobic cavity of β-cyclodextrin, but is placed in the space between the dimeric host molecules (Fig. 4).

Table 1
Crystal data and structure refinement for the β-cyclodextrin–fenchene complex.

Empirical formula	$C_{52}H_{106}O_{45}$
Formula weight	1450
Temperature (K)	100
Wavelength (Å)	0.71013
Crystal system, space group	Orthorhombic, $C222_1$
Unit cell dimensions	
a (Å)	19.2031 (7)
b (Å)	23.8837 (8)
c (Å)	32.5540 (11)
Volume (Å^3)	14930.6 (9)
Goodness-on-fit on F^2	1.089
R indices [Fo > 4sig (Fo)]	$R_1 = 0.105$; $wR_2 = 0.257$

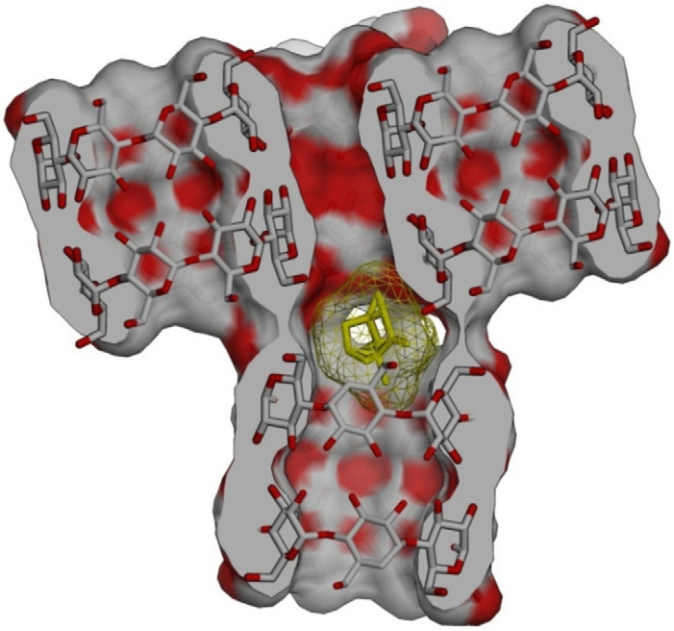

Fig. 4. View of the β-cyclodextrin–fenchene complex.

The guest molecule is disordered and is observed to occupy two sites (as it is shown in Fig. 5). The residual electron density, that can be observed, suggests the possibility of existence of the third

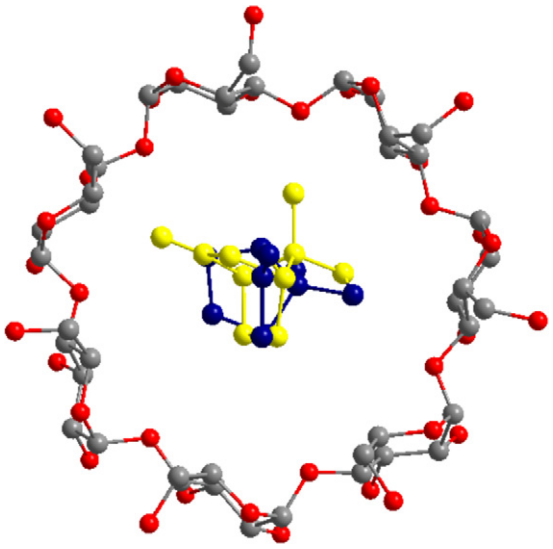

Fig. 5. The graphic presentation of β-cyclodextrin with fenchene, showing the guest compound in two positions.

additional low occupancy guest position, which however is very difficult to localize.

4. Conclusions

The 1:1 complexes of both (+/−) camphene and fenchene with β-cyclodextrin were obtained and their stability constants were calculated. The method for separation of camphene and fenchene via recrystallization with β-cyclodextrin was proposed. Moreover, the crystal structure of β-cyclodextrin with fenchene was obtained showing the formation of exclusion complex with the disordered guest molecule.

Acknowledgement

This research was partly financed by the European Union within the European Regional Development Fund (POIG.01.01.02-14-102/09).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.07.072.

References

Aree, T., & Chaichit, N. (2003). Crystal structure of β-cyclodextrin–benzoic acid complex. *Carbohydrate Research*, *338*, 439–446.

Arun, R., Ashok, K. C. K., & Sravanthi, V. V. N. S. S. (2008). Cyclodextrins as drug carrier molecule: A review. *Scientia Pharmaceutica*, *76*, 567–598.

Astray, G., Gonzalez-Barreiro, C., Mejuto, J. C., Rial-Otero, R., & Simal-Gandara, J. (2009). A review on the use of cyclodextrins in foods. *Food Hydrocolloid*, *23*, 1631–1640.

Asztemborska, M., Bielejewska, A., Sybilska, D., & Duszczyk, K. (2000). Comparative study on camphor enantiomers behavior under the conditions of gas–liquid chromatography and reversed-phase high-performance liquid chromatography systems modified with α- and β-cyclodextrins. *Journal of Chromatography A*, *874*, 73–80.

Asztemborska, M., Nowakowski, R., & Sybilska, D. (2000). Separation ability and stoichiometry of cyclodextrin complexes. *Journal of Chromatography A*, *902*, 381–387.

Asztemborska, M., Sybilska, D., Nowakowski, R., & Perez, G. (2003). Chiral recognition ability of α-cyclodextrin with regard to some monoterpenoids under gas–liquid chromatographic conditions. *Journal of Chromatography A*, *1010*, 233–242.

Bledsoe, J. O. (1997). Terpenoids. In *Kirk–Othmer encyclopedia of chemical technology* (p. 833).(4th ed.). New York: Wiley.

Caira, M. R., Griffith, V. J., & Nassimbeni, L. R. (1996). X-ray structures of 1:1 complexes of L-menthol with β-cyclodextrin and permethylated β-cyclodextrin. *Supramolecular Chemistry*, *1*, 119–124.

Castanheiro, J. E., Fonseca, I. M., Ramos, A. M., & Vital, J. (2008). Acetoxylation of camphene catalysed by beta zeolite. *Catalysis Communications*, *9*, 2205–2208.

da Silva, M. J., & Gusevskaya, E. V. (2001). Palladium-catalyzed oxidation of monoterpenes: Novel tandem oxidative coupling-oxidation of camphene by dioxygen. *Journal of Molecular Catalysis A: Chemical*, *176*, 23–27.

da Silva, K. A., Kozhevnikov, I. V., & Gusevskaya, E. V. (2003). Hydration and acetoxylation of camphene catalyzed by heteropoly acid. *Journal of Molecular Catalysis A: Chemical*, *192*, 129–134.

Ebmeyer, F. (2002). Theoretical investigations towards an understanding of the α-pinene/camphene rearrangement. *Journal of Molecular Structure (TEOCHEM)*, *582*, 251–255.

Findik, S., & Gündüz, G. (1997). Isomerization of a-pinene to camphene. *Journal of the American Oil Chemists Society*, *74*, 1145–1151.

Grandeury, A., Petit, S., Gouhier, G., Agasse, V., & Coquerel, G. (2003). Enantioseparation of 1-(p-bromophenyl)ethanol by crystallization of host–guest complexes with permethylated β-cyclodextrin: Crystal structures and mechanisms of chiral recognition. *Tetrahedron: Asymmetry*, *14*, 2143–2152.

Hbaieb, S., Kalfat, R., Chevalier, Y., Amdouni, N., & Parrot-Lopez, H. (2008). Influence of the substitution of β-cyclodextrins by cationic groups on the complexation of organic anions. *Materials Science and Engineering C*, *28*, 697–704.

Jung, M., Schmalzing, D., & Schurig, V. (1991). Theoretical approach to the gas chromatographic separation of enantiomers on dissolved cyclodextrin derivatives. *Journal of Chromatography A*, *552*, 43–57.

Knoll, W., Bobek, M. M., Giester, G., & Brinker, U. H. (2001). 4-Aziadamantan-1-amine: Synthesis, reactions and cyclodextrin complexes. *Tetrahedron Letters*, *42*, 9161–9165.

Lämmerhofer, M. (2010). Chiral recognition by enantioselective liquid chromatography: Mechanisms and modern chiral stationary phases. *Journal of Chromatography A*, *1217*, 814–856.

Neoh, T. L., Koecher, K., Reineccius, G., Furuta, T., & Yoshii, H. (2010). Dissociation characteristic of the inclusion complex of cyclomaltohexaose (α-cyclodextrin) with 1-methylcyclopropene in response to stepwise rising relative humidity. *Carbohydrate Research*, *345*, 2085–2089.

Purnell, J. H. (1966). *Gas chromatography* (p. 3). London: Institute of Petroleum.

Schurig, V., & Juza, M. (1997). Approach to the thermodynamics of enantionmer separation by gas chromatography. Enantioselectivity between the chiral inhalation anesthetics enflurane, isoflurane and desflurane and a diluted γ-cyclodextrin derivative. *Journal of Chromatography A*, *757*, 119–135.

Sheldrick, George M. (2008). A short history of SHELX. *Acta Crystallographica. Section A*, *A64*, 112–122.

Spencer, J. N., He, Q., Wu, Z., & Fetter, E. (1998). Complexation of inorganic anions and small organic molecules with alpha-cyclodextrin. *Journal of Solution Chemistry*, *27*, 1009–1019.

Tao, Y.-T., Yeh, W.-L., Tu, C. L., & Chow, Y. L. (1991). Acidity effects on light promoted carbonylation of olefins in the presence of cobalt catalysis. *Journal of Molecular Catalysis A: Chemical*, *67*, 105–115.

Tiwari, M., & Kakkar, P. (2009). Plant derived antioxidants—geraniol and camphene protect rat alveolar macrophages against *t*-BHP induced oxidative stress. *Toxicology In Vitro*, *23*, 295–301.

Wang, E. U., Lian, Z.-X., & Cai, J. (2007). The crystal structure of the 1:1 inclusion complex of β-cyclodextrin with benzamide. *Carbohydrate Research*, *342*, 767–771.

Whittaker, D. (1972). *Chemistry of terpenes and terpenoids* (p. 11). London: Academic Press.

Wu, H., Liang, H., Yuan, Q., Wang, T., & Yan, X. (2010). Preparation and stability investigation of the inclusion complex of sulforaphane with hydroxypropyl-β-cyclodextrin. *Carbohydrate Polymers*, *82*, 613–617.

Yuasa, Y., Watanabe, T., Nagakura, A., Tsuruta, H., King, G. A., III, Sweeny, J. G., et al. (1992). An improved synthesis of (S)-aspartyl-(7,7-dimethylnorborn-2R-yl)-(S)-alanine methyl ester, a new high intensity artificial sweetener. *Tetrahedron*, *48*, 3473–3484.