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Experimental and Theoretical Study on the Inclusion Compounds of Aroma Components with β-Cyclodextrins

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Inclusion equilibria between four aroma components suspected to be allergen and various β-cyclodextrins were investigated. The association constants of inclusion complexes were measured in aqueous solution by UV/VIS spectroscopy by competition method with methyl orange and direct titration. The data indicated the formation of 1:1 inclusion compounds. In addition, a theoretical study using MM3 force field was conducted and the computed formation energy, e.g. the stability of the complexes, were in good agreement with the calculated formation constants.

Keywords: Allergen; β-cyclodextrin; Inclusion compounds; UV-visible; Molecular modelling

INTRODUCTION

European legislation requires fragrance products to be evaluated for their content in 24 components suspected to be allergens. The assay of these compounds is quite difficult because of the complexity of the fragrance concentrate or cosmetic derivatives [1,2]. Within this scope, the use of cyclodextrins (CDs) could be proposed to enhance the solubility of the allergen.

Cyclodextrins are a family of cyclic oligosaccharides that are composed of α-1,4 linked glucopyranose subunit [3]. Cyclodextrins are produced from starch by enzymatic degradation [4,5]. The most common cyclodextrins are of three types: α-, β- and γ-cyclodextrin referred to as first generation or parent cyclodextrins. α-, β- and γ-cyclodextrins are composed of six, seven and eight glucose units respectively. β-cyclodextrin (BCD) is the most useful cyclodextrin because it is the most accessible cyclodextrin and the cheapest but it is also the less soluble in water [6]. Some chemically modified β-cyclodextrins, more soluble in water or in organic solvent are commercially available like hydroxypropyl-β-cyclodextrin (HPBCD), randomly methylated β-cyclodextrin (RAMEB) and partially methylated crystallised β-cyclodextrin (CRYSMEB). The structures of these β-cyclodextrins are shown in Fig. 1.

These macrocyclic carbohydrates have a hollow truncated shape with an apolar internal cavity. They can form host-guest complexes with a large variety of solid, liquid and gaseous organic compounds by a phenomenon of molecular complexation [7]. Inclusion in cyclodextrins exerts a profound effect on the physicochemical properties of guest molecules as they are temporarily locked or caged within the host cavity, giving rise to beneficial modifications of the guest molecule properties [8]. These properties are: reactivity, solubility enhancement, control of volatility, stabilisation of labile guest or controlled release of drugs and flavours. Therefore, cyclodextrins are used in many fields of chemistry [8–18].

Some researches regarding the uses of cyclodextrins in the perfume and cosmetic industries have been reported [4,8,19–22]. Applications to cosmetics include solute stability, release control, malodor masking and possible surfactant reduction to produce product irritancy. Their main drawback is a problem encountered with any carrier material, i.e. their selectivity toward the numerous compounds that constitute an aroma. As a consequence, some molecules may be poorly retained whereas others are strongly trapped [23]. Hence, understanding how the fragrance material interacts with the CDs in aqueous solution is essential.

In this paper, the complexation behaviour of four β-cyclodextrins (BCD, HPBCD, RAMEB and

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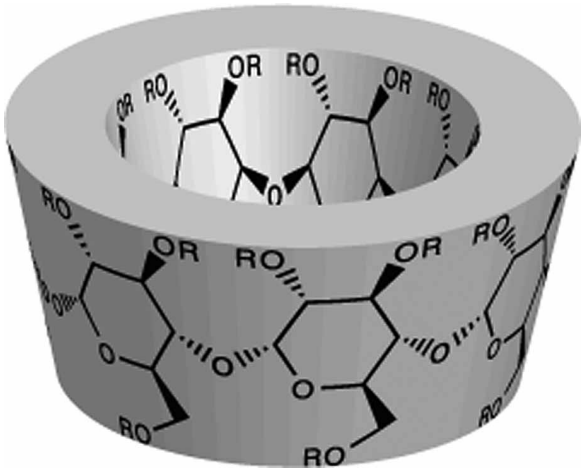

FIGURE 1 Chemical structure of β-cyclodextrins: native: R=H; HPBCD: R=CH_2–CHOH–CH_3 (*0.8 OH groups modified per glucopyranose unit*); CRYSMEB: R=CH_3 (*0.7 OH groups modified per glucopyranose unit*); RAMEB: R=CH_3 (*1.8 OH groups modified per glucopyranose unit*).

CRYSMEB) and four aroma compounds suspected as allergen e.g. eugenol (EUG), isoeugenol (IEUG), benzyl alcohol (AB) and anisyl alcohol (AA) (Fig. 2) was investigated in aqueous solution by UV-Visible spectroscopy. A theoretical study by molecular modelling has been realised by MM3 method to obtain complexation energies. These values are compared with the obtained formation constants.

RESULTS AND DISCUSSION

Experimental Study

In order to evaluate the inclusion ability of the four β-cyclodextrins, we first used a UV-visible competition method to study the inclusion compounds occurring with the four guests. Such a method is based on the spectral variation observed upon addition of each guest on a solution containing

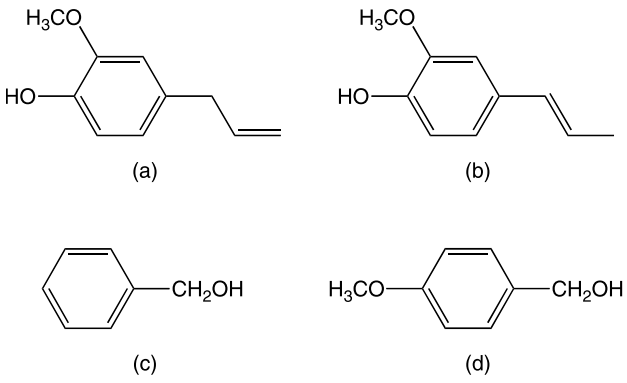

FIGURE 2 Molecular structure of Eugenol (a), Isoeugenol (b), Benzyl alcohol (c) and Anisyl alcohol (d).

both CD and methyl orange. It implies that the CD/MO complex should be characterised. A titration has thus been realised for the four CDs and the obtained data are in agreement with a 1:1 complex ratio. The association constants are consistent with other data for MO and BCD [24]. When applying the competition method, one can observe an increase of absorption (Fig. 3), which indicates the formation of inclusion compounds.

The obtained spectral variations are also in agreement with the 1:1 host/guest ratio, as could be expected from the analogous results for other substituted phenol or benzene derivatives [25]. The formation constants are calculated by an algorithmic procedure and are reported in Table I.

To the best of our knowledge, very few stability constants have been reported for the inclusion of fragrance with cyclodextrins [26–30]. For eugenol, two determinations have been performed, one with HPBCD determined by static headspace [26] and one with BCD determined by direct UV/VIS spectroscopy [27]. If the first one is in the same order of magnitude than our study ($270\,M^{-1}$), the second is much more higher ($396000\,M^{-1}$). This last value is surprising since, generally, the formation constants of phenol derivatives are less than $10^4\,M^{-1}$ [25,31]. Besides, the addition of a methoxy group to a phenol derivative should not lead to such difference in the formation constant: as calculated by Suzuki, the difference in free energies of complexation between phenol and the two methoxyphenol isomers is only of 0.75 kJ/mol [32]. Hence, in order to confirm our results, we performed a direct titration with eugenol and BCD using the same procedure described for CD/MO. As can be seen on the absorption spectra (Fig. 4), the absorption of eugenol decreases in intensity upon addition of various amounts of cyclodextrin. The corresponding association constant, calculated by an algorithmic treatment, is equal to $325\,M^{-1}$ and confirms the one obtained by competition.

Concerning the other guests, the values obtained for isoeugenol and eugenol are close one to the other, which was expected from to the fact that these two compounds differ only by the position of the double bond. For benzyl alcohol, a previous study reports a formation constant of $50\,M^{-1}$ with BCD [28], which is consistent with our values. Finally, as mentioned for phenol, the addition of a methoxy group is not expected to change the stability of the complex in a great manner. This is also true if we compare the formation constant of benzyl alcohol and anisyl alcohol (4-methoxybenzylalcohol).

Theoretical Study

We calculated the stabilization energy ΔE due to the inclusion of guest in the inner cavity of the four

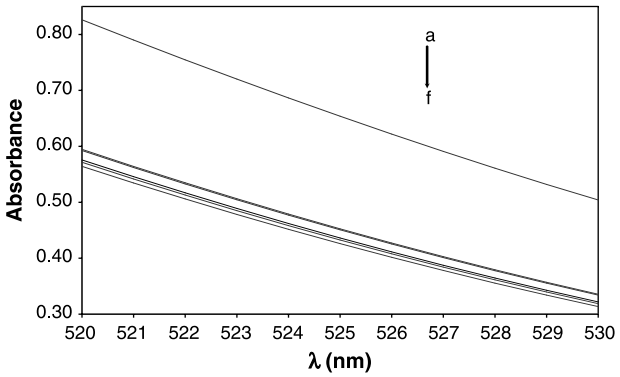

FIGURE 3 Absorption spectra for solutions containing (a) methyl orange 0.1 mM, (b) methyl orange 0.1 mM, BCD 0.5 mM and eugenol 1 mM; (c) methyl orange 0.1 mM, BCD 0.5 mM and isoeugenol 1 mM; (d) methyl orange 0.1 mM, BCD 0.5 mM and anisyl alcohol 1 mM; (e) methyl orange 0.1 mM, BCD 0.5 mM and benzyl alcohol 1 mM; (f) methyl orange 0.1 mM and BCD 0.5 mM.

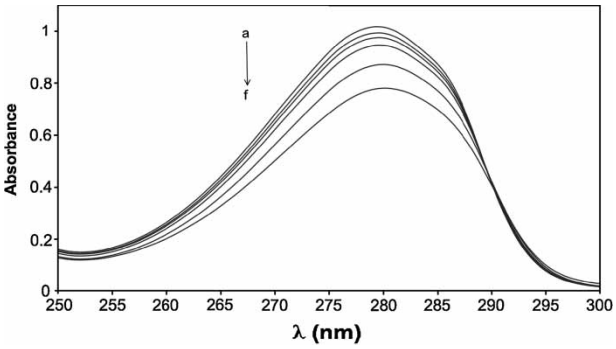

FIGURE 4 UV/VIS absorption spectra of eugenol (0.25 mM) in the absence (a) and in the presence of various concentration of BCD (from b to f 0.25 mM, 0.5 mM, 1 mM, 2.5 mM and 5 mM respectively).

β-cyclodextrins. The corresponding values and conformations are presented in Table II and Fig. 5 respectively.

Structural studies have been carried out by using different methods with some substituted phenols and β-cyclodextrin [33,34]. The proposed structures for eugenol and benzyl alcohol are similar to the ones found by molecular modelling. The most stable conformation of benzyl alcohol with BCD is the one with the hydroxyl group near the secondary hydroxyl rim of the BCD. For eugenol the phenolic hydroxyl is outwards while the phenyl group is inside the cavity, and the double bond present in the apolar region as described by Divakar [33].

For all four β-cyclodextrins the experimental and theoretical studies show that benzyl alcohol forms the less stable complex, a phenomenon which can be attributed to the high hydrophilic character of this compound (aqueous solubility of 40 g/L at 298 K). In addition, the correlation between experimental and theoretical data is rather good for the four guests, as can be illustrated by the correlation coefficients between the logarithm of the formation constants and the energetic stabilisation predicted for the most stable conformer: 0.917 for BCD, 0.921 for HPBCD, 0.885 for RAMEB and 0.922 for CRYSMEB. This result suggests that molecular modelling may be a useful tool for studying the complexation of allergens which are too poorly soluble in water to be analysed by experimental techniques.

TABLE I Formation constant (M^{-1}) obtained by the competition method

	MO	EUG	ISO	AB	AA
BCD	2500	322	304	63	107
HPBCD	5373	445	452	54	156
RAMEB	15942	521	547	55	125
CRYSMEB	3273	401	240	57	130

MATERIALS AND METHODS

Chemicals

Eugenol, isoeugenol, benzyl alcohol, anisyl alcohol, methyl orange, sodium hydroxide and potassium dihydrogenophosphate (Aldrich) were all of analytical reagent grade and were used as received. HPBCD and CRYSMEB were provided by Roquette Frères (Lestrem), BCD and RAMEB were purchased from Wacker Chimie S.A. (Lyon). Deionised water was used throughout this work.

Visible Spectra

Spectra were recorded using a Perkin Elmer Lambda 2S double beam spectrometer and a quartz cell with optical path length of 1.00 cm at 298 K. The control of temperature was realised by the use of a thermostated bath linked to the cell holder (accuracy: ± 0.1°C). All compounds were dissolved in phosphate buffer at pH 5.8.

Formation Constant Determination

Evaluation of CD inclusion capacity towards allergen has been carried out by use of UV-Visible spectroscopy.

Direct Titration Method

First, the CD/MO systems are characterised by a direct titration method. For a 1:1 molar ratio complex the calculation of formation constant K_f was

TABLE II Computed energy of complexation, ΔE (kcal/mol)

	ΔE (kcal/mol)							
	Eugenol		Isoeugenol		Benzyl Alcohol		Anisyl Alcohol	
	E1	E2	E1	E2	E1	E2	E1	E2
BCD	−14.55	−14.72	−13.06	−15.37	−7.05	−6.41	−12.80	−10.14
HPBCD	−16.19	−10.61	−16.98	−17.09	−12.74	−13.23	−16.14	−10.50
RAMEB	−13.93	−14.86	−15.87	−16.64	−12.05	−10.23	−14.51	−12.67
CRYSMEB	−14.30	−14.76	−14.75	−14.47	−12.33	−11.71	−14.23	−13.23

developed as follows:

$$MO + CD \rightleftharpoons CD/MO$$

$$K_f = \frac{[CD/MO]}{[MO][CD]}$$

$$K_f = \frac{[CD/MO]}{([MO]_T - [CD/MO])*([CD]_T - [CD/MO])}$$

$$[CD/MO] = -\frac{1}{2}\sqrt{\left[\left(\frac{1}{K_f} + [CD]_T + [MO]_T\right)^2 - 4[CD]_T[MO]_T\right]} + \frac{1}{2}\left(\frac{1}{K_f} + [CD]_T + [MO]_T\right)$$

where K_f and T stand for formation constant and total respectively. For a given value of K_f, [CD/MO] is known and the spectral characteristic of the complex can be calculated. The algorithmic treatment was applied to the first derivatives of UV spectra in order to avoid any spectral influence of diffraction phenomena [25].

Spectral Displacement Method

Applying a spectral displacement method with MO in its basic form allows the determination of the association constants of allergen with the CDs. 1:1 equilibrium between MO, CD and guest (G) is described as follows:

$$MO + CD + G \rightleftharpoons CD/MO + G \rightleftharpoons CD/G + MO$$

While concentrations of MO and CD are kept constant, the addition of G implies an absorbance increase, proportional to the expulsion of MO from the CD cavity. The formation constant CD/G can therefore be deduced from this absorbance difference. An algorithmic method was used for the data treatment. Its principle consists in the calculation of the concentration of the complexes by considering

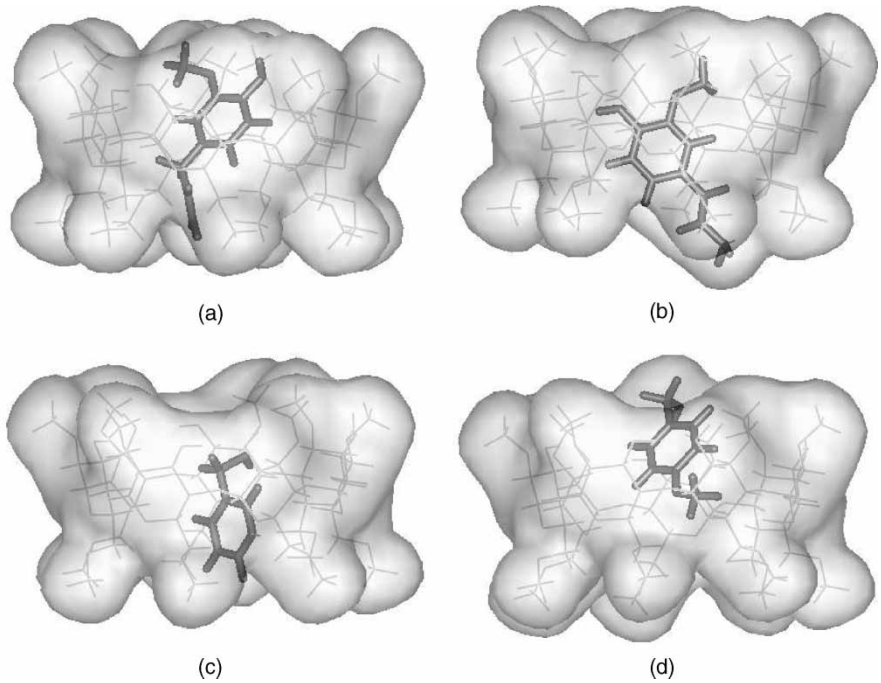

FIGURE 5 Representation of the conformation of host-guest complexes: Eugenol (a), Isoeugenol (b), Benzyl alcohol (c) and Anisyl alcohol (d).

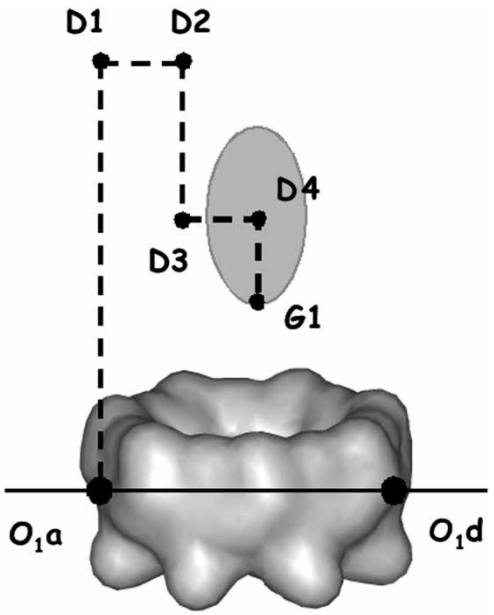

FIGURE 6 Representation of docking protocol with the four dummy atoms (D1, D2, D3 and D4).

the two equilibria successively in an iterative way [25]. As a consequence, the obtained binding constant is not depending on the presence of methyl orange, thus leading to results which are comparable to non competitive methods. Spectra were recorded between 520–530 nm for a MO concentration fixed at 0.1 mM. This wavelength range corresponds to the optimal spectral variation between the free and complexed forms of MO.

Molecular Modelling

The cyclodextrin hosts were based on a non distorted monomeric β-cyclodextrin with C7 symmetry. Guest molecules were initially retrieved from the data provided by the Structural Data Base System of the Cambridge Crystallographic Data Center, and then energy minimised with MM3 force field. The various structural manipulations were made using the CAChe Library [35] on PC-Computer.

Inclusion Compounds Conformation

The docking of each guest into the β-CD unit has been performed using four dummy atoms (Fig. 6). The first one (D1) is perpendicular to the mean plane P of the glucosidic oxygens, the second one (D2) is placed on an axis parallel to this plane, the third one (D3) is on an axis perpendicular to the plane P. The last dummy is placed on an axis parallel to D1-D2, as the centroïd of the guest. This dummy D4 is then virtually attached to one atom of the guest (G1).

For each substituted guest, both regioselective forms have been taken in consideration. Then, three parameters were varied to explore the conformational space of the inclusion compound: the distance between host and guest (distance D2-D3), the orientation of the aromatic ring inside the host cavity (dihedral D1-D2-D3-D4), and its tilt angle (dihedral D2-D3-D4-G1). For this purpose, a multi-conformational search (integrated in CAChe) has been employed with the MM3 force field. During this search, the cyclodextrin host is kept rigid, while the guest freedom is freely allowed. Indeed and contrary to the structure of the studied guests, the cyclodextrin conformation is controlled by many geometric parameters so that random variations may be observed during the search if the cyclodextrin geometry is not locked: since such exploration of the conformational space of the cyclodextrin is not

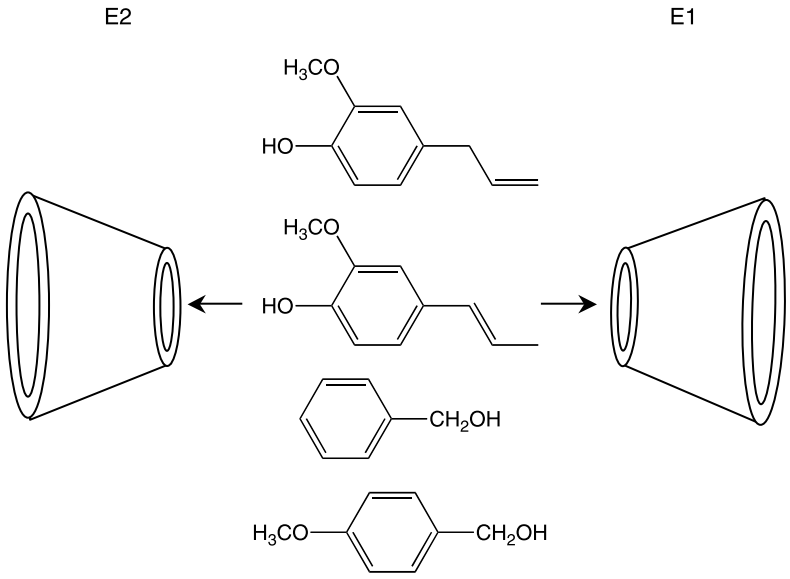

FIGURE 7 Representation of the two types of inclusion complexes (E1 and E2) used for the docking.

exhaustive but has a considerable influence on the energetic parameter, comparison from one guest to another may be biased if no constraint is applied to the cyclodextrin cavity. The most stables structures obtained by this procedure are then energy minimised without any constraint. The difference (ΔE, kcal/mol) between total energy of the inclusion complex and the sum of their individual components in their optimized ground states was then used as the theoretical parameter to evaluate the inclusion capacity of the cyclodextrin host.

Two general types of inclusion complexes marked by E1 and E2 according to the two available docking possibilities were explored (Fig. 7).

CONCLUSION

We have performed an experimental and theoretical study for the inclusion complexes of four aroma components suspected to be allergen and four β-cyclodextrins. The experimental values of the constants were in good agreement with the computed complexation energies, ΔE, from molecular mechanics modelling of the inclusion process. Since a lot of the 24 compounds suspected to be allergenic are poorly soluble, molecular modelling may be the only way to estimate the stability of the inclusion complexes. The extension of our study is thus to compute the complexation energy of the 20 remaining allergens with the β-cyclodextrins in order to have a better knowledge of the affinity of cyclodextrins for these compounds.

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