Release of the *Prays oleae* pheromone as a consequence of supramolecular structure: study of the β-cyclodextrin-(*Z*)-tetradec-7-en-1-al complex by X-ray crystallography and NMR spectroscopy in the solid state and in solution

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The structure of the β-cyclodextrin-(*Z*)-tetradec-7-en-1-al complex in aqueous solution and in the solid state, as well as the release profile of the (*Z*)-tetradec-7-en-1-al (sex pheromone of the olive pest *Prays oleae*) from the solid complex was investigated, in an effort to correlate the supramolecular structure with the macroscopic property of spontaneous liberation of the pheromone. It was observed that in solution a 2 : 1 host : guest complex prevails, having the guest in a curled configuration. In the crystal structure of the complex, two β-CD molecules forming head-to-head dimers and packed in channels enclose one guest molecule whose methyl terminal aliphatic chain curls at the *cis*-double bond and runs along the intradimer interface. In the space between host molecules there is also entrapped an additional pheromone molecule, also visible in the IR spectra, which is heavily disordered. The guest inside the cavity is disordered over two sites and exhibits mobility, especially at the methyl and carbonyl end-groups, which is also confirmed by solid state NMR experiments. Thus, there are two types of guest molecules in the crystalline complex, one inside the β-CD cavity and another trapped and held loosely outside the cavity. The release behavior, studied by NMR, shows that the "outside" pheromone is liberated from the solid initially at a fast rate, which reaches very low levels when almost half of the guest molecules have been released. The other half, molecularly encapsulated in the β-CD cavity, is well stabilized.

Introduction

Cyclodextrins (CDs) are widely used molecular hosts able to solubilise in water a variety of guest molecules. Their torus-shaped cavity is sufficiently apolar to induce the inclusion of the hydrophobic moieties of molecules in an aqueous environment. Of all CDs, β-CD (cyclomaltoheptaose) has the most symmetrical cavity due to the optimum distances of the intramolecular H-bonds at the secondary hydroxy face of the macrocycle.[1] As a consequence, it readily forms head-to-head dimers in the crystalline state, held together by strong H-bonds between the O3 hydroxys.[2,3] The elongated cavity of the dimer can be a host to long aliphatic guest molecules.[3–8] The mode of inclusion depends on the guest's length. For aliphatic chains with up to 9 carbon atoms, the host : guest ratio is 2 : 2 *i.e.* two guests are accommodated inside each dimer.[4] For longer guests, with 10–16 carbon atoms, complexes with a host : guest ratio of 2 : 1 are formed.[3,5–9] In the latter case the aliphatic chain usually runs along the entire length of the dimer. However, this is not always the case. In the β-CD complex of (*Z*)-dodec-9-en-1-ol[10] the chain bends at the double bond carbon atom C9 and runs parallel to the intradimer interface. A similar situation is observed presently in the β-CD inclusion complex of (*Z*)-tetradec-7-en-1-al. The aliphatic chain runs along the 7-fold axis of one monomer up to the double bond at the height of the interdimer interface, where it bends and then follows the periphery of the latter.

(*Z*)-Tetradec-7-en-1-al is the main pheromone component of the olive pest *Prays oleae*. In our efforts to find an efficient formulation for the slow release of the pheromone in order to control the population of the insects, its encapsulation in β-CD was performed. Subsequently, its behavior was studied with respect to protection from oxidation and the release of the guest molecule and structure of the inclusion complex was studied by NMR spectroscopy both in solution and the solid state. The crystalline structure was determined by X-ray diffraction at 100 K. In a freshly prepared inclusion complex the host : guest ratio is 1 : 1, whereas in freshly prepared crystals for the X-ray structure determination it is 1 : 0.75–0.80 as determined by NMR. Stability studies of (*Z*)-tetradec-7-en-1-al in the β-CD complex show that only about 50% of the pheromone content is protected by inclusion against oxidation. The structural evidence explains the above behavior by suggesting two types of guests in the crystal. One inside the β-CD cavity and therefore protected, and another on the outside, in the space between host molecules, usually occupied by water molecules.

Experimental

X-Ray crystal structure determination †

Crystals, in the form of colorless diamond-shaped thin plates stuck together in multiple layers, were obtained by slow cooling of a saturated solution containing a 1 : 5 mixture of β-cyclodextrin and (*Z*)-tetradec-7-en-1-al (Vioryl S. A., Greece) for a period of five days. The multiple plates were separated and a single crystal was used for data collection. Low temperature

† CCDC reference number 176834. See http://www.rsc.org/suppdata/p2/b1/b111632k/ for crystallographic files in .cif or other electronic format.

Table 1 Crystal data and structure refinement

Empirical formula	$2(C_{42}H_{70}O_{35})\cdot 1.4(C_{14}H_{26}O)\cdot 21.1H_2O$
Formula weight (M)	2944.18
Crystal system/space group	Triclinic/$P1$
a/Å	15.475(7)
b/Å	15.466(8)
c/Å	15.720(4)
α/°	101.856(2)
β/°	101.909(2)
γ/°	103.769(3)
V/Å^3	3444.9(3)
Z	1
D_c/g cm^{-3}	1.4
Reflections (unique)/($F_o > 4\sigma(F_o)$)	25138/21255
Goodness of fit on F^2	1.053
R_1, $wR(F^2)$ [$F_o > 4\sigma(F_o)$]	0.0699, 0.1965

data were collected using the synchrotron radiation light source at the EMBL BW7A beamline at the DORIS storage ring, DESY, Hamburg (Table 1). The selected crystal of dimensions 0.6 × 0.4 × 0.2 mm^3, covered with oil and mounted on a hair fiber loop was frozen to 100 K. Three sets of data were collected: (a) a high resolution set (0.65 Å) of 91 frames of rotation $\phi = 2°$ with an image plate detector MAR 30 cm, (b) a medium resolution set (0.95 Å) of 50 frames $\phi = 3°$ and (c) a low resolution set (1.75 Å) of 46 frames of rotation $\phi = 4°$ with an image plate detector MAR 18 cm. The complete data set was processed and scaled using the programs DENZO and SCALEPACK,[11] respectively. The completeness of the data collection was 91.8% and the R_{merge} was 4.1%. The estimated errors of the unit cell dimensions were calculated by the least-squares method from the cell dimensions of the high-resolution frames. The structure was solved by isomorphous molecular replacement of the β-CD glucosidic skeleton atoms of the β-CD-*tert*-butyltoluene complex.[12] The refinement was performed using the program SHELX-97.[13] The rest of the cyclodextrin atoms as well as the water and guest molecules were located from difference electron density Fourier maps. Despite the high resolution of the data, the electron density corresponding to the guest was continuous, especially at the primary faces of the dimer CD cavity and at the space between them, indicating extensive mobility or disorder. However, two models of the guest could be built, although the possibility of some other conformation of similar geometry cannot be excluded. The two separate models of the guest at sites, related by a 2-fold non-crystallographic axis with some of the atoms superimposing, were subsequently improved by fitting into the difference electron density corresponding to them using the graphics program "O".[14] The occupancies of the sites were refined to almost 50% and they were set to that value since they are complementary. The refinement continued anisotropically for all the host atoms and the water molecules as well as for the fixed positions and temperature factors of the guest. Almost all of the hydrogen atoms bonded to carbon atoms, as well as some of the hydroxy groups, of the macrocycle have been identified and refined. Hydrogen atoms not found were generated geometrically and were refined in a riding model. Thermal parameters of all hydrogen atoms were set to 1.25 of the corresponding atoms to which they were bonded. Towards the end of the refinement, it was evident that a continuous electron density was developing between the dimers and parallel to their long axis. Some of this density had been attributed to water molecules. Based on this electron density, it was possible to build models for two pairs of the guests, the molecules of each pair were related by the pseudo 2-fold axis. Occupancies were refined to values close to 10% and were fixed to that value. Isotropic thermal parameters of the atoms of all guest sites were refined at the very end of the refinement, which converged to $R_1 = 6.99\%$ for the $F_o \geq 4\sigma(F_o)$ reflections. The crystal data and refinement details are summarized in Table 1.

NMR and IR spectroscopy

Solution ^{1}H NMR spectra were acquired at 250.13 MHz on a Bruker AC 250 spectrometer. Titration of a 4.8 mM solution of β-CD in D_2O (0.525 mL) containing 5% MeOH-d_4, with a MeOH-d_4 solution (0.002 mL) of (*Z*)-tetradec-7-en-1-al and recording of the chemical shift changes of the cavity protons provided the data for the mole ratio diagram. Methanol was used to increase the solubility of the complex in water and keep the solution clear throughout the titration. Two-dimensional ROESY experiments were acquired on a 500 MHz Bruker Avance DRX spectrometer at 298 K with presaturation of the residual water resonance and a mixing (spin-lock) time of 350 ms at a field of ~2 kHz. Solid state ^{13}C CP/MAS NMR spectra were acquired at a frequency of 45.27 MHz on a Bruker CXP-180 NMR spectrometer equipped with a Doty Scientific MAS NMR probe. Spinning frequencies were ~5 kHz, RF amplitudes were 50 kHz, and CP contact times 1–3 ms. Accelerated "aging" of the samples was achieved by exposing the solid samples, sealed in black plastic bags, to a steady stream of ambient temperature air supplied by a hair dryer until the pheromone content was halved (about 8 h), as analysed by ^{1}H NMR. IR spectra (KBr pellet) were collected on a Nicolet Magna-550 FT–IR spectrometer.

Results and discussion

Stoichiometry and structure of the β-CD-(*Z*)-tetradec-7-en-1-al complex by NMR spectroscopy in aqueous solution

The stoichiometry of the complex in solution was determined by the mole ratio method. Fig. 1 shows quick shielding of the

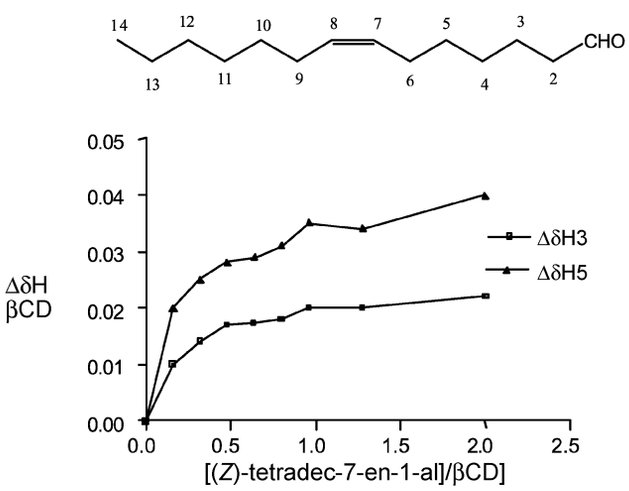

Fig. 1 Mole ratio diagram of the chemical shift changes of β-CD cavity protons during titration with (*Z*)-tetradec-7-en-1-al in D_2O containing 5% MeOH-d_4, [β-CD] = 4.8 mM.

β-CD cavity protons at the beginning of the titration with (*Z*)-tetradec-7-en-1-al and a flattening of the slope of the titration curve at a molar ratio of around 0.5. This suggests a prevailing 2 : 1 host : guest stoichiometry, although participation of some 1 : 1 complexes is very likely, since the change of chemical shifts continues well after the [host] : [guest] ratio reaches 0.5. The structure in solution and the mode of association of the aldehyde with the host were studied by running several 2D ROESY spectra at various host : guest ratios, either in D_2O or in the presence of 5% CD_3OD without spinning. The samples were allowed to settle in the NMR tubes, which resulted in partial precipitation of the complex at the bottom of the tube and phase separation of the insoluble aldehyde at the top of the sample. The complex that remained in solution afforded the dipolar interactions shown in Fig. 2. Strong interactions of both cavity protons (H3 and H5) of β-CD were observed with the

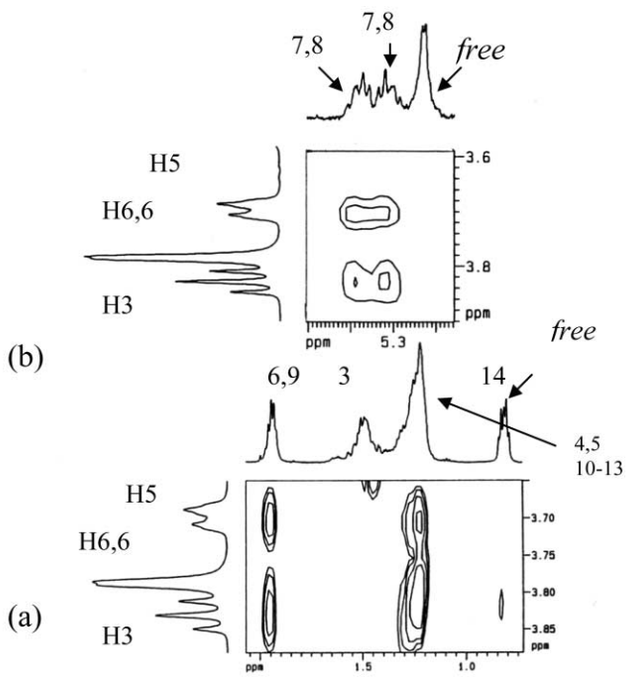

Fig. 2 Parts of the ROESY map in D_2O at 300 K. The β-CD protons correlate with (a) the aliphatic chain region of (*Z*)-tetradec-7-en-1-al and (b) the double bond region of (*Z*)-tetradec-7-en-1-al.

double bond protons at C7 and C8 (Fig. 2(b)), with their respective neighbouring methylenes at C6 and C9 and with the methylenes one bond further, *i.e.* at C5 and C10 (Fig. 2(a)), the latter appearing together with the methylene groups at C4 and C11–C13. There was no observable interaction of the CHO terminal or the adjacent methylene groups at C2 and C3 with the cavity. A weak correlation of the methyl group with the secondary side of the β-CD (H3) was observed (Fig. 2(a)). The above suggest that there is increased mobility of the two ends of the guest, especially at the aldehyde side, and good contacts are not established. This fact rather rules out the possibility of having a fully extended guest molecule along a β-CD dimer (rotaxanated structure), in which the end groups, restricted by the narrow primary side of the host, would give rise to observable interactions with that side.[15,16] In the present case, therefore, the pheromone must be curled. Strong correlations observed around the *cis*-double bond (Fig. 2(b)), indicate that this portion of the molecule is well located inside the cavity. What is unusual, is that we observe two types of double bonds (Fig. 2(b)) belonging to two different guest molecules, since the nearly equal multiplets at 5.40 and 5.35 ppm neither couple in the COSY nor interact dipolarly in the ROESY spectra. Both types of double bond protons show correlation with the β-CD cavity protons, therefore they arise from a distinct positioning of the double bond of two complex molecules in the cavity. The equal population of the two guests suggest complexes of "equivalent" structures and the same association constant. A possible explanation would be two diasteromeric complexes, due to the chirality of the β-CD, arising for example, from either a clockwise or a counterclockwise helical turning of the pheromone backbone imposed by the *cis*-double bond. Finally, in all samples examined, *free* pheromone was readily observed in the solution along with complexed, which afforded no correlation with the cavity protons (Fig. 2(b), right multiplet) and was preserved for more than a week. The above experiments suggest that for the guest of the title complex two bent, rather than extended structures, prevail in solution and the atoms near the double bond are tightly held within the β-CD cavity, whereas the ends, especially the carbonyl group, exhibit greater mobility, and the methyl group is located at the secondary side. It seems that the presence of free pheromone in solution is somehow stabilized by the presence of β-CD. The stoichiometry is primarily 2 : 1.

Spontaneous release of the guest molecule from the complex in the solid state

Dissolution of a freshly prepared solid complex in dimethyl sulfoxide and integration of the host and guest ^{1}H NMR signals yielded a 1 : 1 stoichiometry. The apparent contradiction of the results between the solution and solid phase was resolved when solid samples of the complex were examined by ^{1}H NMR over an extended period of time. It was found that the guest molecule was spontaneously released from the powder (Fig. 3)

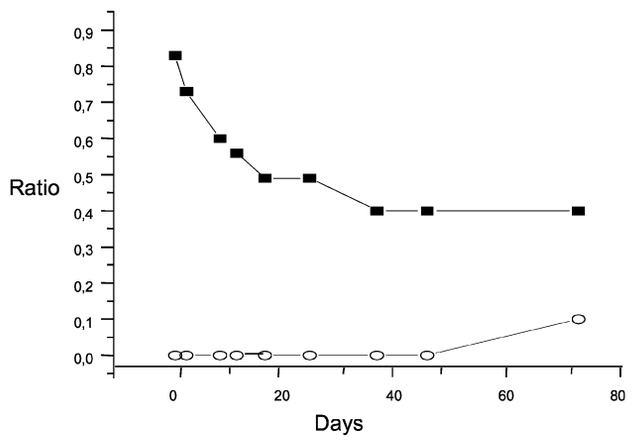

Fig. 3 Release profile of β-CD-(*Z*)-tetradec-7-en-1-al complex of initially 1 : 1 ratio. The complex was protected from photooxidation by a black plastic bag, permeable by the volatile pheromone; (■) [(*Z*)-tetradec-7-en-1-al] : [β-CD]; (○) [(*Z*)-tetradec-7-en-1-oic acid] : [(*Z*)-tetradec-7-en-1-al].

and, at the same time, part of it was oxidized into the corresponding acid.[7] In an attempt to investigate the rate of release under realistic (field) conditions, sealed black plastic bags (to eliminate photooxidation) containing samples of the 1 : 1 complex were placed outdoors at a 5–6 m height above ground. The contents were analysed at intervals by ^{1}H NMR. The remaining pheromone was measured as its corresponding ratio with β-CD. The formation of the oxidation product, (*Z*)-tetradec-7-en-1-oic acid, was also monitored (Fig. 3). We observed a decrease in the pheromone content, apparently lost to the surrounding air, up to about 40% in the first 40–45 days, without detectable oxidation. After that time, formation of some (*Z*)-tetradec-7-en-1-oic acid became visible, however the pheromone content did not vary significantly. This is in contrast to the same experiment conducted in transparent plastic bags where after 40 days ~40% was oxidised. This experiment shows that about half of the aldehyde is easily released and therefore has to be loosely held, whereas the other half does not escape and transforms into the acid very slowly. This latter half must then be stabilised by entrapment inside the β-CD cavity. The release results, therefore suggest two types of pheromone molecules.

Solid state NMR and FT–IR experiments

Solid-state NMR experiments were performed on freshly prepared polycrystalline complexes as well as aged samples (see Experimental section). Guest lines are easily observable and well structured (Fig. 4) alongside the strong β-CD lines (~62, 73, 81 and 104 ppm). The peaks that are most easily assigned and interpreted are the doubled aldehyde peaks in the carbonyl region (~200 ppm) and a triplet peak in the methyl region (~14 ppm), with intensities about 1 : 2 : 1. Therefore, there are two types of carbonyl groups but four types of methyl groups, two of them of identical frequency. The small splittings suggest that differences in the local environment are minor. The NMR

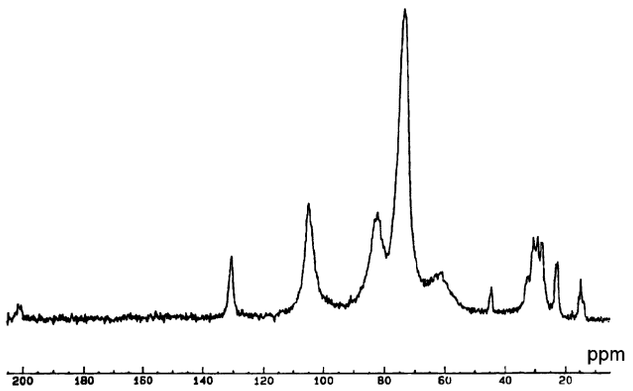

Fig. 4 ^{13}C CP-MAS solid state NMR spectrum of freshly prepared β-CD-(*Z*)-tetradec-7-en-1-al complex.

spectrum was repeated after the sample was "aged" and did not change remarkably. This suggests that the observed lines arise from guest molecules trapped in the cyclodextrin torus that are tightly held and resist oxidation. This would require the component that is easily lost to be essentially invisible in this NMR experiment and could be explained by the fact that the "outside" guest molecules have very different dynamic properties (frequencies and/or amplitude of motions) that make cross-polarization unfavourable. The "outside" guest molecules must be highly disordered, and the disorder may well be dynamic, thus reducing the dipolar couplings required for efficient cross-polarization.

In addition, one would also expect the "inside" and "outside" carbonyls to have very different chemical shifts (at least a few ppm different) because of the vastly different environments inside and outside the cyclodextrin cavity. The observed splitting of the guest lines must arise from slightly different local environments for the guests inside the CD channel.

The IR spectrum (Fig. 5) of a 1 : 1 complex of β-CD-(*Z*)-tetradec-7-en-1-al showed two weak but visible carbonyl peaks at 1726 and 1713 cm^{-1}. This indicates two different aldehyde molecules in the solid. The weakness of the peaks is justified if we consider that even for a 1 : 1 complex the total amount of pheromone is about 16% by weight. When the sample was aged the IR spectrum did not show the carbonyl peak at 1713 cm^{-1}.

The above experiments have thus shown that the 2 : 1 complex that initially prevails in solution, crystallises along with one more aldehyde molecule, enclathrated in the crystal lattice.

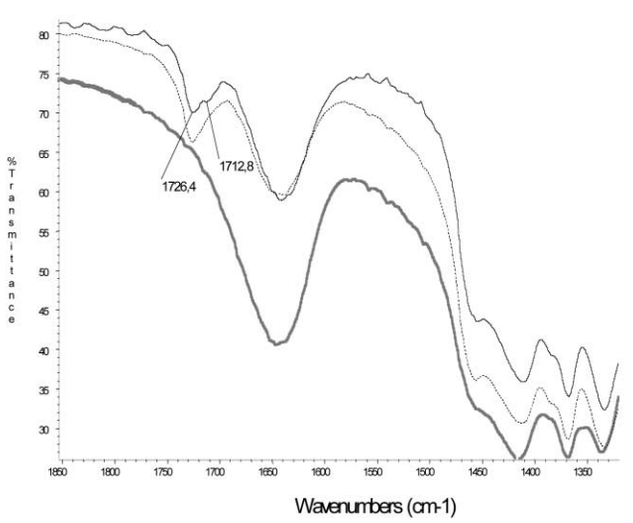

Fig. 5 Partial IR spectrum of β-CD (bottom trace), freshly prepared 1 : 1 complex of β-CD-(*Z*)-tetradec-7-en-1-al with two weak but visible carbonyl peaks (top trace) and an "aged" sample where the pheromone content had been lost up to 50% (middle trace).

Molecular structure of the β-CD-(*Z*)-tetradec-7-en-1-al complex

In order to correlate better the above behavior with the structure of the complex in the crystalline state, we undertook the crystal structure determination of the complex with high resolution X-ray data.

The complex crystallizes as a head-to-head dimer of two β-CD molecules, related by a pseudo 2-fold axis and labeled as A and B, that encloses one guest molecule disordered over two sites, of 50% occupancy each. As mentioned already, the electron density corresponding to the guest atoms as well as the thermal parameters indicate mobility of the guest. The numbering scheme for the host molecule is given in Fig. 6;

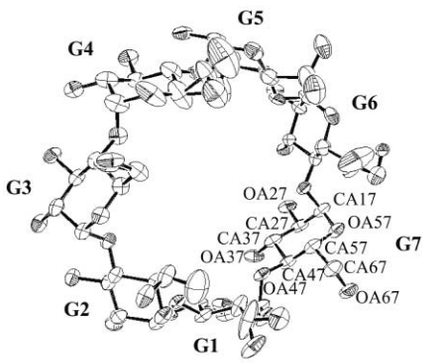

Fig. 6 ORTEP diagram of β-cyclodextrin, host A, showing the numbering scheme.

C(A or B)*mn* and O(A or B)*mn* denote the *m*th atom within the *n*th glucosidic residue (G*n*) of the crystallographically independent β-CD molecules A and B. The guest numbering scheme is shown in Fig. 1. Two pairs of additional sites of (*Z*)-tetradec-7-en-1-al molecules of low occupancy (10% each) are located in the crystal lattice, between β-CD dimers, the molecules of each pair are related also by a pseudo-crystallographic 2-fold axis (Fig. 7). Final atomic coordinates and thermal parameters of the structure have been deposited as a CIF file.

The guest molecule inside the cavity (disordered guest molecules a and b), unlike most of the aliphatic guests with 10–16 carbon atoms in β-CD complexes studied so far,[3,5–9] does not span the entire length of the β-CD dimer cavity. While the

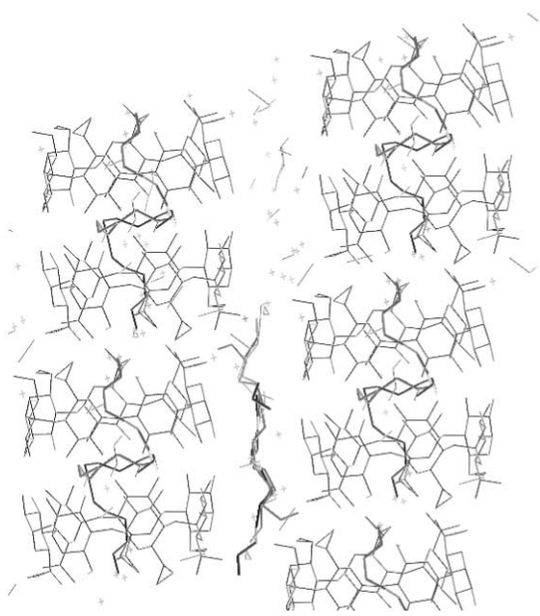

Fig. 7 The packing of the β-CD-(*Z*)-tetradec-7-en-1-al complex. Stick lines represent guest molecules. The four sites of the "outside" guest are shown by stick lines of varying width and shades of grey.

aldehyde groups of both disordered sites reside in the primary faces and the adjacent methylene groups of the aliphatic chains extend along the 7-fold axis of each β-CD monomer, the second half of the molecule curls and runs parallel to the cylindrical surface formed by the hydrogen-bonded secondary hydroxy groups of the β-CD monomers in the intradimer interface (Figs. 7 and 8(a)). The environment of the end groups of the

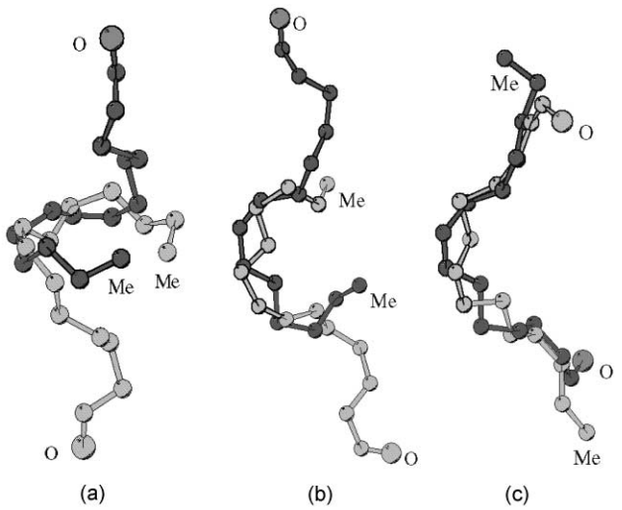

Fig. 8 Detailed view of the guest's conformation: (a) guests a and b (*inside* the β-CD cavity), (b) guests c and d (*outside* the cavity) and (c) guests e and f (*outside* the cavity).

disordered guest a and b does not differ significantly. Carbon atoms C(1) are found at slightly different distances from the host molecules ((C1a)–O(B66) = 4.0 Å, C(1b)–O(A66) = 3.8 Å) which can account for the observed difference in the position of the carbonyl resonances in the solid state NMR spectra. The only interaction of the carbonyl oxygen atom with the host is that with the inward pointing disordered atom O(66c) (O(a)–O(B66c) = 3.1 Å, O(b)–O(A66c) = 3.0 Å). The part of the alkyl chain of the guest molecules that curls in the middle of the β-CD dimer exhibits some short contacts between the calculated H-atoms of the guests and O-atoms of the hosts in the range 2.5–3.0 Å and 2.7–3.0 Å for guests a and b, respectively (directionality[17] of the interactions is good: C–H–O angles are in the range 125–168°). No interactions between the end methyl group and the host molecules are observed.

The existence of a guest molecule outside the cavity in the space between host molecules is uncommon in β-CD complexes. As it has been shown from the NMR studies, uncomplexed (*Z*)-tetradec-7-en-1-al is present in aqueous solution and it is stabilized in the presence of β-CD. Thus it is possible that an association existing in aqueous solution might have resulted in the entrapment of the aldehyde in the crystal lattice. This is also supported by the differences in the IR spectra of recently prepared and aged samples of the complex. Recently, it has been reported that in the complex of (3-*O*-acetyl-2,6-di-*O*-methyl)-β-CD with butyl acetate[18] one guest molecule is located in the intermolecular space between host molecules. The molecule of (*Z*)-tetradec-7-en-1-al outside the host cavity is disordered over four sites (guest molecules c,d,e,f) related in pairs by a 2-fold pseudo-crystallographic axis. One conformation is rather extended ((pair c and d; Fig. 8(b)), while in the other ((pair e, f; Fig. 8(c)) the aldehyde end of the molecule is bent. The aldehyde oxygen atom is hydrogen-bonded to the primary hydroxy groups pointing towards the exterior of the β-CD cavity, O_{guest}–OH host distances ranging between 2.6–2.9 Å. Some short distances observed between guest carbon atoms and the oxygen atoms of the hosts' secondary hydroxy groups, (average distance 3.0 Å), must be considered only indicative, because due to the low occupancy

Table 2 Direct hydrogen bonds between β-CD dimers (I, II): rows in bold indicate additional H-bonds not observed in isomorphous β-CD inclusion complexes where no disorder of the primary hydroxy groups is observed

I	II	$O_I \cdots O_{II}$/ Å	C–$O_I \cdots O_{II}$/°	$O_I \cdots O_{II}$–C/°
O(A25)	O(B27)[a]	2.740	113.0	105.2
O(A27)	O(B25)[b]	2.728	106.0	112.9
O(A63a)	O(A67)[c]	2.896	126.2	111.9
O(A63b)	**O(A67)**[d]	**2.739**	**133.6**	**114.4**
O(B63a)	O(B67)[d]	2.928	125.6	113.4
O(B63b)	**O(B67)**[e]	**2.738**	**132.8**	**113.4**
O(A63a)	O(B61a)[e]	2.857	95.1	110.8
O(A63a)	**O(B61b)**[c]	**2.895**	**125.1**	**104.6**
O(A61a)	O(B63a)[f]	2.651	116.0	108.8
O(A61a)	**O(B63b)**[b]	**2.848**	**112.9**	**96.8**
O(A61b)	**O(B63a)**[b]	**2.790**	**102.0**	**138.0**
O(A61b)	**O(B63b)**[b]	**2.894**	**104.6**	**124.6**
O(A64a)	O(B64a)[g]	2.708	108.4	107.4
O(A64a)	**O(B64b)**[a]	**2.772**	**101.0**	**94.0**
O(A64b)	**O(B64a)**[a]	**2.797**	**93.6**	**99.7**
O(A64b)	**O(B65b)**[a]	**2.547**	**128.9**	**116.5**
O(A65b)	**O(B64b)**[a]	**2.562**	**117.7**	**131.5**
O(A61b)	**O(B66b)**[a]	**2.765**	**114.6**	**111.3**
O(A66b)	**O(B61b)**[a]	**2.774**	**110.6**	**113.7**

[a] $x, 1-y, z$. [b] $1-x, y, z$. [c] $1+x, y, z$. [d] $x, 1-y, z$. [e] $1+x, y, 1+z$. [f] $x, 1+y, 1+z$. [g] $x, y, 1+z$.

and the fact that disordered water oxygen atoms occupy positions close to those of the external guest, the models of the guest molecules could not be refined well and some atoms have high temperature factors.

The β-CD dimers pack in the channel mode (Fig. 7).[2,19] Generally, the channels are held together with direct hydrogen bonds between the primary hydroxy groups of adjacent dimers along the channel (Table 2) and possibly between the guest molecules, as in the case of β-CD with aliphatic monocarboxylic acids with 12–16 carbon atoms.[5] In the title complex no interaction takes place between guest molecules of adjacent dimers, but the number of direct hydrogen bonds among the primary hydroxy groups is increased. It is rather unusual that in the present structure all but three primary hydroxys of the macrocycles are disordered exhibiting not only the usual (−)-*gauche* and (+)-*gauche* orientation pointing away from the β-CD cavity and towards it, respectively, but additional conformations as well (O(66) atoms of both monomers exhibit triple disorder with two conformations pointing to the outside and one to the inside of the cavity). Exceptions are atoms O(B62), O(A67) and O(B67) that have the outwards pointing conformation only. For the doubly disordered hydroxy groups the occupancy factors of the two orientations are often very close. An explanation of the extensive disorder at the primary faces might be that it allows for more H-bonds with the guest inside the cavity and between macrocycles along the channel as well. The latter will strengthen the attachment of the β-CD dimers, thus compensating for the lack of interactions between the guest molecules. Indeed, the direct H-bonds between the primary faces along the channels are not only the usual O(A64a)–O(B64a) ((+)-*gauche* orientation only)[7] but both orientations of hydroxys O(64) due to the additional disorder of the methylene carbon C(64), as well as between certain orientations of hydroxys O(61), O(65) and O(66) as indicated in Table 2.

There are 21.1 water molecules per β-CD dimer, distributed over 51 sites, only three of which are fully occupied and H-atoms were located for two of them. The rest are disordered therefore, it has been difficult to locate their corresponding hydrogen atoms. Water molecules, at 50 locations, form direct hydrogen bonds with the hydroxy groups of the macrocycle. The number of water sites is higher in the present structure

at 100 K, as compared with the isomorphous complex of the β-CD with aliphatic monocarboxylic acids determined at room temperature.[5,7] However, the water molecules found at the isomorphous RT structures were also present at low temperature, most of them disordered over more than two positions. The water molecules have been labeled according to the number of the closest hydroxylic oxygen atom to which they are H-bound. It is assumed that distances O–O(water) of 2.40–3.14 Å and angles C–O–O(water) of 95–139° indicate H-bonding. Nearly all water molecules are tetrahedrally coordinated and form hydrogen-bonding networks that involve, besides the hydroxy groups, other water molecules.

The glucose units of the host have the usual $^4C_1$ chair conformation and the cavity of the macrocycle is symmetrical due to the strong intramolecular hydrogen bonding between O3*n*–O2(*n* + 1) atoms (average distance 2.80 Å, range 2.73–2.84 Å). The deviations from planarity of the glucosidic oxygen atoms of the β-CD molecules are very small (0.001–0.038 Å). The dihedral angles between the glucosidic O4 plane and the planes formed by the atoms O(4*n* − 1), C(1*n*), C(4*n*) and O(4*n*) of the glucose units (tilting angles) are small, between 5.2–13.2°. The O(3*n*) oxygen atoms, which are not H-atom donors in the intramolecular hydrogen bonding process, are donors in the intermolecular hydrogen bonds between the O(A3*n*)–O(B3(8 − *n*)) hydroxy oxygen atoms that connect monomers A and B in order to form the dimers, as has been determined previously.[3] These intermolecular H-bonds have an average distance O(A3*n*)–O(B3(8 − *n*)) of 2.78 Å and angles O(A3*n*)–O(B3(8 − *n*))–C(3(8 − *n*)) and C(A3*n*)–O(A3*n*)–O(B3(8 − *n*)) of 117.3 and 117.3°, respectively.

Conclusions

This report examines in detail the complex formed between β-cyclodextrin and the sex pheromone of the olive pest *Prays oleae*, (*Z*)-tetradec-7-en-1-al, in aqueous solution and in the crystalline state and correlates its unusual structural features with the release behavior of the pheromone from the solid complex.

NMR studies in aqueous solution showed mainly a 2 : 1 host : guest stoichiometry. Two distinct but equivalent inclusion structures are present in solution with the methyl group located at the secondary side of the host. In both, the end groups of the guest exhibit great mobility, whereas the portions of the pheromone molecule near the *cis*-double bond are well localized in the cavity and the second half of the aliphatic chain (C9–C14) is curled. Free (*Z*)-tetradec-7-en-1-al is also persistently observed in aqueous solution, which has no correlation with the host's cavity.

In the crystalline state two β-CD molecules form head-to-head dimers that enclose one guest molecule disordered over two sites and packed in channels. Additional guest molecules enclathrated between β-CD hosts are heavily disordered (four sites of approximately 10% occupancy each). The molecular structure of the complex reveals two uncommon features for the guest molecule: (a) the methyl terminal part curls at the *cis* double bond and runs along the intradimer interface and (b) there is a guest molecule outside the cavity of the macrocycle. The guest inside the β-CD cavity exhibits mobility as indicated by its electron density map and the temperature factors. The crystal structure reveals also disorder for all but three primary hydroxy groups in the β-CD dimer which is unusual and is attributed to the effort by the host molecules to stabilize the disordered carbonyl moiety of the guest as well as to increase the number of interactions among the primary hydroxys along the channels. The solution NMR experiments gave, therefore a clue as to the mechanism of crystallization, that is the complex of the 2 : 1 host : guest ratio prevails in aqueous solution and crystallizes entrapping in the lattice one more aldehyde molecule existing free in solution. The entrapped "outside" pheromone is confirmed by the presence of an additional carbonyl peak in the IR spectra of the solid complex. Solid state NMR spectra, however, only detect the encapsulated pheromone inside the β-CD cavity and confirm the findings of the crystal structure of disordered guest, whereas the highly mobile and disordered "outside" guest is invisible.

The studied complex possesses the unique property of spontaneously releasing the guest molecule from the solid state. The release profile with respect to time showed that about half of the aldehyde is easily released and also easily oxidized, unless it is protected from the light by external means. The observed release behavior comes as a direct consequence of the molecular structure and can be readily understood in terms of the structural features described: the released pheromone is the one held loosely in the crystal lattice formed by the host molecules, whereas the molecules encapsulated in the cavity of the β-CD dimers are well stabilized.

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