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Inclusion complexes of cyclomaltoheptaose (β-cyclodextrin) and its methylated derivatives with the main components of the pheromone of the olive fruit fly

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ABSTRACT

The inclusion complexes of cyclomaltoheptaose (βCD) and heptakis(2,3,6-tri-*O*-methyl)cyclomaltoheptaose (TM-βCD) with the four major components of the pheromone of the olive fruit fly (*Dacus oleae*), namely 1,7-dioxaspiro[5.5]undecane, (−)-α-pinene, nonanal, and ethyl dodecanoate, and the complex of heptakis(2,6-di-*O*-methyl)cyclomaltoheptaose (DM-βCD) with 1,7-dioxaspiro[5.5]undecane were studied. The complexes were characterised in the solid state by differential scanning calorimetry and X-ray powder diffraction. In aqueous solution, the structure of the complexes was investigated by ^{1}H NMR spectroscopy. In solution, 1,7-dioxaspiro[5.5]undecane, (−)-α-pinene, and nonanal enter the cavity of the cyclo-oligosaccharides. Association constants for some of these complexes were also measured. The complexes of ethyl dodecanoate did not provide evidence of their structure in solution. This was attributed to the existence of negligible amounts of these complexes in water due to the combined effects of low solubility and low association constant.

INTRODUCTION

The cyclomalto-oligosaccharides (cyclodextrins, CDs), which form inclusion complexes with a variety of guest molecules, have been used as slow release dispensers for volatile or unstable biologically active compounds[1]. Previous work in this laboratory has shown[2,3] that complexation of 1,7-dioxaspiro[5.5]undecane (**1**) with cyclomaltohexaose, cyclomaltoheptaose, and cyclomalto-octaose (α-, β-, and γ-cyclodextrins, respectively) resulted in a dramatic decrease in the volatility of **1**, and consequently in control of the rate at which this pheromone component is released. The spiroketal **1** together with (−)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene [(−)-α-pinene] (**2**), nonanal (**3**), and ethyl dodecanoate (**4**) are the main components of the pheromone of the olive fruit fly (*Dacus oleae*), and are found in the ratios 3 : 1 : 0.3 : 1 in the natural pheromone extract[4,5]. The present work is focused

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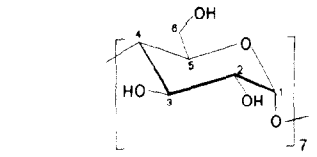
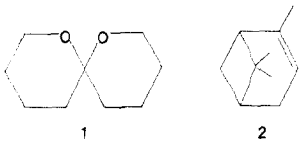


β-CD



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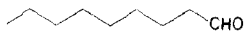

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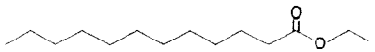

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on the complete characterisation of the structure and properties of the complexes of βCD with all pheromone components. The study also extends to inclusion complexes of heptakis(2,6-di-*O*-methyl)cyclomaltoheptaose (DM-βCD) with spiroketal **1** and heptakis(2,3,6-tri-*O*-methyl)cyclomaltoheptaose (TM-βCD) with **1**, **2**, **3**, and **4**.

EXPERIMENTAL

General.—β-Cyclodextrin was purchased from Fluka Chemie AG (water content, 10–13%) and was used as received. 1,7-Dioxaspiro[5.5]undecane was a gift from Vioryl (Kato Kifissia, Athens, Greece), heptakis(2,6-di-*O*-methyl)cyclomaltoheptaose, (−)α-pinene, nonanal, and ethyl dodecanoate were obtained from Aldrich Chemical Company, and heptakis(2,3,6-tri-*O*-methyl)cyclomaltoheptaose was kindly donated by Professor G. Tsoucaris (Universite de Paris SUD, Chatenay Malabry). A Varian 3300 Gas Chromatograph with a DBU capillary column connected to a Vista 401 integrator was employed. Thermograms were obtained using a Perkin–Elmer DSC 7 differential scanning calorimeter, using vented Al pans. Typical conditions were: temperature range, 50–250°C; scanning rate, 10°C/min; sample weight, 3–10 mg; baseline optimisation was performed before each run. X-ray diffraction photographs of powdered samples were taken on a Debye–Sherrer camera with CuK_α radiation. NMR spectra were recorded on a Bruker AC 250 spectrometer in 99.9% D_2O (Sigma Chemical Company). Typical conditions were 16 K data points with zero filling; sweep width, 1.5 kHz, giving a digital resolution of 0.18 Hz/point; pulse width, 2 μs (90° pulse 10.8 μs);

acquisition time, 2.7 s. Gaussian enhancement was used for the displayed spectra (GB = 0.2, LB = −1). The chemical shifts were related to the water peak at 4.8 ppm. For the measurement of the association constants, to 1 equiv of cyclodextrin in D_2O were sequentially added 0.2, 0.4, 0.6, 0.8, and 1 equiv of guest. The actual concentrations were 5.1 mM βCD for 1,7-dioxaspiro[5.5]undecane, 3.5 mM βCD for nonanal, 11.5 mM TM-βCD for 1,7-dioxaspiro[5.5]undecane, 10.6 mM TM-βCD for nonanal, and 11.8 mM TM-βCD for (−)-α-pinene. Spectra were recorded 15–30 min after each addition at constant temperature (308 K for βCD complexes and 298 K for all others). The measured $\Delta\delta$ values of cyclodextrin protons, the corresponding host–guest concentrations, and approximate values for K_{assoc} and $\Delta\delta_{compl}$ were the input for a computer program (COMPLEX) which calculated the association constants for each case using the SIMPLEX algorithm[6].

General procedure for the preparation of the complexes.—The cyclodextrin was suspended (βCD) or dissolved (DM-βCD and TM-βCD) in water and heated with stirring to 65°C. Three equiv of the liquid guest were added in portions to the clear solution (βCD) or suspension (DM-βCD and TM-βCD) and the whole was left to stir at that temperature for 30 min. The temperature was allowed to decrease slowly to room temperature, and the resulting suspension was left to stir overnight. The white solid was collected by vacuum filtration, air-dried for 1 day, and then further dried in a desiccator over P_2O_5 or under vacuum, depending on the complex.

GLC-determinations. — The complex (10 mg) was stirred in a 1:1 mixture of water and $CHCl_3$ (total 6 mL) for 2 h. The two layers were separated, the aqueous phase was washed with $CHCl_3$ (3 × 2 mL), and the $CHCl_3$ extracts were combined. To a measured volume of the dried ($MgSO_4$) organic solution was added the appropriate internal standard, and 1 μL of the mixture was injected into the chromatograph. The quantity of the guest was thus measured exactly. Each measurement is the average of at least four runs. Values deviating by more than 1% were rejected. For the determination of 1,7-dioxaspiro[5.5]undecane, the internal standard used was *cis*-5-decenyl acetate, and for the remaining guests, 1,7-dioxaspiro[5.5]undecane.

RESULTS AND DISCUSSION

Characterisation of the inclusion complexes in the solid state.—Differential scanning calorimetry (DSC) indicated true complexation. Thus, in all cases, the DSC thermograms of the complexes were different in the number and the position of peaks, compared to the DSC curves of both mechanical mixtures of CD–guest and of the parent compounds. A more reliable and widely used method for the characterisation of the complexes in the solid state is X-ray diffractometry of powdered samples. The diffraction diagrams showed that the reflection pattern of each complex was dramatically different from that of the parent cyclodextrin. The fact that all guests were liquids simplified the qualitative characterisation of the

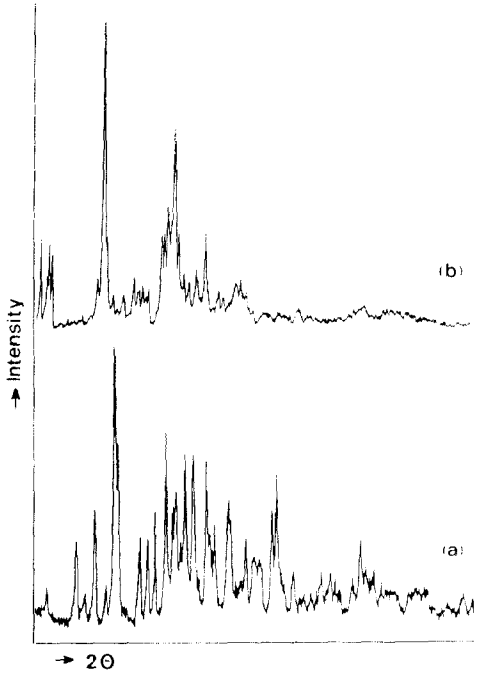

Fig. 1. X-ray powder diffraction diagrams of (a) βCD and (b) βCD–ethyl dodecanoate.

complexes by this method. Figs. 1 and 2 illustrate the diagrams of the complexes formed by ethyl dodecanoate, whose behaviour in solution is exceptional, as will be discussed below.

Stoichiometry of the inclusion complexes.—The percent content of included guest in each complex was measured by gas chromatography. A small amount of the completely dried complex (see Experimental) was stirred in water, and the guest was extracted with $CHCl_3$. The solution was injected into a GLC capillary column, and the amount of the guest in each complex was determined against a known quantity of a suitable internal standard. Table I shows the percent guest content and also compares these values with theoretical values calculated for 1 : 1 CD–guest complexes. It is evident that in all cases, except for the TM-βCD–ethyl dodecanoate complex (which was found to be in the ratio 1 : 2), the values found agree with those calculated. Some loss of the very volatile complexed molecules during their extraction and possibly some coprecipitation of the parent CD during isolation of the complex probably account for values consistently lower than the theoretical. Independent confirmation of the stoichiometry of the complexes was obtained from the integration of their ^1H NMR spectra in Me_2SO. In this manner, the complexes of nonanal, for which the GLC results were not reproducible, were also found to be 1 : 1.

Characterisation of the complexes in solution.—^1H NMR spectroscopy is commonly used for the investigation of the nature of the complexes in solution. It is

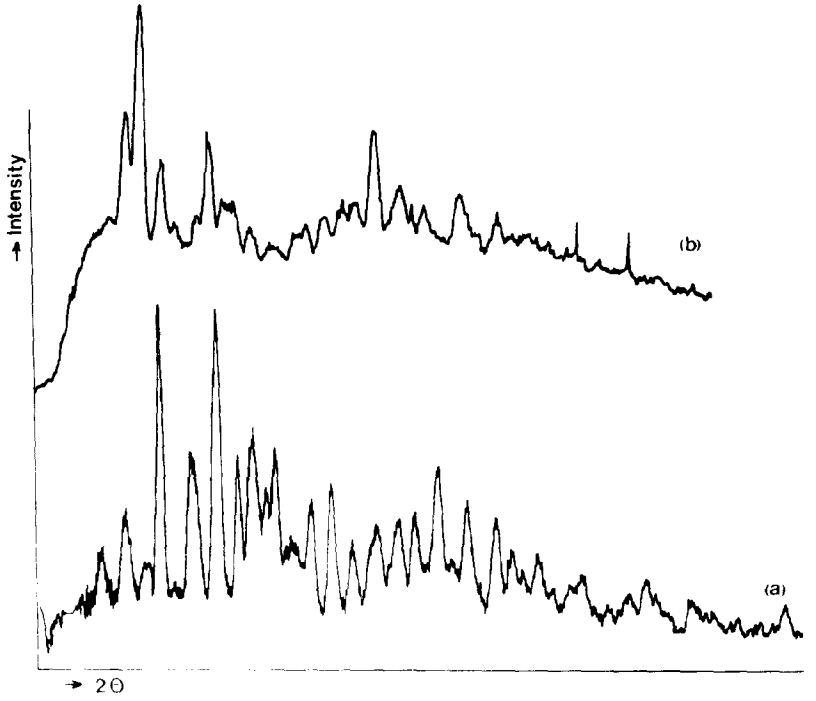

Fig. 2. X-ray powder diffraction diagram of (a) TM-βCD and (b) TM-βCD–ethyl dodecanoate.

not applicable, however, in cases where the complexes precipitate as soon as they are formed. For our soluble complexes, the observed resonances were the time-averaged peaks of pure and complexed βCD (fast exchange regime on the NMR time scale) at 298 K.

(a) Complexation with βCD. βCD has a torus shape formed by seven glucose residues, with a cavity[1] having a diameter of ~ 6 Å, which can accommodate a guest molecule without covalent bonding. Protons located inside the cavity (H-3 and H-5) are expected to experience large chemical shift variations upon inclusion of the guest, whereas those residing outside (H-1, H-2, and H-4) should undergo minimal changes. Thus, observation of the NMR spectra of the individual com-

TABLE I

Guest content (%) in the complexes (calculated/measured)

CD	Guest		
	1	**2**	**4**
βCD	12.1/9.2	10.8/7.5	16.7/19.8
DM-βCD	10.5/7.8	–	–
TM-βCD	9.8/7.6	8.7/8.8	13.8/30.9 [a]

[a] Indicates a 1:2 complex.

plexes and comparison with that of βCD provide clear answers about inclusion. Specifically, the ^{1}H NMR spectrum of βCD–**1** in D_2O showed significant upfield shifts of H-3 and H-5 (Table II), whereas the frequencies of H-1, H-2, and H-4 were not altered. Meanwhile, the frequency of the peaks due to spiroketal **1**, which are centred at 1.6 ppm, undergo a 0.15-ppm downfield shift accompanied by an expected change of appearance of the multiplet, compared to the spectrum of **1** in D_2O (limited solubility). Similar observations were made with βCD–nonanal, with the exception that H-4 undergoes a chemical shift variation larger than H-1 and H-2 (Table II). The resonances of nonanal move collectively downfield by 0.03 ppm (CHO) and 0.05 ppm (CH_3). The complex βCD–(−)-α-pinene is very insoluble in water, but the very small amount that dissolves showed a spectrum in which chemical shift variations were evident for H-3 and H-5 only. All these observations lead to the conclusion that 1,7-dioxaspiro[5.5]undecane, nonanal, and (−)-α-pinene all enter the βCD cavity. Since H-5 experiences larger shifts than H-3 (Table II), it is reasonable to deduce that the guests approach the narrower primary side of the cone, and therefore affect H-5 more. The fact that nonanal induces some shift change on H-4 indicates that this long molecule, having the aliphatic chain in the cavity, possibly extends out to the primary side, causing deshielding effects due to the carbonyl group on this proton. Similar NMR studies with ethyl dodecanoate did not show variation in the positions of the βCD proton signals. In an attempt to see the formation of a complex in situ, spectra were recorded 1, 2, and 18 h at 308 K after the addition of an excess of ester **4**, but still no shift changes were observed while a white solid (the complex) was eventually formed.

(b) Complexation with DM-βCD. Methylation of βCD at O-2 and O-6 practically does not affect the size and shape of the cavity. DM-βCD has the same conformation as βCD, because the hydrogen bonding between HO-3 and O-2 is not disrupted and thus the nearly circular shape of the structure is conserved[7]. On the other hand, dimethylation enhances the solubility of the cyclodextrin in water, which offers a major advantage for many applications. Preparation of very pure DM-βCD is not an easy task. Methylation with dimethyl sulphate, according to a modified procedure[7], gave a product which did not differ much from the commer-

TABLE II

Chemical shift variations (Δδ) in ppm of the cyclodextrin protons induced by the guests at 298 K

Complex	H-1	H-2	H-3	H-4	H-5
βCD–**1**	0.001	0.003	0.035	−0.002	0.053
βCD–**2**	0.002	−0.001	0.030	0.001	0.048
βCD–**3**	−0.002	0.003	0.073	−0.022	0.122
TM-βCD–**1**	0.021	0.021	0.034	0.022	[a]
TM-βCD–**2**	0.065	0.070	0.145	0.076	[a]
TM-βCD–**3**	0.023	0.024	0.052	0.021	[a]
DM-βCD–**1**	0.016	[a]	0.040	[a]	0.056

[a] Obscured by other peaks.

cial sample (see Experimental). It has been reported that this and other methods give a mixture of products[8] with a broad distribution of the degree of substitution[9]. For our purposes, we decided to use the preparation from Aldrich (98%). Under the circumstances, reliable measurement of the association constant was not feasible but the complex with spiroketal **1** was prepared. Its ^{1}H NMR spectrum and a series of experiments in which the spectra of solutions of DM-βCD in D_2O containing increasing concentrations of **1** were taken indicate that 1,7-dioxaspiro[5.5]undecane entered the cavity, since H-3 and H-5 moved significantly upfield (Table II).

(c) Complexation with TM-βCD. Permethylated β-cyclodextrin is soluble in water, and so are its inclusion complexes[1]. Its cavity is somewhat distorted from the circular shape, because of the steric hindrance caused by the methyl groups which results in higher flexibility of the macrocycle[10] compared to the rigid βCD. All TM-βCD complexes were soluble in D_2O at 298 K. For each complex, the ^{1}H NMR spectrum in a solution of D_2O was recorded and compared to that of TM-βCD. As shown in Table II, the guests induced upfield-shifting for all protons; the shift changes experienced by H-3 were, however, much more substantial. In the 250-MHz spectrum, H-5 is hidden under the H-6′ resonance; therefore, its exact position cannot be located. The resonances of spiroketal **1** underwent a small downfield-shifting upon inclusion, as with βCD. The methyl group signal of nonanal shifted downfield by 0.073 ppm, whereas the corresponding shifting for the aldehyde group was 0.020 ppm. Finally, all resonances of (−)-α-pinene experienced an approximately equal downfield shifting of $\sim$ 0.2 ppm. The conclusion is that the cavity has been occupied by the guests in all these cases. (−)-α-Pinene has a more compact structure and consequently a tighter fitting in the cavity, compared to 1,7-dioxaspiro[5.5]undecane, as judged from models, which could partially account for the larger shift effects induced by the former guest. On the other hand, nonanal seems to enter from the aliphatic side, leaving the carbonyl group on the outside. The fact that all CD protons undergo changes may be accounted for by the flexibility of TM-βCD. It is known[10,11] that, in the solid state, two of the trimethylglucose residues are tilted so that O-2 and O-3 point to the inside of the torus; in the other five residues, they point outwards. In solution, time-averaged resonances corresponding to only seven protons are observed and no differentiation of the glucose residues can be made, presumably due to fast internal motion of the individual residues. When a guest molecule is included in

TABLE III

Association constants of cyclodextrin complexes in water and calculated chemical shifts of the corresponding complexes

	βCD–**1** [a]	βCD–**3** [a]	TM-βCD–**2** [b]	TM-βCD–**3** [b]
$K(M^{-1})$	1020	2050	1500	730
δ_{compl}(H-3)	0.051	0.091	0.187	0.080

[a] Measured at 308 K. [b] Measured at 298 K.

the cavity, the above mentioned distortions of the conformation of TM-βCD most probably become amplified, so that all CD protons become exposed to the new environment, but the inner ones (H-3 and H-5) are most affected. As with βCD, the spectrum of TM-βCD–ethyl dodecanoate (**4**) did not show any differences compared to that of TM-βCD alone. As before, when an excess of **4** was added to a dilute solution of TM-βCD, no spectral changes were observed even after 2 days at room temperature and 1 h at 35°C, whereupon a few crystals appeared. Although the solubility of this complex is better than that of βCD–**4**, its association constant is probably low, and thus the percentage of the complexed ester in D_2O very small to result in any changes of the spectrum. This was confirmed by repeating the experiment, using a concentrated TM-βCD solution and excess of ester, which resulted in the formation of a bulky solid, without any changes in the spectrum.

Determination of association constants.—The equilibrium established after formation of a 1:1 complex and the associated binding constant are described by: CD + G = CDG and $K = [CDG]/[CD][G]$,

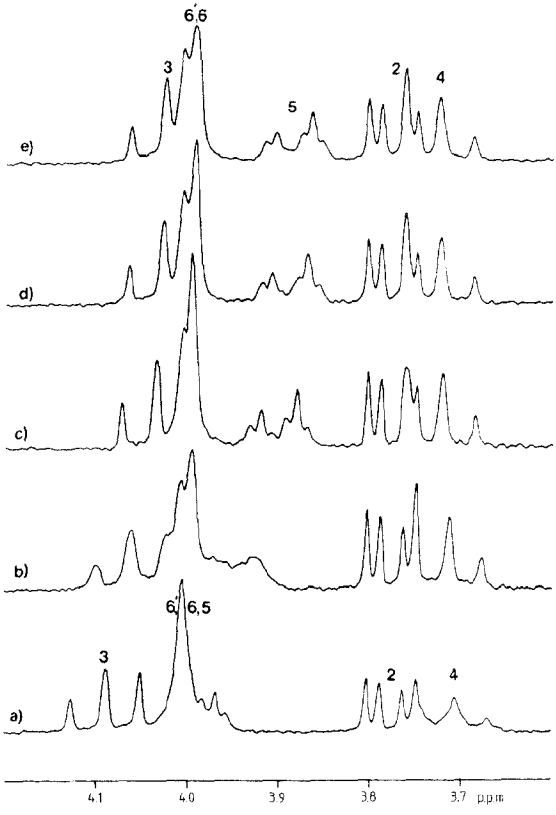

Fig. 3. Partial 250-MHz NMR spectra of (a) βCD, and βCD–nonanal in ratios (b) 5:2, (c) 5:3, (d) 5:4, and (e) 5:5.

where [CD] is the concentration of cyclodextrin, [G] is the concentration of the guest, and [CDG] is the concentration of their complex in the solution. Under conditions of fast exchange, the observed δ is given by:

$$\delta_{CD,obsd} = (\delta_{CD,compl}[CDG] + \delta_{CD,free}[CD])/([CD] + [CDG]).$$

Combination of all the above equations gives

$$[G] = \Delta\delta_{CD,obsd}/\Delta\delta_{CD,compl}\left([CD] + \left[K(1 - \Delta\delta_{CD,obsd}/\Delta\delta_{CD,compl})\right]^{-1}\right).$$

The curve-fitting computer method (COMPLEX) which was utilised works with reasonable estimates of $\Delta\delta_{CD,compl}$ and K, and the actual data for the total concentrations of host, guest, and $\Delta\delta_{CD,obsd}$. Several limitations were imposed by the experimental facts: (*a*) low solubility of the βCD complexes with **1** and **3**, largely circumvented by measuring at 308 K; (*b*) total precipitation of the βCD complex with **2**; (*c*) very low solubility of the guests in the aqueous medium; (*d*) generally small $\Delta\delta$ values, which were too small for accurate calculation of K in

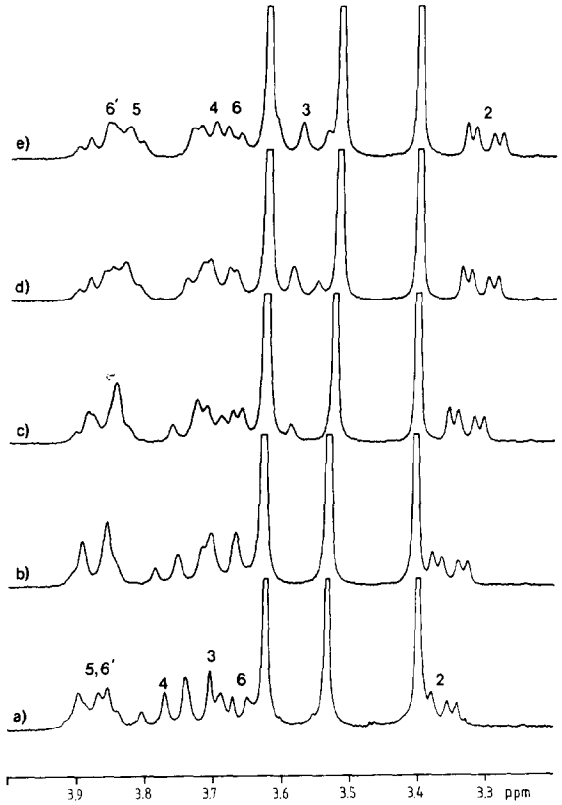

Fig. 4. Partial 250-MHz NMR spectra of (a) TM-βCD, and TM-βCD–(−)-α-pinene in ratios (b) 4:1, (c) 4:2, (d) 4:3, and (e) 4:4.

the case of TM-βCD–**1**. Even with these restrictions, NMR was the only method applicable in our case. Therefore, incremental addition of the guests to CD solutions (see Experimental) and recording of the spectra gave the data for estimation of the binding constants and also a clear picture of how the position of CD protons were affected by the sequential addition of the guest. Figs. 3 and 4 show representative examples. The values of K found in this manner are shown in Table III. For each calculation, the data from more than one proton were used and the results were averaged. Thus, TM-βCD forms a weaker complex with nonanal than βCD, whereas comparison is not possible for the remaining guests. In conclusion, all pheromone components **1–4** form inclusion complexes with βCD and its methylated derivatives, and could therefore be used as possibly more effective pheromone carriers for the control of the olive fruit fly population[2,3] than βCD–**1** alone.

ACKNOWLEDGEMENT

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