Positive or Adverse Effects of Methylation on the Inclusion Behavior of Cyclodextrins. A Comparative NMR Study Using Pheromone Constituents of the Olive Fruit Fly

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The complexation of α-, β-, γ-, tri-*O*-methyl-α-, tri-*O*-methyl-β-, and tri-*O*-methyl-γ-cyclodextrin (CD) with several constituents of the sex pheromone of the olive fruit fly, 1,7-dioxaspiro[5.5]undecane (**1**), nonanal (**2**), and ethyl dodecanoate (**3**), was studied in aqueous solution by NMR spectroscopy. Inclusion was clearly evident from the one-dimensional ^1H and ^{13}C spectra, whereas the proposed mutual disposition of the host and guest molecules was derived from two-dimensional ROESY spectra and the respective stoichiometries. The structures of the complexes in conjunction with their measured association constants allowed for comparison of the behavior of the cyclodextrins. The complexing ability was thus significantly enhanced by methylation in the case of α-CD, moderately reduced for β-CD, and largely diminished for γ-CD. The retention of the nearly symmetrical CD shape combined with the enlargement of the hydrophobic cavity by methylation is considered to be responsible for the properties of tri-*O*-methyl-α-CD. The increased cavity deformation upon enlargement of the cavity diameter, however, further supported by the skeletal distortions detected for the larger methylated macrocycles, overrules the beneficial effects of the cavity increase, resulting in poorer performance for tri-*O*-methyl-β-CD and an even poorer performance for tri-*O*-methyl-γ-CD. Moreover, the methylated cyclodextrins share common features in their inclusion behavior: all undergo conformational changes and all bind exclusively from their secondary side as opposed to normal cyclodextrins, which may use their primary side, too, depending on the guest.

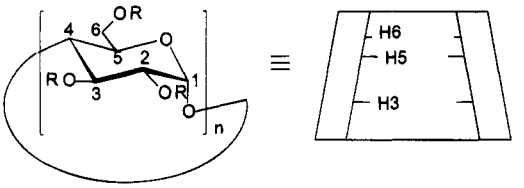
Introduction

Cyclodextrins (CDs) are cyclomaltooligosaccharides composed of six, seven, or eight D-glucopyranose units (α-, β-, γ-CD, respectively, Scheme 1).[1] Their hollow structure enables them to host a variety of molecules (guests) partially or entirely in their hydrophobic cavity.

These inclusion complexes exist both in aqueous solution and in the solid state. Permethylation of the hydroxyl groups of the natural CDs leads to the methylated derivatives, TMCDs, whose inclusion complexes possess enhanced solubility in water,[1] an important property for many applications.[1,2] Systematic investigations of the influence of permethylation of CDs on their inclusion properties are not known.[1] Increased complex stability has been observed, however, with TMα-CD[3] and reduced[3–5] or increased and reduced[6] stability with TMβ-CD, whereas TMγ-CD has not been studied. In the present work, the influence of methylation of CDs on the complex-forming ability in aqueous solution is investigated with the aid of structurally diverse guests. Racemic 1,7-dioxaspiro[5.5]undecane (**1**), an asymmetric molecule consisting of two perpendicularly disposed rings, and nonanal (**2**) and ethyl dodecanoate (**3**), both linear molecules, are used as guests (Scheme 2). These compounds, together with α-pinene (**4**), whose complexation with CDs is the subject of a separate study, are the major constituents of the natural sex pheromone blend of the olive fruit fly (*Dacus oleae*).

Previous work in this laboratory,[2] aimed at controlled release of the volatile substances **1**, **2**, and **4**, has demonstrated their inclusion into β-CD and TMβ-CD. For ester **3**, there had been no evidence for inclusion in

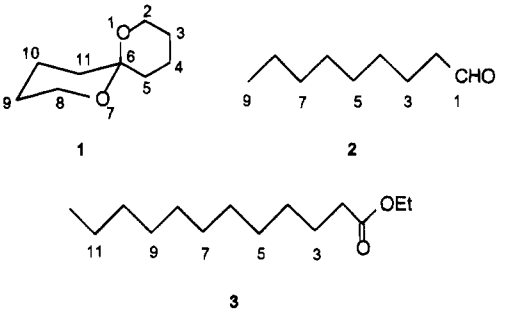
Scheme 1



n = 6, 7, 8

R = H or Me

Scheme 2



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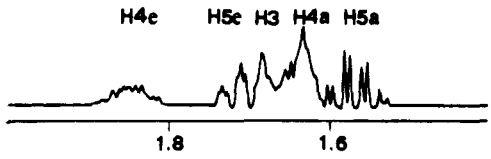

Figure 1. Partial ^1H NMR spectrum (600 MHz) of spiroacetal **1** in D_2O (20 mM) in the presence of TMα-CD (20 mM) at 298 K.

aqueous solution by ^1H NMR spectroscopy; however, formation of the complex was verified in the solid state by other techniques.[2] The present study further explores the inclusion processes of the natural CDs and the corresponding TMCDs in aqueous solution utilizing one-dimensional (1D) ^1H and ^{13}C NMR spectroscopy. The intermolecular proximity and the orientation of the guests are investigated with the aid of two-dimensional (2D) rotating frame NOE experiments (ROESY).

Results and Discussion

Modes of Inclusion from NMR Chemical Shifts. Insertion of a guest molecule into the hydrophobic cavity of a cyclodextrin results in the modification of the NMR frequencies of the signals of both the host and the guest. Observation of the NMR signals in the CD region in the presence of **1**–**3** supplied information on the nature of inclusion. Typically, the inner proton resonances (H3 and H5, Scheme 1) were mostly affected in the ^1H NMR spectra, whereas the induced skeletal changes were reflected mainly in the ^{13}C NMR spectra. On the contrary, observation of the guest signals in the presence of CDs was of qualitative value only, due to reduced solubility of molecules **1**–**3** in aqueous solution and much weaker signals (compared to CDs), owing to their low weight percentage (10–20% for 1:1 or 2:1 stoichiometries). In all cases studied, the ^1H and ^{13}C signals of the oligosaccharides follow the fast exchange condition, giving average shifts of complexed and free host in aqueous solution. Therefore, the values of the chemical shift changes ($\Delta\delta$) observed in a series of CDs upon inclusion of the same guest molecule reflect the degree of intermolecular proximity as well as the strength of association.

^1H NMR Spectra of the Guest Molecules. The assignment of the spectra of **1**–**3** is a prerequisite for a structural study of their complexes. Spiroacetal **1** has a complex ^1H NMR spectrum in D_2O with two groups of peaks around 3.7 and 1.7 ppm. The region at 3.7 ppm is totally or partially covered by the CD signals (depending on the CD) in the respective complexes; however, it can be safely ascribed to *H2(8)* (guest atoms denoted by italics). A dispersion of peaks of **1** due to complexation is generally observed in the presence of a cyclodextrin. This effect is most pronounced with TMα-CD, and for this complex, assignment of the signals around 1.7 ppm was carried out with HETCOR on the basis of reported ^{13}C assignments[7] (Figure 1).

In general, the resonances of **1** subject to the largest displacements upon complexation with the CDs were those arising from *H3(9)* and *H4(10)*, indicating end-type interaction of **1** with the cavity. The ^1H NMR spectra of nonanal and ethyl dodecanoate are similar, displaying the expected signals in the aliphatic region. In the presence of TMα-CD, all signals of **2** undergo displacements to higher frequencies with simultaneous dispersion of the group of methylene peaks, suggesting experience of a different environment along the chain. The largest $\Delta\delta$ values ($\Delta\delta = \delta_{free} - \delta_{obs}$, ppm) were observed for the groups *COH*, CH_3, and $C(8)H_2$ (0.150, 0.195, and 0.203 ppm, respectively). In the presence of all other CDs, $\Delta\delta$s were small and the methylene groups remained as one signal, whereas duplication of the methyl signal was observed, possibly due to two different inclusion modes of the methyl group. Ethyl dodecanoate (**3**) showed interaction (and measurable $\Delta\delta$s) only with TMα-CD, and the displacements observed were similar to nonanal, with the additional feature of slow exchange between complexed and uncomplexed guest.

^1H NMR Spectra of CDs. The ^1H NMR spectra of free TMCDs in aqueous solution have already been assigned in detail.[8] The assignments for TMCD complexes were all confirmed with COSY experiments. Table 1 summarizes the $\Delta\delta$s observed for the ^1H NMR spectra of the title CDs in the presence of **1**–**3**. We therefore observe that interaction of **1** with the CDs results in displacements of H3 and H5, a clear indication of inclusion. The values of the $\Delta\delta$s increase slightly from α-CD to β-CD but dramatically on going to γ-CD. Additionally, $\Delta\delta$s(H5) are larger than $\Delta\delta$s(H3) due to the rigid conical shape of CDs. Nonanal (**2**) with β-CD shows behavior similar to that shown above.[2] However, **2** and **3** cause atypical effects on the proton resonances of α-CD, the "outer" protons also being affected, indicating an interaction in solution not involving inclusion. No frequency variations were observed in the spectra of γ-CD in the presence of the linear molecules **2** or **3**, indicating the absence of complexes in solution.

In the chemical shift variations induced on TMCD protons by each guest, some general features can be identified. Thus, large shift changes of H3 and H5 verify inclusion, and $\Delta\delta$s(H3) greater than $\Delta\delta$(H5) indicate guest binding from the secondary side. Unlike normal CDs, the "external" protons of TMCDs are also affected but to a much lesser extent, suggesting that changes in the macrocyclic conformation of the hosts occur during inclusion. It is known that flexibility is gained by methylation of the hosts, owing to the disruption of the intramolecular hydrogen bonding responsible for the rigid shape of the parent CDs.[9] $\Delta\delta$ values decrease on going from TMα-CD to TMγ-CD, reaching 0 for the linear molecules **2** (with TMγ-CD) and **3** (with TMβ-CD[2] and TMγ-CD). Note that, in the whole series of hosts, inclusion in solution of **3** is observed with TMα-CD only (Figure 2).

The displacements of the TMα-CD protons observed with linear **2** and **3**, larger than those observed with the bulkier spiroacetal **1**, project the spatial requirements for inclusion in this cavity. Finally, the values of the coupling constants J_{56} and $J_{56'}$ do not change with inclusion, suggesting retention of the conformation about the C5–C6 bond, that is, two to three glucose units in the gauche–trans arrangement and the remaining units in the gauche–gauche arrangement.[8]

Summarizing, we have seen that in solution we observe inclusion complexes of **1**, the corresponding $\Delta\delta$ values of CDs increasing in the order α-CD < β-CD < γ-CD,

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Table 1. Complexation-Induced Chemical Shift Differences[a] of CD Protons in D_2O at 298 K

complex	H1	H2	H3	H4	H5	H6	H6′	Me2	Me3	Me6
α-CD/**1**	0.000	−0.010	−0.023	−0.008	0.042	0.000	0.000	–	–	–
α-CD/**2**	−0.013	0.015	−0.030	−0.054	0.007	*b*	*b*	–	–	–
α-CD/**3**	0.000	0.011	0.004	−0.011	0.005	*b*	*b*	–	–	–
β-CD/**1**	0.003	0.003	0.035	−0.003	0.053	*b*	*b*	–	–	–
β-CD/**2**	−0.002	0.003	0.073	−0.022	0.122	*b*	*b*	–	–	–
γ-CD/**1**	0.019	−0.004	0.101	0.006	0.119	*b*	*b*	–	–	–
TMα-CD/**1**	0.037	0.077	0.267	0.065	0.140	0.005	−0.030	0.019	0.000	0.016
TMα-CD/**2**	0.049	0.101	0.359	0.078	0.125	0.035	−0.030	0.032	0.033	0.017
TMα-CD/**3**	0.042	0.102	0.327	0.047	0.065	0.050	0.008	0.027	−0.051	0.017
TMβ-CD/**1**	0.038	0.027	0.073	0.040	0.042	0.000	−0.017	0.009	0.008	0.002
TMβ-CD/**2**	0.022	0.025	0.048	0.019	0.021	0.014	−0.007	0.005	0.004	0.001
TMγ-CD/**1**	0.014	0.007	0.022	0.007	0.015	*b*	*b*	0.005	−0.006	0.003

[a] The maximum shift changes were observed with a 2−3-fold excess of the guest, using the cyclodextrin solutions described in the Experimental Section. $\Delta\delta = \delta_{free} - \delta_{obs}$, ppm. [b] The chemical shifts of these protons could not be derived with sufficient accuracy; however, the shift displacements were negligible.

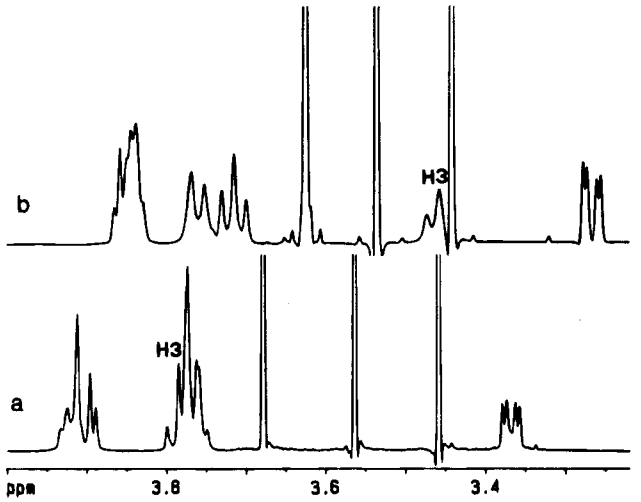

Figure 2. Partial ^1H NMR spectrum (600 MHz) in D_2O at 298 K of (a) TMα-CD (20 mM) and (b) TMα-CD/ethyl dodecanoate (20 mM/12 mM).

Table 2. Complexation-Induced Chemical Shift Differences[a] of CD Carbon Atoms in D_2O at 298 K

complex	C1	C2	C3	C4	C5	C6	Me2	Me3	Me6
α-CD/**1**	−0.25	−0.02	−0.11	−0.28	−0.09	0.02	–	–	–
α-CD/**2**	−0.56	−0.13	−0.47	−0.02	−0.03	0.49	–	–	–
β-CD/**1**	−0.27	−0.05	−0.20	−0.19	−0.18	0.13	–	–	–
β-CD/**2**	−0.24	−0.05	−0.20	0.06	−0.19	0.23	–	–	–
γ-CD/**1**	−0.37	−0.56	−0.20	−0.35	−0.17	0.08	–	–	–
TMα-CD/**1**	−1.09	−0.49	−0.34	−1.87	−0.17	0.22	0.30	−1.00	0.15
TMα-CD/**2**	−1.55	−0.82	−1.03	−2.31	−0.14	0.47	0.28	−1.38	0.15
TMα-CD/**3**	−1.56	−0.85	−0.96	−1.98	−0.18	0.58	0.24	−1.19	0.03
TMβ-CD/**1**	−0.78	−0.24	−0.02	−1.38	−0.17	0.10	0.02	−0.40	0.05
TMβ-CD/**2**	−0.38	−0.12	−0.04	−0.65	−0.13	0.10	0.02	−0.23	0.03
TMγ-CD/**1**	−0.25	0.03	−0.08	−0.49	−0.16	0.03	0.03	−0.32	0.03

[a] The maximum shift changes were observed with a 2−3-fold excess of the guest, using the CD solutions described in the Experimental Section. $\Delta\delta = \delta_{free} - \delta_{obs}$, ppm.

parallel to the increase of the cavity diameter. On the other hand, **2** complexes only with β-CD and **3** with none. Within the permethylated series, $\Delta\delta$ values increase in the opposite sense, TMγ-CD ≪ TMβ-CD < TMα-CD, for all guests. In general, among all CDs, TMα-CD has the largest shift displacements.

^{13}C NMR Spectra of CDs. The shift changes of the CD carbon atoms[8] upon interaction of the guests are shown in Table 2. The assignments for all complexes were confirmed with HETCOR experiments. Shifting of all signals to higher frequencies except for C6, Me2, and Me6 is observed for all complexes. The largest values are observed with TMα-CD.

The $\Delta\delta$s of normal CDs are small, being most significant for C1, C4, and C3. For β-CD/**1** and β-CD/**2**, shift changes of C5 and C6 are also observed, whereas for γ-CD/**1**, C2 is displaced appreciably and C5 moderately. The deshielding of C1 and C4, which are located at the interglucose linkages, suggests the occurrence of changes in the angle C1−O4−C4′. The other $\Delta\delta$s indicate that **1** approaches β-CD from both sides and α-CD and γ-CD from the secondary side, whereas **2** is totally enclosed in β-CD and simply approaches α-CD.

In the spectra of TMCDs, the magnitude of all $\Delta\delta$s follows the order TMα-CD > TMβ-CD > TMγ-CD. The largest displacements are uniformly observed for the resonances of C4, C1, and Me3, in that order, for all complexes studied, which constitutes a common and most prominent feature of TMCDs. The remarkable $\Delta\delta$s of C4 and C1 indicate sizable changes in the macrocyclic conformation. Apparently, serious reorganization of the host molecules takes place upon complexation, referring to probable tilting of some glucose units relative to their previous "free" state, allowed by the inherent flexibility of the host molecules.[9] Permethylation imposes stringent steric requirements on the secondary side of TMCDs which are relieved by rotation of Me3 so that it points to the interior of the cavity. The significant deshielding of Me3 on interaction with the guests is a definite sign of inclusion from the secondary side.

Concluding this section, we have seen from the ^{13}C shift changes that, unlike normal CDs, TMCDs behave similarly during inclusion, varying only in strength.

Stoichiometry of the Complexes and Association Constants. The host/guest ratio in each complex was determined using a combination of two independent methods: (i) dissolution of the prepared solid complex[2] in a solvent capable of dissolving both components, such as DMSO-d_6, and integration of the respective ^1H NMR signals; and (ii) titration of a cyclodextrin solution in D_2O with the neat guest and plotting of the resulting $\Delta\delta$s vs molar ratio[10] (Figure 3). The results are shown in Table 3. Both methods failed for TMγ-CD/**1**, which did not precipitate, and the observed $\Delta\delta$s of a titration run were very small. Method ii was not used for β-CD/**1** and β-CD/**2**, for which titration resulted in immediate formation of a precipitate.

The association constants (see the Experimental Section) for the 1:1 complexes were calculated by computer fitting of the $\Delta\delta$ values against concentration.[2] Such

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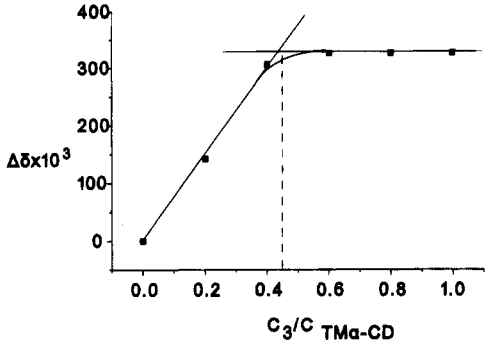

Figure 3. Diagram of $\Delta\delta$(H3) (ppm) of TMα-CD as a function of the mole ratio $C_3/C_{TM\alpha\text{-}CD}$.

Table 3. Stoichiometries and Association Constants (K) of Complexes in D_2O at 298 K

complex	stoichiometry	K_{assoc}
α-CD/**1**	2:1	small[a]
β-CD/**1**	1:1	1160 M^{-1}
γ-CD/**1**	1:1	560 M^{-1}
TMα-CD/**1**[b]	1:1	≅8000 M^{-1}
TMβ-CD/**1**	1:1	310 M^{-1}
α-CD/**2**	2:1	small[a]
β-CD/**2**	1:1	2380 M^{-1}
TMα-CD/**2**	2:1	10^7 M^{-2}
TMβ-CD/**2**	1:1	730 M^{-1}
α-CD/**3**	2:1	small[a]
TMα-CD/**3**	2:1	10^7 M^{-2}

[a] The large curvature of the mole ratio diagram suggests a low value of K. [b] A 2:1 complex may be present.

fitting was impossible with TMα-CD/**1**, suggesting the participation of other stoichiometries (such as 2:1) in solution. Mole ratio diagrams can be used when the maximum signal displacement corresponding to pure complex ($\Delta\delta_0$) can be measured, applicable only for the TMα-CD complexes. For TMα-CD/**1**, deviation from the 1:1 stoichiometry was implied since, from different experimental points, varied values of K were calculated, which, however, were high. In the plots of TMα-CD with **2** and **3**, the shallow break points (e.g Figure 3) did not permit the exact calculation of K_{assoc}.

Overall, in aqueous solution, TMα-CD associates very strongly with all guests (Table 3), which explains the large $\Delta\delta$ values measured for the complexes. On the other hand, β-CD complexes with **1** and **2** 3 times stronger than TMβ-CD. Lastly, the association of **1** is weaker with γ-CD than with β-CD; therefore, the large shift displacements measured in the former case are due to total inclusion, whereas in the latter case, the smaller shifts indicate that **1** is mostly outside the β-CD cavity. The significant contribution of hydrophobic interactions to the formation of the complexes is supported by the fact that complexation in other solvents (DMSO-d_6 and $CDCl_3$) was not observed.

2D ROESY Spectra. The intermolecular NOE data were obtained from ROESY experiments. The ROESY sequence[11] is preferred to the classical NOESY experiment, since, for cyclodextrins and their derivatives, NOEs are almost 0 at room temperature, owing to the fact that $\omega\tau_c \cong 1$.[12] In the ROESY spectra, artifacts which possess phase properties different than genuine NOE cross peaks

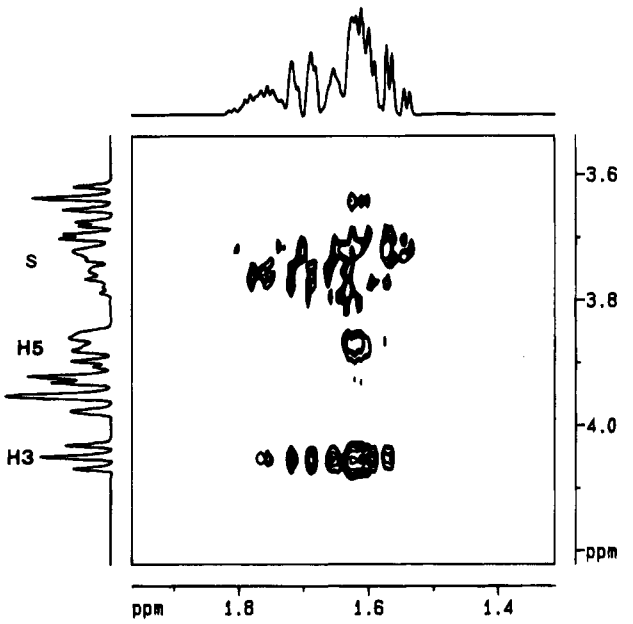

Figure 4. Partial contour plot of the ROESY spectrum of the α-CD/**1** complex in D_2O (7 mm/14 mM at 500 MHz); s = spiroacetal *H2(10)*.

are frequently observed between resonances within a common J-coupling network, thus being easily distinguishable.[13] Intermolecularly, as in the case of CD inclusion complexes, these COSY-type peaks are not observed; however, false cross peaks with the same phase as genuine interactions may arise due to a two-step HOHAHA and NOE pathway.[13] A good estimation of the extent of these artifacts can be made in the case of cyclodextrin inclusion complexes, where cross peaks which have no conformational reasons to exist appear between guest protons and CD H4 (Figures 4–6). They, therefore, arise mostly from scalar transfer from H3 and/or H5, these protons being the most prone to interact dipolarly with the guest.

Table 4 shows the dipolar correlations between CD protons (H) and guest protons *(H)*. For the sake of clarity in the description of the ROESY spectra, the numbering of only one spiroacetal ring is used. Thus, H3 of α-CD shows strong interactions with all spiroacetal protons and H5 with *H3* and/or $H4_{ax}$ (Figure 4). This suggests that the guest molecule is located at the secondary side with only a small part of it included. Therefore, taking into account the 2:1 stoichiometry, the structure shown in Scheme 3 is proposed, in compliance with the small shifts observed in the ^1H and ^{13}C NMR spectra.

In the complex of β-CD with **1**, correlation peaks are observed between H3, H5, and H6,6′ with *H3* and/or $H4_{ax}$ only (Figure 5), indicating that the guest is not deeply inserted into the cavity and that there is complexation from both sides (Scheme 3). This structure, too, is in line with the observed small shifts in the 1D spectra.

The next larger macrocycle is γ-CD with which spiroacetal shows strong correlations. Thus, there are interactions of H3 with all protons of **1** and of H5 with *H3* and/or $H4_{ax}$. In this complex, H6 is partially obscured by H3; therefore, we cannot be sure of dipolar interactions to

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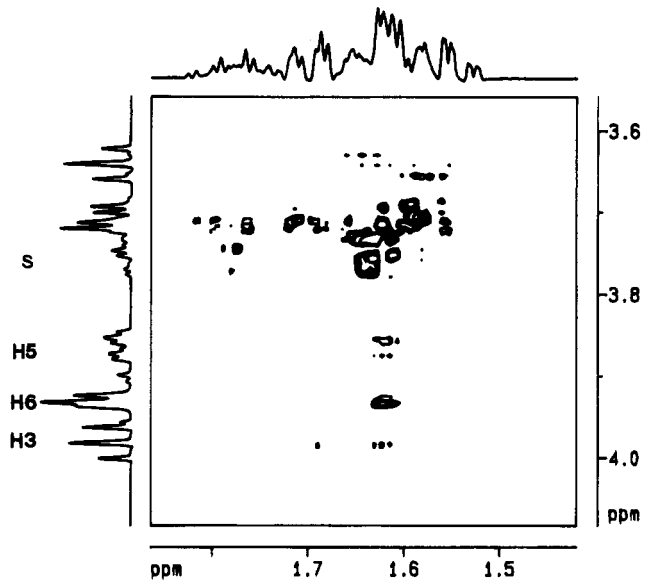


Figure 5. Partial contour plot of the ROESY spectrum of the β-CD/**1** complex in D_2O (2.5 mM/5 mM) at 500 MHz; s = spiroacetal *H2(10)*. The additional peaks of **1**, compared to Figure 1, could be due to enantiomeric discrimination, as has previously been observed.[14]

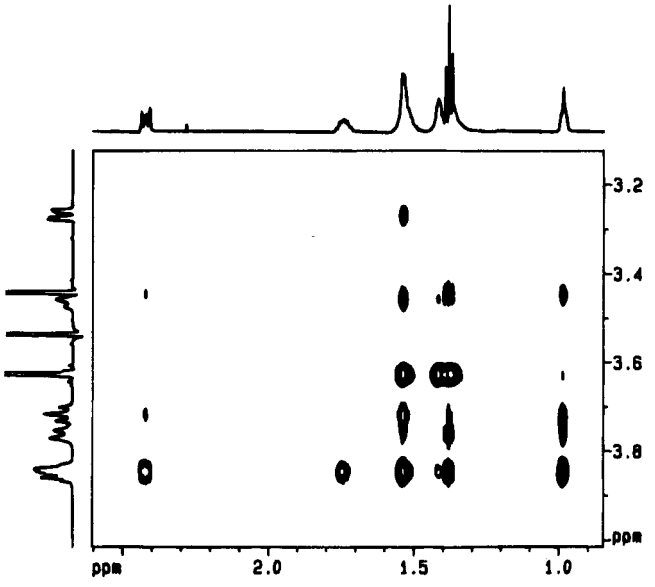


Figure 6. Partial contour plot of the ROESY spectrum of the TMα-CD/**3** complex in D_2O (20 mM/12 mM) at 500 MHz.

the protons at the primary side. The sizable ^1H NMR shift changes observed for this complex and the large γ-CD cavity diameter allow for a structure in which at least one ring of **1** is inside the macrocycle (Scheme 3), in agreement with the statement above that the large shifts are a consequence of total inclusion and not of a high *K*.

The most important dipolar interactions between **1** and TMα-CD are the correlations of Me3 with all guest protons and of H3 and H5 with only *H3* and/or $H4_{ax}$. A large part of **1** is therefore left outside the secondary side of the cavity (Scheme 3), and the very large shifts measured in the 1D spectra corroborate the suggestion that *K* is very high. Next, we observe strong dipolar interactions between all spiroacetal protons with Me3 (and not Me2 or Me6) and H3 and with the nearly overlapping signals of H5 and H6 of TMβ-CD. The guest, therefore, resides at the secondary side of the cavity of TMβ-CD (Scheme 3). The interactions, on the other hand, observed with protons of the primary side are attributed to the flexibility and the distortions of the macrocyclic skeleton of TMβ-CD and not to deep penetration, since, in the latter case, large shifts in the 1D spectra would be observed.

The basic features of the intermolecular rotating frame NOEs observed between TMα-CD and nonanal are the correllations of Me6 and H6 and H6′ with the terminal groups, whereas H3 and H5 correlate with almost the entire chain. Taking into account the 2:1 stoichiometry, an arrangement as in Scheme 4 is suggested for the complex, which accounts also for the observation that the group of methylene resonances of nonanal splits in the presence of TMα-CD (vide supra). A similar situation is observed for the TMα-CD/**3** complex, as shown in Figure 6, and consequently, a similar structure is proposed for this complex.

Between β-CD and nonanal, interactions of H3 and H5 with *Me(H9)* and most of the methylene groups in the chain are observed. H6,6′ shows the same correlation, with additional cross peaks with the β-methylene group *(H3)*, suggesting inclusion with the *CHO* group emerging from the primary side. Finally, for TMβ-CD/**2**, the CD protons (Table 4) correlate with the larger part of the aliphatic chain. The characteristic interaction of Me6 with *Me(H9)*, on the other hand, suggests inclusion in a direction opposite to that described above, that is, *CHO* exiting the secondary side (Scheme 4). Moreover, coiling of the backbone of nonanal inside the cavity or even insertion of the methyl end into a neighboring CD cannot be ruled out on the basis of the ROESY cross peaks.

Comparison of Host–Guest Interactions in CDs and TMCDs. Within the series of normal CDs, NMR data show that **1** does not fit into the α-CD cavity, its dimensions evidently being too small for this guest, and is loosely held in the cavity, as reflected by the small K_{assoc}, in spite of the 2:1 stoichiometry. The cavity of β-CD is larger, and although its internal available volume is theoretically capable of exact and complete inclusion,[15] **1** is not deeply inserted into it. This indicates that the inherent rigidity of β-CD is an obstacle to deep penetration, which would require some flexibility of the host. The suggested approach of **1**, on the other hand, from the primary side also is not surprising since double inclusion via different guest orientations has been proposed from X-ray structures of this[16] and other complexes.[17] The wider γ-CD allows for an easier accommodation of **1**, although it is not as tight. TMα-CD has the best complexing ability of all CDs. Therefore, **1** complexes more tightly with TMα-CD, although most of the molecule is not included. In the TMβ-CD cavity, on the other hand, in spite its deeper inclusion, **1** is only weakly complexed, whereas in TMγ-CD, it just approaches the secondary side. Methylation enlarges the cavity of the macrocycles, makes the environment around it hydrophobic, and allows for increased adaptability of the oligosaccharides toward a guest, through enhanced flexibility. These features work advantageously for TMα-

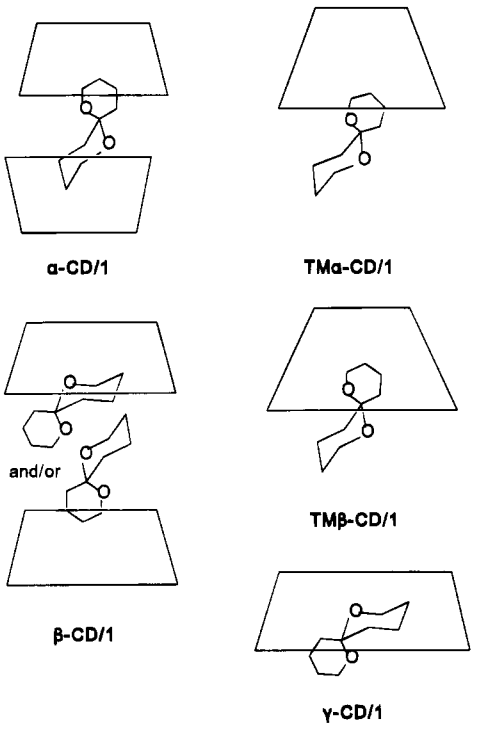
(15) Rysanek, N.; Le Bas, G.; Villain, F.; Tsoucaris, G. *Acta Crystallogr.* **1992**, *C48*, 1466.
(16) Rysanek, N.; Le Bas, G.; Villain, F.; Tsoucaris, G. Submitted for publication in *Acta Crystallogr.*
(17) Hamilton, J. A.; Sabesan, M. N. *Acta Crystallogr.* **1982**, *B38*, 3063.

Table 4. ROESY Cross Peaks in the Complexes

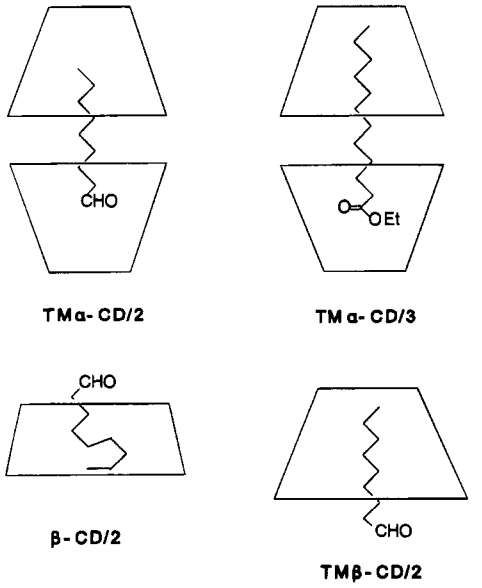
complex	CD protons				
	H3	H5	H6,6′	Me3	Me6
α-CD/**1**	all	*H3* and/or $H4_{ax}$	–	–	–
β-CD/**1**	*H3* and/or $H4_{ax}$	*H3* and/or $H4_{ax}$	*H3* and/or $H4_{ax}$	–	
γ-CD/**1**	all	*H3* and/or $H4_{ax}$	–[a]	–	–
TMα-CD/**1**	*H3* and/or $H4_{ax}$	*H3* and/or $H4_{ax}$	–	all	–
TMβ-CD/**1**	all	all	–[b]	all	–
TMα-CD/**2**	*H2* to *H9*[c]	*H2*, *H9*[c]	*H2*, *H9*	*H2* to *H9*	*H9*
β-CD/**2**[a]	*H4* to *H9*	*H4* to *H9*	*H3* to *H9*	–	–
TMβ-CD/**2**	*H4* to *H9*	*H4* to *H9*	*H4* to *H9*	*H4* to *H9*	*H9*
TMα-CD/**3**	*H3*, *H11*, *H12*, *EtO*	all	*H12*, *EtO*	*H4* to *H10*	*H12*, *Et*O

[a] Assignment uncertain due to partial overlapping of H6,6′ by H3. [b] Assignment uncertain due to overlapping of H3 by H6′ and H5 by H6. [c] *H3* to *H7* show weak interactions.

Scheme 3



Scheme 4



CD; however, their adverse effects are evident in TMβ-CD and, even more, in TMγ-CD, in which distortions of the macrocyclic skeleton are observed. NMR data in aqueous solutions have already indicated that severe changes in the macrocyclic conformation take place on going from TMα- to TMγ-CD.[8] Literature X-ray data of TMCD complexes show that TMα-CD largely retains the symmetrical shape of α-CD, having the O2−O3 side somewhat enlarged, whereas the O6 side is narrower.[9] These features are even stronger for TMβ-CD, which is also remarkably distorted from the regular shape of β-CD with significant tilting of the glucopyranose residues.[9] Crystallographic data are not available for TMγ-CD; however, serious deformation is anticipated in this case. The distorted structure is therefore responsible for the reduced complexing ability of TMβ-CD and (even more) TMγ-CD. Lastly, **1** enters TMCDs only from the secondary side.

Quite analogously, toward the linear guests **2** and **3**, TMα-CD has the strongest complexing ability for reasons such as those discussed above. The narrowing of its already limited primary side by methylation does not allow for deep insertion of the methylene or carbonyl groups and must be responsible for the 2:1 stoichiometry. These molecules form weak (Table 3) and rather insoluble complexes with α-CD, indicating that most of the chain is in contact with the bulk solvent. On the other hand, the large dimensions of γ- and TMγ-CD are inappropriate for such linear molecules since no stabilizing interactions can be established. The dimensions of β-CD and TMβ-CD are suitable for nonanal, but the complex with the latter is weaker. Again, the reduced complexing properties of TMβ-CD and the complete absence of such features for TMγ-CD can be attributed to their distorted shape but also to their large dimensions compared to the linear guests. Finally, TMα-CD complexes with **1**−**3** strongly and α-CD weakly, β- and TMβ-CD complex more strongly with **2** than with **1**, whereas for γ- and TMγ-CD, this order is reversed.

Conclusions

TMα-CD has the best complexing ability of all CDs studied. Among normal CDs, β-CD has better complexing properties than α- or γ-CD, due to its suitable dimensions for the guests examined. TMβ-CD complexes less strongly than β-CD, whereas TMγ-CD is able to complex very weakly with spiroacetal only. The above results lead to the conclusion that methylation makes TMα-CD the best host but reduces the complexing ability of TMβ-CD and reduces the complexing ability of TMγ-CD even more, due to the skeletal distortions. Moreover, all TMCDs complex from the secondary side, and all undergo large conformational changes of the macrocyclic skeleton during complexation, the above constituting a uniform behavior of TMCDs. The present results suggest

that increasing the solubility of the host molecule is not a sufficient prerequisite for improving the solubilizing properties of the natural CDs toward a certain guest. A combination of the possibly incompatible effects of solubility, affinity, and molecular adaptation should be considered for the selection of the optimal host dedicated to a given guest.

Experimental Section

Materials. The α- and γ-CDs were purchased from Jansen, β-CD was purchased from Fluka, TMCDs were purchased from CYCLOLAB (Hungary), and all were used as received, except for the NMR experiments at 500 and 600 MHz, for which lyophilization was carried out prior to the preparation of the sample. 1,7-Dioxaspiro[5.5]undecane and nonanal were obtained from Vioryl (Kato Kifissia, Athens, Greece), and ethyl dodecanoate was obtained from Sigma Chemical Co.

NMR Measurements. The 1D experiments were carried out and processed with Gaussian enhancement as reported previously[2] on either a Bruker AC 250 MHz or a Bruker AMX 500 or 600 MHz instrument. On the 250 MHz instrument, 2D COSY (60° pulse, digital resolution of 1−2 Hz/point) and HETCOR (digital resolution of 3−6 Hz/point) spectra were obtained using the standard Bruker software. The ROESY experiments were run on the 500 and 600 MHz instruments, utilizing the software supplied by Bruker, with a digital resolution of 0.6−1.2 Hz/point, a 300 ms spin-lock time, and a field strength of 3−4 kHz. In all 2D experiments, relaxation delays of 0.5−1 s were used.

Determination of Stoichiometry. Integration of the ^{1}H NMR signals of host/guest in DMSO-d_6 solutions was used for all complexes except for TMγ-CD/**1**. The complexes were prepared from aqueous solutions as described before.[2] The results were checked with the titration method, in which the concentrations of CDs in D_2O used were as follows: 20 and 10 mM α-CD with **1** and **2**, respectively; 12 mM γ-CD with **1**; 20 mM TMα-CD with each of **1**, **2**, and **3**; and 11.5 and 10.6 mM of TMβ-CD with **1** and **2**, respectively. The guests were added in successive 0.2 equiv until the observed chemical shift changes ($\Delta\delta_{H3}$ and $\Delta\delta_{H5}$) were negligible or a precipitate was formed. The mole ratios were 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 in all cases, whereas for TMβ-CD with **1** and **2**, TMα-CD with **1**, and γ-CD with **1**, addition exceeded 1 equiv, reaching 1.4 or even 2.4.

Calculation of Association Constants in D_2O. The data from the stoichiometry measurements were used. The concentrations of β-CD were 5 and 3.5 mM with **1** and **2**, respectively.

For 1:1 complex formation between A and B in solution A, and if a signal of A is observed by the NMR, the following equations can be written:

$$A + B \rightleftharpoons AB \qquad K = \frac{[AB]}{[A][B]}$$

$$C_A = [A] + [AB] \qquad C_B = [B] + [AB]$$

$$\alpha_0 = [A]/C_A \qquad \alpha_1 = [AB]/C_A \qquad \alpha_0 + \alpha_1 = 1$$

C_A and C_B being the total concentrations of A and B, respectively. If δ_A and δ_{AB} are the chemical shifts of the observed nucleus in sites A and AB, then, under fast exchange in the NMR time scale,

$$\delta = \alpha_0\delta_A + \alpha_1\delta_{AB} \quad \text{or} \quad \Delta\delta = \alpha_1\Delta\delta_0$$

$\Delta\delta$ being the observed chemical shift displacement of the signal of A and $\Delta\delta_0$ being the corresponding difference between pure A and pure complex AB. A combination of the above results in

$$[AB] = \frac{\Delta\delta}{\Delta\delta_0}C_A \qquad [A] = C_A - \frac{\Delta\delta}{\Delta\delta_0}C_A \quad \text{and} \quad [B] = C_B - \frac{\Delta\delta}{\Delta\delta_0}C_A$$

If $\Delta\delta_0$ is experimentally measured, then the concentrations of A, B, and AB and therefore K can be calculated (e.g. TMα-CD/**1**). In the general case,

$$\frac{1}{\Delta\delta} = \frac{1}{K(\Delta\delta_0 C_B - \Delta\delta C_A)} + \frac{1}{\Delta\delta_0}$$

If $\Delta\delta_0$ is unknown, the equation becomes nonlinear and is solved with the aid of a computer program. For the 1:1 complexes, the fitting program COMPLEX[2] was used.

The equations for 2:1 stoichiometry are derived taking the formation constant,

$$2A + B \rightleftharpoons A_2B \qquad K = \frac{[A_2B]}{[A]^2[B]}$$

When A is in excess and with the valid assumption that K is large (as indicated from the mole ratio diagrams in the cases of TMα-CD with **2** and **3**), the concentration [AB] may be considered negligible. In analogy to the 1:1 case, we can write

$$C_A = [A] + 2[A_2B] \qquad C_B = [B] + [A_2B]$$

$$\alpha_0 = [A]/C_A \qquad \alpha_2 = 2[A_2B]/C_A \quad \text{and} \quad \alpha_0 + \alpha_2 = 1$$

and under fast exchange,

$$\Delta\delta = \alpha_2\Delta\delta_0$$

which combined with the above gives

$$[A_2B] = \frac{\Delta\delta}{\Delta\delta_0}\frac{C_A}{2} \qquad [A] = C_A - \frac{\Delta\delta}{\Delta\delta_0}C_A \quad \text{and} \quad [B] = C_B - \frac{\Delta\delta}{\Delta\delta_0}\frac{C_A}{2}$$

Using the value of $\Delta\delta_0$ and substituting experimental $\Delta\delta$ values close to the break point, the concentrations [A], [B], and [A_2B] can be calculated and consequently so can K.

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