(+)- and (−)-α-Pinene as chiral recognition probes with natural cyclodextrins and their permethylated derivatives. An aqueous NMR study

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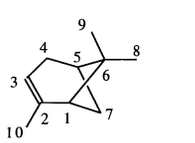
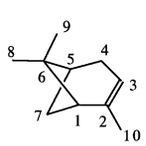
The binding of (1*R*)-(+)- and (1*S*)-(−)-α-pinene to α-, β- and γ-cyclodextrin and to the corresponding permethylated derivatives TMα-, TMβ- and TMγ-cyclodextrin, has been studied in aqueous solution by NMR spectroscopy. The stoichiometries and the association constants have been measured. The signals of both (+)- and (−)-α-pinene were found to follow the slow exchange, whereas those of the cyclodextrins the fast exchange regime, indicating that the type of exchange is independent of the magnitude of binding constants, which vary along the series, and also a result of other factors, in addition to the associated rate constants. The structures of the respective complexes have been derived from 2D ROESY experiments. The cyclodextrins preferentially bind with the (1*S*)-(−)-α-pinene, but only α-CD exhibits remarkable enantioselectivity. These observations show clearly that formation of a hydrogen bond or the presence of an aromatic ring, previously invoked as some of the requirements for enantioselectivity with CDs, are not necessary. In addition to enantioselectivity, site selectivity was also observed for α- and TMα-CD with the preferred (1*S*)-(−)-enantiomer, the stoichiometry being 2:1 in both cases.

Cyclodextrins (CDs) and their synthetic derivatives are cyclic oligosaccharides which form inclusion complexes with various molecules. Their complexing ability, combined with their natural chirality, renders them capable of enantiomeric discrimination, due to the formation of diastereomers from a racemic mixture of a guest. Stationary phases incorporating natural and derivatized CDs and mobile phases with dissolved CDs as modifiers have been recently applied for resolution of optical antipodes in chromatographic techniques.[1] The field is being investigated actively with respect to analytical applications, and GC columns have already become commercially available. In parallel, CDs have been used as chiral solvating agents for optical purity measurements in NMR spectroscopy.[2] Our previous work has shown that CDs can be used as chiral shift reagents in optical purity determination of α-pinene (**1**),[2d] due to discrete and concentration independent NMR signals of enantiomers upon complexation with several CDs, including α-CD, hexakis(2,3,6-tri-*O*-methyl)-α-CD (TMα-CD) and heptakis(2,3,6-tri-*O*-methyl)-β-CD (TMβ-CD).



(1*R*)-(+)-α-pinene



(1*S*)-(-)-α-pinene

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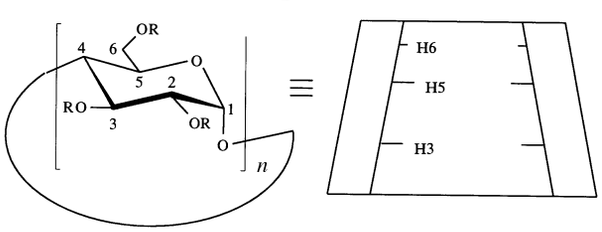


α–, β–, γ–CD; *n* = 6,7,8; R = H
TMα–, TMβ–, TMγ– CD; *n* = 6,7,8; R = Me

Scheme 1

Several requirements for chiral recognition have been proposed for β-CD[3] and also extended to α-CD complexes;[3c] however, these have been applied to highly functionalised molecules. With our non-polar guests, further work was necessary for a better understanding of the mechanism and the requirements for chiral recognition with CDs. Here, we investigate in detail the complexation process of the title CDs with enantiomeric (1*R*)-(+)-**1** and (1*S*)-(−)-**1**, the stability of the complexes formed and their possible structure in aqueous solution by ^{1}H and ^{13}C NMR and intermolecular NOE studies. Special attention is paid to binding constants as well as to exchange processes.

Results and discussion

In the following text, the atoms of the CDs will be denoted with plain characters (H, C) and those of **1** with italicized characters (*H*, *C*)

Variations of ^{1}H NMR signals of CDs with (+)- and (−)-1

Addition of either (+)- or (−)-α-pinene to aqueous solutions of cyclodextrins resulted in modification of their ^{1}H NMR spectra,[4] which were all assigned with the aid of 2D COSY experiments. Examination of the observed chemical shift changes (Table 1) gives a first indication of the nature of host–guest interactions. In the case of α-CD with either (+)- or (−)-α-pinene, the H2 resonance undergoes the largest shift to lower frequency while those of H3 and H5, protons located inside the cavity,[5] experience smaller shifts to higher and lower frequencies, respectively. The only notable difference between the two diastereoisomeric complexes is the larger shift of H2 with the (−)-enantiomer. The above indicate that in both complexes, the guest is situated mostly outside the secondary side of the cavity, with such an orientation that the double bond exerts deshielding effects on H3. With β- and γ-CD, both enantiomers induced displacement of the H3 and H5 resonances only, suggesting formation of inclusion complexes which were, however, both of

Table 1 Chemical shift changes ($\Delta\delta = \delta_{free} - \delta_{compl}$, ppm) of cyclodextrin protons in D_2O solution in the presence of (+)-**1** and (−)-**1** at 298 K

	α-CD		β-CD		TMα-CD		TMβ-CD	
	(+)-**1**	(−)-**1**	(+)-**1**	(−)-**1**	(+)-**1**	(−)-**1**	(+)-**1**	(−)-**1**
H1	0.013	0.017	0.002	0.002	0.010	0.011	0.084	0.081
H2	0.040	0.054	0.000	0.001	0.020	0.021	0.088	0.085
H3	−0.031	−0.030	0.020	0.030	0.071	0.077	0.177	0.174
H4	−0.009	−0.009	0.001	0.001	0.012	0.014	0.098	0.095
H5	0.027	0.035	0.040	0.048	0.030	0.033	0.068	0.064
H6a	−0.014	−0.015	*a*	*a*	0.003	0.003	0.008	0.005
H6b	−0.016	−0.019	*a*	*a*	−0.011	−0.012	−0.028	−0.025
Me2	—	—	—	—	0.006	0.006	0.031	0.028
Me3	—	—	—	—	0.008	0.008	0.007	0.005
Me6	—	—	—	—	0.003	0.003	0.011	0.008

[a] The chemical shifts of these protons could not be derived with sufficient accuracy; the shift displacements, however, were negligible.

Table 2 Chemical shift changes ($\Delta\delta = \delta_{free} - \delta_{compl}$, ppm) of cyclodextrin carbons in D_2O solution in the presence of (+)-**1** and (−)-**1** at 298 K

	α-CD		TMα-CD		TMβ-CD	
	(+)-**1**	(−)-**1**	(+)-**1**	(−)-**1**	(+)-**1**	(−)-**1**
C1	−0.33	−0.45	−0.24	−0.24	−1.39	−1.34
C2	−0.12	−0.17	−0.14	−0.14	−0.51	−0.50
C3	−0.11	−0.15	−0.12	−0.12	−0.34	−0.31
C4	0.01	0.01	−0.40	−0.40	−2.33	−2.15
C5	−0.14	−0.17	−0.01	−0.01	−0.22	−0.21
C6	0.04	0.05	0.07	0.07	0.21	0.15
Me2	—	—	0.06	0.06	0.01	0.02
Me3	—	—	−0.21	−0.21	−0.83	−0.78
Me6	—	—	0.02	0.02	0.12	−0.11

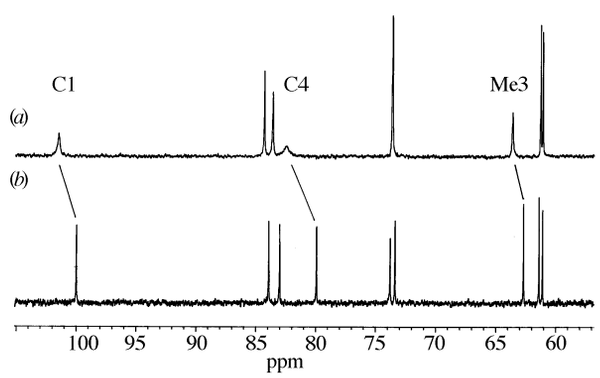


Fig. 1 Partial ^{13}C NMR spectrum (125 MHz) in D_2O at 298 K of (*a*) TMβ-CD (15 mM) and (*b*) TMβ-CD–(−)-α-pinene (15 mM–15 mM)

very low solubility (< 2 mM). The very small $\Delta\delta$ values observed in the case of γ-CD (0.011 and 0.014 ppm for H3 and H5, respectively, not included in Table 1) indicate very weak association. Examination of the ^1H NMR spectrum of TMα-CD in the presence of either enantiomer shows a large shielding of H3 and smaller of H5 and H2. Among the two methylene proton signals, only that of H6b, which points inwards,[4] has been slightly displaced to a higher frequency, for both guests. These observations indicate a partial inclusion of the guest from the secondary side. The interaction of either (−)-**1**[6] or (+)-**1** with TMβ-CD results in similar displacements of the oligosaccharide signals. Specifically, in both cases significant shielding of all signals is observed, except for H6b, which is deshielded. The larger dislocations experienced by the signals of H3 and H5, indicate inclusion in the TMβ-CD cavity. The shifting, on the other hand, observed for the signals of H1, H2 and H4, can be attributed to a distortion of the macrocyclic conformation upon inclusion, permitted by the lack of intramolecular hydrogen bonding in the host.[6,7] Finally, no chemical shift changes were observed in the spectrum of TMγ-CD in the presence of either (+)-**1** or (−)-**1**, indicating absence of complex formation.

Variations of ^{13}C NMR signals of CD with (+)- and (−)-1

The chemical shift variations of CD carbon signals[4] due to complexation (Table 2) usually reflect the induced skeletal changes of the oligosaccharides upon binding of the guest. Each resonance has been assigned using HETCOR experiments to avoid misinterpretation of the observed displacements. Small chemical shift changes were observed in the ^{13}C NMR spectrum of α-CD in the presence of each of the two enantiomers. The resonances of C4 and C6 were unaffected, while the remainder moved to higher frequencies, C1 undergoing the largest dislocation. The very low aqueous solubility of the corresponding adducts with β- and γ-CD did not allow for detailed studies in the solution. Each of the enantiomers induces the same kind of shift variations on TMα-CD and on TMβ-CD carbons, which differ only in amplitude, and are smaller for TMα-CD. All carbons experience deshielding, except for C6, Me2 and Me6 (Fig. 1). The shift changes are most significant for C1 and C4, indicating, as stated before,[7] a distortion of the macrocyclic ring and a possible tilting of some of the glucose units, more evident for TMβ-CD. Regarding the methoxy groups, only Me3, which points inwards, experiences a significant shift. Finally, no frequency variations were observed in the spectrum of TMγ-CD, upon addition of either enantiomer. All the above suggest that the two enantiomers are included partially in the cavity of α- and TMα-CD and entirely in the cavity of TMβ-CD.

Interestingly, strong broadening is observed for C4, C1 and, to a lesser extent, Me3 of TMβ-CD, upon association with each of the enantiomers at 298 K. However, with (−)-α-pinene, there is an additional reduction of intensity of the remaining carbons, except for Me2, which points outwards. As this reduction disappears when a relaxation delay of 1 s is inserted, it must arise from an increase of T_1 of the relative carbons and as a consequence, a decrease of their correlation times. This indicates that the internal motion in the pyranose ring of TMβ-CD becomes more rapid upon association with (−)-α-pinene. Increase of the T_1 of the carbons of TMβ-CD has also been observed in complexes formed with methyl orange,[8] while in the same study, the relaxation times of the carbons of α-, β-, γ- and DMβ-CD decreased. Since the broadening of C1, C4 and Me3 in both diastereomers did not disappear with the insertion of larger relaxation delay times (up to 4 s) it must result from intermediate exchange-rate conditions between slightly different conformations of the adducts. Attempts to reach the slow exchange regime by lowering the temperature and/or running the spectra at higher magnetic field resulted in disappearance of the signal of C4 and a slight splitting of the other two broad signals in both complexes. The above indicates that complexation of TMβ-CD with both enantiomers, breaks down the apparent seven-fold symmetry of the carbon skeleton of the oligosaccharide. This indicates a distortion of the macrocyclic conformation and points particularly to variations in the

Table 3 Chemical shifts (ppm) of α-pinene protons (δ_{free}) and the corresponding variations ($\Delta\delta = \delta_{free} - \delta_{compl}$) due to complexation with CDs in D_2O solution at 298 K

		Δδ					
		α-CD		TMα-CD		TMβ-CD	
	δ_{free}	(+)-**1**	(−)-**1**	(+)-**1**	(−)-**1**	(+)-**1**	(−)-**1**
H1	1.948	−0.246	−0.207	−0.196	−0.189	−0.139	−0.127
H3	5.188	−0.481	−0.423	−0.268	−0.269	−0.214	−0.192
H4	2.252	−0.325	−0.292	−0.073	−0.110	−0.121	−0.163
H4′	2.190	−0.157	−0.154	−0.062	−0.062	−0.156	−0.156
H5	2.093	−0.268	−0.331	−0.256	−0.255	−0.176	−0.221
H7	2.348	−0.355	−0.345	−0.208	−0.231	−0.206	−0.206
H7′	1.216	−0.317	−0.319	−0.071	−0.095	−0.121	−0.138
Me8	1.294	−0.317	−0.294	−0.188	−0.187	−0.161	−0.148
Me9	0.880	−0.356	−0.347	−0.201	−0.193	−0.155	−0.149
Me10	1.660	−0.302	−0.339	−0.190	−0.180	−0.144	−0.132

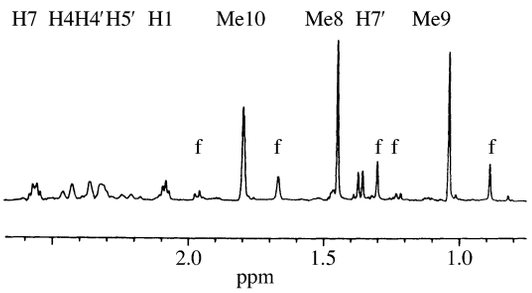


Fig. 2 Partial ^{1}H NMR spectrum (500 MHz) of (−)-α-pinene in D_2O (20 mM) in the presence of TMβ-CD (15 mM) at 298 K

glucosidic angle C1–O–C4′ as well as in the respective inter-glucose torsion angles, as has been reported previously.[7]

Variations of ^{1}H NMR signals of α-pinene with CDs

The ^{1}H NMR spectra of either (+)- or (−)-α-pinene in the presence of each CD showed, in addition to the signals of free **1**, new lines at higher frequencies for each proton due to complexed **1**. The positions of the signals of free and complexed **1** were concentration independent; therefore the slow exchange regime is encountered for the protons of α-pinene**.** As a consequence, the observed chemical shift differences Δδ (Table 3) between free and complexed species reflect directly only the degree of intermolecular proximity and not the magnitude of the binding constants. Assignment of the signals (Fig. 2) was carried out with COSY, DQS and HETCOR experiments and was in agreement with reported NMR parameters of **1** in other solvents.[9,10] The very low solubility of the corresponding adducts with β- and γ-CD did not allow studies of the guest in the solution, whereas with TMγ-CD complex formation was not detected, as mentioned above.

The largest shift variations of ^{1}H NMR signals of (+)-**1** or (−)-**1** were observed with α-CD, suggesting that the fit is very tight. In those complexes, the signals of *H3*, *H7*(*exo*) and *Me9* of **1** were most affected, while that of *H4′*(*endo*) underwent the smallest displacement. With TMα-CD, large shift changes were observed for all protons except for *H4*, *H4′* and *H7′*(*endo*), indicating that this part of the molecule is probably not included. Finally, the resonances of all protons of both enantiomers were subject to large displacements upon interaction with TMβ-CD, suggesting total inclusion in the cavity. For both enantiomers, the magnitude of the observed Δδ values follows the order α > TMα > TMβ implying a similar order for the intermolecular proximities between **1** and the respective hosts.

Determination of the stoichiometry

The mole ratio method[11] was used to derive the stoichiometry of the complexes of α-, TMα- and TMβ-CD. This method was applied for the signals of the CDs (fast exchange) and also for the signals of α-pinene (slow exchange). Thus, the concentration of CDs was held constant while the total guest concentration was varied. The induced chemical shift changes (Δδ) of the CD protons and/or the integrals of the signals of complexed **1** were plotted as a function of mole ratio.[7] Both Δδ and the respective integrals reached a maximum value when the stoichiometric point was just passed. The derived stoichiometry was 2:1 for the complexes with α- and TMα-CD and 1:1 with TMβ-CD, for both enantiomers (Table 4). Confirmation of these results was obtained by dissolution of the solid complexes in $(CD_3)_2SO$ and integration of the signals in the ^{1}H NMR spectra. This last method yielded 1:1 stoichiometry for both enantiomers with β- and γ-CD.

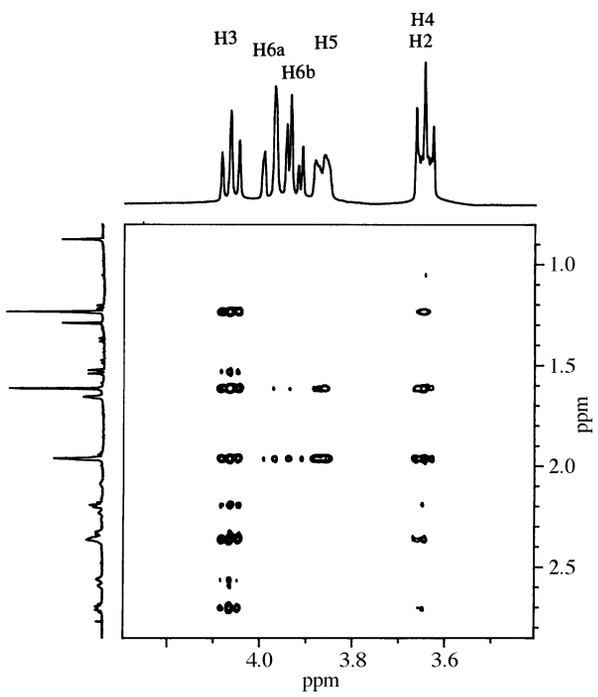


Fig. 3 Partial contour plot of the ROESY spectrum of α-CD–(+)-α-pinene (7 mM–7 mM) at 500 MHz. The cross-peaks observed with H2 and H4 are presumably due to transfer from H3 and H5.[7]

Intermolecular NOEs

Dipolar correlation spectroscopy in the rotating frame (ROESY)[12] was used in order to determine the spatial proximity between hosts and guests (Table 5). The dipolar interactions observed in the case of α-CD with either the (+)- or the (−)-enantiomer showed that *Me8* and *Me10* enter the cavity (cross peaks of both with H5) from the secondary side, since there are

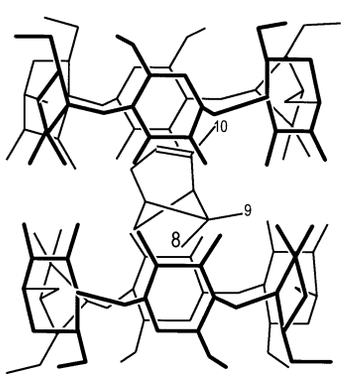
Table 4 Stoichiometries and association constants (K_{assoc}) for CD–**1** complexes in D_2O solution at 298 K

	α-CD		β-CD	γ-CD	TMα-CD		TMβ-CD	
	(+)-**1**	(−)-**1**	**1**[a]	**1**[a]	(+)-**1**	(−)-**1**	(+)-**1**	(−)-**1**
Stoichiometry	2:1	2:1	1:1	1:1	2:1	2:1	1:1	1:1
K_1/dm^3 mol^{-1}	2000	4500			350	520	1000	1300
K_2/dm^3 mol^{-1}	2000	3600			350	410	—	—
K_{assoc}	4×10^6	1.6×10^7	—	—	1.2×10^5	2.1×10^5	1×10^3	1.3×10^3

Table 5 ROESY cross-peaks between host (H) and guest (*H*) protons in the complexes in D_2O solution

	α-Pinene				
CD	α-CD–(+)-**1**	α-CD–(−)-**1**	TMα-CD–**1**[a]	TMβ-CD–(+)-**1**	TMβ-CD–(−)-**1**
H3	All	All except *H4*	*Me8–Me10*	All except *H7*	*Me8–Me10, H1, H3, H5*
H5	*Me8, Me10*	*Me8, Me10*	*Me8, Me10*	All	All except *H4, H4′, H7′*
H6a, H6b	*Me10*	*Me10*	—	*Me8–Me10, H4, H4′, H5, H7′*	*Me8–Me10*
Me2	—	—	—	*Me8–Me10*	*Me8–Me10*
Me3	—	—	All	All	All
Me6	—	—	—	*Me8–Me10, H4 H4′, H7′*	*Me8–Me10*

cross peaks between H3 and all α-pinene protons (Fig. 3). We can, therefore, identify two binding sites for **1** (*Me8* and *Me10*), since adverse steric interactions do not allow insertion of the entire molecule in the α-CD cavity. The only difference between the two diastereoisomeric adducts is the absence of cross peaks between *H4* and *H3* in α-CD–(−)-**1**. Considering the 2:1 stoichiometry, the realistically drawn structure shown in Scheme 2



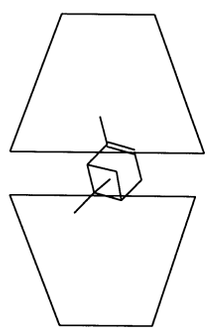
α-CD–(–)-**1**

Scheme 2

is proposed for both complexes. Most of the molecule remains outside the cavity, thus accounting for the large decrease of solubility relative to α-CD, and affects the H2 protons located close to the rim of the secondary side.

With TMα-CD, the two diastereomers gave the same dipolar correlation maps (Table 5). Cross peaks were observed between H3, H5 and *Me8*, *Me10* of either (+)- or (−)-**1**, whereas H6a and H6b gave no correlations. Here, also, we identify two binding sites, *Me8* and *Me10*. In addition, Me3 showed dipolar interactions with all pinene protons, suggesting inclusion from the secondary side. Despite the higher flexibility and the possibly wider secondary rim of TMα-CD, most of the molecule is outside the cavity. The two binding sites are inserted less deeply in TMα-CD than in α-CD, possibly due to steric hindrance of the methoxy groups. As a consequence, the fitting is not as tight as with α-CD. The loose binding indicates the much smaller association constants *K* (see below). Combination of the above data with the 2:1 stoichiometry of the complexes leads to the proposed structure in Scheme 3.

The correlation maps of the complexes of TMβ-CD confirm



TMα-CD–**1**

Scheme 3

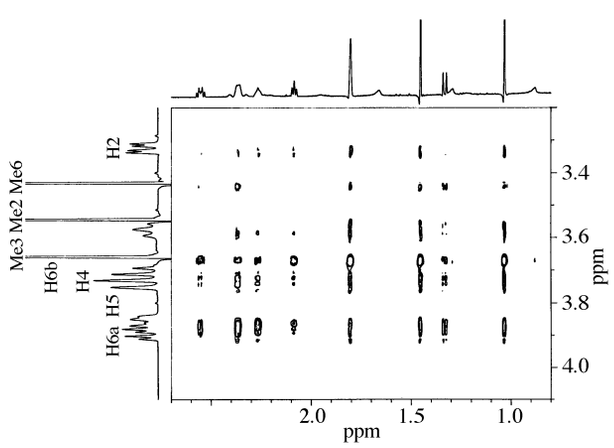
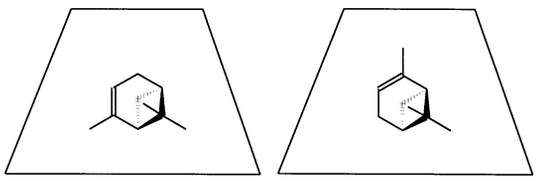


Fig. 4 Partial contour plot of the ROESY spectrum of TMβ-CD–(+)-α-pinene (15 mM–20 mM) at 500 MHz. The cross-peaks observed with H2 and H4 are presumably due to transfer from H3 and H5.[7]

that total inclusion of (+)- and (−)-**1** has occurred. The characteristic of these correlation maps is that the three methyl groups of α-pinene have cross peaks with all the protons of TMβ-CD (Fig. 4), suggesting that each enantiomer is included in more than one way. The geometry of the dominant structures (Scheme 4) of the two complexes can be elucidated by the extra cross peaks observed between *H4*, *4′* and *H7′* of (+)-α-pinene and H5, H6a, H6b and Me6 of TMβ-CD, which are absent in the case of (−)-α-pinene. These are simply static models of the two diastereomers; however they can be considered as averaged



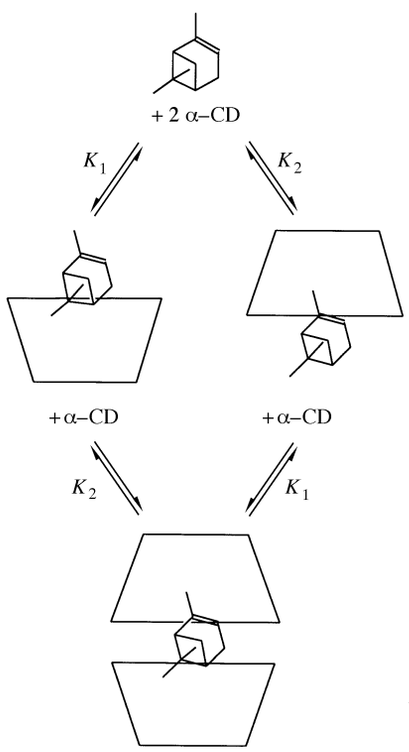
TMβ-CD–(+)-1 TMβ-CD–(−)-1

Scheme 4

representations of a number of mutually exchanging structures, as the NOE and the other NMR data suggest.

Association constants of the complexes

In all complexes, the slow exchange regime is encountered for the signals of (+)- and (−)-α-pinene, since two separate resonances in concentration-independent frequencies are observed for each guest proton. As the areas of the signals are proportional to the concentrations of the free and complexed species, the binding constants are calculated with the additional knowledge of the total concentrations[11] (Table 4). The above method was used (see Experimental) to derive the binding constants (K_1 and K_2, Scheme 5) of each binding site of α-pinene



Scheme 5

(*Me8* and *Me10*) with α- and TMα-CD. The association constant of TMβ-CD with (+)-α-pinene was also obtained using the same curve-fitting computer method (COMPLEX)[5] as with (−)-α-pinene.[6] This procedure requires observation of shift changes of protons in the fast exchange regime (TMβ-CD in our case) upon varying concentrations of host and/or guest. The *K* values are in accordance with the ratios of complexed (−)-**1**:(+)-**1** derived from the ^{1}H NMR signals of racemic α-pinene upon association with the same CDs reported previously[2d] (the ratios were 2, 1.10 and 1.05 for α-, TMα- and TMβ-CD, respectively). The values of binding constants reflect the enantioselectivity of these CDs which follows the order α > TMα > TMβ.

Enantioselectivity

α-Pinene does not fulfil some of the previously proposed requirements for chiral recognition by CDs, namely hydrogen bond formation during association and/or presence of an aromatic ring.[3] Few examples of enantioselectivity exhibited by CDs without the aid of hydrogen bonding have been reported,[13] in one case at least one aromatic ring being present.[13a] The present study shows that selective binding occurs in spite of the lack of functional groups in α-pinene, and suggests that steric factors dominate the observed enantioselectivity. In addition, in all cases CDs show a preference for (−)-α-pinene, indicating that the geometry of this enantiomer fits better in the asymmetric CD cavity. Furthermore, the fact that the two binding sites of the (−)-**1** have different values K_1 and K_2, while those for (+)-**1** are the same, supports the dependence of recognition on shape selectivity. Among the CDs studied, only α-CD exhibits a marked chiral recognition in aqueous solution, although most of the skeleton of α-pinene remains outside the cavity. The binding, however, is characterised by a very tight fitting, as the $\Delta\delta$ values indicate. In contrast, with TMα- and TMβ-CD, the fitting is not as tight. TMβ-CD showed reduced enantioselectivity, even though the two enantiomers are totally included. The high flexibility of TMβ-CD allows for changes of the macrocyclic conformation upon complexation, in order to better accommodate the guest in the cavity. Consequently, the non-selective binding of TMβ-CD with the two enantiomers can be attributed to flexibility, whereas the chiral recognition observed with α-CD can be related to its rigid structure. From all the above, tight fitting seems to be essential for achieving high enantioselectivity.

Chemical exchange

The exchange regime observed on the NMR timescale has been related to the binding constants of complex formation.[11,14] Specifically, it has been proposed that high values of K_{assoc}, as well as tight fitting, usually result in slow exchange conditions. In the present study, the exchange regime on the NMR timescale is slow for the α-pinene and fast for the CD signals, although K_{assoc} values vary among the complexes (Table 4). This leads to the conclusion that there is no direct relationship between the observed exchange rate conditions on the one hand, and the strength of association and the type of fitting, on the other.

Furthermore, the rate constants of the complex formation reactions have been considered responsible for the type of observed chemical exchange,[11,14] although others[15] have pointed out that the relation between NMR measured rate constants to those of the chemical process associated with the exchange is not straightforward. Indeed, if one assumes that the exchange regime is the result of the reaction rate constant (k), which is associated with the lifetime (τ) of a nucleus in the complexed state ($k = 1/\tau$), then the two complex forming molecules should belong to the same regime. The relationship, however, $\tau > \sqrt{2}/\pi\Delta\nu$ for the slow exchange results in $\tau \gg 0.015$ s, a value derived from the signals of **1** at 250 MHz, but for the fast exchange, where the inequality reverses, $\tau \ll 0.005$ s, derived from the CD signals, even at 500 MHz. Our observations lie in the spirit of the recently published work by Green and coworkers[15] that the rate constants measured by NMR methods differ from those of the chemical process giving rise to the exchange.

Experimental

Materials

The α- and γ-CDs were purchased from Jansen, β-CD from Fluka, and TM-CDs from CYCLOLAB (Hungary) and 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. (+)- and (−)-α-Pinene were obtained from Sigma Chemical Company, and were used without purification.

NMR measurements

The 1D experiments were carried out in unbuffered D_2O solutions and processed with Gaussian enhancement as reported previously[6,7] on either a Bruker AC 250 MHz, or Bruker AMX 500 or 600 MHz instruments. 2D COSY (60° pulse, digital resolution 1–2 Hz/pt in *F*2) and HETCOR (digital resolution 3–6 Hz/pt in *F*2) spectra were carried out on the 250 MHz instrument. The ROESY experiments were run on the 500 and 600 MHz instruments, using the standard Bruker software as described before.[7]

Determination of stoichiometry

In the titration method[7] the solutions of CDs in D_2O were used of the following concentrations: 7 mM of α-CD; 10 mM of TMα-CD; 15 mM of TMβ-CD. The neat (+)-**1** or (−)-**1** was added in successive 0.2 equiv. until the observed chemical shift changes ($\Delta\delta_{H3}$ and $\Delta\delta_{H5}$) were negligible and the integrals of complexed **1** were constant or a precipitate was formed. The mole ratios were 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, in all cases and additionally 1.6 for TMβ-CD. The results were verified by integration of the ^{1}H NMR signals of host/guest in $(CD_3)_2SO$ solutions of the prepared complexes.[6] This method was also used for the complexes of β- and γ-CD, the very low solubility of which did not allow use of the titration method. The preparation of the complexes was carried out in aqueous solutions as described before.[6]

Calculation of association constants

Slow exchange would normally allow calculation of K_{assoc} by simple integration of the signals due to complexed and free species, but here the situation was more complicated. The stoichiometries of α-CD–**1** and TMα-CD–**1** are both 2:1, and *Me8* and *Me10* had been identified as the different binding sites of each complex. One can calculate K_1 and K_2 for each site, if it is assumed that the two sites bind independently. By denoting the host CDs as H and the associated molecule as XY, X and Y being the two binding sites, we derive eqns. (1) and (2).

$$H + XY \rightleftharpoons HXY, \qquad K_1 = \frac{[HXY]}{[H][XY]} \qquad (1)$$

$$H + YX \rightleftharpoons HYX, \qquad K_2 = \frac{[HYX]}{[H][YX]} \qquad (2)$$

If C_{CD} and C_1 are the total concentrations of CDs and **1**, then we have eqn. (3).

$$C_{CD} = [H] + [HXY] + [HYX], \text{ and}$$
$$C_1 = [XY] + [HXY] = [YX] + [HYX] \qquad (3)$$

Integration of the signals of *Me8* in free and bound states gives a ratio I_{xy}, and of those of *Me10* gives I_{yx}. Then, eqn. (4)

$$\frac{C_1 - [HXY]}{[HXY]} = I_{xy} \text{ and } \frac{C_1 - [HYX]}{[HYX]} = I_{yx} \qquad (4)$$

holds from which [HXY] and [HYX] were calculated and therefore [H], [XY] and [YX] and finally K_1 and K_2. The association constants of 1:1 complexes of TMβ-CD were measured as described previously[6] using the specific concentrations described in the stoichiometry determination (see above). The final association constants were the mean values of three measurements.

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References

1 (*a*) M. L. Hilton and D. W. Armstrong, in *New trends in Cyclodextrins and Derivatives*, ed. D. Duchêne, Editions de Santé, Paris, 1991, ch. 15, pp. 517–549; (*b*) W. A. Köning, in *New trends in Cyclodextrins and Derivatives*, ed. D. Duchêne, Editions de Santé, Paris, 1991, ch. 16, pp. 551–594.
2 (*a*) K. Uekama, T. Imai, F. Hirayama, M. Otagiri, T. Hibi and M. Yamasaki, *Chem. Lett.*, 1985, 61; (*b*) D. Greatbanks and R. Pickford, *Magn. Reson. Chem.*, 1987, **25**, 208; (*c*) A. Casy and A. Mercer, *Magn. Reson. Chem.*, 1988, **26**, 765; (*c*) A. Botsi, K. Yannakopoulou, E. Hadjoudis and B. Perly, *J. Chem. Soc., Chem. Commun.*, 1993, 1085.
3 (*a*) D. W. Armstrong, T. J. Ward, R. D. Armstrong and T. E. Beesley, *Science*, 1986, **232**, 1132; (*b*) J. A. Hamilton and L. Chen, *J. Am. Chem. Soc.*, 1988, **110**, 5833; (*c*) K. B. Lipkowitz, S. Raghothama and J. Yang, *J. Am. Chem. Soc.*, 1992, **114**, 1554.
4 A. Botsi, K. Yannakopoulou, E. Hadjoudis and B. Perly, *Magn. Reson. Chem.*, 1996, **34**, 419.
5 F. Djedaini and B. Perly, in *New trends in Cyclodextrins and Derivatives*, ed. D. Duchêne, Editions de Santé, Paris, 1991, ch. 6, pp. 215–246.
6 A. Botsi, K. Yannakopoulou and E. Hadjoudis, *Carbohydr. Res.*, 1993, **241**, 37.
7 A. Botsi, K. Yannakopoulou, E. Hadjoudis and B. Perly, *J. Org. Chem.*, 1995, **60**, 4017.
8 M. Suzuki, J. Szjetli and L. Szente, *Carbohydr. Res.*, 1989, **192**, 61.
9 R. J. Abraham, M. A. Cooper, J. R. Salmon and D. Whittaker, *Org. Magn. Reson.* (*Magn. Reson. Chem.*), 1972, **4**, 489.
10 K. Laihia, E. Kolehmainen, P. Malkavaara, J. Korvola, P. Monttori and R. Kauppinen, *Magn. Reson. Chem.*, 1992, **30**, 754.
11 K. Connors, *Binding Constants*, Wiley, New York, 1987.
12 A. Bothner-By, R. Stephens, J. Lee, C. Warren and R. Jeanloz, *J. Am. Chem. Soc.*, 1984, **106**, 811.
13 (*a*) K. Kano, M. Tashumi and S. Hashimoto, *J. Org. Chem.*, 1991, **56**, 6579; (*b*) N. Rysanek, G. Le Bas, F. Villain and G. Tsoukaris, *Acta Crystallogr., Sect. C*, 1992, **48**, 1466.
14 J. Feeney, J. G. Batchelor, J. P. Albrand and G. C. K. Roberts, *J. Magn. Reson.*, 1979, **33**, 519.
15 M. L. H. Green, L. Wong and A. Sella, *Organometallics*, 1992, **11**, 2660.

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