Journal of Inclusion Phenomena and Molecular Recognition in Chemistry **26:** 209–218, 1996.

^{1}H NMR Spectroscopic Study of the Interaction Between Cyclodextrins and Bicyclo[3.3.1]nonanes#

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(Received: 3 January 1996; in final form: 24 May 1996)

Abstract. The formation and structure of inclusion complexes of 2,6- and 2,9-substituted bicyclo[3.3.1]nonanes with α- and β-cyclodextrin (CD) has been investigated by high-resolution ^{1}H NMR spectroscopy. α- and β-CD were found to form 1 : 1 inclusion complexes and the binding constants were estimated from titration studies. 2D ROESY experiments provided insight into the structure of the complexes.

Key words: Cyclodextrins, bicyclo[3.3.1]nonanes, complexation, NMR.

1. Introduction

Cyclodextrins (CDs) are a class of cyclic cyclomaltooligosaccharides built up from glucopyranose units. Commonly CDs consist of six, seven, or eight α-(1→4)-linked D-(+)-glucopyranose units and are named α-, β- and γ-CD, respectively. The CD molecule has a conical shape, the interior being moderately non-polar. The relatively hydrophobic cavities of the doughnut-shaped CDs are known for their ability to bind organic molecules in solution and in the crystalline state. The complexation driving forces have been attributed to hydrophobic interactions, van der Waals–London dispersion forces and hydrogen bonding. The shape and the internal diameter of the CD cavity and the size of the guest molecules, i.e. a close match, is of primary importance for complex formation. There is a growing interest in the study of CD inclusion complexes since the interaction between host and guest molecules may provide useful tools in molecular recognition, substrate-receptor interactions and, in general, supramolecular chemistry [1].

The interaction of CDs with a number of organic structures has been studied in recent years [2]. Among the organic compounds exibiting the strongest binding

* Authors for correspondence.
# Presented in part at the 8th International Cyclodextrin Symposium, Budapest, March 30–April 2, 1996.

to the CDs are substituted adamantanes [3]. The bicyclo[3.3.1]nonanes studied here are conformationally flexible structures which have one carbon atom less than adamantane and also act as hosts to form host–guest inclusion compounds with different molecules [4]. This investigation on the interaction of CDs with bicyclo[3.3.1]nonanes was carried out to gain insight into the bicyclononanes-CDs complex formation and structure, to clarify forces involved in this interaction as well as the role of the conformational changes, if any, of the guest molecules. The potential of these complexes to interact differentially with both enantiomers in racemic mixtures of bicyclo[3.3.1]nonanes is discussed.

2. Experimental

2.1. Materials

α- and β-Cyclodcxtrin (Aldrich) were used as received and freeze dried before use. Literature methods were used to prepare bicyclo[3.3.1]nonane-2,6-dione (**1**), *endo,endo*-bicyclo[3.3.1]nonane-2,6-diol (**2**) and *endo*-2-*anti*-9-bicyclo[3.3.1]nonanediol (**4**) [5,6]. Monoacetate **3** was prepared from **2** by acetylation of 0.025 mol with an equimolar amount of acetic anhydride in dry tetrahydrofuran under reflux for 2h. Solvent was evaporated *in vacuo* to dryness and the residue was dissolved in benzene and filtered off. The benzene layer was concentrated and purified by column chromatography (Al_2O_3, eluent: chloroform) yielding **3** with R_f 0.28 (1.1g, 22%) as a white solid; m.p. = 65–66 °C (from cyclohexane–ether); IR (nujol), ν 3350 br, 1745, 1070, 1036 cm^{-1}; ^1H NMR (D_2O), δ 4.96 (m, 1H, H—OAc), 3.92 (m, 1H, H—OH), 2.1 (s, 3H, CH_3), 2.02–1.93 (m, 2H), 1.90–1.83 (m, 2H), 1.80–1.50 (m, 8H, methylene envelope); mass-spectrum (*m/z*, %): *m/z* 180 (9%, M^+), 138 (30, M—AcOH), 120 (100), 105 (15), 92 (38), 79 (50).

2.2. Proton NMR Spectra

All ^1H NMR spectra were recorded at 303 K on a Bruker AMX500 spectrometer equipped with a Eurotherm controller for temperature regulation. The complexes were obtained and the titrations were performed by adding variable amounts of the substrates to a cyclodextrin solution in D_2O and *vice versa*. The concentrations of substrates and the CDs varied between 1–10 $\times$ 10^{-3} M. The solutions were thoroughly mixed and allowed to equilibrate for several minutes in the probe before the spectrum was acquired. The chemical shift of H-1, virtually unaffected by inclusion of the guest, was used as a reference point for the $\Delta\delta$ values. For the 1D spectra, typically 128 FIDs of 32 K each, separated by 2s relaxation delay were collected, for a spectral width of 6024 Hz. Processing consisted of multiplication with a squared sinebell function shifted by $\pi/2$ followed by Fourier transformation and baseline correction using standard UXNMR software from Bruker. For the ROESY spectra the standard pulse sequence as proposed by Bax and Davis [7] was used. Although the carrier was put in between the CD and substrate resonances and

the spinlock was performed with a field strength of only 2.5 kHz, the intramolecular NOEs were masked by HOHAHA crosspeaks in both substrate and CDs. The only exceptions were NOEs to the anomeric protons which are well-resolved from the remainder of the CD protons. Typically, 256 FIDs of 2K data points, 32 scans each with mixing times varying between 50 and 500 ms were accumulated using TPPI [8] for phase sensitive acquisition in t_1. The analysis was based on the 100 ms data. Processing consisted of zero filling in t_1, multiplication by a $\pi/2$ shifted squared sinebell in both time domains followed by Fourier transformation and fifth order polynomial baseline correction in F2 in F1. The final 2K × 2K data matrix yielded a resolution of 2.94 Hz per point.

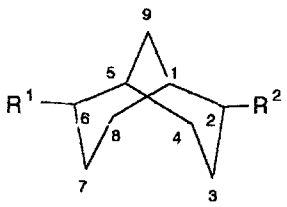
2.3. Stability Constants K

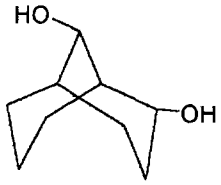
The inclusion stability constants and the estimated associated errors were calculated from ^{1}H NMR titration data using a non-linear least squares fit of the data to a modified version of the Benesi–Hildebrand equation [9] within the program SigmaPlot (Jandel Scientific).

3. Results and Discussion

A wide variety of techniques have been employed in the detection and quantification of CD inclusion complexes. Compared to other techniques NMR spectroscopy provides a powerful method to study inclusion phenomena because both guest and host molecules are simultaneously observed. The complex formation is expected to induce shifts of the resonance of the cyclodextrin protons, especially of H-3 and H-5, which are directed towards the interior of the cavity. Furthermore, 2D ROESY spectra can provide direct information on the disposition of the guest molecule relative to the host cavity in solution.

The ^{1}H NMR spectra of the mixtures of α- and β-CD with derivatives of bicyclo[3.3.1]nonanes **1**–**4** were analyzed and compared to the spectra of pure CDs and bicyclic compounds to investigate the formation of inclusion complexes. The inclusion of guest molecules in the α- and β-CD cavity in aqueous solution was supported by the changes in the ^{1}H NMR spectra of both partners, more specifically in their chemical shift values. The occurrence of chemical shift changes without line broadening allows one to characterize the interaction as being fast on the NMR time scale. Furthermore, the fact that significant chemical shift changes occur only for hydrogens on the inner surface (H-3 and H-5) of the CDs, and not for those on the outer surface (H-1, H-2, H-4, and methylene H-6) allows one to establish unambiguously that these changes are the result of inclusion complex formation and not the result of non-specific interactions between both partners.





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1 $R^1=R^2=-O$
2 $R^1=R^2=OH$
3 $R^1=OH$, $R^2=OCOCH_3$

Interaction of dione **1** and diol **2** with the two CDs in solutions produced different effects. After addition of α-CD to the solution of dione **1** an upfield shift (0.064 ppm) was observed for the H-3 proton of the α-CD which is located in the wide opening of the truncated cone. Practically no induced shift was observed for the H-5 proton which is at the narrower side. Downfield shifts for the protons of the included dione **1** substrate molecule were observed, e.g. 0.04 ppm for H_1 and H_4 as well as 0.02 ppm for H_9 (Table I). From these data and from the Job plots constructed for the induced shift data (cf. [9b]) for this complex it follows that the substrate molecule does not penetrate deeply into the α-CD cavity and the complex has 1 : 1 stoichiometry.

Because of the closer match of the β-CD cavity size and the shape of bicyclo[3.3.1]nonane (both ca. 7 Å), a stronger host–guest interaction was expected in this case and the influence of the β-CD on the proton chemical shifts of both partners was studied.

Significant chemical shift changes were observed for the substrates **1-4** and β-CD protons (Figure 1). The upfield shift of H-5 was larger than of the H-3 proton in dione **1**, i.e. 0.12 and 0.064 ppm, respectively. This observation is consistent with a deeper insertion of the bicyclic framework in the β-CD cavity than in α-CD cavity. The protons of the guest dione **1** showed also significant downfield shifts (0.11–0.12 ppm).

The inclusion of the diol **2** has a larger effect on the H-3 shift than on the H-5 shift of β-CD (0.054 and 0.03 ppm, respectively) showing that the penetration of diol **2** is presumably less deep in the CD cavity compared to the insertion of dione **1**. The proton signals of the bicyclic framework display comparatively large shifts of >0.1 ppm. In the 2D ROESY spectra all but the anomeric proton H-1 of β-CD are involved in dipolar contacts with substrate protons. These observations may be accounted for by considering a more complex interaction of the diol **2** with β-CD than simple inclusion whereby capping of the CD entrance occurs. This has heen reported for CD complexes involving some alkanediols and other bifunctional compounds [10]. Additional support for the capping by the diol **2** comes from the results of complexation with monoacetate **3**, which was obtained by monoacetylation of **2**, thus rendering the molecule more hydrophobic. Now

Table I. ^{1}H NMR chemical shifts (ppm) displacements ($\Delta\delta_{lim}$) resulting from the inclusion of **1–4** in α- and β-cyclodextrins and inclusion association constants K at 30 °C.

$\Delta\delta_{lim}$					
			Guest + host		
Nucleus	**1** + αCD	**1** + βCD	**2** + βCD	**3** + βCD	**4** + βCD
Guest					
H_1	0.05	0.12	0.21		0.21
H_4					0.12
H_9	0.02	0.11	0.105		
H_2				0.07	
H_6				0.08	
CDs					
H-3	−0.64	−0.064	−0.054	−0.07	−0.045
H-5	−0.01	−0.12	−0.03	−0.13	−0.01
K (M^{-1})	60 ± 11	345 ± 12	71 ± 14	195 ± 10	13.5 ± 4

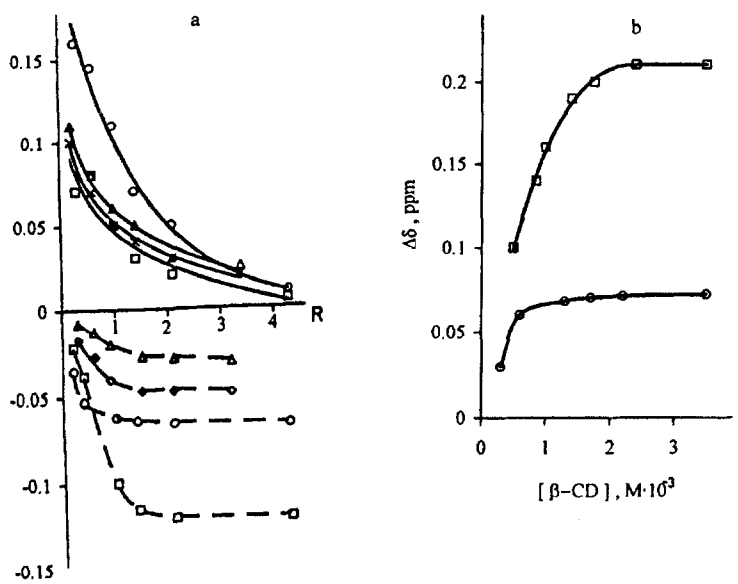

Figure 1. (a) Solid lines: plot of the chemical shifts of the dione **1** H_1 (△) and H_9 (×), and diol **2** H_1 (○) and H_9 (□) protons as a function of guest-to-host ratio (R) in aqueous solution at constant host concentration (2.5×10^{-2} M and 4.0×10^{-2} M, respectively). Dashed lines: graph of the chemical shifts of the β-CD inner protons H-3 (◇ and ○) and H-5 (△ and □) as a function of R for the complexes of **2**–β-CD and **1**–β-CD, respectively; (b) ^{1}H NMR chemical shift differences for the **3** H_1 (□) and for **4** H_2 (○) protons as a function of [β-CD] ([**3**] = 2.1×10^{-3} M, [**4**] = 2.9×10^{-2} M).

the H-5 shift is much larger (0.13 ppm) compared to the shift of H-3 (0.07 ppm), indicating that **3** is deeper in the cavity. No changes were detected for the chemical shifts of the outer CD protons.

In the case of 2,9-diol **4** the resonances of H-3 and H-5 of CD showed small induced shifts (0.045 and 0.01 ppm, respectively); however, relatively large shifts were observed for the H_1 proton (0.21 ppm) and some other protons of the bicyclic molecule. Unfortunately, the resonances of protons at C↑OH (positions C_2 and C_9) overlap with β-CD resonances, precluding a more detailed analysis.

It has to be noted that downfield shifts were observed for most of the bicyclic ring protons of **1–4** ($\Delta\delta \geq 0.01$–0.2 ppm); however, some signals, mainly of the protons adjacent to substituents, overlap with CD signals and do not allow quantitative determination of all induced shifts over the whole range of R. The most representative shifts and those used for the further calculations of K are presented in Table I.

The inclusion of the different guest molecules were further studied by 2D ROESY spectroscopy. Indeed, the close match in the size of the cavity leads one to expect that several protons of the guest molecule can be located within 3–4 Å of each other, allowing these spatial proximities to be revealed by the NOE effect. As the interaction between both partners is fast on the NMR time scale, a straightforward interpretation of the observed NOEs is possible [11]. However no intermolecular NOEs could be detected in 2D NOESY spectra recorded with various mixing times, while intramolecular NOEs were weak and positive and weak and negative for the guest and host molecules, respectively. This can be rationalized from the rotational correlation time τ_c associated with the molecular weight of the β-CD and the complex which approaches the spectrometer frequency. This leads to the situation where the NOE has vanishing intensity [12]. In fact the observation of weak negative NOEs for CD is mainly the result of the high β-CD concentration and the degeneracy of the glucopyranose resonances. Therefore, 2D rotating frame NOE spectroscopy or ROESY was used as ROE is always positive irrespective of τ_c. In the case of the substrate **1** the protons of the guest molecules show intramolecular cross peaks within the substrate molecules and intermolecular cross peaks with the H-3 and H-5 protons of the host molecule (Figure 2). The intermolecular NOEs are found between H-3 and $H_{1(5)}$ as well as between H-3 and $H_{9syn(anti)}$ (the parentheses show the homotopic protons related through the C_2 axis). The same NOEs, although weaker, are found to occur with H-5 of the β-CD. Thus the protons at carbon atoms 1(5) and 9 are closer to H-3 than they are to H-5. The much weaker NOEs were observed at the other end of the bicyclic molecule, i.e. between $H_{3(7)endo,exo}$ and both H-3 and H-5. In the case of **2** essentially all β-CD protons display intermolecular NOEs of more or less equal intensity with most of the 2,6-diol resonances. A notable exception is the H-1 anomeric proton of the CD which seems to be located more than 3 Å from the guest molecule protons. As H-1 in β-CD is located on the outside and is furthest from the entrance, the absence of intermolecular ROEs does not disagree with the capping of the β-CD entrance

by **2** as described above. Although the ROESY spectra support the formation of inclusion complexes and allow a delineation of the location of the guest molecules in the host, the pseudo C_7 symmetric cavity of the β-CD as well as the C_2 symmetry of the guest molecules **1** and **2** does not allow a more quantitative elaboration of the intermolecular distances and lead to the only conclusion that the guest molecules enter into the β-CD cavity from the large side to give a symmetrical location of the guest molecule in the complex (Figure 2).

The molar ratios and the stability constants for the inclusion complexes formed between bicyclononanes and the α- and β-CDs were determined from ^{1}H NMR titrations in D_2O at 33 °C. The limiting changes in the chemical shifts of the CD proton resonances upon inclusion of the guest were determined by a titration of CDs with an excess of substrates and *vice versa* (Figure 1). Upon consecutive additions of β-CD to a solution of monoacetate **3** and 2,9-diol **4** the resonances for the bicyclic protons displayed downfield shifts (Figure 1b). During the course of the titration the H-5 and H-6 resonances of CD merged. To determine the stoichiometry of the CD-substrate complexes the $\Delta\delta$ values for the β-CD and bicyclic structures were plotted as a function of guest-to-host molar ratio (R). The observed changes of induced shifts $\Delta\delta$ are observed with increasing R until the value of $R = 1$ for protons of CDs (Figure 1a) and of substrates when adding the CD to the solution of substrates (Figure 1b). The variation of the ^{1}H chemical shifts over the range of R values considered is consistent with the formation of a 1 : 1 inclusion complex. The limiting values of $\Delta\delta$ at large R correspond to the chemical shifts of the substrate–CD adduct.

The $\Delta\delta$ data can be used to estimate the association constant K for the substrate-CD adducts. In the absence of any information about the activity coefficients, only an apparent association constant can be determined. The complex association constants were obtained by a modification of the Benesi–Hildebrand equation [9b] using the expression

$$K = \frac{\Delta\delta_i}{C_0(\Delta_{\text{lim}} - \Delta\delta_i)},$$

where $\Delta\delta_i$ represents the observed shift, e.g. the difference between the chemical shift of the free guest and the same guest in the presence of CD, C_0 the total host concentration and Δ_{lim} the limiting chemical shift of the guest in a complexed state with the reference to an uncomplexed state.

Only values for protons that show the largest chemical shift changes were used for the K calculation. The condition for using the Benesi–Hildebrand equation is that the host must be present in excess. The inclusion stability constants were determined from simultaneous fits to the equation of at least three different proton resonances data and are presented in Table I. Each of these resonances was elaborated by incorporation into a standard non-linear least-squares fitting program. These constants are on the rather low side; however, they are of the same magnitude as for some other alicyclic substrates [13,14].

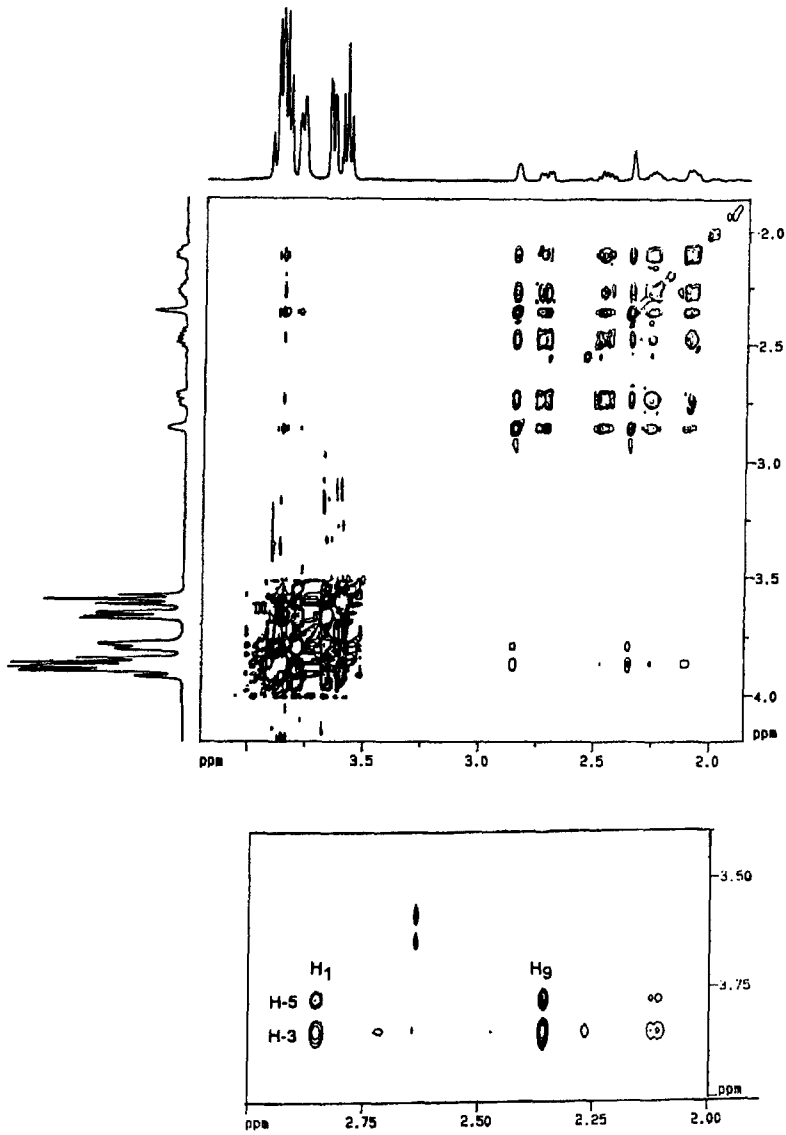

Figure 2. A part of the 500 MHz 2D ROESY spectrum with a spin lock time of 200 ms of the complex of β-CD with dione **1** observed in D_2O solution at 35 °C illustrating the intermolecular NOEs between protons of the host and guest molecules. The cross-peaks connecting the resonances of the H-3 and H-5 protons of β-CD to those of H_1 and H_9 are indicated in the enlarged part of the spectrum.

D-Glucopyranosyl residues in CDs are equivalent due to the presence of a pseudo C_n symmetry axis and thus CDs are gyrochiral molecules (there is no symmetry element of the second kind, e.g. mirror plane). As can be expected, the resonances of each D-glucopyranoside ring overlap to yield one set of glucopyranosyl reso-

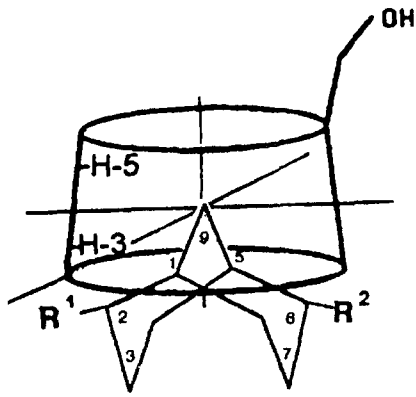

Figure 3. One of the symmetry related locations of the bicyclononane molecule in the β-CD cavity as deduced from intermolecular NOEs and chemical shift data.

nances in the NMR spectra. The 2,6-dione **1** is a chiral molecule possesing a C_2 symmetry axis and adding a racemic mixture to CD should give two diastereomeric complexes, which can display different chemical shifts and may have different stability constants. However, the fast exchange conditions blur everything into an averaged picture. We do not see duplication of the NMR resonances, thus the chemical shifts are a weighted average of the free state and both complexes and the reported stability constants should be interpreted accordingly. Lowering the temperature to 153 K in D_2O–CD_3OD solution did not yield slow exchange conditions under which duplication may be expected to occur. Thus the thermodynamic discrimination between the enantiomers of substrates is insignificant, and thereby a difference in the interaction between β-CD and the (*R*) and (*S*) enantiomers was not observed. The inclusion complexes reported cannot be expected to be of use as chiral resolving agents for the guest molecules studied here.

In conclusion, an inclusion complex formation between bicyclo[3.3.1]nonanes and cyclodextrins has been demonstrated on the basis of the ^{1}H NMR titration experiments. The structure and the binding constants of the complexes have been estimated and their insertion into the cavity relative to one another has been discussed on the basis of chemical shift arguments and the observation of intermolecular NOEs.

4. Acknowledgement

We wish to thank Prof. G. van Binst for encouragement and discussions.

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