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Complexation of [2.2]paracyclophane with β- and γ-cyclodextrins studied by HPLC and NMR

Research Article

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Abstract: The NMR spectra of [2.2]paracyclophane with β- or γ-cyclodextrin in DMF-d_7 at room temperature do not show significant complexation, while HPLC of the complexes in mixed H_2O:alcohol solvents demonstrate complexation with different stoichiometries. At 243 K in DMF solution the H3 and H5 NMR signals of γ-cyclodextrin (but not β) exhibit complexation-induced chemical shifts denoting complex formation. According to HPLC, at room temperature the [2.2]paracyclophane complex with β-cyclodextrin in 20% H_2O:EtOH exhibits 1:2 stoichiometry with $K_1 = 1\times10^2 \pm 2$, $K_2 = 9.0\times10^4 \pm 2\times10^3$ ($K = 9\times10^6$) while that with γ-cyclodextrin in 50% H_2O:MeOH exhibits 1:1 stoichiometry with $K_1 = 4\times10^3 \pm 150$ M^{-1}. Thermodynamic parameters for both complexes have been estimated from the retention time temperature dependence. For the β-cyclodextrin complexation at 25°C ΔG^0_{CD} is -39.7 kJ mol^{-1} while ΔH^0_{CD} and ΔS^0_{CD} are -88.2 kJ mol^{-1} and -0.16 kJ mol^{-1} K^{-1}. For γ-cyclodextrin, the corresponding values are ΔG^0_{CD} = -20.5 kJ mol^{-1}, ΔH^0_{CD} = -33.5 kJ mol^{-1} and ΔS^0_{CD} = -0.04 kJ mol^{-1} K^{-1}.

Keywords: *Cyclodextrin cyclophane complexes • HPLC • NMR • Thermodynamic parameters*

1. Introduction

Cyclodextrins (CD) are macrocyclic sugars built of glucopyranoside units (Scheme 1) [1,2]. They selectively form inclusion complexes with various guest molecules in their cavities. These complexes are among the most studied supramolecular systems in view of their numerous applications [1,3-9] in food, cosmetic, textile [10], agrochemical [11] and other industries. Several applications in molecular devices have been proposed [12]. They are also interesting for basic research into the driving forces for complexation and serve as enzyme models [13]. On the basis of X-ray studies, CDs were long thought to have a structure of rigid truncated cones, but it is now recognized that they are flexible and the cone structures are average ones [14,15].

The solutions contain a dynamic mixture of free and complexed species. The latter may have varying stoichiometry (see below). Free host, guest and the 1:1 and 1:2 complexes that may be present in solution are shown schematically in Fig. 1. In the latter complex, the guest is held inside a capsule formed by two cyclodextrins with several hydrogen bonds between their 2-OH and 3-OH groups. In a polar solvent, these hydrogen bonds between the CD molecules are formed and broken rapidly and hydrogen bonds with the solvent take part in the dynamic equilibria among the species shown in Fig. 1.

NMR is one of the main methods in studies of CD inclusion complexes [16]. Signals from CD H3 and H5 protons pointing into the cavity are especially sensitive to the aromatic guest presence. Averaging due to rapid exchange between free and complexed guest causes small but significant shifts of H3 and H5 providing proof of complex formation. Another more significant proof is provided by NOE and ROE, as irradiation of CD H3 and H5 affects the signals of the nearby guest protons.

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The forces responsible for CD complex formation with hydrocarbons are not large, consisting mainly of weak but numerous attractive nonbonding interactions and the solvophobic effect. Different techniques can provide valuable information, but their different experimental conditions (solubility, timescales, *etc.*) can lead to apparently contradictory results. Different modes of entrance of nitrophenol into the permethylated α-CD cavity in the solid state and in solution illustrate the latter point [17].

A few examples of CD complexes of the same guest with different stoichiometries studied by one experimental technique have been documented [18-23]. However, to our best knowledge, no thermodynamic data for complexation have been reported. In this communication, HPLC measurements for the complexation of [2.2]paracyclophane (**1**) with β- and γ-CD (**2**, **3**) are presented together with ^{1}H NMR spectra measured at 303 and 243 K for complexes with β-, γ- and δ-CD (**2** – **4**). The chromatographic studies include determination of stability constants and stoichiometry for the complexes with β- and γ-CD at room temperature in the mixed (alcohol/water) solvents at room and a few elevated temperatures, allowing us to obtain thermodynamic parameters for the complexes' formation.

2. Experimental procedure

2.1. Samples

[2.2]paracyclophane of 99% purity was supplied by Alfa Aesar; β-CD and γ-CD were bought from Chinoin. δ-CD preparation has been reported [24,25].

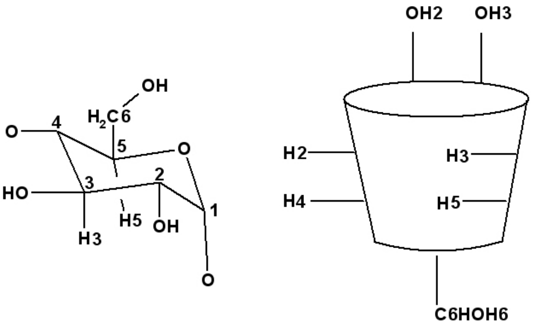
2.2. NMR

The complexation of cyclophane (**1**) with β-, γ-, and δ-CD (**2** – **4**) was investigated in DMF solution at 303 and 243 K. All NMR spectra were obtained on a Bruker 500 Avance spectrometer at 500.13 MHz using about 15 mg of CD. TMS was the internal standard. Acquisition parameters for 1D spectra were: spectral width 7500 Hz, number of data points 64 K, acquisition time 4.36 s, relaxation delay 2 s. The NOESY and ROESY spectra were run in phase-sensitive mode (States-TPPI). Acquisition parameters: spectral width 2500 Hz, acquisition time 0.41 s, relaxation delay 3 s, number of scans 40, spin-lock time 200 ms, number of time incremented spectra 400, number of data points 2 K in both domains. Standard Bruker software was used for acquisition and processing.

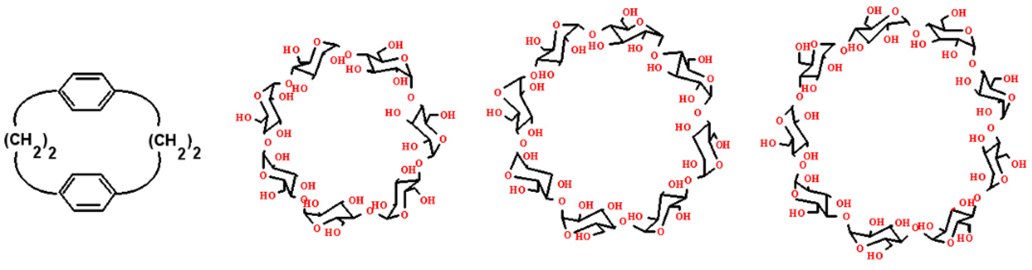
2.3. HPLC

The mobile phase was a water/alcohol mixture with an appropriate CD concentration (for γ-CD: 5×10^{-5} – 2×10^{-2} M in 50% v/v of methanol, and for β-CD: 1×10^{-3} – 1.5×10^{-2} M in 20% v/v ethanol). The column was XTerra MS C18, 5 mm, 4.6×150 mm from Waters (Vienna, Austria). To determine CD complex stoichiometry and stability changes of the analyte retention factor with CD concentration were analyzed on the basis of the model described below.

A temperature study was carried out to estimate thermodynamic parameters. The column used was Jupiter 5 μM C4 300A 150×1 mm from Phenomenex. The mobile phase was 50% MeOH or 30% EtOH for the system without cyclodextrin. Otherwise it was 50% MeOH with 1.5 mM γ-CD or 20% EtOH plus 1.5 mM γ-CD or 1.5 mM β-CD, flow 0.04 mL min^{-1}. The range of temperatures was 20-45°C.



Scheme 1. Glucopyranoside unit with the atom numbering (left) and a schematic view of CD with H3 and H5 protons pointing inside the cavity while OH2, OH3 are on the wider rim and C6HOH6 on the narrower.



1 **2** **3** **4**

Formulae 1 - 4

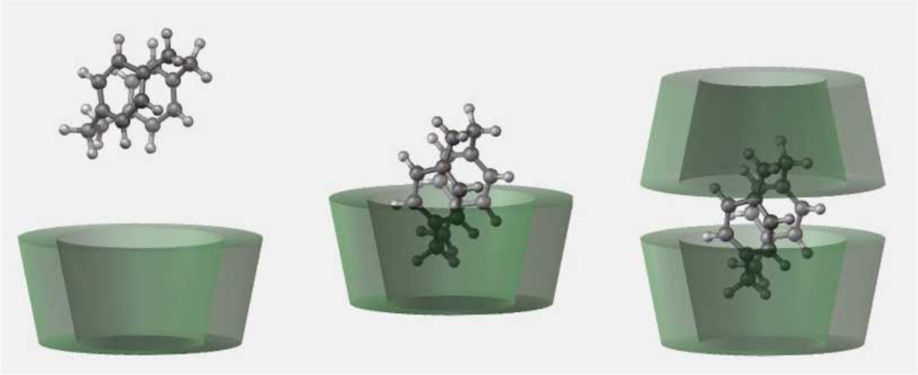


Figure 1. Schematic views of free [2.2]paracyclophane and cyclodextrin (left) and their 1:1 (middle) and (1:2) (right) complexes.

2.4. Method

2.4.1. HPLC Estimation of binding constant

To establish the CD complex stoichiometry and stability, changes of the guest retention factor with CD concentration were followed. The data were analyzed on the basis of the model described earlier [23,26,27].

The solute retention factor k_1 in the chromatographic system described by the model may be defined by:

$$k_1 = \frac{k_0}{1+\sum_{i=1}^{n}(\prod_{i} K_i)\cdot[\mathrm{CD}]^i} \quad (1),$$

where k_1 and k_0 are the retention factors observed with and without CD and [CD] is the concentration of CD in the mobile phase.

The stability constants were fitted to Eq. 2 by a non-linear least square procedure

$$\frac{1}{k_1} = \frac{1}{k_0} + \frac{K_1[\mathrm{CD}]}{k_0} + \frac{K_1K_2[\mathrm{CD}]^2}{k_0} + \frac{K_1K_2K_3[\mathrm{CD}]^3}{k_0} + ... \quad (2)$$

The shape of a plot of 1/*k* *versus* [CD] provides information on the complex stoichiometry and stability constants since for 1:1 stoichiometry the plot is linear, while for higher stoichiometry it becomes parabolic. Moreover, of two species the one forming more stable complexes with a complexing agent present in the mobile phase should be eluted faster from the column.

2.4.2. Thermodynamic estimation

In a system without CD the van't Hoff expression is given by Eq. 3:

$$lnk_0 = \frac{-\Delta H^0}{RT} + \frac{\Delta S^0}{R} + ln\varphi \quad (3)$$

where k_0 is the retention factor, ΔH^0 and ΔS^0 represent enthalpy and entropy changes of the solute of transfer between the mobile and stationary phases, *R* is the gas constant, *T* is the absolute temperature and ϕ is volume phase ratio of the stationary to mobile phase.

To estimate changes of enthalpy of complexation in the system with CD the following equations can be used:

for 1:1 stoichiometry [22]

$$lnk_1 = \frac{-(\Delta H^0 - \Delta H^0_{CD})}{RT} + \frac{\Delta S^0 - \Delta S^0_{CD}}{R} + ln\varphi - ln\left(\frac{1}{K_1} + [CD]\right) \quad (4)$$

and for 1:2 stoichiometry [23]

$$lnk_1 = \frac{-(\Delta H^0 - \Delta H^0_{CD})}{RT} + \frac{\Delta S^0 - \Delta S^0_{CD}}{R} + ln\varphi - ln\left(\frac{1}{K_1K_2} + \frac{[CD]}{K_2} + [CD]^2\right) \quad (5)$$

where:

k_1 is the retention factor in the system with CD, K_1 and K_2 are the stability constants for 1:1 and 1:2 stoichiometry, ΔH^0_{CD} and ΔS^0_{CD} are standard enthalpy and entropy changes of complex formation in the mobile phase, and [CD] is the mobile phase CD concentration.

The enthalpy changes ΔH^0 of transfer of the solute between the mobile and stationary phase, were calculated from Eq. 3, while ΔH^0_{CD} values were determined from Eqs. 4 and 5 for γ-rim β-CD, respectively. The difference in the changes of free energy of complexation (at 25°C) were determined according to Eq. 6 for the 1:1 complex and Eq. 7 for the 1:2 complex

$$\Delta G^0_{CD} = -RTlnK_1 \quad (6)$$

or

$$\Delta G^0_{CD} = -RTlnK_1K_2 \quad (7).$$

The entropy changes of complexation were determined from:

$$\Delta G^0_{CD} = \Delta H^0_{CD} - T\Delta S^0_{CD} \quad (8).$$

3. Results and discussion

3.1. NMR

^{13}C NMR spectra and T_1 measurements for the free hosts (**2 – 4)** and their complexes with [2.2]paracyclophane (**1)** at 303 and 243 K in DMF-d_7 solution provide no clear evidence of complexation. Similarly, no complexation-induced shifts could be detected in the room temperature ^{1}H NMR measurements, except for the signal of CD H3 (where the complexation effect is very small) (Table 1), and only one set of intermolecular NOE interactions between H3 and H5 γ-CD protons with both aromatic and aliphatic protons of the guest could be found. The latter observation is not surprising since the molecular weight of the possible complex is located in the range where the NOE effect is close to zero. The NOE observation was confirmed by the ROESY experiment. Notably, the correlations with the aromatic cyclophane protons are stronger than those with the aliphatic ones. On the other hand, considerable complexation induced shifts have been found for the **1@3** complex at low temperature. For the **1@2**complex the ^{1}H NMR manifestations of complexation are much weaker; no complex at 303 K and a very weak one at 243 K were detected.

3.2. HPLC

In view of the small amount of δ-CD available, chromatographic measurements (Figs. 2, 3) could be carried out only for hosts **2** and **3**. The HPLC titrations (Fig. 2) show that the **1@3** complex has 1:1 stoichiometry (B) while for the **1@2** complex the stoichiometry is significantly larger (A). Stability constants were estimated to be $K_1 = 4\times10^3 \pm 150$ M^{-1} for γ-CD in 50% H_2O:MeOH mixture and $K_1 = 1\times10^2 \pm 2$, $K_2 = 9.0\times10^4 \pm 2\times10^3$ ($K = 9\times10^6$) for β-CD in 20% H_2O:EtOH. The temperature dependence of the retention times allowed us to determine thermodynamic parameters (Table 2).

In the last table, ΔH^0 represents enthalpy changes of transfer of the solute between the mobile and stationary phase, ΔH^0_{CD}, ΔS^0_{CD} and ΔG^0_{CD} are the standard enthalpy, entropy and free energy changes of complex formation. The K and ΔG values indicate that the 1:2 **1@2** complex is much stronger than the 1:1 **1@3** one. This is probably due to hydrogen bonds between two cyclodextrins forming the capsule in which the guest **1** is held. The latter complex is schematically presented in Fig. 1 (right) without showing the hydrogen bonds explicitly. Such highly dynamic bonds can be reliably studied only by neutron diffraction at low temperatures.

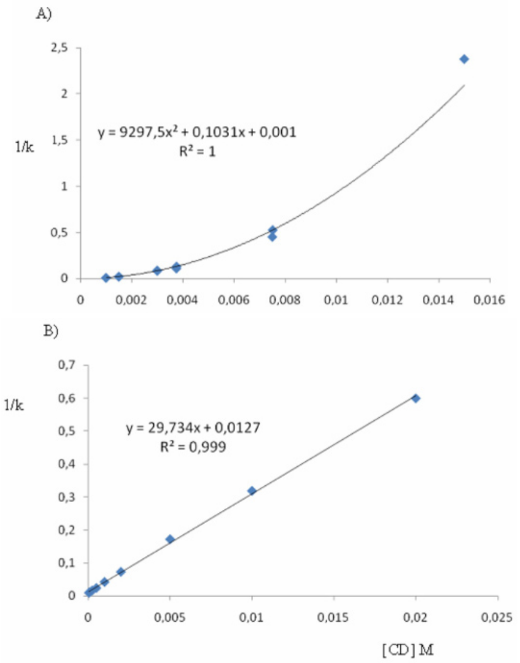


Figure 2. Dependence of the reciprocal of the retention factor (1/k) versus CD concentration (M) giving the complex stoichiometry and stability constants; parabolic for 1:2 stoichiometry for the complex with β-CD (A) and linear for the 1:1 γ-CD complex stoichiometry (B).

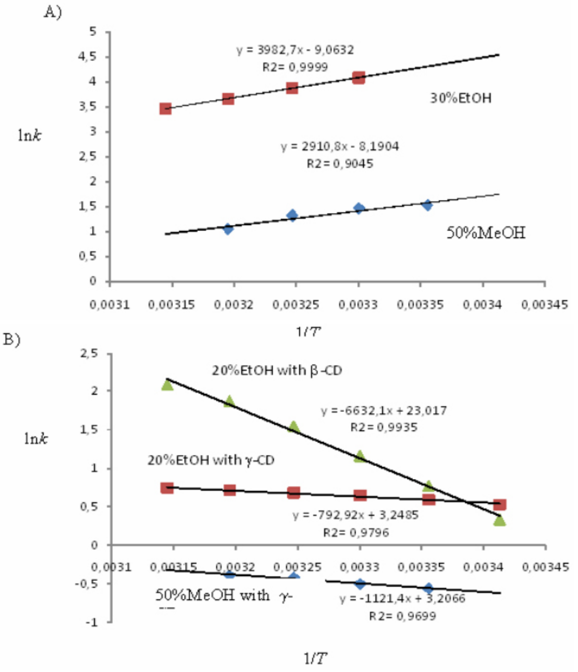


Figure 3. Thermodynamic parameter determination: Ln k of cyclophane as a function of 1/T (A) without cyclodextrin, (B) with cyclodextrin.

Table 1. Chemical shifts for free CD protons and those in the complex at room temperature and 243 K.

	303 K		243 K		303 K		243 K		303 K		243 K	
	2	**1@2 (Δ)**	**2**	**1@2 (Δ)**	**3**	**1@3 (Δ)**	**3**	**1@3 (Δ)**	**4**	**1@4 (Δ)**	**4**	**1@4 (Δ)**
H1	4.95	4.94 (0.01)	4.97	4.97 (0.0)	5.01	5.01 (0.0)	5.03	5.05 (-0.02)	5.08	5.08 (0.0)	5.11	5.12 (-0.01)
H2	3.45	3.45 (0.0)	3.45	3.45 (0.0)	3.46	3.47 (-0.01)	3.46	3.48 (-0.02)	3.45	3.45 (0.0)	3.44	3.44 (0.0)
H3	3.84	3.83 (0.01)	3.83	3.85 (-0.02)	3.79	3.82 (-0.03)	3.79	3.85 (-0.06)	3.80	3.80 (0.0)	3.79	3.79 (0.0)
H4	3.48	3.47 (0.01)	3.49	3.49 (0.0)	3.49	3.50 (-0.01)	3.51	3.53 (-0.02)	3.53	3.53 (0.0)	3.56	3.55 (0.01)
H5	3.77	3.76 (0.01)	3.78	3.77 (0.01)	3.71	3.72 (-0.01)	3.68	3.72 (-0.04)	3.69	3.70 (-0.01)	3.66	3.66 (0.0)
H6	3.83	3.83 (0.0)	3.83	3.84 (-0.01)	3.81	3.81 (0.0)	3.79	3.80 (-0.01)	3.80	3.80 (0.0)	3.79	3.79 (0.0)

Table 2. Stoichiometry, stability constants (M^{-1}) and thermodynamic parameters (kJ mol^{-1}) for the complexes of **1** with **2** and **3**.

Solution	**Cyclodextrin**	**Stoichiometry**	$K_1{}^a$ **or** $K_1{}^*K_2{}^b$	$-\Delta H^o$	$-\Delta H^o{}_{CD}$	$-\Delta G^o{}_{CD}$	$T\Delta S^o{}_{CD}$
H_2O:MeOH (50:50)	No CD			24.2			
	γ-CD	1:1	$4\times10^{3,\,a}$		33.5	20.5	-13.0
H_2O:EtOH (70:30)	No CD			33.1			
H_2O:EtOH (80:20)	β-CD	1:2	$9\times10^{6,\,b}$		88.2	39.7	-48.6
	γ-CD				39.6		

4. Comparison of the results from NMR and chromatographic measurements and conclusions

Thermodynamic parameters for cyclodextrin complexes are usually determined by microcalorimetry [28] and to our best knowledge there are no such data for complexes of different stoichiometry formed by the same host and guest. Due to the different solvents in which NMR and HPLC measurements were carried out, different results were obtained for the complexation of [2.2]-paracyclophane **1** with β- and γ-CDs **2** and **3** at room temperature. For **1@2**, a relatively strong complex was observed with HPLC and a lack of complexation under the experimental conditions of the NMR measurements. For the **1@3** complex, proton chemical shift manifestations of complexation are very weak at room temperature (but significant in NOESY and ROESY spectra) and considerable at 243 K, while the same complex studied at room temperature by chromatography was of medium strength. The temperature dependence of retention times allowed us to obtain thermodynamic parameters for the complexes **1@2** and **1@3**.

The impressive differences in complexation observed using NMR and chromatographic techniques are ascribed to different experimental conditions (solvent, temperature, concentrations). Unfortunately, in view of the very low guest solubility the measurement of the complexes using the same solvent in both methods was not possible.

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