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A Study of *β*-Cyclodextrin Inclusion Complexes with Progesterone and Hydrocortisone Using Rotating Frame *Overhauser* Spectroscopy

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Summary. Inclusion complexes of β-cyclodextrin with two steroid derivatives, progesterone (pregn-4-ene-3,20-dione) and hydrocortisone (11,17,21-trihydroxy-pregn-4-ene-3,20-dione), were studied in the liquid state by NMR spectroscopy. The complex formation process was monitored by intermolecular dipolar interactions between ^{1}H signals in the hydrophobic β-cyclodextrin cavity (H-3 and H-5 of the α-glucose units) and the steroid moiety in ROESY spectra. The data revealed that progesterone is fully immersed in the β-cyclodextrin cavity; however, complete inclusion of the hydrocortisone molecule was prevented by the polar hydroxyl groups on its surface.

Keywords. Cyclodextrin; Inclusion complexes; Steroids; NMR spectroscopy; ROESY.

Introduction

Cyclodextrins represent a unique family of naturally occurring organic compounds [1]. In these molecules six, seven, or eight α-glucose units are connected by $\alpha(1 \rightarrow 4)$ glycosidic bonds to form a cyclic oligomer (α-, β-, and γ-cyclodextrin) (a in Scheme 1). The deformed cylinder shape (b in Scheme 1) and the relatively rigid conformation of these compounds are a requisite for the formation of inclusion complexes [2, 3] with small molecules. This ability initiated many applications ranging from the pharmaceutical industry [4, 5] through cyclodextrin mediated organic reactions [6] to the field of supramolecular chemistry [7].

Several experimental methods are now available to study the complex formation process, but they are restricted to rather indirect or qualitative experiments. Among these, electron spectroscopy [8], circular dichroism [9, 10], and fluorescence spectroscopy [11] are the most important ones. Although X-ray crystallography provides absolute structural information, it can only be used for solid-state structural studies [12–14]. In NMR spectroscopy, the chemical shift change upon complex formation has been used to monitor this process [15] and widely used to obtain quantitative information about the thermodynamics of the host-guest

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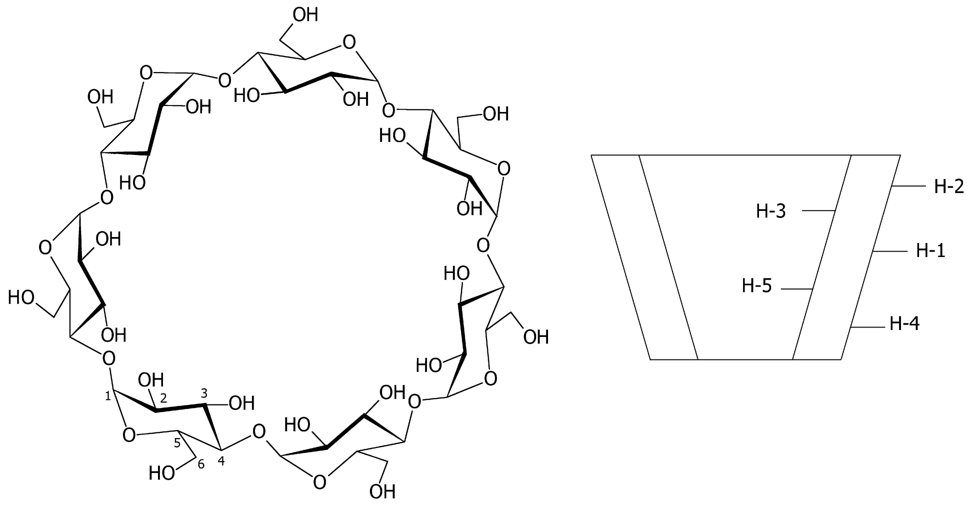


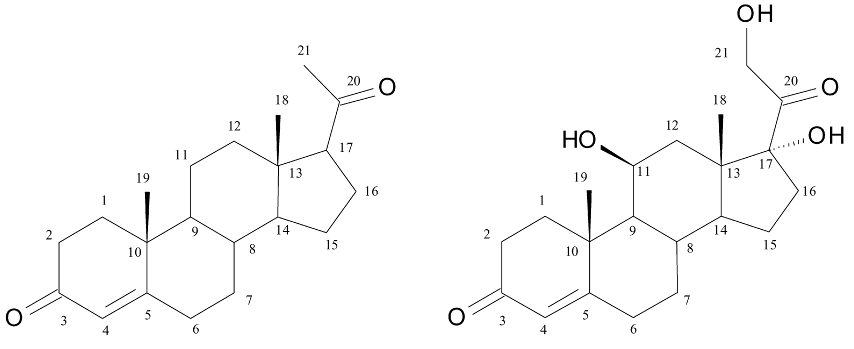
a **b**

Scheme 1

interaction [16–18]. The H-3 and H-5 protons of the α-glucose units in the β-cyclodextrin molecule are placed inside the hydrophobic cavity (b in Scheme 1) and hence are the most sensitive probes of any immersion process.

Dipolar interactions between protons in the substrate and H-3 and H-5 of the cyclodextrin host provide more detailed structural information. One widely accepted method to measure dipolar interactions in complicated systems is the two-dimensional NOESY experiment [19], however NOESY is impractical for our purpose at this field strength for medium sized ($MW \approx 1000$) molecules, since the cross-peak intensities go through zero as the molecular size increases.

Rotating frame *Overhauser* spectra [20] are superior to investigate such systems [21] because the enhancements are always positive, regardless of the molecular size [22]. The complex formation process was studied with two model compounds, progesterone (**1**) and hydrocortisone (**2**) (Scheme 2). These steroids are important



1 **2**

Scheme 2

hormones and play significant roles either in pregnancy or have anti-inflammatory activity, affect carbohydrate, protein, and fat metabolism, and the growth of white blood cells as well as the retention of salt and water in the body. The two derivatives were selected on the basis of their different hydrophilic properties: **1** represents a relatively hydrophobic molecule, whereas the three hydroxyl groups on the surface of **2** render the molecule more hydrophilic. It was assumed that this difference would result in differences in the complex formation process with the hydrophobic β-cyclodextrin cavity.

Results and Discussion

ROESY experiments were applied to detect intermolecular interactions between the guest molecules and β-cyclodextrin in *DMSO* solution. The two-dimensional ROESY spectrum (Fig. 1) of the β-cyclodextrin-progesterone complex showed intermolecular correlations of several steroid signals to H-3 and H-5 of the α-glucose units in the cyclodextrin ring. The signal assigned to H-3 is a triplet at 3.64 ppm in the ^{1}H NMR spectrum; it is oriented axially and has two large axial–axial three-bond couplings to the neighboring protons H-2 and H-4. H-5 is a multiplet at 3.56 ppm and has only one large axial–axial coupling to H-4 and two smaller couplings to the two protons at position 6. ROE correlations were observed to H-18 (at 0.57 ppm), H-19 (at 1.14 ppm), H-21 (at 2.06 ppm), and H-16 (at 1.61 ppm).

Since correlations were found to both H-3 and H-5 of the cyclodextrin host, it can be concluded that during the complex formation process the progesterone

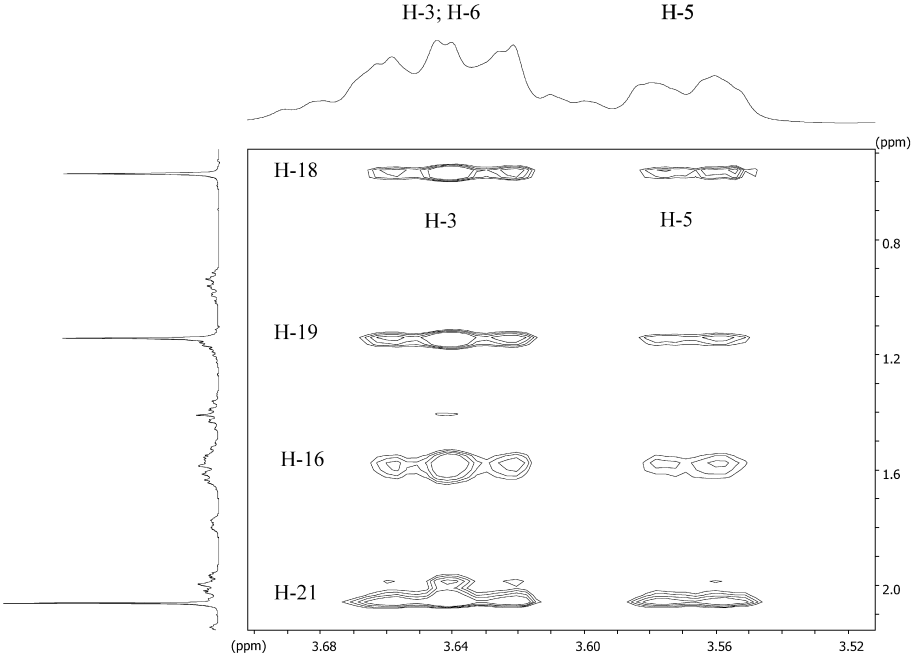


Fig. 1. Expanded region of the two-dimensional ROESY spectrum of the β-cyclodextrin complex of **1**

molecule is fully immersed in the cavity of the host molecule. The ROESY spectrum of **2** did not show these characteristic correlation patterns.

Since one of the signals (H-18) of the progesterone molecule was well separated in the ^{1}H NMR spectrum, a selective ROE experiment was carried out to investigate its dipolar interactions. From the different procedures developed to

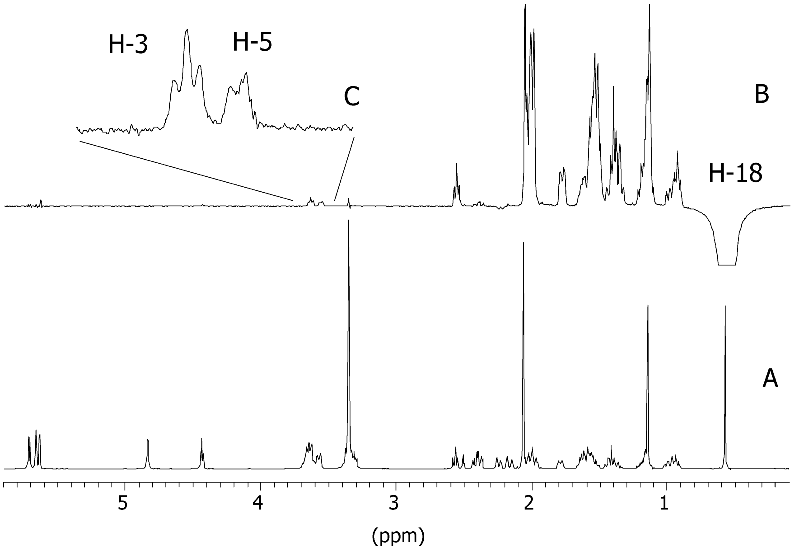


Fig. 2. One-dimensional ^{1}H NMR spectrum of the cyclodextrin complex of **1** (A), selective one-dimensional ROE spectrum (B), and expanded and magnified region of H-3 and H-5 (C)

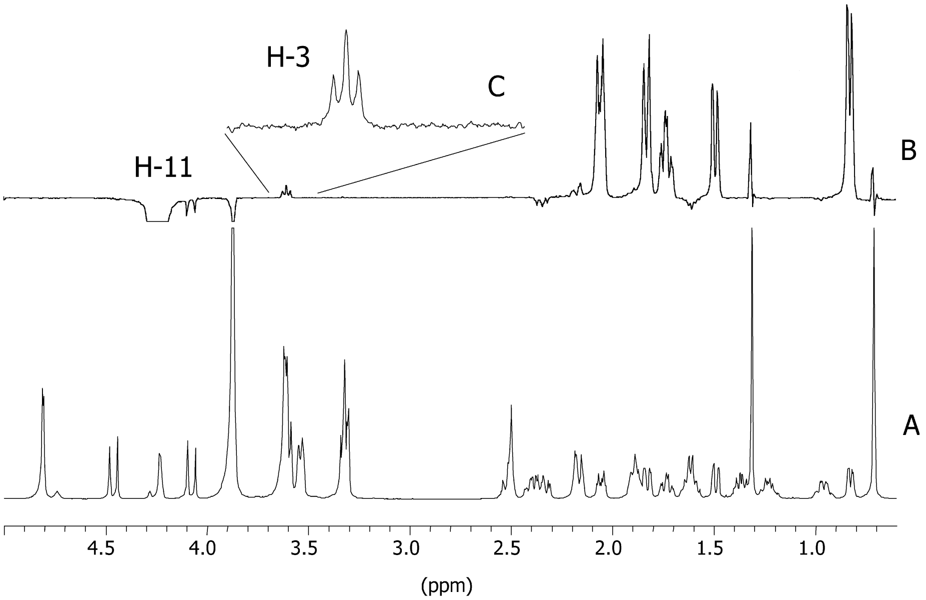


Fig. 3. One-dimensional ^{1}H NMR spectrum of the β-cyclodextrin complex of **2** (A), selective one-dimensional ROE spectrum (B), and expanded and magnified region of H-3 (C)

achieve selective excitation [23–27], a double-pulsed field gradient spin-echo (DPFGSE) sequence was chosen in a selective one-dimensional ROESY experiment to excite H-18. The resulting spectrum is shown in Fig. 2B.

The one-dimensional selective ROESY spectrum provided intramolecular enhancements (1.0–2.5 ppm in figure 2B) within the steroid moiety; in addition, two more characteristic ROE signals were observed to H-3 and H-5 (Figure 2C) of the α-glucose in the β-cyclodextrin. The intensity of these signals is much lower but can be identified readily from the spectrum. The only correlation that was found in the β-cyclodextrin/hydrocortisone complex resulted from excitation of H-11 of the steroid moiety in the selective ROESY experiment (Fig. 3).

Among the intramolecular ROE signals (0.7–2.0 ppm in figure 3B), only one intermolecular ROE (to H-3 of the α-glucose in the β-cyclodextrin; Fig. 3c) was observed which points to a partial immersion of the guest in the cyclodextrin cavity.

Conclusions

The complex formation of two natural steroid derivatives with β-cyclodextrin was investigated. Progesterone (**1**) was found to be immersed deeply in the cavity, as dipolar interactions between its protons with H-5 and H-3 of the cyclodextrin were observed. Partial complex formation was detected in the case of **2**, because only one ROE signal appeared (to H-3, which is close to the wider base of the cyclodextrin cone). Complete complex formation is prevented when the size of the substrate is larger than the size of the cavity, or in case of a hydrophilic substrate like hydrocortisone (**2**).

Experimental

The β-cyclodextrin (Aldrich) was used without further purification. 0.6 cm^3 *DMSO*-d$_6$ were used as solvent for the NMR experiments. Samples were prepared with 22 mg of β-cyclodextrin, 22.5 mg **1** (Steraloids), and 30.0 mg **2** (Steraloids). Spectra were recorded using a Bruker Avance DRX 500 NMR spectrometer (11.7 T) equipped with a 5 mm inverse probe head with z-field gradient capability. All experiments were carried out at room temperature.

ROESY experiments

The standard two-dimensional ROESY pulse sequence was used with a low-power spin-lock pulse. The relaxation delay was set to 2.0 s, and a 5.0 ppm wide chemical shift range was detected with the carrier frequency placed at 3.23 ppm. Complex data (4 k) were collected in 512 increments with 48 transients each. The spin-lock field strength (γB_1) was 3200 Hz with a mixing time of 500 ms. Phase-sensitive two-dimensional time-domain data were recorded and processed according to the TPPI protocol. A pure squared cosine window function was used in both dimensions prior to zero filling and *Fourier* transformation.

Selective excitation was carried out using a double-pulsed field spin echo sequence with a combination of selective *Gaussian* pulses and 1 ms long sine-shaped z-pulse field gradients. The relaxation delay was 2.0 s, the pulse length of the 90° hard pulse was 9 μs. The pulse length of the two selective 180° pulses was 80 ms, and a *Gaussian* function was used to shape these pulses. The strength (γB_1) of the spin-lock field was 3200 Hz with a mixing time of 500 ms. Four sine-shaped *z*-gradient pulses were applied during the course of the pulse sequence with a gradient ratio of 40:40:7:7. 2 k scans were recorded in a 8 k complex data set. A *Lorentz* window function with 1.0 Hz line-broadening was used prior to the *Fourier* transformation.

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