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Complexation of bile acids with 2-hydroxypropyl-β-cyclodextrin: a ^{13}C-NMR study

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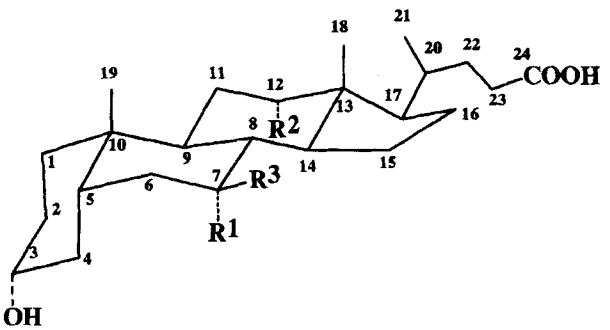
The interaction of chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholan-24-oic acid) 1; cholic acid (3α,7α,12α-trihydroxy-5β-cholan-24-oic acid) 2, deoxycholic acid (3α,12α-dihydroxy-5β-cholan-24-oic acid) 3 and ursodeoxycholic acid (3α,7β-dihydroxy-5β-cholan-24-oic acid) 4 with 2-hydroxypropyl-β-cyclodextrin HPβCD (partially functionalized) is studied by measuring the changes in ^{13}C-NMR chemical shifts induced by complexation in methanol-d_4. The alkyl chain of the bile acids enters the cyclodextrin cavity. The interaction of 1 and 4 with HPβCD is stronger, in this solvent, with respect that of 2 and 3.

INTRODUCTION

Cyclodextrins are known to form a variety of inclusion complexes and association compounds.[1] In the pharmaceutical field, they are widely used to improve the oral bioavailability of poorly soluble drugs.[2] To avoid toxic effects of parenteral application of β-cyclodextrin,[3] new cyclodextrin derivatives have been developed. Among them, hydroxypropylated cyclodextrins have lower hemolytic effect toward human erythrocytes than their natural parents.[4]

Some bile acids are successfully employed in the therapy of liver disease[5] and in cholesterol gallstone dissolution.[6] In patients affected by severe cholestasis or having undergone liver transplant, parenteral administration of bile acids could be useful, but both their low solubility and hemolytic properties preclude this route of administration. Thus, the complexation of bile acids with 2-hydroxypropyl-β-cyclodextrin could allow safe parenteral application. In fact, both the increase in drug solubility[7] and the reduction of the hemolysis induced by bile acids[8] were recently demonstrated by 2-hydroxypropyl-β-cyclodextrin (HPβCD) complexation.

In the present work, a NMR study of the complexation of chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholan-24-oic acid, **1**), cholic acid (3α,7α,12α-trihydroxy-5β-cholan-24-oic acid, **2**) and deoxycholic acid (3α,12α-dihydroxy-5β-cholan-24-oic acid **3**) (all from Sigma), with 2-hydroxypropyl-β-cyclodextrin (HPβCD, average MW 1380, average molar substitution: 0.6, Aldrich) is reported. A comparison with ursodeoxycholic acid (3α,7β-dihydroxy-5β-cholan-24-oic acid)[7] **4** is also made. Their behavior towards β-cyclodextrin has already been studied through ^{1}H-NMR.[9] However, to study the HPβCD inclusion complex, the ^{13}C-NMR approach seem to be more effective than the ^{1}H analogue to



1 : R^1 = OH, R^2 = H, R^3 = H;
2 : R^1 = OH, R^2 = OH, R^3 = H;
3 : R^1 = H, R^2 = OH, R^3 = H;
4 : R^1 = H, R^2 = H, R^3 = OH.

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provide an insight in the complex structure, as HPβCD is usually available as a mixture of isomers.

RESULTS

The NMR classical approach to the study of inclusion complexes of cyclodextrins is mainly based on the shifts of the protons of the host or of the guest induced by complexation and on 2D-NMR experiments such as nuclear Overhauser enhancement correlation spectroscopy (NOESY) and its rotating frame analogue (ROESY).[10–12] The ^{1}H-NMR spectrum of the HPβCD under study, reported in Figure 1a, consists of very broad resonances. This is due to the presence of a number of isomers and to slow internal motions.[13] The assignment of ^{1}H-NMR signals would require a variable temperature multidimensional NMR study. The problem is further complicated by both the complexity of the ^{1}H-NMR spectra of the biliary acids (e.g. that of **1** in Figure 1b) and the overlapping of the signals from the bile acids and HPβCD in the complexes. Figure 1c reports the ^{1}H-NMR spectrum of the complex between HPβCD and **1**. The few isolated ^{1}H signals, generally the two H(23)'s, CH_3(18), CH_3(19) and CH_3(21),[14] show very low shifts upon complexation[15] and the changes in HPβCD signals are small and hard to evaluate. On the other hand, we cannot expect large shifts induced by systems lacking aromatic rings or paramagnetic species. Some selected differential steady-state NOE measurements on the **4**/HPβCD complex were carried out but not appreciable intermolecular NOEs were detected, presumably because $\omega\tau_c \approx 1$ under our conditions. A ROESY experiment (mixing time 140 ms, spin-lock field 3500 Hz) allowed the detection of very low, and surely not intramolecular, cross peaks between CH_3(18) and CH_3(21) or CH_3(19) (overlapped signals) and HPβCD's signals at 3.87 and 3.72 ppm.

Alternatively, ^{13}C-NMR chemical shifts can be utilized to obtain information on complexation owing to their sensitivity to the changes in the molecular environment.[16] In particular, it is well known that the insertion of a molecule, or of part of it, into the inner cavity of α-cyclodextrin causes a change in the dielectric constant of the medium surrounding the molecule and produces a typical behavior in the ^{13}C-NMR chemical shifts: the signals of the included carbons move to high field (negative shift), whereas those close to the wider cyclodextrin rim move to low field (positive shift).[10]

The proton-decoupled ^{13}C-NMR spectra of HPβCD, of **1** and of the complex **1**/HPβCD are reported in Figure 2a–c respectively. The spectrum of HPβCD consists of broad lines and changes are seen after complexation, but their interpretation is not straightforward. The carbon spectrum of bile acids is instead rather simple and consists of 24 lines whose assignments are known[14] and whose changes upon complexation can be easily monitored. The most significant $\Delta\delta(^{13}C)$ are reported in Table 1. The carbons of the polycyclic region far from the alkyl chain show small shifts (mainly $< \pm$ 0.03 ppm) with some exceptions.[17]

DISCUSSION

Analysis of the data (Tab. 1) shows remarkable trends: the carbon signals that undergo the largest shifts are all localized near, or on, the alkyl chain. The values of these shifts are very similar to those usually reported for other inclusion compounds.[18] Moreover, the carbonyl carbons are shielded in the complex with respect to the free acid (even if only slightly in **3**) and a progressive deshielding is evident along the alkyl chain, starting from C24, with a maximum at C21.[19] This behavior is very similar to that found for molecules complexed by α-cyclodextrin[10] suggesting the insertion of the alkyl chain into the inner cavity of HPβCD. On the other hand, the high $\Delta\delta(^{13}C)$ on C16 and C18 point out that the interaction extends to the cyclopentane ring. Similar results were obtained from a recent ROESY study on the complex **4**/β-cyclodextrin[20] in D_2O. The small shifts of the remaining carbons indicate a weak interaction of the other parts of

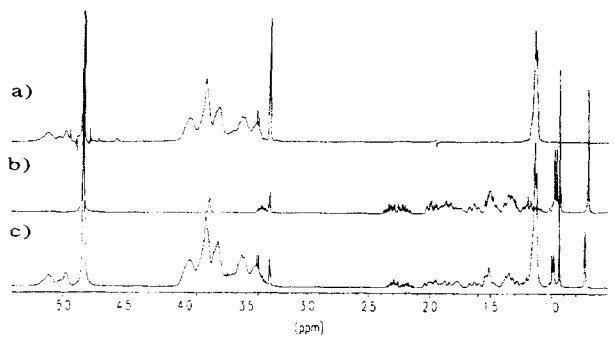

Figure 1 ^{1}H-NMR spectra of (a) HPβCD, (b) chenodeoxycholic acid **1**, (c) complex **1**/HPβCD; the highest signal is that of residual OH.

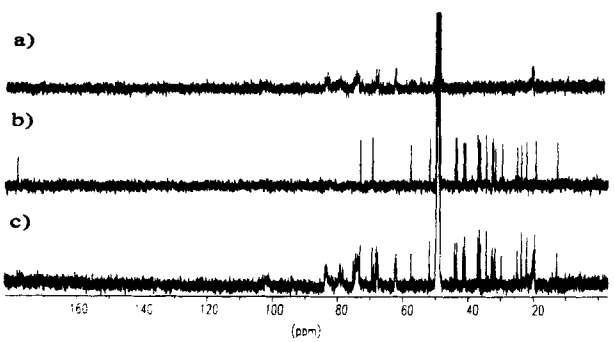

Figure 2 ^{13}C-NMR spectra of (a) HPβCD, (b) chenodeoxycholic acid **1**, (c) complex **1**/HPβCD; the highest signal is that of methanol-d_4.

Table 1 Shifts ($\Delta\delta^a$) in ^{13}C-NMR signals induced by complexation with HPβCD on derivatives **1–4**.

						$\Delta\delta^a$						
guest	C12	C13	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24
1	+0.06	+0.08	+0.05	+0.23	−0.14	+0.23	+0.02	+0.02	+0.41	+0.09	−0.08	−0.18
2	+0.01	+0.01	+0.01	+0.04	−0.01	+0.08	+0.01	−0.03	+0.14	+0.05	0	−0.04
3	0	0	+0.01	+0.05	0	+0.06	+0.02	+0.02	+0.09	+0.04	+0.06	−0.01
4	+0.11	+0.06	+0.07	+0.21	−0.08	+0.21	+0.02	+0.03	+0.30	+0.09	−0.05	−0.07

$^a\Delta\delta = \delta_{complexed} - \delta_{free}$

the molecules with HPβCD. The shifts caused by complexation in **2** and **3**, even those on C21, C18 and C16, are smaller than in **1** and **4** suggesting a weaker affinity of the formers with HPβCD in methanol-d_4 solution. The weaker interaction of **2**, but not that of **3**, with respect to **1** and **4**, for HPβCD parallels that for unsubstituted β-cyclodextrin in water.[9] We agree with Tan and Lindenbaum in ascribing this different behavior to the presence of a hydroxyl group on C12 close to the complexation site, but for derivative **3** no indication of a complexation involving the *cis*-decaline extreme of the molecule is derived from $\Delta\delta(^{13}C)$ in methanol-d_4 solution.

In conclusion, when the interaction of a molecule with a spectrally complex species, such as a partially functionalized cyclodextrins, is to be studied, the analysis of the $\Delta\delta(^{13}C)$ of the guest can be a good alternative of the ^{1}H-NMR approach. Information on the type of interaction and, only qualitatively, on the strength of complexation can be derived. In the present case, the interaction of bile acids with HPβCD is localized at one extreme of the molecule, that bearing the alkyl chain. The model proposed by Inoue[10] for α-cyclodextrin seems to gain a more general validity, even though in this respect further investigations are needed.

MATERIALS AND METHODS

Derivatives **1–3** were used as received from the manufacturer and the lyophilized inclusion complexes were prepared as described by ref. 7 for **4**. ^{1}H and ^{13}C-NMR spectra were obtained at 300 K (unless stated) using a Bruker AMX-400 WB spectrometer operating at 400.13 and 100.61 MHz, respectively, on *ca.* 10^{-2} M CD_3OD solutions which, in the case of the complexes, were left to equilibrate for several days.[21] δ values (ppm) refer to Me_4Si. Typical digital resolution was ± 0.1 Hz for ^{1}H-NMR spectra and ±0.3 Hz for ^{13}C-NMR spectra.

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