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Complexation of 3α,7α,12α-trihydroxy-5β-cholan-24-amine by β- and γ-cyclodextrins

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Abstract

The binding constants, standard molar enthalpy, Gibbs free energy, and entropy changes were determined for the formation of inclusion complexes between 3α,7α,12α-trihydroxy-5β-cholan-24-amine, $C_{24}NH2$, and β-cyclodextrin and γ-cyclodextrin. The stoichiometry of both complexes is 1:1 in agreement with previously reported results for other trihydroxy bile salts. The equilibrium constant values for the formation of the inclusion complex are similar as well. The structure of the $C_{24}NH2$/γ-cyclodextrin complex was studied by ROESY experiments. These results suggest that B, C and D-rings of the steroid skeleton, as well as the side chain, interact with the cyclodextrin cavity, while the A ring of the steroid nucleus remains outside the cavity.
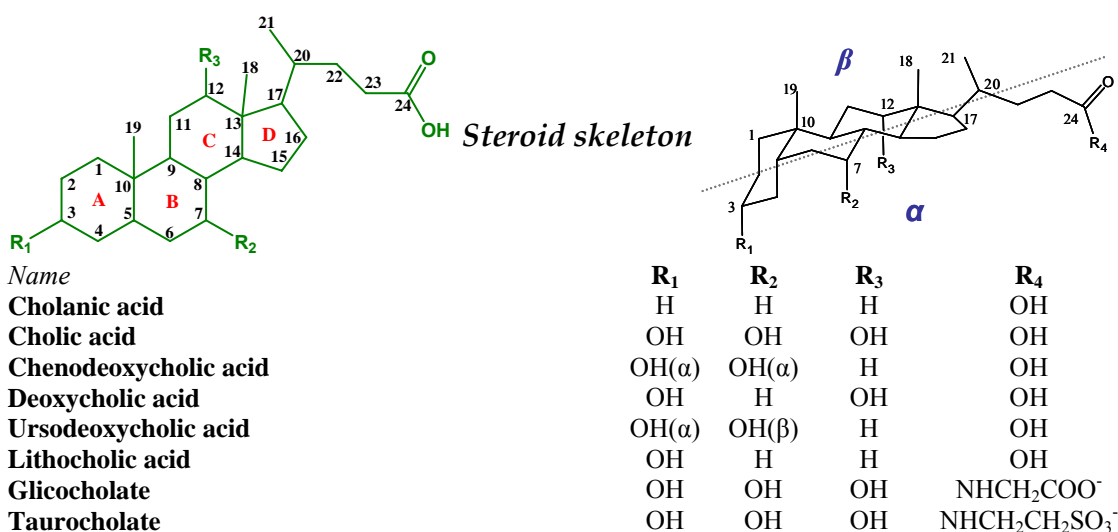
Introduction

Bile salts are biological surfactants which display a great variety of different biological functions.[1] Figure 1 shows the structure and functional groups in common natural bile acids. Bile acids have a hydrophilic side (α), where the hydroxyl groups are located (with the exception of ursodeoxycholic acid), a hydrophobic side (β), where the methyl groups are located, and a side chain carrying the carboxylic group.[2] They have a three-axial chirality.[3] Dispersed in water, these amphiphiles form different types of aggregates in which the hydrophobic faces are shielded from water and the hydrophilic groups are oriented towards the solvent.

[1] Hofmann, A. F., *News Physiol. Sci.*, **1999**, *14*, 24.
[2] Monte, M. J.; Marin, J. J. G.; Antelo, A.; Vázquez Tato, J. *World J. Gastroenterol.* **2009**, *15*, 804.
[3] Miyata, M.; Tohnai, N.; Hisaki, I. Acc. Chem. Res. **2007**, *40*, 694.

Name	R_1	R_2	R_3	R_4
Cholanic acid	H	H	H	OH
Cholic acid	OH	OH	OH	OH
Chenodeoxycholic acid	OH(α)	OH(α)	H	OH
Deoxycholic acid	OH	H	OH	OH
Ursodeoxycholic acid	OH(α)	OH(β)	H	OH
Lithocholic acid	OH	H	H	OH
Glicocholate	OH	OH	OH	$NHCH_2COO^-$
Taurocholate	OH	OH	OH	$NHCH_2CH_2SO_3^-$

Figure 1.- Structure of the most important bile acids and their glycine and taurine conjugates.

Cyclodextrins (CDs) are cyclic oligosaccharides built up from 6, 7, or 8 glucopyranose units, linked through α-1,4 glucosidic bonds, named α-, β-, or γ-CD, respectively (Figure 2). They are truncated cone-shaped, the central cavity being hydrophobic, where various kinds of organic molecules (guests) can be trapped thus forming inclusion compounds. This allows the solubilisation in water of poorly soluble compounds as drugs[4] and cholesterol.[5] Other sterols, as bile salts, also form inclusion compounds with cyclodextrins.[6,7] The guests enter by their side chain into the cavity of the cyclodextrin by its secondary rim. However, the oxidation of the 3-hydroxyl group of sodium cholate, NaC, to its 3-ceto derivative, Na3CC, affecting the hydrophobic character of the compound (cmc values being 20 mM and 15 mM for NaC[8] and Na3CC, respectively) modifies this behaviour and Na3CC, in comparison to NaC, changes the side of its entrance into the γ-CD cavity. [9]

[4] T. Loftsson, M. E. Brewster and M. Masson, *Am. J. Drug Delivery* **2004**, 2, 175–261.

[5] Alvarez, M.; Antelo, A.; Jover, A.; Meijide, F.; Gancedo, C.; Galantini, L.; Vázquez Tato, J. *J. Inclusion Phenom. Macrocycl. Chem.* **2009**, *63*, 309.

[6] Cabrer, P. R.; Alvarez-Parrilla, E.; Meijide, F.; Seijas, J. A.; Rodríguez Núñez, E.; Vázquez Tato, J. *Langmuir* **1999**, *15*, 5489.

[7] Ramos Cabrer, P.; Alvarez-Parrilla, E.; Al-Soufi, W.; Meijide, F.; Rodríguez Núñez, E.; Vázquez Tato, J. *Supramol. Chem.* **2003** , *15*, 33.

[8] Coello, A.; Meijide, F.; Rodríguez Núñez, E.; Vázquez Tato, J. *J. Phys. Chem.* **1993**, *97*, 10186.

[9] Tellini, V. H. S.; Jover, A.; Novo, J. V. T.; Meijide, F.; Garcia, J. C.; J. V. Tato 8th International Electronic Conference on Synthetic Organic Chemistry. ECSOC-8. 1-30 November 2004. http://www.lugo.usc.es/~qoseijas/ECSOC-8/ (F004)

<div align="center">(a)   (b)   (c)</div>
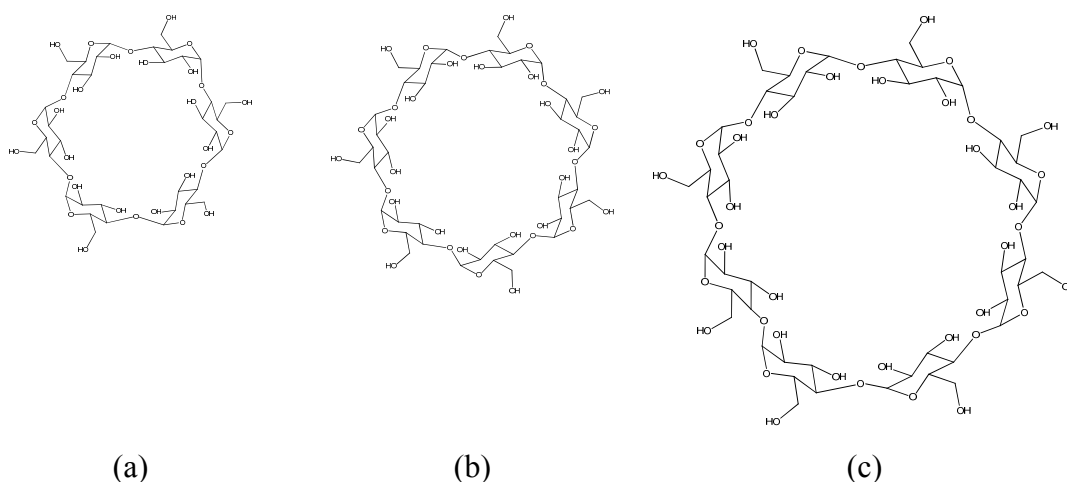
Figure 2.- Structures of cyclodextrins. (a) α-CD. (b) β-CD. (c) γ-CD.

The main aim of the present communication is to check whether the lateral chain functional group of the guest can or cannot modify the side by which it enters into the hydrophobic cavity of the γ-CD. For this purpose, the 3α,7α,12α-trihydroxy-5β-cholan-24-amine, $C_{24}NH2$, in which the 24-carboxylate group of sodium cholate (NaC) is substituted by an amine group (Figure 3). The complexation process between $C_{24}NH2$ and β-CD is also studied. Since the steroid body of bile salts is too big to enter into the α-CD cavity[7] this cyclodextrin will not be considered. ITC and NMR techniques have been used.
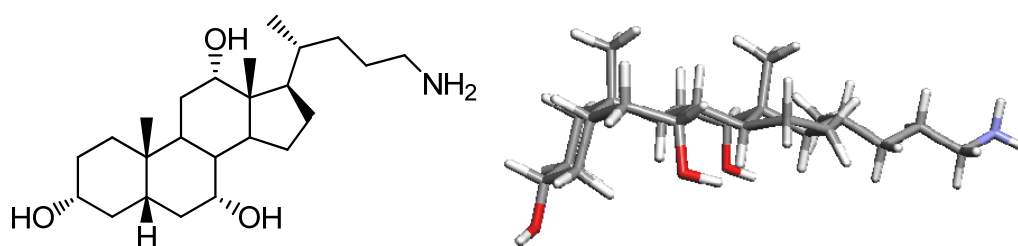

Figure 3.- Structure of 3α,7α,12α-trihydroxy-5β-cholan-24-amine (*$C_{24}NH2$*).

Results and discussion

To avoid any influence of the demicellization process of $C_{24}NH2$ on ITC measurements, the concentration of this surfactant was kept below its cmc value.[10] Thus experiments were carried out with an initial concentration of 0.5 mM for $C_{24}NH2$ in the measurement cell, while CDs concentration in the syringe was 10mM. Figure 4 shows

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[10] Fini, A.; Fazio, G.; Roda, A.; Bellini, A. M.; Mencini, E.; Guarneri, M. *J. Pharm. Sci.* **1992**, *81*,726.

two examples of calorimetric enthalpograms for the titration of $C_{24}NH2$ with β–CD and γ–CD.

(a)   (b)
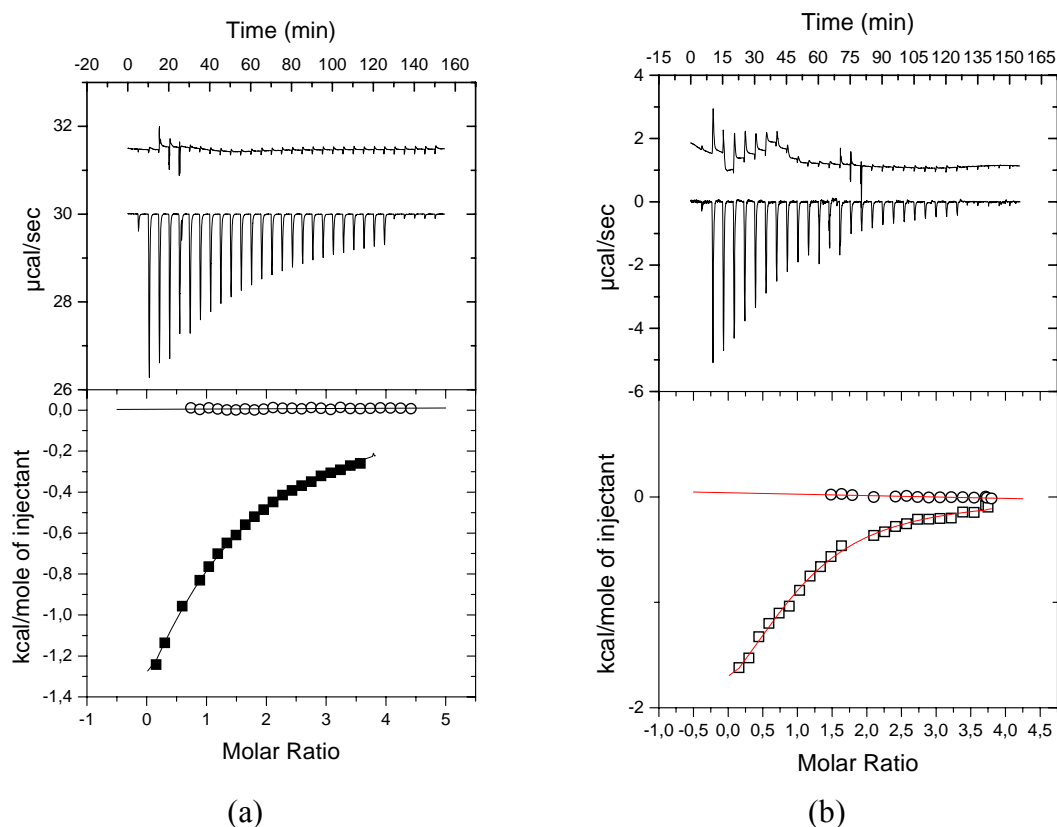
Figure 4.- Enthalpograms observed upon injecting (a) 10 μL aliquots of β-CD solution (10mM) into the sample cell containing a 0.5 mM solution of $C_{24}NH2$. (b) Idem with γ–CD. Experiments were performed at 30 °C.

The experimental results were fitted to the "one set of binding sites" model.[11] Table 1 shows the thermodynamic parameters deduced from this analysis.

Table 1.- Thermodynamic parameters from ITC experiments. Experimental data were fitted to the one set of binding sites model. Experiments were carried out at; T = 30,01ºC and pH= 7,55 (phosphate buffer, 50mM) to ensure ionization of the amine group of $C_{24}NH2$.

Host	n	$Log(K_S / M^{-1})$	$\Delta G^0 / kJmol^{-1}$	$\Delta H^0 / kJmol^{-1}$	$T\Delta S^0 / kJmol^{-1}$
β-CD	0.98 ± 0.07	3.00 ± 0.06	-17.4 ± 0.3	-24.8 ± 0.4	3.1 ± 3.9
γ–CD	1.00 ± 0.04	$3.61 \pm 0,8$	-20.4 ± 0.9	-12.5 ± 0.7	12.4 ± 0.8

The value obtained for *n* indicates that $C_{24}NH2$ forms inclusion complexes with both cyclodextrins with a 1:1 stoichiometry, in agreement with which was observed for the complexation of other trihydroxy bile salts.[7] Equilibrium constants are of the same order of magnitude as well. It must be noticed that the contribution of the enthalpy to

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[11] *Origin . ITC Data Analysis in Origin, Tutorial Guide*, versión 5.0, **1998**

the free energy for the $C_{24}NH2/\gamma$–CD system is half the value for the $C_{24}NH2/\gamma$–CD one. This is probably related to the higher diameter of γ–CD leading to a looser structure of the guest inside the host. In fact bile salts enter deeper inside into the γ–CD cavity than into the β-CD one.[7] This less favourably enthalpy contribution is overcompensated by the entropy term. The larger positive value observed for the $C_{24}NH2/\gamma$–CD system is probably related to a release of a lager number of water molecules from the cavity of γ-CD in comparison to β-CD.

The structure of the $C_{24}NH2/\gamma$–CD complex was studied by NMR experiments, particularly COSY (Figure 5) and ROESY (Figure 6) experiments. Here we will only comment on the last one. Hydrogen atoms of the surfactant will named with "P" and those of the cyclodextrin by "H".
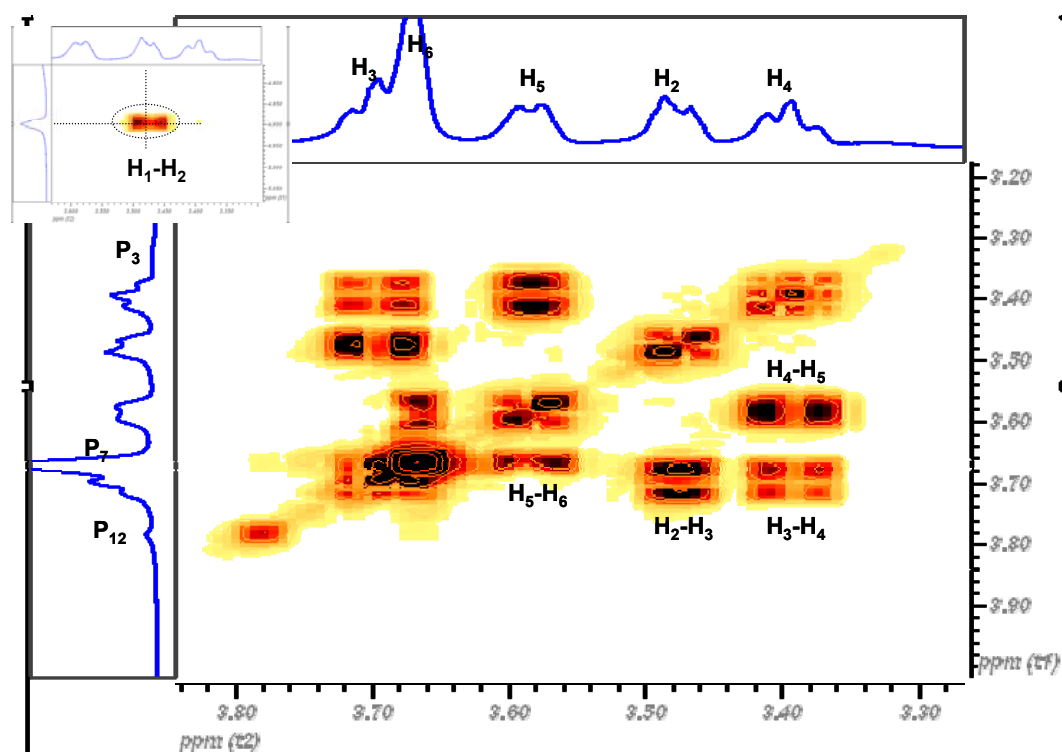

Figure 5.- COSY spectrum of $C_{24}NH2/\gamma$CD system..

Figure 6 shows that: (i) the protons of methyl group P21 (on the side chain of the surfactant) has strong cross peaks with both H3 and H5; (ii) this is also the case P18 (on the D-ring) and P19 (linked to the C10 carbon atom, common to A- and B-rings); (iii) P17, P16 and P15 (steroid D-ring), as well as P22 and P23 (side chain) have cross peaks with H3; (iii) P6 and P8 (B-ring) have only interactions with H3; (iv) no protons on the A-ring have cross peaks with any protons of the cyclodextrin. This information, together with the fact that H3 is close to the secondary hydroxyl edge of the

cyclodextrin while H5 is closer primary one, suggest that the surfactant enters into the cyclodextrin cavity by its secondary rim reaching the B-ring of the bile acid derivative (Figure 7) as normal bile salts do.
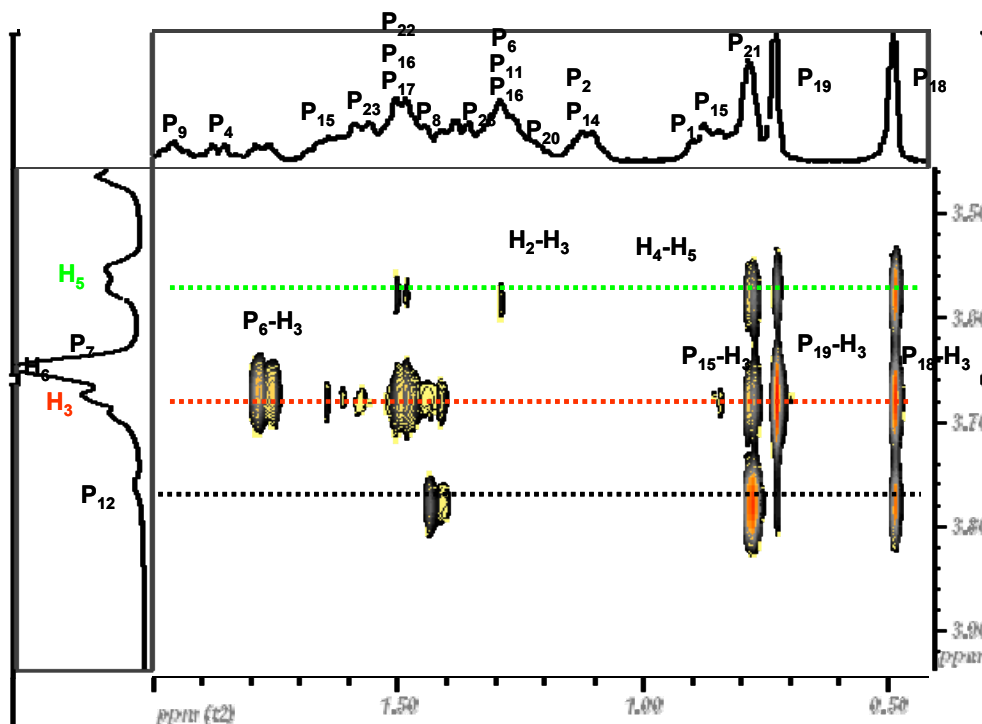
Figure 6.- ROESY spectrum of $C_{24}NH2/\gamma CD$. The region of mostly bile salt chemical shift is depicted at the x-axis and the region of CD chemical shift at the y-axis.
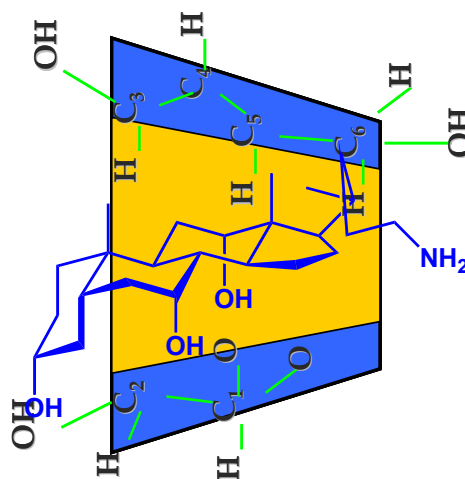
Figure 7.- Schematic representation of the 1:1 $\gamma CD /C_{24}NH2$ complex, deduced from ROESY cross-peak interactions.

Conclusion

The change of the carboxylic group located at the lateral chain of ordinary bile salts does not modify their associative behaviour towards cylodextrins.

Experimental section

ITC measurements. The description of the thermodynamic background for ITC experiments can be found elsewhere[3] and experimental procedure in the MicroCalc calorimeter instructions. ITC experiments were carried out at 30.00 (0.01 °C). Experimental titration curves were analyzed with the MCS Origin ITC 5.0 program delivered with the instrument. Average values of the thermodynamic parameters and their standard deviations were calculated from 2-3 experimental runs.

General NMR Procedure: ^{1}H, ^{13}C, DEPT 135 NMR spectra and Rotating-frame Overhauser effect spectroscopy (ROESY) experiments were recorded on a Bruker AMX spectrometer at 500 MHz at 298.1 K. Conditions for ROESY were as follows: [γ-CD] = 5.24 mM, [$C_{24}NH2$] = 5.01 mM, pD= 7.36, phosphate buffer concentration of 0.2 M. Sample was kept 24 h before measurement for equilibration; relaxation delay 0 s, mixing time = 300 ms; spectral width = 10 ppm. All NMR experiments were carried out in D_2O.