

Full Research Paper

Inclusion Compounds of Dehydrocholic Acid with Solvents

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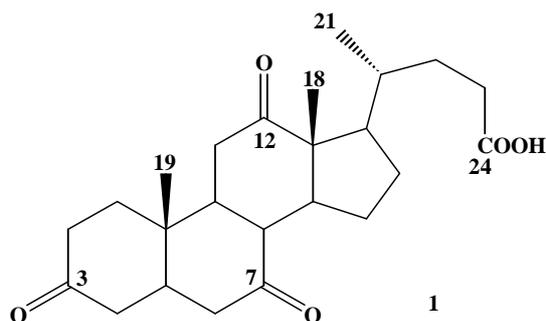
Abstract: The host-guest inclusion of various organic solvents within dehydrocholic acid has been studied and the selectivity of enclathration determined by competition experiments.

Keywords: Dehydrocholic acid, host-guest, inclusion compounds, selectivity

1. Introduction

The process of selective enclathration has been studied in a variety of host-guest systems and bile acids have shown a particular ability in the inclusion of organic guest molecules as aliphatic and aromatic hydrocarbons, alcohols, ketones, esters, nitriles, epoxides, amides etc [1]. In most cases, as for example for cholic, deoxycholic, chenodeoxycholic, amide derivatives and norbile acids, the inclusion aptitude of host molecules has been confirmed in the crystalline state [1]. In recent years we have studied the inclusion ability of dehydrocholic acid **1** for the resolution of organic racemates [2] of aryl methyl sulfoxides [3] and cyclic amides [4], including the precise definition of the different structures involving the sole host or the host-guest assemblies [5].

In this frame, it was found that dehydrocholic acid may be obtained in two polymorphic forms, α and β , the latter being thermodynamically more stable [6]. The α -form crystallizes in such a way that two independent molecules are linked in dimers by two hydrogen bonds between the carboxylic acid groups at C-24, whereas in the β form two hydrogen bonds are formed between the carboxylic acid functionality and the carbonyl group at C-3.



We now present the results of the inclusion process occurring between dehydrocholic acid **1** in its α -form and different solvent molecules i.e acetone (AC), dimethylsulfoxide (DMSO), dimethylformamide (DMF), N-methyl-2-pyrrolidinone (NMP). Competition between couples of these guests with **1** and the related selectivities have been determined [7].

2. Results and Discussion

2.1 Inclusion compounds

The host compound **1** was prepared by oxidation of cholic acid with the Jones reagent [8], followed by crystallization from acetone. The host prepared in this way contains ordered acetone molecules in a ratio **1**:acetone of 2:1, as obtained by ^1H NMR analysis. The guest acetone could be quantitatively removed from the structure by heating under vacuum at 140 °C to form **1** in the polymorphic α -form. The X-ray structure previously obtained for **1** put in evidence the absence of channels which characterize the crystal structure of many steroid compounds [5a,b]; consequently this form of **1** must have high flexibility to alter its structure to accommodate guest species. Following these findings we have prepared inclusion compounds of dehydrocholic acid with a variety of organic solvents, that accommodate within **1** in assemblies of variable host:guest ratio as shown in Table 1.

Table 1. Guests, inclusion ratio and selected resonances for inclusion in dehydrocholic acid **1**.

Guest	Host :guest ratio	Selected ^1H NMR resonances ^a
AC	2 : 1	δ 1.09 (s, 3H, 18-H3); 2.19 (s, 6H, COCH ₃)
DMSO	1 : 1	δ : 1.08 (s, 3H, 18-H3); 2.68 (s, 3H, SCH ₃)
DMF	1.2 : 1	δ 1.08 (s, 3H, 18-H3); 3.00 (s, 3H, NCH ₃)
NMP	1.1 : 1	δ 1.08 (s, 3H, 18-H3); 3.44 (dd, J= 7.1 Hz, 2H, NCH ₂)

^a resonances used to determine the host:guest ratio, for numbering see structure **1**.

Worthy of note in all cases the inclusion is reversible and the empty powder obtained after evacuation under vacuum of the various guests exhibits a X-ray powder diffraction (XRPD) pattern with peak positions and intensity identical to that of the original structure of **1** α -form [5a], indicating that the inclusion process and consequently the structural change are fully reversible, equation (1) for the DMSO case. The inclusion compounds are formed by direct absorption of the guest in solution into the crystal lattice of solid dehydrocholic acid.



In Figure 1 are reported the XRPD of **1** with and without DMSO. In particular, pattern A refers to **1** α -form [5a], pattern B to the **1**·DMSO inclusion derivative which shows a different but well-defined chclathrate α' -phase compared to A, and pattern C obtained upon heating at 170 °C at 12 mmHg for 4h.

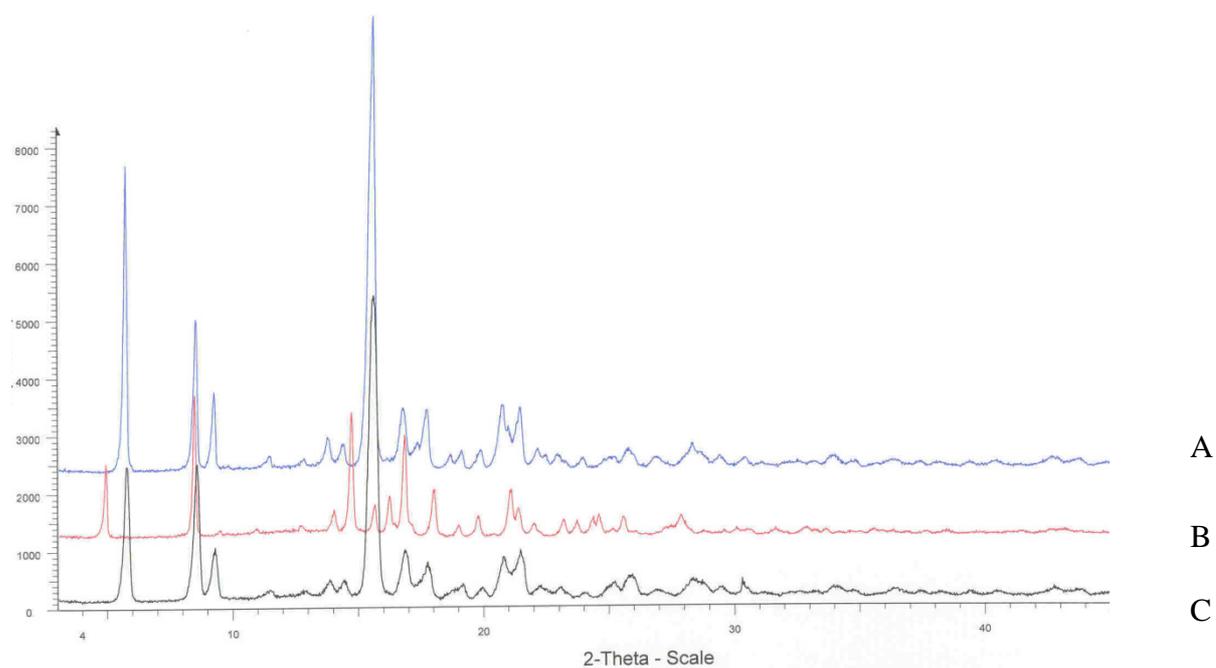


Figure 1. X-ray powder diffraction (XRPD) pattern of **1** as α -form (A), included with DMSO (B) and after removal of the DMSO included guest (C).

The reversibility of the process is confirmed and these studies demonstrated that the XRPD patterns in the presence and absence of the guest are an effective means of monitoring structural changes that may be occurring upon guest absorption-desorption [9].

2.2 IR analysis

The IR analysis of the inclusion derivatives, obtained in KBr pellets, evidenced a shift in the characteristic vibrational modes of the sulfoxide and carbonyl groups of the different solvents, upon

complexation, significantly moved to lower wavenumbers with respect to free SO and CO absorptions, Table 2.

Table 2. Assignment and wavenumbers of the vibrational bands (KBr) of free dehydrocholic acid **1**, DMSO, DMF, NMP and the related inclusion compounds.

Compound	Guest	ν_{XO} (cm^{-1})	1 ν_{CO} (cm^{-1})
1			1707
DMSO	1040	ν_{SO}	
1 · DMSO	1025	ν_{SO}	1707
DMF	1666	ν_{CO}	
1 · DMF	1649	ν_{CO}	1719, 1700
NMP	1667	ν_{CO}	
1 · NMP	1650	ν_{CO}	1719, 1705

In agreement with previous interpretations [10] the lowering of the wavenumber of the sulfoxide and carbonyl moieties absorption upon inclusion is a clear indication that coordination within the bile acid host occurs, also for these new complexes, through the sulfoxide or carbonyl groups (guest)O...H-O(host) hydrogen bonds, following a weakening of the XO bond strength, as depicted in Figure 2.

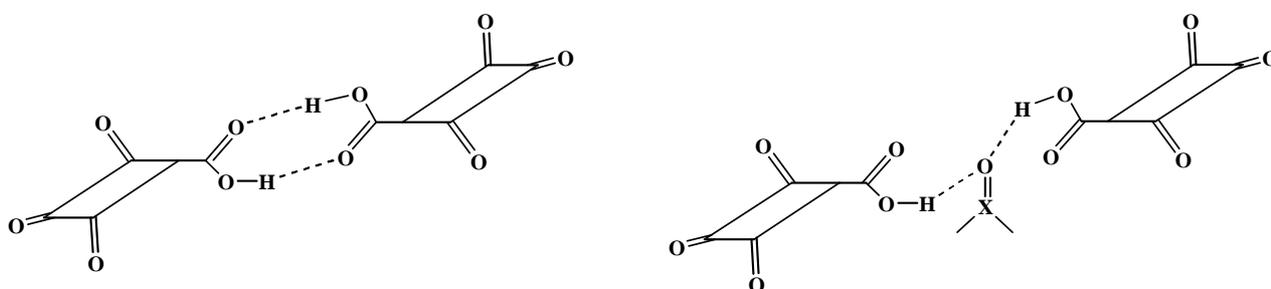


Figure 2. Assembly of **1** α -form as obtained by X-ray analysis and proposed assembly for inclusion solvents in **1**.

2.3 Thermal analysis

Thermal analysis (TG) and simultaneous thermal differential analysis calorimetry (STDA) were carried out on a Mettler Toledo TGA/STDA 851. The pertinent results, collected for the inclusion compounds: **1**·0.5 AC (clathrate A); **1**·DMSO (clathrate B); **1**·0.9 NMP (clathrate C); **1**·0.83 DMF (clathrate D) are shown in Figure 3. The related curves indicate that inclusion compounds **1**·0.5 AC, **1**·DMSO and **1**·0.9 NMP decompose in a single step in the mass loss curve (TG), whereas the STDA analysis showed a first endothermic signal due to guest loss, followed by a endothermic signal due to the melt of the host, Figures 3 A-C.

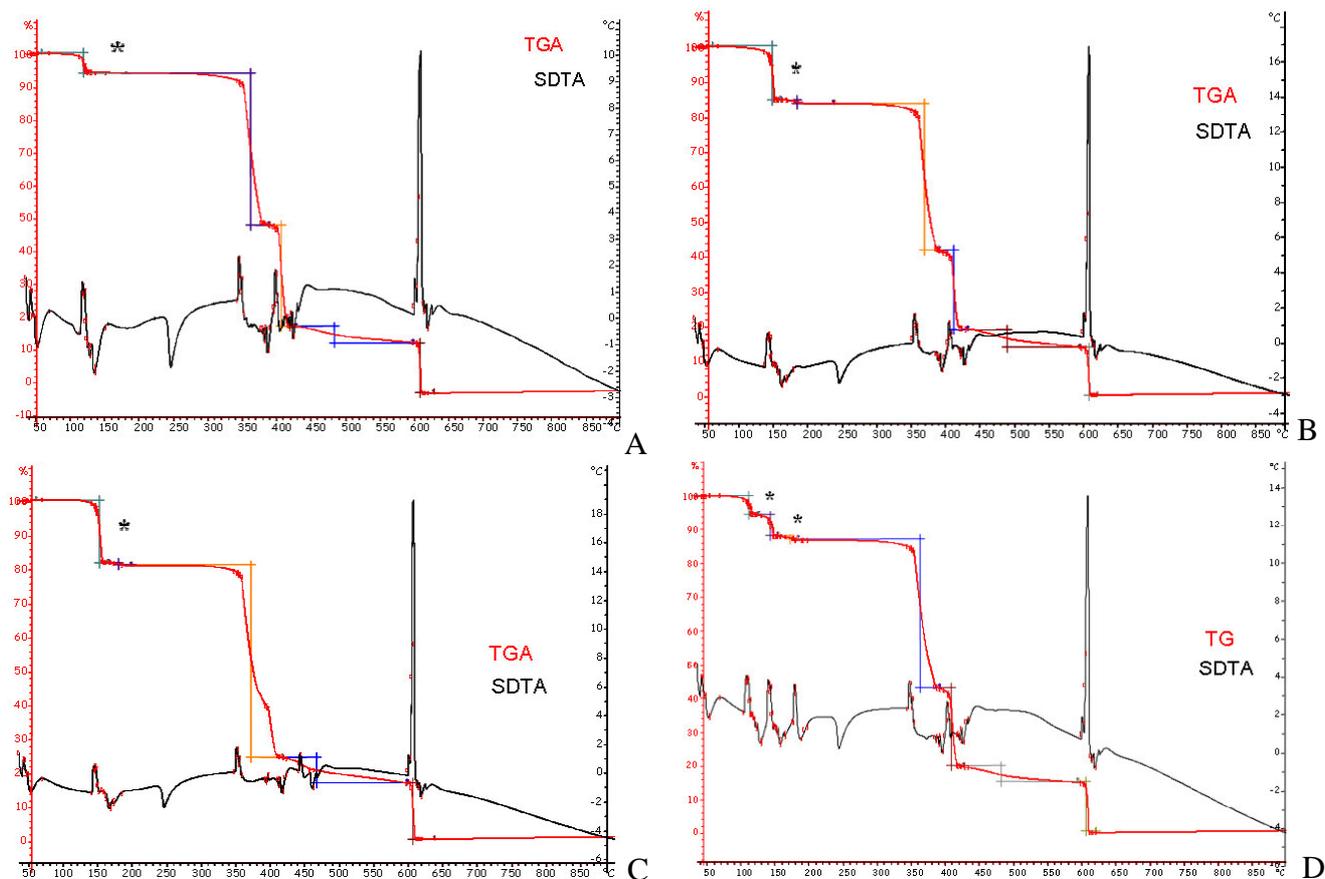


Figure 3. TG and STDA analysis of 1·0.5 AC (clathrate A); 1·DMSO (clathrate B); 1·0.9 NMP (clathrate C); 1·0.83 DMF (clathrate D); asterisks denote the weight loss step(s).

Table 3. Thermal analysis result for the inclusion compounds.

Inclusion compounds	Obs. weight loss from TG (%)	Calc. weight loss from NMR (%)	Guest release T_{on} °C	Host melting T_m °C	Bp of pure guest T_b °C	$T_{on}-T_b$
1·0.5 AC (clathrate A)	6.0	6.7	86.2	244	56	30.2
1·DMSO (clathrate B)	15.5	16	155	243	189	-34
1·0.9 NMP (clathrate C);	18.5	18.3	160	243	202	-42
1·0.83 DMF (clathrate D)	5.44	13.1	122	244	153	-31
	6.48		146			-7
	1.06		184			31

Calculated (NMR), observed mass losses and onset temperatures for guest release (T_{on}) as well as the difference between T_{on} , the boiling point of pure guest (T_b) and the melting point (T_m) of host for each inclusion compound are given in Table 3 [11]. The decomposition of 1·0.83 DMF (clathrate D) is

more complex compared with previous cases. The TG curve in fact has three distinct steps but the mass losses do not correspond to a simple stoichiometry and STDA shows three distinct endothermic signals for the guest loss as well as one for the host melt. To note that in the first three cases the observed mass losses from TG traces are in good agreement with the calculated value for that particular host:guest ratio.

2.4 Competition experiments

Here we described the competition experiments between NMP and DMF, DMSO and DMF, DMSO and NMP for the host dehydrocholic acid. These experiments were carried out in solid-liquid biphasic system as follows. A series of 11 vials was made up with mixture of two guest, dissolved in a mixture diethyl ether / ethyl acetate 80/20, such that the mole fraction of a given guest varied from 0 to 1. The insoluble host was added to each mixture keeping the total guest : host ratio at 20 : 1. The vials were sealed and left at room temperature for 24 hours; the inclusion compounds were filtered off, washed with diethyl ether and dissolved in chloroform. These solutions, as well as the mother liquors, were analysed by gas chromatography. The results are shown in Figure 4 A-C, in which X represents the mole fraction of a given guest in the liquid mixture and Z that of same guest entrapped in the crystals [12]. The diagonal line represent zero selectivity.

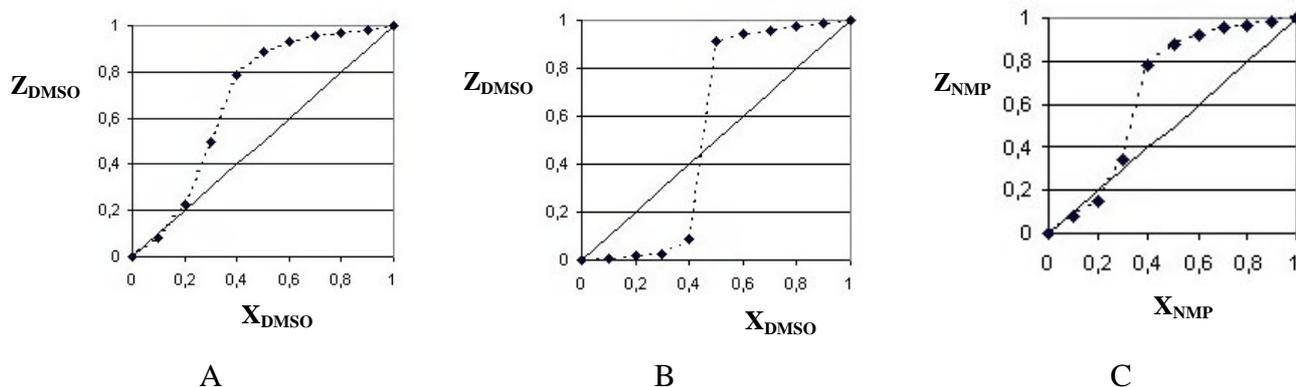


Figure 4. Competition experiments of two different guests with respect to dehydrocholic acid host. (A): selectivity of dimethyl sulfoxide vs dimethylformamide; (B): selectivity of dimethyl sulfoxide vs N-methyl-2-pyrrolidinone; (C) selectivity of N-methyl-2-pyrrolidinone vs dimethylformamide.

The results of Figure 4A show that DMSO is preferentially enclathrated over DMF when its mole fraction X_{DMSO} is > 0.2 ; before this value the selectivity is close to zero. Similarly, Figure 4C, NMP is preferentially enclathrated over DMF when its mole fraction X_{NMP} is > 0.3 . The competition experiments carried out between DMSO and NMP showed a different behaviour, Figure 4B, being the selectivity dependent on the concentration of the guest. The coordination of DMSO is, in fact, strongly favoured when its mole fraction is ≥ 0.5 , while enclathration of NMP is preferred below this limit.

In summary, a multiple technique approach applied to organic inclusion compounds confirmed to be particularly useful for the study of complex systems. In the present case XRPD, IR, TG and STDA analysis were used to investigate the inclusion process occurring between dehydrocholic acid **1** in its α -form and different solvent molecules i.e acetone (AC), dimethyl sulfoxide (DMSO),

dimethylformamide (DMF), N-methyl-2-pyrrolidinone (NMP). Dehydrocholic acid has a remarkable ability to reversibly bind guest molecules from solid-liquid biphasic. Competition between couples of these guests with **1** and the related selectivities have been determined.

3. Experimental Section

¹H NMR spectra were recorded in CDCl₃ solution with a Varian Gemini 300 spectrometer operating at 300 MHz, with TMS as external standard. IR spectra, were run with a Nicolet 510P spectrometer operating in Fourier transform mode. X-ray powder diffraction analyses were recorded with Bruker D8 advance diffractometer. Thermal analyses were carried out on a Mettler Thermo-gravimetry and on a Toledo TGA/STDA 851. GC was performed with Fisons 9000 series instrument, equipped with a capillary Megadex DETTBS column. AC, DMSO, DMF, NMP were p.a. quality and used as obtained from the supplier.

Preparation of inclusion compounds by absorption method. Crystals of **1** (100 mg, 0.25 mmol) were dipped in a solution (2 mL) of appropriate guest (5 mmol) in diethyl ether/ethyl acetate (80/20) at room temperature. After 24 h the solid was collected by filtration, washed with diethyl ether (5 mL), dried in air and analyzed for the presence of respective guest by ¹H NMR analysis.

Competition experiments were carried out between pairs of guests as follows: 50 mg (0.125 mmol) of **1** was added to each solution of a series of 11 vials made up with mixture of two guest in diethyl ether/ethyl acetate 80/20 such that the mole fraction of a given guest varied from 0 to 1. The total guest-host ratio was kept at 20:1 in each case. The vials were sealed and solid-liquid mixtures left at room temperature for 24 hours. The inclusion compounds were filtered off, washed with diethyl ether and dried at room temperature. About 5 mg of crystals of various inclusion were dissolved in chloroform (2 mL); the solutions were analyzed by gas chromatography. The ratio of the guest compounds are calculated from the integration of the peaks after calibration.

Acknowledgements

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