Polymorphism of dehydrocholic acid: crystal structure of the β-phase and guest-mediated solid phase conversion

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Dehydrocholic acid may be obtained in two polymorphic forms: α and β. The complete characterization and the crystal structure of the β-phase (obtained by unconventional powder diffraction methods) are described and discussed. Conversion of the α-form into the thermodynamically more stable β-polymorph may be obtained through a room temperature solvent-mediated process. On the other hand, transformation of the β-form into the α-form is realized through two distinct pathways: *via* a temperature-mediated process or as a guest-mediated solid state conversion, without the intervention of an amorphous state. *R*-(+)-Methyl *p*-tolyl sulfoxide is the guest of choice for this unusual β-to-α transformation.

Introduction

Polymorphism (a kind of supramolecular isomerism) addresses the existence of multiple crystalline phases for the same 'species'.[1,2] Since the properties of a given solid strictly depend on its structure, polymorphic forms possess different physical and even chemical properties.[3] In this respect, polymorphism has particular importance in pharmaceutical research and development, since differences in thermodynamics (and kinetics) can affect the stabilization, solubility and bioavailability of a given drug.[4] Selected examples include, among others, indomethacin,[5] foconazole,[6] lomeridine[7] and candesartan cilexetil.[8]

Dehydrocholic acid (3,7,12-triketo-5β-cholan-24-oic acid, **1**, hereafter called DHA and shown in Chart 1) is an unnatural bile acid having the cholanoic acid structure. This derivative, usually obtained from cholic acid *via* oxidation of the C3, C7 and C12 hydroxyl groups, possesses important therapeutic and chemical applications.[9] From a pharmaceutical point of view, DHA is used as a choleretic and for the treatment and prevention of gallstones.[10,11] In supramolecular chemistry, this bile acid was found to act as a host molecule for the inclusion, and consequent optical resolution, of different guests, like alkyl aryl sulfoxides[12,13] and cyclic amides.[14]

In the course of our studies on the inclusion of organic sulfoxides,[13] we determined the crystal structure of DHA, obtained by crystallization from ethyl acetate. This phase, hereafter referred to as the α-form, is characterized by the presence of conventional dicarboxylic acid dimers (Scheme 1).

Here we report on the preparation and the complete characterization of a new polymorph of DHA, referred to as the β-form, by scanning electron microscopy (SEM), IR and solid-state NMR spectroscopy, differential scanning calorimetry (DSC), thermogravimetry (TG) and X-ray powder diffractometry (XPRD). It is noteworthy that its detailed crystal and molecular structure, determined by *ab initio* XRPD methods on a *laboratory* instrument, highlights, with respect to the α-form, important conformational changes of the carboxylic side-chain and a new (unexpected) supramolecular motif. In addition, the host-guest properties of the β-form and a guest-mediated solid state β-to-α conversion are described and discussed.

Results and discussion

Preparation of β-DHA and comparison with the α-form

Dehydrocholic acid in its α-form is a stable polymorph obtainable by standard crystallization[11] from solvents like ethyl acetate, acetone, water–acetone mixtures or acetonitrile. The β-form may be obtained, through a solvent-mediated

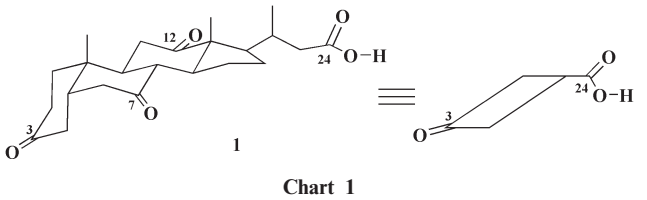
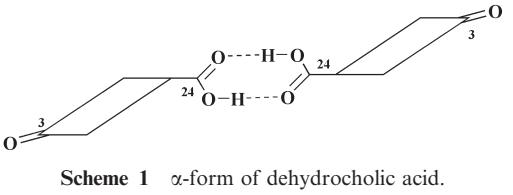


Chart 1



Scheme 1 α-form of dehydrocholic acid.

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transformation, by means of prolonged contact of the solid α-form with water–acetic acid (1:1) or water–HCl solutions (at pH 3–4) under stirring [eqn. (1) in Scheme 2]. The time required for the conversion is either 3 or 40 days, depending on the solvent. On the other hand, the β-form may be reconverted into the α-homologue by heating above 228.5 °C [eqn. (2) in Scheme 2 and below]. This thermal process is not reversible.

The relative stability of the two forms may be assessed by suspending α-DHA in water–acetic acid (1:1) or water–HCl solutions and following the modifications of the mixture with time. What is experimentally observed is the progressive transformation of the α-form into the β-form, thus confirming the latter as the thermodynamically stable polymorph. Additional support for these findings may be found in the solubility data, which clearly puts into evidence a higher solubility of the α-form with respect to the thermodynamically more stable β-polymorph (0.79 *vs.* 0.55 g, respectively, in 100 mL ethyl acetate).

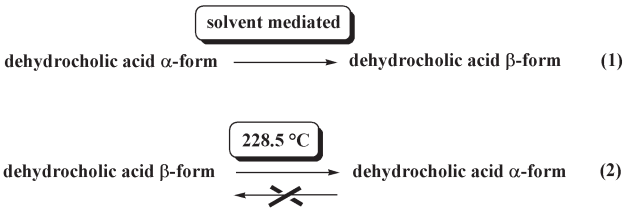
Physico-chemical characterization of α- and β-DHA

The morphology of the two polymorphs, determined by SEM analysis, is shown in Fig. 1. Crystals, or crystal aggregates, of the α-form show 'smoother' faces than those of the β-polymorph, the latter having an almost spherical shape.

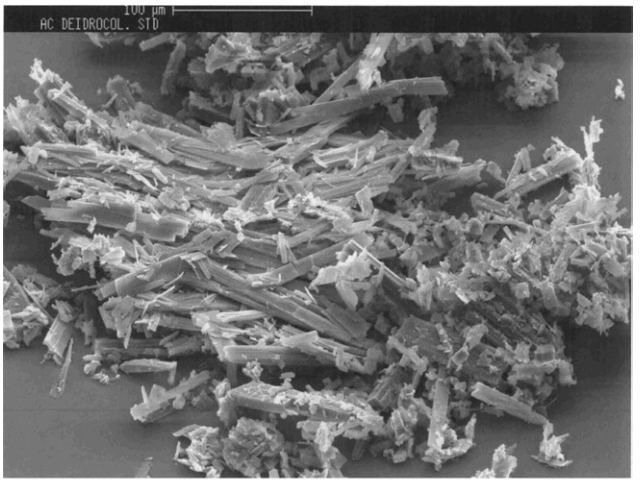
The IR spectra of both the α- and β-forms are shown in Fig. 2. In the former, a strong absorption band due to the stretching vibrations of the carbonyl groups is centred at 1708 cm^{-1}, which shifts and splits into three bands at 1751, 1719 and 1706 cm^{-1} in the latter. The absorption at 1751 cm^{-1} may be assigned to the C=O carboxylic group. In addition, a broad intense O–H stretching band in the 3300–2500 cm^{-1} region is displayed by the α-polymorph as the result of the strong intermolecular hydrogen bonding of the tail-to-tail arrangement shown in Scheme 1. This broad band is replaced by a much sharper signal at 3361 cm^{-1} in the IR spectrum of the β-form, likely suggesting a different H-bond pattern.

A further characterization of the two polymorphs has been obtained by DSC and TG analyses. The DSC trace of the α-form, shown in Fig. 3(a), consists of a single endothermic peak at 243 °C, ascribable to the melting of the compound. In contrast, that of the β-form, Fig. 3(b), shows two endothermic peaks at 228.5 and 243 °C. In the 20–320 °C temperature range, the simultaneous TG analysis of both forms showed no significant weight loss. Accordingly, the overall process of Fig. 3(b) may be interpreted as the β-to-α phase transition followed by the melting of the α-polymorph. Selected examples of a similar phenomenon refer to indomethacin[15] and TKS159.[16] The thermal behaviour of the β-form is irreversible.

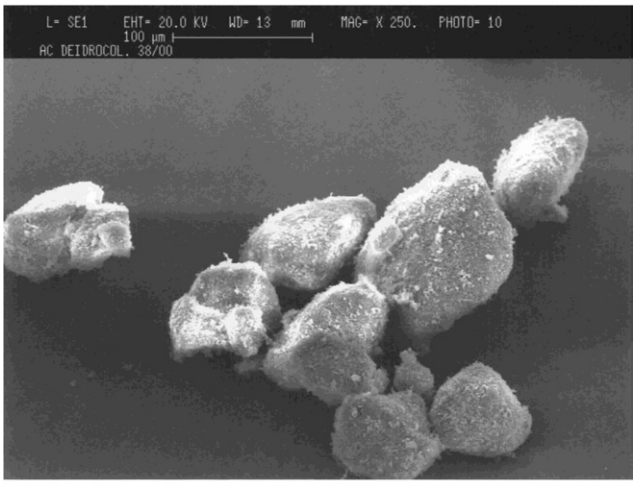
The solid state ^{13}C NMR spectra of the two forms are shown in Fig. 4. The 160–220 ppm resonance range presents the most distinguishing features to characterize the polymorphs. The β-form shows a single peak at 173.3 ppm, ascribable to the carboxylic carbon, and three distinct resonances, assignable to the carbonyls, at 209.6, 212.5 and 213.3 ppm [Fig. 4(b)]. The absence of other signals indicates a crystal formed by a sequence of molecules possessing an identical spatial arrangement. On the contrary, the α-derivative shows two



Scheme 2 Transformations of the α- and β-forms.



(a)



(b)

Fig. 1 Morphology of the two DHA polymorphs, as determined by SEM analysis: (a) α-form and (b) β-form.

signals that may be assigned to the carboxylic carbons (180.5 and 181.0 ppm) and four resonances attributable to the carbonyl carbon atoms. These findings agree with the presence, in α-DHA, of two slightly different (and crystallographically independent) molecular units.

Fig. 5 shows the X-ray powder diffraction patterns of the two forms. Sharp and intense X-ray diffraction peaks, for both the α- and the β-forms, indicate their highly crystalline nature. While the structure of the α-form is known from conventional single-crystal X-ray analysis, that of the β-form, in the absence of single crystals, had to be retrieved from polycrystalline samples only. Thus, on the basis of the experience accumulated by some of us in recent years,[17] we decided to tackle its complete crystal structure determination by *ab initio* XRPD methods.

Crystal structure of β-DHA

Crystals of β-DHA are monoclinic, space group $P2_1$, with two symmetry related molecules per unit cell. As in the α-polymorph (for which a summary of crystal data is synoptically reported in Table 1)[11] and in the 1:1 inclusion product with *R*-(+)-methyl *p*-tolyl sulfoxide (hereafter referred as *p*TolSOMe@DHA), the shorter cell axis (*ca.* 6.8 Å) is related to the packing of DHA molecules on top of each other, the 'average' reference plane being that of the B, C and D steroidal rings.[18] This fact alone shows that it is the shape of such a corrugated molecule that strictly imposes the overall packing in

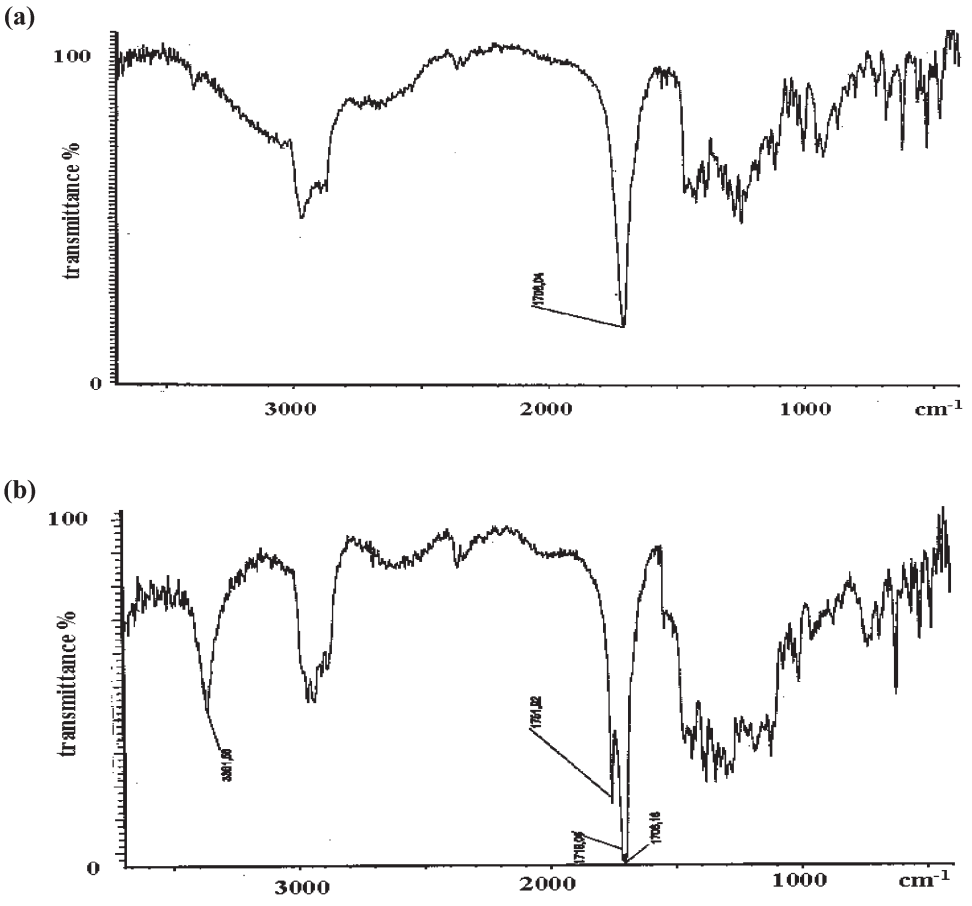


Fig. 2 IR spectra (KBr pellets) of the two DHA polymorphs: (a) α-form and (b) β-form.

the different phases, irrespective of the conformational freedom of the carboxylic side chain, which, as shown in Table 2 (see Fig. 6 for labelling), adopts markedly different conformations: while the ψ_1 dihedral angles approach 180° in all cases (their values being related to the steric hindrance suffered by the branching methyl on the chain), the other angles (particularly ψ_2 and ψ_3) can adopt a variety of values.

Interestingly, the overall tgtt (t = traus, g = gauche) conformation found for β-DHA (see Table 2 and Fig. 6) leads to a bending of the whole chain, which, differently from those found in α-DHA (ttgt and tttt) and in *p*TolSOMe@DHA (ttgt), does not lean outwards in search for a hydrogen-bonded partner 'in plane' with its steroidal skeleton. Accordingly, the pseudo-centrosymmetric dimer present in α-DHA and depicted in Scheme 1 does not exist in β-DHA and the prototypical pairing of complementary $(RCOOH)_2$ functions through two strong H-bonds is avoided. Indeed, in β-DHA, the swinging OH group nicely connects to a ketonic oxygen atom (O1) of an adjacent molecule, symmetry related by the twofold screw axis parallel to the *b* axis (O4···O1 2.722 Å) (see Scheme 3). This also means that, down *b*, *columns* of DHA molecules are helically packed and that only weak(er) interactions are shared between neighbouring molecules in the *ac*plane. At variance, in the α-phase, DHA molecules give rise to *isolated* dimers (Fig. 7).

The effect of this different *supramolecular* arrangement (*i.e.* 0D *vs.* 1D in α- and β-DHA, respectively) is clearly shown by the rather different (by more than 5%!) crystal densities (see Table 1), DSC traces and solubility properties (*vide supra*), which are *all* consistent with β-DHA as the room temperature *thermodynamically stable phase*. Interestingly, this implies a competition between global and local factors, such as efficient packing *vs.* hydrogen bond topology and molecular conformation. Incidentally, the reported structural features of β-DHA clearly explain the spectroscopic observations depicted above,

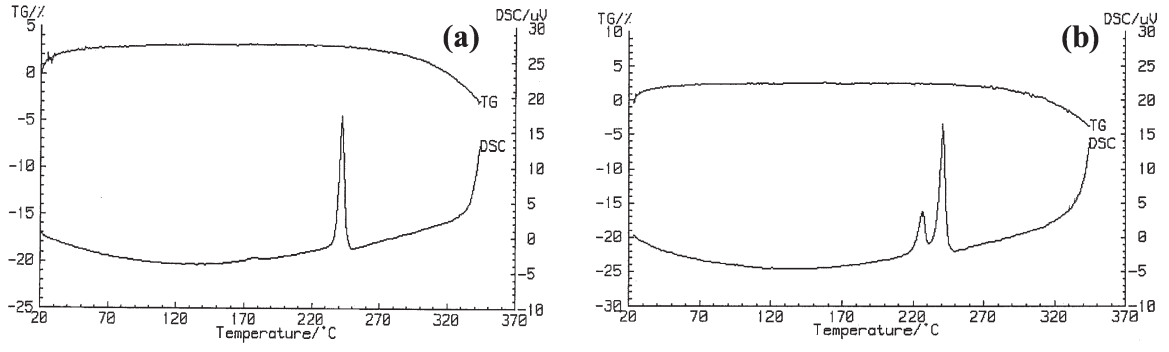


Fig. 3 TGA (top) and DSC (bottom) traces of the two DHA polymorphs: (a) α-form and (b) β-form.

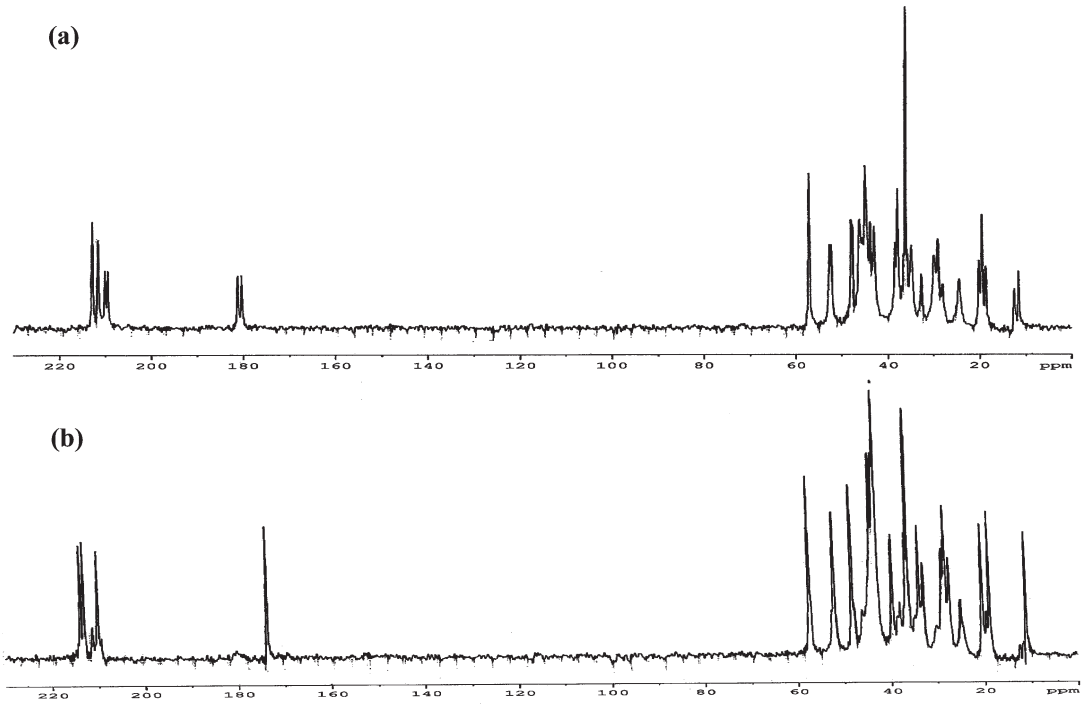


Fig. 4 Solid state CP-MAS ^{13}C-NMR spectra of the two DHA polymorphs: (a) α-form and (b) β-form.

such as the location and the multiplicities of the solid state ^{13}C NMR carbonylic/carboxylic resonances.

Guest-mediated solid state β → α conversion

As extensively described in previous paragraphs, dehydrocholic acid may be obtained as two stable polymorphic forms (α and β) that may interconvert one into the other by solvent-mediated or temperature-mediated process (Schemes 2 and 4). Furthermore, the α- and β-forms spontaneously absorb[12] *R*-(+)-methyl tolyl sulfoxide guest molecules (from racemic mixtures) affording identical inclusion complexes, as proved by XRPD. In the inclusion compound, the sulfoxide molecules are accommodated in the crystal lattice between the DHA molecules by formation of hydrogen bonds of the >S=O···HOC(=O)R type.[13] From the *p*TolSOMe@DHA inclusion product the included guest sulfoxide may be released by heating (166.9 °C),[19] affording the original α-form.

Therefore, dehydrocholic acid β-phase crystals exhibit guest-responsive structural changes, with conversion into the α-form

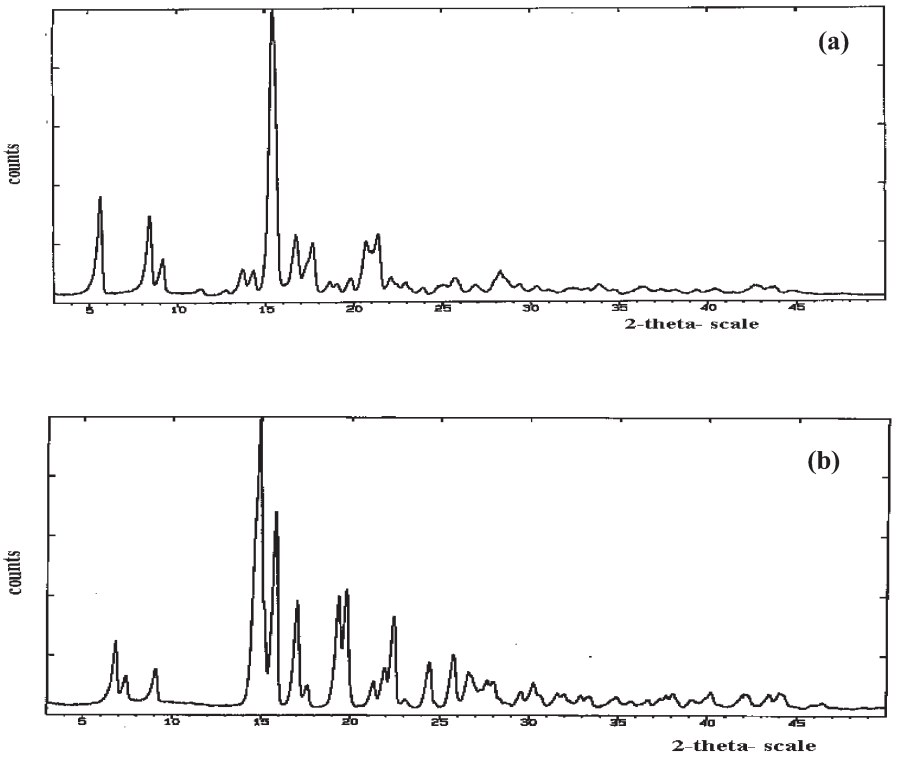


Fig. 5 XRPD traces (Cu Kα radiation) of the two DHA polymorphs: (a) α-form and (b) β-form.

Table 1 Synoptic collection of crystal data for the two DHA polymorphs

DHA polymorph	α	β
Formula	$C_{25}H_{34}O_5$	$C_{25}H_{34}O_5$
FW	402.53	402.53
Crystal system	Triclinic	Monoclinic
Space group	*P*1	$P2_1$
a/Å	6.8583(4)	12.0762(5)
b/Å	10.5646(10)	6.8366(3)
c/Å	15.7879(17)	13.1029(5)
α/°	78.25(1)	90
β/°	86.68(1)	101.069(2)
γ/°	89.29(1)	90
U/Å^3	1118.1(2)	1061.64(8)
Z	2	2
D_{calcd}/g cm^{-1}	1.196	1.259
Method	Single-crystal XRD	Powder XRD
Reference	13	This work

without decomposition or transition through amorphous states. Such a guest-responsive crystal-to-crystal transformation means that the molecules of dehydrocholic acid can move, in particular rotate, while retaining their gross structure. To our knowledge, this unusual transformation has been reported only for the inclusion crystals of cholic acid,[20] aromatic triads[21] and porphyrinogen.[22]

In conclusion, a new crystalline form of dehydrocholic acid, referred to as the β-form, has been obtained, fully characterized and compared with the known α-form of the same derivative. Conversion of the α into the thermodynamically more stable β-polymorph may be obtained by a room temperature solvent-mediated process. Of particular interest is the conversion of the β-form into the α-derivative, achievable *via* two distinct pathways: a temperature-mediated process or a guest-mediated solid state conversion, without the intervention of an amorphous state. Worthy of note, the *converging* sets of analytical data, collected prior to the complete crystallographic study, have been *fully* rationalized on the basis of the structural model obtained from powder diffraction; thus, we have demonstrated that this method, although lacking 'atomic resolution' (in the single-crystal technique sense), can still afford important structural information, particularly if coupled to pre-existent 'flexible' models known from conventional diffraction studies (on polymorphs or solvates), structural databases or even computational methods.

Experimental

Synthesis of β-DHA

The β-form may be obtained, through solvent-mediated transformation, by means of a prolonged contact of the solid α-form with water–acetic acid (1:1) or water–HCl solutions (at pH 3–4) under stirring. The time required for the conversion is 3 or 40 days, depending on the solvent.

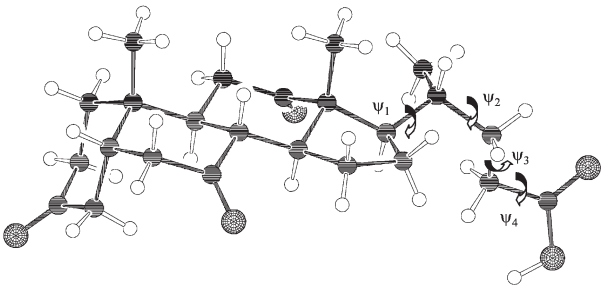
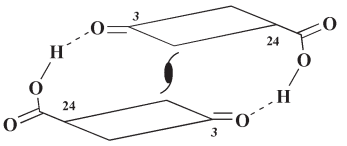


Fig. 6 Drawing of the molecular structure of β-dehydrocholic acid. Carbon atoms as solid spheres, oxygen atoms starred.



Scheme 3 β-form of dehydrocholic acid.

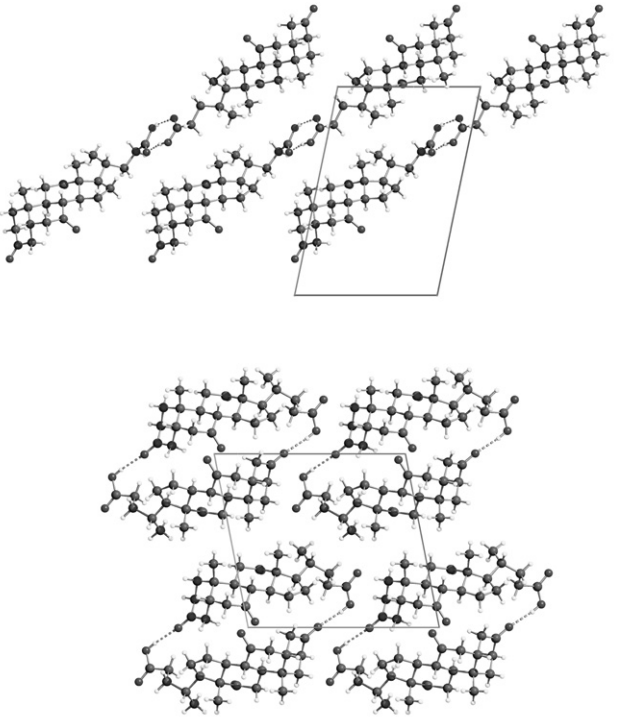
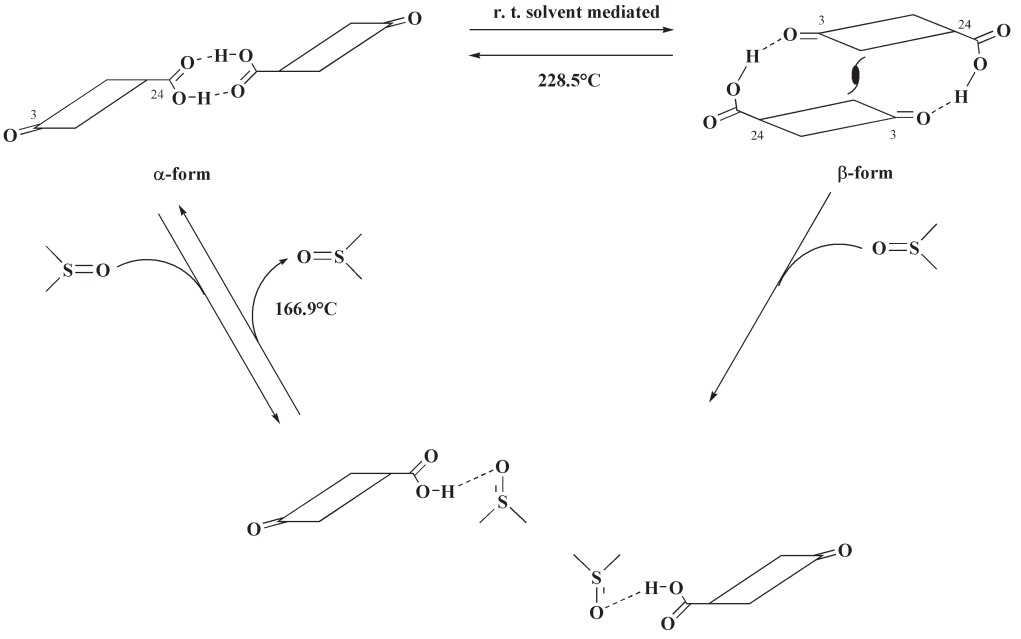


Fig. 7 Crystal packing of the (top) α- and (bottom) β-DHA polymorphs viewed down the [100] and [010] axes, respectively. Horizontal axes are *b* and *a*, respectively. The intermolecular hydrogen bonds are shown as broken lines.

Table 2 Torsional angles (°) and conformations of the carboxylic side chains of DHA in the α- (with two crystallographically independent molecules, A and B) and β-polymorphs, as well as in the crystals of the 1:1 inclusion derivative *R*-(+)-methyl *p*-tolyl sulfoxide@DHA.

Molecule	ψ_1[a]	ψ_2[b]	ψ_3[c]	ψ_4[d]	Conformation	Reference
α(A)	−175.4(8)	−163.1(9)	77.2(13)	−165.5(11)	ttgt	13
α(B)	179.6(9)	−154.5(10)	−152.1(12)	−169.2(12)	tttt	13
β	175.3(2)	71.8(3)	155.0(4)	139.4(6)	tgtt	This work
Inclusion product	−172.5(3)	−165.1(3)	80.4(4)	172.3(3)	ttgt	13

[a] C13–C17–C20–C22. [b] C17–C20–C22–C23. [c] C20–C22–C23–C24. [d] C22–C23–C24–O(H)



Scheme 4 Conversion and interconversion of the α- and β-forms.

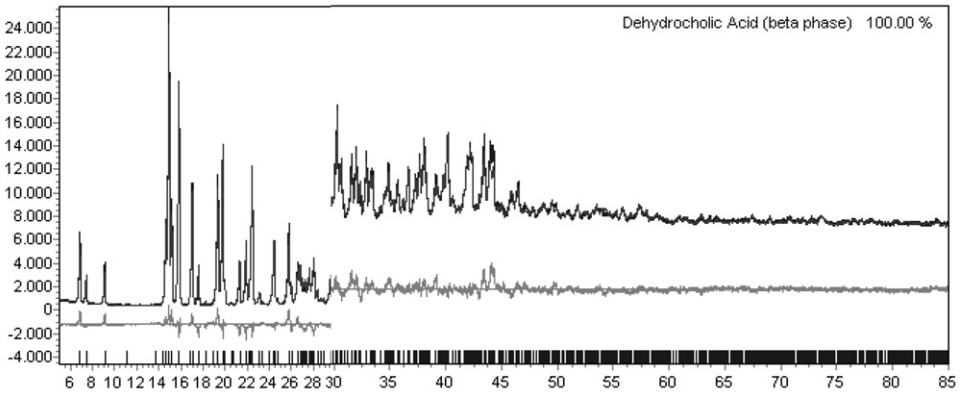


Fig. 8 Rietveld refinement plot for β-dehydrocholic acid. Horizontal scale, $2\theta/°$; vertical scale, counts. Difference plot and peak markers are at the bottom. The section above 30° (2θ) has been magnified (5×).

Characterization

^{1}H NMR spectra were obtained with a Varian Gemini 300 spectrometer and high-resolution ^{13}C magic angle spinning (MAS) NMR spectra were measured with a Bruker MSL-300 operating at 75.5 MHz. Differential scanning calorimetry (DSC) and thermogravimetry (TG) analyses were performed on a Simultaneous Thermal Analyzer STA 409 instrument (Netzsch). IR spectra, in KBr, were obtained with a Nicolet 510P spectrometer operating in Fourier transform mode.

Dehydrocholic samples were examined in a scanning electron microscope (SEM; Cambridge, model Stereoscan S360) operating at 20.0 kV and equipped with a secondary electron detector. The crystals were coated with gold using a Sputter Coater S150 (Edwards) at 10^{-2} mm Hg.

Formation of the *p*TolSOMe@DHA inclusion product

One equivalent of DHA (α- or β-form) was added to 3 equiv. of pTolSOMe dissolved in diethyl ether–ethyl acetate (70:30). After 24 h the solid was filtered, washed several times with the same solution, air dried and analyzed by ^{1}H NMR.

Ab initio X-ray powder diffraction analysis

The white powders were gently ground in an agate mortar and then deposited in the hollow (0.5 mm deep) of a zero-background quartz monocrystal plate. Diffraction data (Cu Kα_1, $\lambda = 1.5406$ Å) were collected on a horizontal scan Seifert MZVI diffractometer, equipped with parallel (Soller) slits, a primary beam curved Ge(111) monochromator, a Na(Tl)I scintillation detector and pulse height amplifier discrimination. The generator was operated at 40 kV and 30 mA. The receiving slit was 0.2 mm. Nominal resolution for the present set-up is 0.07° 2θ (FWHM) for the Si(111) peak at 28.44° (2θ). A long scan was performed over $5° < 2\theta < 85°$, with $t = 30$ s and $\Delta 2\theta = 0.02°$, and used for structure solution and refinement.†

Indexing, using TREOR,[21] of the low angle diffraction peaks suggested a primitive monoclinic cell of approximate dimensions $a = 13.08$, $b = 6.82$, $c = 12.05$ Å, $\beta = 101.06°$ [$M(20)^{21} = 27$; $F(20)^{21} = 57$ (0.011, 36)]. Systematic absences indicated $P2_1$ as the probable space group, later confirmed

† Crystallographic data for the beta phase of dehydrocholic acid, including fractional atomic coordinates, have been deposited with the Cambridge Structural Database, CCDC 213098. See http://www.rsc.org/suppdata/nj/b3/b307145f/

by successful solution and refinement. The structure solution was initiated by the simulated annealing technique implemented in TOPAS,[21] using a DHA fragment identical to one of the DHA molecules found in the α-polymorph (free to translate and rotate), flexible at the four torsional angles of the carboxylic side-chain. The final refinements were performed by the Rietveld method with the aid of TOPAS, with peak shapes described by the fundamental parameters approach and an isotropic crystal size broadening factor of Lorentzian contribution. The background was modelled by a polynomial function, while further systematic errors were corrected with the aid of a sample-displacement shift and a preferred orientation model (010 pole, in the March–Dollase[21] formulation); a single isotropic displacement parameter was also refined. Scattering factors were taken from the internal library of TOPAS. Final agreement factors R_p, R_{wp} and R_{Bragg} were 0.071, 0.095, 0.064, respectively, for 4000 data points collected in the $5° < 2\theta < 85°$ range. The final Rietveld refinement plot is shown in Fig. 8.

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