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# Crystal structure of 3β,12α-dihydroxy-5β-cholan-24-oic acid (iso-deoxycholic acid)

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**Abstract**

Iso-deoxycholic acid (3β,12α-dihydroxy-5β-cholan-24-oic acid, iso-DCA), the 3β hydroxy epimer of deoxycholic acid (DCA), was synthesized from deoxycholic acid. The crystals, obtained by recrystallization from *p*-xylene, are orthorhombic, space group $P2_12_12_1$, with $a = 7.3232(6)$, $b = 10.5938(16)$ and $c = 28.2957(18)$ Å. The crystals contained no inclusion molecules, and thus differed from those of DCA when recrystallized from *o*-, *m*- and *p*-xylene. This absence of solvate molecules could explain the reduction of ≈3 Å in the *b*-axis length in iso-DCA when compared with that of DCA with xylene guests. 

*Keywords*: Crystal structure; Bile salts; Iso-deoxycholic acid

## 1. Introduction

Bile salts are key biological surfactants in vertebrates. A common bile acid is cholic acid (3α,7α,12α-trihydroxy-5β-cholan-24-oic acid, CA), which is converted in vivo by bacterial enzymes to deoxycholic acid (3α,12α-dihydroxy-5β-cholan-24-oic acid, DCA). The perspective structural formula of cholic and deoxycholic acids illustrate that their hydroxy groups lie beneath the plane of the steroid skeleton and its protruding methyl groups lie above it. Bile salts thus contain an hydrophobic side, an hydrophilic side and a short hydrophilic tail [1]. Therefore, they possess a planar polarity and in aqueous solution form aggregates above their critical micellization concentration. The aggregates form mixed micelles with insoluble, polar lipids. In bile, the mixed micelles consist of bile salts, phospholipids and cholesterol. In intestinal contents, the mixed micelles contain bile salts, fatty acids and monoglycerides [2]. The self-aggregation behavior of bile salts has been studied by different techniques in different experimental conditions [3–15], but there is not yet an agreement about the mechanism of formation and structure of the aggregates formed. Three main structures have been proposed [1,16–18]. Those proposed by Giglio et al. [17] for several bile salts in aqueous solution have been constructed based on principles derived from their crystal structures.

Solutions of sodium deoxycholate (NaDC) present a particular behavior. At pH values close to neutrality

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it forms gels [19–24], a characteristic not described for other bile salts with the exception of chenodeoxycholic acid at high salt concentrations [25]. Hydrophobic interactions and hydrogen bonding between hydroxyl groups play an important role in this aggregation [26,27]. The 3β hydroxy epimer has an hydroxy group directed to the apolar part of the molecule, where the methyl groups are located; the global polarity being considerably affected with respect to the original compound. Therefore, it would be of interest to study how this change in the configuration of the 3-hydroxy groups affects the physicochemical properties of the bile salt (aggregation properties, gel formation, crystal formation, etc.). It is also of interest to test whether this change influences the formation of crystalline inclusion compounds, as has been described for cholic [28–37] and deoxycholic acids [38–45]. To this end, we have synthesized iso-DCA and solved its crystal structure.

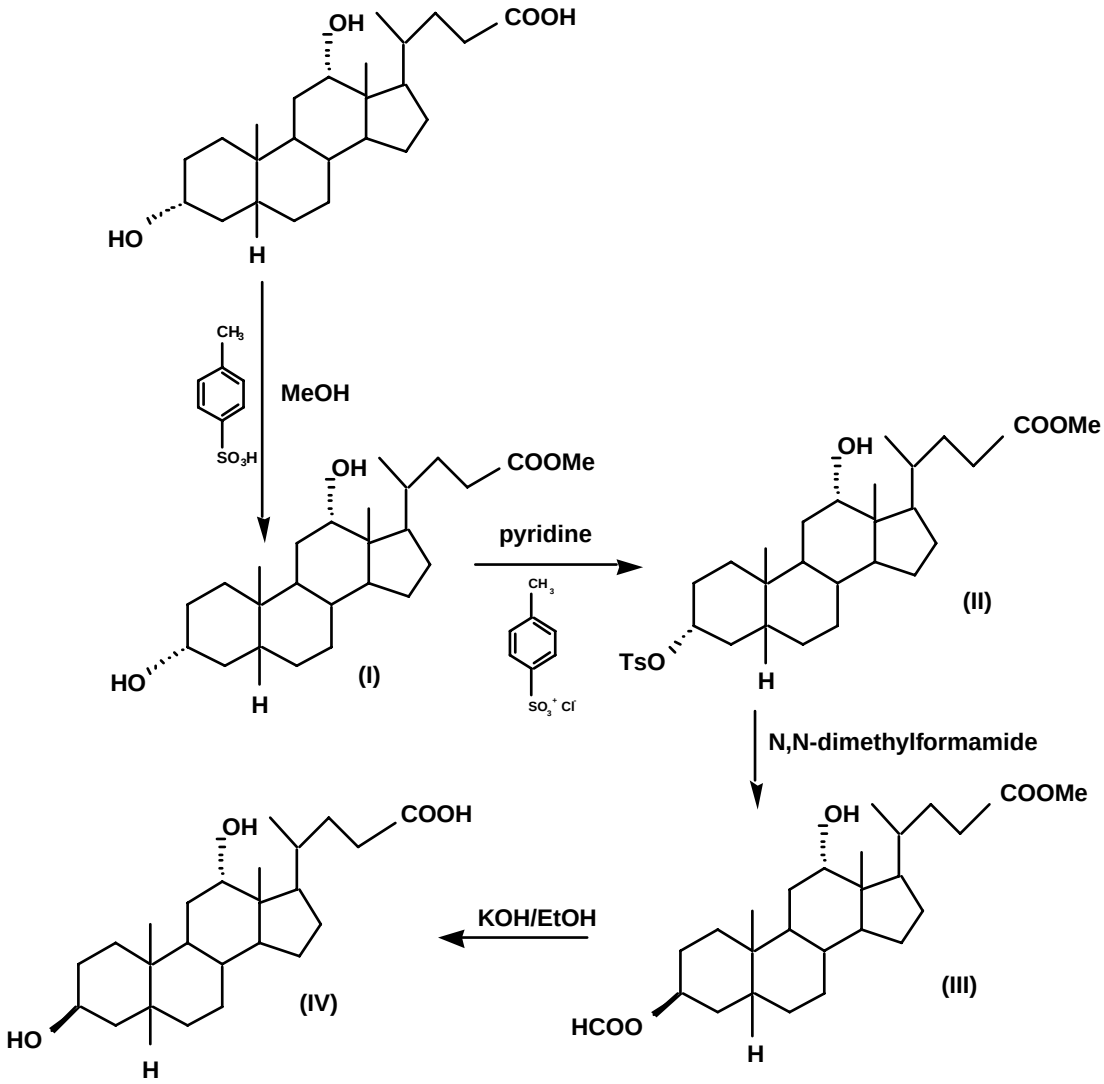


Fig. 1. Scheme of the synthesis of iso-DCA.

Table 1
Crystal data, data collection and refinement

| | |
|---|---|
| Empirical formula | $C_{24}H_{40}O_4$ |
| Formula weight | 392.56 |
| Temperature (K) | 291(2) |
| Wavelength (Å) | 1.54184 |
| Crystal system, space group | Orthorhombic, $P2_12_12_1$ |
| A (Å) | 7.3232(6) |
| B (Å) | 10.5938(16) |
| C (Å) | 28.2957(18) |
| α | 90° |
| β | 90° |
| γ | 90° |
| Volume (Å$^3$) | 2195.2(4) |
| $Z$, calculated density (g cm$^{-3}$) | 4, 1.188 |
| Absorption coefficient (mm$^{-1}$) | 0.619 |
| F(000) | 864 |
| Crystal size (mm) | 0.40 × 0.20 × 0.20 |
| θ range for data collection (°) | 3.12–74.18 |
| Index ranges | $-9 <= h <= 0$, $0 <= k <= 13$, $0 <= l <= 35$ |
| Reflections collected/unique | 2576/2576 [$R$(int) = 0.0000] |
| Absorption correction | Psi-scan |
| Maximum and minimum transmission | 0.977 and 0.953 |
| Refinement method | Full-matrix least-squares on $F^2$ |
| Data/restraints/parameters | 2576/0/255 |
| Goodness of fit on $F^2$ | 1.088 |
| Final $R$ indices [$I > \sigma(I)$] | $R1 = 0.0423$, $wR2 = 0.1093$ |
| $R$ indices (all data) | $R1 = 0.0590$, $wR2 = 0.1188$ |
| Absolute structure parameter | 0.0(4) |
| Extinction coefficient | 0.0041(4) |
| Largest diff. Peak and hole | 0.293 and −0.162 e.A$^{-3}$ |

Table 2
Atomic coordinates ($\times 10^{-4}$) and equivalent isotropic displacement parameters ($A^2 \times 10^{-3}$) for iso-DCA. $U_{(eq)}$ is defined as one third of the trace of the orthogonalized $U_{ij}$ tensor

| | $x$ | $y$ | $z$ | $U_{(eq)}$ |
|---|---|---|---|---|
| O(25) | 5260(3) | 1230(2) | 1753(1) | 52(1) |
| O(26) | 1185(3) | 5368(2) | 3415(1) | 47(1) |
| O(27) | −608(4) | 7472(3) | 5938(1) | 75(1) |
| O(28) | −3536(4) | 7199(2) | 6114(1) | 66(1) |
| C(1) | 1988(4) | 1818(3) | 2335(1) | 41(1) |
| C(2) | 3213(4) | 2753(3) | 2085(1) | 45(1) |
| C(3) | 5177(4) | 2301(3) | 2073(1) | 45(1) |
| C(4) | 5840(4) | 1941(3) | 2562(1) | 42(1) |
| C(5) | 4584(4) | 1048(2) | 2829(1) | 37(1) |
| C(6) | 5347(4) | 784(3) | 3326(1) | 43(1) |
| C(7) | 5089(4) | 1897(3) | 3661(1) | 41(1) |
| C(8) | 3090(4) | 2307(2) | 3686(1) | 33(1) |
| C(9) | 2403(3) | 2649(2) | 3184(1) | 32(1) |
| C(10) | 2563(4) | 1496(2) | 2846(1) | 35(1) |
| C(11) | 488(4) | 3249(3) | 3189(1) | 38(1) |
| C(12) | 191(4) | 4275(2) | 3561(1) | 35(1) |
| C(13) | 757(3) | 3801(2) | 4056(1) | 32(1) |
| C(14) | 2784(4) | 3407(2) | 4022(1) | 33(1) |
| C(15) | 3376(4) | 3237(3) | 4536(1) | 42(1) |
| C(16) | 2241(4) | 4216(3) | 4812(1) | 45(1) |
| C(17) | 824(4) | 4787(3) | 4466(1) | 36(1) |
| C(18) | −479(4) | 2701(3) | 4200(1) | 42(1) |
| C(19) | 1332(4) | 399(3) | 3003(1) | 47(1) |
| C(20) | −995(4) | 5141(3) | 4708(1) | 43(1) |
| C(21) | −2388(5) | 5714(4) | 4366(1) | 65(1) |
| C(22) | −699(4) | 6032(3) | 5131(1) | 51(1) |
| C(23) | −2304(5) | 6077(3) | 5465(1) | 57(1) |
| C(24) | −2199(5) | 6976(3) | 5868(1) | 47(1) |

## 2. Experimental

The synthesis of the 3β,12α-dihydroxy-5β-cholan-24-oic acid (iso-deoxycholic acid, iso-DCA) was carried out following that reported in literature with minor modifications. It begins (see Fig. 1) with the preparation of the methyl ester of DCA (I) [46]. To a solution of this bile acid (4 g) in 120 ml of absolute methanol was added *p*-toluenesulfonic acid (520 mg). After stirring for 12 h at room temperature, the reaction was complete (followed by TLC $CHCl_3/CH_3OH$ 9:1). The reaction mixture was slowly dripped over ice chips with stirring, forming a precipitate. This precipitate was washed, vacuum filtered and then dissolved in ether. The ether phase was washed with dilute base (0.5 M NaOH), water, dried with sodium sulfate, concentrated down to dry powder with the rotavapor, and then dried under vacuum for a few hours.

Methyl 3α-tosyloxy-12α-hydroxycholanate (II) was prepared [47] by addition at room temperature of *p*-toluenesulfonyl chloride (1.83 g) and dry pyridine (20 ml) to the methyl ester (I) (3 g); yield is nearly quantitative. The reaction was monitored by TLC ($CHCl_3/CH_3OH$ 9:1) and was completed in two days. Work up of the reaction involves neutralization of pyridine with HCl 1 M (260 ml) dripped in an ice bath, then vacuum filtered and dissolved in ether. The ethereal phase was washed three times with water until pH 7, dried and evaporated.

The obtained product was dissolved in *N*,*N*-dimethylformamide (80 ml) at a constant temperature of 77°C and the reaction, monitored by TLC (toluene/acetone 7:3), showed complete disappearance of the tosylate in 60 h [48]. The reaction content was slowly dropped in ice and water, and the precipitate of methyl

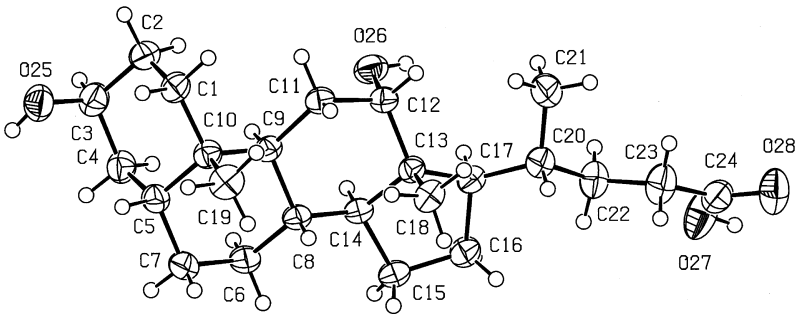


Fig. 2. Perspective view of the molecule, showing 70% probability ellipsoids for the non hydrogen atoms and the numbering scheme of the atoms in the molecule.

3β-formate 12α-hydroxy cholanate (III) extracted with ether and dried.

Compound III was hydrolyzed by the slow addition of KOH 10% (w/w) (40 ml) and ethanol (95%) (40 ml) [42]. The reaction, monitored by TLC ($CHCl_3/CH_3OH$ 9:1), was complete in 3–4 h. Ethanol was evaporated and the remaining solution acidified. The precipitate of iso-DCA (IV) was filtered and dissolved in ether. The ether phase was washed with water until the water phase was neutral. The ether was then dried, filtered and evaporated. Purification on a silica gel 60 Merck column (particle size 0.040–0.063 mm) with $CHCl_3/CH_3OH$ 8.5–9.5:1.5–0.5 as mobile phase. Crystals were obtained by re-crystallization from *p*-xylene.

### 2.1. X-ray data collection and reduction

Crystal data, data collection procedure and refinement details are reported in Table 1, and fractional atomic coordinates in Table 2. A colourless prismatic crystal of iso-DCA was mounted on a glass fiber and used for data collection. Cell constants and an orientation matrix for data collection were obtained by least-squares refinement of the diffraction data from 25 reflections in the range of $22.794° < \theta < 42.642°$ on an ENRAF-NONIUS CAD4 automatic diffractometer. Data were collected at 291 K using $CuK_\alpha$ radiation ($\lambda = 1.54184$ Å) and the omega-scans mode and corrected for Lorentz and polarization effects [49]. A semi-empirical absorption correction (Psi-scans) was made [50].

### 2.2. Structure solution and refinement

The structure was solved by direct methods [51] which revealed the position of all non-hydrogen atoms, and refined on $F^2$ by a full-matrix least-squares procedure using anisotropic displacement parameters [52]. All hydrogen atoms were located in difference map and included as fixed contributions riding on attached C atoms with isotropic thermal parameters 1.2 or 1.5 times those of the respective C and O atoms. The absolute structure for the compound was chosen according to the Flack parameter [53]. Inspection of $F_c$ and $F_o$ values indicated that a correction for secondary extinction was required ($F_c^* = kF_c[1 + 0.001 \times F_c^2\lambda^3/\sin(2\theta)]^{-1/4}$), where $k$ is the overall scale factor, and $x$ (extinction parameter) refined to 0.0041(4) in the final run. Atomic scattering factors were taken from *International Tables for X-ray Crystallography* [54]. Molecular graphics are from PLATON [50] and SCHAKAL [55]. A summary of the crystal data, experimental details and refinement results are listed in Table 1.

## 3. Results and discussion

Fig. 2 represents a schematic view, showing a crystal structure similar to that reported for other bile acids with the same ring junctions in *cis* configuration. As can be seen in Fig. 3, the packing diagram for iso-DCA shows an orthorhombic crystal system with the space group $P2_12_12_1$. This is the same crystal system and space group as reported for cholic acid

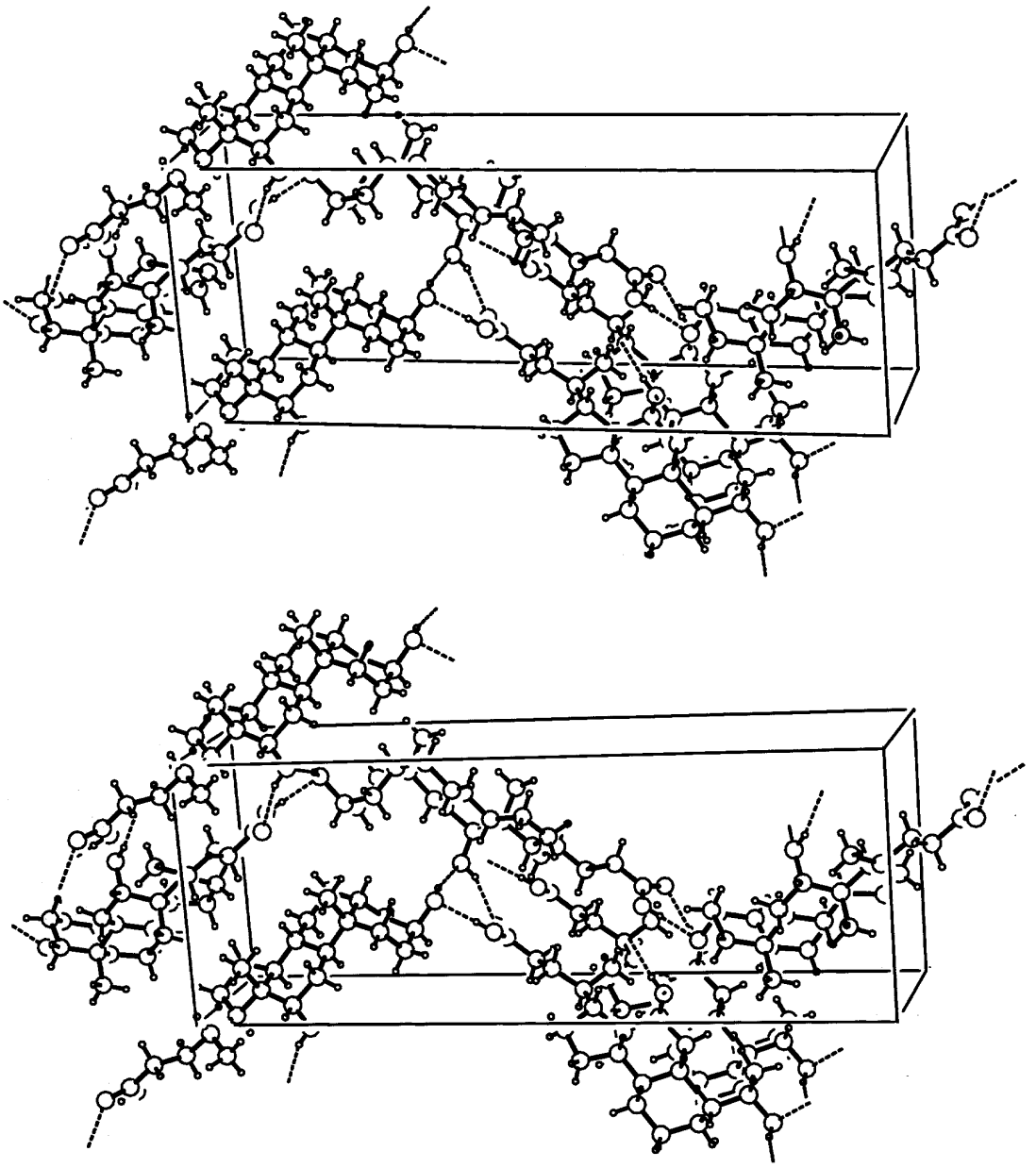

Fig. 3. View of the crystal structure of the molecule. The origin of the unit cell is in the rear of the lower left corner, with *a* pointing toward the reader, *b* from left to right and *c* upward.

crystals containing no guest molecules [56]. However, the crystal structure of DCA has been solved only for crystals containing different inclusion compounds and not for the acid devoid of solvate molecules. Therefore we cannot make a comparison with our data.

The crystallographic structure for DCA recrystallized from xylenes was established by Gallese et al. [57]. Table 3 summarizes the results from Gallese et al. compared with those obtained in this work for iso-DCA. The crystal systems for DCA are respectively monoclinic for *o*- and *p*-xylene and orthorhombic for *m*-xylene. This crystal system was also found for

Table 3
Comparison of some crystal data for deoxycholic and iso-deoxycholic acid recrystallized from xylenes

| Crystal/solvent | DCA/*o*-xylene | DCA/*m*-xylene | DCA/*p*-xylene | iso-DCA/*p*-xylene |
|---|---|---|---|---|
| Space group | $P2_1$ | $P2_12_12_1$ | $P2_1$ | $P2_12_12_1$ |
| *a* (Å) | 7.24 | 7.20 | 7.27 | 7.32 |
| *b* (Å) | 26.17(unique axis) | 13.69 | 13.38(unique axis) | 10.59 |
| *c* (Å) | 13.51 | 25.75 | 27.08 | 28.29 |
| β (°) | 90.9 | 90.00 | 91.00 | 90.00 |
| Host/guest ratio | 2:1 | 2:1 | 2:1 | |

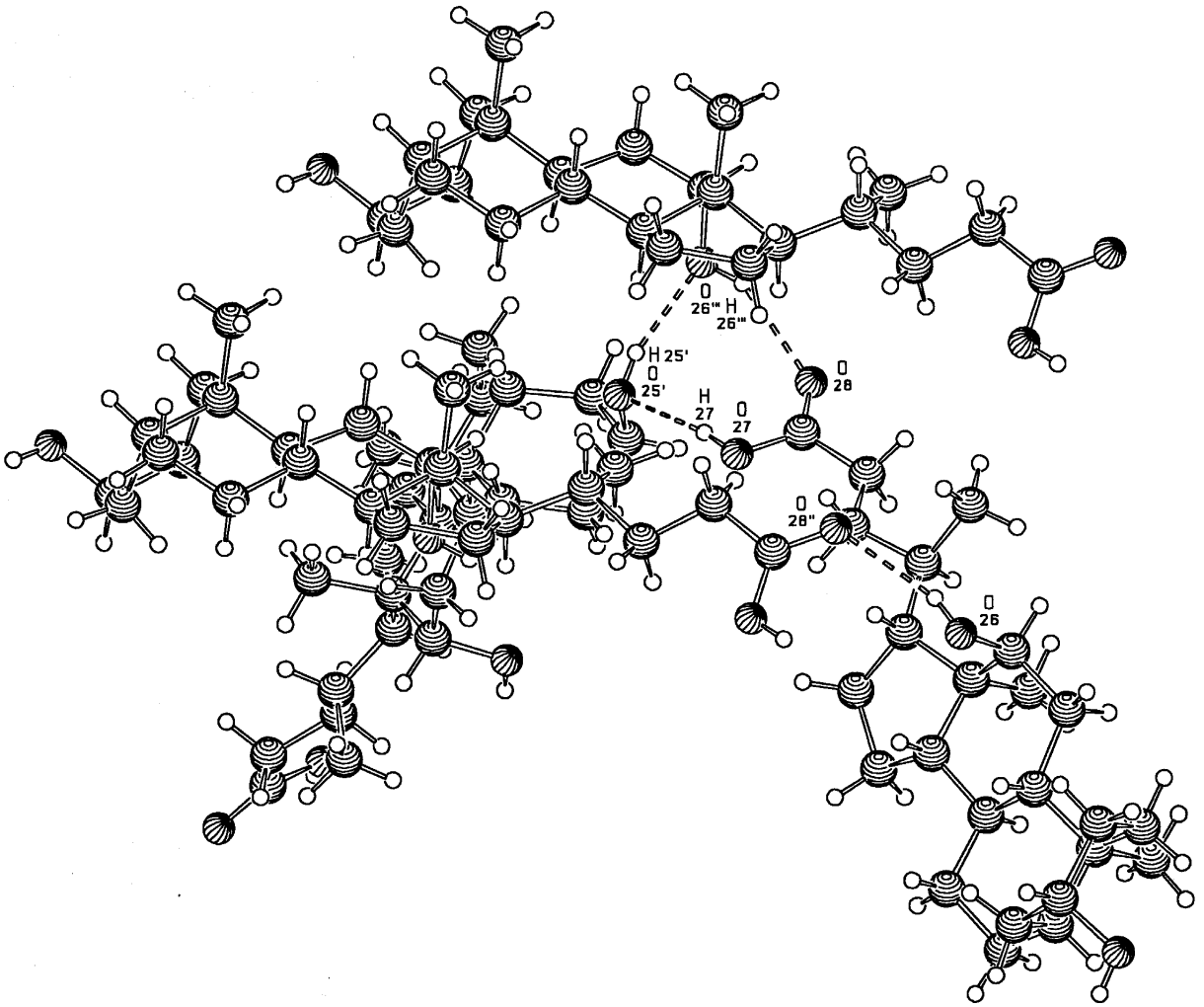


Fig. 4. View of a hydrogen-bonded molecular group. Some intermolecular hydrogen bonds are shown by dashed lines.

Table 4
Hydrogen bonds for iso-DCA

| D–H···A | d(D–H) (Å) | d(H···A) (Å) | d(D···A) (Å) | < (DHA) (°) |
|---|---|---|---|---|
| O(26)–H(26)···O(28)[a] | 0.82 | 2.01 | 2.800(3) | 161.3 |
| O(27)–H(27)···O(25)[b] | 0.82 | 2.16 | 2.908(3) | 152.3 |
| O(25)–H(25)···O(26)[c] | 0.82 | 1.88 | 2.697(3) | 171.5 |

[a] Symmetry transformation used to generate equivalent atoms: $-x+1, y-1/2, -z+1/2$.
[b] $x+1/2, -y+3/2, -z+1$.
[c] $-x+1/2, -y+1, z+1/2$.

iso-DCA in *p*-xylene. Moreover, both epimers belong to the α group, because of the short length of one of their axes (≈7.3 Å). The main difference between them is the length of *b*-axis, which is ≈3 Å smaller in the epimer of DCA. It may be caused by the absence of solvent molecules in the crystal network of iso-DCA. On the other hand, the length of *c*-axis is ≈2.5 Å higher in iso-DCA than in DCA.

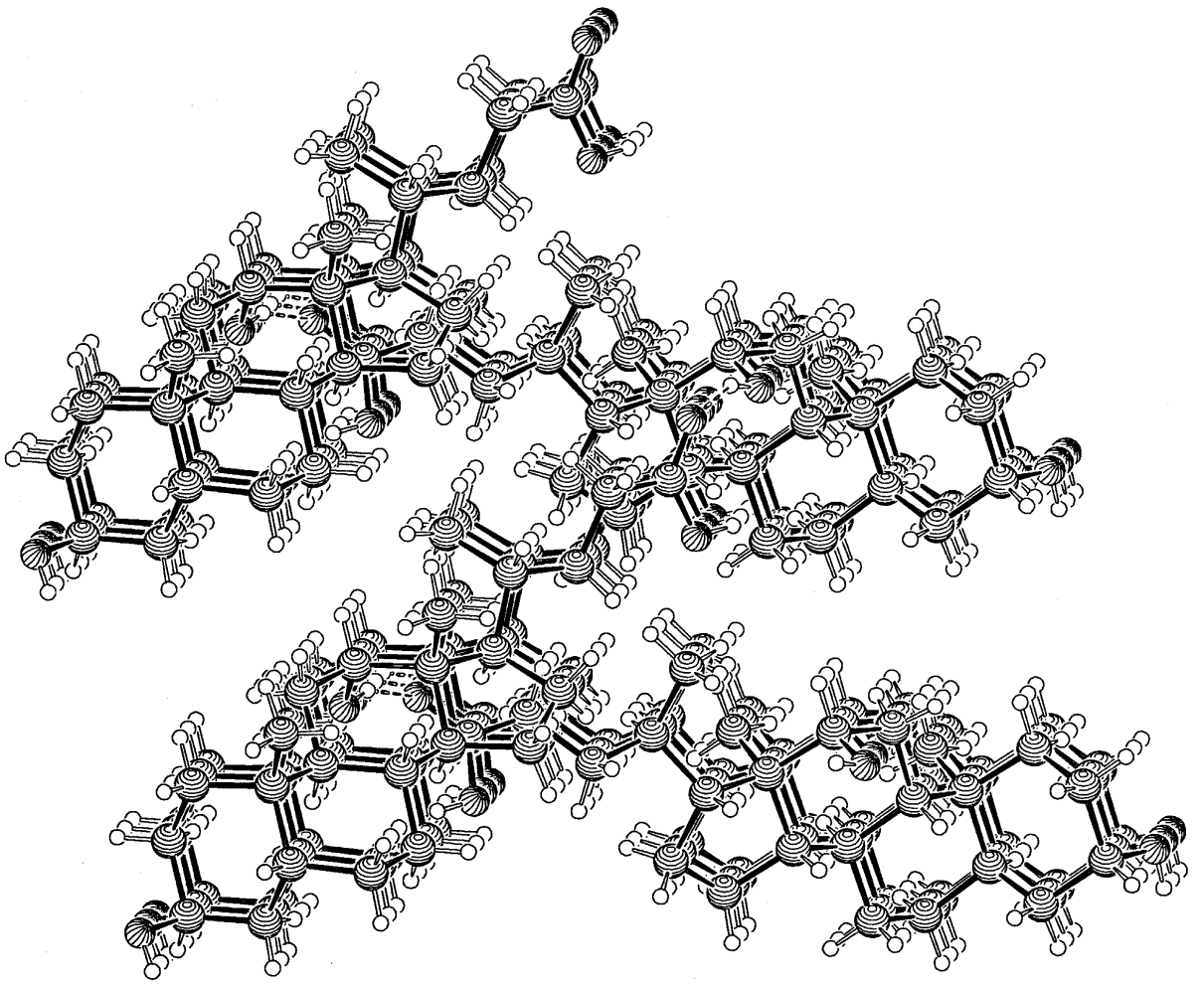

Fig. 5. View showing the self-organization of the molecules along the *b*-axis.

This fact can be attributed to the different spatial orientation of the hydroxyl group at C-3 in both epimers.

Fig. 4 shows the hydrogen-bonded molecular group. Three bile acid molecules are connected involving the carboxylic O and OH of the first, the OH at 12α of the second and the OH at 3β of the third one (O(28)–H(26), O(26)–H(25), O(25)–H(27)). On the other hand, the OH at 12α of this molecule interacts with the oxygen atom of the carboxylic group of a new one (H(26)–O(28)). Bond lengths and angles of these interactions are summarized in Table 4, and the self-organization of the molecules can be seen in Fig. 5.

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